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Annapolis, MD

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Abstract
This document contains abstracts and papers of the oral and poster presentations offered at the 31st annual U.S. Department of Agriculture interagency research forum on invasives species held in Annapolis, MD, in January 2023. The forum, sponsored by the Forest Service, Animal and Plant Health Inspection Service, National Institute of Food and Agriculture, and Agricultural Research Service, illustrates the depth and breadth of studies supported by the agencies and their cooperators.

Cover graphic by Melody Keena, USDA Forest Service, Northern Research Station.

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January 10-13, 2023
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Annapolis, MD

Compiled by:
Katherine A. McManus
Therese Poland
Nathan Havill

Sponsored by:

U.S. Forest Service Research & Development

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U.S. Department of Agriculture

FOREWORD

This meeting was the 31st 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 Agricultural Research Service. The Group's original goal of fostering communication and providing a forum for the overview of ongoing research among the Agencies and their cooperators continues to be realized and facilitated through this meeting. This proceedings documents the efforts of many individuals: those who organized and sponsored the meeting, those who provided oral and poster presentations, and those who compiled and edited the contributions. The proceedings illustrates the depth and breadth of studies being supported by the agencies and their many cooperators and demonstrates the benefits and accomplishments that can result through the spirit of collaboration.

Acknowledgments

The program committee would like to thank the four USDA agencies for their continued support of this meeting, and the Management and Staff of the Graduate Annapolis. Thanks to Melody Keena for providing the cover artwork, "We're back", and Vince D'Amico for poster coordination.

Program Committee

Michael McManus, Jian Duan, Joseph Elkinton, Nathan Havill, Scott Pfister, Therese Poland, Talbot Trotter

Local Arrangements

Katherine McManus, Therese Poland

Proceedings Publication

USDA Forest Service Northern Research Station
Registration Assistance provided by Kristine LaVigna

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Kristine LaVigna and Vince D'Amico for facilitating Forum registration

Vince D'Amico for poster coordination

Melody Keena for cover artwork

Management & Staff of the Graduate Annapolis Hotel

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GLOBALIZATION AND THE ACCIDENTAL SPREAD OF INVASIVE INSECTS

Cleo Bertelsmeier [1], Aymeric Bonnamour [1], Olivia Bates [1], Jelena Bujan [1], Eckehard Brockerhoff [2], Jérôme Gippet [1], Andrew M. Liebhold, and Sébastien Ollier[1]

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ABSTRACT

Aim
Globalization has resulted in the accidental movement of thousands of species around the world. Even though it has been recognized for a long time that humans introduce species, researchers have focused on factors directly linked to the biology of organisms rather than human-mediated dispersal. Here, we studied the global spread of alien insects, which may impact native biodiversity and generate high economic costs. Our aim was to make use of a massive dataset of global distributions and border interceptions of to investigate how alien insect species spread as accidental consequence of increasingly globalized trade.

Methods
We used spatial statistics and time series analyses to test the effect of three features of trade (temporal dynamics, the trade networks and types of commodities traded) on global flows of alien ant species.

Results
Our findings lead to several major conclusions: (1) Spread dynamics of ants over the two centuries have been strongly influenced by variations in trade openness and have followed two waves of globalization. (2) Global flows of species around the world differ greatly by taxon and are linked to trade networks. (3) Precise trade metrics are needed to link invasions with global trade. (4) Introduction pathways were often complex and included secondary spread from areas where the species has been previously introduced, contributing to an acceleration of global spread. (5) The pet trade in exotic pets such as insects provides new opportunities for species introductions.

Conclusions
Our results demonstrate the importance of understanding socio-economic drivers of invasions to better understand the spread dynamics of ants at a global scale.

PRESENTATIONS
Research Reports

CHANGE IN THE SEASONAL TIMING OF FLIGHT OF WINTER MOTH IN EASTERN NORTH AMERICA REINFORCES REPRODUCTIVE ISOLATION IN A CLINAL HYBRID ZONE

Jeremy C. Andersen[1], Jennifer L. Chandler[1,2], Nathan P. Havill[2], and Joseph S. Elkinton[1]

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ABSTRACT

The European winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae), was accidentally introduced to North America on at least four separate occasions, where it has been hybridizing with the native Bruce spanworm, *O. bruceata* Hulst, at rates of nearly 10 percent per year. Both species are known to respond to the same sex pheromones and to produce viable offspring, but whether or not they possess allochronic differences in regard to the seasonal or diel timings of their mating flights are unknown. Therefore, to examine seasonal differences, we collected adult male moths weekly along two transects in the northeastern United States and genotyped individuals using polymorphic microsatellite markers; and for diel differences, we compared real-time pheromone trap counts based on infrared counters to those from a simple experiment where pheromone traps were replaced at 09:00 and 15:00 hrs daily and the total numbers of individuals counted at each collection event. For the seasonal dataset, we then estimated the cumulative proportions of each species on each calendar day, and our results indicate that there are significant differences between the species in the seasonal timing of flight. Analyses of day vs. nighttime flight indicate that temperature and time of day influence winter moth and Bruce spanworm flight; though there is nighttime overlap of the flight of these two species, winter moth flies almost exclusively during the night with little flight occurring when temperatures are below freezing, whereas Bruce spanworm flight occurs during the day and night with a shift toward daylight hours as temperatures decrease. Combined, these two types of allochronic differences likely are acting to reinforce reproductive isolation between these two species, resulting in a stable hybrid zone. In addition, our results indicate that the late-season flight observed by winter moth compared to Bruce spanworm may be limiting its inland spread in the northeastern United States as a result of increased exposure to extreme winter events.

BEECH LEAF DISEASE: WHAT'S GOING ON? AND WHAT DOES THE FUTURE LOOK LIKE?

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ABSTRACT

Beech leaf disease was first noted as an unknown ailment of American beech, *Fagus grandifolia*, in Lake Metroparks near Cleveland, OH, in 2012. The disease is characterized by very uncommon symptoms for a forest tree disease. It is now accepted that a foliar nematode, *Lytilenchus crenatae mccannii* (LCM), which is closely related to one earlier described in Japan (*L. crenatae*) on Japanese beech (*F. crenata*) is, at the very least, a necessary condition for disease development, if not singularly sufficient to cause disease, making it the first case worldwide of a major forest disease associated with a foliar nematode. Due to LCM's similarity to a Japanese species of no particular importance in Far East Asia, and the explosive nature and specific dynamics of the North American epidemic, it is currently hypothesized that the nematode is a non-native pathogen that was initially introduced in the Cleveland area. The disease eventually leads to abortion of new buds and subsequent failure to produce new leaves, leading to progressive decline and death over several years, especially of younger understory trees. The disease has now spread throughout the Northeast and threatens the entire distribution range of this iconic and ecologically significant tree species, from the Great Lakes to Georgia, and East of the Mississippi to New England.

Furthermore, it has been demonstrated that European beech, *F. sylvatica*, is also susceptible, which has put Europe on edge as they are dealing with several concurrent forest disease epidemics of major significance. In this talk I will provide an excursus of the discovery and spread of the disease, significant research development in the last 10 years, and an informed opinion on where we are heading with this emerging forest epidemic.

PROGRAMMATIC UPDATE ON ASIAN LONGHORNED BEETLE (*ANOPLOPHORA GLABRIPENNIS*) IN SOUTH CAROLINA

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ABSTRACT

In May 2020, an infestation of Asian Longhorned Beetle (*Anoplophora glabripennis*) (ALB), an invasive wood boring beetle that attacks various hardwood trees, was discovered in Charleston County near Hollywood, South Carolina. South Carolina is the sixth state to detect an ALB infestation, and the first in the southern United States. The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), in cooperation with Clemson University's Department of Plant Industry (DPI), forms the ALB Cooperative Eradication Program and is implementing quarantine and control strategies to eradicate ALB from South Carolina.

Since the initial detection, over 6,991 infested trees have been detected, 4,356 infested trees and 2,799 high risk trees have been removed, and over 180,349 tree surveys have been conducted. Public reception to the ALB Eradication Program has been overwhelmingly positive with most landowners cooperating with tree survey and tree removal activities and opting for high-risk host removal which is the preferred strategy for eradication. However, eradication efforts in South Carolina have faced many unique challenges due to the subtropical climate, various field hazards, difficult or inaccessible wetland terrain, and extreme weather. Research studies have been on-going in collaboration with APHIS-PPQ Science and Technology, Clemson University, and North Carolina University, focusing on population dynamics, phenology, and alternative management strategies.

ADVANCES IN BIOLOGICAL CONTROL OF THE POLYPHAGOUS SHOT HOLE BORER, *EUWALLACEA FORNICATUS*

Christine Dodge[1,2], Zachary Lee[2,3], Deena Husein[1], Aaron Weber[2,3], Yi-Chang Liao[1,4], Paul Rugman-Jones[1], Richard Stouthamer[1], and Juli Gould[2]

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ABSTRACT

The polyphagous shot hole borer (Coleoptera: Curculionidae: Scolytinae) is one of several species of ambrosia beetle (Xyleborini) in the cryptic *Euwallacea fornicatus* species complex. Native to various regions in Southeast Asia, members of the *E. fornicatus* species complex has become invasive in several countries across the globe, where they threaten agricultural production, natural landscapes, and urban forests. The polyphagous shot hole borer (PSHB) has the widest invasive range, which includes Israel, South Africa, Australia, and the United States. Three of the four known species in the complex are invasive in the United States, in California, Florida, and Hawaii. Members of the *E. fornicatus* species complex harbor fungal symbionts that are mild to severe plant pathogens, causing the plant disease *Fusarium* dieback in over sixty species of hardwood tree. Due to the beetles' cryptic lifestyle, chemical control is difficult to achieve, and cultural control options are limited. Here we describe our progress in implementing a biological control program for the *E. fornicatus* species complex. *Eucosmophorus* sp. (Braconidae), *Phymastichus* sp. (Eulophidae), and *Plastanoxus* sp. (Bethylidae) are parasitoids associated with PSHB in Taiwan. Since late 2021, we have coordinated monthly shipments of PSHB-infested wood from Taiwan to collect and rear parasitoids. We obtained significant numbers of *Eucosmophorus* sp. and *Phymastichus* sp. from these efforts and were able to rear *Phymastichus* sp. in a laboratory colony for six generations over a six-month period. We developed a parasitoid rearing microcosm consisting of a living bolt of wood artificially infested with laboratory-reared PSHB. While this method appears sufficient for rearing *Phymastichus* sp., our starting number of wasps was ultimately too low to maintain the colony. While a significant number of *Eucosmophorus* sp. were obtained from shipments, attempts to rear them in the microcosm were unsuccessful. In addition to obtaining parasitoids for research, receiving monthly shipments of infested wood from Taiwan provided insight into the parasitoids' annual life cycles, which we will use to inform all future collection efforts. This research is the necessary first step toward implementing a biological control program for the *E. fornicatus* species complex, and to our knowledge represents the first effort at using parasitoids for biological control of an economically important ambrosia beetle.

