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FOREWORD

This meeting was the 26th in a series of annual USDA Interagency Research Forums that are sponsored by the Forest Service, Animal and Plant Health Inspection Service, National Institute of Food and Agriculture, and Agriculture Research Service. The Group’s original goal of fostering communication and providing a forum for the overview of ongoing research among the Agencies and their cooperators is being realized and facilitated through this meeting. This proceeding documents the efforts of many individuals: those who organized and sponsored the meeting, those who provided oral and poster presentations, and those who compiled and edited the contributions. The proceedings illustrates the depth and breadth of studies being supported by the agencies and their many cooperators and demonstrates the benefits and accomplishments that can result through the spirit of collaboration.

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PRESENTATIONS
Keynote Address
ECO-EVOLUTIONARY INTERACTIONS OF DEER, EARTHWORMS, AND INVASIVE PLANTS TRANSFORM FOREST COMMUNITIES

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ABSTRACT

All habitats of the Northeast and their inhabitants are currently facing an unprecedented challenge by needing to simultaneously respond to global climate change, invasive species, habitat fragmentation and increasing deer herds. To the casual observer it is not immediately clear how blatant disturbances such as windstorms interact with more “subtle” changes during succession, range expansion of native species or the increasing abundance of introduced and invasive species. All organisms experience these changes simultaneously and the important question is whether their behavioral repertoire and adaptability is able to appropriately respond. Which species can be assumed safe? Which species will likely go extinct? And what is the role of humans not only as facilitators of global change but also of stewards of our natural heritage? Long-term investigations detailing the interplay of deer, worms and invasive plants consistently implicate high abundances of white-tailed deer as the drivers of native plant species declines in eastern forests. While earthworms have discernable negative effects on native plant species and litter food webs, invasive plant species appear passengers rather than drivers of ecosystem change. Our evaluations of multiple stressors and their effects on native plants show that plants are also responding by changing their phenology but without significant reductions in the white-tailed deer, the future of forests in the northeast is bleak.
UNDERSTANDING INVASION PATHWAY RISKS AND EFFECTS OF MITIGATION MEASURES

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ABSTRACT

Invasions of forest pests are a major concern worldwide, and detections of new insect pests and pathogens are a regular occurrence in many countries. The ongoing increase in international trade is a main cause of this but our knowledge of the role of different invasion pathways, the level of propagule pressure of potential invaders, and our ability to prevent invasions are limited. Here I use a combination of information on pest detections trends, border interception data, and international trade data to examine the effects of phytosanitary measures designed to mitigate pathway risks. A case study on measures for one comparatively well-understood pathway, ISPM No. 15 (an international standard regulating treatments of wood packaging material used in international trade) was shown to be effective in reducing invasions to some extent. Although non-compliance issues may need more attention, these findings are encouraging and suggest that improvements are also possible with other high-risk pathways such as the live plant trade and “hitchhiker pests” in shipping containers. However, the effectiveness of mitigation measures may partly be overwhelmed by increases in international trade and associated propagule pressure.

INTRODUCTION

Biological invasions are affecting more and more ecosystems worldwide. Forests in North America have been the subject of particularly many invasions but this is clearly a significant global problem. Although most invaders are relatively benign, some are high-impact insect pests and pathogens that can kill host trees and transform ecosystems by causing changes in tree species composition and, potentially, affect the delivery of forest ecosystem services. A compilation of high-impact tree pests in the US listed 62 insects and 16 pathogens (Aukema et al. 2010). Well-known examples include gypsy moth (Lymantria dispar) and the pathogens that cause chestnut blight (Cryphonectria parasitica) and Dutch elm disease (Ophiostoma ulmi and Ophiostoma novo-ulmi). More recent high-impact invaders affecting forests include emerald ash borer (Agrilus planipennis), Asian longhorned beetle (Anoplophora glabripennis), Xyleborus glabratus and its symbiotic pathogen Raffaelea lauricicola (causing laurel wilt), and several Phytophthora species such as Phytophthora ramorum. Other countries have also experienced numerous invasions of insect pests and pathogens affecting trees and forests (e.g., Roques 2009, Brockerhoff et al. 2010).

Most serious invasions represent species translocations between continents, placing them into an ecological context without a shared coevolutionary history. The ever-increasing international trade of a wide range of commodities is considered the main cause, along with the transport of personal effects and intentional introductions. In this paper, I will provide a short review of the history of phytosanitary regulation and the effects on pathway risks, using a combination of information on pest detections trends, border interception data, and international trade data.

A BRIEF HISTORY OF PHYTOSANITARY MEASURES

The recognition of the severe impacts of invasive species and the role of international trade were barely rec-
ognized until the late 1800s. Early attempts to mitigate invasion threats to plant health include the New Zealand ‘Orchard and Gardens Pests Act, 1896’, which enacted measures to prevent the introduction of diseases affecting Orchards and Gardens, to provide for the eradication and to prevent the spread of such diseases. In the United States the ‘Plant Quarantine Act’ was passed in 1912 to regulate inspections of imported plant material and quarantines, and in 1919 ‘Quarantine 37’ was implemented which prescribed inspection and fumigation of imported plants, prohibited the importation of soil with plants, and led to a considerably reduced volume of plant imports. However, these measures were later eased in the United States in favor of more liberal trade regulations. By contrast, New Zealand continued to maintain stricter quarantine regulations, which was probably facilitated by New Zealand’s remote island situation and an appreciation that New Zealand was free of most known pests and diseases.

In the 1990s there was increasing international harmonization of phytosanitary regulations. In 1995, the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (known as ‘SPS Agreement’) provided a multilateral framework to facilitate both free trade and safe trade (in terms of mitigating pest risks). Two years earlier, the first of a series of ‘International Standards for Phytosanitary Measures’ (ISPMs) by the International Plant Protection Convention (IPPC) appeared. However, the best known of all the ISPMs is probably ISPM No. 15 (‘Regulation of wood packaging material in international trade’; specifying heat or fumigation treatments). Ever since the establishment of Asian longhorned beetle in New York City in 1996, the use of wood packaging materials (such as pallets) in international trade was recognized as a high-risk pathway that needed mitigation urgently. Therefore, the implementation of ISPM No. 15 and its almost worldwide adoption is a great success in itself. However, considerable variation remains among countries regarding phytosanitary legislation and regulations of some pathways (Eschen et al. 2015).

ASSESSING THE EFFECTS OF PHYTOSANITARY MEASURES

An analysis of temporal patterns of invasions of forest pests and pathogens suggest that regulations such as Quarantine 37 were effective, at least to some degree, as there appeared to be a reduction in detections of new pests and pathogens in the United States from the 1930s to the 1980s. In the New Zealand record, no reduction in establishments is noticeable over this period. However, it is difficult to determine effects of phytosanitary measures because pest detections depend on the extent of detection efforts and because of time lags between establishment and detection. Such time lags may be 20 years or more, even for highly damaging species (Siegert et al. 2014). Nevertheless, the relative increase in pest detections in the United States since the 1990s is consistent with the substantial increase in international trade since the 1980s that may have overwhelmed the effectiveness of our phytosanitary measures. In fact, an extrapolation of USDA’s interception rates, inspection effort and trade trends suggests that pest arrivals may have increased despite our efforts to improve phytosanitary measures. At least since 2000, New Zealand has not seen such an increase in detections, and this may be a result of New Zealand’s comparatively stricter approach to biosecurity that has been in effect for several decades.

Although actual establishment events of invasive species are rarely documented, information about the pathways and commodities that are likely to be the most ‘risky’ can be discerned from known host associations and from border interception records that are held by national plant protection organizations (e.g., Brockerhoff et al. 2006, Haack 2006, McCullough et al. 2006, Liebhold et al. 2012). Based on this information, wood packaging and other solid wood items clearly are key pathways for wood borers and bark beetles which confirm the importance of ISPM No. 15. For most other types of forest pests and pathogens, international trade in live plants (nursery stock, plants for planting), appears to be the most important pathway (Liebhold et al. 2012).

Propagule pressure (i.e., pest arrival rates) is widely acknowledged to be a key driver of invasions (Simberloff 2009). Therefore, to evaluate whether phytosanitary measures and ISPMs are effective at achieving the intended reduction in invasion risks, comparing actual propagule pressure and associated risks of establishment before and after implementation of an ISPM is a desirable methodology. In order to do this, information on changes in propagule pressure (or a suitable proxy measure) and relationships with establishment probability are needed (Brockerhoff et al. 2014, Leung et al. 2014). Despite the somewhat haphazard nature of inspections of imports and
recording of interceptions, interception records are the best available information on propagule pressure and pest arrivals associated with international trade, and the relative frequency of different species. Furthermore, subsets of interception records, such as USDA APHIS’ Agriculture Quarantine Inspection Monitoring (AQIM), are indeed recorded from random cargo inspections and systematic sampling plans, and therefore suitable for before-after comparisons to evaluate phytosanitary measures. In the case of ISPM No. 15, our analysis of AQIM interception data provided an estimate of a 52% decrease in infestations of wood packaging materials after the ISPM’s implementation (Haack et al. 2014, Leung et al. 2014). As the relationship between a species’ propagule pressure and its probability of establishment is not linear, and because there are hundreds of borer species that are moved on wood packaging pathways, it is important to consider effects of a phytosanitary measure across entire species pools rather than individual species (Brockerhoff et al. 2014, Leung et al. 2014). This information was used to evaluate the effectiveness of ISPM No. 15 and to conduct a cost-benefit analysis, weighing up the costs (including treatment costs and impacts on trade) as well as the benefits in terms of reduced future pest damages (Leung et al. 2014). This showed that although the implementation of ISPM No. 15 is costly, cumulative net benefits are estimated to exceed $11 billion by 2050 (Leung et al. 2014), demonstrating that phytosanitary measures are worthwhile economically.

CONCLUSIONS

In conclusion, analyses of establishment and interception data can be useful to evaluate pathway risks and the effects of phytosanitary measures mitigating risks of biological invasions. In the absence of other information on pest arrivals with international trade, it would be desirable to enhance the recording of inspection and interception data, ideally such that the data are more amenable to statistical analysis and interpretation. There are excellent opportunities for international collaboration and sharing of data for mutual benefit. As a case study of the effects of a phytosanitary policy, the analysis of the benefits and costs of ISPM No. 15 showed that it is clearly worthwhile, although there appear to be issues with non-compliance. Other important pathways that need more attention are the live plant trade and the movement of “hitchhikers” with shipping containers which are both challenging as there are no silver bullets to mitigate pathway risks.

ACKNOWLEDGMENTS

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LITERATURE CITED


PRESENTATIONS
Shaping our Understanding and Response to Emerging Forest Pathogens
ASH DIEBACK IN EUROPE - A NOVEL FUNGAL PATHOGEN THRIVES

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ABSTRACT

_Hymenoscyphus fraxineus_ (synonym _H. pseudoalbidus_ Queloz et al. (2011), anamorph: _Chalara fraxinea_ Kowalski (2006)) is an invasive fungal pathogen on _Fraxinus_ spp. in Europe, especially on common ash _F. excelsior_ (Gross et al. 2014a). The disease was initially observed in Eastern Poland and subsequently spread almost through the entire distribution range of _F. excelsior_ in Europe (McKinney et al. 2014). Symptoms of the disease include (i) brown leaf spots and necrotic lesions along leaflet veins and rachises (including the petiole); (ii) necrotic lesions along stems, branches and root collars (iii) wilting of single shoots; (iv) crown dieback and formation of epicormic shoots (Gross et al. 2014a; Husson et al. 2012). The causal agent was detected in 2001 and subsequently described as the new hyphomycete species _Chalara fraxinea_ (Gross et al. 2014a). Later, the pathogenicity of the species was confirmed and connected to the teleomorph _Hymenoscyphus albidus_, which was known from Europe for a long time, but never linked to disease (Gross et al. 2014a). This fueled discussions about disease etiology and led to the identification of _H. pseudoalbidus_, a cryptic sister species of _H. albidus_, which was verified as the causal agent of the epidemic (Queloz et al. 2011).

The disease cycle starts with the release of ascospores from apothecia which are produced during summer on leaf remnants of _F. excelsior_ in the litter. Ascospores are distributed by wind and infect ash leaves (Gross et al. 2014a). The early infection process inside the leaves is still not well understood. Stems are infected via leaf petioles but this happens relatively rarely because most leaves are shed before stem infection. Inside the stem the pathogen preferentially colonizes starch-rich tissues including the medullary sheath, phloem, para-tracheal parenchyma and ray parenchyma (Gross et al. 2014a). Rapid axial and subsequent radial expansion of the infection leads to the death of bark tissues and to the development of characteristically cinnamon-brown to dark-brown necrotic bark lesions (Gross et al. 2014a). If shoots are girdled during summer, the distal part starts to wilt and die. Trees react with the formation of substitutive shoots in the next growth period which are infected again when the fungus starts to sporulate in summer. The pathogen overwinters in leaf petioles and rachises in the litter. To protect its thallus and substrate, the fungus produces a black pseudosclerotal layer underneath epidermal tissues of petioles, which is composed of tightly packed and strongly melanized fungal hyphae, (Gross and Holdenrieder 2013; Gross et al. 2014a). Conidia of the asexual form likely act as spermia (i.e. fertilization of apothecia), and are not involved in the infection process (Kirisits et al. 2009; Gross et al. 2012b).

Recent investigations suggested that _H. fraxineus_ originates from East Asia where it apparently has a wide distribution range (Gross and Han 2014, in review). Population genetic investigations in Europe found a low genetic, yet high genotypic diversity among isolates. The latter is supportive for a distribution by sexually produced ascospores (Bengtsson et al. 2012; Gross et al. 2012a). The low genetic diversity and population differentiation, both at inter- and intra-population scales, was confirmed in a large scale population genetic study that incorporated over 1000 strains distributed all over Europe (Gross et al. 2014b). The study revealed the signature of a severe genetic bottleneck. On the contrary, populations from Japan showed a higher genetic diversity, as expected to be found in the native range (Gross et al. 2014b).

In Asia, four nearly-cryptic species of _H. fraxineus_ and _H. albidus_ were recently identified (Gross and Han 2014, in review; McKinney et al. 2014; Gross et al. 2014, accepted article). Their biology is only vaguely known: _Hymenoscyphus koreanus_ and _H. occultus_ have, so far, only been detected on Korean ash _F. chinensis_ subsp.
rhynchophylla in South Korea (Gross and Han 2014, in review). Hymenoscyphus linearis has been found on F. platypoda in Japan (Gross et al. 2014, accepted article). Finally, H. albidoïdes has been found on leaves of Picrasma quassioides in China. A pathogenic behavior of these species has never been reported so far and it is likely that they live as endophytes in the leaves of their respective host trees.

The level of ash resistance against the pathogen has been assessed in various studies (reviewed in McKinney et al. 2014). A very low frequency of highly resistant ash genotypes were found but it could be shown that resistance is heritable. However, the term tolerance is more appropriate in this case because infections also occur on “resistant” trees but infections stay restricted to the leaves and do not proceed to the stem. The heritability of the trait encouraged resistance breeders in Europe and several breeding programs were launched (see various contributions in Fraxback 2013). The host range of the pathogen is restricted to Fraxinus spp. However, there is very little data about the susceptibility of different ash species. Narrow-leafed ash Fraxinus angustifolia is similarly affected as F. excelsior (Kirisits et al. 2010). Drenkhan and Hanso (2010) investigated a small set of ornamental ash species and found that Black ash F. nigra trees were badly affected, Green ash F. pennsylvanica trees were moderately affected and White F. americana and Manchurian ash F. mandshurica trees were least affected. The flowering ash F. ornus is apparently very tolerant (Gross et al. 2014a; Kirisits et al. 2009).

Conclusion: Recent investigations have identified several important aspects of the ash dieback disease that are relevant for quarantine and control measures. In Europe, new introductions from the native range must be avoided to prevent pathogen evolution and to preserve the few highly tolerant F. excelsior genotypes. In North America, where the highly susceptible F. nigra is native and other susceptible ash species occur, efficient quarantine measures are needed. This also accounts for other parts of the world where susceptible ash species were introduced as ornamental or forest tree species (e.g. Australia). Moreover, the finding of several other closely related species of H. fraxineus shows that there is a risk of additional introductions of Hymenoscyphus species, which might spill over to indigenous hosts or hybridise with resident Hymenoscyphus species, potentially fostering pathogen evolution.

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INVASIVE OR NATIVE VECTORS? THE GENETIC STRUCTURE OF OAK TREES AND THE OAK WILT DISEASE VECTORS

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ABSTRACT

Japan has lost a vast number of oak tree forests, particularly stands of Quercus crispula, due to wilt, at a rate of approximately 2,000 hectares/year. The ambrosia beetle, Platypus quercivorus, is the vector of oak wilt Raffaelea quercivora. The range of the damaged area has been expanding, but where the beetles originated from remains unknown. Although the disease was first reported in the 1930s and its emergence has been sporadically reported, no epidemical symptoms were established before the 1980s. There are two hypotheses for this phenomenon. One is invasion and the other is the forest structural change hypothesis. The former implies that global climate change has pushed the beetle’s distribution northward such that it is now attacking Q. crispula, which is a typical species of northern cool temperate forests in Japan. The latter suggests that changes in the management of oak forests (i.e., the abandonment of firewood use) increased the prevalence of mature trees that are vulnerable to the disease.

Our research group set two objectives to elucidate the cause of the oak wilt outbreak. Our first objective was to map the spatial population structure of P. quercivorus in an area damaged by oak wilt disease. The second was to infer dispersal of P. quercivorus populations, to gain an understanding of the expansion mechanisms of the disease. Using five highly polymorphic microsatellite loci, 605 individuals from 14 sampling sites were assessed.

Population differentiation ($F_{ST} = 0.047$, $G'_{ST} = 0.167$) was moderate and two major clusters were detected by several methods, dividing the samples into north-eastern and south-western populations. A similar genetic divergence was reported in the host oak trees. Because isolation by distance was detected for the entire population and also within the north-eastern populations, migration was considered to be limited between neighboring populations, and most populations were suggested to be in genetic equilibrium of genetic drift and gene flow. Therefore dispersal between populations should be restricted in neighboring areas.

Because the genetic boundaries coincide, we suggest that the geographical structuring of the beetle was formed by co-evolution with the host species. Thus, we found that the oak wilt disease is dispersed by endemic vectors. Knowledge of the genetic structuring of the beetle populations could reveal their population history and distinguish whether they are invasive or native.
ELUCIDATING THE ROLES OF MULTIPLE BIOTIC AGENTS IN THE DEVELOPMENT OF THOUSAND CANKERS DISEASE WITHIN THE NATIVE RANGE OF JUGLANS NIGRA

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ABSTRACT

Thousand cankers disease (TCD) is an emerging disease of Juglans species that has caused significant concern in the eastern USA due to the high susceptibility of eastern black walnut (J. nigra L) to the disease. J. nigra is the most valuable timber species grown in the USA with the majority of timber plantations being found in the eastern states. TCD results from aggressive attack of walnut bark tissues by the walnut twig beetle (WTB) (Pityophthorus juglandis Blackman) that also transmits the associated canker pathogen Geosmithia morbida Kolařík. Bark cankers develop around thousands of WTB holes and galleries. Coalescence of cankers leads to branch dieback and death that can result in tree mortality. Other insect pests and canker pathogens are known to colonize J. nigra, particularly trees that have suffered damage or stress. A synthesis of key results from a series of related studies on J. nigra conducted in four eastern states offer insights into several insect species and plant pathogenic fungi that we hypothesize play a role in the development of TCD.

A number of insect species were found to attack stressed and TCD-symptomatic black walnut. In 2011, multiple trap trees were created (by chainsaw girdling) during spring on 27 sites in Indiana and Missouri. Insects were emerged from stem and branch samples taken from the trees following felling in late summer. Over 16,800 insects in three taxonomic groups were obtained. Ambrosia beetles (15 species) comprised 96.3% of the collection, weevils accounted for 3.4%, and bark beetles the remainder, 0.3%. The most prevalent species within each taxonomic group, i.e. Xylosandrus crassiusculus (Motschulsky), Xyleborinus saxesenii (Ratzburg), Pityophthorus laatus Eichhoff, Stenomimus pallidus (Boheman), and Himatium errans LeConte, did differ by state. Prevalent species were broadly distributed within the study area or are known to be distributed throughout the native range of eastern black walnut. Between 2012 and 2014 in Tennessee, branches of walnut were girdled and harvested four or five months later. Insects commonly emerged from branch samples include Chrysobothris fémorata (Olivier) (s. str.), C. sexsignata Say, Neoclytus acuminatus (Fabricius), Lepturges confluens (Haldeman), Liopinus alpha (Say), as well as limited collections of Cossonus sp., Himatium errans and S. pallidus weevils, and X. crassiusculus and X. saxesenii. In 2014, four TCD symptomatic J. nigra in Butler County, Ohio, were felled in September and insects emerged from 32 stem and 32 branch sections over three months. Eight species (n=143) were emerged only from main stem sections of two trees. X. crassiusculus, X. saxesenii and S. pallidus accounted for 96% of the collection.

Several fungal pathogens were found on TCD-symptomatic and non-symptomatic walnut trees. Fungus isolations were attempted from insect and pathogen-type damage on branches of TCD-symptomatic and asymptomatic J.
**Juglans nigra** in Knoxville, Tennessee, in 2012. Seven damage types were characterized and included stains, cankers and insect-associated damage. The fungi isolated from representatives of the damage types were categorized as saprophytes, woody plant pathogens, insect pathogens, fungal parasites, and stain fungi. Three reported black walnut pathogens (*G. morbida*, *Fusarium solani* (Mart.) Appel & Wollenw. Emend. Synd. & Hans., *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not.) were commonly isolated from branch segments and each fungal species was obtained from several damage types. In 2014, the same three pathogens also were commonly isolated from three different types of cankers found on branches and stems of the same TCD-symptomatic trees in Ohio that were used for the insect study. Co-occurrence of two or three of the species in one canker was common.

