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Spotted lantern fly, a new pest from Asia

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FOREWORD

This meeting was the 28th 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 continues to be realized and facilitated through this meeting. This proceedings documents the efforts of many individuals: those who organized and sponsored the meeting, those who provided oral and poster presentations, and those who compiled and edited the contributions. The proceedings illustrates the depth and breadth of studies being supported by the agencies and their many cooperators and demonstrates the benefits and accomplishments that can result through the spirit of collaboration.

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PRESENTATIONS

Keynote Address

THE ROLE OF SPECIES INTERACTIONS IN FOREST INVASION

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ABSTRACT

Across the globe, actions of humans have changed abiotic and biotic conditions to favor the success of invasive species. These include 1. modifying invaders' dispersal patterns (e.g., humans transport species beyond their historical geographic ranges), 2. altering environmental suitability (e.g., eutrophication, changed land use, fragmentation, changing temperature or rainfall) and 3. disrupted long-standing biotic interactions within native communities (e.g., loss of predators and enemy release, trophic downgrading, trophic cascades, plant-soil feedbacks, changes in competitive hierarchies following environmental change). In my talk, I focus on disrupted native species interactions that are occurring in forests across the globe driven by overabundant ungulates (e.g. Vavra et al. 2007) and invasive species (Mitchell et al. 2006) and present results from my lab demonstrating the linkage between overabundant white-tailed deer, exotic invader success and native plant performance declines.

Data were collected in a long-term (2003-2016) replicated deer exclusion/deer access paired plot experiment in a Pennsylvania forest and used to assess the effects of deer on the demographic performance of both native and invasive herbaceous species, focusing on both native species that are highly palatable or highly unpalatable to deer. These results are compared to analyses of data collected in similar deer exclusion experiments in Virginia and to those from studies in natural forested sites in Pennsylvania that experience a range of deer browse intensity. Further, field and controlled environment experiments were conducted to test the role mutualism disruption of the AMF-native plant nutritional mutualism by the allelochemicals released by garlic mustard (Alliaria petiolata M. Bieb (Brassicaceae) into the soil.

Our data from the paired plots and natural forests in Pennsylvania indicate that white-tailed deer (Odocoileus virginianus Zimmerman; henceforth deer) are dramatically changing species interactions and abiotic conditions in the forest to favor invasive species. Demographic and vital rate analyses of the deer-preferred native forest herb species (large white trillium, Trillium grandiflorum [Michx.] Salisb. (Liliaceae), red trillium T. erectum L. (Liliaceae), false Solomon's seal Maianthemum racemosum L. Link(Ruscaceae) indicate that these species' population growth rates and vital rates decline significantly where deer are abundant (Knight et al. 2009; Kalisz et al. 2014; Brouwer et al. 2015). Even the highly unpalatable native herb, Jack-in-the-pulpit (Arisaema triphyllum [L.] Schott (Araceae)) and five other unpalatable native species, show negative effects in areas where deer are abundant in Pennsylvania and Virginia, despite the fact that they are not browsed by deer (Heckel et al. 2010; Burke et al. 2011). Declines in vital rates or size of these unpalatable species shown to be due to indirect negative effects that overabundant deer have on forest soil quality. Where deer are most abundant, their effects are strong enough to cause divergence in the life history traits in Jack-in-the-pulpit populations (Heckel and Kalisz 2016).

In contrast, the invader, garlic mustard, exhibits significant increases in population growth rate where

deer are overabundant, but declines where deer are excluded (Kalisz et al. 2014), in part because deer suppress the biotic resistance of the native plant community and diminish its competitive strength against garlic mustard invasion. Further, deer find garlic mustard unpalatable (Kalisz et al. 2014; Averill et al. in review), increasing its edge as a forest invader. However, where deer are excluded, the population growth rate of garlic mustard declines significantly (Kalisz et al. 2014). In addition, in these areas where heavily deer browse the native understory community and remove significant amounts of the herb layer leaf area, light levels at the forest floor are higher, and garlic mustard exhibits higher photosynthetic rates than in areas where it deer area excluded, further the invader's success (Heberling et al. in press). Together our data demonstrate that deer facilitate the invasion success of garlic mustard through suppression of biotic interactions in the ground layer of the forest and altered abiotic conditions.

We demonstrated that the invader, garlic mustard, releases allelochemicals into the forest soil that are toxic to bacteria and fungi, both harmful and beneficial strains in the soil (Cantor et al. 2011). Since ~80% of the understory forest herb community are partners with arbuscular mycorrhizal fungi (AMF) (e.g. Brundrett and Kendrick 1988) in a nutritional mutualism (i.e. AMF supply water and soil nutrients to plants, plants supply carbohydrates to AMF), we predicted that where it invades forests, garlic mustard's allelochemicals will have significant negative effects for the herbaceous layer of the forest through AMF mutualism disruption (Hale and Kalisz 2012). We found that garlic mustard disrupts the AMF-native plant root nutritional mutualism in the forest soil through the release of allelochemicals (Cantor et al. 2011). This mutualism disruption caused declines in growth and other vital rates in false Solomon's seal, a native herbaceous species (Hale et al. 2011; Brouwer et al. 2015). In greenhouse experiments with this same species, we show that growth and photosynthetic rate are significantly reduced by garlic mustard's allelochemical, similar to a non-systemic fungicide application (Hale et al. 2016). Thus, the combined effects of overabundant deer and invasive species can combine to significantly negatively impact the performance of the forest understory. While we focus on the forest herbaceous perennials, undoubtedly, these negative effects extend to the advance regeneration woody seedling layer that forms nutritional mutualisms with ectomycorrhizal fungi (EMF) and exchange carbon for soil nutrients and water.

Together the results of my lab and others point to the need for co-management of deer and invaders. Efforts to mitigate the damage caused by human mediated effects on forests could include increasing the number of predators on deer, including human hunting effort or other methods to reduce deer herd sizes, removal of invaders, restoration of mutualism through forest soil inoculation with beneficial AMF and EMF spores, protection and restoration of native species and application of rotational fencing techniques to periodically relieve over-browsing pressure on native species.

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PRESENTATIONS

Northeastern Biocontrol Regional Project (NE-1332)
Honoring 27 Years of Leadership by Dick Reardon in Research
and Implementation of Biological Control of Forest Pests

OUTRUNNING THE INVASIVE SPECIES TREADMILL: INTEGRATING BIOLOGICAL CONTROL AND OTHER MANAGEMENT TECHNIQUES TO RESTORE INVADED SITES

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ABSTRACT

The goal of many weed biological control programs in natural areas is to restore native plant communities and/or ecosystem services to a pre-invasion level. In some cases, biological control alone may achieve this objective; however, in others integration of biological control with additional management techniques is necessary. Successful control of one weed species may result in replacement by another, the invasive treadmill effect. Integrating biological control with herbicides, mechanical control, fire, grazing or other techniques to manage invasive weeds may successfully control the target weed and prevent the invasive treadmill effect. Mile-a-minute weed [*Persicaria perfoliata* (L.) H. Gross] is an aggressive annual vine that has invaded the eastern United States. Successful biological control of mile-a-minute weed by the weevil *Rhinoncomimus latipes* Korotyaev has led to replacement by other invasive weeds in some sites.

Biological control was successfully integrated with native plantings in two small-scale field experiments in this system to restore plant communities. One experiment combined biological control with plantings of a native seed mix that included warm and cool season grasses and an annual and a perennial forb. Native plant richness and diversity increased in these plots after three years. The second experiment integrated weevil releases with a single application of a pre-emergent herbicide and plantings of a native forb and tree. Mile-a-minute seedling counts were significantly higher in the no-herbicide plots in 2009, as well as 2010 and 2011, a legacy of the one-time treatment in 2009. After two years, the cover of native plants in the integrated treatment plots was greater than 80%. In 2015, the number of mile-a-minute seedlings no longer differed between the herbicide and no-herbicide plots and were very low across all treatments. Six years post-treatment one site does not need additional management, mile-a-minute cover has increased at a second site, and one of the replacement weeds, Japanese stiltgrass [*Microstegium vimineum* (Trin.) A. Camus] has increased at a third site. In both experiments, the integration of management techniques reduced the abundance of the target weed, promoted recruitment of additional native plant species that were not included in the plantings, and at least temporarily prevented dominance by other invasive plants compared to non-planted control plots. Sites will vary, and customized and adaptive management strategies will be required.

BIOLOGICAL CONTROL OF EMERALD ASH BORER: CAN NATURAL ENEMIES PROTECT NORTH AMERICAN ASH?

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ABSTRACT

After the inadvertent introduction of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire, Coleoptera: Buprestidae) from Asia to the United States in the 1990s (Siegert et al. 2014), this destructive beetle spread widely and degraded ash communities (*Fraxinus* spp.) over much of the north central and northeastern North America. The EAB biocontrol program started shortly after the pest's detection in southeast Michigan in 2002 (Bauer et al. 2015). This involved foreign exploration and introduction of specialist natural enemies of EAB from its native range. To date, the egg parasitoid *Oobius agrili* Zhang and Huang (Encyrtidae) and the larval parasitoid *Tetrastichus planipennisi* Yang (Eulophidae) from China have established in many regions of North America where they were released (Bauer et al. in press). While the role of *O. agrili* in reducing EAB population growth requires further evaluation (Abell et al. 2014), we have found that *T. planipennisi* currently plays a significant role in protecting ash saplings and small trees (DBH <12 cm) in long-term study sites in southern Michigan (Duan et al. 2013, 2015a ; JJD, LSB and RGVD, unpublished data). This reduction in EAB larval density allows for continued growth and survival of ash saplings and small trees and is likely to spread geographically as populations of *O. agrili* and *T. planipennis* increase in density and disperse beyond the initial release sites.

As ash trees grow larger, however, the outer bark on lower trunks becomes too thick for EAB- larval parasitism by *T. planipennisi* because of its short ovipositor (2.0 to 2.5 mm) (Duan and Oppel 2012; Abell et al. 2012). To protect larger diameter ash trees, we recommend releases of the braconid larval parasitoids *Spathius agrili* Yang from China to southern regions of the U.S. and *S. galinae* Belokobylskij from the Russian Far East to northern regions (Duan et al. 2015b; USDA-APHIS/ARS/FS, 2016). Ovipositors of these parasitoids are about twice as long as that of *T. planipennisi*, allowing them to successfully parasitize EAB larvae under the thicker bark on large diameter ash trees. A second species of egg parasitoid, *O. primorskyensis* Yao & Duan (Encyrtidae) from the Russian Far East (Yao et al. 2016; Larson and Duan, 2016), which is currently being evaluated for release as an EAB biocontrol agent, may be needed in some regions.

As densities of EAB decline following the first invasion wave in North America, future studies will be needed to evaluate the (1) growth and survival rates of ash in aftermath forests with self-sustaining and increasing biocontrol agent populations, (2) persistence of key ash-dependent invertebrates (Wagner and Todd 2016), and (3) repetition of basic population work in new regions as EAB invades different regions of the U.S., which are climatically and ecologically distinct from the northern areas on which this discussion was based.

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NATIVE *SIREX-AMYLOSTEREUM-DELADENUS* MEET INVASIVE *SIREX NOCTILIO*

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ABSTRACT

Sirex noctilio is an invasive wood borer from Eurasia/North Africa, obligately associated with a white rot fungus (usually *Amylostereum areolatum)*, and it principally attacks pine trees. *Sirex noctilio* has invaded pine plantations around the Southern Hemisphere since approximately 1900. Phytotoxic venom (from glands within female abdomens) plus the white rot fungus *Amylostereum areolatum* (vectored in female mycangia), are both injected into trees by *S. noctilio* females, and these work together to kill pines, thus allowing *S. noctilio* larvae to develop. *Sirex noctilio* appears to prefer attacking suppressed pines (Haavik et al. 2016) although when population densities are high, healthy trees can be successfully attacked (Madden 1975).

A specimen from an established population of *S. noctilio* was first collected in North America in 2004 in Fulton, New York. Since then, this spreading invasion has become the first example where *S. noctilio* has been introduced to a region where pines and siricid communities are native. Our laboratory has been studying the interactions between *Sirex noctilio*, the strains of fungus that it uses and the nematodes associated with it in North America. Along with these studies we have studied the sympatric native communities using pines: *Sirex nigricornis*, *Amylostereum areolatum* or *Amylostereum chailletii* and *Deladenus proximus*. Very little is known about the biology and ecology of *S. nigricornis* in the northeastern US although *S. nigricornis* is generally known as a much less aggressive species than *S. noctilio*, regarding use of pines. In Europe, *S. noctilio* was reported as ovipositing only in standing wood (Spradbery & Kirk 1978). While *S. nigricornis* attacks suppressed pines (Hartshorn et al. 2015) as does *S. noctilio*, in Arkansas this native will also oviposit into wood on the ground, from recently cut trees (Hartshorn 2012).

A major method for control of *S. noctilio* in the Southern Hemisphere has been use of the Sopron and then Kamona strains of the nematode *Deladenus siricidicola* which sterilize female *S. noctilio* in Australia. In particular, we have been asking whether the strain of *D. siricidicola* presently used for biological control in the Southern Hemisphere (Kamona) would impact the native *S. nigricornis*, if this nematode strain was used for control of *S. noctilio* in the US.

From 2007-2015, a very different aspect of this system has become obvious during our studies. While *S. nigricornis* co-infests some trees with S. noctilio, the numbers of these natives are much lower than numbers of *S. noctilio* emerging from co-inhabited trees. In New York State, the flight and oviposition period for *S. noctilio* is mostly in July and August, while most *S. nigricornis* adults fly in September and early October. Therefore, *S. noctilio* oviposits in suppressed trees before *S. nigricornis* is flying. Also, relative to the sizes of females, the venom glands in *S. noctilio* are much larger than venom glands in *S. nigricornis*. While studies have characterized the venom of *S. noctilio* (Bordeaux et al. 2014) and have shown that it is phytotoxic (Coutts 1969; Fong and Crowden 1973; Bordeaux et al. 2014), we know nothing about the contents of the smaller venom glands in S. nigricornis.

Sirex noctilio that emerge in the northeastern US are significantly larger than *S. nigricornis* and will therefore lay more eggs (see Haavik et al. 2016b). In addition, we have found that about half of *S. nigricornis* we have sampled from red pine in New York State have a 2 year life cycle while only 5-25% of *S. noctilio* develop within wood for 2 years.

The information above is based on sampling and rearings from pines with resin beads which are characteristic of *S. noctilio* infestations. To date, however, we have not investigated use of dead pines by *S. nigricornis* in the northeast. Such studies are necessary in order to evaluate the population densities of *S. nigricornis* that might be developing in wood not acceptable to or used by *S. noctilio*.

In summary, the native *S. nigricornis* appears to be outcompeted by *S. noctilio* when utilizing trees normally acceptable to *S. noctilio* in the northeastern US. The resulting low densities of these native *Sirex* makes it difficult for us to study the potential for non-target impacts of parasitic nematodes that could be used for *S. noctilio* control in the US.

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PRESENTATIONS

Invasive Organisms in Native Ecosystems:
Effects on Structure and Function

RESPONSE OF GROUND-DWELLING ARTHROPODS TO TEMPORAL PATTERNS OF ASH MORTALITY CAUSED BY EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) has resulted in widespread, nearly simultaneous formation of canopy gaps in forests as ash trees die. Canopy gaps increase light availability and alter the environment on the forest floor, including increased soil temperature, soil moisture, and understory vegetation growth. Ash snags fall after several years as they uproot or snap along the bole, and high volumes of coarse woody debris (CWD; large branches and logs) can accumulate on the forest floor, depending on ash density. These changes to the forest floor environment have the potential to impact populations of ground-dwelling invertebrates.

Based on these patterns of ash mortality, we predict that the magnitude of effects of canopy gap formation and accumulation of ash CWD on ground-dwelling invertebrate communities will vary inversely over time (Perry and Herms 2016a). Canopy gaps are predicted to have the greatest impact during the early stages of EAB-induced ash mortality that decline over time as they close by growth of suppressed understory and surrounding canopy trees. The amount of CWD on the forest floor is predicted to be low initially and increase over time as standing dead snags fall. Therefore, effects of ash CWD on ground-dwelling invertebrate communities are predicted to be greatest during advanced stages of EAB-induced ash mortality.