The content of this paper reflects the views of the authors(s), who are responsible for the facts and accuracy of the information presented herein.

SYNCHRONY OF EGG HATCH WITH BUDBURST DRIVES POPULATION DYNAMICS OF WINTER MOTH IN EASTERN NORTH AMERICA

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ABSTRACT

We developed a nonlinear model to predict the date of winter moth, *Operophtera brumata* L, egg hatch as a function of temperature from January to April based on laboratory experiments wherein, we reared eggs at different temperatures (5,10, 15, 20 and 20+°C) in growth chambers until they hatched. The eggs were held in batches at 1° C where they accumulated age but did not grow and were then placed in the growth chambers at one- or two-week intervals. The laboratory data showed that the daily growth rate of eggs (1/number of days to hatch) increased exponentially up to about 20° C, whereupon growth rate declined dramatically, and the eggs all died at about 26° C. This pattern of increase and decline of growth rate depending on temperature is universal across many insect taxa. Our data also showed that growth rates increase steadily as the eggs aged at 1° C. Using data from these lab experiments and observation of hatch of eggs deposited by naturally occurring winter moths at various sites in eastern Massachusetts in 2012 and 2013, we optimized an exponential growth model with an age-dependent response to temperature that predicts timing of egg hatch with temperature data from nearby weather stations. We showed that hourly temperatures in the field from mid-November to mid-April rarely exceeded 20°C, so we focused our model on the exponential growth that occurs below that temperature.

We coupled this model with a previously developed model of budburst of northern red oak, Q*uercus rubra* L, to study the effect of the synchrony of hatch with budburst of the most common tree species that winter moth feeds on in New England. With these models we predicted hatch and budburst from spring and winter temperatures over a 10-year interval from 2006 to 2016 during which we collected yearly data on winter moth pupal density at multiple sites in eastern Massachusetts. We showed that hatch typically preceded budburst by approximately one week, but early spring caused hatch to precede budburst by as much as two weeks. Substantial asynchrony with budburst was correlated with a decline in winter moth density, as measured by yearly estimates of pupae per m^2. These analyses support earlier studies in Europe and Canada which suggests that synchrony of egg hatch with budburst is a major driver of population change in winter moth.

EXPERIMENTAL EVIDENCE FOR BARK MEDIATED FEEDBACKS BETWEEN SCALE INSECTS AND FUNGI AS A DRIVER OF BEECH BARK DISEASE DYNAMICS

Jeff R. Garnas[1] and Kenneth Z. Windstein[1]

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ABSTRACT

Beech bark disease of American beech arises from the interaction between the invasive felted beech scale, *Cryptococcus fagisuga*, and native *Neonectria* fungi, specifically *N. faginata* and *N. ditissima.* Along the advancing front of the disease, *Neonectria* depends entirely on feeding by *C. fagisuga* to gain access to and/or successfully capture resources within beech phloem. However, in the later stages of disease (i.e., the aftermath forest), correlations between scale insect and *Neonectria* abundance have been observed to switch from strongly positive to slightly negative (Garnas et al. 2013). We hypothesized this switch from facilitation (at least of fungi by the scale insect) to moderate antagonism between disease agents in the aftermath forest, as mediated by changes in the physical structure of beech bark and as a consequence of prior BBD infection. We further hypothesized that co-infection with both *N. faginata* and *N. ditissima* (shown to be common across the range of BBD; Morrison et al. 2021) would result in the formation of larger cankers relative to those produced by either of the two fungi when inoculated separately, and that scale insects would moderately antagonize fungal growth.

To test our hypotheses, we performed two controlled establishment/inoculation experiments using 80 mature beech trees across two size classes (10-20 cm DBH and 20-30 DBH) and bark response/ prior infection categories (smooth and rough). Scale insects were established by collecting and placing two densities of eggs (~500 and 1200) under foam pads affixed to trees and covered in Tyvek for 11 months. In August 2020, foam pads were removed and the total area colonized by scale insect quantified. On the same trees, starting in October 2019, 12 *Neonectria* inoculations per tree were performed in a two-way design: fungal inoculation (control [agar], *N. faginata, N. ditissima,* and both fungi) × local scale insect density (zero, low, high). For this experiment, 6mm plugs of mycelium were extracted from the growing edge of cultures plated on malt yeast extract agar and placed in a superficial wound of similar size excised from the bark of beech with a biopsy punch. For the co-inoculation treatment, both *Neonectria* species were placed in the tree at approximately equal proportions. After 10 months, cankers were carefully examined and the total area of active lesion growth, host response zone, and dead necrotic tissue quantified.

Our experiments yielded a suite of interesting results, and analyses are ongoing. Here we focus on scale insect establishment rates and on lesion growth and host response across fungal inoculation treatments. Scale insect colonization was uniformly low (<1percent total cover) on rough bark irrespective of tree size and number of eggs placed. Large, smooth-barked trees showed similarly low rates of establishment, again unaffected by the density of eggs placed. On small, smooth trees, however, we saw considerably higher establishment (1.9 times and 2.6 the mean for all rough and for large smooth trees, for low and high egg placement densities, respectively). Thus, rough-barked trees (i.e., those that have survived and responded to earlier BBD attack) represent low-quality habitat for scale insects as do large, smooth trees, which very likely have elevated genetic resistance to attack. In contrast, small, smooth-barked trees with average levels of resistance and with bark that was as yet unaltered by BBD, represented the highest quality resource from the perspective of scale insects.

Our fungal inoculations revealed that *N. faginata* produced lesions that were on average nearly 1.5 times larger than those produced by *N. ditissima* inoculations. Lesion formation for the control treatment (agar plugs) was negligible. Co-inoculation with both fungi resulted in intermediate lesion formation, leading us to reject the hypothesis of synergistic effects between these two species at a small spatial scale. Finally, the host defensive response zone that formed around each localized infection was considerably (1.5 times) larger for *N. ditissima* than *N. faginata*, suggesting that beech trees may be better able to detect and/or defend against *N. ditissima* infection. Overall, lesion growth was moderately greater on larger trees across all inoculations and lower on rough-barked trees, but only for *N. ditissima.* Local scale insect densities had little effect on fungal lesion growth.

This study represents an important first step in experimentally testing mechanisms of bark mediated feedbacks between scale insects and fungi in the beech bark disease system. Enhanced knowledge on this front will help explain observed variation in disease severity across the landscape and may suggest key modifications to suggested management approaches. For example, the preservation of trees with moderate BBD-induced defects that may have otherwise been tagged for removal may in fact represent a reasonably healthy cohort where symptom severity is mitigated by structural changes in the bark.

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PERFORMANCE AND IMPACT OF THE INVASIVE BEECH LEAF-MINING WEEVIL, *ORCHESTES FAGI*, ON ITS NATIVE HOST (EUROPEAN BEECH) VS. NEW HOST, AMERICAN BEECH

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ABSTRACT

The beech leaf-mining weevil, *Orchestes fagi* (Coleoptera: Curculionidae) is a common pest of European beech in its native range in Europe, where it occasionally erupts in outbreak populations that typically last 1-3 years. Heavy infestations can reduce growth rate of European beech and beech nut production but do not kill trees. *Orchestes fagi* was discovered on American beech in Halifax, Nova Scotia in 2012 and was likely present there as early as 2005 based on anecdotal reports. Beech weevil populations have been consistently high in Nova Scotia for more than a decade and up to 95 percent mortality of mature American beech has been recorded in permanent sample plots set up in 2014 (Sweeney et al. 2020). Defoliation increases the susceptibility of American beech to infection by decay fungi (Tubbs and Houston 1990) so it is likely that defoliation by the weevil year-after-year is weakening American beech and mortality is caused by decay fungi. *Orchestes fagi* may be inadvertently spread by human transport of logs and firewood because the adult is present most of the year and overwinters in large numbers on the trunks of American beech cankered by beech bark disease as well as on red spruce and red maple (Morrison et al. 2017). The weevil was recently reported as established in Sackville, New Brunswick and Charlottetown, PEI (Klymko & Anderson 2022).

The prolonged outbreak of *O. fagi* in Nova Scotia and greater apparent damage in American *vs.* European beech may be due to escape from natural enemies, fewer defenses in the naïve American beech than in its native host, or both. The weevil is host to many species of parasitoids in its native Europe (Péré et al., 2011); surveys conducted in France and Switzerland in 2019 recorded an average of 50percent larval/pupal parasitism (Marc Kenis, personal comm.). In contrast, we have observed only very low rates of parasitism in Nova Scotia to date (Goczal & Sweeney unpublished data).

In spring of 2022, we conducted a sleeve cage study in Halifax to test the hypotheses that the naïve host, American beech, has fewer defenses against herbivory by the weevil than does European beech. If this were true, we predicted that, at the same attack densities, damage to foliage would be greater in American- than in European beech. Because mature leaves are significantly larger on American beech than European beech they should also provide more space and food for larval development, so we also predicted that reproductive success (number of new adult progeny/number of mating pairs introduced to the sleeve cage) would be greater in American- than in European beech. In mid-April, we placed sleeve cages on three branches in each of 12 American and 12 European beech trees; each sleeve cage contained seven tight closed buds. On the same day we collected moss that contained overwintering *O. fagi* adults from the trunks of beech trees in Halifax and transported them in a sealed cooler to the Atlantic Forestry Centre (AFC) containment facility. The adult *O. fagi* were sexed and stored individually in plastic vials at 3-4°C until the second week of May when beech buds began to burst, and we returned to Halifax and introduced mating pairs to the sleeve cages.

On each of the 24 trees, we put two mating pairs in one sleeve, four mating pairs in another sleeve, and zero mating pairs in the remaining sleeve to serve as a no-feeding control. We collected the sleeves in late June when the new generation of adults had emerged and processed the sleeves/branches in the FC containment facility, recording the following variables: 1) number of live leaves (still attached to the branch); 2) number of dead branches (< 6 live leaves); 3) number of larval mines; and 4) number of next generation adults (total adults in sleeve cage minus the mating pairs introduced). We divided the number of next generation adults by the number of mating pairs to provide an estimate of reproductive success. We used generalized linear models to test the effect of host tree species, attack density, and their interaction on our response variables, using the model: y = species + attack density + species*attack density + tree (species), where species and attack density were fixed effects and tree within species was a random effect. Models were run with Gaussian, Poisson, and negative binomial distributions; we report results from the model with the distribution that best fit the data as measured by the lowest value of corrected Aikaike's Information Criterion (AIC).