*G. morbida* spores were found on bodies of two insect species, *P. juglandis* and *S. pallidus*. The frequencies of *G. morbida* isolation and its DNA presence on WTBs obtained from TCD trees on single sites in California, Colorado, Tennessee and Virginia were documented in 2011 as part of a larger study on mycoflora associated with WTBs. DNA of *G. morbida* was detected on 100% of assayed WTBs, but isolation success was variable ranging from 35 to 100%, depending on the collection source. *G. morbida* also was detected on 3 of 21 *S. pallidus* that emerged from two girdled *J. nigra* trees on one site in Indiana by serial dilution plating or by DNA assay as part of the 2011 trap tree study. The fungus was not detected on any of 105 *S. pallidus* collected from 11 other sites in Indiana and subsequently assayed.

In summary, we hypothesize that a suite of insect species colonizes *J. nigra* as TCD develops but the particular species involved may vary by state, if not site by site. WTB is still considered the initial and most aggressive insect colonizer. We also hypothesize that *F. solani* and *B. dothidea* contribute to canker development and branch death on TCD trees. Because *G. morbida* is a weak plant pathogen, the roles of these two opportunistic pathogens relative to *G. morbida* warrant investigation. Lastly, a weevil species *S. pallidus* was found to carry *G. morbida* in Indiana and has since been emerged commonly from TCD-symptomatic trees in Ohio. We hypothesize that the relative importance of *S. pallidus* as a *G. morbida* vector is minor compared to *P. juglandis*, but further research on this question also is warranted.
PRESENTATIONS
Ambrosia Beetles: The Fungi They Transmit & Subsequent Impacts
AMBROSIA BEETLES: OVERVIEW OF BIOLOGY AND IMPACTS

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ABSTRACT

Ambrosia beetles have always been among the most common insects in forests, but it was not until outbreaks of invasive tree-killing species that this group received increased attention of entomologists, tree growers and regulators. Consequently, research on the beetle ecology and management is relatively young. This talk summarized current knowledge about the beetles and important gaps in knowledge that still need to be filled.

In the first part, the basics of ambrosia beetle biology and diversity were reviewed. Ambrosia beetle is an ecological designation, not a taxonomic group. All ambrosia beetles are wood borers that live in an obligate symbiosis with ambrosia fungi. The beetles bore tunnels into tree tissues but they do not eat wood; instead it is the highly adapted fungal mutualist that spreads its mycelium into the ambient wood, extracts nutrients, and delivers them to the beetle and its progeny. There are over 3,000 species of ambrosia beetles around the world, originating in 12 different lineages. Although these lineages employ the same ecological strategy, they are all slightly different in their diversity, relationship to fungi, host tree utilization, and other factors. Importantly, only a very small minority of species can be considered pests, the vast majority are harmless wood-degraders.

One group – the Xyleborini – are the most species-rich group of ambrosia beetles, and they also contain most of the pest species. Therefore they are the best known ambrosia beetles. Xyleborini have arrhenotokous reproduction, in which males are haploid and are produced from unfertilized eggs. Furthermore, all Xyleborini are inbreeders – there is usually only a single male per family, and most of matings involve that male and his sisters. Outcrossing may occur in some species but it’s rare. Because Xyleborini are the best known and most common ambrosia beetle group, their reproduction is often mistakenly thought of as typical of ambrosia beetles, but in reality most other ambrosia beetles are neither haplo-diploid nor inbreeders.

In the second part of the talk, several lesser-known aspects of ambrosia beetles were discussed. For example, while ambrosia beetles as a whole are known as tree-killing pests, there are actually several different ways in which a tree can be killed by these insects, or ways how tree death can be facilitated by them. 1) Most putative ambrosia beetle tree-killers actually only act as synergists of predisposing stress factors, most often excess of water. 2) Ambrosia beetles often attack trees with a prior infection by a true pathogen. The pathogen is typically not diagnosed, which results in an observation that the tree was killed by the beetles. 3) Some species are capable of a mass attack which slowly kills a tree. In most cases this is not a rapid and coordinated attack as in pine bark beetles, but rather a slow accumulation of individual galleries on an already weakened tree. 4) There is one, and only one known case of an ambrosia beetle that kills trees by inoculating them with a pathogenic fungus – the redbay ambrosia beetle Xyleborus glabratus. Its primary symbiont Raffaelea lauricola triggers wilt in Lauraceae and has caused a massive die-offs of those across the Southeastern US.

One other aspect that is known among specialists but needs to be communicated to end-users of ambrosia beetle information (such as agency identifiers) is the difficulty of delimiting species in the haplo-diploid and inbred Xyleborini. Some xyleborine species are notoriously difficult to identify, but lack of morphological characters is only a part of the reason. It is also partly because of unclear boundaries between inbred phylogenetic lineages. In most of biology, species names correspond to predictable morphological and ecological features of an organism, but
such limits are difficult to establish in inbred populations that lack mechanisms for maintaining species cohesion.

Another aspect of the ambrosia symbiosis that remains understudied is the identity of ambrosia fungi. Over 70% of genera of ambrosia beetles have never been explored for their fungal symbionts. Of those that were, only a fraction has been studied well, using reliable methods, and those were mostly the pest species. Virtually nothing is known about fungal associates of tropical beetles that have not yet been recorded as invasive.

The third part of the talk was devoted to aspects of the ambrosia beetle research and management about which we know little or nothing. It was discussed that two strategies need to be employed on large scale to avoid or mitigate future destructive invasions. First, pre-invasion assessment of ambrosia beetles is possible to the extent that the fungi can be isolated in the native region and tested for their effect on naïve tree species in regions where the insect vectors are likely to establish in the future. A project on pre-invasion assessment of Asian ambrosia beetles, currently conducted by our group at the University of Florida, was mentioned as an example. Second, to mitigate epidemics of tree diseases caused by woodborer-pathogen consortia, resistance breeding was recommended as a viable practice. A successful case of breeding of redneys for resistance against Raffaelea lauricola was described. The project is also being conducted at the University of Florida.

Lastly, it was suggested that researchers seeking for solutions to invasions of beetle-fungus complexes may need to explore new generation of technologies. Traditional approaches, such as biocontrol or IPM often do not work, and if they do it is only on smaller scales of managed orchards. To address epidemics of forest tree deaths caused by foreign pests and pathogens spreading through natural areas, researchers may need to explore technologies such as manipulating the host tree’s responses or the pathogen’s virulence using molecular tools and landscape deployment. Such tools have been used with success in other disease scenarios, and many of the molecular methods are available as off-the-shelf kits. It was proposed that, to employ those in ambrosia beetle management, two main obstacles will need to be overcome: inertia in the research community and lack of funding.
ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, carries a phytopathogenic symbiont, *Raffaelea lauricola* T.C. Harr., which causes laurel wilt, a lethal vascular disease of some Lauraceae species. Both *X. glabratus* and *R. lauricola* are natives of Asia that recently invaded much of the coastal plain of the southeastern USA. This new beetle-disease complex has decimated vast areas of native trees of the Lauraceae in the southeastern USA, and is now threatening the avocado industry in south Florida. In recent years many efforts have been made aiming to identify semiochemicals for early detection of this beetle. Males of *X. glabratus*, like many other ambrosia beetles, are flightless and never leave the beetle galleries where they mate with sibling or parental females. Females mate before engaging in dispersal and host seeking behaviors, making long range attraction between sexes unnecessary and unlikely. Efforts concentrated in identifying economical kairomone-based attractants using essential oils high in sesquiterpenes. Distilled cubeb oil lures were identified as an effective tool for detection of *X. glabratus*, with a field life up to 3 months due to extended, low release of attractive sesquiterpenes, primarily α-copaene and α-cubebene.

Fortunately for the avocado industry *X. glabratus* has not colonized commercial avocado groves in south Florida. However, laurel wilt was detected in commercial avocado systems in south Florida in February 2012; since then, over 6000 avocado trees have been destroyed. Recently, however, the *R. lauricola* pathogen has been found in or on at least 7 other ambrosia beetles present in south Florida, two of which (*Xyleborus ferrugineus* Fabricius and *Xyleborus volvulus* Fabricius) have been demonstrated under greenhouse conditions capable of transmitting *R. lauricola* to avocado trees. To date very few *X. glabratus* have been detected in the commercial avocado production area but, thousands of other ambrosia beetles have. Thus it appears in the agricultural area ambrosia beetles other than *X. glabratus* are the main vector of laurel wilt. This has complicated the situation in that *X. glabratus* beetle attacked healthy trees but generally these others ambrosia beetles attack environmentally (e.g., lightning and flooding) and/or biologically (e.g., root rot) stressed trees.

The current laurel wilt and ambrosia beetle control strategy in commercial avocados includes aerial detection of trees symptomatic for laurel wilt, ground verification of diseased trees and immediate destruction (i.e., uprooting, chipping, and burning) of infected trees. This has been demonstrated to be effective in reducing the spread rate of laurel wilt. For example, in one orchard a laurel wilt positive avocado tree was confirmed and the owner declined to destroy the tree, six months later, over 95 additional trees were dead. In other orchards, owner/operators have implemented the immediate tree destruction strategy and limited spread of the disease.

Research has demonstrated chipping infested avocado wood significantly reduces ambrosia beetle survival. Ambrosia beetles are attracted to avocado wood chips and treating wood chips and surviving tree surfaces with insecticide may reduce ambrosia beetle populations for 7-14 days. However, this may not be very effective during the rainy season (i.e., May through September historically). More recently, one line of research has shown promise in controlling ambrosia beetle populations. Several commercial strains of entomopathogenic fungi have shown...
highly infective to ambrosia beetles associated with avocado. Current efforts aim to identify the best application strategy to improve the efficacy of the entomopathogens.

More research is needed to understand the role of *X. glabratus* and other resident ambrosia beetles in the epidemiology of laurel wilt in commercial avocados. This disease could potentially reach other avocado producing areas in the US and in Central and South America.
PRESENTATIONS
Northeast Biocontrol Regional Project (NE-1332):
Updates on Regional Biocontrol Projects
DISPERSAL AS A REGULATOR OF OUTBREAKING WINTER MOTH (LEPIDOPTERA: GEOMETRIDAE) POPULATIONS IN MASSACHUSETTS

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ABSTRACT

Winter moth (Operophtera brumata, L.) is an invasive, polyphagous geometrid that has caused regular defoliation of deciduous trees in Massachusetts since its introduction in the 1990s. Winter moth population ecology has been well studied in its native range in Europe, as well as from two prior, accidental introductions into North America: to Nova Scotia in the 1940s and 1950s and to British Columbia in the 1970s. Previous work has suggested that winter moth populations are regulated at low densities by pupal predation, and that larval mortality is not density dependent in low-density populations, but may be density dependent in high-density populations. Density dependent mortality of winter moth larvae has been observed in Massachusetts, but no clear cause of mortality has been implicated.

The potential for larval mortality due to cannibalism, predation, and dispersal were investigated through three experiments: (1) a field experiment excluding predators from late instar caterpillars, (2) a lab experiment manipulating the rearing density of early instar caterpillars, and (3) monitoring larval densities on sample trees of three species (Malus domesticus, Acer rubrum, and Quercus rubra) over the feeding season. These experiments indicated that predation and cannibalism were unimportant (predation: treatment $\chi^2=0.14$, df=2, p=0.93, cannibalism: present >9% of cups), and that strongly density dependent mortality of larvae occurs due to density dependent dispersal of early instars from opening buds (density: $\chi^2=13.19$, df=1, p<0.001).

Larvae exhibited a varying dispersal response to conspecific density depending on host plant species (species: $\chi^2=20.54$, df=2, p<0.001). This together with longterm data, which show that pupation weight declines significantly with increasing host plant defoliation, indicates that density dependent dispersal may be mediated by induced host plant defenses. This hypothesis was tested though an experiment testing the response of caterpillars to defoliated as compared to undamaged foliage. The study showed that winter moth larvae disperse at a significantly higher rate from previously defoliated foliage than undamaged foliage (treatment $\chi^2=33.1993$, df=1, p<0.001). These four experiments together suggest that density dependent larval dispersal of early instars, probably mediated by an induced decline in foliar quality, can explain previously observed regulation of larval winter moth populations in Massachusetts.
MICROSCLEROTIA APPLIED IN HYDROMULCH TO CONTROL ASIAN LONGHORNED BEETLES

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ABSTRACT

The entomopathogenic fungus *Metarhizium brunneum* Petch strain F52 (Hypocreales: Clavicipitaceae) is able to produce environmentally persistent microsclerotia, which are sclerotized hyphal bundles averaging about 200 microns. Microsclerotia are made by different fungi as persistent, environmentally tolerant structures. Incorporating these desiccation-tolerant microsclerotia (Mb MS) plus a carrier into hydromulch [a mixture of water + wheat straw mulch] and adding a psyllium tackifier, represents a novel, easy-to-use and environmentally-friendly mycoinsecticide that can be sprayed onto the trunks of forest or orchard trees to control insect pests. The premise is that hydromulch holds moisture that provides more time for Mb MS to produce infective conidia, which in turn will infect Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), adults.

To date, we have shown that Mb MS in hydromulch using psyllium as a tackifier and applied with paint sprayer equipment can significantly reduce beetle fecundity and survival when beetles are exposed to sprayed logs and bark surfaces after a lag period during which infective spores are produced. To measure the effect of the formulation on beetle fecundity (offspring produced), Mb MS-containing hydromulch was sprayed onto bolts of wood and pairs of beetles in containers with high or low humidity were exposed for two-week oviposition periods. In the high humidity treatment, fecundity was highest for the controls (18.3 viable offspring/female) versus 3 viable offspring/female for beetles exposed to Mb MS-containing hydromulch. We also wanted to know whether beetles could be infected by the fungus applied to 40 cm long sugar maple logs when the beetles walked twice across the applied surface. The results of three completed replicates indicated that treatments in which logs had hydromulch applied with Mb MS were more effective at killing beetles than those logs to which only Mb MS was applied without hydromulch. The microsclerotia on logs that received Mb MS-hydromulch produced more conidia and the median survival times of exposed beetles were lower (18-22 d) compared with microsclerotia on logs that received only Mb MS and no hydromulch that produced fewer conidia, which increased the beetle survival times (median survival 23-26 d).

We have also shown that hydromulch can persist for up to 100 days in the field by conducting experiments using pieces of bark on which Mb MS + hydromulch had been sprayed, which were placed in a woodlot for different lengths of time, with the study beginning 8 times during the 2014 summer. Spore production continues to increase over a 30 day period depending on moisture (rainfall, RH) and temperature, as well as time. The highest spore production was achieved when hydromulch had been out on trees for at least 20 days. Overall beetle mortality when exposed to wood pieces in the various sprayed replicates ranged from 36-93%, depending on the production of conidia by microsclerotia within the hydromulch, which was ultimately dependent upon the weather. Female beetles that were exposed to wood pieces in the best replicate, where moisture and temperature were highest, survived for 22 days and males 26 days.

We hypothesize that repeated contact by beetles with the Mb MS + hydromulch, can increase the rate of infection, causing beetles to die faster. This may be achieved by combining male produced-pheromone blend and lures with...
the hydromulch, which is the next step in the development of this product. Further, by improving the water holding capacity of the hydromulch, one can increase the spore production of the fungus. This may be achieved by incorporating additional fibres such as modified papers (e.g., by-products of paper towel material) into the hydrostraw, or additives such as carboxymethyl cellulose, which can increase spore production, or polymers that grab water from the atmosphere (e.g., Hydretain®, Ecologel Solutions LLC). As a next step, the key is to find the best combination of constituents that maximises spore production but reduces the overall cost of the product.
DEVELOPING A SNP-ARRAY TO MEASURE PRE- AND POST-INTRODUCTION GENETIC DIVERSITY AND LEVELS OF HYBRIDIZATION BETWEEN TWO HOST RACES OF KNOTWEED PSYLLID, CANDIDATES FOR THE BIOLOGICAL CONTROL OF JAPANESE KNOTWEED

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ABSTRACT

Three species of invasive knotweed currently cause extensive damage to riparian and roadside habitats by out-competing native plants and altering community structures, reducing habitat quality for wildlife, and changing stream-flow dynamics. The psyllid *Alaphara itadori* is currently being evaluated as a candidate biological control agent in the United States and Canada for controlling populations of knotweed after its successful introduction to the United Kingdom. Populations of *A. itadori*, however, differ in their host preferences and virulence on different species of knotweed, and thus two host-specific strains are being proposed for introduction. These strains will have overlapping distributions once introduced; therefore hybridization is likely between individuals after introduction and could have an important impact on the sustainability of this biological control program. Therefore, we developed a single nucleotide polymorphism (SNP) array to identify individuals to parental and hybrid classes. We test the ability of this array to correctly identify individuals and compare the results from of two commonly used population genetic software packages. We find that our SNP array correctly identified all individuals to parental and hybrid classes using both the Structure and NewHybrids software packages, though NewHybrids assigned individuals with greater probability. Finally we comment on how hybridization may affect the sustainability of this biological control program.
BIOLOGICAL CONTROL OF PHRAGMITES AUSTRALIS: AN UPDATE

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ABSTRACT

Introduced Phragmites australis (common reed), one of the most troublesome plants along the Atlantic Coast of North America, has become invasive across North America, impacting many interior waterways, including the Platte River and the Mississippi Delta. Invasions are associated with decreases in plant diversity and reduced habitat quality for fish and wildlife.

In addition to this invasive lineage which was probably introduced to the northeast coast of the USA from Europe early in the 19th century, there are two other distinct lineages in North America; the widely distributed native Phragmites australis americanus, and P. australis berlandieri found in the Gulf and Pacific Coasts of the US.

In the Northeast and Mid-Atlantic regions of the US, introduced P. australis has nearly entirely replaced the native subspecies. Native populations are more common in the Midwest, Southwest, and Gulf Coast states. However, these populations may be declining, potentially accelerated by local introduction of introduced P. australis.

Introduced P. australis grows in a wide range of habitats including wetlands, marshes, riverbanks, roadsides, ditches and other watercourses. Natural area managers spend millions of dollars attempting to reduce threats to birds, amphibians, plants and fish in fresh and saltwater wetlands. Phragmites invasions along watercourses increase evapotranspiration, a concern in the arid West and Midwest. Extensive stands of introduced P. australis block river water flows, causing extensive flooding. In urban and recreational areas, introduced P. australis often obscures views, reducing aesthetic and property values - particularly along the Atlantic Coast. And similar to Arundo donax, it increases fire danger if dry reed beds are ignited. Consequently, there is strong interest in controlling introduced P. australis in urban, rural, agricultural and in natural areas throughout the USA.

In 1998 biological control specialists from the University of Rhode Island, Cornell, and CABI – Switzerland began a program for biological control of invasive P. australis. Of the 28 insect herbivores found to currently exist in North America, only five are native to this continent and they are not having a significant impact on invasive P. australis. Following literature reviews, field surveys, and biological investigations in Europe, four of the 175 known European herbivores of P. australis were selected for evaluation as possible biological control agents in North America. Through host range testing at URI and additional behavioral tests in Switzerland, we have identified two agents: Archanara geminipuncta and A. neurica (Lepidoptera: noctuidae) which offer promise to substantially reduce populations of introduced P. australis without adverse impact on nontarget species or the native subspecies of this plant. Strong ovipositional preferences for introduced P. australis, combined with a lack of suitable overwintering sites on native plants, leads us to conclude that these Archanara species would not harm native populations, but in fact offer the best long term hope for their survival through avoiding competitive exclusion by populations of introduced P. australis. We are preparing a release petition for submission to USDA for release of these two agents toward the goal of reducing the size and negative impacts of monocultures of invasive P. australis in North America.
PRESENTATIONS
Current Research on *Sirex noctilio* and Eastern North American *Sirex*
WHAT WE NOW KNOW OF NATIVE SIREX IN LOUISIANA:
10 YEARS AFTER INITIAL INVESTIGATIONS

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ABSTRACT

Following the detection of *Sirex noctilio* Fabricius in North America in 2004, annual monitoring and study of *Sirex* and their associated organisms has been conducted in Central Louisiana. These investigations have been aimed at providing a better understanding of the potential threat that *S. noctilio* poses to the vast and valuable pine forests of the South, and to develop effective survey and detection methods and possible management strategies. Over the years, these efforts have relied on the support and expertise of many technicians, students, and scientists at various state and federal research institutions. Below is a brief summary of the progress accomplished over the past decade from various cooperative and collaborative efforts regarding native *Sirex*.

In Louisiana, very little was known about *Sirex* spp. (and their associated organisms) in 2004, beyond limited collection records that existed. Since native *Sirex* are not considered pests, it’s not surprising they hadn’t been previously studied in greater detail. Close attention to the by-catch of the 2004 fall pheromone trapping survey for southern pine beetle (*Dendroctonus frontalis* Zimmerman) on National Forests in western gulf states revealed infrequent catches (one to two specimens) of adult female *S. nigricornis* Fabricius, and, at that time, *S. edwardsii* Brullé, occurring during mid-October to late November. Trapping methods consisted of 12-unit Lindgren funnel traps baited with a 185 g UHR polysleeve of Hercules® steam-distilled wood turpentine, mimicking volatiles from a damaged *Pinus* sp. tree, and a brown plastic pouch containing two tubes of frontalin, the aggregation pheromone of SPB. This preliminary finding pointed towards the potential that *Sirex* may be attracted to host volatiles and possibly pheromones of primary colonizers, such as bark beetles. Thus in 2005 we participated in an Early Detection and Rapid Response trap trial for *Sirex*. Four types of traps were investigated: a 12-unit Lindgren funnel trap; an Intercept™ panel trap; a high-low, double-sided, black, sticky panel trap; and a chemically debilitated *P. taeda* L. trap tree created by applying Vapam-DMSO into basal hatchet frills. Chemically debilitated trap trees had a blank 12-unit funnel trap and a high-low sticky panel trap alongside opposite sides of the lower bole. All other trap types were free-hanging and baited with a UHR turpentine sleeve and UHR ethanol (ETOH) sleeve (Synergy Semiochemical Corp.). Four replicates of each treatment were installed in early June in a 22-yr old *P. taeda* stand that had been thinned in April (<2 months prior). Following the first collection of female *S. nigricornis* and *S. edwardsii* on Nov.10, the experiment was removed for administrative reasons and relocated on Nov. 17 to nearby a sawtimber-sized stand in which thinning operations were completed the week prior. A total of only 44 female native *Sirex* were collected over the course of the year-long trial involving 16 traps. Though 66% (29) of the *Sirex* were collected in the multiple funnel traps, and none from the baited sticky panel traps, there was no significant difference between the panel and funnel trap types.