Results of studies conducted during early stages of ash mortality in Ohio, and late stages of ash mortality in southeast Michigan were consistent with these predictions. When canopy gaps were presumably at their maximum size and levels of downed CWD were low, ground beetle (Coleoptera: Carabidae) assemblages were altered by gap formation, but not low levels of CWD (Gandhi et al. 2014, Perry and Herms 2016b). Gandhi et al. (2014) documented decreased ground beetle activity-abundance and diversity, and distinct assemblages in hydric forest stands dominated by black ash (*Fraxinus nigra* Marsh.) compared to mesic and xeric stands dominated by green (*Fraxinus pennsylvanica* Marsh.) and white (*Fraxinus americana* L.) ash, respectively. Perry and Herms (2016b) reported decreased activity-abundances of two common ground beetle species, *Cyclotrachelus convivus* (LeConte) and *Pterostichus stygicus* (Say) in canopy gaps, but no effects of low levels of CWD. Perry and Herms (2016a) observed decreased ground-dwelling invertebrate richness and diversity in newly formed gaps, which was driven by decreased activity-abundances of harvestmen (Opiliones), ground beetles, scarab beetles (Scarabaeidae), camel crickets (Rhaphidophoridae), and three families of springtails (Hypogastruridae, Isotomidae, and Sminthuridae).

During advanced stages of EAB-induced ash mortality, Ulyshen et al. (2011) documented higher densities of invertebrates near ash CWD, but small canopy gaps had no effect. Abundance of earthworms (Annelida), spiders (Araneae), harvestmen, isopods (Isopoda), millipedes (Diplopoda), beetles (Coleoptera), and springtails (Collembola) increased with proximity to as CWD. Subsequent studies in these plots found that total activity-abundance, evenness, and diversity of ground-dwelling invertebrates was

higher near recently fallen ash logs than more decayed logs, but again no effect of small canopy gaps was observed. Invertebrate community composition was similar near logs of the same decay class, but variation in composition increased away from downed woody debris.

Results of these studies are consistent with the predicted inverse temporal relationship between the effects of canopy gaps and accumulation of ash CWD caused by EAB-induced ash mortality on ground-dwelling invertebrate communities (Perry and Herms 2016a), and suggest that disturbance from EAB may have persistent impacts on forest communities.

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PRESENTATIONS

Update on the Response to Spotted Lanternfly
and Supporting Research in Pennsylvania

UPDATE ON SPOTTED LANTERNFLY IN PENNSYLVANIA

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ABSTRACT

An update of a program to contain and eradicate Spotted lanternfly (HEMIPTERA: FULGORIDAE: *Lycorma delicatula* (WHITE)) which was implemented in Pennsylvania by the Pennsylvania Department of Agriculture is summarized. Egg mass scraping was found to be effective to kill the egg stage, tree bands were found to be effective in killing first through third instar nymphs, and fourth instar nymphs and adults required active capture techniques. Adults were found to be associated primarily with tree of heaven (*Ailanthus altissima* (Philip Miller)Swingle) in late summer, so a strategy of tree of heaven removal and trap tree establishment was employed. Combined control measures including egg mass scraping, tree banding, and active capture performed by volunteers and paid crews killed 1,972,951 spotted lanternflies by the end of 2016. Spotted lanternfly was detected at 2,815 individual points on 956 separate properties since 2014. Many new sites were detected as a result of public reports which were 89% accurate. The insect is now known from six counties (Berks, Bucks, Chester, Montgomery, Lehigh, and Northampton) and 74 municipalities in Pennsylvania. A quarantine restricting the movement of the pest and conveyances was extended the new areas of detection. Surveys at 7,565 other locations in Pennsylvania were negative as were trace forward surveys in other states. Tree removal and trap tree establishment are taking place at several properties that were found to have the highest populations or were smaller remote populations. Though not yet quantified, visual evidence indicates that spotted lanternfly mortality is extremely high on treated properties. Property management is not keeping pace with the insect's expansion.

Excellent cooperation led to the early detection of and rapid response to this new invasive threat. Continued cooperation from the agencies and researchers involved, the local public officials, local businesses, and especially the owners of affected properties, who have in many cases volunteered, has been crucial for the response and containment to the introduction of this new pest.

DNA-BASED INVESTIGATIONS OF THE SPOTTED LANTERNFLY, *LYCORMA DELICATULA* (WHITE)

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ABSTRACT

To better understand the origin of the Spotted Lanternfly, *Lycorma delicatula* (White*)* in Pennsylvania, and to characterize its potential impact, three subprojects were conducted as summarized below.

1. Identification of the origin of *L. delicatula* in Pennsylvania using microsatellite sequencing: Six microsatellite loci were sequenced for 32 individuals from the Pennsylvania population of *L. delicatula*, 25 individuals from each of three Korean populations, 19 individuals from China, and 2 individuals from Vietnam. Results indicate 1) the Pennsylvania population appears to represent a single introduction, and 2) the Pennsylvania population is strongly differentiated from all of the other sampled populations, and as such, the origin of this invasion is not yet known. Novel microsatellite loci are currently being developed in order to provide more informative genetic loci that show greater variation within the Pennsylvania population, in order to allow for tracking of population structuring and detection of subsequent introductions. The novel microsatellite loci will also be used to genotype a more extensive sampling of *L. delicatula* within its native range, and hence, serve to better estimate the origin of the current invasion.

2. Characterization of the microbial associates harbored by *L. delicatula*: A region of the bacterial 16S rRNA gene was amplified and deep sequenced on the Illumina MiSeq platform to characterize the bacterial communities harbored in the abdomen and proboscis of 10 *L. delicatula* individuals from the Pennsylvania population. Bacteria from the proboscis did not amplify for any individuals. Abdominal bacterial communities were dominated by three obligate endosymbionts, *Sulcia muelleri, Vidania fulgoroideae*, and an uncharacterized Gammaproteobacterium, consistent with other sampled outgroup species of Fulgoridae. However, principal components analysis indicated *L. delicatula* was distinctly different from other sampled Fulgoridae and Dictyopharidae. In order to determine whether these results perhaps reflect a unique bacterial community within the gut due to feeding on *Ailanthus altissima* (Miller) Swingle, bacterial communities harbored by *L. delicatula* nymphs feeding on grape were sequenced. The composition of the bacterial communities harbored by grape-feeding nymphs was nearly identical to that of adults. To test the hypothesis that the bacterial communities harbored by *L. delicatula* are distinctive in being less diverse due to a founder effect (i.e., genetic bottleneck), bacterial communities were compared for *L. delicatula* individuals sampled from the species' native range (China) to those sampled from two invasive ranges (Pennsylvania and Korea). Preliminary results are consistent with this hypothesis as the bacterial communities harbored by *L. delicatula* in China were significantly more diverse than those sampled from Pennsylvania and Korean populations.

3. Characterization of changes in microbial communities on plants associated with *L. delicatula* feeding and honeydew deposition: A region of the bacterial 16S rRNA gene and a region of the eukaryotic 26S rRNA gene were amplified and deep sequenced to characterize the bacterial and fungal communities harbored in a frothy substance collected from *A. altissima* trees heavily infested with *L. delicatula*

in Pennsylvania. Bacterial communities from six froth samples were dominated by two bacterial genera, *Acetobacter* and *Leuconostoc*, both of which are aerobic fermenting bacteria capable of converting sugars to acetic acid and lactic acid, respectively. Analysis of fungal communities indicated that the most abundant species detected was *Geotrichum candidum*, a ubiquitous yeast, also capable of aerobic fermentation. These microbes may be feeding upon sugars present in *L. delicatula* honeydew and/or seeping tree sap. Further investigation is underway to examine a broader range of tree species for evidence of froth production in order to better understand the potential scope and impact of this unusual phenomenon.

SPOTTED LANTERNFLY AND GRAPEVINES: DAMAGE ASSESSMENT AND CHEMICAL CONTROL

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ABSTRACT

Spotted lanternfly (*Lycorma delicatula* (White)) is a polyphagous planthopper that has the potential to become a serious pest of many agricultural commodities in Pennsylvania. Previous studies indicate spotted lanternfly has a preference for tree of heaven (*Ailanthus altissima*) and grapevine (*Vitis vinifera*), which could endanger Pennsylvania's more than $20.5 million grape industry. Feeding from this insect reduces overall plant vigor, increases disease susceptibility, and attracts secondary pests. In addition, these planthoppers secret a sticky, sugar-rich liquid called "honeydew" which accumulates at the base of their feeding area and encourages sooty mold growth and the formation of fungal mats. Korean research indicates sooty mold growth on grapevines blocks sunlight absorption, increases labor costs, and decreases fruit marketability. Private and commercial vineyard owners in the Berks Co., PA quarantine area did not report spotted lanternfly in their vineyards in 2014 and 2015. In 2016, these owners did report spotted lanternfly populations on their grapevines, particularly in rows adjacent to forested areas. Adult insects were commonly observed on wild grapevines in courting pairs or clusters. Feeding competition between adult females was recorded in the field and in our laboratory colonies. This coming season, we plan to perform insecticidal bioassays in a commercial vineyard to test the efficacy of insecticides registered for grapevines. We will continue to observe and record spotted lanternfly behavior on cultivated and wild grapes within the quarantine zone. Lastly, we are currently taking temperature readings to verify degree-day models for *L. delicatula*. Results from these studies will be made available to grape growers to inform them about the potential impact of this pest and aid them in making pest management decisions.

KAIROMONES AND TRAP TECHNOLOGY FOR THE SPOTTED LANTERNFLY, *LYCORMA DELICATULA* (HEMIPTERA: FULGORIDAE)

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ABSTRACT

The spotted lanternfly (SLF) *Lycorma delicatula* (Hemiptera: Fulgoridae) is a newly invasive pest in eastern Pennsylvania where attempts are underway for its eradication. It is a univoltine phloem feeder whose primary host is tree-of-heaven *Ailanthus altissima*, but which has been found to feed on numerous other hosts including species of *Acer, Actinidia, Alnus, Aralia, Arctium, Betula, Cornus, Elaeagnus, Malus, Metaplexis, Morus, Phellodendron, Platanus, Populus, Prunus, Quercus, Rhus, Robinia, Rosa, Rubus, Salix, Styrax, Toona,* and *Vitis*. Recently, we also recorded large numbers feeding on Chinaberry *Melia azedarach* trees in Anhui province in China. They are invasive in Korea where they are a serious pest of grape, causing damage by feeding in large aggregations causing plants to wilt, and producing copious amounts of honeydew and sooty mold which ultimately blocks photosynthesis.

In order to assist in the eradication efforts, improved survey and detection tools are needed. At the start of this project in the spring of 2015, the best survey tool available was a brown sticky band made by a company in Korea. Bands are wrapped around tree trunks and SLF get captured as they walk across the surface as they migrate up the tree. Although these bands were able to capture 1st instar, 2nd instar, and 3rd instar nymphs, the 4th instar nymphs and the adults were able to avoid capture. In addition, no lures existed for SLF, although spearmint oil was reported as an attractant by Korean researchers. Therefore, our goal was to identify attractants and improve upon current detection tools. The goal of this project is the discovery of novel kairomones and the improvement of trapping technology for SLF. In support of this work, laboratory rearing capabilities and behavioral bioassays needed to be developed and conducted concurrently with host plant volatile collections and field trapping.

Observations on biology. Since the primary host of SLF is *A. altissima*, initial volatile collections and literature review focused on odors from *Ailanthus* and compared them to odors from grape. SLF were field collected in PA and brought to the Otis Laboratory insect containment facility where they were reared and used in behavioral bioassays. Nymphs were housed in cages with a choice of fresh cuttings of *Ailanthus* and a potted grape plant. Feeding by 1st and 2nd instars was observed on both host species, however, 3rd and 4th instars appeared to ignore the grape plant and fed only upon *Ailanthus* cuttings, and those that didn't have access to *Ailanthus* cuttings died. This suggested there might be an obligatory host shift to *Ailanthus* around the 3rd instar. Because of the possibility of different host preferences by different instars, we attempted behavioral bioassays and field testing of attractants for all stages. Additionally, in the end of August 2015 when we field-collected adults from aggregations on *Ailanthus* to use in laboratory bioassays, it was discovered that nearly all of the 400 adults collected from the aggregation were female. It was

later discovered that aggregations of males were on other species, and that the aggregations on *Ailanthus* gradually became more mixed in sex ratio over the mating season (see abstract by Michael Domingue, "Semiochemicals and mating behavior of adult spotted lanternfly"). For this reason, the adult stage was categorized into early (same-sex aggregations), mid (mating and mixed-sex aggregations), and late (oviposition), recognizing that adults at different stages of development are in different physiological states and would potentially respond differently to semiochemicals. Early adults seem to be feeding heavily on *Ailanthus* and would likely still be attracted to kairomones, whereas mid adults are finding mates and late adult females are ovipositing.

Host plant volatiles. Volatile collections were conducted in the summers of 2015 and 2016 on *Ailanthus*, wild grape, and Chinaberry and analyzed by gas chromatography coupled with mass spectrometry. In addition, literature was searched for volatiles for these three species and compared to our results. From these, several compounds of interest were selected based on abundance or unique presence in *Ailanthus*. Most compounds were available commercially, and one sesquiterpene (A) had to be synthesized.

Behavioral bioassays. Custom designed Y-shaped Teflon bioassay plates of two sizes were used to test nymphs and adults for attraction to compounds in the laboratory in the summers of 2015 and 2016. Lures for bioassays were made by placing 1 ul of compound in a 0.25 ml microtube and piercing a hole in the cap. These were placed upwind in one arm of the bioassay, and an empty microtube was placed in the other arm as a control. Seven odors were tested and of these, three (an alcohol, an ester, and a sesquiterpene) were found to be highly attractive in the Y-plate to one or more stages of SLF. The same compounds were also tested using 10 ul, but this reduced attraction. Spearmint oil was not significantly attractive.

Field studies in PA in 2015. Three field studies were attempted in PA in 2015. The first study targeted 1st and 2nd instars, and tested five odors on brown sticky bands from Korea, as well as blank brown sticky bands, and blank clear sticky bands (by Alpha Scents). Within each block, the seven treatments were deployed to *Ailanthus* trees that were within approximately 7 m from each other. Ten blocks of treatments were set up at 10 field sites within the quarantine zone. The five odors to be tested were formulated into lures by Alpha Scents and contained: 1) spearmint oil, 2) an acetate from *Ailanthus*, 3) an alcohol from *Ailanthus,* 4) a blend of the alcohol, acetate, and a sesquiterpene (B) from *Ailanthus* (ratio 2:1:1), a blend used for brown marmorated stink bug (BMSB). The study was replicated at ten field sites. The alcohol caught the most SLF (11.6 per trap), followed by the *Ailanthus* blend (8.2 per trap), the BMSB blend (6.6 per trap), no lure (4 per trap), the acetate (3.3 per trap), spearmint oil (3.1 per trap), and the clear band with no lure (1.5 per trap). However, there was a large amount of variation in the field and there were no significant differences.

The second field study was disrupted by eradication efforts. The third field study targeted adults, which we knew would not get caught on the brown sticky bands from Korea. Therefore, we tested two types of traps, the brown sticky bands from Korea and the purple prism sticky trap for emerald ash borer, wrapped around tree trunks. On both traps we tested five treatments: the alcohol from *Ailanthus,* the ester from *Ailanthus,* a blend of the alcohol and ester (1:1), and a commercially available sesquiterpene blend that contained small amounts of the attractive sesquiterpene (A), and blank controls. The purple prism traps caught more SLF adults than the brown sticky bands in all cases. The alcohol+ester and the ester alone caught the most SLF adults, followed by the alcohol, the sesquiterpene blend, and the control.

Field studies in China and PA in 2016. Twenty field studies were conducted on all SLF stages in three locations in China in the summer of 2016, a Chinaberry plantation at a southern site in Anhui province, and urban *Ailanthus* landscaping trees at two northern sites in Beijing. Five of these studies were also replicated in PA as well. Several of the same tests were conducted in two locations to improve results. Tests can be categorized as follows:

- Main attractants (alcohol, ester, sesquiterpene, control)
- Dose response to the ester (control, 1/2x, 1x, 2x)
- Blends of ester and alcohol (100:0, 75:25, 25:75, control)
- Trap technology (four tests)
- Attract and kill (two tests)

In 2016, lures were formulated in-house, and we conducted release rate studies to compare them to the Alpha Scents lures used in 2015. Results showed that the release rates of the in-house lures were much lower than those made by Alpha Scents in 2015. The sesquiterpene lure, which was not developed until 2016, released about 16 mg/d initially under lab conditions at 22 C and decreased to about 5 mg/d after two weeks. Although not significantly different, there was a trend that the sesquiterpene lure caught more SLF than the ester or the alcohol lures which released much less material than in 2015. When the dose was doubled by using two ester lures, the release rate exceeded that in 2015 and we caught significantly more SLF compared to the controls. Future work will focus on improving lure formulation for higher release rates.