The results supported our prediction that foliar damage would be greater in American than European beech. The numbers of live leaves, dead branches, and larval mines were all significantly greater in American- than European beech. Our prediction of greater reproductive success in American vs. European beech was also supported but only at the lower attack density. Reproductive success was significantly affected by tree species, attack density, and their interaction. At the attack density of 2 mating pars per 7 buds, reproductive success was significantly greater (1.7X) on American beech than on European beech. However, at the higher attack density, reproductive success decreased significantly in American beech and was no different than that in European beech. Reproductive success in European beech was not affected by attack density. Edwards et al. (2021) found a strong positive relationship between the amount of leaf-tip necrosis in American beech and the amount of adult feeding by *O. fagi,* and showed that most of the damage occurred when adults fed on young developing leaves around the time of budburst. Their findings and our results suggest that the drop in reproductive success at high weevil attack density was due to less food and space for larval development as a result of defoliation associated with adult feeding damage. Although we have observed American beech trees almost totally defoliated by the weevil in parts of Nova Scotia, our higher attack density of 4 mating pairs per 7 buds inside the sleeve cages probably occurs only rarely under natural conditions where adults are free to disperse and feed on any number of developing leaves.

Our results clearly demonstrate that *O. fagi* causes greater defoliation in American beech than in European beech, and suggest the damage is caused by excessive damage to the young developing leaves by adult feeding. However, further research is necessary to determine the mechanism(s) explaining the difference in foliar damage between the native and naïve host. The higher reproductive success of *O. fagi* in American beech at the lower attack density suggests it may be a more suitable host than European beech, but whether that is due to larger leaves or fewer herbivore defenses remains to be determined.

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VARIATION IN SPOTTED LANTERNFLY RESPONSE TO TEMPERATURE ACROSS THE INVADED RANGE

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ABSTRACT

Validated phenology models based on the responses of spotted lanternfly (SLF, *Lycorma delicatula*) to a broad range of temperatures and hosts are needed for predicting when monitoring for SLF needs to occur, when the right stage is present for application of control methods, and to identify the regions of the US most at risk for invasion by SLF populations. Founder effects resulting from small introductions and disjunct dispersal into regions with novel conditions can lead to rapid selection and potential local and regional adaptation as an invasive species spreads. Under these conditions phenology models will need to incorporate these shifts to estimate how these adaptations may shift the potential range of SLF. In the process of conducting phenology studies using SLF populations from a range of locations, and integrating cold snaps into analyses, we have seen evidence that SLF populations may be adapting to colder climates in two ways: the timing of egg hatch and the response of nymphs to different temperatures. The response of these parameters to temperatures varied by population source. To expand on this finding, we collected SLF egg masses from 20 geographically distinct populations distributed across a range of plant hardiness zones (5B-7B) to identify population-level differences in temperature-dependent development rates. We assessed hatch timing using 2 temperature regimes (constant 15°C and an alternating regime that mimicked Scranton, PA), and evaluated the development of first and third instars at both 15 and 25°C constant temperature regimes for each population. Newly hatched nymphs (first instars) from each of the populations were reared in groups of 40 on tree of heaven (*Ailanthus altisima*) in small cages until they molted or died. All newly molted nymphs were weighed within 24 hours. When individuals reached the third instar they were set up in large cages in groups of 15 and held with both tree of heaven and grape vines at 20-25°C. Mean time in instar and days to death were calculated for each population and for both first and third instars. We also assessed the distribution of developmental times both within and between populations to determine the variation present and the potential for adaptation.

There was substantial variation in the timing of hatch at 15°C (constant) between populations. Populations from the 6B plant hardiness zone (the middle of the temperature gradient sampled) hatched the fastest, and speed of hatch declined for populations from both progressively colder and warmer zones. The differences in speed of hatch were consistent for populations from these sites when sampled over multiple years. Under an alternating temperature regime there was also substantial variation in hatch but the overall differences between populations were reduced and hatch became more synchronous within individual egg masses. Percentage hatch tended to be higher for egg masses held in the alternating regime compared with those held at constant temperatures. Field observations indicate populations closer to the core of the infestation (plant hardiness zone 6B) hatch earlier. Spring temperatures may in part explain this, but there are likely other factors influencing the variation in hatch timing.

There was substantial variation across the invaded range—and in some cases within a population—in the duration of the first instar at 15°C. The variation in duration of the first instar at 15°C was relatively stable across time from individual sites that were sampled more than one year. There was less variation across the invaded range in first instar duration at 25°C. Time in third instar also varied among populations at both temperatures, but the populations of first instars

that grew the fastest at 15°C did not have the fastest third instar development. There were no clear correlations between duration of the instars and plant hardiness zones. More analysis is needed of the data to determine what may be driving the variation. We have collected from some of the same populations and expanded the geographic range from which we collected to further study the variation and its stability.

Plant hardiness zones (climate) do not fully explain the variation, although there are trends. Other aspects of climate and ecological factors (e.g. hosts, age of infestation, etc.) associated with the sites need to be evaluated to see if they explain the variation that was observed. However, just knowing there is substantial variation that might provide some populations an advantage if moved to other locations should be considered when dealing with new infestations. For example, populations with either earlier hatch or more rapid development at colder temperatures may be able to complete development faster and essentially lengthen the growing season so they could establish in cooler climates. All findings from this work will be used to improve the individual-based phenology model we are working on and help to predict the potential geographic invasion range for SLF.

PLANT COMMUNITY DYNAMICS FOLLOWING INVASIVE SPECIES MANAGEMENT DEPEND ON INVASION INTENSITY AND FOREST SITE CHARACTERISTICS

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ABSTRACT

Globally, temperate deciduous forests are threatened by invasion of non-native plant species. As human populations continue moving to urban areas, there is a growing need to understand urban forests and their capacity for regeneration, and to evaluate the relative successes of restoration strategies. Studies have shown urban forest canopies consist mainly of native species, while understory composition contains many non-native invasive species. In the eastern United States, *Rosa multiflora* (Thunb.), hereafter "rose", is a dominant shrub invader in forests, which often forms dense thickets that reduce sunlight availability in the understory, where decreased native plant diversity and abundance is observed. Management and restoration of invaded forests are often difficult, but desirable, especially when invasion intensity is still low. However, little is known about how invasion and urbanization pressures influence the seedling and herbaceous layers in these forests over time, and few studies have examined the relative success of different management strategies under varying invasion intensities.

The objectives of this study were to assess the responses of the herbaceous and seedling communities to non-native species, investigate the potential for native tree and forest regeneration, and determine if non-native species were increasing in abundance and/or dominance across 10 forest sites experiencing a range of plant invasion. Additionally, we conducted an invasive plant removal experiment in three forest sites experiencing different invasion intensities with the objective of restoring native plant biodiversity while preventing secondary invasion. We utilized three management strategies: invasive plant removal, removal followed by native seed addition, and removal plus native seeds and invasive stems applied as mulch. We investigated the similarity between seed bank species composition and existing vegetation before and after removal to assess the potential for passive restoration.

Native richness was highest, and non-native richness lowest, in the herbaceous plant layer. Native woody species were becoming less dominant within sites, and those that were no longer present in the mature tree/shrub canopy layer were not replaced in the seedling layer. Two seasons after removal, we found that simply removing rose increased native species richness, floristic quality index (FQI), and native shrub abundance in our medium invasion site, and total species richness in our low and medium invasion sites. Compared to removal alone, native seed addition, with and without mulch, resulted in larger increases in native richness and FQI at all sites, larger increases in native shrub abundance in our medium invasion site, and larger reductions in non-native shrub abundance in our low and medium invasion sites. Following removal, species similarity between seed bank and vegetation improved for all three sites.

These results indicate that while the herbaceous layer seems to resist invasion more than other layers, small forests are becoming more invaded, and displaced native species increase in abundance at slower rates than non-native species. Invasion and urbanization influence forest plant communities and the potential for native regeneration, but responses differed by forest strata. Furthermore, our results indicate that removal of rose alone increased native plant biodiversity in the medium invasion scenario, but the seed bank may not provide a large native species pool. Additional management strategies lead to improved outcomes, especially in our most invaded forest, demonstrating the need to conduct multiple plant removal treatments across forests with varying site conditions and plant invasion intensity to improve management recommendations.

TRENDS IN WOOD BORER DETECTIONS IN WOOD PACKAGING MATERIAL SINCE THE U.S. FULLY IMPLEMENTED ISPM 15

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ABSTRACT

Wood packaging material (WPM) is a known pathway for wood boring insect introductions. To reduce the risk of introducing woodboring pests into new regions via WPM, the International Standards for Phytosanitary Measures No. 15 (ISPM 15) was created by the International Plant Protection Convention. ISPM 15 requires all WPM used in international trade made from solid wood to be either heat treated or fumigated following an approved treatment schedule. The U.S. began implementing ISPM 15 in 2005, and by July 2006 the standards were fully enforced for all WPM entering the U.S. In 2009, ISPM 15 was amended to require WPM to be constructed from de-barked wood, with a tolerance for any residual bark being limited to less than 3-cm wide, or if wider than 3-cm, having an area less than 50 cm^2. To evaluate the effectiveness of ISPM 15 in reducing the risk of wood borer introductions into the U.S., we compared wood borer detection rates at U.S. ports of entry during the period just prior to ISPM 15 (2003-2004) to the periods when: A) ISPM 15 was fully implemented with no bark restrictions (2007-2009), and B) bark restrictions were added (2010-2020). Detection rates for each period were calculated using the APHIS AQIM database. These data are randomly collected by U.S. Customs and Border Protection and used by APHIS PPQ to evaluate the risk of different pest introduction pathways. We found that woodborer detections decreased from 0.34 percent of inspected consignments with WPM prior to ISPM 15 implementation, to 0.18 percent during the first three years after full ISPM 15 implementation (2007-2009) and similarly to 0.22 percent after bark restrictions were implemented (2010-2020). Decreases in woodborer detection rates also varied among different survey categories and the top three U.S. trading partners. For example, WPM in the survey categories "Perishables" and "Italian Tiles" had the largest decreases in detection rates, while "General Wood Packaging Materials" had the smallest decrease. Also, detection rates decreased most in imports from Mexico and Italy, compared to China. The most frequently detected woodborers were in the family Cerambycidae and the subfamily Scolytinae. We discussed the risk WPM still poses for woodborer introductions given current detection rates and possible reasons why decreases in detection rates were not as significant as anticipated.

More details of this study can be found in (Haack et al. 2022) such as crafting and pallets, is recognized as a high-risk pathway for introduction of bark- and wood- infesting insects (borers).