Given the results above, a Special Technology Development Project (STDP) was launched in 2006 to evaluate various trap lures and alternative methods of creating trap trees at different times. Eight treatments were deployed in August of 2006, with three replicates of each treatment, at each of five different *P. taeda* stands which had all been thinned earlier in the year. Intercept panel traps were used throughout the experiment. Trap treatments...
consisted of: *Sirex* lure (70% α-pinene, 30% β-pinene) alone; *Sirex* lure + ETOH lure; *Sirex* lure + ipsdienol & ipsenol lures; *Sirex* lure + ETOH lure + ipsdienol & ipsenol lures; early season dicamba trap tree; late season Vapam+DMSO trap tree; and late season dicamba trap tree. From the 120 traps, only 96 female *S. nigricornis* were captured over the course of the season. In 2007, the STDP project was moved to forested areas adjacent to pine processing mills due to their affinity for attracting large concentrations of insects associated with pine. With three reps of each treatment (8) at just two mill sites (48 traps total), the seasonal catch of *S. nigricornis* females was nearly doubled (184 females) from the previous year. Although the mean *Sirex* lure + *Ips* lures (5.3/trap) was significantly better than the *Sirex* lure alone (3.0/trap) and *Sirex* lure + ETOH + *Ips* lures (3.2/trap), no treatment provided any meaningful improvement in trap catch. A concurrent experiment at another pine processing mill used 10 pairs of panel traps to test whether or not treating the trapping surface with a dry Teflon lubricant (via aerosol spray) could increase *Sirex* trap retention. It did not, though it did significantly increase the catch of a variety of other pine-associated insects, including the *Sirex* parasitoid *Ibalia leucospoides ensiger* Norton.

Without any real gains or success in survey/detection efforts, in 2008 we explored whether or not select sesquiterpenes might be attractive to *Sirex*. In a stand in which thinning had just been completed the week previous, 18 interpret panel traps were established in Oct., arranged in groups of three, maintaining 30 m. distance between traps. Each group consisted of one blank trap, a second trap baited with the *Sirex* lure, and a third baited with both a Manuka oil lure and a Phoebe oil lure. Surprisingly, the blank traps caught the most *Sirex* (20) followed by the sesquiterpene lure treatment (17), and those with the *Sirex* lure catching the fewest (8). However, it was noted that blank traps near or above fresh green *Pinus* Sp. slash piles appeared to catch the most *Sirex*. Fortunately, a pine sawtimber thinning operation had commenced in route to the trap site, and on November 20th one of the authors stopped to inspect the fresh (hours old) pine tops being left in the woods, and observed tens of adult female *S. nigricornis* ovipositing in fresh *P. taeda*, *P. echinata* Mill., and *P. palustris* Miller tops. The same site was revisited periodically and female *Sirex* activity noted as late as Dec. 11. The tops were left in place in the woods until Sep. of the following year when they were cut up and brought into a variety of rearing chambers. The subsequent emergence from this material in the fall of 2009 yielded 513 *S. nigricornis* (including 209 males, the first we’d witnessed), as well as hundreds of *I. l. ensiger*, with rates of parasitism ranging from 18-58%.

Live specimens from rearing results were sent to specialists for examination for potentially parasitic nematodes. Nematodes were subsequently detected within the population of *S. nigricornis* in Louisiana, and later identified as *Deladenus proximus* Bedding, having both a parasitic and fungus-feeding life cycle similar to *D. siricidicola* Bedding, the biocontrol agent used against *S. noctilio*.

In 2009, we also attempted to mimic the attractive and productive logging tops observed in 2008 by felling, bucking (into 1.2 m lengths), and stacking pulpwood-sized (15-20 cm dbh) *P. taeda* trees, and stacking alternating rows of cut logs next to the residual top. We created 4 such log stacks just prior to the peak of flight activity (Nov. 3), 4 more two weeks later (Nov. 18), and 4 standing trap trees treated with dicamba on Sep. 23, all in a pine stand adjacent to a pine plywood mill. Adult female *S. nigricornis* were noted arriving and utilizing the log stacks within hours of their creation. Rearing results demonstrated the success of the technique, and the importance of the timing of the creation of such log stacks for attracting females and generating offspring. The early cut date yielded on average almost 10X more *Sirex* and >10X more *I. l. ensiger* per tree than similar log stacks created two weeks later. Individual log stacks from a single tree yielded highs of 419 *S. nigricornis* and 186 *I. l. ensiger*. In addition, the standing dead dicamba-treated trees, which were felled in September 2010 and also brought into rearing tents, by and large failed to produce any emerging *Sirex*, with only one of four trees producing a total of 8 *S. nigricornis*.

Given the success of creating attractive oviposition and rearing material via the felling, bucking and stacking of trees next to residual top material, in 2010 we conducted a trap trial to evaluate the attractiveness of fresh pine vs. the *Sirex* lure, vs. blank traps. Ten intercept panel traps of each treatment were established on Oct. 20 in a recently completed pine sawtimber thinning operation. Traps were arranged in linear fashion and randomly assigned.
treatment within each of the ten replicates. Fresh pine lures were created by felling small loblolly pine (ca. 10 cm dbh), cutting the stem into 30.5 cm lengths, and splitting each bolt repeatedly into billets resembling kindling. Twelve to 15 split pine billets were placed in a nylon mesh bag, along with boughs of fresh foliage. Bags were then zip-tied to trap stand poles and located horizontally adjacent to the trap. Pine bag lures were replaced with fresh bags and Sirex lures replaced with new ones on 10/28, 11/8, and 11/23. Over 300 female S. nigricornis were caught from this single site between Oct. 20 and Dec. 17, with the fresh pine bags catching significantly more on average (11.3) compared to traps with the Sirex lure (1.8) or blank traps (0.6). Fresh pine bag lures appeared to be appreciably more attractive than Sirex lures for at least 18 days.

About this time it became apparent there was confusion about what type of symbiotic fungi the native Sirex might be carrying. Populations from Louisiana which had yet to be closely examined were believed to carry only the Amylostereum chialletii (Pers.) Boidin fungus. Initial examination of a specimen submitted to a pathologist at Louisiana State University was surprisingly diagnosed as carrying Amylostereum areolatum (Chiallet ex Fries) Boidin, the same fungal symbiont of S. noctilio. Subsequent sampling of native S. nigricornis in Louisiana, by two separate entities, confirmed that individuals carried either A. areolatum or A. chialletii, and that both species were relatively common among the native population of Sirex nigricornis.

By 2011, there was also a question as to the species status of S. edwardsii Brullé, and reports of forthcoming evidence from molecular DNA analysis, that S. edwardsii was the same species as S. nigricornis, and simply just a color morph of the latter. From our observations and studies since 2004, we had collected data as if they were two distinct species, and noticed no differences in the biology and behavior between the purported species (e.g., timing of adult flight activity and use of identical host material). Utilizing a mating technique from the Cornell Univ. lab, reciprocal crosses (S. nigricornis males X S. edwardsii females, and S. edwardsii males X S. nigricornis females) of unmated adults from rearing chambers were conducted in 20 ml clear polystyrene sample cups on a lab bench under artificial light. Successfully mated pairs (8 of the former and 11 of the later) were then released with like pairs into a screened cage secured to the upper surface of a freshly felled and uninfested P. taeda bolt ca. 1.2 m in length. Following adult mortality, cages were removed and tagged logs were then stored in an outdoor screen tent to prevent attack and infestation from other organisms for 10 months. In Sep. 2012 logs were moved to individual rearing drums. Subsequent emergence of both male and female adult offspring from both crosses demonstrated successful mating and breeding, offering further support that the two former species should be considered a single species, S. nigricornis.

By the close of 2011 we still had not detected any of the known hymenopteran parasitoids of S. nigricornis previously recorded in the southeastern U.S., other than the commonly encountered I. l. ensiger, whose adult flight period overlaps with that of Sirex. With the log stacks of fresh cut pine stems providing successful rearing material, in 2012 we extended rearing operations long past complete emergence of Sirex and Ibalia in the hope of finding other natural enemies that may have attacked infested log stacks during the 10 months they remained in the woods prior to being tented for brood emergence. Beginning in Feb. and extending through Mar., 35 adult specimens of the Sirex parasitoid Rhyssa howdenorum (Townes) emerged within rearing tents located outdoors but within an open-sided shed, as well as a single specimen of R. linoelata (Kirby). Blank intercept panel traps over log stacks in the field created in the fall of 2011 also caught wild R. howdenorum in Mar. – Apr. 2012. Similar results were obtained in 2013, suggesting that both Rhyssa species are early to mid-instar parasitoids in Louisiana.

From the years of annual monitoring collaborators have developed a degree-day model for S. nigricornis flight in Central Louisiana that supports the consistent observation that adults are active from late Sept. through Dec., and when applied to two years of test data, only over or under predicted observed percent emergence (i.e., 10%, 50%, & 90%) by as little as 0 days, and at most 11 days. With the development of the attractive/effective ‘fresh’ pine bag lure the relative effectiveness of various trap types could also finally be determined. Trapping experiments comparing 12-unit funnel traps vs. intercept panel traps (in 2011), and additionally (in 2013) vs. modified funnel traps (a 12-unit Lindgren funnel trap with shortened funnels providing enlarged bottom openings and no nesting overlap between funnels when deployed), all baited with fresh pine bag lures, replaced at two-week intervals,
were conducted in Louisiana (as well as by collaborators elsewhere for *S. noctilio*). Results clearly demonstrated that there were no significant differences among the three trap designs tested for catching *S. nigricornis*.

Lastly, over the course of ten years of study in Central Louisiana it has become obvious that there is a well-established, abundant, and diverse community of organisms utilizing dying or recently dead pines. This community includes various primary tree colonizers such as pine bark beetles; and in addition to *S. nigricornis*, numerous secondary xylophagous insects such as woodborers, weevils and ambrosia beetles. Also present are various wood decay fungi, including two species associated with *Sirex*; and numerous predators and parasites, including at least three hymenopteran parasitoids and a native parasitic nematode of *S. nigricornis*. Competition and natural enemies appear to be factors limiting native *Sirex* populations, and this existing community seemingly presents a formidable challenge to the future success of *S. noctilio* in the South.
A NEW INVASIVE SPECIES-SIREX NOCTILIO IN CHINA

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ABSTRACT

*Sirex noctilio* F. (European wood wasp) is a worldwide significant forestry invasive species originated from Europe and northern Africa. It is a tree killing borer of *Pinus* species. In July 2013, *S. noctilio* was first identified following its recovery by Beijing Forestry University from an artificial *Pinus sylvestris* var. *mongolica* forest in Du Meng, Heilongjiang Province, China.

Firstly, a rapid molecular identification technology was developed, which can be applied to distinguish *S. noctilio* and its relative species occurred in China without sequencing, and then demonstrated to be applicable for all life stages.

Secondly, according to host selection experiment, the maximum quantity of oviposition was found on *P. tabuliformis*, not *P. sylvestris* var. *mongolica*, which indicates that *S. noctilio* may pose high risk to *P. tabuliformis*, a native Chinese pine and widely distributed in northern part of China.

Finally, through field investigation, it is confirmed that virgin or newly emerged females of *S. noctilio* can lay eggs without mating. Their mating period is very limited (only found 10 times in our study). They usually mate in 10:00AM to 14:00PM under clear weather. Mating lasts for about 1.5 min. Mating behavior can only be found to carry on the gauze (seldom on the bolts or on the ground), which may lie in the fact that the male prefer staying in a higher position.
SIREX POPULATION DYNAMICS IN ONTARIO:
TOP-DOWN VS. BOTTOM-UP FACTORS

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ABSTRACT

Since its discovery in 2004, the non-native European woodwasp, Sirex noctilio F., has so far not been a serious pest in North America. Similarities between pine forests in North America and the native range of S. noctilio are thought to explain this. Similarities include heterogeneous, fragmented forest composition and/or a rich community of subcortical associate insects (natural enemies + competitors). Using experimental and observational approaches, we examined factors at different trophic levels that could potentially limit Sirex spp. (S. noctilio and the native congener, S. nigricornis) in Ontario. Potential factors were: availability of susceptible (and very susceptible = overtopped) pine, levels of parasitism, and presence of associate insects. Survey of parasitism at 17 sites revealed that parasitoids were not likely limiting Sirex, and at only one out of six sites did parasitism increase between 2007 and 2014 in a pattern suggestive of regulation. Life table analysis revealed that associates can limit Sirex in an experimental setting. In that same experiment, endoparasitoids exhibited a functional response to availability of Sirex larvae, likely due to increased searching ability. However, survivorship curves from experimental and natural cohorts indicated that factors that act early during Sirex development (e.g. tree resistance, indirect competition through fungal associates) are paramount in dictating woodwasp survival. We found some evidence that Sirex is limited by the availability of overtopped pine in Ontario, but many pines were not initially suitable hosts for developing larvae. Several successive years of attack may be necessary to kill overtopped pines in Ontario. It is possible that high Sirex densities, at the site level, are required to kill pines and illicit a response (functional or numeric) from natural enemies. Both bottom-up and top-down factors likely limit Sirex in Ontario.
STERILIZATION OF SIREX NIGRICORNIS F. (HYMENOPTERA: SIRICIDAE) BY A NATIVE PARASITIC NEMATODE, DELADENUS SP. (NEMATODA: NEOTYLENCHIDAE)

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ABSTRACT

Sirex nigricornis is a wood wasp commonly collected in late summer and fall in pine stands of the eastern United States and Canada. It is not considered an economically important pest and there are many native parasites and predators which provide significant natural control. One of these parasites is Deladenus proximus (Nematoda: Neotylenchidae), a nematode that can parasitize wood wasps or feed on the symbiotic fungus of Sirex, Amylostereum (Basidiomycota: Russulales) while free-living in pines. In 2004, a non-native wood wasp, S. noctilio, was discovered in New York and has since spread to seven states and much of southern Ontario. Deladenus proximus has already been found parasitizing S. noctilio in the northeastern U.S., implicating it as a potential control agent of the non-native. Our objectives were to evaluate the effects of geographic location, forest management activity, and nematode infection on native wood wasp size and fecundity and to begin to explore the potential for biological control of S. noctilio using native nematodes. Over 400 S. nigricornis females were collected using black panel traps baited with host volatiles in the fall of 2012, 2013, and 2014 in three geographic locations in Arkansas: the Ozark National Forest, Ouachita National Forest, and south Arkansas. Two forest management regimes were also represented in each of these geographic locations: unmanaged (no thinning or burning for 15+ years) and managed (thinned within three months of initial trap erection). Collected females were measured using an ocular micrometer (body length (mm) and pronotum width (mm)) and dissected. After dissection, number of eggs (fecundity) was counted and proportion of eggs infected with nematodes was estimated by examining a random subsample of eggs from each ovary. Effects of geographic location, forest management activity, and nematode infection on female body size and fecundity were tested. Geographic location and forest management activity did not significantly affect female body size or fecundity. However, nematode infection resulted in significantly smaller females that contained significantly fewer eggs. We believe these effects on S. nigricornis female size and fecundity, combined with the nematode’s ability to infect S. noctilio, make it a potential agent for applied biological control of S. noctilio. Artificial infection of S. noctilio to evaluate these effects would help test the feasibility of this idea.
ESTABLISHING HOST-PARASITOID LINKAGES AMONG SIREX NOCTILIO,
S. NIGRICORNIS, AND NATIVE PARASITOIDS THROUGH
MOLECULAR TECHNIQUES

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ABSTRACT

Sirex noctilio F. (Hymenoptera: Siricidae) is an invasive forest pest across the Southern Hemisphere and has been established in the northeast for over a decade. Thus far, its damage has been minimal and mortality has been restricted to stressed and weakened Scots (Pinus sylvestris L.) and red pine (Pinus resinosa Aiton). Its limited impact may be due to biotic interactions with native bark beetles, congeners, and hymenopteran parasitoids. However, establishing and quantifying trophic linkages is complicated by its subcortical larval development and complex life cycle. We used DNA barcoding techniques to investigate trophic linkages among S. noctilio, a native pine siricid (Sirex nigricornis L.), and a suite of shared native hymenopteran parasitoids. We developed primers designed to amplify Sirex-specific and S. noctilio-specific regions of the cytochrome oxidase I gene to identify tissue from siricid larvae, parasitoid gut content, and cadavers associated with parasitoids. We also used a restriction digest enzyme to separately identify tissue of S. nigricornis. We felled infested trees from sites across New York and Pennsylvania that varied in time since S. noctilio invasion. Larvae of siricids, parasitoids, and associated cadavers were collected by dissecting infested material with a log splitter. We successfully identified 85% of all samples. We had the greatest success in identifying siricid host from the gut content of ibaliid and rhyssine parasitoids in the larval life stage (74% and 92%, respectively). Hymenopteran parasitism was not significantly different among regions where S. noctilio had recently-invaded or was long-established.
INVASIVE AND ENDEMIC SIREX/AMYLOSTEREUM ASSOCIATIONS

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ABSTRACT

Samples collected in 2004 contained the first *Sirex noctilio* from what turned out to be an established population of this invasive near the southeastern edge of Lake Ontario (Hoebeke et al. 2005). The next year *S. noctilio* was found on the northern shore of Lake Ontario, in Canada (de Groot et al. 2006). Although *S. noctilio* had already been introduced in many locations in the Southern Hemisphere it had never before become established in an area where pines, its principal host trees, or other *Sirex* and their associated symbionts and natural enemies were native. In northeastern North America, three species of *Sirex* are native: *Sirex nigricornis* which usually attacks pines, *Sirex nitidus* which usually attacks spruce and *Sirex cyaneus* which usually attacks firs. Based on host trees, there was the greatest chance that *S. noctilio* would meet up with *S. nigricornis* as larvae of both of these species develop within pine.

Our laboratory has been studying the potential for interactions between the native and the invasive *Sirex* in North America. We have already found that females of *S. noctilio* and *S. nigricornis* will lay eggs that can successfully develop within the same pine trees (Hajek et al. 2013). Ryan et al. (2012) also reported rearing both of these species from the same pine trees.

*Sirex* are obligately associated with the white rot fungus *Amylostereum*, and this fungus must be present for successful egg eclosion and larval development. In the past, it was assumed that *Sirex* species were very specific about the species of symbiotic fungi with which they were associated. Before the establishment of *S. noctilio* in northeastern North America, it was assumed that this species was only ever associated with *A. areolatum* and that this fungal species was not native to North America. However, we’ve found that *S. nigricornis* females carry either *A. chailletii* or *A. areolatum* in their mycangia. The fact that from 13.5-35% of *S. nigricornis* in Louisiana (far from the area where *S. noctilio* is established) carry *A. areolatum* demonstrates that this fungal species is in fact native to North America (Hajek et al. 2013; Olatinwo et al. 2013). *S. noctilio* specimens collected in Canada were shipped to South Africa where it was found that a very small percentage of *S. noctilio* females (3.5%) can be found to carry *A. chailletii* in their mycangia (Woodring et al. 2013).

Our rearings have demonstrated that during summer, *S. noctilio* emerges over a much longer period that is significantly earlier in the season than *S. nigricornis* (first to last emergence from trees cut from mid-April to July 10, 2012: *S. noctilio* July 3-October 8; *S. nigricornis* August 27-October 12) (studies were conducted in an indoor but unheated *Sirex* rearing facility in Ithaca, NY). Peak emergence in our rearings in 2012 was in August-July for *S. noctilio* and late August-September for *S. nigricornis*. This general pattern of most *S. noctilio* emerging before *S. nigricornis* and over a longer period is in agreement with findings in Ontario by Ryan et al. (2012) for *S. noctilio* and *S. nigricornis* and Haavik et al. (2013) for *S. noctilio*, although these latter studies were conducted further north from our rearings in Ithaca, NY.

To investigate co-occurrence of these species within the same trees, we conducted experimental choice studies. We investigated whether drilling by ovipositing females of *S. noctilio* or *S. nigricornis* might be
influenced by the prior presence of several strains of *A. areolatum* (one native and one invasive) or *A. chailletii* in wood. We inserted fungal plugs within bolts of red pine (*Pinus resinosa*) one week ahead of releasing mated females of either *S. noctilio* or *S. nigricornis* within tents containing bolts and we subsequently quantified the oviposition drills made in each bolt. We found that there were definitely differences in drilling by these two species. No treatment resulted in complete avoidance by either *Sirex*. *S. noctilio* drilled most in control logs (that had no fungus already in them), next most frequently in logs inoculated with the invasive *A. areolatum* (that we think was introduced with *S. noctilio* from Europe) and least in bolts inoculated with *A. chailletii*. *S. nigricornis* drilled most in bolts inoculated with the *A. areolatum* strain that we think was introduced with *S. noctilio* from Europe. While neither *Sirex* completely avoided wood colonized by any *Amylostereum*, they adapted distances from plugs for drilling in a way consistent with the numbers of drills in different treatments.