Trap technology. Several types of sticky bands were tested and compared with the Korean brown sticky bands. A Chinese packing tape caught as many or more SLF than the Korean sticky bands, but did not fare as well or last as long in the sun and weather. An American company, Web-Cote, produced a light brown sticky band which was acquired late in the season and compared. This sticky band captured 30 times more adult SLF than the Korean sticky bands. Future work will be conducted to test it against other stages of SLF.

Phenology. Time to first emergence was recorded for the three field sites and is depicted in the figure below. At the southern-most site of Anhui, first emergence of 1st instar SLF preceded the Beijing site by about 3 weeks, and PA by about a month. By adulthood, the two China sites had converged but were still ahead of PA by several weeks.

NATURAL ENEMIES OF THE SPOTTED LANTERNFLY IN ASIA AND NORTH AMERICA

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ABSTRACT

Spotted Lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), is a sporadic pest of Tree-of-Heaven, *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae). The natural distribution of *L. delicatula* includes most of China (especially northern China), Taiwan and Vietnam. As an exotic pest, *L. delicatula* invaded Korea in 2004, Japan in 2009, and the U. S. in 2014. In North America, it is now found in six counties (Berks, Bucks, Chester, Lehigh, Montgomery, and Northampton) in eastern Pennsylvania, with more than 2,000 km^2 under state quarantine. Host species in its native range include more than 70 woody plants and vines in 25 families, such as apple, birch, grape, cherry, lilac, maple, poplar, and stone fruits (Zhou 1992, Kim et al. 2011, Dara et al. 2015). Feeding by nymphs and adults on phloem tissue causes oozing wounds on trunks and branches, resulting in wilting or death of the branches. Nymphs and adults also excrete large amounts of honeydew, attracting ants, bees, hornets, and promoting the growth of sooty mold. Significant damage has been recorded in vineyards in Korea, and it is a potential threat to the grape and fruit industries in Pennsylvania and beyond in North America (Barringer et al. 2015, Dara et al. 2015).

Natural enemies of *L. delicatula* in its native range of Asia include a solitary egg parasitoid, *Anastatus orientalis* Yang & Choi (Hymenoptera: Eupelmidae) (Choi et al. 2014, Kim et al. 2011, Yang et al. 2015); and a solitary ecto-parasitoid on nymphs, *Dryinus browni* Ashmead (Hymenoptera: Dryinidae) (Yan et al. 2008). In North America, a few generalist predators such as *Apoecilus cynicus* (Say) (Hemiptera: Pentatomidae) and *Arilus cristatus* (L.) (Hemiptera: Reduviidae) have been reported attacking *L. delicatula* adults in the field (Barringer and Smyers 2016).

Containment strategy since its discovery in North America has been focused on mechanical /chemical host tree removal and population suppression through egg scraping, tree banding and insecticide treatments. Should the eradication efforts fail, biological control could be the management option for *L. delicatula* population in natural landscapes where management with insecticides may be too expensive and/or environmentally undesirable. We carried out a study in in eastern and northern China 2016 to explore for potential biological control agents, and to survey of its natural enemies in Pennsylvania.

In North America, we selected four study sites (R1, R2, OD, and LU) near the initial introduction in Berks County in Pennsylvania for field surveys and collections. Egg masses and live nymphs of *L. delicatula* were collected from March to April and in July respectively and brought back to the quarantine laboratory for rearing of potential parasitoids. A total of 252 egg masses (7,846 eggs) were collected from those four study sites, with most egg masses found on black birch, black cherry, *Ailanthus*, and sweet birch trees. We found adults of an egg parasitoid on the surface of some *L. delicatula* egg masses at two

study sites (OD and LU) during the field collections. Adults of the same species were also reared out in the laboratory from *L. delicatula* eggs collected from the OD site. This parasitoid was later confirmed as *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) (Liu and Mottern 2017). This parasitoid utilized 6.8% of the egg masses from that site, with 19.9% of the eggs from those egg masses parasitized. A total of 1,185 2nd - 4th instar *L. delicatula* nymphs were also collected from those four study sites. An unidentified Dryinidae (Hymenoptera) was found attacking a 4th instar nymph.

In China, exploration surveys were carried in four provinces in spring 2016. A total of 11 sites were surveyed, including Daxing, Yongdinghe, and Haidian in Beijing; Tangshan, Yongqin, and Chende in Hebei; Yantai in Shandong; and Baodi, Wuqin, Waihuan, and Guanggang in Tianjin. A total of 286 egg masses and 529 nymphs of *L. delicatula* were collected. Native egg parasitoid, *Anastatus orientalis* was recovered from nine of the 11 sites, with an egg mass parasitism of 5.5 to 92.3%, and overall egg parasitism of 0.4 to 26.0%. A native nymphal parasitoid, *Dryinus browni,* was found attacking a few 2nd-3rd instar *L. delicatula* nymphs in Beijing and Shandong.

Ooencyrtus kuvanae is an egg parasitoid of gypsy moth (*Lymantria dispar* (L.) (Lepidoptera: Erebidae)) from Japan. It was introduced into the Unites States in 1908 for gypsy moth biological control. Successful establishment was achieved in Massachusetts by 1911. Since then, millions of *O. kuvanae* have been released throughout the areas infested by gypsy moth in North America (Brown 1984). Release and subsequent recovery of *O. kuvanae* in Pennsylvania occurred between 1969 and 1971 (Smilowitz and Rhoads 1973). *Anastatus orientalis* utilizes 30.4% of *L. delicatula* egg masses, with 40.2% of all eggs being parasitized in the field in China (Yang et al. 2015). Both species have the potential to be important agents for *L. delicatula* biological control in North America. Projects are underway in 2017 to study the seasonal abundance of *O. kuvanae* in the field, to expand field surveys at more sites for potential natural enemies in Pennsylvania, to continue foreign exploration surveys in China for additional natural enemy species, and to evaluate the potential of *A. orientalis* as an introduced parasitoid for *L. delicatula* biological control in North America.

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SEMIOCHEMICALS AND MATING BEHAVIOR OF ADULT SPOTTED LANTERNFLY

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Abstract

The spotted lanternfly (SLF), *Lycorma delicatula,* (Hemiptera Fulgoridae) is an invasive pest to the Korea and the United States of Asian origin. The primary adult hosts are *Ailanthus* trees, and secondary adult hosts are grapevines (*Vitis*). Little is known about its adult behavior with respect to mating or dispersal. Here we performed studies to elucidate movement by adults in relation to host plants and disturbance. We also collected volatiles of the adult insects for potential examination of semiochemical attraction.

First we noted weekly changes in their presence and sex ratio on host plants at four sites in a quarantine zone in Burks County, PA. Just after adult emergence, the larger *Ailanthus* hosted nearly exclusively females, while smaller *Ailanthus*, grapevines (*Vitis*), a Willow (*Salix*), and rarely other tree species, had high proportions of males. By the fifth week, greater proportions of males were observed on the larger *Ailanthus* trees. Pairings of males and females were observed most frequently on the smaller *Ailanthus* and *Vitis*.

Next we performed experiments in which female adult SLF were disturbed from feeding on the trunks of large *Ailanthus* trees. After adult females were disturbed using a short plastic stick, they typically flew initially southerly away from the tree line toward a sunlit field, but then flew northeasterly back toward the tree line. Additionally, the female insects were manually taken from trees after which the experimenter's hand was slowly opened, palm upwards. The females immediately opened their wings in an apparent aposomatic display. They would then either immediately fly toward the sunlit open field, or remain on the open hand for a prolonged period before closing their wings. If they did leave after closing their wings they flew away from the field toward the *Ailanthus* tree line.

An experiment was performed where adult SLF were caged onto *Ailanthus* trees using black plastic screening. In the control treatment no insects were included in the screen. In the second treatment, ten females were included. In the final treatment, ten females were secured under the screen. The three treatments were duplicated on six trees. On half the trees yellow stick traps were used and on the other half red ones were deployed. At each weekly check it was noticed that nearly all of the insects died under the screening. Most were found at the edges of the screen, in positions that suggested they were strongly attempting to escape. Small numbers of adult SLF were found on the sticky traps, but there were no significant differences among treatments.

We collected volatiles from 4th instar nymphs and adults by suspending solid phase microextraction (SPME) fibers adjacent to feeding aggregations. The fibers were then injected and thermally disorbed into a Hewlett-Packard 5973 GC-MS instrument (Hewlett-Packard, Palo Alto, CA, USA). This instrument provides the capability of matching the mass spectra of chromatographic peaks that of a standard reference library. After examining the chromatographic peaks and their mass spectra, it became readily

apparent that the control and SLF-exposed SPME fibers collected very similar odors.

We collected volatiles from SLF on Ailanthus branches. For this experiment group of SLF adults were enveloped in Tedlar sampling bags (Sigma Aldrich). An air stream created using a portable push-pull air delivery system (Volatile Assay Systems, Rennselaer, NY) was drawn through the bags across an insert containing Porapak-Q absorbent. The filters were eluted with dichloromethane and injected into the Hewlett-Packard 5973 GC-MS instrument for analyses. The collections were performed weekly. The first treatment was a control with the collection bag placed over *Ailanthus* leaves without any insects. The other treatments included the addition of five females, five males, or a mixture of three males and two females. We identified 12 compounds of interest, including three ketones, one alcohol, three aldehydes, one acetate, one alkane hydrocarbon, and three acids. However, no obvious trends were observed with respect to certain compounds only being produced by particular treatments.

EFFICACY AND LONGEVITY OF DINOTEFURAN BARK SPRAYS TO CONTROL THE SPOTTED LANTERNFLY

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ABSTRACT

An infestation of spotted lanternfly (*Lycorma delicatula*) was detected for the first time in the United States in late 2014 in eastern Berks County of southeastern Pennsylvania. The infestation is currently known to be present on hundreds of properties within 6 counties, with the heaviest infestations centered on the initial point of detection. Recent finds in new municipalities continue to be located adjacent to existing quarantine locations. The strategy to eliminate this pest is multifaceted and includes the following: establishment and enforcement of quarantines, intensive surveys using tree sticky bands, various research efforts (host range, trapping), outreach and extension activities that include volunteer efforts (egg mass scraping and tree banding), and chemical control.

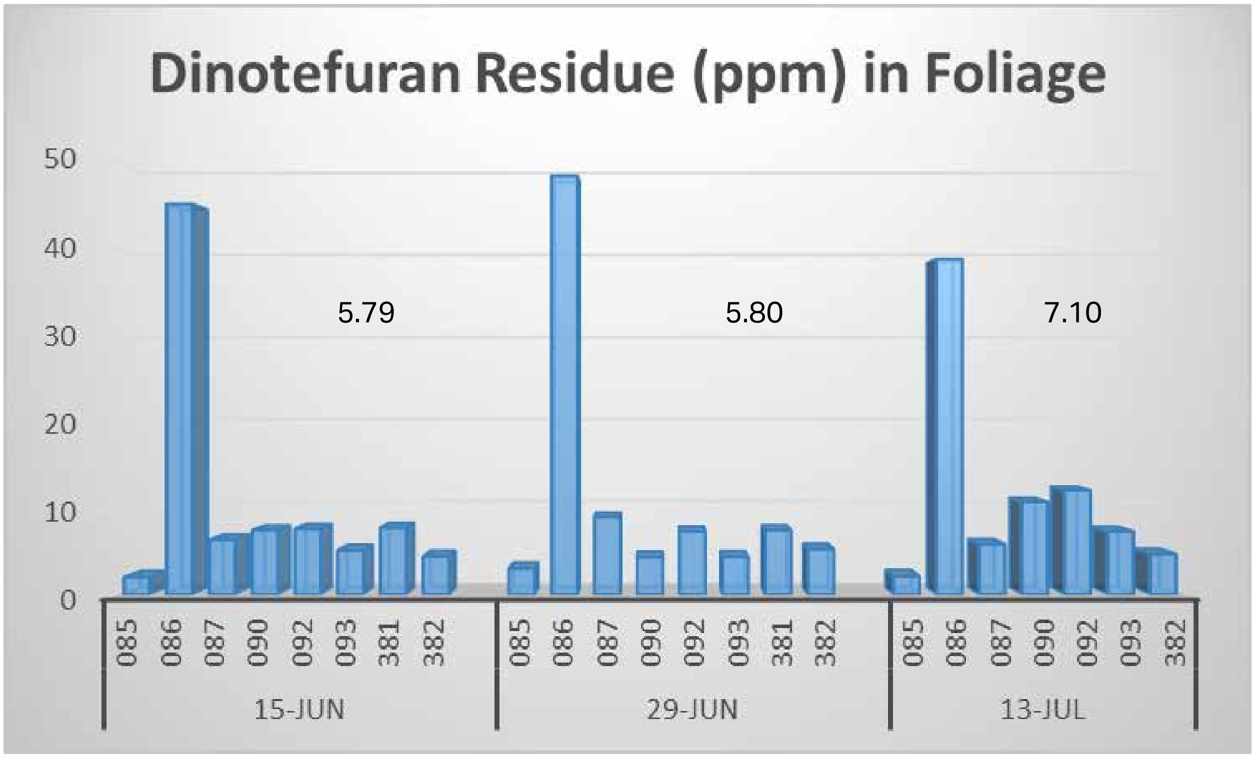
Tree of heaven (*Ailanthus altissima*) is a key host where all life stages of the insect congregate. Prior to the 2016 field season, seventy-five parcels of land were identified and cleared of *Ailanthus*, except for several trees on each parcel that were retained as trap trees. Treatment of trap trees began in late May, 2016 using a bark spray application to the lower portion of the tree trunk using a systemic insecticide (dinotefuran, Transtect 70 WSP).

Observations of these trees during the summer verified that the treatments were quite effective, with dead lanternfly nymphs and adults readily found beneath treated trees. There is a concern, however, about proper timing and continued efficacy of the treatments considering EPA label restrictions of one application per year and that adults are active between mid-July and a hard frost (December or later in Pennsylvania, depending on the year). Tree foliar residues of dinotefuran can be readily detected using a commercially available ELISA kit. Foliage samples were collected between mid-June through October and were processed and analyzed in the lab to quantify dinotefuran residue. The majority of samples were collected from trees treated in late May to early June. Samples were also collected from a few trees that were treated in late in the fall and from trees that had been treated at ½ of the maximum labeled rate.

Dinotefuran residue was determined for all foliage sample collections, even in late October when leaf senescence was beginning to occur (Figure 1). There was a decrease in residue detected over time, with the collections in September and October differing from both of the June and the July samplings (One-Way ANOVA p=0.003; Tukey HSD Q=4.33). Spotted lanternfly nymphs and adults were sensitive to the pesticide applications, with abundant cadavers present throughout the summer and fall; poisoned adults exhibited wing-flaring while resting on the tree trunks (Figure 2). Trees treated with a fall bark spray application had minimal to low levels of dinotefuran.

Future plans for this work will investigate treatment timing and efficacy of a ½ rate application and a tank mixture of dinotefuran and imidacloprid. The goal is to provide the eradication program with data to determine the longevity and efficacy of the treatments so that optimal timing and impact of the applications is achieved.

Figure 1. Dinotefuran residue detected in *Ailanthus* foliage over time. Numbers above the bars are the average residue value for all of the trees for each date, but excluding tree 086.