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RESULTS OF A WORLDWIDE TRAPPING PROGRAM USING GENERIC LURES TO DETECT CERAMBYCID INVADERS AT ARRIVAL ON OTHER CONTINENTS

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ABSTRACT

Globalization is resulting in an exponential increase in the introduction of non-native insects into other continents, of which a growing proportion are emerging species yet never found outside their native range and not included in the quarantine lists. Since they are unexpected, their detection at arrival at ports of entry could not rely on specific attractants. Deployment of traps baited with broad-spectrum semiochemical lures at ports-of-entry could be one of such early detection tools. Given the difficulty of predicting which species may arrive, such lures should be efficient even at low population densities and should ideally attract multiple species from different taxa. Recent progresses in chemical ecology of cerambycids revealed that a number of genera, tribes or even subfamilies from different parts of the world are sharing, at least partly, similar components of aggregation-sex or sex pheromones. Therefore, we designed a blend mixing 8 of these pheromones, which are used by largely different cerambycid taxa either associated to conifers or broadleaved trees. Since no antagonistic effects were shown among the blend components, such a multi-pheromone blend was expected to result in an effective, generic attractiveness to multiple related species.

During 2018–2021, we conducted a worldwide field trial programme using traps baited with this 8-pheromone blend. A total of 1296 traps were deployed at 298 sites covering simultaneously or sequentially European countries, Chinese provinces, and some regions of the USA, Canada, Australia, Siberia, and Martinique island. A total of 78,321 long-horned beetles were trapped, representing 376 species from 8 different subfamilies. Captures comprised 60 tribes, with 10 tribes including more than 9 species trapped on different continents. Some invasive species were captured on both the native and invaded continents. Sixteen species were trapped at more than 1000 individuals, 58 ones at more than 100 individuals, and 84 ones at more 50 individuals, suggesting that such species have a high probability to be detected upon arrival. This demonstrates the potential of the multi-pheromone lure to constitute an effective tool for the detection of 'unexpected' cerambycid invaders accidentally translocated outside their native ranges. A database of ca. 400 cerambycid species susceptible to be trapped by the multilure blend is thus available, with a convenient genericity for early detection of non-natives belonging to tribes such as Clytini, Callidinii, Monochamini, Acanthocini, Acanthoderini, Prionini, etc. It must also be noted that only 8 of the 16 species trapped at more than 1000 individuals have their pheromone already identified, and it is very likely that the blend included major pheromone components of the 8 other species.

Further tests were carried out in 2020-2021 in France and China to test for the effect of increasing to 10 the number of cerambycid pheromones in the blend. The 10-component blend especially included the pheromone of two recent invaders in Europe, the red-necked longhorned beetle *Aromia bungii* and the velvet longhorned beetle, *Trichoferus campestris*. It allowed the novel detection of *T. campestris* in France, and large captures of *A. bungii* in its native China without altering the previous catches in both countries. Thus, increasing the number of blend constituents is a way to be capable of detecting other tribes, given no antagonistic effects. However, it appears likely unrealistic to expect catches of species with unique pheromones (e.g. *Rosalia alpina*) unless their pheromones are part of generic blends.

NOVEL PATHWAYS FOR TRANSPORT OF FUNGAL PATHOGENS: TWO CASE STUDIES

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ABSTRACT

Fungal pathogen transport is facilitated by human activities and climate change. This dispersal is leading to novel interactions with hosts, with increasing frequency of negative impacts. Here, two case studies are presented. We surveyed imported wooden handicraft products for viable of fungi sold in six retail stores in Florida, United States. Studies were conducted in two experiments using culture-based methods, followed by DNA identification of the isolates. Sampling was achieved by taking samples from the inside of wood, using sterilized instruments and collecting sawdust and or small wood chips that were then used for plating on several semi-selective media types. Many of the tested products were observed to have bark present, evidence of decay or current or previous fungal growth, suggesting the potential for survival of a diverse array of fungi even if biosafety protocols are being followed. Forty-seven unique isolates were identified and classified into functional groups and biogeography. Among the fungi recovered were non-endemic plant and human pathogens as well as mycotoxin-producers. A high proportion of species isolated were reported to be either thermophilic or halophilic, suggesting adaptability and selection through current wood treatment protocols that utilize heat (60 ^{o}C) and/or fumigation with methyl-bromide. Our study demonstrates high survival and diversity of fungi in wood products originating from at least seven countries on three continents. This research suggests that current federal guidelines for imports of wooden goods are insufficient to prevent introduction of fungi in wooden products and demonstrates the necessity to improve safeguards at both points of origin and entry for biosecurity against introduction from invasive fungal species in these commodities. A second study examines the role wildfire smoke plays in transporting fungal pathogens. Although living microbes have been documented in wildfire smoke previously, the diversity, origin, function, and potential impacts of these microbes are largely unknown. Using culturing and Illumina sequencing from smoke samples obtained from drone-based filters, we observed a wide diversity of plant and human pathogens. These results suggest wildfire smoke may play an important, unexplored role in the long-distance dispersal of fungal pathogens.

UNINTENTIONAL BIOLOGICAL CONTROL IS INCREASING COMMON FOR INVASIVE INSECTS

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ABSTRACT

Unintentional introductions of insect natural enemies (predators and parasitoids of pests) apparently exceed species introduced intentionally. Several factors favor this: a general surge in international trade; lack of surveillance for species that are not associated with live plants or animals; inability to intercept tiny organisms such as egg parasitoids or entomopathogens; huge invasive host populations in source and/or receiving areas that allow rapid establishment; and lack of aggressive screening for pests already established. Available databases for non-native fauna in several continents and islands show varying but relatively high proportions of unintentional versus planned intentional introductions, in particular for the well-defined natural enemy group, parasitoids in the order Hymenoptera.

Weber et al. (2021) profile a number of recent frequent and surprisingly rapid accidental natural enemy introductions, including insect parasitoids, entomopathogens, and insect herbivores of invasive weeds. Examples of these natural enemies and the pests they attack include:

1. egg parasitoids of brown marmorated stink bug (*Halyomorpha halys* (Stål) in North America and Europe;
2. larval parasitoids of spotted wing drosophila (*Drosophila suzukii* (Matsumura)) in North America;
3. mymarid egg parasitoids of glassywinged sharpshooter (*Homalodisca vitripennis* (Germar)) in Oceania;
4. the leaf beetle *Ophraella communa* (Coleoptera: Chrysomelidae) consuming common ragweed (*Ambrosia artemisiifolia* L.) in southern Europe and east Asia; and
5. the entomopathogenic fungus *Entomophaga maimaiga* Humber attacking spongy moth (*Lymantria dispar* (L.)) in North America.

These examples and others raise important issues of the role of systematics and genetic considerations for unintentional introductions. They also highlight the role of the bridgehead effect, the common phenomenon of invasive establishment and subsequent secondary invasions from original invaded areas, first documented in the predatory lady beetle, *Harmonia axyridis* (Pallas) (Lombaert et al. 2010). In contrast, the <u>receptive bridgehead effect</u> (Weber et al. 2021) occurs when hosts (typically pests, either herbivorous insects or weeds) have invaded new territory, and their abundance "sets the table" for natural enemies invading, often by the same routes of entry, to attack the established invasive hosts.

Unintentional biological control brings with it several disadvantages:

1. genetics are uncontrolled (there may be multiple introductions);
2. best candidates are not chosen logically;
3. generalists may be favored, increasing negative nontarget effects;

a. pathogens & hyperparasitoids are not screened out, and lastly, it:

b. damages our illusion of control and destroys our wishful thinking!

On the positive side, unintentional biological control:

1. can be rapid and cost-free;
2. does not require official approvals and accompanying paperwork;
3. there are several examples of introductions that (if purposeful and allowed) would be judged successful;
4. it is occasionally superior to prior intentional efforts;
5. can showcase the power of introduction biological control.

We need to recognize that we live in an imperfect world where accidental introductions are now common, and play a major role in introducing natural enemies. We need to continue and direct resources to prevent, detect, intercept, and eliminate accidental introductions of *all* types, treating all invasive species as a biosecurity threat. Taking a more common-sense approach to regulating intentional biocontrol introductions, however, recognizes that the perfect can be the enemy of the good. Unintentional introductions call into question the value of the typically reactive and protracted response for introduction (classical) biological control, which adventive unintentional introductions have a high probability to displace or disrupt. With the increased focus on guarding against nontarget effects of introduction biological control, comes the cost of delay and reduction in number of projects carried out (Hajek et al. 2016). While this may in some cases prevent negative ecological consequences, criticisms of long-past classical biological control mistakes are today largely misplaced.

Practical introduction biocontrol should strike a balance to solve problems as much as it should seek to avoid creating new problems. With increased barriers to introductions come the prospect of prolonged and even irreversible ecological and economic disruption from pest damage, pesticide applications, and lost ecological services. Along with delay comes the increasing likelihood that accidental introductions of potentially suboptimal natural enemies occur, removing the chance to address pest invasions in a timely manner through best scientific practices. Conversely, the model of *proactive biological control* – preparation (even pre-approval) for natural enemy introductions if and when pests invade and establish (Hoddle et al. 2018) – has the advantage of short-circuiting these potential negative effects, improving prospects for success of introductions. Of course, we cannot foresee all potential invasions, so pest detection and interception of remain key barriers to invading pests.

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The content of this paper reflects the views of the authors(s), who are responsible for the facts and accuracy of the information presented herein.

PRESENTATIONS
Emerald Ash Borer

EMERALD ASH BORER (EAB) EARLY DETECTION IN EUROPE: HAVE WE GOT THE TOOLS TO FIND IT QUICKLY ENOUGH TO CONTAIN IT?

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ABSTRACT

Emerald ash borer, EAB, (*Agrilus planipennis* Fairmaire) has become a major mortality factor of ash trees in both North America and in Russia and Ukraine. In these cases, the pest is presumed to have arrived on traded wood or wood products. Following the discovery of EAB in Michigan in 2002, extensive surveys revealed that the pest was already well established and had probably been present since the early 1990s.

Evidence of widespread impacts in newly invaded regions has raised the prospect that EAB could add further mortality to the already badly affected ash populations in Europe infected by ash dieback fungus, *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya. A previous Euphresco project (PREPSYS https://www.forestresearch.gov.uk/research/prepsys/) considered the risks to Europe from both EAB and its close relative bronze birch borer, BBB, (*Agrilus anxius* Gory). Arising from that project, an outline European Toolbox for EAB/BBB was produced and is reproduced below.

From research and management experiences in North America and Russia, considerable progress has been made in development and implementation of survey and management tools for EAB. Notably, selective use of insecticides linked to biological control make potential EAB population management in the long term a viable prospect.