Finally, we were interested in why *S. nigricornis* made so many fewer drills than *S. noctilio* during the choice study. We know that the moisture level of the wood strongly influences oviposition behavior of *S. noctilio* and we expect that this would also be true of *S. nigricornis*. However, the moisture levels of the wood used for our oviposition studies was within the levels cited by Morgan & Stewart (1966) as acceptable by *S. noctilio* for laying eggs. Based on results by Ryan et al. (2012b), it is possible that *Pinus resinosa*, the pine species used in our studies is not a preferred host of *S. nigricornis* although this host tree is clearly accepted by *S. noctilio*. Finally, we hypothesized that drilling by *S. nigricornis* could have been less than *S. noctilio* because the former has fewer eggs to lay. However, the numbers of eggs within females of similar sizes were very similar, if not greater for *S. nigricornis*.

Based on results from these studies, most adult *S. noctilio* adults had emerged from trees before *nigricornis*. *S. noctilio* females were very ready to drill in trees where no fungus was present already. Conversely, the later emerging *S. nigricornis* did not avoid trees where *S. noctilio* had already inoculated *Amylostereum*.

**REFERENCES**


PRESENTATIONS
Research Reports
ADDRESSING OLD CHALLENGES WITH NOVEL APPROACHES – PLANNING COST-EFFECTIVE SURVEILLANCE OF INVASIVE FOREST PESTS UNDER A TIGHT BUDGET

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ABSTRACT

Decision makers tasked with planning the surveillance of invasive species often have to rely on uncertain knowledge about the spread capacity of an invader of interest, and face the dilemma of scarce resources available to conduct surveys but the aspiration to cover all possible pathways of invasion. Often, surveillance planning relies on the estimates of invader’s propagule pressure (which is a well-recognized guiding principle in planning survey and management actions for emerging invasive pests). We present a pest surveillance model based on a somewhat different principle, the Maximum Expected Coverage Problem (MECP), which meets an important decision-making objective: it maximizes coverage of potential pathways of species entry from already-infested areas, and, in turn, fully captures the high-threat infested sources (as opposed to propagule pressure-based models, which focus on uninfested destination sites). The new model shows more stable performance under tight budgets compared to the survey allocations based on the propagule pressure concept.

We demonstrate the MECP approach by analysing pathways of the spread of the emerald ash borer (Agrilus planipennis Fairmaire), a major pest of ash trees in North America, with infested firewood that may be carried by visitors to campgrounds in central Canada and the U.S. Midwest. The underlying surveillance model was based on a pest transmission network that involved campers traveling from approximately 6500 infested or quarantined locations in central Canada and the U.S. to uninfested campgrounds in three Canadian provinces (Ontario, Quebec and Manitoba) and three U.S. states (Michigan, Minnesota and Wisconsin).

Overall, the new approach offers a workable strategy for dealing with typical uncertainty about the human-mediated spread of invasive species, and makes the planning of pest surveillance campaigns less subject to possible errors in the invader’s spread estimates. The concept can be applied in many practical situations where surveillance planning must be done under uncertainty and tight budget constraints.
RISK-BASED NATIONAL SAMPLING SURVEYS TO IMPROVE OUR ABILITY TO DETECT THE EMERALD ASH BORER AND EUROPEAN GYPSY MOTH BEYOND THE KNOWN INFESTED AREA

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ABSTRACT

The sampling efforts of two invasive exotic insects, the emerald ash borer (\textit{Agrilus planipennis} Fairmaire, Coleoptera: Buprestidae) and the European gypsy moth (\textit{Lymantria dispar dispar} (Linnaeus), Lymantriidae), have both recently taken on a national scope with the sample scheme becoming more centrally directed based on a new methodology. This methodology has combined preexisting information on locations of successful detection with expert knowledge of the invasive ecology of each of these two insects, resulting in an annual map product over the conterminous United States describing the detection likelihood and risk for each of the above insects. From these risk map products a prescribed set of 1km squares was created. These 1km squares occurred predominantly in areas of higher risk. This process has been applied to the sampling of emerald ash borer (EAB) for three years and was expanded last year to include the European gypsy moth (EGM) as well. Validations are presented for the latest risk maps and show for the 2014 season that the risk map products provided effective concentration of sampling efforts into areas of high detection success. This was found to be true not only when examining detection success within the prescribed 1 km squares (p < 0.0001), but also when evaluating additional detection successes within a 5 km radius of each prescribed square (p < 0.0001). This process illustrates a new methodology for the optimal distribution of sampling resources, one which will continue for the sampling of EAB and EGM and which can also be applied to other sampling contexts.
INVASIONS BY TWO NON-NATIVE INSECTS ALTER REGIONAL FOREST SPECIES COMPOSITION AND SUCCESSIONAL TRAJECTORIES

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ABSTRACT

While invasions of individual non-native phytophagous insect species are known to affect growth and mortality of host trees, little is known about how multiple invasions combine to alter forest dynamics over large regions. In this study we integrate geographical data describing historical invasion spread of the hemlock woolly adelgid (Adelges tsugae) and beech scale (Cryptococcus fagisuga) with regional forest inventory data collected by the US Forest Service’s Forest Inventory and Analysis program to quantify the individual and combined impacts of these pest species. This analysis indicates that regional impacts of these insects on their hosts occur surprisingly slow but act to change regional forest succession pathways. Because beech and hemlock commonly co-occur in eastern North American forests, invasions by the two pest species are altering the current and future composition of large forest regions through their impacts on these two late-successional species. Such results demonstrate how forest insect invasions can profoundly modify forest dynamic processes, resulting in long-term changes in forest ecosystems.
FIRST OUTCOMES OF THE EPPO STUDY ON WOOD COMMODITIES AND ASSOCIATED PEST RISK

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ABSTRACT

The EPPO Study initially called ‘Study on non-manufactured wood commodities’ was initiated by the EPPO Panel on Phytosanitary Measures in 2014 because little information was available and no classification and definitions existed for wood commodities such as wood chips and ‘waste’ wood. First objective of this Study was targeted to classify wood commodities other than round wood, sawn wood and firewood. The first draft document was prepared by an expert subcontracted by EPPO (Mr Kucinskas, Lithuania). Then, the special Expert Working Group (EWG), composed of EPPO and NAPPO experts, met at the EPPO headquarters in Paris (2014-10-07/10) to discuss this draft. The EWG decided to change the title of the Study to ‘The EPPO Study on wood commodities other than round wood, sawn wood and manufactured items’. It considered existing phytosanitary terms and definitions of the ISPM 5 Glossary of Phytosanitary Terms for wood commodities (e.g. ‘round wood’, ‘sawn wood’, ‘bark’, and ‘processed wood material’) and discussed classification and definitions for those wood commodities which are not covered by the Glossary. The EWG considered recommendations received on the draft document from IFQRG (International Forest Quarantine Research Group), EFSA (European Food Safety Authority) and ISO/TC 218 Timber group, and decided for phytosanitary reasons to subdivide all possible wood commodities into the following categories:

- Harvesting residues (stays on place after felling trees)
- Round wood (is taken from the forest)
- Processing wood residues (received at processing round wood)
- Bark (received at debarking round wood)
- Sawn wood (received by processing round wood)
- Wood chips (received by chipping different wood products)
- Hogwood (received by crashing different wood products)
- Processed wood material (received by processing wood with glue and/or heat)
- Manufactured wood items (items made of wood)
- Post-consumer scrap wood (stays after the life of items)

Following the recommendations of EFSA and ISO/TC 218 Timber, the EWG avoided the use of the term ‘waste’ or ‘wood waste’ in the Study because most wood residues at different stages of wood consumption now tend to
be used for different purposes and are no longer ‘waste’. For ‘firewood’, the EWG concluded that it refers to the final use of wood commodities, which could be any of the above mentioned commodities, or in many cases ‘round wood’.

The EWG developed definitions for those abovementioned wood commodities which were missing from the Glossary and established their links with terms used in the ISO system, in the IPPC Study on Electronic Certificates and in Customs codes. For each of the identified wood commodities (and some subcategories), the EWG considered the initial material from which they derive, most common intended uses, transport practices, pests which are likely to be associated with the material and survive on/in it, and possible phytosanitary measures. The general pest risk presented by each subcategory of wood commodities of concern was assessed in a very preliminary way. It was decided that the risk mainly depends on freshness of the commodity (which could be expressed through intracellular moisture content), bark presence and size of pieces or particles.

The finalised document will be sent to the EPPO Panel on Quarantine Pests for Forestry, and then for consultation with forest experts in the EPPO region before being presented to the EPPO Working Party for Phytosanitary Regulations for approval.
EUROPEAN BEECH LEAF MINING WEEVIL: A NEW INVASIVE ESTABLISHED IN NOVA SCOTIA

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ABSTRACT

The beech leaf mining weevil, Orchestes fagi (L.), (Coleoptera: Curculionidae) is a widespread pest of beech, Fagus sylvatica L., in Europe, and was discovered in Halifax, Nova Scotia in 2012, causing extensive damage to the foliage of American beech, Fagus grandifolia Ehrh. Orchestes fagi is the third European species of leaf-mining weevil confirmed as established in North America—prior introductions include the elm flea weevil, Orchestes alni (L.), and a willow/poplar leaf miner, Isochrominus sequensi (Stierlin). The weevil has a 1-year life cycle, overwintering as an adult and emerging in early spring coinciding with budburst on beech. Adults enter beech buds just as they start to burst and feed on developing beech leaves, making tiny holes that expand to “shot holes” when the leaves fully develop. Females lay eggs in the mid-rib on the underside of developing beech leaves and the larvae create a mine that extends to the leaf margin. After three larval instars, the weevil pupates inside the leaf. From egg to adult takes only about 35 days, with the new generation of adults emerging in June. When weevil densities are high, many leaves die and shrivel during development, giving the crown a scorched appearance; this type of damage has been common in Halifax for the last 3–4 years. Although larval feeding is restricted to beech, adults have been observed feeding on a number of other hosts in Europe, including raspberry and apple. In Nova Scotia, however field observations and no-choice bioassays by Moise et al. (poster abstract, these Proceedings) suggest O. fagi adult feeding in Nova Scotia is restricted almost entirely to beech. Surveys (visual, branch beating, and yellow sticky traps) conducted by the Canadian Food Inspection Agency in 2012 (Nova Scotia), 2013 (Ontario, Quebec, and all three Maritime provinces), and 2014 (Maritime provinces) suggest the weevil is still confined to Nova Scotia where it is distributed within 30 km of Halifax, widespread on Cape Breton Island, with satellite infestations in Chester and Wolfville, NS. Here we report results from studies to determine the risk of O. fagi being artificially spread in the movement of logs and firewood, to develop survey tools, and to test TreeAzin® stem-injection as a control method.

A survey of potential overwintering substrates in April 2013 found that densities of overwintering O. fagi adults were greatest on the boles of trees, including beech, red spruce (Picea rubens Sarg), and red maple (Acer rubra L.), with much lower densities in the crowns of spruce and in the duff. Bark roughness significantly affected mean density of O. fagi adults. Beech that were heavily cankered as a result of beech bark disease had high densities of weevils, as did rough-barked maple and spruce, whereas smooth barked trees had much lower numbers. These results indicate that movement of logs and firewood from infested areas may increase the rate of spread of O. fagi.

In an effort to develop lures for surveillance of the weevil, volatiles from the leaves, buds, and wood of beech as well as the effluvia of adult weevils, were analyzed by gas chromatography/mass spectrometry. Several candidate
compounds were identified and synthesized from host tissues (e.g., beta caryophellene, alpha cubebene) and weevils (methyl-branched alkanes and alkenes, fatty acid esters) and tested for physiological (electroantennograms) and behavioral responses (y-tube olfactometer, field trapping tests). Antennal response was observed to most compounds but no significant attraction has been observed to more than a dozen compounds and blends tested to date in the lab or the field. Yellow sticky traps captured significantly more adults than non-sticky boll weevil traps but still did not detect weevil presence as well as branch beating. Some promising compounds identified in expanding beech buds have been synthesized and will be tested in lab and field bioassays in 2015.

Field tests of TreeAzin® stem-injection for protection of beech foliage were conducted in Halifax in 2013 and 2014. Injections that were done prior to- or at the time of budburst significantly reduced larval survival but injections done 2 weeks after budburst were not effective. However, pre-budburst treatment reduced larval feeding damage in only one of two years and did not reduce adult feeding damage in either year. In 2015, we will assess the level of foliage protection in trees injected in the fall of 2014.
GENETIC STRUCTURE OF A PALEARCTIC DEFOLIATOR,
THE GYPSY MOTH (LYMANTRIA DISPAR, LEPIDOPTERA: EREBIDAE),
INFERRED FROM MICROSATELLITE DATA

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ABSTRACT

The gypsy moth (Lymantria dispar Linneaus 1758) is among the most destructive defoliating pest insects in the world. Its current distribution encompasses forests throughout the entire temperate region of the northern hemisphere. The gypsy moth is native to Europe and Asia and was introduced to North America from Western Europe less than 150 years ago. Given its Holarctic distribution and great economic importance, we examined the population structure and evolutionary history of L. dispar and considered how geographic factors have shaped patterns of genetic variation in this species. With an extensive sampling of 1738 moths throughout the Holarctic and using allele frequencies from nine microsatellite loci, we revealed that the overall genetic diversity is highest in Asian populations, intermediate in European populations, and lowest in North American populations. This result is consistent with the hypothesis that gypsy moth originated in Asia and with the fact that gypsy moth was only recently introduced to North America through a few founding individuals. For the global picture of the population structure, we identified four genetic clusters representing four distinct geographic areas using both Bayesian and distance methods. These clusters correspond to three named subspecies (L. d. asiatica, L. d. dispar, and L. d. japonica) that occupy geographically distinct regions. Introduced North American populations represent a unique cluster, presumably a consequence of an initial population bottleneck and subsequent allele frequency change. The Ural Mountains are thought to be the phylogeographic break between L. d. dispar and L. d. asiatica. But we found that specimens identified as L. d. asiatica from east of the Urals cluster with L. d. dispar. In East Asia, the subspecies L. d. asiatica and L. d. japonica overlap and form an extensive hybrid zone in northeastern China, the Russian Far East, and the Korean Peninsula. Lymantria dispar japonica probably has dispersed across the Korea Strait to hybridize with L. d. asiatica. Our study provides the fundamentals for appropriate responses to control populations based on their genetic structure and facilitates the development of approaches for recognizing new introductions.
AZALEA LACE BUG, *STEPHANITIS PYRIOIDES* (HEMIPTERA: TINGIDAE), A THREAT TO FOREST SHRUBS?

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ABSTRACT

Azalea lace bug (AZLB), *Stephanitis pyrioides* (Scott), is an Asian species known to be established in eastern North America for almost a hundred years. This sucking insect damages hosts by extracting chlorophyll from the leaves, sometimes causing severe chlorosis, which may reduce host vigor. Although sometimes severe, damage in the East has tended to be sporadic, with the most severe effects on plants in full sun. All previously known hosts are in the family Ericaceae, the heath family. Severe damage appeared limited to vulnerable species and varieties of azaleas and rhododendrons.

Prior to 2008, the western extent of the North American distribution of AZLB appeared to be eastern Texas. AZLB was found in Washington state in 2008 and in Oregon in 2009. The behavior of AZLB in Oregon appears markedly different from its behavior in the East. The overwintering stage in the East is the egg while there is evidence that in Oregon all life stages may overwinter. Eggs, immatures, and adults were found alive on plants in December and January following extended periods of subfreezing weather. Whereas severe infestations are largely associated with plants in full sun in the East, extensive and intensive damage in Oregon occurs regardless of the degree of exposure to sun. Damage from AZLB is apparently much more severe than is normal for the East. Almost complete chlorosis of entire plants and plantings now occurs throughout metropolitan areas in the northern Willamette Valley. Damage can be so severe that vulnerable hosts often die. Highly susceptible species and varieties are now being removed from landscape plantings and may not be offered by retail nurseries. Populations of AZLB can be so large that these insects become nuisance pests by entering homes by the hundreds. Whereas infestations in the East appear limited to gardens, nurseries, and ornamental plantings, severe infestations have been found in several heavily wooded Oregon parks that are reasonable approximations of native forest.

Another disturbing difference from the East is that numerous novel hosts for AZLB have been found in Oregon, based upon both field observations and host trials conducted in environmental chambers. These include three novel families, thirteen novel genera, and twenty-five novel species. This list doesn’t include numerous previously unrecorded hybrid varieties of azaleas and rhododendrons. Some of these hosts are natives of the East. Perhaps the most disturbing aspect of these novel hosts is that several, such as salal, huckleberry, and western azalea, are native shrubs that can be important components of Oregon and western forests. Another concern is that both species of kalmiopsis, a genus with limited distribution in southwestern Oregon and northwestern California, have shown severe damage in ornamental plantings. Plants of both species were killed during host plant trials, despite receiving good care.

It appears that AZLB may become an ecological threat as well as a horticultural pest, at least in the West. If the levels of damage currently seen in more managed settings are expressed in forest and shrub lands, wildfire risk may become greater due to increased fuel accumulations of dead leaves and plants. If vulnerable native shrub vigor is reduced and mortality increased, resources for herbivores may be affected. Availability of berries from hosts such as huckleberries and salal may be reduced. Berries from these plants are important foods for wildlife and humans. Kalmiopsis, a genus already threatened by limited distribution and patchy habitat, may become yet more threatened. Since the known infestation in the West is recent and information is somewhat limited, these concerns are speculative. Further monitoring and study must be done to ascertain whether the azalea lace bug will remain primarily a problem for horticulture or if it will become a new forest pest as well.
SPOTTED LANTERNFLY: A NEW PEST DETECTED IN PENNSYLVANIA

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ABSTRACT

Spotted lanternfly (HEMIPTERA: FULGORIDAE: Lycorma delicatula (WHITE)) was detected on September 22, 2014 in Eastern Berks County, Pennsylvania. Pennsylvania Game Commission Employees reported damage to tree of heaven (Ailanthus altissima (Philip Miller)Swingle) and the presence of an unknown insect to the Pennsylvania Department of Agriculture Entomology Division. An immediate site visit yielded several hundred specimens of the unknown insect which were identified as L. delicatula, a pest new to North America. Existing literature details that the lanternfly is native to China, India, and Vietnam and was introduced into Japan and South Korea. In South Korea, L. delicatula spread rapidly and is considered to be an invasive pest of grape (Vitis spp.) and other species. The insect completes one generation per year, overwintering as a mass of 25-50 foam covered eggs from October to May. From May through August, the lanternfly completes four nymphal instars feeding on up to 71 species of plants. In August adults congregate primarily on A. altissima where the feed, mate, and lay eggs prior to dying. Eggs can be laid on any hard surface.

Possible pathways for the introduction and additional spread of the pest were identified. A quarantine has been established in Pennsylvania, and the pest has been designated as reportable/actionable by the USDA. Several community meetings were conducted in the affected area. A new pest advisory group was formed, and plans are being developed for eradication of this new pest. A delimiting survey indicates the pest is present over an area of 40 square kilometers. Interviews of local residents and the presence of old egg masses indicate that the infestation is two to three years old. The Pennsylvania Department of Conservation and Natural Resources, Bureau of Forestry, Division of Forest Pest Management is doing aerial sketch mapping of A. altissima in the affected area. A pest alert, scraper cards and a quarantine checklist were produced. Photographs are available for use through Forestry Images, and reference specimens have been provided to several states and museums. The Pennsylvania Department of Agriculture is working with the affected community and several cooperating state and federal agencies to develop eradication strategies. Plans are under way to modify existing gypsy moth control monitoring techniques to gage population size. Emergence and host range information will be investigated. The community will be engaged in egg mass scraping and tree banding control programs. Chemical control options are being evaluated and local vineyards and orchards have been inspected.

Excellent cooperation has led to the early detection of and rapid response to this new invasive threat. Thank you to the Pennsylvania Game Commission, the Pennsylvania Department of Conservation and Natural Resources, the Pennsylvania Department of Environmental Protection, the USDA, the USFS, Berks County Government, the Pennsylvania State University, and the citizens and businesses of Berks County.
THE QUEST FOR ASH RESISTANCE TO EMERALD ASH BORER: TOWARDS A MECHANISTIC UNDERSTANDING

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ABSTRACT

Since emerald ash borer (EAB), Agrilus planipennis, was discovered in North America in 2002, it has killed many millions of ash trees in North America, and ash mortality now exceeds 99% near the epicenter of the invasion in southeast Michigan (Klooster et al. 2014). The development of EAB-resistant ash trees will be critical for restoration of ash in natural and urban forests. Goals of our collaboration are to identify, breed and screen ash germplasm for EAB resistance and silvicultural traits; and identify mechanisms of EAB resistance to facilitate breeding and screening.

In Asia, EAB does not devastate its endemic hosts, which suggests that Asian ashes are inherently resistant by virtue of their coevolutionary history with EAB. In a common garden study, patterns of ash decline and mortality were largely consistent with this hypothesis. Manchurian ash had the highest rate of survival and little canopy decline. The high EAB resistance of this Manchurian ash population of seedling origin is consistent with that observed previously for the clonal Manchurian ash cultivar ‘Mancana’ (Rebek et al. 2008), which suggests that EAB resistance is a species-level trait. Fraxinus x ‘Northern Treasure’ ash, which is a Manchurian (Asian) x black ash (North American) hybrid, had similarly high survival and low canopy decline, suggesting introgression of Manchurian ash resistance genes into the hybrid. However, this pattern contrasts sharply with that observed by Rebek et al. (2008), who found ‘Northern Treasure’ ash to be highly susceptible to EAB. One potential explanation for these divergent conclusions is that there may be taxonomic confusion surrounding this cultivar that needs to be resolved.