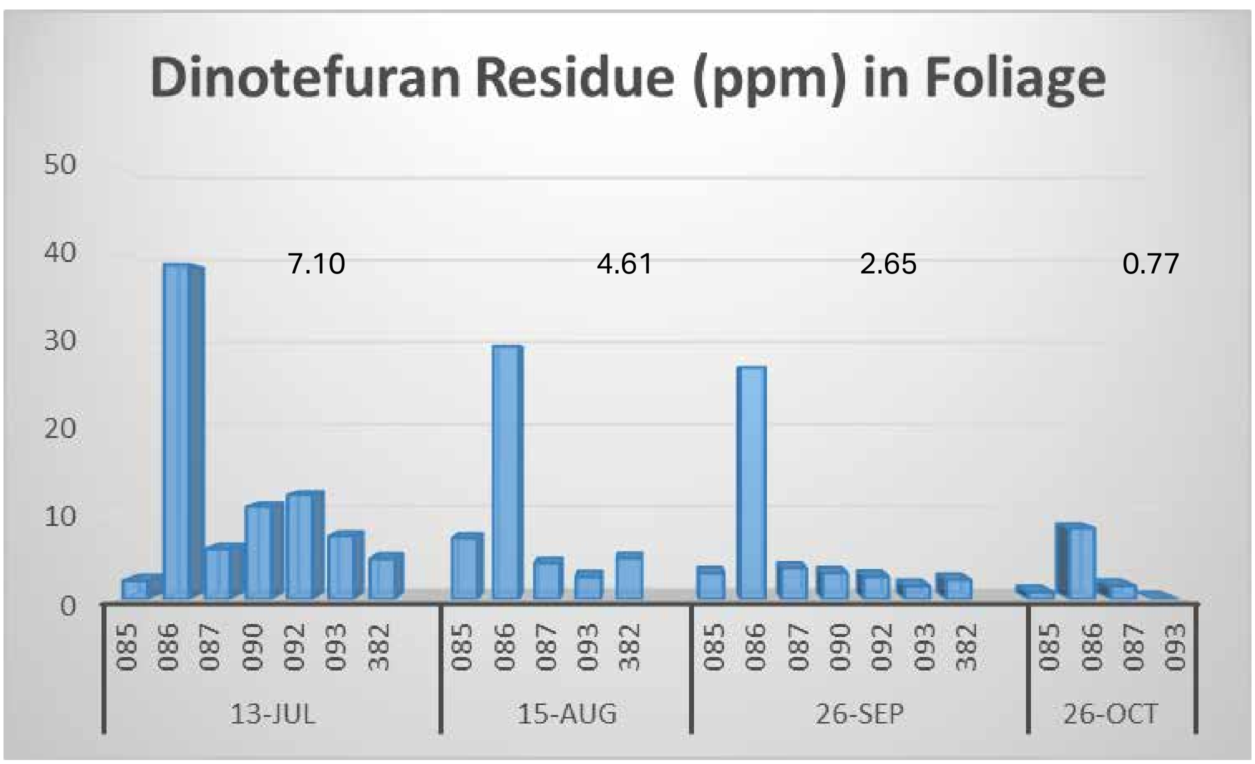


Figure 2. Adult mortality is evident from this picture of a treated tree in August. Wing-flaring of adults indicates pesticide poisoning.

PRESENTATIONS

Research Reports

THE NEW EU PREPSYS PROJECT: WORKING TO UNDERSTAND AND MANAGE THE RISKS OF EXOTIC BUPRESTID BEETLES.

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ABSTRACT

International trade provides multiple pathways for movement of tree pests, through the wide range of products transported globally compounded with the massive volumes traded each year. Whilst these international trade pathways provide the opportunity for new pests to move to and establish in new locations, their further spread is facilitated by internal movement of goods and by accidental transfer of contaminated host material.

Among the increasing lists of pests that have established outside their native ranges, emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), a native of SE Asia, has caused devastation of indigenous ash trees in North America and in the European part of Russia. From the extensive research carried out in North America, commencing in the USA followed by Canada, supplemented by substantial research in the native range in China, there is a rapidly accumulating knowledge base on the biology and management of this important pest. More recently, EAB has caused extensive ash mortality in Moscow and is spreading west, with no ecological or physical barriers, to neighbouring European countries. The track record of both extensive damage and demonstrated capacity to spread rapidly in exotic locations is, therefore, of considerable concern to European countries, particularly since ash is already under threat from the ash dieback fungus *Hymenoscyphus fraxineus*. Arising from this concern, Pest Risk Analyses (PRAs) have been carried out both at country levels and through the European and Mediterranean Plant Protection Organisation (EPPO). These have helped to identify the principal pathways for movement of EAB in international trade, with emphasis on plants for planting and raw wood.

The clear threat posed by EAB has also prompted the carrying out of risk assessments on other bark and wood boring pests and resulted, following an EPPO-sponsored PRA, in the addition of bronze birch borer (BBB), *Agrilus anxius*, to the EPPO and European Union lists of threats. Since BBB is native to North America, the experiences of researchers and land managers in dealing with this pest is critical to furthering European risk assessment for BBB. Both EAB and BBB have been added to the newly developed UK Plant Health Risk Register (https://secure.fera.defra.gov.uk/phiw/riskRegister), a database of pests and pathogens that now totals over 900 species.

Arising from the European-wide concern about EAB and BBB, a partnership has been developed under the Euphresco programme in a new project entitled *Risk-based strategies to prepare for and manage invasive tree borers* with the acronym PREPSYS (Pest Risk Evaluation and Pest management SYStems). PREPSYS is a 3-year project which commenced on 1 October 2016 and includes the following partners:

- Forest Research, FR (UK): Coordinator - Prof Chris Quine, Prof Hugh Evans, Dr Mariella Marzano and Dr David Williams.
- Federal Research and Training Centre for Forests, Natural Hazards and Landscape, BFW, Department of Forest Protection (Austria): Dr Gernot Hoch and Dr Ute Hoyer-Tomiczek
- Department of Agriculture Food and the Marine, Teagasc (Ireland): Dr Gerry Douglas and Dr Rachel Wisdom
- Nederlandse Voedsel-en Warenautoriteit, NVWA (The Netherlands): Dr Antoon Loomans and Dr Martijn Schenk.
- United States Department of Agriculture, Animal and Plant Health Inspection Service, USDA APHIS (USA): Dr Leslie Newton, Dr Laurene Levy and Dr Wendy Jin.

Aims of PREPSYS

Although there is increasing information on EAB and, to a lesser extent BBB, further work especially from a European perspective is required to both anticipate and to react to incursions of the pests. PREPSYS will, therefore, address key questions and gaps in our knowledge on the pests' biology, dispersal and economic/environmental impacts.

The overall objective is to provide research outputs to underpin contingency planning, policy development and policy communication through assessment of the entry, establishment, spread, impact and management of the pests, with the main emphasis on reducing the likelihood of their establishment and, as a worst case, in coping should the pests succeed in establishing populations in Europe. The key overall question that the research should address is: 'How can we best prepare for and manage the risks and impacts of EAB and BBB?'

The specific questions that will be considered within PREPSYS include:

- What are the potential risks and impacts for the two pests both in their countries of origin and where they have established in new locations?
- What are the pathways for movement of the pests and how can we better protect against the risk of introduction?
- How can we improve early detection both on the pathways and at their end points?
- What are the biological characteristics and pest population sizes that are likely to lead to successful breeding and establishment in a new location?
- What are the rates of natural spread and can we improve prediction of spread, both natural and by human assistance?
- How can we develop or improve cost-effective and socially acceptable management and control approaches and tools (including biological control)?
- How can we best communicate and implement policy and engage effectively with stakeholders (including policy makers, woodland owners and managers, academia and the public, importers and the wider nursery industry) to develop the best approaches?

In each case, the starting point is to identify the state of knowledge internationally and with specific reference to European conditions and identify and prioritise knowledge gaps.

The need for international collaboration

Apart from the inputs by the project partners, it is recognised that PREPSYS needs to collaborate widely and draw on expertise in countries already dealing with either EAB or BBB or both species (e.g. for EAB - China (native zone for the pest), Russia and USA (invaded zones for the pest)). As part of this knowledge-gathering phase, PREPSYS is analysing management approaches and their effectiveness and will use this knowledge to extrapolate to European conditions within a risk-based systems approach. The project outputs will analyse all information sources to develop best practice in pathway management, early detection, contingency outcomes and longer-term sustainable management practices. This accumulated knowledge will inform policy-making at national and EU levels and will draw on lessons learnt from other similar national and international projects.

In order to foster collaboration the project has established both an Expert Forum and a Stakeholder Group. We are keen to include new members in the two collaboration mechanisms with the aim of providing benefits to all who contribute.

Expert Forum Membership

Scientists, forest and woodland managers, plant health practitioners, pest management companies and practitioners. The aim is sharing of knowledge, ideas and, where possible, joint research. If you are able to join the group could you please email Prof Hugh Evans - hugh.evans@forestry.gsi.gov.uk.

Stakeholder Group Membership

Policy makers, woodland owners and managers, academia and the public, importers and the wider nursery industry and other interested parties as appropriate.

The successes of any management interventions are often influenced just as much by social, political and organisational issues as they are by technical innovations and constraints. The aim is to explore policy communication and implementation methods and consider ways to best engage with stakeholders on preventative and control options in advance of pest introductions and if the pests succeed in establishing. If you are interested in joining the Stakeholder Group please contact Dr Mariella Marzano – mariella.marzano@forestry.gsi.gov.uk.

A specific call for material to train dogs to detect EAB

A key part of anticipating and reacting to pest incursions is early detection and this is one of the important aims of PREPSYS. Through Dr Ute Hoyer-Tomiczek of our Austrian partner BFW, work is being carried out to train dogs to detect EAB, building on the success of the group in training dogs to detect Asian longhorn beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Dr Hoyer-Tomiczek is keen to obtain uncontaminated specimens of different life stages of EAB and can provide specific instructions on collection, preservation and shipping of specimens to Austria. If you can help please contact her by email - ute.hoyer@bfw.gv.at.

Sharing of information: A key aim and deliverable from PREPSYS

Information gathered during the project will be shared both by direct communication and through the project website on www.forestry.gov.uk/fr/prepsys. Further information can also be found on the Euphresco website - www.euphresco.net. Euphresco is a valuable resource for sharing information and research on plant health generally. It was initiated as a network of research programme owners and programme managers funded under the 6th and 7th EU Framework Programmes from 2006 to 2014. It has now grown to 69 members from 55 countries in 5 continents and, thus, has a wide international coverage and appeal. Both Canada and the USA are members, which is also reflected in both direct and Expert Forum membership of the new PREPSYS project. We, therefore, invite you to collaborate on this new project so that knowledge and best practice on both EAB and BBB can be shared to support planning and management to combat these important pests.

Acknowledgements

This paper represents the PREPSYS partnership and I would like to thank the project partners for their support and input to this paper.

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CASTING YOUR NET WIDELY: BROADLY TARGETED EXOTIC SPECIES SURVEYS

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ABSTRACT

Most surveys for exotic invertebrates are narrowly targeted, largely due to constraints on funding, taxonomic expertise, and available identification tools. There are significant benefits to more broadly targeted surveys, including examination of by-catch that is normally discarded. These benefits include detection of new exotic pests not on target lists (some of our worst recent exotic insect species were never recognized as significant potential pests), detection of new "benign" exotics (which may become pests), acquiring new range data for recent exotic pests as well as for indigenous and long-established exotic species, and elucidation of active exotic species introduction pathways (including those formerly unrecognized).

Oregon Department of Agriculture (ODA) is fortunate to have the taxonomic expertise to support broadly targeted surveys, enabling ODA to reap the benefits of this tactic. For instance, ninety-nine species of exotic terrestrial invertebrates have been documented from Oregon in the past ten years, primarily through ODA's surveys. Of these species, 54% were initially detected by broadly targeted survey or as by-catch, some of which were new to North America. Among such species were eight known significant pests. Analysis of probable introduction pathways revealed almost 80% of the exotic species detected in the last ten years were associated with live plants. Almost half of these species had origins in Europe, indicating that the European live plant pathway is active. The average rate of detections over the past ten years was remarkably constant, 9.9 species per year, underscoring the need for continued exotic species surveillance.

ODA broadly targeted surveys have included the Exotic Terrestrial Plant Pest (ETPP) survey (focused on transportation hubs) and the Exotic Wood Boring Insect (EWBI) survey. Both detected exotic species new to regions ranging from western Oregon to North America. ETTP detected twenty-one exotic species, including seven significant pests, and EWBI detected at least nineteen exotic species, including ten significant pests. Among the latter was *Xylosandrus crassiusculus*, an ambrosia beetle that is a major orchard, horticultural, and landscape threat. Early detection via broad-based EWBI survey enabled eradication of this pest from the Oregon locality where it was introduced. Via by-catch examination, ODA has documented many new exotic species, including the initial detection of brown marmorated stink bug (*Halyomorpha halys*) in Oregon, the initial detection of ash whitefly (*Siphoninus phillyreae*) in Oregon, and many new country records for spotted wing drosophila (*Drosophila suzukii*). Early ash whitefly detection enabled implementation of biocontrol efforts before this pest became widespread. Broadly targeted exotic invertebrate species surveys have proven extremely useful to Oregon.

ASSESSMENT OF SYSTEMS APPROACH FOR THE PHYTOSANITARY TREATMENT OF WOOD INFESTED WITH INVASIVE WOOD BORERS

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ABSTRACT

Systems approaches are beginning to be adopted for the phytosanitary treatment of export lumber. Use of systems approaches requires extensive knowledge of the levels of reduction of pests of concern at each stage in the production pathway for the exported lumber. However, it has not been possible to obtain statistically validated levels of reduction for most woodboring pests in milled lumber because of relatively low densities of these organisms. The large outbreak of emerald ash borer (*Agrilus planipennis*; EAB) in eastern North America has provided an opportunity to evaluate the systems approach because the large number of individuals in an infested tree can be used to generate statistically valid datasets of the risk reduction at each stage in the production process (Harvest, Milling and Heat Treatment). EAB is usually present in infested ash at high densities a few years after a stand is attacked, and large volumes of infested ash are relatively easy to obtain in regions where the insect is established (e.g., southern Ontario, Canada)

Previous studies have assessed the effect of heat treatment on survival and subsequent emergence of EAB. In the most complete study, testing larvae at all developmental stages and at multiple times and temperatures found that EAB did not survive beyond 56° C in small bolts and in small research kilns. The results are consistent with observations from firewood or bolt-sized pieces of infested ash processed in larger kilns, where survival (as measured by emergence post-treatment) was not observed for temperatures above 65° C for durations of at least 60 minutes. This suggests that treatment guidelines that meet these minimum standards will likely sanitize EAB infested wood. However, it is possible that some EAB may survive phytosanitary treatment temperatures due to insufficient heating. This risk can be mitigated if the surviving adults are non-viable (e.g., they cannot mate, cannot disperse or cannot lay eggs). No study has examined the effect on EAB of sub-lethal exposure to phytosanitary treatment.

In the fall of 2015 we harvested infested ash in southern Ontario, Canada and milled 100 cm long by ca. 5 cm thick (ca. 2”) slabs from these trees. These slabs were treated in a research kiln at FPInnovations in Québec (Quebec) to one of three temperatures (48° C; 56° C; 71° C) for 30 minutes, then placed into rearing drums and the surviving EAB were allowed to emerge. After emergence had completed all the slabs were dissected to determine the fate of all un-emerging insects. All EAB that did emerge were reared until death and their size, lifespan and fecundity recorded.

We found that no EAB emerged from slabs treated to 56° C or 71° C. Dissection of the slabs showed that all samples contained the same number of insects thus we can conclude that no EAB survived above a

temperature of 56° C. The same number of EAB emerged from the 48° C treatment as from unheated control slabs. When we compared the viability of insects from the 48° C treatment to those from the controls we found no statistically significant differences in lifespan, fecundity or size. This result suggests that there were no sublethal effects at the 48° C treatment.

Future work will evaluate the survivorship and viability of insects in slabs treated between 48° C and 56° C with the intent of eliciting sublethal effects in the resulting EAB. A companion study planned for 2017 will evaluate other aspects of the systems approach. Specifically we will test the effect of milling on reduction of woodborer density in infested trees. The results of this study should have short term impacts on the treatment standards for ash lumber. Over the longer term this study should provide parameters for estimating the risk reduction associated with production and phytosanitary treatment for other wood boring insects of trade importance.