In Europe we are particularly concerned with early detection of pioneer populations of EAB, and, to this end, we have had a small project to examine potential surveillance regimes to spot new arrivals quickly. In assessing potential early warning systems, we have been mindful of the unfortunate fact that, in virtually all cases where new infestations of EAB have been found, the pest has already been present for several years. Parallels can be drawn with the experiences in Colorado and Oregon where EAB must have arrived through human transportation, there was full awareness of the threat and yet initial detection of the pest was several years after it established a pioneer population. Attempts to apply the optimal surveillance modelling approaches developed in North America for Europe have been hampered by difficulty in gathering suitable data on trade movements and the relatively poor ash inventory data at municipal scales. It is also apparent that much of the literature and technology developed to manage EAB is concerned with survey and delimitation of known populations of the pest. For example, in Europe EFSA has developed a 'survey card' for EAB which includes statistically defined survey efforts, but which are triggered by the first find of the pest. Indeed, most monitoring strategies rely on establishment of trapping regimes to track existing rather than to detect completely new infestations. Thus, the surveillance elements in the toolbox below have a range of detection regimes that delimit and, to a certain extent, quantify known populations of EAB.

Methods for survey therefore exist but have not been sufficient to enable detection of infestations within the first generation after a new arrival. Risk-based survey strategies based on likely pathway end points, sizes of population centres, main transport routes and proximity to ash resources indicate that placement of trapping systems should be concentrated in urban centres with good ash inventory. However, as is apparent in all modelling approaches to optimal surveillance, resource availability is the major constraint to setting up an early warning system. Taking account

of the fact that coloured traps (green or purple) with lures appear to have a very limited attraction range (perhaps as low as 25 m), even allowing for adult flights in new infestations accumulating to 4 km per year, there would need to be many thousand traps in a major city (for example Felixstowe is a major English port with an area of 17000 ha) even with trap density as low as one per hectare. It also appears that soon after a new infestation is established, population growth tends to be slow with usually a 2-year life cycle which would result in fewer infested trees and fewer adults likely to be caught in traps. In North America an accelerated rate of development has been reported once populations build up. However, modelling of degree-days for Europe suggests that a one-year cycle would be likely across most of the available ash resources. While these findings indicate a slow population build up, the need for early detection remains high but it is likely that resource constraints will limit the scale of trap deployment. Additional measures such as citizen science and awareness campaigns might provide early warning capacity, but it is not clear whether this can aid surveillance if most of the criteria for detection rely on damage symptoms and presence of D-shaped exit holes, woodpecker damage, etc. By the time these symptoms become apparent, substantial damage and EAB population increase is likely to have taken place.

At this stage in development of early warning systems the unfortunate conclusion is that resource limitations are likely to result in small pioneer populations being missed such that infestations are likely to be detected several years after the first attacks. In Europe, the situation is likely to be exacerbated by the presence of stressed and damaged trees arising from the extensive infections from *Hymenoscyphus fraxineus*. Further research into optimized early warning regimes is required and would benefit from collaboration.

PROOF OF A GENETIC BASIS FOR EAB-RESISTANCE: AN UPDATE ON BREEDING RESEARCH

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ABSTRACT

'Lingering' ash trees are healthy mature trees that have survived long-term emerald ash borer (EAB) infestation that causes mortality of up to 100 percent of mature ash trees (Knight et al. 2013). The frequency of lingering green ash (*Fraxinus pennsylvanica*) and white ash (*Fraxinus americana*) is estimated to be ~ 0.01 percent across the core infestation zone and surrounding counties in SE MI and NW OH (Romero-Severson and Koch 2017). From 2007 to 2012, ten white and forty-two green ash were selected, replicated through grafting, and challenged in greenhouse bioassays with 400 EAB eggs per meter square of bark area (methods described in Koch et al. 2015 and Kelly et al. 2020). Dissection of the trees eight weeks post infestation revealed that the proportion of larvae killed by tree defenses is significantly affected by the genotype of tree, i.e. certain lingering ash trees can kill EAB larvae better than other genotypes. The best performing lingering white ash killed 23 percent of the larvae, while the best performing lingering green ash killed 46 percent of the larvae compared to an average of 1.3 percent and 0.9 percent tree-killed larvae in unselected genotypes and known susceptible cultivars of white and green ash, respectively.

Heritability is the degree to which genetics influences the expression of EAB-resistance vs. environmental factors, providing the foundational evidence that breeding can produce improved populations. Using the ratio of variances method to calculate clonal repeatability (Roberds and Strom 2006), heritability for proportion of tree-killed larvae was estimated at 0.45 for white ash and 0.15 for green ash. Heritability estimates based on family structure are more precise, so controlled cross-pollinations using combinations of 12 female and 13 male lingering green ash parents were performed to produce 27 full-sibling families. Bioassays were performed on a total of 773 cross progeny from these families and used to determine that more than 60 percent of the expression of EAB-resistance in green ash has a genetic basis, indicating that improvement through breeding is possible.

Although EAB egg bioassays demonstrate that some lingering ash are capable of mounting a genetically controlled defense response against EAB, additional investigation is needed to determine if these results extend beyond white and green ash. There have been reports that black ash (*Fraxinus nigra*) is more susceptible and blue ash (*Fraxinus quadrangulata*) is more resistant than green or white ash, but the variation in response to EAB within these species is not well understood because they are less common, and it took EAB longer to infest their core ranges. To date, the NRS breeding program has accessioned only three black ash and one blue ash that met the criteria of 'lingering' ash for field selection. To further explore variation of response to EAB larvae in these species, we obtained seedlings grown from unselected single mother tree seed lots, collected as part of conservation efforts by the US Forest Service National Tree Seed Lab. Additional black ash seedlings were a gift of the Saint Regis Mohawk Tribe from seedlings grown for their conservation efforts.

To assess EAB resistance within and between these half-sibling families, potted seedlings were subjected to the standard eight-week bioassay, with hand dissections and scoring eight weeks after egg application (12 eggs per seedling at 400 eggs/m^2). Black and blue ash seedlings had

similar results. On average, 24 percent of the larvae in black ash seedlings were killed by host defenses (tree-killed larvae, TK) while 69 percent developed into late instar larvae (L3 or L4, LL). Blue ash families averaged 22 percent tree-killed and 67 percent late larvae. Both species compared favorably to an unselected green ash family which averaged 4 percent tree-killed and 96 percent late larvae. Figure 1 shows individual family means and illustrates that there is variation within and between families in both black and blue ash and both species perform better than an unselected green ash family, shown for comparison purposes. Individual black and blue ash seedlings were identified that killed more larvae than the best performing lingering green ash selection (45 percent larvae killed). Taken together, these data indicate that there may be more variation in response to EAB in black and blue ash than green and white ash. This may make it possible to breed for EAB-resistant black and blue ash proactively, by identifying resistant parents based on seedling progeny performance, instead of waiting for the majority of the trees to die before selecting trees.

One-year bioassays would avoid the need for the labor intense dissections used in eight-week bioassays, and seedlings can be grown outside (with bird exclosures to prevent woodpecker predation/damage) to increase capacity. To assess a pilot one-year bioassay, a subset of the black and blue ash families were infested the same as eight-week bioassay but allowed to grow until the next summer. One year after eggs were applied, we combined counts of EAB exit holes, and exit holes from *Tetrastichus planipennis* (a parasitoid that lays eggs in late instar larvae), as a measure of overall EAB damage. While the dataset is too limited to calculate correlations between the eight-week and one-year bioassays (Figure 2; only five families were in both experiments), there are still apparent trends. Families with higher numbers of late instar larvae at eight weeks also tend to have higher numbers at one year (regardless of species). Interestingly, in blue ash higher tree-killed larvae was positively associated with survival, while higher tree-killed in black ash did not always result in increased survival.

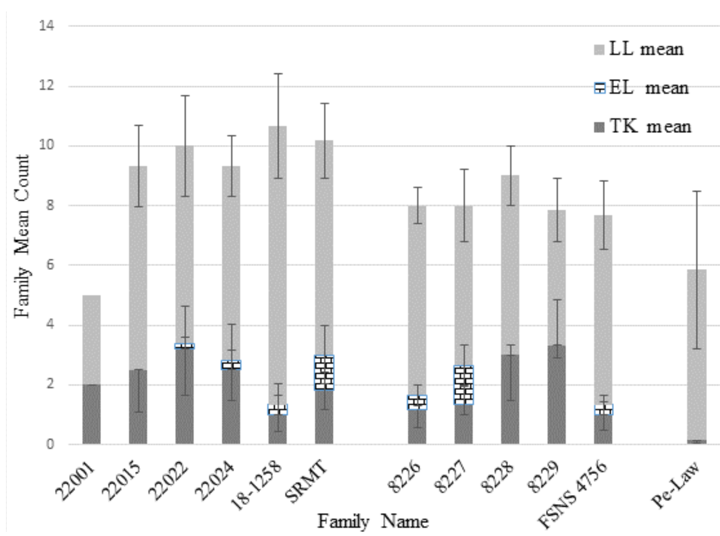

Figure 1.— Family means and standard errors from eight-week bioassays. Counts show black and blue ash have similar distributions of larvae outcomes with more tree-killed and early larvae than a comparable green ash family from a different experiment. Dark bar is family mean tree-killed, hatched bar is family mean early larvae (EL, L1 and L2 combined), light grey bar is family mean late larvae (LL, L3 and L4 combined). Note that family 22001 is raw count as it has only one seedling, other families have 3-6 seedlings.