Most North American species and cultivars in the common gardens study experienced complete or nearly complete mortality, with green ash cultivars, black ash, and Oregon ash declining more rapidly than white ash cultivars. Blue ash has survived at a higher rate in the common garden than other North American species, but by 2014 had lower survival and greater canopy decline than Manchurian ash. Decline and mortality of blue ash has increased over time, suggesting that surviving trees in the plot may ultimately succumb to EAB. The European species and cultivars evaluated in the common garden also experienced high decline and mortality, including F. ornus, F. excelsior ‘Aureafloria’, and F. angustifolia subsp. oxycarpa ‘Raywood,’ which suggests that EAB has the potential to cause widespread economic and ecological impacts in Europe as it continues to spread in Russia and beyond.
Because the devastating impact of EAB on its host is due to larval feeding, we have focused much of our research on mechanisms that affect larval density, growth, and survival, including female oviposition preferences and phloem chemistry. In field studies, we evaluated the role of female oviposition preference as a determinant of interspecific variation in ash resistance to EAB (Rigsby et al. 2014). The “mother knows best” hypothesis predicts that ovipositing females should choose hosts on which their offspring will best perform, maximizing their own fitness. We found that susceptible green, white, and black ash consistently received more eggs than resistant Manchurian ash, and that preference for ash species was independent of tree size and vigor. These observations are consistent with the “mother knows best” hypothesis (Rigsby et al. 2014), and suggest that oviposition preferences may contribute to interspecific patterns of host resistance observed in previous studies (e.g. Rebek et al. 2008, Whitehill et al. 2012).

Eyles et al. (2007) identified constitutive phenolic compounds in phloem of the resistant Manchurian ash cultivar ‘Mancana’ that were not present in the more susceptible green and white ash, including several hydroxycoumarins and two phenylethanoids, and suggested that they might represent potential EAB resistance mechanisms. However, in subsequent studies in which species comparisons were more phylogenetically controlled, these compounds were detected in highly susceptible black and European ash at concentrations comparable to or higher than in the closely related Manchurian ash (Whitehill et al. 2012). This strongly suggests that these hydroxycoumarins and phenylethanoids are, in fact, not responsible for the high resistance of Manchurian ash. The pinoresinol dihexoside and a tentatively identified coumarin derivative were the only phenolic compounds detected that were unique to Manchurian ash. Recent experiments have shown, however, the phenolic profile of Manchurian ash much more highly bioactive than that of black ash when consumed by EAB larvae, as they were oxidized to a greater degree by enzymes such as peroxidases that are expressed at higher levels in Manchurian ash. As a result, midgut tissues of larvae feeding on Manchurian ash experienced much higher levels of oxidation stress than did larvae feeding on black ash, even though the phloem phenolic profile of the two ash species is similar (Whitehill et al. 2012). In a comparative study of phloem proteomes of resistant and susceptible species, we found that other proteins implicated as defenses in other species were also constitutively over-expressed in Manchurian ash relative to green, white, and black ash, and thus might also contribute to high EAB resistance of Manchurian ash. These include a PR-10 protein, phenylcoumarin benzyl ether reductase, an aspartic protease, and ascorbate peroxidase (Whitehill et al. 2011).

As another approach towards identifying resistance mechanisms of Manchurian ash, experimental manipulations were used to alter performance of EAB larvae, and to relate larval performance to associated phytochemical changes in the phloem. Manchurian and black ash were exposed to variable water regimes and challenged with egg inoculations to elicit induced responses to larval feeding (Chakraborty et al. 2014). Growth of EAB larvae was lower on Manchurian ash, which provides evidence that antibiosis, as well as ovipositional non-preference, contributes to its high EAB resistance. EAB larval feeding induced higher concentrations of pinoresinol A in Manchurian than black ash, which may also contribute to higher resistance. Larvae grew faster on drought stressed Manchurian ash trees, which is consistent with the role of EAB as a secondary colonizer of stressed trees in its native habitat.

In another study, methyl jasmonate was applied to susceptible North American and resistant Asian ash species to determine if it can elicit induced responses in bark that enhance resistance to EAB (Whitehill et al. 2014). MeJA application decreased adult emergence in susceptible ash species to levels achieved by insecticide application. Concentration of the phenolic compound verbascoside sharply increased after MeJA application to green and white ash. When incorporated in an artificial diet, verbascoside decreased survival and growth of EAB neonates in a dose-dependent fashion. Lignin and trypsin inhibitors were also induced by MeJA, and analogs of both compounds reduced growth of EAB larvae in artificial diets (Whitehill et al. 2014). Application of MeJA prior to EAB attack may have potential for enhancing resistance of susceptible ash species by inducing increased concentrations of verbascoside and other defensive compounds (Whitehill et al. 2014).

We have initiated a breeding program based on hybridization of resistant Asian ash and susceptible North American ash (following the successful chestnut blight breeding program) (Koch et al. 2012), and have begun screening rare native genotypes that continue to survive (“lingering ash”) where overall ash mortality is very high to see if they are truly resistant (Knight et al. 2012, 2013). We have made progress in overcoming barriers to hybridization of Asian
and North American ash (Koch et al. 2012), and several extensive common garden plantations have been established in Delaware and Wooster, Ohio to evaluate EAB resistance and silvicultural characteristics of additional Asian species, Asian-North American hybrids, and lingering ash selections for use as parental lines to expand the breeding program. We have also developed bioassays to screen young plants for resistance, which will expedite breeding activities.

REFERENCES


RAPID WHITE OAK MORTALITY IN MISSOURI:
A COLLECTION OF CAUSES

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ABSTRACT

An unprecedented mortality event, principally associated with white oak (Quercus alba), has been observed in the Missouri Ozarks since 2010, with a pronounced occurrence in 2012. In order to understand the etiology of this event, we investigated the scope of the mortality using surveys provided to landowners and foresters. On-site studies were conducted to search for a number of putative mortality agents. From surveys completed by citizens and foresters, the oak mortality has been observed in Iowa and Arkansas as well as Missouri. Anecdotal information and observations suggest that the mortality is associated with lower slope positions and in areas of the Ozarks, mortality is highest along stream systems. Two field study sites were selected in the Missouri Ozarks in which we collected data from overstory species composition, site conditions, vigor and mortality estimates. Trees were assessed vigor ratings, and we examined trees for presence of Hypoxylon. Soil samples from the bases of trees were baited to determine presence of Phytophthora and both intensive study locations revealed that Phytophthora cinnamomi was present. Additionally, bolts from trees were used to determine insect emergence for possible ambrosia and bark beetles associated with the event. Within the first few months, Xyloborinus gracilis dominated the guild of emerging beetles, but emergence will be evaluated for a total of 9 months. Armillaria present on the sites were sampled; identification is being verified using molecular identification techniques.

The mortality patterns are consistent with observations elsewhere where Phytophthora has been involved as a mortality agent. Phytophthora cinnamomi was recovered from soils next to trees with and without symptoms, suggesting this is a more widespread concern than first recognized. Moreover, dendrochronology data from the sites reveal that trees have been growing unusually slowly for approximately two decades. Anomalous climate patterns, particularly unusually heavy precipitation years and highly variable and extreme temperatures may also be implicated as conditioning factors for Phytophthora and the other associated biotic agents. Research will continue for several months to sample emerging ambrosia beetles, resample for Phytophthora, and to more closely examine the relationship between tree growth, climate and mortality.
POSTERS
EMERSON ASH BORER [EAB] Agrilus planipennis Fairmaire (Coleoptera: Buprestidae) – is an example of a destructive invasive insect which abruptly expanded its initial East-Asian area in the 1980–1990s. This species is now the main pest of ash (Fraxinus) trees in the USA and Canada and is quickly spreading over 11 administrative regions of European Russia. It is very important to determine a list of possible host plants of A. planipennis in order to produce a pest risk assessment of invasion of this pest over the territory of Central and Western Europe.

In its native area – North-Eastern Asia – this buprestid attacks only dying trees of the East-Asian ash species Fraxinus chinensis and F. mandshurica whereas healthy trees of these species are highly resistant to the pest. No examples of resistant ash species have been found in North America.

Documentary data are presented for the first time on infestation by the emerald ash borer of all three European ash species that are established at the Main Botanical Garden of the Russian Academy of Sciences in Moscow, Russia. At the first time This pest was first recorded at the garden in 2011. During the period of 2010–2014, EAB killed from 70 to 100% of trees of European ash species: Fraxinus excelsior, F. angustifolia (= F. oxycarpa) and F. ornus. During the same period, from 81 to 90% of specimens of North American ash species (F. pennsylvanica and F. americana) were killed by this buprestid. Simultaneously, dead trees of Asian species F. mandshurica and F. chinensis (= F. rhynchophylla) showed no signs of EAB infestation.

This case study is a good example of the value of using the concept of ‘sentinel trees’. Arboretas that contain collections of non-native plants may serve as ‘ecological traps’ for local pests and pathogens – potential invasive organisms in the source regions of introduced plants.

This work was supported in part by the Russian Fund for Fundamental Research (grant # 14-04-01235a) and COST Action FP1401.
**IMIDACLOPRID AND ITS METABOLITE OLEFIN: MULTIPLE YEAR PERSISTENCE IN EASTERN HEMLOCK FOLLOWING TREATMENT FOR HEMLOCK WOOLLY ADELGID**

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**ABSTRACT**

Hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), has caused widespread mortality in eastern hemlock populations. The front of adelgid infestation has rapidly progressed through the Great Smoky Mountains National Park (GRSM), and to suppress HWA populations imidacloprid treatments have been applied to many hemlocks in GRSM.

The efficacy of single imidacloprid treatments for HWA population suppression was assessed four to seven years after application in hemlock trees. The concentrations of imidacloprid and its metabolite olefin were determined using liquid chromatography-mass spectrometry. Since olefin is over ten times more toxic to insects than imidacloprid its persistence is of particular interest.

Imidacloprid and olefin are present in hemlock tissue four to seven years after a single imidacloprid treatment, and HWA population suppression is still occurring. Four years after treatment, average olefin concentrations are still greater than the LC₅₀ (i.e., the lethal concentration to kill 50% of the population) for HWA. Olefin concentrations are below the LC₅₀ six and seven years after treatment. Continued HWA suppression could be the result of a synergistic effect of low imidacloprid and olefin concentrations present in hemlock tissue. Knowledge of this combined long-term effect of imidacloprid and olefin persistence in hemlock and the continued HWA suppression can enhance GRSM’s control program by informing the timing of retreat treatments. Reducing the frequency of retreat treatments would reduce the pesticide input into the ecosystem, minimize potential non-target impacts, and reduce costs. The longevity of imidacloprid treatments due to the synergistic activity of imidacloprid and olefin can provide both financial and environmental benefits to HWA control programs.
DEFINING, MAPPING, AND RANKING PORT ENVIRON TO PREVENT PEST INTRODUCTIONS

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ABSTRACT

The U.S. receives a staggering amount of imported commodities from many different countries that move rapidly through ports of entry to associated locations along the trade pathway. This poses a significant risk for the introduction of invasive pest species and creates a challenge for plant health regulatory officials to determine the areas most susceptible to new pest incursions. To address this challenge, we developed a method to define, map and rank these areas, which we call Port Environs. We mapped the density of relevant location types as a continuous Port Environ to represent where imported material may pose a risk. In addition to density, risk also depends on the type and volume of the imported commodity, how long a commodity remains at a location, the pest’s environmental suitability, and the consequences associated with pest introduction. To address these components of risk we are quantifying import volume using U.S. Census data, estimating the amount of host material using Forest Service and USDA data, and determining consequences using economic analyses. This quantification of risk will allow us to develop a multiple-scale resource allocation calculator for pest monitoring in Port Environs. This process of defining, delimiting, and ranking Port Environs will allow us to address recent changes in trade and transport patterns and serve as a guidance tool for plant health regulatory officials.
INTERACTIONS BETWEEN NATIVE PREDATORS AND AN INTRODUCED PARASITOID IN THE CONTROL OF WINTER MOTH (OPEROPHTERA BRUMATA) IN THE NORTHEASTERN U.S.

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ABSTRACT

A tachinid parasitoid, Cyzenis albicans, has been introduced to the northeast United States to control the outbreak of winter moth (Operophtera brumata). A large change in winter moth densities with only a relatively small percent parasitism (35%) was documented at one of the release sites. In Nova Scotia in the 1950s and British Columbia in the 1970s, predation of winter moth pupae increased following parasitoid establishment. It appears that mortality from parasitism and predation is greater than if the two factors were acting independently. We tested three hypotheses proposed by Roland (1990) as to why this may be: (1) Mortality due to parasitoids is enough to bring the winter moth population down to a level that can be effectively controlled by generalist ground predators, (2) Parasitized pupae experience lower mortality rates than do the unparasitized pupae, (3) The presence of C. albicans induces a numerical response in generalist ground predators because C. albicans is available in the spring. We found little support for the three hypotheses: There was (i) no evident predator saturation threshold, (ii) only an early season difference between parasitized and unparasitized pupae, and (iii) limited support of a predator numerical or functional response to the present of C. albicans. We, however, found a native Ichneumonid wasp (Pimpla aequalis, ID by D. Wahl) parasitizing deployed pupae (1-33%) in areas where winter moth is well established. We suggest that the aggregation of this wasp after winter moth establishment may be responsible for the apparent synergistic effect. Further studies will be conducted this coming season (2015) to evaluate parasitism rate across a winter moth density and establishment gradient.
NUCLEOPOLYHEDROVIRUS AND MICROSPORIDIA IN WINTER MOTH (OPEROPHTERA BRUMATA) AND BRUCE SPANWORM (O. BRUCEATA) POPULATIONS IN THE NORTHEASTERN U.S.

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ABSTRACT

The winter moth (WM, Operophtera brumata), a polyphagous geometrid affecting mainly deciduous tree species, was accidently introduced to the northeastern United States from Europe in the 1990s. Although WM has been in a continuously outbreaking population since its introduction, the native congener, Bruce spanworm (BSW, O. bruceata), rarely exhibits outbreaks. We propose that this difference in population dynamics exists because BSW is experiencing a different set of pathogens, which may exist at a higher prevalence or may be more virulent. Field collected WM and BSW larvae were reared in the lab and percent mortality was noted for 2013 and 2014 collections. Cadavers were examined microscopically for evidence of microsporidia (possibly Nosema sp.) and nucleopolyhedrovirus (NPV) infections. To better understand the phylogenetic relationship between the NPVs found in WM as compared to BSW, NPV isolates were analyzed using PCR, amplifying the polyhedron and the p74 gene. Resulting bands were gel-extracted and Sanger sequenced. The BSW polyhedron sequences were compared to that of WM (OpbrNPV) available on GenBank. The p74 sequences were aligned using MUSCLE and Mega6 was used for phylogenetic analysis. To test for the possibility of cross infection, WM eggs were surface sterilized, temperature induced to hatch, moved to diet for 7-10 days, starved for 4-20 hrs, and then fed on diet infected with WM or BSW NPV. Moribund larvae were analyzed for NPV using species-specific polyhedron gene primers. Of 433 BSW larvae collected in 2013, 38% did not survive to pupation while only 1.3% of the 15,677 WM larvae died in this same interval. There was no evidence of density dependent mortality. In 2014, 14% of 170 BSW collected and 5% of 11,646 WM did not survive to pupation. Across both years, BSW had a higher rate of microsporidian infection than WM (33% of cadavers compared to 0.4% in 2013 and 20% compared to 1.9% in 2014) while WM experienced a higher prevalence of NPV than BSW (32% of cadavers as compared to 1.7% in 2013 and 40% compared to 20% in 2014). The polyhedrin sequence from BSW was only 82% identical to that of OpbrNPV while the p74 phylogenetic analysis found the BSW NPV to be most closely related to that of WM. Together this indicates that the NPV infecting these insect species are different but only recently diverged. In the cross infection trials, BSW NPV was unable to infect WM larvae. In conclusion, WM and BSW are experiencing different pathogens and at a different rate. Understanding the activity of epizootics in these closely related species may provide valuable insight into possible biological control agents of WM.
TOTAL RECORDS OF VELVET LONGHORN BEETLE

TRICHOFERUS CAMPESTRIS FALDERMANN
(COLEOPTERA: CERAMBYCIDAE) FROM UTAH

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ABSTRACT

The velvet longhorn beetle (Trichoferus campestris Faldermann) is a potential threat to Utah’s urban, orchard, and riparian wood land areas. Field sampling and actions taken in association with the detection efforts by the Utah Department of Agriculture and Food (UDAF) are necessary to maintain productive commercial tree fruit production, and healthy urban forests. Expanded detection trapping along natural waterways and orchards indicate that the velvet longhorn beetle (VLB) distribution, first discovered in South Salt Lake City in July 2010, is widespread in Salt Lake and Utah counties. The insect was first detected in North America in the province of Quebec, Canada in 2002 and 2006 (Grebennifov et al. 2010). The VLB has been found in warehouse settings in Ohio (2009), Rhode Island (2006), New Jersey (2007, 2013), and Illinois (2009) (Blackwood 2010), it has also been detected in Colorado (2013), and New York (2014). It spreads into new areas through infested wood packing material that accompanies a wide variety of imported commodities such as: building supplies, machinery, tools, glass, tiles, etc. (Cavey 1998). Published reports from the European Plant Protection Organization, CABI and Global Pest Disease Database have been summarized in the USDA-APHIS-National Identification Services Plant Pest Risk Assessment (1998). The conclusion of this literature review is that VLB is polyphagous and prefers to attack apple (Malus), and mulberry (Morus) in its native range. In Utah, VLB larva, pupae, and adult life stages have been recovered through destructive sampling from peach and cherry (Prunus).
VALIDATING THE EXPAT SPREAD MODEL
WITH EUROPEAN GYPSY MOTH

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ABSTRACT

The Exotic Pest Assessment Tool (EXPAT) is a generic model to predict the spread of invasive arthropod, vertebrate, plant diseases, and weeds. Originally developed to justify the costs of pre-border measures to prevent introductions of invasive species, EXPAT is yet to be validated with any observed data on pest spread. We validated the model using the observed spread of European Gypsy Moth (EGM) in Wisconsin from 2000-2012 under the Slow-the-Spread program. To model EGM spread in EXPAT, we used information on pest specific parameters: growth rate, diffusion, initial infested area, climatic suitability, human mediated movement, and host density. After 10,000 iterations in a Monte Carlo simulation the modeled average infested acreage in 2012 was 5,807,157 and the maximum and minimum was 12,879,280 and 2,718,803 acres, respectively. In comparison, the observed affected area was 4,484,404 acres by 2012. When examined on average for each year between 2000 and 2012, the cumulative infested acreage of the model was 21% less than the observed spread. In its current form, the curve of the predicted spread grows at an exponential rate whereas the observed spread is linear. This leads to an under-estimation early, e.g., between 2000 and 2005, and an over estimation towards the end of spread scenario, e.g., between 2005 and 2012. We will develop spread equations including exponential, logistic, linear and logarithmic to be better tailored to pest groups. Validation of the right model for each pest group will lead to more accurate predictions. Finally, we will continue to expand the EXPAT validation effort by examining with additional pests and pathogens for with there is observed spread data.
THE NON-STERILIZING STRAIN OF *DELADENUS SIRICIDICOLA* (TYLENCHIDA: NEOTYLENCHIDAE) AND ITS DEVELOPMENT ON DIFFERENT STRAINS OF *AMYLOSTEREUM* (BASIDIOMYCOTA: RUSSULALES)

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**ABSTRACT**

The nematode *Deladenus siricidicola* Kamona, which sterilizes *Sirex noctilio* females, has been extensively and successfully used as a biological control agent for control of this woodwasp in the Southern Hemisphere. Curiously, a non-sterilizing (NS) strain of *D. siricidicola* is commonly found in North America and it is thought that the NS strain was introduced with *S. noctilio* when this woodwasp was introduced to North America. Finding an appropriate biological control agent for *S. noctilio* in North America has been challenging due to the existence of native species of *Sirex* woodwasps that are not considered pests but are part of the decomposer communities in forests. Therefore, evaluation of biological control agents requires studies of host specificity of the nematodes.

For this experiment, we evaluated the NS strain of *D. siricidicola*, which is poorly understood and is a potential competitor of *D. siricidicola* Kamona. *D. siricidicola* has two forms: a form that parasitizes *S. noctilio* and a mycophagous form that feeds on the *Sirex* fungal symbiotic *Amylostereum*. The goal of this study was to compare associations between the NS and Kamona nematodes and different isolates of the symbiotic fungus. We evaluated the ability of the nematodes to develop and reproduce when feeding on different isolates of *Amylostereum* associated with *Sirex* in North America.

We found that both strains of the nematode grew better when feeding on the three isolates of *A. areolatum* that are assumed to have been introduced to North America with *S. noctilio* and they did not grow on *A. chailletii*. Comparing the growth of the two nematode strains on different fungi, we saw significant differences for *A. areolatum* BD, which favored *D. siricidicola* NS rather than Kamona growth.
GENETIC VARIATION WITHIN GYPSY MOTHS FROM CHINA AND RELATEDNESS TO GYPSY MOTHS FROM OTHER WORLD AREAS

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ABSTRACT

The gypsy moth is a destructive defoliator with a broad geographic range and is one of the most recognized pests of forests and ornamental trees in the world. Asian Gypsy moths (AGM) are considered to pose a greater threat to North America than European gypsy moths (EGM), because of a broader host range and females capable of flight. To evaluate genetic variation in Chinese gypsy moths, Inter Simple Sequence Repeat (ISSR) markers were used. In addition, DNA Barcoding sequences (658 bp mtDNA COI) with restriction site genotyping (NlaIII and BamHI) were utilized to compare mitochondrial sequence variation within and among 9 Chinese gypsy moth strains and 7 from other areas.