FIRST RESULTS OF A MULTILURE TRAPPING PROGRAM TO DETECT EXOTIC CERAMBYCIDS AT PORTS OF ENTRY IN FRANCE

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ABSTRACT

First tests using multiple lure combinations were carried out in France since 2014 in order to develop trapping methods to detect xylophagous beetles, especially cerambycids, in ports-of- entry. In 2014, the trapping efficiency of two multilure blends were compared using black cross-vane traps coated with teflon in 20 different forests belonging to different bioclimatic regions of France. The first blend combined Fuscumol, Fuscumol Acetate, Geranyl acetone and Monochamol diluted in isopropanol, the second one combined 3-hydroxy-hexan-2-one, 2-methylbutanol, 2R*,3S*-hexanediol, and Prionic acid also diluted in isopropanol, whereas a control trap contained only isopropanol. These trappings allowed to catch a total of 2433 cerambycids corresponding tor 55 species with a rather good generic effect for 4 subfamilies and 6 tribes at least, which differed according to each multilure blend but corresponded to the specific sex and/or sex-aggregation attractivity of each compound indicated in Hanks and Millar (2016). Relatively few cerambycid species (<15) were caught by the control traps. The captures of bark beetles were also limited (<100) whatever the trap.

Based on these preliminary results the PORTRAP project, funded by the French Ministry of Agriculture, tested the efficiency of the same blends in 11 ports-of-entry in 2015 and in 12 in 2016. From 2015 on, a mixture of (-) α-pinene and ethanol was added to the first blend in order to get a better efficiency for bark beetles. A total of 12 species of cerambycids were trapped in these ports during the first year, of which 2 alien ones never recorded in Europe (*Xylotrechus altaicus* and *Uraecha angusta,* both originating from Asia), whilst 32 species for 305 individuals were caught in 2016, including numerous specimens of the exotic *Xylotrechus smei* in several ports. Bark beetles were also trapped in large numbers, including exotic platypodid species.

The final objective being to minimize as much as possible the trapping efforts for an early detection at arrival, the trapping efficiency of a combination of the two previous multilure blends hang on the same trap was also compared to the one of each blend in 17 forests in 2016. At site level, traps baited by both blends captured significantly more cerambycid species than those baited by each blend separately. No significant difference was observed between traps baited by both blends and the sum of the captures by each blend separately. These results have to be confirmed but clearly suggest that a multilure blend combining the 10 above compounds have limited repellency effects and could be considered for trappings at ports-of-entry. Since 2014, these trappings allowed to capture 100 cerambycid species, i.e. 41% of the species recorded in France (241 spp.), with a relatively good generic effect since the captures included 100% of the species from 7 cerambycid tribes and more than 50% of those from other 15 tribes.

Hanks L.M. and Millar J.G. (2016) Sex and aggregation-sex pheromones of cerambycid beetles: Basic science and practical applications. *Journal of Chemical Ecology* 42:631- 654

AREA-WIDE PEST MANAGEMENT OF THE INVASIVE EMERALD ASH BORER: RECENT PROGRESS AND CHALLENGES

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ABSTRACT

The premise of area-wide pest management (AWPM) is based on the principle that "*uniform suppressive pressure applied against the total population of the pest over a period of generations will achieve greater suppression than a higher level of suppression on most, but not all, of the population each generation*" (Knipling 1992). Historically, the AWPM concept has been applied since 1800s and many successful examples for managing serious economic pests over large spatial scales can be found in the literature (see review in Faust 2008).

The emerald ash borer (EAB), *Agrilus planipennis*, is an invasive pest that has killed hundreds of millions of North American ash trees in forest and landscape settings. First detected in Michigan in 2002, EAB has now spread to 31 U.S states and two Canadian provinces via long-distance movement of infested materials by people and short-distance dispersal flights, particularly by mature females (Mercader et al. 2012). Sales of ash, once a major component of nurseries, have collapsed across the U.S. and Canada, and the use of ash in the forest product industry is threatened. Municipal governments in affected states sustain the high costs associated with removal and replacement of dead or dying trees, or protecting landscape ash on public lands with insecticides. Costs of treating or removing even half of the ash trees growing on municipal property in urban and suburban communities were projected to exceed $20 billion per year by 2019 (Kovacs et al. 2010). In addition, more than eight billion ash trees occupied diverse forest habitats across the U.S., accounting for nearly 150 million ft^3 of timber nationwide. Besides economic losses from EAB infestation, widespread ash mortality in natural forests will cascade through ecosystems, directly and indirectly affecting biological diversity (Wagner and Todd 2015), nutrient cycling and other ecological processes, and facilitating invasion by non-native plants (Knight et al. 2013, Jennings et al. 2016a).

Early eradication efforts were quickly abandoned in the U.S., largely due to the difficulty of identifying and delimiting new EAB infestations when its densities are low. Current strategies have shifted toward integrated management to (1) reduce the rate of EAB population growth in infested areas and (2) slow the spread of EAB to non-infested areas. These strategies include integration of ongoing detection efforts through artificial traps and public outreach (through actions by local task forces), regulations to reduce risk of human transport of EAB-infested materials, systemic insecticide treatments to protect trees, physical destruction of infested trees, including the use of artificially girdled trees to serve as "trap" trees (McCullough et al. 2015; Mercader et al. 2015, 2016), and in some areas, biological control via release of

parasitoids collected in EAB's native range (Bauer et al. 2015, Duan et al. 2015, Jennings et al. 2016b).

Although quarantines, inspections and related regulatory activities can prevent most human-assisted EAB movement, these will not have an impact on slowing natural range expansion. Insecticide treatments, such as trunk injection of systemic insecticides (emamectin benzoate, azadirachtin), basal trunk sprays (dinotefuran), or soil applications (imidacloprid, dinotefuran) can protect ash trees in the landscape. Costs and environmental concerns, however, prevent widespread use in forested areas. Two of the three introduced Asian parasitoids (*Tetrastichus planipennisi* and *Oobius agrili*) have established stable populations in several locations following releases between 2007 and 2013 in Michigan and other states (Abell et al. 2014; Duan et al 2015, Davison et al. 2015, Jennings et al. 2016b). These species, along with some native natural enemies, have substantially reduced EAB population growth in some locations (Duan et al. 2015). In addition, a new Russian parasitoid (*Spathius galinae*) was approved for release in 2015 (USDA APHIS 2015). However, releases of these parasitoids have, so far, been limited to inoculative efforts. No efforts involving augmentative releases of these biocontrol agents nor native parasitoids have been attempted, nor has the integration of biocontrol and selective insecticides been implemented.

The AWPM project funded by the USDA Agricultural Research Service was initiated in collaboration with USDA APHIS, USDA Forest Service, University of Massachusetts and Michigan State University in 2015. The long-term goal of this AWPM project is to decrease EAB economic and ecological impacts in urban, suburban and natural forests. The project involves development and implementation of integrated pest management strategies for EAB in two distinct regions – the upper Midwest region and Northeast region. The midwestern region includes infested areas of Lower Michigan (the original epicenter of invasion) and adjacent areas in Indiana and Ohio. In this region, characterized as the "core" of the EAB invasion wave, mortality of untreated landscape ash trees and overstory ash in many forested areas can range from 80 to 100% (Klooster et al. 2014; Smith et al. 2006). Recent data show EAB populations persist in the core, even in southeast Michigan where the invasion began. While densities decline after the invasion wave peak, EAB are colonizing ash regeneration saplings and trees that escaped from the peak invasion wave. Forests in invaded sites where young ash are abundant were selected as study areas (sites) for the proposed work in this region. In contrast, the northeast region is the leading edge of new EAB infestations, including those in New York, Massachusetts, Connecticut, and New Hampshire. In much of this region, EAB invasions are still at an early stage, consisting of several discontinuous populations. Study sites in this region were selected near the leading edge of EAB invasion in urban landscapes and adjacent forested areas where ash is abundant.

In both regions, similar approaches to managing EAB are employed but goals differ somewhat. In the **Midwest** region, the focus is on reducing and slowing growth of EAB populations, conserving existing ash and facilitating recruitment of ash regeneration. Tactics to achieve this goal included (1) treatment of selected trees with trunk injections of emamectin benzoate and (2) augmentative releases of introduced EAB parasitoids at relatively high temporal and spatial frequencies. In contrast, EAB management in the **Northeast** region focuses on preventing further spread of isolated EAB populations to non-infested natural areas primarily by (1) encouraging municipal and private property owners to use effective, systemic insecticides to protect valuable ash trees and (2) using inoculative release and establishment of biocontrol agents at the leading edge of invasion areas.

To date, data on ash inventory and EAB mortality rates have been collected in both regions. These data have confirmed our key assumptions about EAB density and stage-specific mortality rates that are dramatically different in the recently invaded forest (Northeast Region) vs. the aftermath forest (Michigan). In addition, control tactics to slow EAB, including larval and egg parasitoid releases and insecticide treatment of selected trees in both regions were applied in 2016. However, the unexpected shortage of funding has not only hindered the implementation of the program at large regional scales, but also cut short the evaluation of the impact of the AWPM program for protection of North American ash from EAB invasion.

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TRACKING THE *ENTOMOPHAGA MAIMAIGA* "DEATH CLOUD"

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ABSTRACT

Entomophaga maimaiga Humber, Shimazu, and Soper is an important fungal pathogen infecting gypsy moth (*Lymantria dispar* [L.]). This fungus actively ejects infective spores (conidia) after host death, and this spore type is involved in the long-distance spread of the pathogen. Long-lived soilborne azygospores (resting spores) are also produced and overwinter to produce infective spores in subsequent years. Rapid long-distance spread of *E. maimaiga* has been documented, but little is known about the aerobiological factors that promote spread or the variation in airborne conidia across seasons and years. The goal of this study was to develop effective field collection methods to measure the quantity of *E. maimaiga* conidia being deposited over space and time. We investigated gypsy moth outbreaks with epizootics in Pennsylvania in June and July of 2016. We applied highly specific and sensitive quantitative PCR (qPCR) methods (previously developed for *E. maimaiga* resting spores in soil) to detect conidia deposited within wet-cup spore traps containing a non-toxic preservative. Using four-day time periods beginning with third instars and continuing through pupation, we detected variable amounts of target DNA that had fallen from the air. The amounts of DNA detected corresponded to spore counts ranging from 0 to 62 spores. Conidial DNA declined over the period as gypsy moth larvae pupated, because pupae are not common sources of conidia. Defoliation is an indicator of high gypsy moth population density, and therefore defoliation could be associated with conidial production. DNA quantity in traps was negatively correlated with the distance to the nearest defoliation in the previous year (2015) as well as to defoliation in the current year. We obtained site-specific weather data from PRISM Climate Group (Oregon State University, http://prism.oregonstate.edu). DNA quantity was not correlated with the sum of local rainfall one day before plus during the trapping period. Rainfall can affect aerobiology through spore production (increasing numbers of conidia ejected) and wet deposition (deposition in rainfall), so more fine-scaled data is needed to tease out the effects of rainfall. This preliminary study developed methods that will be useful in future studies, and eventually will contribute to predictive modeling of epizootics of *E. maimaiga*.

POSTERS

BENEFITS OF LOOKING OUTSIDE THE TARGET LIST

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ABSTRACT

Conventional survey methods are a valuable tool for seasonality information, new distribution records, and host associations using existing infrastructure. Retention of bycatch from these samples can stretch resources further yielding a trove of valuable information beneficial to both the agency, cooperators, and the scientific community. Minimal monetary input is required as existing survey infrastructure is already invested. Pennsylvania's Department of Agriculture has had a broad range of discoveries looking outside survey target lists, projected species ranges, and identifying unusual specimens. In the past 6 years PDA and collaborators have accumulated 44 new state records while averaging 97,000 a year identifications, the majority of which are non-reportable. This poster serves to highlight some examples of those projects and results while hopefully providing what may be a catalyst for possibilities for other agencies or laboratories.

EFFECTS OF SUGAR RESOURCE USE AND QUALITY ON EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) PARASITOID LONGEVITY

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ABSTRACT

Four parasitoids are currently being released in a classical biological control program against emerald ash borer (EAB), *Agrilus planipennis* Fairmaire. One of these parasitoids, *Tetrastichus planipennisi* Yang, has been establishing well at many release sites, however, *Spathius agrili* Yang has not, which prompts considering factors contributing to successful biological control programs. One very important factor is available nutrient resources, which can contribute to survival, increased longevity and fecundity, as well as egg maturation. Many parasitoids feed on floral and extrafloral nectar, while others may utilize honeydew. The nutritional ecology of *S. agrili* and *T. planipennisi* is largely unknown and considering this may improve long term establishment of EAB parasitoids. Objectives of this research were to determine differences in preference of *S. agrili* and *T. planipennisi* among floral, extrafloral, or honeydew over other sources and to quantify their longevity with varying food sources. Food sources were chosen based on prior work quantifying potential parasitoid food sources at release sites in New York, Maryland, and Michigan with successful establishment. Sources evaluated were: aphid honeydew, Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch) flowers, Morrow's honeysuckle (*Lonicera morrowii* Gray), both floral and extrafloral sources, border privet (*Ligustrum obtusifolium* Seibold & Zucc.) floral and extrafloral, black cherry (*Prunus serotine* Ehrh.) extrafloral nectaries only, black locust (*Robinia pseudoacacia* L.) floral and extrafloral, white ash (*Fraxinus americana* L.) extrafloral only, and controls of honey, water, and nothing.

To measure potential preference among sources, individual parasitoids were presented with a food source and time spent feeding at each was recorded. To quantify longevity, a group of parasitoids were presented with a single food source and survival recorded daily until all were dead. Nectar, honeydew, and honey feeding increased parasitoid longevity compared to water and blank controls, with honey increasing lifespan the most. Increased longevity can increase host-finding time and may be important in fecundity and brood survival. When testing preference among food sources, both *S. agrili* and *T. planipennisi* fed longer on more nutritive sources, feeding on honey longest, followed by extrafloral and floral sources. This suggests possible visual or chemical attractiveness of these nutrient sources. There were no significant differences in feeding time among plant species, except for *S. agrili*, which fed significantly longer on white ash extrafloral nectaries than on any other extrafloral or floral sources.

Our results suggest floral and extrafloral resources at release sites may be an important factor in establishment of EAB parasitoids. Further research is required as it this, among other factors, is likely an important factor to consider prior to parasitoid release.

KUDZU BUG IN TENNESSEE: A FUNGAL SURPRISE

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ABSTRACT

The kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), is native to the Old World and was found in northern Georgia (USA) in mid-October 2009. It is the only member of the family Plataspidae in the continental U.S. and has been documented in 13 states and the District of Columbia thus far. *M. cribraria* are attracted to legumes, particularly kudzu, *Pueraria montana* var. *lobata* (Willd), and soybean, *Glycine max* (L.) Merrill, where they extract vascular fluids from stems, petioles, and leaves. Although it may reduce biomass of kudzu plants, this invasive insect has also been shown to reduce yield in soybean crops. The impact of kudzu bug on kudzu growing in and around forests is not known.

During population monitoring of *M. cribraria* in Tennessee in 2015, a fungal pathogen infecting immatures and adults in kudzu was observed. This pathogen was identified as *Beauveria bassiana* (Balsamo) Vuillemin: a well-documented, generalist pathogen that has been known to infect *M. cribraria* in both its native and invasive ranges.

Beginning in September 2015, a six-week study was conducted at two locations in eastern Tennessee to monitor prevalence of this pathogen on, and mortality of, *M. cribraria* due to infection by *B. bassiana* on kudzu. Kudzu stems and foliage containing infected and non-infected *M. cribraria* were clipped, bagged, and taken to the laboratory and later processed. During the study, total infection of adults was only 33.4% while total infection of immatures was 75.5%. *B. bassiana* was ultimately found in three additional counties in eastern Tennessee in 2015. Regular sampling for kudzu bug phenology in 2014 never revealed signs that the pathogen was present, even though sampling occurred at the same time that the pathogen was discovered in 2015.

This study is an indicator of seasonal impacts of *B. bassiana* on immature and adult life stages of *M. cribraria*. Research to monitor *B. bassiana* on a wider scale throughout Tennessee will continue, as well as efforts to assess its impacts on populations of kudzu bug.

THE NEW YORK INVASIVE SPECIES RESEARCH INSTITUTE: WORKING TO BRIDGE THE GAP BETWEEN INVASIVE SPECIES RESEARCH AND MANAGEMENT

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ABSTRACT

Based out of Cornell University's Department of Natural Resources, the New York Invasive Species Research Institute (NYISRI) has the mission to coordinate invasive species research to help prevent and manage the impact of invasive species in New York State and beyond. NYISRI serves to improve the scientific basis of invasive species management through facilitating and promoting innovation, communication and collaboration between the scientific research community, natural resource and land managers, state offices and partner organizations.