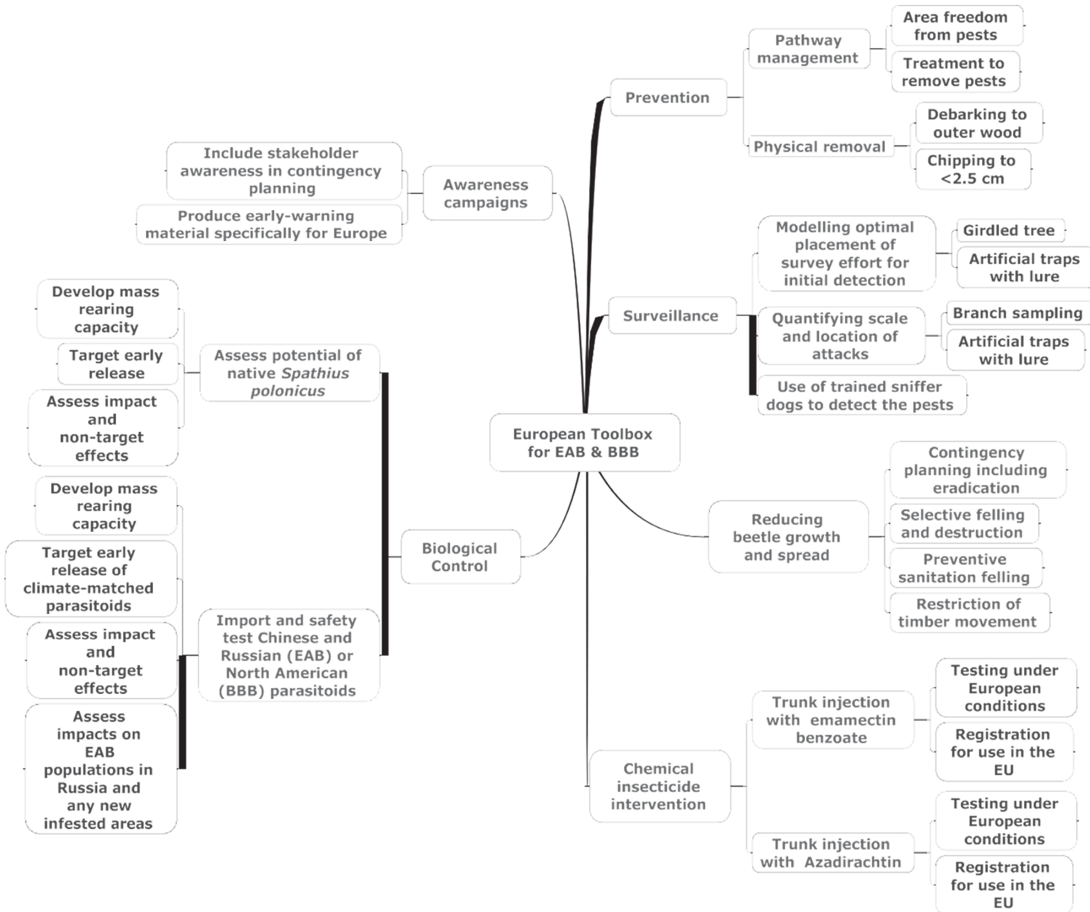

Figure 2.—Family means and standard errors from one-year bioassays. Black and blue ash have similar proportions of EAB and parasitoid exit holes, but more blue ash survive at one year. Light grey is the family mean number of EAB exit holes, dark grey is family mean number of *Tetrastichus* exit holes (indicative of late larvae). Counts of alive over total seedlings are in parenthesis.

These results are consistent with field observations that black ash seem to be killed at lower EAB density than other species (Nate Siegert, personal communication). However,

it is important to note that 1 out of 50 of the black ash seedlings survived, higher than the estimate of survival of green ash in the field. Our data are also consistent with reports of

higher survival rates of blue ash in field studies (Tanis and McCullough 2012). Even so, we half of the blue ash trees had very low or zero tree-killed larvae in the eight-week bioassay, and 45 percent of the blue ash seedlings died in the one-year bioassay. Our pilot study indicates that there is a higher frequency of resistance in the small number of

families tested, but more research is needed to determine the variation of various populations across the range of the species. In addition, previous research studies indicate that adult preferences play a role in driving lower EAB attack rates in natural stands (Peterson et al. 2015; Poland et al. 2009). Efforts should be made to capture both mechanisms of resistance (host tree-killed larvae and adult preferences) in blue ash breeding programs.

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PERSISTANCE OF ASH A DECADE AFTER INITIAL EMERALD ASH BORER INFESTATION IN NEW YORK

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ABSTRACT

Emerald ash borer (EAB, *Agrilus planipennis,* EABFairmaire) was first detected in western New York (NY) in 2009 and a second infestation was detected in eastern NY the following year. Research sites in both regions were established in 2012/2013 at which time ash trees were tagged for long term monitoring. Trees were grouped into "large" (≥ 4.0 cm DBH in 2013) and "small" (< 4.0 cm DBH in 2013) size classes based on the likelihood of EAB infestation. Tree health characteristics including canopy condition were assessed in 2013, 2017, and 2021. Mean mortality of large ash trees in both regions has been extensive (63.5–89.3 percent) while mortality of small trees was significantly less severe (21.1–56.3 percent). The majority of dead small trees in 2017 did not show signs of EAB infestation, indicating death from other causes. Mortality rates remained consistent between the 2017 and 2021 sampling events with the exception of a significant decrease in mortality for large trees in eastern NY by 2021. In both 2013 and 2017 a high percentage of trees in both size classes showed evidence of canopy decline. Death of these trees by the subsequent sampling interval accounted for most of the observed mortality. The percentage of living trees showing signs of decline as of 2021 has greatly decreased in both regions for both size classes. In the period from 2017 to 2021 the number of trees with no canopy decline remained constant or increased for both size classes in western NY, and for large trees in eastern NY. The number of healthy small trees in eastern NY decreased only slightly during the same interval. Ash regeneration was assessed first in 2012/2014 and again in 2021. Density of ash seedlings (0–0.5 m tall) decreased non-significantly during this interval, while density of small saplings (0.5–1 m tall) increased non-significantly. Density of saplings (>1 m tall, <2.5 cm DBH) increased in both regions, but only significantly in eastern NY. Results indicate the persistence of both established and regenerating ash in forest stands infested with EAB for over a decade. The lack of declining trees in 2021 may indicate a shift in EAB invasion dynamics.

INTEGRATING BIOCONTROL AND INSECTICIDE TREATMENTS IN URBAN FORESTS AND BLACK AND GREEN ASH FORESTS

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis,* EAB*)* (Coleoptera: Buprestidae), is a destructive invasive forest pest causing widespread mortality of ash trees in North America. This presentation will highlight research on two ongoing integrated pest management studies in both urban and forested areas. Both studies involve integrating trunk injections of ash trees and releases of biological control agents for widespread control of EAB. The urban study was established in 2015 in Syracuse, New York, Naperville, Illinois and Boulder, Colorado. *Tetrastichus planipennisi*, *S. galinae*, and *O. agrili* were released and have established in all three cities. *Spathius agrili* did not establish. Both *T. planipennisi* and *S. galinae* have increased and dispersed widely across both the release and control plots in multiple cities. EAB densities in all three cities have fallen to low levels, below 10 larvae/m^2 phloem. Treated trees have remained healthy while most untreated trees have died. Woodpecker predation (21-67 percent) and parasitism (18-46 percent) have remained important sources of mortality for EAB even as EAB densities have declined to low levels. The forested IPM study is just beginning and has been set up in three green ash plots is 2020 and three black ash plots in 2021 in New York. The goal is to conserve larger reproductive ash trees with trunk injections while biocontrol agents establish. Trees will remain treated until the EAB population crashes and then a subsection of trees will be removed from treatment.

USDA EMERALD ASH BORER BIOCONTROL PROGRAM: PARASITOID PRODUCTION, RELEASE, AND RECOVERY

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ABSTRACT

Since its discovery in Michigan in 2002 emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae) has spread to 36 states and is causing widespread mortality of ash trees and substantial economic and ecological impacts. The USDA has been developing a robust biological control program for this pest since the first releases of biological control agents in 2007. The EAB Biocontrol Rearing Facility currently produces four parasitoids for the biological control of EAB, *Oobius agrili,* a solitary egg parasitoid and three gregarious larval parasitoids, *Tetrastichus planipennisi, Spathius agrili,* and *Spathius galinae*. This presentation will cover research updates from the EAB Biocontrol Rearing Facility on developing an artificial diet for EAB and improving methods for cold storage of *Spathuis galinae*. As of 2022 releases have been done at over 950 sites, in 31 states, D.C., and four Canadian Provinces. The program has worked with 268 cooperators and 206 non-PPQ cooperators. Over 8 million parasitoids have been released including over 500,000 in 2022. Parasitoids have been recovered in 23 states and two Canadian provinces.

USING MACHINE-LEARNING MODELS TO IDENTIFY CROWN CLASS CONDITION OF EAB-INFESTED FORESTS

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ABSTRACT

As an increasing number of invasive insect pests are introduced to landscapes due to global trade transport and geographic range shifts, there is a greater need for research and study on alternative methods of control beyond insecticides and pesticides. A classical biocontrol program was developed and implemented by the U.S. Department of Agriculture (USDA) against the most destructive invasive beetle *Agrilus planipennis* (emerald ash borer, EAB) shortly after EAB was first detected in the U.S. in 2002. The EAB biocontrol program has since resulted in releases and establishment of several introduced biocontrol agents across over 350 counties of 32 EAB-infested states. These introduced EAB biocontrol agents included one egg parasitoid *Oobius agrili* and three larval parasitoids *Spathius agrili, Spathius galinae* and *Tetrastichus planipennisi*. While recent field studies have observed successful establishment of and as well as significant EAB suppression by these introduced agents in some earlier release sites, the contribution of the EAB biocontrol program to ash tree recovery and regrowth is currently not known. As ash trees are the host species of the EAB and the most vulnerable targets of the EAB, understanding the effect that this biocontrol program may have on ash trees is critical to assessing the success of the program. This project focuses on assessing the ecological impacts of the current EAB biocontrol program on ash tree survival, recovery, and regrowth to canopy trees in North America where the introduced biocontrol agents have been released in the last two decades. Collaboration with the USDA Agricultural Research Service (ARS) attempts to answer this question: **What methodologies can we use to assess the impact of parasitoid wasps on the EAB, and what effects do these wasps have on native ash forests after their deployment?**

To accomplish this, this project has three aims:

Aim 1. To establish whether machine learning (ML) models and drone photography can serve as a new fieldwork methodology to identify crown class conditions and forest canopy cover in EAB-infested forests.

Aim 2. To determine whether current field methodologies of transect surveys and crown class estimation of native ash tree forests are adequate in estimating EAB damage and recovery.

Aim 3. To assess whether the crown class conditions of EAB-infested forests are recovering/regenerating over time given parasitoid/biocontrol deployment.

As the EAB and other invasive pests disperse throughout North America, monitoring of these species is critical, especially in newly infested regions that have populations of vulnerable native species. Many common methods such as transect surveys are used in ecology to monitor and detect invasive species. These methods are often limited by the number of workers, the physical conditions in the field, and the sheer geographic size of many field sites. Uses of drone imagery and machine learning represents an opportunity for enhanced research and detection capabilities that can serve as a fieldwork supplement or even substitute in certain cases.

Drone Imagery Methods

This project uses aerial drones to collect imagery of native ash forests in New England. Field collection of drone images use a commercial drone supplied by the USDA-ARS in Delaware. Images are taken from 50-meter and 75-meter altitudes above the forest canopy to standardize image resolutions and compare the effect of resolution on model performance. Field sites are areas that have either been infested by the EAB and have had a deployment of parasitoid wasps as a biocontrol method in the past, or a control site where no infestation of EAB and no releases of parasitoids have occurred. After collection, drone images are individually processed for any vulnerable trees, which will be classified on a qualitative 1-5 scale of crown condition. These images will be used in machine learning to develop a model capable of predicting forest crown class conditions for ash trees.

Field Transect Survey Methods

Field sampling procedures follow methods depicted in Duan et al 2017. To compare the machine learning model performance with field data, 50m x 2m transect surveys are collected at each field site, estimating crown class condition, number of EAB exit holes, and other signs of infestation. At each site, a Hip Chain Distance measurer is used to delineate the transect and sample any living ash tree (DBH > 5 cm). Field sites are determined by previous records of EAB sightings and releases of biocontrol agents filed in the mapBioControl database, as well as monitoring efforts from the USDA-ARS. This allows us to ground-truth the ML model and evaluate its accuracy and predictive capabilities compared to current field methodologies.