In ISSR analysis, 102 polymorphic loci were observed; overall genetic diversity (Nei’s, H) was 0.2357, while the mean genetic diversity within geographic populations was 0.1845 ± 0.0150. The observed genetic distance among the eight populations ranged from 0.0432 to 0.1034. Clustering analysis (using UPGMA) revealed a correlation between the genetic relatedness among populations and their geographic proximity. Analysis of molecular variance demonstrated that 25.43% of the total variability (Fst = 0.2543, P < 0.001) was attributable to variation among geographic populations. Three mtDNA marker haplotypes were identified using the restriction sites and two Chinese strains had both the AGM and EGM haplotypes. Using the 658bp COI sequence, the overall average pairwise nucleotide sequence divergence using K2P was 0.005. The sequence divergence between the Asian subspecies and the European subspecies was three times greater than the variation within subspecies (mean = 0.9%, range = 0.2% to 1.7%). Using a TCS haplotype network, 26 COI haplotypes were found. The network and a neighbor-joining tree using the barcoding sequences indicated a clear delineation between gypsy moth subspecies. However, some moths from two Chinese strains formed a separate branch in the EGM subspecies cluster in both analyses. The results suggest there is more variation in the AGM than originally thought or some EGM have been introduced into China. This may complicate efforts to identify AGM introductions and require changes in exclusion programs. This research provides crucial genetic information needed to assess the distribution and population dynamics of this important pest species of global concern.
GLOBAL PEST AND DISEASE DATABASE

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ABSTRACT

The Global Pest and Disease Database (GPDD) is a comprehensive archive of exotic pest information. It serves as a secure electronic storehouse of scientific information about potentially invasive pests of concern that are exotic to, or have limited distribution in the United States. The data contained in this system can be further analyzed and used for 1) prioritizing pre-emergent pest threats to the U.S., 2) developing off-shore mitigation strategies, 3) focusing domestic exotic pest surveys and domestic port activities, and 4) supporting pest risk assessments. Data is collected and compiled from public and secure electronic sources (websites and databases), primary literature, expert correspondence, and internal APHIS documents. The GPDD currently houses over 5,000 pest profiles. Due to the sensitive nature of some sources, users have individual role-based access levels and data restrictions.
A CROSSING STUDY TO EVALUATE INVASIVE EUWALLACEA NEAR FORNICATUS POPULATIONS IN CALIFORNIA AND FLORIDA

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ABSTRACT

Several introductions have occurred of an ambrosia beetle that, based on morphological traits, was identified as *Euwallacea fornicatus*, the Tea Shot Hole Borer (Coleoptera: Curculionidae: Scolytinae) (TSHB). One detection occurred in Miami-Dade County in Florida in 2002, another in Los Angeles County in California in 2003, and another in Israel in 2005. Because they are morphologically indistinguishable, the three were initially thought to be the same species. But the population in Israel has caused severe damage to the avocado industry there, whereas the population in Florida has not caused as much damage. Recent molecular work has determined that the populations in Los Angeles and Israel are genetically identical, but the beetles in Miami-Dade differ genetically, as do their fungal associates, to the point that they are considered separate species. A new common name was coined for the populations in Israel and Los Angeles, the Polyphagous Shot Hole Borer (PSHB), due to the extensive list of host trees they attack and infect with their fungal associate, *Fusarium euwallaceae*. However, they have yet to be officially named as a new species.

Most of the biological differences between TSHB and PSHB are unknown, but it is important to understand if they are indeed reproductively isolated since they are now both present in the United States and there is a chance that the two populations could spread and coincide.

To determine whether they are truly two reproductively isolated species, we conducted a crossing experiment and evaluated the fecundity and survival of their hybrid offspring.

By conducting crosses between PSHB and TSHB, and evaluating the fecundity of hybrids, we tested whether these two populations of morphologically indistinguishable beetles are reproductively isolated. Because these beetles are haplo-diploid, fertilized eggs become females, and unfertilized eggs become males. We found that although they were able to hybridize, the first hybrid generation resulted in fewer female offspring and more male offspring, suggesting some degree of incompatibility, perhaps in the form of reduced mating, unsuccessful fertilization, or failed development. The hybrids that were most successful at reproducing were those that mated with their siblings, which is the normal reproductive strategy for these beetles. Subsequent generations failed to produce many offspring at all, or only females. Egg production did not decrease in hybrid generations, but numbers of larvae, pupae, and adult females dropped with every generation. By the third generation most failed to reproduce. This study confirmed that the two populations of *Euwallacea near fornicatus* in Los Angeles County, CA and Miami-Dade County, FL are reproductively isolated, and we conclude that they are, indeed, two distinct species.
THE SUITABILITY OF *SPATHIUS GALINAE* FOR BIOCONTROL INTRODUCTION AGAINST EMERALD ASH BORER: HOST SPECIFICITY TESTING AND CLIMATIC MATCHING

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Classical biological control has long been used for sustainable management of exotic arthropod pests in agricultural and forest ecosystems. Because it involves the introduction and establishment of non-native predators or parasitoids, however, potential non-target risks from this ecologically-based pest control technology need to be thoroughly assessed prior to regulatory approvals for environmental releases. In addition, climatic suitability of the receiving environment to the introduced biocontrol agent will critically affect the subsequent establishment and successful suppression of the target pest. In the present study, we evaluated the host specificity of a newly described parasitoid, *Spathius galinae*, a natural enemy of the invasive emerald ash borer (EAB), *Agrilus planipennis*, against 15 native North American wood-boring insects from seven families of three different Orders. We also conducted climatic matching analysis between the Russian Far East, where the parasitoid originated, and different regions of North America, where the parasitoids would be released. Among the 15 species of non-target wood-boring insects tested, *Spathius galinae* only attacked one species other than the EAB, and that was the gold spotted oak borer, *Agrilus auroguttatus*. The rate of parasitism was, however, considerably less (41%) on the non-target host than on the EAB (71%) under test conditions that strongly favored parasitism. *Spathius galinae* did not attack any of the other three species infesting red oak, nor did they attack any of the other *Agrilus* or any of the three non-*Agrilus* species infesting ash. This level of host specificity is quite high, and is higher than that of *S. agrili*, which was approved for release against the EAB. The climate matching analysis indicates that Northern U.S. and part of South Canada where EAB have established or have the potential to establish appear to have climatic matching index (>0.7) to the Russian Far East, where both EAB and *S. galinae* occur and have co-evolved (Fig. 1). This indicates that *S. galinae* may be better adapted for establishment in the northern U.S.

**Fig. 1** Climatic matching index between the Russian Far East and North America
INFLUENCE OF MATING AND AGE ON SUSCEPTIBILITY OF ASIAN LONGHORNED BEETLES TO A FUNGAL PATHOGEN

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ABSTRACT

Insect susceptibility to a pathogen challenge can be influenced by a variety of physiological factors, including the age of the insect and its reproductive history. Immune defense is generally considered costly and there can be tradeoffs between immunity and other physiological traits such as reproduction, development and longevity in invertebrates as well as mammals. Additionally, as insects age their ability to combat a pathogen challenge can decrease through a process referred to as immunosenescence.

The fungal pathogen *Metarhizium brunneum* Petch is being developed to control the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), an invasive wood-borer native to China and Korea that was initially detected in North America in 1996. If these invasive woodborers were to become established they have the potential to cause extensive damage to urban and natural forests in North America as well as Europe. Entomopathogens are used for biological control of a variety of pests and physiological traits can influence the efficacy of these products for control. Female *A. glabripennis* take 1-2 weeks to mature after eclosion and both sexes can be long-lived; therefore, we wanted to determine how age, maturation and mating would impact *A. glabripennis* susceptibility to *M. brunneum*.

We inoculated young (6-7 days-old) virgin, mature (27-33 days-old) mated and virgin, and older (57-71 days-old) mated and virgin adult beetles with lethal doses of *M. brunneum* and monitored them daily for death. We determined whether beetles of different ages and mating status were resisting or tolerating the pathogen by quantifying the numbers of *M. brunneum* cells in the hemolymph on days 5 and 9 after inoculation. Bioassay results indicated that young male beetles are more susceptible to *M. brunneum* infection than mature or older male virgin beetles. Young male and young female beetles were more likely to have detectable levels of fungal cells in their hemolymph on day 9 than older beetles. Additionally, our results suggest there is a cost to reproduction, at least for mature beetles of both sexes, which died from *M. brunneum* infections more quickly than older beetles or unmated mature beetles. However, mated beetles were just as likely to have detectable levels of fungal cells in their hemolymph compared with unmated mature and old beetles of both sexes.
IMPROVING DETECTION TOOLS FOR EAB: EFFECT OF HOST, AGE OF TRAPS AND KILLING AGENT

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ABSTRACT

Green multifunnel traps, coated with fluon, a fluoropolymer, had been found to be comparable to or better than purple prism traps in terms of both trap catch and detection, the ability of a trap to catch at least 1 beetle. As part of an ongoing project to improve survey tools for the emerald ash borer (EAB), several field assays were conducted in high EAB population density sites in southeastern Michigan, as well as in low EAB population sites along the edge of the currently known infestation.

Multifunnel traps, while more expensive than prism traps are designed to be re-used from one year to the next. However, it is unknown whether the green pigment in the trap could fade over time, diminishing the trap’s attractiveness to EAB. Also, since fluon is applied externally to the traps, the slick surface of the trap could also diminish. In a multifunnel trap durability / aging assay, we compared new traps (2014) with traps that had been placed in the field for one (2013), two (2012) or three field seasons (2011). Traps placed in the field since 2011 and 2012 caught significantly few EAB than traps placed in the field in 2013 and 2014. One reason for this may have been a switch-over from fluoning traps by hand using a sponge (which can lead to an uneven coating) to having the manufacturer pre-fluon traps (using a dipping method) prior to shipment. We will continue this project in 2015.

In 2013 and 2014, as part of an ongoing detection tools comparison conducted along the edges of the currently known EAB infestation a trapping assay was conducted to compare trap catch and detection rates of EAB with four trap / lure combinations on ash as well as several non-hosts. Trapping on non-host was conducted to answer two questions that have been raised about surveying for EAB: 1) if an ash tree is not readily available to surveyors does placing the trap on another species affect trap catch and detection rates and 2) can these traps that have been developed for EAB be used for surveying other woodborers (ie. in a CAPS survey). The four traps were 1) an unbaited purple prism trap, 2) an unbaited purple multifunnel trap, 3) an unbaited green multifunnel trap, and 4) a green multifunnel trap baited with a 3Z-hexenol lure (50 mg/d release rate). In 2013 traps were placed on four tree genera: 1) Fraxinus, 2) Acer, 3) Pinus and 4) Quercus (red oak group only). In 2014, five tree groups were compared: 1) Fraxinus, 2) Betula, 3) Populus, 4) Quercus (red oaks) and 5) Quercus (white oaks).

Overall, baited green multifunnel traps had higher detection rates than unbaited purple multifunnel traps but were comparable to unbaited green multifunnel and purple prism traps. In 2013, of the sixteen replicates of traps placed in maple, red oak and pine, seven, eight and eight did not have a single detection recorded among any of the traps, compared to 15 of 16 ash replicates (in the same areas) recording at least one positive catch. The 2014 EAB and all non-target catch data from 2013 and 2014 data is still being summarized and analysis will begin soon.
Besides being conducted in 5 states in 2013 and 8 states in 2014, this comparisons were also conducted in China, Russia and Poland (with adjustments made for locally available tree genera). In 2013, 3717 buprestids in 17 species (including 12 *Agrilus* species) and 60 cerambycids in 11 species were caught in Poland.
WALNUT ALERT: EXPANSION OF A REGIONAL OUTREACH PLAN TO SLOW THE MOVEMENT OF THOUSAND CANKERS DISEASE

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ABSTRACT

Black walnut (Juglans nigra), which is native to the eastern United States, is grown for its nuts and for its high quality wood. In 2010, a new threat to black walnut was discovered in its native range – this threat is known as thousand cankers disease (TCD). This disease is caused primarily by a fungal pathogen, Geosmithia morbida (Ascomycota: Hypocreales: Bionectriaceae), which is vectored by the walnut twig beetle, Pityophthorus juglandis (Coleoptera: Curculionidae: Scolytinae). TCD has caused branch dieback and death of thousands of trees in the western United States (Colorado, Idaho, New Mexico, Oregon, Utah, Washington, etc.), where it is grown as an ornamental or nut tree outside its native range. Its discovery in the native range of black walnut has caused concerns to the wood products industry, wood carvers, nut producers, landowners, and consumers. Tree decline and mortality also have been observed in the native range of black walnut. Because the industry and the general public are not aware of this disease, its symptoms, its management, or the consequences of its presence, a Regional Outreach Plan – WALNUT ALERT – was initiated in 2013 to improve awareness and knowledge about TCD. This Plan was expanded in 2014 to enhance efforts to develop and distribute educational materials related to TCD in the native range of black walnut, as well as to train students to be more knowledgeable in the workforce. This knowledge expansion through outreach materials and education is essential to better limit the spread and impact of TCD and to protect and preserve black walnuts. For more information or to access outreach materials, please visit www.tcdgroundzero.com.
ARTIFICIAL DIET FOR ADULTS OF THE NON-NATIVE WOOD-BORING BEETLE ANOPLOPHORA GLABRIPENNIS (CERAMBYCIDAЕ)

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ABSTRACT

Little is known about sustaining adult twig-feeding cerambycids on artificial diet in the laboratory. Gardiner (1970) observed adults of one Lamiinae species feeding and ovipositing in blocks of larval diet, but gave no further details. Our objective was to formulate a semi-artificial diet for twig-feeding ALB (Anoplophora glabripennis) adults that provides essential nutrients for normal adult life expectancy, mating behavior, and fecundity.

Peeled maple bark (Acer pensylvanicum L.) was frozen and lyophilized before being ground into powder for inclusion in the diet. Fresh or lyophilized bark was also used to make a crude water extract. Our starting diet was based on the larval diet reported by Gardiner (1970) as a putative food source for adult cerambycids. The crude water extract of maple bark as well as pure maple syrup were added due to previous observations of marked increase in feeding by adult ALB on non-host material when they were applied. The diet was dispensed into plastic cylindrical molds (12 X 100 mm) to approximate the shape of a twig.

Ten teneral adults of each sex were randomly assigned to treatment or control. Mating pairs were held in environmental chambers at a constant 25 ±5⁰C and 65 ±10% RH during the course of the experiment. The light:dark cycle was 16:8 to simulate summer months when ALB is most active. Twigs were replaced every 4–7 days and the artificial diet every 2–3 days. Eggs were collected 1–2 times a week for the duration of the trial.

Mated female beetles offered striped maple twigs cumulatively laid 315 eggs over a 2-month period compared with 355 eggs laid by females offered semi-artificial diet. The average oviposition rate by twig-fed females (63.00 ± 17.85, n=5) was not statistically different from the rate of females fed semi-artificial adult diet (71.00 ± 28.79, n=5) (t=0.55, d.f.= 8; p=0.5969). Egg hatch rate of females fed twigs did not significantly differ from females fed semi-artificial diet. We anticipate this artificial diet will have applications for other adult twig-feeding beetles beyond ALB, especially within the cerambycid subfamily Lamiinae.

ANCIENT AND MODERN COLONIZATION BY HEMLOCK ADELGIDS

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ABSTRACT

Most importations of non-native insects do not result in successful establishment. Beyond the impacts of climate, habitat suitability, and propagule pressure, there may be population level characteristics that make a species more likely to invade. These include high genetic diversity, large geographic range, broad host range, and asexual reproduction, but there is little consensus about which of these is important predictors of invasion success. For this reason, details about invasive species’ historical biogeography, genetic differentiation, diversity, and life history in their native and introduced ranges are valuable; not only for managing a particular pest, but for understanding what makes a species a successful invader.

We used microsatellite markers and mitochondrial DNA sequences to examine genetic variation of hemlock adelgids from throughout their native and introduced ranges. With a more extensive sample of adelgid specimens and higher resolution markers than previous studies, this study aims to provide a more comprehensive look at the historical biogeography and introduction history of this invasive species.

Genetic clustering analyses recovered four major groups that are consistent with previous studies: one in China, two in Japan associated with different hemlock species, and one in western North America, and suggest that additional lineages might exist in Taiwan, Ulleung Island, Korea, and in eastern China. Some populations in China and Japan are holocyclic, with host alternation, as evidenced by similar adelgid genotypes on hemlock and spruce, and with sexual reproduction, as evidenced by linkage equilibrium.

Adelgids in eastern North America are members of a single clonal lineage that originated from southern Japanese hemlock (Tsuga sieboldii) and tigertail spruce (Picea torano) in southern Japan. They all share the exact same genotype, except for some individuals in Massachusetts that differ by just a single microsatellite mutation. Adelgids in western North America are distinct from other sampled populations, are more diverse than those found in eastern North America, and have very high observed heterozygosity, a signature of ancient asexuality. This all suggests that there is a separate anholocyclic lineage endemic to that region. Reconstruction of the biogeographic history of the Western lineage suggests a complex history of ancient colonization between Asia and western North America across the Beringian Corridor.
EFFECTS OF LIGHT AND WATER AVAILABILITY ON THE PERFORMANCE OF HEMLOCK WOOLLY ADELGID (ADELGES TSUGAE)

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ABSTRACT

Eastern hemlock (Tsuga canadensis [L.] Carriere) is a dominant shade-tolerant tree in northeastern United States that has been declining since the arrival of the hemlock woolly adelgid (Adelges tsugae Annand). Determining where A. tsugae settles under different abiotic conditions is important in understanding the insect’s expansion. Resource availability such as light and water can affect herbivore selectivity and damage. We examined how A. tsugae settlement and survival were affected by differences in light intensity and water availability, and how adelgid affected tree performance growing in these different abiotic treatments. In a greenhouse at the University of Rhode Island, we conducted an experiment in which the factors light (full-sun, shaded), water (water-stressed, watered), and adelgid (infested, insect-free) were fully crossed for a total of eight treatments (20 two-year-old hemlock saplings per treatment). We measured photosynthesis, transpiration, water potential, relative water content, adelgid density and survival throughout the experiment. Adelgid settlement was higher on the old-growth foliage of shaded and water-stressed trees, but their survival was not altered by foliage age or either abiotic factor. The trees responded more to the light treatments than the water treatments. Light treatments caused a difference in relative water content, photosynthetic rate, transpiration and water potential, however, water availability did not alter these variables. Adelgid did not enhance the impact of these abiotic treatments. Further studies are needed to get a better understanding of how these abiotic factors impact adelgid densities and tree health, and to determine why adelgid settlement was higher in the shaded treatments.
BEECH SCALE IN PART OF ITS NATIVE RANGE (CAUCASUS MTS., GEORGIA) AND IN AN INVATED AREA (MASSACHUSETTS, USA)

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ABSTRACT

The Caucasus Mountains in the country of Georgia are part of the native range of beech scale (Cryptococcus fagisuga) and Massachusetts (United States) is part of the invaded range of this species. As background to determine if the native range of this scale might be a source of natural enemies useful for correcting the ecological damage caused by beech scale in North America to American beech (Fagus grandifolia) comparative scale densities were measured in both locations in natural forest stands of F. grandifolia in Massachusetts and F. orientalis in Georgia. Scale densities were found to be 45.4-fold higher per unit area of bark in Massachusetts on F. grandifolia than in the country of Georgia on orientalis. Also, F. orientalis trees at sample sites in Georgia were 2.9-fold larger in DBH and much healthier that were F. grandifolia trees in Massachusetts. Beech scale, Cryptococcus fagisuga (Hemiptera: Eriococcidae), phenology and survival were investigated in part of its native range (Caucasus Mts., Georgia) and in an invaded area (Massachusetts, USA). The scale was found to be bivoltine in the Caucasus Mt. region but univoltine in Massachusetts, despite nearly identical growing seasons (as measured by cumulative day degrees). In Georgia, the scale overwintered as adults, while in Massachusetts, settled first instar crawlers overwintered. In both Georgia and Massachusetts, protective cages increased scale survival over a year-long period. However, the effect was not large and was attributed to generalist predators, not specialized natural enemies. No support was found for the hypothesis that specialized natural enemies might exist in the scale’s native range that might be imported for biological control of the pest in the United States. Rather it is recommended that efforts to select for resistance in American beech lines offer the best chance to restore healthy stands of American beech in North American forests.
ASSESSMENT OF WALNUT TWIG BEETLE
(PITYOPHTHORUS JUGLANDIS) COLONIZATION OF TREATED
WALNUT LOGS AND NURSERY STOCK SAPLINGS

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ABSTRACT

Eastern black walnut (Juglans nigra L.) is currently threatened by thousand cankers disease. The disease is caused by an invasive insect-pathogen complex comprising the walnut twig beetle (Pityophthorus juglandis Blackman) and the associated fungal pathogen Geosmithia morbida. In recent decades, the beetle and pathogen’s range has greatly expanded likely owing to the transport of infested walnut wood. Understanding potential anthropogenic pathways of further introduction and spread of this beetle and pathogen is crucial to the effective management of thousand cankers disease. This study assessed the beetle’s colonization habits in treated J. nigra logs and in young, small diameter J. nigra nursery stock. Pityophthorus juglandis were reared from steam heated and methyl bromide fumigated logs that had been baited with a pheromone lure and hung in actively infested walnuts in eastern Tennessee. Reproduction appears to have taken place within the treated material. Surprisingly, beetles were also recovered from kiln-dried lumber samples with bark intact, however, there was no evidence of successful reproduction in the material. Adult P. juglandis attacked nursery saplings when caged directly onto stems. Trees from four basal diameter classes ranging from 0.5 to 2.0 cm were attacked, however, beetles displayed a preference for larger diameter stems. Based on this study, steam heated and methyl bromide fumigated logs should not be exposed to P. juglandis following treatment to ensure no beetles are transported on walnut logs. These data also support quarantine restrictions on walnut nursery stock. Nursery saplings are a viable resource for sheltering beetles and present a threat to the nursery industry.
NEW DEVELOPMENTS WITH EMERALD ASH BORER PARASITOIDS

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ABSTRACT

To date, three species of parasitoids have been released in North America for classical biological control of emerald ash borer (EAB), Agrilus planipennis Fairmaire. This includes one egg parasitoid (Oobius agrili Zhang and Huang), and two larval parasitoids (Spathius agrili Yang, and Tetrastichus planipennisi Yang). Here we provide a brief overview of the release and recovery of these three parasitoids in North America, as well as an assessment of their impact on EAB populations. We also present an update on the outcome of recent foreign exploration for potential new biological control agents of EAB.