NYISRI partners with New York's eight Partnerships for Regional Invasive Species Management (PRISMs), iMap Invasives, the NYSDEC Invasive Species Coordination Unit, NYS Parks, and the many researchers across disciplines who are willing to share their expertise to improve invasive species management on the ground.

The institute has initiated a number of projects to engage managers and researchers. A few examples are: the development of a NYS invasive species priority setting tool, the establishment of the Northeast Regional Invasive Species and Climate Change (RISCC) Management working group, research on the biological control of water chestnut and project to assess the success of invasive plant control efforts.

One of NYISRI's main platforms for sharing information is our website (nyisri.org), which is updated regularly. The site features numerous resources including: a searchable invasive species expert database and invasive species manager database; a blog on recent relevant invasive species research; invasive species research news; a monthly digest of invasive species research publications; posts on Twitter @ NY_ISRI with up-to-date news and research; and a biological control information page. NYISRI also coordinates monthly invasive species webinars and coordinates an annual invasive species conference at Cornell University with recordings available on our website.

NYISRI's success in connecting invasive species research and management is dependent on the expertise and collaboration of our partner managers and researchers. We are always looking to learn about new research, better understand manager needs and find synergies among them. If you are interested to learn more about NYISRI, to blog or present about research findings for a manager audience, better understand manger research needs, or engage in manager-researcher partnerships, we welcome you to join our expert database (http://www.nyisri.org) or contact NYISRI's director Carrie Brown-Lima for more information (cjb37@cornell.edu).

ARTIFICIAL DIET SYSTEM FOR ADULT EMERALD ASH BORERS, OBLIGATE LEAF-EDGE FEEDERS

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ABSTRACT

An artificial diet is being developed for adults of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) to facilitate mass production of its biocontrol agents. Adult EAB feed naturally only on ash leaves, which are not available in winter at the EAB biocontrol rearing facility in Michigan. Prior and current work revealed that adults are obligate leaf-edge feeders that cannot feed on materials thicker than ~500 µm. All nutrients needed by adult EAB (complete proteins, sugars, other carbohydrates, vitamins, minerals, sterols, and phospholipids) must be provided in a format that is palatable, bioavailable, and stable. Keeping leaf-thin diets hydrated is a major problem, while hydration presents a further challenge in the form of mold growth. The most promising solution is a "flow-through" system to keep the surface dry by coating diet materials, prepared as a slurry, with soy-waxed paper or a plasticized film such as Parafilm™. Tested slurry formulae were made with or without ash leaf powder. An aqueous solution of sugars, vitamins, preservatives, and other candidate components flowed through the coating and slurry by the wicking action of a fibrous foundation. Feeding and frass production occurred on edges of the structure lacking the waxy coating, showing promise for the flow-through system.

DISCOVERY OF PHEROMONES IN SCOLYTINE AMBROSIA BEETLES: THREE INVASIVE CRYPTIC SPECIES IN THE *EUWALLACEA FORNICATUS* SPECIES COMPLEX

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ABSTRACT

Three cryptic species of ambrosia beetles in the *Euwallacea fornicatus* Eichhoff species complex (#1, #2, and #5) were each found to produce a pheromone composed of the same two ketones, 2-Heneicosanone and 2-Tricosanone, but at different ratios. All three species were found to be highly attracted to the synthetic pheromone blend, but only at the ratio that matched their species. Each species was repelled by the ratios of the other two species. To determine whether *E.* sp. #1 beetles used pheromones, volatiles were collected from treatments: Control, Diet, Diet+Fungus, Diet+Fungus+Beetles, Gallery, and Beetles. Volatile profiles were analyzed and examined for GC peaks that uniquely appeared in certain treatments. Two ketones, 2-Heneicosanone and 2-Tricosanone, were present only when beetles were present, and were particularly abundant inside the galleries. Both compounds were found in odors from both sexes. These two compounds were found in all three species, but each species had a different ratio of the two compounds. Synthetic blends were formulated to match natural ratios for each species for behavioral testing. Beetles of each species were highly attracted in a Y-tube to the synthetic ratio that matched their natural ratio, and they were highly repelled by the ratios matching those of the other two species. Conversely, quercivorol was found only in treatments containing fungus, and was attractive to all three species, suggesting it is a fungal kairomone.

ATTRACTION OF THE INVASIVE AMBROSIA BEETLE *EUWALLACEA FORNICATUS* SP. AND RESPONSE TO FOUR STEREOISOMERS OF THEIR FUNGAL KAIROMONE *P*-MENTH-2-EN-1-OL

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ABSTRACT

Initial still-air petri dish bioassays compared attraction of *Euwallacea fornicatus* (Eichhoff) sp. #1 to odors from either diet, diet+fungus, or diet+fungus+beetles. Beetles were attracted to odors that contained the latter two. Analysis of volatiles using GCMS revealed the presence of (1S,4R)-menth-2-en-1-ol (quercivorol) in diet+fungus odors, but not from diet alone. Quercivorol has four stereoisomers, which were individually synthesized, thus enabling behavioral testing on each isomer. We subsequently conducted chemical ecology and behavioral studies on responses to the four stereoisomers of *p*-menth-2-en-1-ol to improve lures containing these stereoisomers. The (1R,4S)-menth-2-en-1-ol isomer, and quercivorol (the 1S,4R isomer), both E isomers, were attractive. The two Z isomers (1R,4R and 1S,4S) were not attractive alone, and when the two Z isomers were combined they became repellent. The Z isomers also removed attraction when combined with either of the attractive E isomers. To optimize attraction, lures should be formulated to maximize the (1R,4S) and (1S,4R) isomers, and minimize the (1R,4R) and (1S,4S) isomers.

PHYSIOLOGY OF THE INVASIVE AMBROSIA BEETLE EUWALLACEA FORNICATUS IN RESPONSE TO FOUR STEREOISOMERS OF *P*-MENTH-2-EN-1-OL, THEIR SYNTHESIS AND ABSOLUTE CONFIGURATIONS

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ABSTRACT

Recently, the invasive ambrosia beetles in the *Euwallacea fornicatus* species complex, (Coleoptera: Curculionidae: Scolytinae), pests of avocado (*Persea americana*) and other woody trees, were discovered in California and Florida. These ambrosia beetles carry different species of mutualistic phytopathogenic fungi, (*Fusarium* spp) which cause *Fusarium* dieback in infected trees. The species in Los Angeles is referred to as the Polyphagous Shot Hole Borer (PSHB). We have identified a kairomone, *p*-menth-2-en-1-ol, from PSHB-infested box elder bolts (*Acer negundo*) and from ambrosia beetle diet tubes supplemented with box elder sawdust. Identifications were aided by coupled gas chromatographic-mass spectrometric (GC-MS) analysis, micro-chemical reactions, chiral GC-MS analysis, GC coupled electroantennographic detection (GC-EAD), and GC and MS comparisons with synthetic standards. All four enantiomers of *p*-menth-2-en-1-ol were synthesized and all four enantiomers showed antennal activity by GC-EAD using female antennae. Comparisons between PSHB-infested box elder volatiles and the synthetic enantiomers by chiral GC-MS analysis showed a single enantiomer present in the PSHB-infested box elder volatiles, which was a perfect match with (1*S*,4*R*)-*p*-menth-2-en-1-ol.

Commercial lures enriched in (1*S*,4*R*)-*p*-menth-2-en-1-ol are available for monitoring this invasive insect pest.

DEVELOPING AND IMPROVING SURVEY METHODS FOR EMERALD ASH BORER AND OTHER BUPRESTIDS

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ABSTRACT

Green multi-funnel traps, coated with fluon, a fluoropolymer, have been found to be comparable to or better than purple prism traps in terms of both trap catch and detection of the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae). As part of an ongoing project to improve survey tools for EAB, we conducted a multi-state comparison of several currently available traps on a variety of host and non-host tree genera. As part of this project we conducted a general woodborer survey comparing buprestid genera trap catch in each of the designs.

In 2013, the following four trap treatments: 1) green, fluoned multi-funnel traps baited with z-3-hexenol, 2) unbaited green, fluoned multi-funnel traps, 3) unbaited, fluoned purple multi-funnel traps and 4) unbaited purple prism traps were placed on one of four host genera: 1) *Fraxinus*, 2) *Pinus*, 3) *Acer* and 4) *Quercus* (red oak group only). Replicates consisted of a single host / non-host genus. In 2014, we placed traps on five host types: 1) *Fraxinus*, 2) *Populus*, 3) *Betula*, 4) *Quercus* (red oaks), 5) *Quercus* (white oaks).

We are still identifying specimens from some replicates, but have completed identifications from 56 of 72 replicates placed in 2013 and 75 of 101 replicates placed in 2014. Here we present preliminary findings from those completed traps. Among the different genera, more EAB were caught on ash than on the non-hosts. Overall in 2013, more EAB were caught on baited, green multi-funnel traps than on the other trap types, and detections were higher on green multi-funnel traps in general than on the purple multi-funnel and prism traps. In 2014, more EAB were caught on green multi-funnel traps and detection rates were higher than on purple traps.

A total of 30 and 27 species respectively have been identified from traps placed in 2013 and 2014. In general, green multi-funnel traps caught more non-EAB *Agrilus* species than purple traps. In 2013 out of 56 traps, 43 green baited multi-funnels, 42 green unbaited multi-funnels, 15 purple prisms and 18 purple

unbaited purple multi-funnels caught *Agrilus* species. In 2014, out of 75 traps, 51 green baited multi-funnels, 52 green unbaited multi-funnels, 30 purple prisms and 36 purple unbaited purple multi-funnels caught *Agrilus* species. Among other buprestid genera, more *Chrysobothris* and *Dicerca* species were caught on purple traps than on green traps, while in 2013 green multi-funnel traps caught more *Anthaxia*.

In 2013, we also tested the same trap and lure combinations in Poland that we tested in the US. Traps were placed on the following host genera: 1) *Fraxinus*, 2) *Quercus*, and 3) *Pinus*, in a total of 10 replicates. In the 10 replicates a total of 2015 *Agrilus* beetles in 11 species were caught. Similar to results found in the US, more beetles were caught on green traps than on purple traps. Among other buprestid genera, more *Phaenops* (*cyanea*) were caught on purple traps than on green traps.

Preliminary results from the general buprestid survey have shown that green multi-funnel traps may provide a useful tool for the capture and detection of other *Agrilus* species besides EAB.

TESTING FORMULATION IMPROVEMENTS FOR USE OF THE ENTOMO-PATHOGENIC FUNGUS *METARHIZIUM BRUNNEUM* AGAINST ASIAN LONGHORNED BEETLES

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ABSTRACT

The Asian longhorned beetle (ALB) infestation in Ohio, first detected in 2011, is still under eradication by USDA. In an ongoing effort by our Cornell lab group to assess the potential for biological control using insect-pathogenic fungi, in 2016 we tested a sprayable formulation of *Metarhizium brunneum* microsclerotia within the Ohio ALB regulated zone, at East Fork State Park. Since ALB might be rare or absent, effectiveness was tested using beetles in our quarantine lab colony. Small wood veneer samples (attached to tree trunks with Velcro®) were sprayed in June and then collected biweekly from June to August for use in bioassays with adult females. Additional samples were used for spore counts, since insect mortality depends on the density of infective spores produced by the microsclerotia.

Spore production peaked at 4 weeks after spraying, resulting in a median ALB survival time (ST50) of 9.5 days after beetle exposure. Samples incubated 2 weeks at 100% humidity after spraying had much greater sporulation than samples in the field, and all beetles died in 6-8 days.

The Ohio formulation included straw hydromulch and hydrogel to increase moisture retention. To test whether the gel provided a significant improvement over hydromulch alone, in a separate set of 4-week field trials in Ithaca, NY, three versions of the microsclerotial formulation were compared (straw, gel, or a mixture with both). There was no significant difference in beetle mortality in the lab exposures, both for samples collected from outdoors after a severe June drought, or from later in the season when precipitation was greater. In the August trial, a mere 2-day exposure of ALB was effective in killing all beetles within 10-11 days.

When rainfall is adequate, any of the three formulations showed some potential for development as a spray application onto tree trunks for use during summer when adult beetles would be active. However, for samples in the field during the drought, the addition of hydrogel to the formulation did not retain moisture long enough for sufficient spore production to kill ALB.

NEW HOLARCTIC SPECIES OF MICROSPORIDIA DISCOVERED IN FIELD AND LAB POPULATIONS OF *HALYOMORPHA HALYS*

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ABSTRACT

The brown marmorated stink bug (*Halyomorpha halys* Stål) is native to Asia but was first discovered in the US in Allentown, Pennsylvania in 1998. Since approximately 2010, it has become a significant agricultural pest with a wide range of hosts. *Halyomorpha halys* colonies being maintained by research institutions began to crash and, upon further investigation, microsporidia spores were discovered in the cadavers of individual insects from those colonies. Preliminary sampling of field populations detected microsporidia as well.

A similar microsporidian species had been reported in green stink bug (*Chinavia hilaris* Say) in the Midwest by Dr. J. V. Maddox in 1979 but never officially described. Microsporidia were also found in brown marmorated stink bugs in Asia. When compared genetically to samples taken from the North American Midwest and from Asia, the microsporidia recently discovered in the colonies of *H. halys* was found to be the same species.

In 2015-2016 a series of bioassays were performed to confirm the pathogenicity of this microsporidia. In these tests, individual egg masses were treated with microsporidia inoculum created from highly infected adults. At high concentrations, newly hatched *H. halys* nymphs became infected with the microsporidia although at low concentrations, infections did not occur.

Additionally, a survey of wild-caught *H. halys*, *C. hilaris*, and other native stink bug species revealed that the microsporidia was present in populations from New York to North Carolina, and that four species were known to act as hosts: brown marmorated stink bug, green stink bug, brown stink bug (*Euschistus servus* Say), and dusky stink bug (*Euschistus tristigmus* Say). All of the known hosts are within the subfamily Pentatominae.

FORECASTING FRUIT FLY RISK FOR EFFECTIVE RESOURCE ALLOCATION

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ABSTRACT

Fruit flies have consistently invaded the U.S. causing considerable economic impacts and incurring significant management costs. Since 2005, there have been at least 50 fruit fly incursions in the US, but annual incursion rates vary. This variation presents significant challenges for regulatory officials tasked with allocating resources on an annual basis. In years with many incursions, like 2015, additional funding is typically necessary to support eradication and monitoring compared to years with few incursions. This spatiotemporal variation in incursion patterns suggests climatological, biological, and anthropogenic differences between years contribute to variations in ports of entry, incursion hot spots, and high risk origin countries. The knowledge and models related to this spatiotemporal variation can be unified to help predict high risk ports of entry and hot spots for upcoming time periods.

Our integrated Medfly Risk Model (MRM) is a decision support tool—based on the spatiotemporal variation in incursion patterns—that will enable stakeholders to make better informed choices on resource allocation. Mediterranean fruit fly "Medfly" (*Ceratitis capitata*) represents an excellent opportunity to begin integrating models because of the wealth of information on the pest and number of individual models already developed. However, other fruit flies also cause considerable impact and might have different spatiotemporal invasion patterns that warrant their own integrative risk based models. In the future, we aim to expand the Medfly Risk Model to include other high risk fruit fly species and develop a mechanism to rapidly disseminate forecasting information to key stakeholders. This information may assist with monitoring ports of entry to improve pest exclusion, field surveillance and monitoring, optimizing eradication strategies, and determining optimal lengths of quarantine.