Machine Learning Methods

To test the accuracy and performance of the models, a suite of machine learning architectures will be compared and tested for the best fit. Model architectures commonly employed in image classification problems will be used, such as ResNet152V2, MobileNet, and Inception. This project uses versions of these models with previous training experience from the ImageNet dataset to determine model training performance using only the drone image dataset versus a model that has already trained from other datasets. To evaluate their success and accuracy, performance metrics such as Area Under Curves – Receiver Operating Characteristic curves (AU -ROC), confusion matrices, and training vs. loss plots will be compared at regular intervals to ensure model improvement.

Current Results

The machine-learning models have been trained on drone imagery collected in the summer of 2022 in New England. Trained on an estimated 3,000 images, the models can adjust their weights and predictions for crown class but still require more data to account for the diversity in crown class structure. This project plans to resolve this with another season of data collection and drone imagery during the summer of 2023 in Oregon, in collaboration with the Oregon Department of Agriculture (ODA). With that, a benchmark ML model capable of predicting forest crown class condition can then be compared to current field methodologies used to detect EAB damage in native ash forests.

INITIAL DETECTION OF EMERALD ASH BORER IN OREGON, AND RAPID RESPONSE

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ABSTRACT

Introduction

Emerald ash borer (*Agrilus planipennis,* EAB), EAB, is one of the most devastating invasive insects in North America. EAB was first introduced to Michigan in the mid-1990s but went undetected until 2002. By 2009, EAB was estimated to have killed 17 million trees, and caused over $25 billion in damage.

In the years since its introduction it has spread to 36 states, reaching from Rhode Island to Oregon, and from Louisiana to Quebec. It's spread is not random. EAB adults fly relatively short distances, once per year. The primary mode of long-range dispersal is human-aided transport in infested wood material. We believe this is how EAB reached Oregon.

On June 30th, 2022, a forester and attentive citizen detected EAB at Joseph Gale Elementary school in Forest Grove, Oregon. The elementary school parking lot had 16 ornamental ash trees planted when the school was built in 2012. All 16 trees showed the unmistakable signs of multi-year infestation. Canopy decline, branch dieback, split bark, D-shaped exit holes, and emerald ash borer adult beetles climbing up the bark. Emerald ash borer larvae feed under the bark of the tree, eating the phloem tissue. This feeding damage girdles the tree from the inside, making early detection very difficult. Infestations are almost undetectable for at least two years.

Response

On the same day as the report, Oregon Department of Forestry was able to visit the site and confirm the report. A sample was sent to Oregon Department of Agriculture (ODA) and U.S. Department of Agriculture (USDA) for taxonomic identification. Over the 4th of July weekend, Oregon Department of Agriculture contacted the elementary school, and requested their arborist destroy the infested trees. On July 5th, ODA began scouting Forest Grove for the presence of EAB. Twenty- six green funnel traps, and two purple prism traps were immediately placed in the area around forest grove by USDA and ODF respectively. ODA created and distributed a visual survey tool for detecting trees infested by EAB. By August, the Oregon EAB Response Task Force was formed, consisting of members from over 40 organizations.

A crew of four seasonal technicians were hired for the winter 2022-2023 season. Emerald ash borer damage is permenant. Bark splits, dead branches, and woodpecker damage are all visible after leaves drop. As of January 8, 2022, the visual survey has surveyed over 6000 ash trees in and around Forest Grove (image 1). In doing so, they have helped to identify the current extent of EAB in Oregon.

Due to how easily emerald ash borer is spread unintentionally in wood, a quarantine was put into effect. Ash (*Fraxinus* spp.), White Fringe Tree (*Chionathus virginicus*) and cultivated olive (*Olea europea*) are all viable hosts of emerald ash borer. Transporting woody material of EAB host plants risks spreading this destructive pest to uninfested areas, including Washington, California, Idaho and the majority of Oregon. As of now, no woody material of EAB host plants may be transported out of Washington County, Oregon under penalty of law.

Planned Control Actions

Based on the previous states' response actions, and outcomes, we do not believe EAB can be eradicated from Oregon. We will be implementing SLow Ash Mortality (SLAM) strategies to mitigate the spread of EAB from Forest Grove, and give growers, land managers, and homeowners as much time as possible to make management decisions. SLAM strategies are intensive, and multifaceted. This includes conventional chemical control, creation of "buffer zone" of trap trees, and biological control using introduced natural enemies.

Chemical Control

Treatment applied correctly at the appropriate time of the year can protect trees with low level of infestation. Generally, trees with greater than 20 percent canopy decline are not considered good candidates for treatment due to the amount of damage already done. If a tree is going to be protected from EAB, treatment needs to occur **before** infestation within the tree has reached this level. Emamectin benzoate, applied via trunk-injection, has provided the highest efficacy of protection against EAB.

"Buffer Zone"

Three strategies to create a "buffer zone" surrounding the known infested area:

1. Spring-girdle trees along a corridor to increase their attractiveness to ovipositing EAB. These "trap trees" will be removed and destroyed in fall.
2. In addition, selected trees in highly attractive positions within the spring-girdled corridor will be girdled and trunk-injected with insecticide to create trap trees lethal to EAB larvae.
3. Selected mature seed-producing trees will be trunk-injected to maintain seed production and regeneration at the site.

Trunk-injection treatments are compatible with biocontrol release at nearby sites closer to Forest Grove. EAB parasitoids require healthy larvae for oviposition and will not oviposit in lethal trap trees. Treatments will utilize emamectin benzoate, applied by independent certified contractors.

Biological Control

Four species of introduced biological control agent have been introduced to North America against EAB. Three species which attack EAB larvae, *Spathius agrili, Spathius galinae*, and *Tetrastichus planipennisi*; and one species that attacks EAB eggs, *Oobius agrili*. Each of these species attacks EAB in a different niche. EAB infesting larger mature trees are heavily targeted by *Spathius* species, while EAB infesting saplings are more heavily targeted by *T. planipennisi*.

Both Gales Forest Grove and Fernhill Wetlands are planned release sites for the introduced biological control agents for EAB. Degree day models suggest that *S. galinae*, *T. planipennisi*, and *O. agrili* will be suitable for release in Oregon. Within each site, we measured the DBH of ash trees to determine specific release locations for each species.

Timeline of Activities:

tree girdling	March-April 2023
trunk injection	April-May
beetle emergence (trap service)	May-Sept
tree removal	Sept-Oct
biocontrol agent release	3 species at 2 selected field sites

trap trees/site = depends on suitable tree location within sites.
trunk-injected trees = up to 10/site
sites = 5

IMPACT OF EAB INVASION STAGE AND POST-RELEASE TIME ON THE PERSISTENCE AND IMPACT OF INTRODUCED EAB LARVAL PARASITOIDS

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ABSTRACT

For classical biological control agents to be effective, it is crucial that they remain in area when the host levels are below threshold levels. Emerald ash borers (EAB), an invasive pest of ash in North America, has a characteristic population crash after their initial invasion into an area and explosive population growth. Parasitoids introduced during the outbreak stage may prevent a second population outbreak after the ash resource recovers. Emerald Ash Borer was first detected in Connecticut in 2012 in five5 contiguous towns in northern New Haven County. We began parasitoid releases in 2013. We started with releases of *Tetrastichus planipennisi* and *Oobius agrili* in two 2 sites. As EAB spread throughout the state, parasitoid releases continued. Each year, new release sites were chosen at the edge of the known EAB infestation range, starting northern New Haven County, and moving out in a radial fashion into surrounding counties Starting in 2016, *S. galinae* was added to the releases. Releases followed U.S. Department of AgricultureUSDA Animal and Plant Health Inspection Service (APHIS) protocol and were conducted at each site for 2 years. In year 3, establishment of the parasitoids was assessed at each site through peeling and/ or sentinel logs.

In this study, we assess the persistence of EAB larval parasitoids after their release and initial recovery. Three specific aspects of parasitoid persistence and impact were investigated: 1. Will EAB larval parasitoids be able to maintain populations in areas where the EAB population has dropped. 2. Will the presence of parasitoids, or rate of parasitism be correlated with EAB larval density and 3. Will ash stand structure be changed by the presence of parasitoids. Three release sites for each of three3 time periods were chosen; three3 sites where parasitoids had been first released 7 - 8 years before the start of the study, three3 sites where first releases were five5 years before then, and three3 sites where releases had started 2-3 years before the start of the study in 2021. For each of these sites a control site, where no releases had been made, was chosen approximately 10 km distant.

For each of the sites we characterized the ash resource using transects, EAB population and parasitism rates by peeling ash trees and parasitoid presence was additionally documented using sentinel logs. We found that the standing ash resource was smallest in the oldest release sites, and largest in the sites where releases had recently ended. Furthermore, the healthy ash remaining in the oldest release sites was significantly smaller in diameter than those in the most recent sites. Forest structure analysis on the 2022 transect data showed that release sites had larger-diameter remaining ash than control sites, suggesting that parasitoids are allowing more mature trees to survive the initial EAB invasion. Finally, in both release and control sites, sapling numbers increased from 2021 to 2022, suggesting potential for regrowth.

At least one of the larval parasitoids of EAB, *Spathius galinae* and *Tetrastichus planipennisi*, was found at 14 of the 18 sites in this study. At eight8 of the sites both parasitoids were detected. One of the oldest release sites had only *T. planipennisi*, and the remaining five5 sites had only *S. galinae* detected. Of the four4 sites without parasitoids, one was a new release site, where releases were completed in 2020. However, at its control site 10 km away, we found *S. galinae* in both 2021 and 2022. One site with no parasitoids detected was a control site for a middle age site, and the remaining two2 were control sites for the oldest sites. At some sites parasitoids were located at the

sites by both peeling bark, and sentinel logs, and at some sites they were only detected with one of the two methods. Notably, *S. galinae* was found at two 2 of the oldest release sites, where the parasitoid was never released.

Density of EAB in release sites reflected the age of the infestation, with the oldest sites having the lowest number of larvae per square meter of phloem and the newest site having the highest number of larvae per square meter of phloem. Combined with the large disparity of available live phloem among the different ages of the research sites, the low density of EAB larvae in the oldest sites means there are very few EAB larvae in these areas. Rate of parasitism of available EAB larvae was 50 percent at the oldest sites, 16 percent at the middle age sites and 40 percent at the oldest sites. Given the small number of EAB larvae recovered, especially in the oldest, and middle age sites, it was not possible to do statistical comparisons of the rate of parasitism among the three3 ages of sites. Peeling is ongoing to better characterize these rates.