The numbers of O. agrili and T. planipennisi released have generally increased each year since the beginning of the EAB Biocontrol Program in 2007, with over 500,000 T. planipennisi being released in 2014 alone. Through 2013, parasitoid recovery efforts had been conducted in 12 states, with over 640 samples (e.g., debarked trees, deployment of yellow pan traps and sentinel logs) collected since 2008. Of these samples, 88 have been gathered from release sites with a further 27 from control sites where no parasitoid releases occurred. Tetrastichus planipennisi has been recovered from eight states, and it has established (i.e., been recovered for two or more years since a release occurred) populations at 13 sites. Oobius agrili has been recovered from six states, and has established at eight sites. In contrast, while S. agrili has been recovered from six states (indicating that this species can successfully overwinter in North America), it appears to only have established at one site. For this reason, the EAB Biocontrol Program decided to restrict future releases of S. agrili to sites below the 40th parallel.

We utilized a life-table approach to assess the impact of larval parasitoids on the growth rate of EAB populations in the field. Stage-specific life-tables were constructed for EAB in Michigan from 2008-2014 (Duan et al., 2014), and in Maryland from 2012-2014. In Michigan, this involved fully debarking 4-6 wild EAB infested trees at each of six sites every fall, and recording the developmental stage and fate (depredated, diseased, killed by tree resistance, parasitized, alive, or exited as an adult) of each EAB individual. We also estimated the phloem density of each tree, which allowed us to estimate EAB population size while controlling for differences in tree area. The life-table analyses in Michigan found that predation and parasitism were the two most important mortality factors for large EAB larvae. Additionally, we found that populations of T. planipennisi had increased over time. This increase in T. planipennisi population size coincided with a reduced number of live EAB larvae found and consequent reduced population growth rate of EAB.
In Maryland, we created experimental cohorts of EAB by attaching 30 eggs to each of five trees at 12 sites in the summer of successive years (2012 and 2013). The following spring after each cohort had been created, we debarked trees to determine the fates of the experimental cohort. We found that larval fate was dependent on the crown condition of host trees and developmental stage of EAB larvae. Tree resistance was the main source of mortality for larvae on healthy trees, but as tree crown condition deteriorated, predation and parasitism became more important. Further, tree resistance generally killed early instars of EAB larvae, but predation and parasitism accounted for a higher proportion of mortality for mature larvae and pupae.

Foreign exploration for new EAB parasitoids from 2008-2012 resulted in the discovery of *Spathius galinae* Belokobylskij & Strazanac around Vladivostok, Russia and an additional un-described species of egg parasitoid (*Oobius* sp., n.). Climate matching models have shown that the climate of Vladivostok is more comparable to the northern US states and Canada than Tianjin, China, where *S. agrili* populations used in the EAB Biocontrol Program originated. Host range testing has shown that the host specificity of *S. galinae* is restricted within the host genus (*Agrilus*) level, and extensive laboratory experiments have found that *S. galinae* also appears to be able to coexist with *T. planipennisi*. Thus, the risks from *S. galinae* to native non-target hosts and existing biological control agents appear to be minimal. Presently, *S. galinae* is undergoing final regulatory approval and may be ready for field releases in the summer of 2015.

**REFERENCES**

SILVICULTURAL AND INTEGRATED MANAGEMENT STRATEGIES FOR
RESTORING HEMLOCK TO DEGRADED
SOUTHERN APPALACHIAN FORESTS – PHASE 1

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ABSTRACT

The ecologically foundational species eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *Tsuga caroliniana* Engelm., are being functionally eliminated from southern Appalachian forests by the hemlock woolly adelgid, *Adelges tsugae* Annand (HWA). HWA management has focused on chemical and biological control, conservation of hemlock genetic resources, and host resistance breeding, however research on the reintroduction of hemlocks to forests where it has been lost has received almost no attention. This poster presents progress made on Phase 1 of a three phase project to develop a hemlock restoration strategy that integrates silvicultural prescriptions with biological and chemical controls for the reintroduction of eastern and Carolina hemlock to southern Appalachian forests. Phase 1 is testing the effects of canopy structure (thinned versus canopy gap), deer exclusion, fertilization, and weed control on the establishment, survival, and growth of planted eastern hemlock seedlings receiving insecticide protection. A total of 12 research plots (each measuring 18x30 m) were established at two sites in western North Carolina (Cold Mountain Game Land and DuPont State Recreational Forest, 6 plots per site) in stands dominated by dead and dying eastern hemlock. Plots were established as pairs with one serving as a “high” light canopy treatment (clearcut/canopy gap) and the other as a “low” light canopy treatment (thinned from below to ~120 ft²/ac). Each plot is divided into two 10x10 m subplots with one surrounded by at 2.5 m high deer exclusion fence and the other remaining open (no fence). Each subplot contains four 5x5 m treatment plots, each with 16 eastern hemlock seedlings (2-3 bare-root transplants; 18-24 in tall) planted at a 1x1 m spacing. The four treatments are weed control, fertilization, weed control + fertilization, and no treatment (control). Seedling health, height, and basal diameter will be measured November-February of each year post establishment. During fall 2014 the research plot boundaries were established, existing woody canopy and regeneration vegetation was inventoried, overstory treatments completed, and seedlings planted. Initial post-planting seedling measurements, insecticide protection treatments, and construction of deer exclusion fences are being completed during winter 2015. Initial weed control and fertilization treatments will be applied during spring and early summer 2015. This research represents the first step towards attempting to restore eastern hemlock to southern Appalachian ecosystems where it has been eliminated.
PHENOLOGY/HOST BASED LANDSCAPE RISK FOR THE ASIAN LONGHORNED BEETLE IN THE CONTERMINOUS U.S.

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\textbf{ABSTRACT}

The Asian longhorned beetle (\textit{Anoplophora glabripennis} Motschulsky) is a wood boring Cerambycid native to East Asia. International movement of the beetle via transport of infested solid wood packing material has resulted in the establishment of populations in Canada, France, Germany, Italy, Austria, The United Kingdom, The Netherlands, and the United States. The beetle is known to feed on at least 43 tree species in 15 genera, including \textit{Acer}, \textit{Populus}, \textit{Salix}, and \textit{Ulmus}. The beetle’s broad host range and potential for transport through trade places large portions of the landscape at risk.

The risks associated with invasive species can be placed into four phases of invasion; transport, establishment, spread, and impact. Previous work has focused on transport, establishment, and spread, and so we focus here on the potential for impact. In the absence of co-evolved natural enemies and host resistance, the potential for impact is largely mediated by the availability of host trees, and the potential for rapid population growth. Here, we calculated the estimated generation time (a corollary of population growth rate) of ALB at a landscape scale based on a phenology model (ALBV). Inputs to the phenology model included physiological thermal requirements for 14 life-stages of the ALB, PRISM derived temperature data for the continental U.S., mediation of ambient temperatures by wood in the host trees, represented with a Newtonian Cooling Model, and FIA data for each of the 43 species and 15 genera known to host ALB.

Compilation of these parameters in a Geographic Information System (ArcGIS V10) results in risk maps denoting the presence and abundance of host material, in combination with the generation time for ALB. Generally, generation time decreased along a north-to-south gradient, as would be expected with decreasing temperatures. Generation times in Florida were less than one year, while to the north, generation times in Maine were as long as 4 years. Host abundance is high in much of the continental United States, with high concentrations east of the Mississippi. Host abundance also follows general north/south gradient, with host abundance higher in the north. The alternating patterns of generation time and host abundance along the latitudinal gradient may produce some landscape-scale balancing of risk factors, though generally risk may be highest in the mid-Atlantic states.
TRACKING REPEATED INTRODUCTIONS OF AN INVASIVE SPECIES: STABLE ISOTOPE EQUILIBRATION IN JAPANESE BEETLES ("POPILLIA JAPONICA") NEWMAN IN OREGON

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ABSTRACT

The Japanese beetle ("Popillia japonica" Newman) is an invasive non-indigenous species that is well established in the eastern US, but has not yet become established in the western US. The Oregon Department of Agriculture has been monitoring cargo flights coming into Oregon from states regulated for the Japanese beetle since 1991. Japanese beetles have been caught in traps at PDX almost every year for more than a decade. Trapping density and treatments to eradicate the insect have increased over the same time period.

Isotope composition of animals reflects their natural history—what they eat, what they drink, and where they travel. Isotope signatures can determine whether an individual is a recent arrival, with an isotope signature of its region of origin, or whether it represents an established population isotopically equilibrated with a new environment. The Japanese beetle presents a case study for tracking movement using stable isotope analysis.

Hydrogen isotope signatures of beetles captured at the Portland International Airport (PDX) reflect eastern US source populations, providing evidence that trapped individuals are new arrivals and not yet established locally. Stable isotopes of hydrogen (δ²H) in beetles shipped from Virginia equilibrate with hydrogen isotope signatures of food and water in Oregon within a five-week period. In a quarantine facility, newly arrived (24 hours) Virginia beetles had significantly higher δ²H values (X = -72.0) than beetles nourished in Oregon for five weeks (X = -144.1). These results provide evidence that Japanese beetles caught at PDX early in the summer months are originating in the eastern US. The δ²H isotope signatures of captured individuals each year verify that intense trapping and control measures are preventing the establishment of a nascent population in Oregon. Thus, stable isotopes offer a powerful tool for assessing the success or failure of management decisions concerning invasive species.
DIFFERENCES IN WING MORPHOMETRICS OF *LYMANTRIA DISPAR* BETWEEN POPULATIONS THAT VARY IN FEMALE FLIGHT CAPABILITY

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**ABSTRACT**

All male gypsy moths, *Lymantria dispar* L., are capable of strong directed flight, but flight in females varies, increasing from west to east geographically across Eurasia. To better understand how the wings differ between female flighted/flightless strains, a wing morphometric analysis of 821 gypsy moths from eight geographic strains (three *L. dispar dispar* L., four *L. dispar asiatica* Vnukovskij, one *L. dispar japonica* Motschulsky) was performed. Body mass, length and width of both fore- and hindwing, wing area, wing aspect and wing loads were measured on both sexes from each strain.

Gypsy moths are sexually dimorphic; females had a higher wing load, larger aspect ratios, bigger wing area, and heavier body mass than males. Wing loads of females, but not males, differed significantly among geographic strains. Wing area varied more than wing aspect in females among the strains, which indicates area and aspect evolved independently. Female fore- and hindwing area are both larger in strains for females with strong directed flight capabilities versus those with no female flight, suggesting both fore- and hindwing areas play significant roles in flight. The relationship between wing area and body mass of females was predictive of their flight capability, but the same relationship in males is not related to female flight capability. The relationship between the total wing area and the body mass may be useful in predicting the flight capability of trapped female moths.

Previous studies demonstrated that flight capability of female gypsy moth has a heritability of 0.60 and forewing length has a heritability of 0.70, both with no evidence of sex linkage or maternal effects. This would suggest that the genes for critical flight characteristics such as wing length are most likely located on the autosomes rather than mitochondrial DNA or the sex chromosomes. Male moths, all of which can fly, didn’t separate into groups based on the female flight capability of the strains suggesting that there has to be one or more genes on the W chromosome (females are the heterogenetic sex in Lepidoptera) that directly regulate flight genes in females or indirectly regulate it by coding for sexually dimorphic body masses. Further research to identify the genes involved in flight is needed to determine exactly how flight is regulated in the two sexes.
EFFECTS OF FOREST DISTURBANCE ON GROUND-DWELLING INvertebrate Dispersal

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ABSTRACT

Traits of species, such as dispersal ability of ground-dwelling invertebrates, influence their response to forest disturbance. The objective of this study was to quantify dispersal of forest floor invertebrates in response to a factorial combination of two disturbance treatments: canopy gap formation and understory vegetation removal. Invertebrate dispersal was quantified using a mark-capture technique where fluorescent powder was applied to the forest floor in three concentric bands differing in distance from plot center. Powder was detected on 18% of invertebrates collected, suggesting limited dispersal of most individuals. Only 1% crossed two bands, and these were dominated by active hunters such as harvestmen and ground beetles. A greater proportion of harvestmen crossed two bands when vegetation was undisturbed, while more spiders crossed one band in canopy gaps. Low dispersal may slow recolonization of ground-dwelling invertebrates following disturbance, thereby affecting community structure ecosystem services.
INVESTIGATING THE FLIGHT CAPABILITIES OF ASIAN LONGHORNED BEETLE, *ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE), WITH COMPUTERIZED FLIGHT MILLS

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ABSTRACT

Flight mill studies were conducted to assess the dispersal capabilities of Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky), adults under different age, mating, and nutritional status. In total, 76 individuals were tethered to computerized flight mills for a 24 hour trial period to collect information on total distances flown, flight times and velocities, and number and duration of flight bouts. The initial results of this study show that ALB adults fly an average of 2 miles within a 24 hour period, but are capable of flying up to 8.5 miles. This information may be useful for defining quarantine zones surrounding areas of infestation. Results of this study (scheduled for completion in 2015) can also help to determine the potential environmental and economic risk of ALB within the invaded regions.
ECOLOGY OF COGONGRASS IN THE SOUTHEASTERN US: WHERE ARE THE GAPS IN OUR UNDERSTANDING?

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ABSTRACT

Cogongrass (Imperata cylindrica) is a highly invasive, warm-season, rhizomatous grass problematic throughout the southeastern US. Cogongrass is considered one of world’s worst weeds, and it detrimentally affects natural and managed forest systems throughout its invasive range. Here, we present a summary of some key findings from our work on the biology of this noteworthy invasion and identify areas where further study may improve management efforts.

This species was reported to have been imported twice from Asia into the Southeast, and results from our genetic analyses are consistent with that assertion. Further, our ecological studies and additional genetic analyses also suggest anthropogenic vectoring as a primary driver of spread in the region. Landscape-scale analyses have shown cogongrass to be very closely associated with roads (as potential dispersal corridors) and indicate some degree of plasticity in this species’ responses to local conditions. In keeping with the phenotypic variation observed, patterns of genetic diversity across the Southeast suggested high genetic diversity and that sexual reproduction occurs more frequently than previously assumed.

Analysis focused on interspecific hybridization with Brazilian satintail (Imperata brasiliensis Trin.) in Florida, sought to confirm if hybridization was an important invasion facilitator throughout the region. However, findings failed to support the existence of a separate congeneric species or of ‘hybrid swarms’ in that state. Broader scale genetic analyses in other southeastern states continued to show consistent patterns of two introductions of distinct exotic material into the US, which appears to have expanded from the sites of initial introduction in Mississippi and Alabama into Florida, Georgia, Louisiana, South Carolina and Texas.

Most cogongrass management relies primarily on repeated chemical treatments of glyphosate and imazapry. Due to the lack of effective management options, research specifically aimed at differential responses of cogongrass populations and/or genotypes to repeated chemical treatment(s) would identify whether herbicide resilience (or resistance) is present, or may develop over time. Additional research can provide important insights into the genetically driven aspects of cogongrass biology and management.
EFFECTS OF ETHANOL AND CONOPHTHORIN RELEASE RATES ON CATCHES OF AMBROSIA BEETLES

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ABSTRACT

To date, >20 species of ambrosia beetles have invaded the USA with several species such as *Xylosandrus crassiusculus* (Motschulsky) and *X. germanus* (Blandford) causing economic damage in fruit orchards and horticultural nurseries. Traps baited with ethanol lures are used in detection programs for bark and wood boring beetles. Tests of conophthorin with ethanol-baited traps have yielded mixed results. One issue is a difference in release rates (RR) for both ethanol and conophthorin from release devices among studies. Our goal was to determine the effect of ethanol and conophthorin RR on catches of three common species (*X. crassiusculus*, *X. germanus* and *Xyleborinus saxesenii*). In 2014, we conducted the same experiment at 6 locations across the USA: (1) Greene Co., Georgia (24 April–24 June); (2) Tippecanoe Co., Indiana (6 May–30 June); (3) Oakland Co., Michigan (9 July – 19 September); (4) Wayne Co., Ohio (15 April–10 June); (5) Clackamas Co., Oregon (15 May – 10 July); and (6) Virginia Beach, Virginia (2 April–28 May). Multiple funnel traps (ConTech Inc., Victoria BC) were used in Georgia, Michigan and Oregon whereas bottle traps were used in Indiana, Ohio and Virginia. AgBio Inc. (Westminster CO) supplied low RR ethanol lures (Lo E) whereas conophthorin and high RR ethanol lures (Hi E) were obtained from ConTech. The RR of ethanol were about 0.065 and 0.25 g/d for Lo E and Hi E devices, respectively, whereas conophthorin was released at about 0.25 mg/d (Lo C). A high RR treatment of 0.75 mg/d (Hi C) for conophthorin was obtained by combining three conophthorin lures in a single trap. We used 10-unit multiple funnel traps in Georgia and Oregon, and 12-unit traps in Michigan. Funnel traps were modified by increasing the center hole of each funnel from 5 cm to 12 cm, allowing placement of all lures within the trap. At each location, we deployed 40 traps, set in ten replicate blocks of four traps per block. Traps were spaced > 8m apart. All cups contained solution of propylene glycol. One of the following treatments was allocated to a trap within each block: (1) Lo E + Lo C; (2) Lo E + Hi C; (3) Hi E + Lo C; and (4) Hi E + Hi C. We obtained larger catches of: (1) *X. germanus* with high ethanol RR at 2 of 4 locations; (2) *X. saxesenii* with high ethanol RR at 3 of 5 locations; and (3) *X. crassiusculus* with high ethanol RR at 2 of 3 locations. The effects of conophthorin release rate are unclear.
IPSENOL, MONOCHAMOL AND α-PINENE: TRAP LURE BLEND FOR MONOCHAMUS SPECIES (CERAMBYCIDAЕ) IN CANADA AND USA

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ABSTRACT

Sawyer beetles, Monochamus spp. (Coleoptera: Cerambycidae) are broadly attracted to volatiles released from pine trees as well as pheromones of Ips bark beetles associated with the same hosts. The generic Monochamus pheromone, “monochamol”, is attractive to several species of Monochamus in eastern USA, particularly when combined with α-pinene. Our objective was to determine the interaction between ipsenol and monochamol on attraction of Monochamus species to traps baited with α-pinene. We compared mean catch of Monochamus spp. in multiple funnel traps baited with four different lure treatments: 1) α-pinene alone; 2) α-pinene + ipsenol; 3) α-pinene + monochamol; and 4) all three compounds. The experiment was replicated at 11 locations across Canada and the USA in 2012-2014. Seven different Monochamus species were trapped: M. carolinensis, M. clamator, M. mutator, M. notatus, M. obtusus, M. s. scutellatus, and M. titillator complex. Traps baited with the combination of α-pinene + ipsenol + monochamol captured the most beetles in 16 of 19 site-years, with significantly greatest mean catch in 11/19 site-years. Results suggest this triple-lure combination would be very effective for surveillance of North American Monochamus species.
HOST-PLANT FEEDING PREFERENCE OF THE INVASIVE BEECH LEAF-MINING WEEVIL IN NOVA SCOTIA

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ABSTRACT

The introduction of alien pest insects can have substantial impacts on indigenous ecosystems. One of the key components driving the success of their establishment is host suitability within the invaded range. For example, the establishment of the exotic beech leaf-mining weevil, *Orchestes fagi* L., on American beech, *Fagus grandifolia* (Ehrh.), in Nova Scotia is likely due in part to its use of beech, *Fagus sylvatica* L., as a host throughout its native European range. However, evidence that adult *O. fagi* feed on several additional plants in Europe suggests that its North American host range may extend beyond a single species. We employed a combination of observational studies and no-choice feeding assays to assess adult feeding damage on a variety of potential hosts (including beech) within its introduced range.

The beech leaf-mining weevil is univoltine. Adults overwinter and emerge in early spring to feed and oviposit on developing beech leaves as soon as buds burst. Larvae create a narrow mine from the mid-rib to the leaf margin undergo three instars, and pupate within the leaf mine. Development from egg to adult takes 30–35 days with the new generation of adults emerging in mid- to late June. In Europe, the newly emerged adults are reported to feed on foliage before seeking overwintering sites between late July and September.