ROGUE ONE IN THE FOREST: A WALNUT TWIG BEETLE STORY

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ABSTRACT

Walnut twig beetle (WTB), *Pityophthorus juglandis*, has a pivotal role in the spread of thousand cankers disease (TCD) and the decline and death of black walnut trees. WTB vectors the fungus *Geosmithia morbida*, which forms numerous cankers on the tree trunk, reducing nutrient flow. WTB and TCD have been documented in urban and rural areas in numerous states, but little is known about their presence in forests. To enhance knowledge and understanding of incidence, distribution, and dispersal of WTB in forests, research was initiated to: 1) document incidence and distribution of WTB in Appalachian forests (eastern TN/western NC), and 2) assess dispersal of WTB within a forest. To assess WTB in forests, 30 pheromone-baited funnel traps were deployed in or near black walnuts in forested areas at 11 locations in eastern Tennessee and western North Carolina. In 2016, 140 WTB were collected from five traps (17% of all traps) in three forested areas [Anderson (n=1), Blount (n=5), and Knox (n=134) Co., TN]. WTB was not collected from 83% of traps (n=25) at eight forested locations. To assess dispersal of WTB in a forest, a study was initiated at the University of Tennessee Forest Resources AgResearch and Education Center (Morgan Co., TN) on 6 June 2016. No symptomatic trees were present, and no WTB were previously collected at this site. Four-funnel traps (n=78) baited with WTB lure were placed in transects every 8 m (8 to 96 m) uphill and downhill from a release site located ca. 560 m from the forest edge. One trap each also was placed 50 m east and west of the release site; transects were replicated three times, ca. 100 m apart. WTB adults (25 female and 25 male per transect) were released on 10 June and again on 31 August 2016. Following releases, traps were monitored every 7 to 8 days; contents were taken to the laboratory and examined. No apparent trend in WTB movement was observed, as WTB were collected at a range of distances (8 to 96 m) in Spring (n=14) and Fall (n=8). Dispersal may be enhanced by close proximity of traps to one another, creating a 'chemical corridor' to assist with navigation to farther traps. Results indicate WTB can disperse to walnut in forests. Further research will determine specific factors that may influence dispersal ability, thereby enhancing management efforts against WTB.

PHENOLOGY AND OVERWINTERING SURVIVAL OF THE INTRODUCED LARVAL PARASITOIDS OF EMERALD ASH BORER IN THE NORTHEAST

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ABSTRACT

Three larval parasitoids (Hymenoptera) have been introduced to the United States for biological control of emerald ash borer (EAB), *Agrilus planipennis*. Two species, *Tetrastichus planipennisi* Yang (Eulophidae) and *Spathius agrili* Yang (Braconidae) were discovered in China and have been released in most states with known EAB infestations. While *T. planipennisi* appears to be establishing well, *S. agrili* does not appear to be doing so in northern states. A third larval parasitoid, *S. galinae* Belokobylskij & Strazanac, was discovered in a more northern range in the Russian Far East, and climate matching suggests this species may be better suited for northern climates in North America.

We conducted phenology studies at three scales in Syracuse, NY (growth chambers, an open-air insectary, and caged trees in the field) to document phenology of all three larval parasitoids and EAB in the northeast in an attempt to determine if asynchrony between parasitoid development and host availability or climate could have an impact on establishment. Parasitoid colonies were started in 2015 with adults obtained from the USDA APHIS EAB parasitoid rearing facility (Brighton, MI). Growth chambers were programmed with sub-cortical temperature data collected from under the bark of ash trees in the field.

We monitored EAB phenology in 2015 and 2016, and detected one and two-year life cycles, with the preferred host size (3rd and 4th instar larvae) present from mid-May to early-Oct. Insectary and growth chamber studies indicated *S. galinae* was well-adapted to the northeast as it emerged before EAB, suggesting it can take advantage of EAB developing with two-year life cycles, and it completed multiple generations in one season. Field studies suggest *S. galinae* is adapted to fall temperatures in the northeast and can reach overwintering stages even when eggs are oviposited later in the fall and temperatures begin to decrease. *Spathius agrili*, which emerged after EAB, partially completed only one generation, and had difficulty overwintering. This parasitoid is from a more southern climate in China and appears to be asynchronous with both EAB development and climate in the northeastern US. Though we had difficulty rearing *T. planipennisi* in the insectary, other studies indicate it is establishing and dispersing well where it has been released in the northeast.

Our results indicate *S. galinae* is a better-suited biocontrol agent than *S. agrili* for the northeast. Continued monitoring of parasitoid phenology should further elucidate factors inhibiting/facilitating establishment of parasitoids for biological control of EAB in the northeast.

COMPARISON OF 14-DAY SURVIVAL AND DEVELOPMENT OF GYPSY MOTH POPULATIONS FROM DIFFERENT GEOGRAPHIC AREAS ON NORTH AMERICAN CONIFERS

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ABSTRACT

North American gypsy moths (*Lymantria dispar dispar*, originally from Europe) are highly polyphagous, with 148 host trees identified as highly susceptible hosts. Asian gypsy moth larvae (*L. dispar asiatica* and *L. dispar japonica*) utilize many of the same host tree species and have been reported to feed on 316 species from 61 orders and 130 genera. Since the Asian subspecies continue to be detected on ships and cargo as well as in pheromone traps near ports, host utilization information is critical to managers for estimating the hosts at risk and potential geographic range in North America. A total of 100 larvae per host (20/container) each from six different geographic areas (4 Asian and 2 European) were reared on 16 different hosts in chambers maintained at 25±1°C and 60% RH with L:D of 16:8. At 14 days, the surviving larvae were counted, individually weighed, and instar was determined. Only two larvae out of 600 survived to 14 days on *Pinus palustris* and *P. taeda*. Survival on *Juniperus virginiana* was < 7% for all populations. Greater than 79% of the larvae from all the populations survived to 14 days on *Quercus velutina*, *Tsuga canadensis*, *Larix occidentalis*, and *Betula populifolia*. Survival on the other hosts was variable among populations, but larval survival of Asian populations was not consistently higher than that of the *dispar* subspecies populations. Several individual larvae attained the fourth instar (predominant instar attained on *Q. velutina*) on *Picea pungens*, *Pinus ponderosa*, *T. canadensis*, and *L. occidentalis* which would suggest that those hosts are suitable for several of the populations. However, weight gain on these hosts was substantially lower than on *Q. velutina*, a preferred host. Weight gain on *Pinus strobus* and *Picea glauca* was very low even compared to *Acer rubrum* (a poor host) despite fairly good survival. Lower weight gain and survival on *Psuedotsuga menziesii* and *P. glauca* were due, at least in part, to a mismatch between tree phenology and hatch. While there was variation in the ability of gypsy moth larvae from different geographic origins to utilize key North American conifers, that variation was not consistent within gypsy moth subspecies. Although development is slower and survival poorer on several of the conifers, the first instars are able to utilize conifers that do not have tough needles (*P. taeda* and *P. palustris*) or contain some compounds that cause metabolic problems or deter feeding (*Abies concolor* and *J. virginiana*). Host phenology will be critical on some conifers since the early instars feed preferentially on only new foliage or buds of several of the conifers evaluated. These results suggest that the measures being taken to prevent establishment of gypsy moths of all subspecies in western North America are warranted given the risk to the economically important conifers found there. The risk of gypsy moth establishment on southern pines appears to be very low, but larger larvae may feed on them.

PHYTOPHTHORA AND *PYTHIUM* SPECIES IN PENNSYLVANIA

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ABSTRACT

We recovered *Phytophthora* and *Pythium* isolates in PA forest streams in 2010-2013. Nine streams were selected for bait monitoring of *Phytophthora ramorum* using mature, healthy leaves of *Rhododendron.* All stream bait locations had tree species common to PA, intact mixed hardwood (Quercus, Carya, Betula, Acer) and conifer (Pinus, Tsuga), with a substantial understory of susceptible foliar hosts consisting of *R. maximum*, *Kalmia latifolia* L. and *Vaccinum* spp. At each location, two nylon mesh bait bags were left in targeted streams for two weeks; three bait periods were implemented in spring and autumn when stream flows were consistent and stream temperatures remained between 15 °C and 22 °C. A total of 224 leaf and 26 leaf disks samples were analyzed for *P. ramorum* using *P. ramorum*-specific primers and all samples came *P. ramorum* negative. Leaf samples were also incubated on PARP, and 241 cultures in total were isolated. Species was determined for 144 isolates by sequencing their ITS regions, which revealed the presence of sixteen *Phytophthora*, *Pythium, and Phytopythium* species.

IDENTIFICATION AND ORIGIN OF INFESTED WOOD PACKING MATERIAL

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ABSTRACT

Despite stringent phytosanitary regulations, solid wood packing material (WPM) is still an important pathway of introduction for exotic wood boring insects. An ongoing cooperative effort between U.S. Customs and Border Protection and USDA Animal and Plant Health Inspection Service (APHIS) is promoting identification of live larvae of cerambycids, buprestids and siricids intercepted at U.S. ports. Data are applied to development of risk maps for pest entry and establishment. This study aimed to identify the most commonly intercepted wood genera used to construct WPM to better understand the pathway of pest introduction. *Pinus* (40%), *Picea* (19%), and *Populus* (20%) are the three most common WPM tree genera intercepted with wood borers. WPM may originate in areas outside the shipment origin; we therefore also explored the possibility of using stable isotopes to ultimately pinpoint its origin. δ^2H and δ^{18}O values were calculated for 20 pine WPM samples from five Eurasian countries. Lacking data on where the pines grew, we designated the countries where phytosanitary treatment marks were applied to WPM as proxies for origin. δ^2H and δ^{18}O isotope profiles were significantly correlated to each other. The combined profile showed some discrimination among regions. δ^2H values accounted for 90% of the observed variation in origins. If a global map of δ^2H isotope values is created from wood originating at precise locations, it will be possible to pinpoint the geographic origins of WPM samples by comparing these values with an isoscape, a predictive geographic model.

EVALUATING PHOTOSYNTHESIS IN URBAN VERSUS RURAL RED MAPLES

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ABSTRACT

Photosynthesis is a fundamental process that trees must perform over fluctuating daily and seasonal environmental conditions. For long-lived trees, the ability to acclimate photosynthetically is a critical adaptive characteristic and is particularly relevant in urban environments, which, due to the urban heat island effect, can be much warmer than surrounding rural areas. In this study, we measured photosynthesis and stomatal conductance of red maple (*Acer rubrum* L.) along an urban-rural gradient in Raleigh, North Carolina, U.S.A. We asked: (1) Do rates of photosynthesis and stomatal conductance differ between sites over the growing season, indicating long-term photosynthetic acclimation? and (2) Do rates of photosynthesis and stomatal conductance differ between sites during experimental temperature increases, indicating the capability for short-term photosynthetic acclimation?

While cities possess a host of environmental conditions that may be stressful to plants, in this study, urban sites did not have a negative effect on red maple photosynthesis. Over the growing season, rates of photosynthesis and stomatal conductance were significantly higher for urban red maples than for suburban or rural trees. When leaf temperatures were experimentally raised, urban trees still maintained higher rates of photosynthesis and stomatal conductance than did suburban or rural trees. These results suggest that red maple is a valuable species for maximizing urban ecosystem services while minimizing economic and management costs. Our results also suggest the stability of this species in rural forests under future climate change. However, warming that promotes photosynthesis may simultaneously exacerbate other abiotic and biotic stresses, such as drought and herbivory, that reduce tree growth and condition. The interactions between abiotic and biotic factors have only recently begun to be investigated in urban landscapes, but are a key component of understanding the long-term performance of urban trees and the potential effects of climate change on rural forests.

EFFECT OF TRAP HEIGHT ON CATCHES OF BARK AND WOOD BORING BEETLES IN A STAND OF WHITE OAK AND SHORTLEAF PINE IN GEORGIA

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ABSTRACT

Detection programs for non-native, potentially invasive species of bark and wood boring beetles typically employ traps placed at breast height. Species composition of insects inhabiting forest canopies are known to differ from those closer to the ground (Dodds 2014 *Can. Ent.* 146: 80-89). Our objective was to determine the effect of trap height on catches of common species of bark and wood boring beetles, and their associates, in a stand of white oak and shortleaf pine in north-central Georgia. In 2014, we conducted two experiments in a mature stand of white

oak and shortleaf pine in northcentral Georgia (Jasper Co.). In both experiments, twelve 10-unit modified multiple-funnel traps were set in six replicate blocks of two traps per block. In each block, one trap was positioned at breast height (Ground) and one trap positioned 18-22 m

above ground (Canopy). Traps were spaced > 8m apart horizontally. Each trap in Expt. 1 was baited with lures releasing a hardwood borer blend (EKD) of ethanol, 3,2-hydroxyketones and 2,3-hexanediol, whereas each trap in Expt. 2 was baited with lures releasing a pine borer blend (ASD) of α-pinene, ipsenol and ipsdienol. Cups contained a solution of propylene glycol. Expt. 1 was conducted 30 May to 9 October 2014 with lure changes on 2 July, 31 July and 1 September, whereas Expt. 2 was conducted 6 August to 9 October without any lure changes. We found that among Cerambycidae, canopy traps were preferred over ground traps by *Acanthocinus obsoletus*, *Monochamus titillator*, *Neoclytus jouteli*, *N. scutellaris* and *Xylotrechus sagittatus*. The reverse was true for *N. acuminatus* and *Xylotrechus colonus*. *Cnestus mutilatus*, *Hylocurus rudis* and *Ips avulsus* were more common in canopy traps whereas *Dendroctonus valens*, *Dryoxylon onoharaenses*, *Hypothenemus rotundicollis*, *Stenoscellis brevis*, *Xyleborinus saxesenii* and *Xylosandrus crassiusculus* were more common in ground traps. Similar patterns were shown by predators and associates.

MONITORING AND SHIP INSPECTION PROGRAM FOR ASIAN GYPSY MOTH (ORDER: LEPIDOPTERA) AND OTHER LYMANTRIA IN THE RUSSIAN FAR EAST, JAPAN, SOUTH KOREA AND CHINA

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ABSTRACT

The genus *Lymantria* (Hübner) contains some of the most destructive forest pests in the world. Asian gypsy moth (AGM), *Lymantria dispar asiatica* Vnukovskij larvae are known to feed on over 500 plant species, including a variety of conifers and hardwoods. In the early 1990s, AGM was introduced into the Pacific Northwest by infested ships originating from ports in the Russian Far East. Unlike the flightless female European gypsy moth, AGM females are capable of flying distances of up to 32 km (20 miles). AGM overwinters in the egg stage and depending on latitude, larval development occurs from May through July with peak adult flight occurring from July through early September. Since 1994, a cooperative agreement with the Russian government has focused on annual AGM detection surveys in and near Russian Far East ports to determine population levels. Analyses of population densities help determine on a seasonal basis, the risk of introduction by ships and cargo coming from or transiting within the Russian Far East. Other monitored lymantriids include the nun moth, *L. monacha* and the pink or rosy gypsy moth, *L. mathura*. Although AGM is not established in North America, monitoring and trapping programs continue to detect AGM introductions periodically. The first detection of AGM occurred on a Russian ship docked in Vancouver, British Columbia in 1991 where egg masses were observed hatching. Pheromone detection trapping also caught AGM adult males in Portland, OR, and Tacoma, WA. Swift regulatory and control actions were put in place, and these localized populations were eradicated. As a result of these eradication programs, the USDA and our Canadian colleagues developed a map of high-risk areas in cooperation with the governments of Russia, Japan, China, and South Korea. These programs minimize the risk of AGM introductions into North America through inspections and certifications of vessels entering North American ports from these locations. Program goals include: 1) continuing to improve communications among stakeholders, the shipping industry, accredited inspection and certification bodies, and National Plant Protection Organizations, 2) increasing the number of maritime vessels arriving with compliant certificates, and 3) developing best regulatory options for increasing program compliance and reducing potential introductions of AGM into North America. Currently, the North American Plant Protection Organization is working to align the elements of the respective AGM programs based on Regional Standards for Phytosanitary Measures (RSPM 33: *Guidelines for regulating the movement of vessels from areas infested with the Asian gypsy moth*), which was implemented in 2012. The USDA, Canadian Food Inspection Agency and Canadian Forest Service continue to emphasize offshore AGM inspection and certification programs that were established with Russia (1994), Japan (2007), South Korea (2010), and China (2011).

LIMITATIONS TO ESTABLISHMENT OF INTRODUCED PARASITOIDS OF EMERALD ASH BORER: A SOUTHERN PERSPECTIVE

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis*, is an invasive wood-boring beetle native to Eastern Asia. It was first found in North America near Detroit, MI in 2002. The primary habitat and food source for EAB is ash (*Fraxinus* spp.), and since its introduction to the U.S. it has been responsible for the deaths of millions of trees. EAB was initially discovered in Tennessee in 2010, and open releases of introduced parasitoids of EAB (*Spathius agrili*, n ≈ 24,000, and *Tetrastichus planipennisi,* n ≈ 84,000) were initiated in 2012. Following these releases, a study was initiated to assess parasitoid establishment via pan trap collections. These assessments will inform biological control activities directed against EAB in the South and emphasize any limitations to their use. Beginning in January 2015 10 pan traps were deployed at release sites. Pan traps consisted of yellow plastic bowls zip-tied to a metal bracket attached to a tree and half filled with diluted propylene glycol antifreeze. Traps were collected once every week in spring and summer, and once every other week in fall and winter. Collections were taken to the laboratory and examined under a microscope. All suspect parasitoids were placed into vials containing 95% ethyl alcohol, and suspect EAB parasitoids were shipped to USDA APHIS PPQ Center for Plant Health Science and Technology for visual identification and molecular confirmation. One adult female *S. agrili* was collected from a pan trap in Hamblen County, TN. Native *Atanycolus* species and *Spathius* species make up the majority of parasitoids collected. Native *S. floridanus* was the most collected parasitoid (67% [67/100] of all parasitoids). No adults of *T. planipennisi* were collected from any site. Pan trapping continues through 2017. While the discovery of *S. agrili* in a release site is promising, as it indicates *S. agrili* can overwinter and reproduce in the wild, several factors could limit the success of this and other introduced parasitoid species in the southeast. Primary limitations to success in the South are: EAB's annual life cycle that is exhibited in the southeastern U.S., extreme temperature fluctuations, especially during winter months, and lack of diapause in *T. planipennisi.* These factors have the potential to affect the phenological synchrony of EAB and its introduced parasitoids in a southern climate. Further study is required to determine the future use of currently deployed parasitoid species, or if new, more tolerant and climate-adapted species should be sought as part of biological control efforts.

DETECTION OF BARK- AND WOOD BORING BEETLES IS INCREASED BY USING A DIVERSITY OF TRAP COLORS AND HEIGHTS AND IS DECREASED BY *Z*-3-HEXENOL

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ABSTRACT

The impact of emerald ash borer on North American ash, combined with the diversity of buprestids worldwide (>2700 *Agrilus* spp.) and the ever increasing volume of global trade suggests there is high risk that additional exotic species of buprestids may become invasive pests in North America. Most surveys for exotic wood borers use black funnel traps placed about 1.5 m from the ground. While these are effective at detecting many species of Scolytinae and Cerambycidae, they detect very few Buprestidae. Our objective was to determine the effect of trap color, trap height and *Z*-3-hexenol on efficacy of detecting species of bark and wood borers, and in particular improve the efficacy of detecting *Agrilus* spp. and other buprestids. Based on emerald ash borer literature, we predicted that detection of buprestid species would be: greater in green or purple traps than in black traps; greater in the tree canopy than in the understorey; and would be enhanced by *Z*-3-hexenol. We also predicted that species composition of target taxa (Buprestidae, Cerambycidae, and Scolytinae) would differ among trap colors and heights, and therefore we would detect more species overall by using more than one trap color and by placing some of the traps in the canopy and some in the understorey. We conducted two field experiments in 2015: (1) a 3 x 2 factorial testing the effects of trap color (black *vs.* green *vs.* purple) and trap placement (tree canopy *vs.* understorey) with all traps baited with a "superlure", conducted at four sites (New Brunswick, Canada; Białowieża, Poland; Georgia, USA; and Jilin, China); and (2) a 3 x 2 factorial testing the effects of the same three trap colors and lure (superlure *vs.* superlure + *Z*-3-hexenol), with all traps in the canopy, conducted at three sites (New Brunswick, Massachusetts, and Ontario). Treatments were replicated 8x in randomized blocks at each site and all traps were coated with Fluon®. The superlure was a multi-lure combination of racemic 3-hydroxyhexan-2-one, racemic 3-hydroxyoctan-2-one, *R*R**-2,3-hexane diols,

E/*Z*-fuscumol, *E*/*Z*-fuscumol acetate, and UHR ethanol. Preliminary results from experiment 1 (New Brunswick and Poland) were presented at the USDA Interagency Research Forum on Invasive Species in January 2016. Here we summarize nearly complete results from both experiments on detection of Cerambycidae (all sites), Buprestidae (all sites but Jilin and Ontario), and Scolytinae (all sites but Jilin, Ontario and Georgia).

In terms of species richness, the number of buprestid species detected per trap was significantly affected by trap color and trap height but not by *Z*-3-hexenol. Green traps in the tree canopy detected the greatest number of buprestid species, followed by purple canopy traps. Mean catch per trap and/or detection rate of individual buprestid species was significantly greatest in: green traps for 10 species (e.g., *Agrilus laticornis, A. obscuricollis*), purple traps for 4 species (e.g., *Agrilus sulcicollis, Dicerca divaricata*), black traps for 0 species, canopy traps for 10 species (e.g., *Agrilus angustulus, Trachys minuta*), and understorey traps for only 1 species (*Dicerca lurida*). Cerambycid species richness per trap was not significantly affected by trap color or height but was significantly reduced by *Z*-3-hexenol. However, mean catch per trap and/or detection rate of several individual cerambycid species was significantly affected by trap color and/or trap height and was greatest in: green traps for 16 species (e.g., *Anoplodera sexgutatta*, *Molorchus minor*), black traps for 12 species (e.g., *Plagionotus pulcher*, *Xylotrechus colonus*), purple traps for 8 species (e.g., *Elaphidion mucronatum*, *Tetropium cinnamopterum*), canopy traps for 16 species (e.g., *Saperda scalaris, Sphenostethus taslei*), and understorey traps for 19 species (e.g., *Prionus insularis, Neoclytus mucronatus*). The addition of *Z*-3-hexenol lures to traps baited with the superlure significantly affected detection of 18 cerambycid species, decreasing catches of 15 species (e.g., *Sarosesthes fulminans*) and increasing catches of only 3 species (e.g., *Graphisurus fasciatus*). Species richness of Scolytinae in traps was not affected by trap color but was significantly greater in the understorey than in the canopy. Trap height significantly affected mean catch per trap and/or detection rate of six species of Scolytinae, three species that were caught mainly in the canopy (e.g., *Xyleborinus saxesenii*), and three species caught mainly in the understorey (e.g., *Xylosandrus germanus*). *Z*-3-hexenol significantly affected detection of only two Scolytine species, increasing catch of *Polygraphus rufipennis* and decreasing catch of *Anisandrus sayi.* Because species composition differed between traps in the canopy and the understorey, the mean total number of species detected per trapping effort was increased by placing half of the traps in the canopy and half in the understorey. For example, in Poland, a mix of four green canopy traps and four purple understorey traps detected 58 species of target taxa compared to only 34 species detected in eight black understorey traps. We conclude that efficacy of detecting exotic and potentially invasive bark- and wood boring beetles at survey sites would be improved by using more than one color of trap and by placing traps in both the canopy and the understorey.

VULNERABILITY OF US SOYBEAN GERMPLASM TO RED LEAF BLOTCH CAUSED BY *CONIOTHYRIUM GLYCINES*

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ABSTRACT

Red leaf blotch of soybeans is a serious disease that causes blotching and defoliation of soybeans (*Glycine max* (L.) Merr.) and a wild legume, *Neonotonia wightii*. The disease was first reported as *Pyrenochaeta glycines*, occurring in Ethiopia. Since then, it has occurred in several Eastern, Central, and Southern African countries and has also been reported from Bolivia. Red leaf blotch has been deemed a potentially devastating disease for US soybean production and thus *C. glycines* is currently listed as one of the USDA Plant Protection and Quarantine (PPQ) Select Agents and Toxins. Red leaf blotch is known to cause serious soybean losses in the field, and it was observed that all commercial US cultivars tested in Zambia and Zimbabwe between 1982 and 1984 were susceptible; no resistance was noted among local lines nor ca. 5500 additional exotic soybean lines tested. Cultivars and breeding lines tested to date have been evaluated in the field in regions where the disease is endemic, requiring resources including land and the presence of natural inoculum sources. A soybean seedling inoculation method was developed to provide a means of screening soybean lines with greater efficiency. To determine conditions for optimal infection, the effects of inoculum density, temperature (20°C and 25°C), and dew chamber incubation period (2-5 days) on disease expression in Williams 82 soybeans were determined. Similar results were obtained for studies performed in dew chambers and in a humidity tent used in a greenhouse environment. Twenty-three soybean genotypes that represent nearly 90% of the genes present in US soybeans were evaluated using the seedling inoculation method, and found to be susceptible. The assay will prove useful in determining the reactions of additional soybean genotypes to red leaf blotch. These results underscore the vulnerability of US soybeans to red leaf blotch and suggest that screening of additional soybean germplasm from diverse sources be performed. If sources of resistance can be identified, such resistance can be incorporated into US soybean varieties in the event that *C. glycines* enters and becomes established in the US.

BRACKETING THE SPATIAL DISTRIBUTION OF RISK WITHIN AN INFESTATION OF THE ASIAN LONGHORNED BEETLE (*ANOPLOPHORA GLABRIPENNIS*)

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ABSTRACT

Mapping the spatial distribution of the risk for the dispersal and expansion of an invasive species often requires detailed information on the biology, ecology, and behavior of the species, information which is not always available. Efforts to eradicate populations of the Asian longhorned beetle (*Anoplophora glabripennis*) highlight some of the challenges presented by limited information on organism biology and behavior. Previous work (Trotter and Hull-Sanders, 2015) demonstrated a graph theory based approach to inferring the dispersal of the beetle using records documenting the locations and infestation levels of individual trees. However, these efforts were limited in two key ways. First, the analyses used did not capture the full range of possible dispersal behaviors exhibited by the beetles, and second, the estimated dispersal probabilities were not integrated over the landscape to account for both the locations and abundances of infested trees. Here, this work is extended to address both of these issues.

To infer beetle movements, the locations and levels of infestation of individual trees were identified by surveys conducted by the US Department of Agriculture Animal Plant Health Inspection Service Asian Longhorned Beetle Eradication Program and State Cooperators, and these values were used to map the infested trees. These data were then combined with two separate and discrete rules regarding the hierarchy of beetle movement to identify connections among trees. Rule set *A* results in numerous long-distance dispersal events, with very little short distance movement, implying this rule set may overestimate the distances beetles move, while rule set *B* allows short movement distances, but fails to capture some longer, though less common dispersal events. Consequently, set *A* likely overestimates dispersal distances, while set *B* underestimates them, resulting in two distinct dispersal kernels, which, when applied to the known locations of infested trees, allows for a bounded estimation of spatial risk on the landscape surrounding known infestations of the Asian Longhorned Beetle.

ASSOCIATIONS BETWEEN *MATSUCOCCUS MACROCICATRICES*, *CALICIOPSIS PINEA*, AND EASTERN WHITE PINE DIEBACK IN THE APPALACHIANS

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ABSTRACT

Eastern white pine (*Pinus strobus* L.), an ecologically important tree in North America, has undergone unprecedented dieback and mortality during the last few decades. Symptomatic trees show branch flagging and dieback, crown thinning, and cankers with pitching (Mech et al. 2013, Schulz 2015). Associated with cankers are a bast scale insect, *Matsucoccus macrocicatrices* Richards and fungal pathogens especially, *Caliciopsis pinea* Peck. To elucidate this novel phenomenon, we assessed the spatial patterns of eastern white pine dieback in 120 plots in the southern Appalachian Mountains (Schulz 2015). More eastern white pine trees are symptomatic in the higher latitudes, and trees in denser stands are more severely impacted. Sapling (2.54-12.45 cm) and poletimber (12.7-22.61 cm) sized trees have greater dieback than sawtimber (≥ 22.86 cm) sized trees. Further, to assess colonization patterns at the tree-level in different age classes, we cut-down 45 trees in northern Georgia. Pole-sized trees harbored the most scale insects. Larger cankers and more scale insects were found on lower than higher branches indicating bottom-up mortality patterns. Branch areas closer to the bole had more scale insects than the ones further away on pole-sized and sawtimber trees. There were no differences in *C. pinea* incidence between age-classes and branch areas in sapling and sawtimber-sized trees. Future studies will focus on the population genetics of *M. macrocicatrices* to elucidate whether it was introduced to the southeastern U.S. (previously it was reported only from the northeastern U.S.); relative contributions of insect and pathogen to canker formations; and monitoring of long-term plots to determine temporal changes in eastern white pine health.

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SHORT-TERM DETECTION OF IMIDACLOPRID IN STREAMS FOLLOWING TREATMENT OF EASTERN HEMLOCK

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ABSTRACT

Since the introduction of hemlock woolly adelgid (HWA) (*Adelges tsugae*) from Japan in the 1950s, millions of eastern hemlock (*Tsuga canadensis*) have died in areas where HWA has established. To preserve remaining hemlock resources, imidacloprid has been applied to hemlock in many HWA-infested areas. However, the ability of imidacloprid to leach into stream waters adjacent to treated hemlock and potentially affect aquatic systems is a concern. The objective of this research is to document the time interval from application to initial detection in streams adjacent to imidacloprid-treated eastern hemlock. Four streams (treatment streams: Black House Branch, Coyle Branch, and Laurel Fork Creek; control stream: Middle Creek) at Big South Fork National River and Recreation Area (BISO) were sampled monthly from May 9, 2014 to May 4, 2015 to screen for potential contamination of stream water by imidacloprid. One additional sample set was collected during a rain event (October 10, 2014). On each sampling date, two 1 L water samples were collected downstream from the imidacloprid-treated area. One water-blank (distilled water from the lab transferred to a bottle at a treatment stream) was conducted each sampling date for quality control. Chemical analysis of samples was conducted by Pyxant Labs, Inc. (Colorado Springs, CO), using liquid chromatography tandem mass spectrometry; level of detection was 25 parts per trillion (ppt). Imidacloprid was detected during only one sampling event (October 10, 2014) from all treatment streams. Maximum concentrations of imidacloprid were 53.3 (Black House Branch), 268.0 (Laurel Fork Creek), and 833.0 (Coyle Branch) ppt. These samples were conducted during a rain event (ca. 4.7 cm the day of sampling). Imidacloprid was not detected in any of the treatment streams on any other sampling date, nor from the control stream. Days since treatment ranged from 184 to 196. These findings document the earliest detections of imidacloprid in streams adjacent to treated hemlock. However, concentrations observed in this study are well below the U.S. Environmental Protection Agency's chronic (1,050 ppt) and acute (34,500 ppt) benchmarks for aquatic macroinvertebrates. Imidacloprid easily photodegrades, thus it may be difficult for concentrations to consistently reach chronic or acute benchmarks in natural systems. While imidacloprid may move to streams within six months of application, the infrequency of detection and low concentrations observed suggests low risks to aquatic macro-invertebrates. Thus, the benefit to maintaining healthy trees may outweigh the potential low risk to stream macroinvertebrates.

IDENTIFICATION OF THE GEOGRAPHIC ORIGIN OF INVASIVE ASIAN GYPSY MOTH USING GENOMIC DATA

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ABSTRACT

As one of the most destructive defoliating pest insects in the world, the European gypsy moth (*Lymantria dispar dispar*, EGM) was introduced to North America from Western Europe around 150 years ago. Currently nearly the entire northeastern portion of the U.S. is under quarantine for this species. In recent years, egg masses of Asian subspecies of gypsy moth (AGM, including *L. d. asiatica* and *L. d. japonica*) have frequently been intercepted on vessels and cargo at US ports of entry. Adult AGM have also been detected during USDA annual domestic surveys. The port and domestic interceptions of AGM is alarming because compared to the established North American population AGM has a wider host range and stronger flight propensity, which could allow them to spread even more rapidly. Identifying the country of origin of invasive AGM provides the data necessary to evaluate trading policies that aim to prevent AGM introduction.

Using Double Digest Restriction-Site Associated DNA (ddRAD) sequencing approach, we developed 55 anonymous nuclear loci and 5 mitochondrial loci and genotyped 1328 gypsy moth samples worldwide. Principal-component analysis showed substantial genetic differentiation between AGM and EGM, with populations from central Asia and western Russia transitioning from the genetic makeup of AGM to EGM. For the first time, we would be able to distinguish populations within AGM into three genetic clusters, which correspond to three geographic regions, China, Japan, and a mixture zone comprising northeastern China, Russian Far East, and the Korea Peninsula. This separation will allow us to assign AGM egg masses and adult moths into one of the three regions. For example, AGM egg masses intercepted at ports in Houston, Texas and Juneau, Alaska were identified as originating from Japan and the mixture zone, respectively. Additionally, among 17 genotyped male moths caught in survey traps in Washington State in 2015, three were confirmed as AGM and assigned to multiple geographic origins.

We have further advanced our understanding of the global population structure of gypsy moth from previous studies based on microsatellite data and mitochondrial sequence data. Our study provided the capability to assign survey or outbreak AGM specimens to putative sources among Asian regions for future identification of introduction pathways.