Contrary to our prediction, and to reports of its behavior in Europe, adult beech weevil feeding damage was almost entirely exclusive to beech, a trend that was consistent across both observational and manipulative feeding assays. Moreover, adults that emerged in the summer (i.e., the next generation that had not yet overwintered) appeared to consume no plant material at all which is likely to have important implications for life history dynamics. Overall, we anticipate that this restricted feeding breadth is likely to result from either nutritional inequality or host phenological asynchrony with indigenous plants other than beech. Although weevils have been observed to use secondary hosts in other capacities such as overwintering sites, its complete dependence on beech as a food resource suggests that impacts on secondary hosts will be extremely minimal.
WHAT ARE WE LEARNING FROM IDENTIFICATION OF LARVAE INTERCEPTED IN WOOD PACKING MATERIAL? 
THE CASE OF AN EMERGING PEST, TRICHOFERUS CAMPESTRIS

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ABSTRACT

Identification of live organisms intercepted at ports elucidates pathways of pest entry, risk of pest establishment, and regulatory failure. An effort begun in 2012 seeks to identify live cerambycids and buprestids intercepted in wood packing materials (WPM) at eight U.S. ports. Live interceptions occur despite phytosanitary regulations for wood before export. The beetles typically arrive as larvae, which are difficult to identify by morphology. The study aims to rear larvae to the adult stage, identify specimens by morphology, and couple identification with a genetic barcode sequence. Reliance on rearing will decrease as the sequence library expands. Among species identified through the study, velvet longhorned beetle (VLB) (Trichoferus campestris [Faldermann]) was intercepted nine times in WPM associated with cargo from China. Populations of this emerging orchard and timber pest recently established in Illinois and Utah, and individuals were detected in nine other states over the past decade. In three of the nine interceptions, cargo with VLB-infested WPM was to be delivered to counties where VLB is established, providing strong evidence for WPM as the pathway of introduction and suggesting a history of regulatory non-compliance by trade partners. Likewise, of the species identified in the study, four of the seven most frequently intercepted are already established in the U.S. Not surprisingly, the data indicate an elevated risk of introduction in areas intended as destinations of infested WPM. The data can inform stakeholders about species at high risk of establishment, areas at high risk of introductions, and high risk trade partners.
PRESERVING ASH REGENERATION AND STABLE AGE STRUCTURE
BY PROTECTING MATURE ASH DURING
THE EMERALD ASH BORER INVASION

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ABSTRACT

Wide-scale ash mortality by emerald ash borer (EAB) (*Agrilus planipennis*) has stopped ash reproduction and new regeneration in southeast Michigan (invasion epicenter), creating an unstable population structure that threatens the persistence of ash in this area. If the same trend occurs as EAB continues to spread, it could functionally extirpate susceptible species range-wide, creating a need to preserve the ash gene pool. Systemic insecticides are highly effective at protecting individual ash trees from EAB and treatment models suggest that treating smaller proportions of ash trees in forests (i.e., 10-20%) can be a viable strategy for conserving the ash populations. In an attempt to implement this strategy, the Five River MetroParks (FRMP) in Dayton, OH, initiated an ongoing program in 2011 to protect 600 mature (reproductive) ash trees with emamectin benzoate every 2 years. Our objectives were to determine whether insecticides can effectively preserve ash reproduction, regeneration, and stable population structure over the course of the EAB invasion. We hypothesize that treating larger proportions of ash trees will maintain a larger mating population than treating smaller clusters, thus preserving reproduction, new regeneration, and stable population structures.

In 2014, we established 24-one hectare quadrats across gradients of treatment intensity (density of treated ash; high, medium, low, or untreated) and EAB impact (percent ash mortality; low, moderate, or severe). Within each quadrat, we assessed canopy decline (scale of 1-5; 1 = healthy, 2-4 = progressive degrees of canopy thinning, 5 = dead) of treated and untreated ash trees, and densities of new (first year seedlings with cotyledons) and established seedlings (<1.5 m tall, without cotyledons) to determine the effects of both EAB impact (EAB-induced ash decline) and treatment intensity on these variables.

Overall, treated ash were healthier (lower canopy rating) than untreated ash, suggesting insecticides are providing direct protection to treated trees. Untreated ash trees became significantly more unhealthy (*p*<0.0001) from low to severe EAB impacts, whereas treated ash canopy rating did not change. Furthermore, new seedling density decreased (*p*<0.003) from low to severe EAB impacts, but established seedling density did not change. However, we found no relationship between treatment intensity and either ash health (treated or untreated) or seedling density (new and established seedlings). We will continue to monitor ash demography over time. We anticipate that as mortality of untreated trees increase and treated trees become an increasingly larger proportion of the surviving ash population, the treatment effects will intensify and the spatial relationship between treated trees and density of ash seedlings will strengthen.
STUDIES ON THE EUROPEAN OAK BORER
AND OTHER AGRILUS IN MICHIGAN

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ABSTRACT

European oak borer and twolined chestnut borer. The European oak borer (EOB) [Agrilus sulcicollis Lacordaire (Coleoptera: Buprestidae)] was first discovered in Ontario in 2008 (Jendek and Grebennikov 2009), and Michigan and New York in 2009 (Haack et al. 2009). Later, specimens in collections were dated back to 1995 in Ontario, and 2003 in Michigan. The discovery of EOB in North America raised concerns about its potential impact on North American oaks (Quercus spp.). We conducted life-history studies on EOB and made comparisons with the native twolined chestnut borer (TLCB) [Agrilus bilineatus (Weber)] in two oak plantings in southern Michigan. We found that EOB adult females were attracted to purple and white traps (sticky cards were 15 cm × 30 cm and coated with clear insect trapping glue), compared to similar green or yellow traps. TLCB adults were most attracted to purple followed by yellow traps. Males of both species showed no significant color preference. EOB flight started and peaked about 2 weeks before TLCB. EOB successfully attacked and emerged from 1-m-long trap logs cut from live Q. alba, Q. robur, Q. rubra, and Q. velutina trees cut and placed in the field in spring prior to adult flight. TLCB only emerged from one Q. alba log. EOB adults oviposited preferentially on Q. alba, Q. robur and Q. rubra trees with browning foliage within one month of girdling, while TLCB preferred girdled trees that still had green foliage. Overall, EOB was most attracted to dying or recently dead hosts, while TLCB was most attracted to hosts that were stressed but still appeared healthy. Therefore, it is likely that EOB will not be a significant oak pest in North America as compared to TLCB. More details of this study can be found in Petrice and Haack (2014b).

Native Agrilus attracted to different trap types and colors. Since the discovery of emerald ash borer (EAB), Agrilus planipennis Fairmaire, in North America in 2002, there have been several studies focused on developing effective traps for monitoring and detecting EAB. An early study by Oliver et al. (2003) found purple and violet to be attractive to many buprestids, and Francese et al. (2005) found purple to be attractive to EAB. Later, shades of green were found to be attractive to EAB as well. Studies on other Agrilus and other buprestids have found variation in color preferences (Domingue et al. 2013; Petrice et al. 2013). During 2011–2012, we conducted studies comparing the attractiveness of native Agrilus to different colors and trap types that had been previously tested on EAB. In one study, we tested light green, purple, white, and yellow sticky traps and found that light green and yellow sticky traps captured the most Agrilus individuals and species. In the second study, we tested sticky traps that were light green, dark green, or purple, and 7-unit funnel traps that were green or black and found that the most Agrilus individuals were captured on light green sticky traps and the least in black funnel traps, while the greatest number of Agrilus species were captured in green funnel traps and the least were captured in black funnel traps. Overall, light green or yellow sticky traps were very effective at capturing the most Agrilus individuals in our studies. Purple and white tended to be the least effective sticky traps. Most of the Agrilus species captured in both studies were smaller species that oviposit on small branches and twigs. This could explain their preference
for green or yellow that likely mimics the color of foliage rather than purple, which may more closely resemble the color of tree bark. Green funnel traps could be an effective alternative to sticky traps given that they captured the most *Agrilus* species and an intermediate number of *Agrilus* individuals. See Petrice and Haack (2014a) and (2015) for more details on these studies.

**REFERENCES**


EASTERN HEMLOCK RESISTANCE TO THE WOOLLY ADELGID: PROGRESS & PROSPECTS

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ABSTRACT

We and our colleagues have conducted a series of experiments assessing the potential for HWA resistance in naturally-occurring eastern hemlock, and a potential mechanism for this resistance. Both rooted and grafted ‘resistant’ tree cuttings had lower HWA settlement than control tree cuttings. Twigs from resistant trees (sampled in situ and growing in a common-garden environment) had much higher terpenoid concentrations in both locations and over time, indicative of high constitutive defenses. These findings confirm naturally-occurring HWA resistance in eastern hemlock, and might provide a useful complement to existing chemical and biological control efforts.
EVALUATION OF GENERIC ATTRACTANTS FOR TRAPPING THE VELVET LONGHORNED BEETLE *Trichoferus campestris*

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ABSTRACT

The velvet longhorned beetle, *Trichoferus campestris* (Faldermann)(VLB; Cerambycidae: Cerambycinae: Hesperophanini), is native to east Asia where it feeds on a wide range of tree species, including orchard and timber trees. Larvae of VLB can be transported in wood packing material, and individuals are frequently intercepted in quarantine facilities. Populations of VLB have established outside of the native range of the species, including near Salt Lake City, UT. Adults are non-descript and nocturnal, and nothing is known of their pheromone-mediated biology, which hinders monitoring and control efforts. We conducted field bioassays testing the response of adult VLB to traps baited with nine “generic” cerambycid attractants and ethanol. These attractants included compounds conforming to the conserved 2,3-alkanediol/hydroxyketone structural motif. Compounds of this chemical motif are male-produced pheromone components of multiple species in the subfamily Cerambycinae. A total of 104 adult VLB were captured in bioassays. Significantly more adult VLB were captured in traps baited with racemic 3-hydroxybutan-2-one or ethanol than in traps baited with other attractants. In addition, histological sectioning revealed subcuticular metathoracic glands present in males, but not females. Glands were connected to pits in the cuticle. The glands and pits are diagnostic for production of volatile pheromones of the 2,3-alkanediol/hydroxyketone motif by males of other cerambycine species.
PHYTOPHTHORA CINNAMOMI AND OTHER BIOTIC AGENTS ASSOCIATED WITH RAPID WHITE OAK MORTALITY IN MISSOURI

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ABSTRACT

Rapid white oak mortality (RWOM) mostly affects dominant and co-dominant white oak trees (Quercus alba) on shallow, lower slopes and in the bottoms of upland drainages in Missouri and Iowa. Entire tree crowns often die within one growing season and retain leaves. A one-year study was performed to identify biotic agents associated with RWOM. Forty-one and 39 plots (0.08 ha) were established at Sunklands Conservation Area (SCA) and Harmon Springs, Mark Twain National Forest (MTNF), respectively. Average crown vigor of white oak trees in each plot was assessed. Trees and soils in high and low vigor plots were sampled for Phytophthora spp., Armillaria spp., and bark and wood-boring insects. Soils at the bases of three tree per plot were collected and leaf-baited for Phytophthora. Suspect Armillaria mycelium from the root of one dead or severely declining tree in each plot was cultured. Suspected Phytophthora and Armillaria isolates were identified by DNA sequence or RFLP analysis, respectively. Insects associated with declining or recently dead white oaks were emerged from main stem and crown logs placed in emergence buckets.

At MTNF and SCA, more than 80% of plots located at the bottom of the slope were rated as low vigor, while 50% or more of plots on the upper slopes were rated as high vigor. P. cinnamomi was detected throughout the MTNF study area and within hillside plots at SCA. P. cinnamomi detection rates ranged from 100% of plots at the bottom of the slope to 50% of plots on the summit at MTNF. Detection rates were lower at SCA with no detections at the bottom of the slope and a 38% detection rate in plots on the upper-half of the slope. Armillaria mellea and A. gallica were detected on the roots of dead white oaks in both high and low vigor plots at SCA. RFLP analyses are continuing for MTNF samples. Xyleborinus gracilis, a wood-boring ambrosia beetle was emerged from 33% of 13 trees felled at SCA and 80% of 5 trees felled at MTNF. No other xylo- or phleophagous beetle emerged from logs from both locations.

Armillaria spp. are commonly associated with oak decline in Missouri while Phytophthora cinnamomi and Xyleborinus gracilis are not. Prior to this study, P. cinnamomi was detected in soils in Warren County, Missouri. It is unknown if P. cinnamomi is present in stands without RWOM. Xyleborinus gracilis is a wood-boring ambrosia beetle native to the SE United States and was first reported in Missouri in 2008. The beetle has been emerged from trees felled at three other RWOM-affected locations. Phytophthora cinnamomi, Armillaria spp., and Xyleborinus gracilis are associated with affected white oaks and may contribute to rapid white oak mortality.
INTERACTIONS BETWEEN EASTERN WHITE PINE (PINUS STROBUS) AND THE RECENTLY DISCOVERED SCALE INSECT-PATHOGEN COMPLEX IN THE SOUTHERN APPALACHIANS

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ABSTRACT

Eastern white pine (Pinus strobus L.) is an ecologically and economically important conifer species across the eastern region of North America. Recently, white pines in the southeastern United States started showing signs of dieback including branch flagging, resinosis, crown thinning, and canker development. Upon closer examination, a scale insect was found and identified as Matsucoccus macrocicatrices Richards. This scale insect is typically found under lichen, or in epiphytic mats, branch crotches, and/or cankers. Since eastern white pine dieback has become more prevalent, it has become critical to understand the white pine, associated fungal pathogen and scale insect complex.

We collected 246 white pine saplings from 41 sites across six states (Georgia, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia). The saplings received an overall health rating of 1-5, where one is a healthy and five is a dead tree. Starting from the top first node, we cut the saplings up to 1 m to the base, and measured small and large end diameters. From each tree we determined the number of scale insects, size and location of cankers, presence/absence of Caliciopsis pinea Peck and Septobasidium pinicola Snell. Preliminary results indicate that there were positive correlations between scales and cankers, cankers and tree dieback, and scales and tree dieback. Caliciopsis pinea was the most prevalent fungal species, making up to 63% of the total canker surface area. We are currently isolating and identifying other fungal pathogens from the cankers. Overall, there appears to be a strong relationship between dieback of white pine saplings, and the presence of cankers and insect-pathogen complex.
ACOUSTIC AND MICROWAVE METHODS FOR PEST DETECTION IN GRAINS

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ABSTRACT

According to the National Invasive Species Council’s National Management Plan, animal and plant communities are being affected by species that are not native to the United States and are brought into the country from abroad. They are a leading cause of biodiversity loss. CBP works with USDA’s APHIS and FWS to enforce laws prohibiting or limiting the entry of invasive species into the USA. The challenge is to detect multiple pests at ports of entry before they make their way into the United States. Currently, CBP Agriculture Inspectors inspect for pests using manual techniques. These methods are labor-intensive and have many drawbacks, including high error margins that can vary depending on the inspector, lighting, and other factors.

This raises the need to develop technologies that allow better detection of invasive species in ports of entry. Our presentation provides a review of acoustic and microwave methods for pest detection in grains and cereal, describes systems developed at Stevens for this purpose, and results of the initial experiments.

Even though acoustic methods have not been applied to the detection of pests and insects in ports of entry, such methods are intensively investigated and used for the detection of insects in stored products, wood, and other substrates. We built an acoustic system based on a high sensitive piezo-ceramic receiver. It was tested for detection of a single maggot in rice and cereal. The maggot movement produced a weak sound that was clearly recorded by the developed setup.

The microwave method is based on the application of a X-Band continuous wave signal to grain placed in a special container and the detection of small phase and amplitude variations of the EM field produced by pest motion. The experimental setup allowed the detection of a single maggot in rice and cereal.

This work has been sponsored by DHS’s Science and Technology Directorate.
ASIAN LONGHORNED BEETLE DISPERsal IN INVADED LANDSCAPES

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ABSTRACT

Background: Once moved to a novel environment, invasive herbivores are often freed from specialized predators and co-evolved plant defenses. As a result of this release from top-down and bottom-up pressures, populations can grow rapidly and may be limited primarily by physical constraints and dispersal behaviors. Since 1996, at least 5 established populations of the Asian longhorned beetle (Anoplophora glabripennis Motschulsky, hereafter ALB) have been discovered in the U.S. and Canada. While ALB populations in Illinois, New Jersey, and parts of the New York City metro area have been eradicated, infestations in Worcester, MA and Bethel, OH present novel challenges as these populations are established in suburban environments which grade rapidly to contiguous forested landscapes.

Here we describe part of a series of studies intended to identify and describe patterns of movement by ALB with the goals of using data collected by the APHIS ALB Eradication Program. Past research on ALB dispersal using mark-recapture studies in the beetle’s native environment have shown that individuals disperse an average of >250 m, and are capable of dispersing >1 km (Bancroft and Smith 2005). Here, using an approach based on graph theory and conservative assumptions of dispersal we have found similar median inferred dispersal distances, and 99th percentile dispersal distances as long as 4 km in Ohio, and 8 km in Massachusetts. While the two infestations yielded similarly shaped dispersal distance-probability curves, distances above the 80th percentile differed by as much as two-fold between locations. Variation in the behavior/biology of founding populations, and/or differences in landscape structure account for these differences, suggesting dispersal risk may vary among populations.

REFERENCES

ENHANCING MANAGEMENT OF HEMLOCK WOOLLY ADELGID BY ESTABLISHING A BIOLOGICAL CONTROL DEMONSTRATION SITE

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ABSTRACT

Since the initial discovery of hemlock woolly adelgid (HWA), *Adelges tsugae*, in the Great Smoky Mountains National Park (GRSM) in 2002, biological control has been an important component of management of HWA in GRSM. Two species of predatory beetle, *Laricobius nigrinus* and *Sasajiscymnus tsugae*, have been released throughout GRSM in over 200 sites, and both species have been recovered from ca. 20% of release sites sampled between 2008 and 2012. Hemlocks are persisting in some of these recovery sites, and both *L. nigrinus* and *S. tsugae* have been observed coexisting in some sites, suggesting that these species may provide a compounding impact on HWA populations. To highlight biological control of HWA in the GRSM, a site where 300 adult *L. nigrinus* were released in February 2007 and recovered in 2009 and 2010 was selected and designated as the HWA Biological Control Demonstration Site. At the Site, three other predators of HWA, *Laricobius osakensis*, *S. tsugae*, and *Scymnus sinuanodulus*, were released in 2013 and 2014, and a species native to the eastern U.S., *Laricobius rubidus*, also occurs there. Large cages were installed on four trees, and initial releases of HWA predators were conducted on these trees. Cages were removed from the two *Laricobius* release trees but remain on *S. tsugae* and *S. sinuanodulus* trees. The Site has been monitored periodically since predator releases using both beat-sheet sampling and emergence trapping. From Winter 2013 through Spring 2014, *L. nigrinus* (n = 88), *L. osakensis* (n = 2), *L. rubidus* (n = 9), and *S. tsugae* (n = 1) were collected. No *S. sinuanodulus* were collected during this period. Monitoring of predator populations will continue at the Site. This Site is intended to serve as an educational and technical resource to land managers to illustrate the benefits of utilizing multiple species of natural enemies to manage this invasive forest pest.
OVERWINTERING OF *SPATHIUS AGRILI* IN A SOUTHERN CLIMATE: FACT OR FICTION?

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**ABSTRACT**

The invasive emerald ash borer (EAB), *Agrilus planipennis*, is a native to eastern Asia whose primary habitat and food source are trees of the genus *Fraxinus*. This invasive pest has caused the death of millions of ash trees in the U.S. since it was initially discovered near Detroit, MI, in 2002. A study investigating the overwintering ability of EAB parasitoids using large cages was initiated in 2013; three green ash trees in Blount Co., TN, were selected for use in this study. Each tree was topped at 6 m from ground level, and the base of the upper trunk and limb sections were placed in plastic 125-liter drums, so that they could be supplied with water throughout the year. A large cage (ca. 7 m tall, 5 m diameter at base) was placed over each of the three trees. In June 2013, adult EAB (50 female and 45 male) were added to the cages, so that they could mate and lay eggs and larvae could begin development within the trees. In August 2013, 100 female *S. agrili* and 100 *T. planipennisi* were released in the cages to parasitize the maturing EAB larvae. All tree material was left in the field throughout the winter to allow parasitoids to pupate/complete their lifecycle. In Spring 2014, the caged trees were felled and sectioned (ca. 75 cm), and the sections were placed in barrels according to their designation as trunk, upper trunk, or limbs. All barrels with wood from this study were monitored throughout the summer to document parasitoid emergence. *S. agrili* adults began emerging from barrels on 4 August and ended on 19 August; sex ratio was 1:1.5 (M:F). No *T. planipennisi* were observed in barrels. These results demonstrate that *S. agrili* can overwinter and survive in Tennessee. Further research will enhance biological control efforts against EAB and minimize adverse impacts of this invasive pest to native ash.
IMPACT OF PLANT DEFENSE AND THE HEMLOCK WOOLLY ADELGID ON THE PREFERENCE AND PERFORMANCE OF HEMLOCK LOOPER

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ABSTRACT

Eastern hemlock (Tsuga canadensis [L.] Carriere) is widespread and ecologically important in the eastern United States. Following the introduction of the hemlock woolly adelgid (Adelges tsugae Annand), hemlocks have rapidly declined. Efforts to understand the cascade of physiological effects following attack provide insights to mechanisms driving tree mortality. The activation of defense pathways alters foliage quality for subsequent attackers. The hemlock looper (Lambdina fiscellaria Guenée) is a chewing lepidopteran native to North America and has lead to significant defoliation of hemlocks in the past. As the looper and adelgid have the potential to co-exist on the landscape, it is useful to explore their interaction. We investigated whether the performance or preference of looper differed given Tsuga spp. foliage infested with adelgid (T. canadensis and T. caroliniana), uninfested (susceptible), or resistant to adelgid. Larvae reared on foliage infested with the adelgid matured more rapidly than those reared on uninfested foliage. However, the number of surviving larvae reared on infested T. canadensis foliage decreased more rapidly over time than larvae reared on other foliage types. Maturation rates and pupae weights of larvae reared on resistant foliage were significantly higher than larvae reared on other foliage types. In a preference experiment, larvae presented with uninfested and resistant foliage consumed significantly more resistant foliage. Differences in rates of looper maturation are likely due to distinct foliar chemical composition. The looper performance on adelgid-infested foliage indicates that the adelgid likely activates the jasmonic acid defense pathway. Future studies to explore the mechanism driving these differences in development and preference of the looper will provide a better understanding of the defense pathways of hemlock.
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