Together our results suggest that ash resources are recovering in wake of the EAB invasion of Connecticut. Larval EAB parasitoids are remaining in 'aftermath' forests and continuing to parasitize the few remaining larvae that are present. We will continue to characterize the dynamics of the parasitoids in relationship to the age of EAB invasion.

PRESENTATIONS
Hemlock Woolly Adelgid

ESTABLISHMENT AND POST-RELEASE RECOVERY OF *LARICOBIUS NIGRINUS AND LARICOBIUS OSAKENSIS* (COLEOPTERA: DERODONTIDAE), RELEASED FOR BIOLOGICAL CONTROL OF *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE), IN THE NORTHEASTERN U.S.

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ABSTRACT

Hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), is a major forest pest in the eastern United States (U.S.) responsible for killing millions of hemlocks (*Tsuga canadensis* and *T. caroliniana*). The U.S. biological control program for HWA has largely invested in the rearing and releasing of *Laricobius nigrinus* (Fender) and more recently *L. osakensis* (Montgomery and Shiyake). Though establishment of *L. nigrinus* has been well-documented in the southern U.S., documentation in the northeastern U.S. is limited. Establishment of *L. osakensis* in the northeastern U.S. has not yet been documented. Release locations in the northeastern U.S. were surveyed for *L. nigrinus* and *L. osakensis* establishment (presence of the F_3 or later generation) to examine the relationship between establishment success and winter temperatures, as winter minimum temperatures are believed to limit the northern range of *Laricobius*. Results suggest that *L. nigrinus* establishment was limited by winter minimum temperatures at sites sampled in USDA Plant Hardiness Zones 5b (-28.9 to –26.1˚C) to 7a (-17.8 to -15˚C) and that as the absolute minimum temperature declines so does the probability of establishment. We found *L. nigrinus* established at sites in Maine, New York, and Pennsylvania, but did not recover any *L. nigrinus* in Massachusetts, New Hampshire, or Vermont. Similarly, we found *L. osakensis* established at sites in New York and Pennsylvania and recovered individuals in Maine, though further sampling is necessary to confirm F_3 generation. No releases of *L. osakensis* had been made in Massachusetts, New Hampshire, or Vermont at the time of this study.

The content of this paper reflects the views of the authors(s), who are responsible for the facts and accuracy of the information presented herein.

PREY-ASSOCIATED GENETIC DIFFERENTIATION IN SILVER FLY PREDATORS OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

Leucotaraxis argenticollis (Zetterstedt) and *L. piniperda* (Malloch) (Diptera: Chamaemyiidae), being evaluated and released as biological control agents for the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), in eastern North America. We developed new microsatellite markers for these species and genotyped 467 *L. argenticollis* and 533 *L. piniperda* specimens feeding on native *A. tsugae* in western North America, as well as on other western and eastern adelgid species. For both species of silver flies, population genetic analyses using these new loci, plus DNA barcode sequences, confirmed east-west allopatric divergence, and uncovered genetic differentiation associated with flies feeding on different adelgid prey species in western North America. This provides evidence for sympatric host-associated genetic differentiation in these species with different genetic lineages specializing on different adelgid prey in the West. This supports the continuing collection of flies from western *A. tsugae* and not from other western adelgid species for safe and potentially more effective release as biological control agents in the east. These new markers will also be useful for monitoring potential hybridization between western and eastern flies after their release.

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PRESENTATIONS

Posters Abstracts

UTILITY OF ENVIRONMENTAL DNA ANALYSIS FOR EARLY DETECTION AND MONITORING OF HWA AND BIOCONTROLS IN EASTERN NORTH AMERICA

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ABSTRACT

Environmental DNA (eDNA) is widely recognized as a source of information about species distribution or presence/absence and has been reported from all parts of the environment (water, soil, living and nonliving surfaces, air). Analysis of eDNA provides potential for early detection and monitoring of pests and other insects.

We designed species-specific qPCR assays to detect a group of insects affecting hemlock trees (*Tsuga canadensis* [L.] Carrière and *T. caroliniana* Engelmann) in eastern North America: the pest, hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera:Adelgidae), and predators *Leucotaraxis argenticollis* Zetterstedt and *Le. piniperda* Malloch (Diptera:Chamaemyiidae) and *Laricobius nigrinus* Fender (Coleoptera:Derodontidae). The pest in eastern North America is the Japanese lineage of *A. tsugae*, and all three predators are permitted to be relocated, following USDA APHIS quarantine protocols, from the Pacific Northwest area where they feed on the native lineage of *A. tsugae* to states in the eastern U.S. In this talk we review the findings of Kirtane et al. (2022) and we present new data from two additional projects.

Samples collected from a 16-acre island in Lake George, New York showed that by the time a visual detection was noticed on just a few trees on the island, eDNA of *A. tsugae* had already spread across the entire island, found on all 16 trees sampled and all canopy strata sampled. There was no difference in mean eDNA quantity (copy number) of HWA at different strata of canopy sampling. The detection rate for visual identification of infestation on the samples (under the microscope) was about 23 percent whereas with eDNA detection it was nearly 90 percent. Similarly significant increase by eDNA detection over visual detection was seen for all three biocontrol predator species on foliage from their native range (Kirtane et al. 2022).

Early detection of *A. tsugae* could help to save hemlock stands by expediting chemical treatment and may also help to understand range expansion patterns of *A. tsugae*. We performed an early detection survey along the southeastern edge of the Adirondack Park in summer 2022. Sites were selected based on known infestation (positive controls, n = 3), lack of infestation (presumed negative controls >4 miles away, n = 5), and distance to closest known infestation (within 1, 2, 3, or 4-mile radius of closest known infestation, n = 5 per category). Based on the finding of Kirtane et al. (2022) we only sampled foliage that was reachable from the ground. We found low signal in 2 of the presumed negative control sites on the southern edge near each other, but not in 3 negative controls far to the north. We found high signal from known infestations. For the remaining 20 sites there were 11 non-detects, 8 low, 1 moderate, and no high detections. There was no apparent relationship with distance, but the results suggest possible influence of prevailing winds on dispersal of eDNA. The results are consistent with the high sensitivity of detection reported in Kirtane et al. (2022).

For biocontrol predators, detection with eDNA represents a new tool for monitoring establishment and spread. This work is the first to our knowledge using eDNA to target a biological control

agent, and results could help inform the overall coordinated strategy for control of *A. tsugae*. We reported detection using foliage from the native range of both *Leucotaraxis* spp. and quantities were weakly correlated with population size of immatures on the branches (Kirtane et al. 2022). Large-scale free releases of *Leucotaraxis* spp. began in New York in 2019, thus providing a new opportunity to try eDNA detection in the introduced range. During spring and summer of 2021, we sampled from 6 sites in New York using a fine-scaled approach of multiple collections over time (n = 3 times), 8 trees/site transected by 20 m-distances from release point, and two canopy heights. The amount of foliage per sample fit into a 500-mL wide-mouthed bottle. Out of 576 samples, there were 69 detections of *Le. argenticollis*. These detections occurred mostly in June and July, synchronous with when they are expected to be feeding on HWA. Detections were spread across 5 out of 6 sites, usually with multiple positive trees per site and in both upper (pole pruner) and lower (reachable from ground) samples. Quantities of eDNA were low overall and we did not calculate density.

We followed up one year later, in 2022, at the 5 sites that had positive eDNA signal by using yellow prism sticky traps at the sites (2-week trapping durations over 8 weeks) and foliage collections for rearing in cages. Unfortunately, these efforts did not yield any specimens of *Leucotaraxis*. Tree health and HWA infestation levels at each site had changed over time in ways that presumably would not favor continued occupancy by *Leucotaraxis*. The signals detected could represent occupancy in 2021 by populations of flies that faded out or moved elsewhere by 2022.

In summary, detection of eDNA by qPCR works for both pest and predators. The eDNA is highly mobile in the environment, therefore it is not necessary to sample in the upper canopy. More work is needed to standardize field controls and quantification methods, and to determine optimal sampling approaches, average eDNA persistence time, relationship of eDNA quantity to insect populations, and follow-up steps to be triggered when HWA is detected at a new site.

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EFFICACY & PERSISTENCE OF INSECTICIDES APPLIED BY VARIOUS METHODS FOR HEMLOCK WOOLLY ADELGID CONTROL IN MICHIGAN

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ABSTRACT

Hemlock woolly adelgid (*Adelges tsugae* Annand), a tiny aphid-like insect native to Japan, is one of the most damaging invasive forest pests in eastern North America. Since it was discovered in 1951 in Virginia, this pest has been spreading across much of the range of eastern hemlock (*Tsuga canadensis* (L.) Carrière). This shade tolerant, long-lived tree is a foundational species that plays an integral role in numerous ecological processes in upland and riparian forests. Hemlocks are an especially important wildlife resource, providing deep shade, critical winter cover, food and habitat for several bird, mammal and aquatic species. Mortality of hemlock following hemlock woolly adelgid (HWA) invasion has affected water quality, nitrogen cycling, forest structure and community composition as well as property values in residential areas.

Localized populations of HWA were initially detected in 2015 in areas of Ottawa and Muskegon counties in western Lower Michigan. Currently, infestations have been identified in three additional counties, all bordering Lake Michigan. More than 173 million hemlock trees grow in Michigan forests, primarily in the northern lower and Upper Peninsula, and thousands more hemlocks have been planted in landscapes. High densities of HWA cause needles and buds to die, canopies to thin and foliage takes on an unhealthy grayish-green color. Healthy trees can succumb to an HWA invasion after only four to 10 years and trees stressed due to drought, poor growing conditions or weakened by other insect pests can die more rapidly.

Managers of HWA suppression and containment efforts, along with arborists, foresters and property owners in the 21 states where this invader occurs require accurate and objective information to efficiently allocate resources and optimize insecticide treatments. Various products and application methods have been used to combat HWA, but most efforts now involve systemic insecticides. Soil applications of imidacloprid have been commonly used in the eastern US with some treatments reportedly persisting up to 7 years, but products, application methods and dosages vary, as do reports of efficacy and persistence. Basal trunk sprays of dinotefuran or in some states, imidacloprid, have been more recently used for HWA control but less is known about persistence of these products and methods.

We evaluated insecticide persistence by quantifying insecticide residues in 236 hemlocks treated by the multi-agency Michigan HWA program in an operational project between 2017 and 2020. Treatments included imidacloprid applied via trunk injection or basal trunk spray, and dinotefuran applied with or without imidacloprid, via basal trunk spray. Density of HWA and canopy condition of treated and untreated control trees were also monitored. Hemlock samples were collected in the spring of 2021 by harvesting terminal shoots from four aspects of the upper and lower canopy of each tree. Collections were dried in individual paper bags and needles and stems separated before being ground into a powder. Needle and stem material were analyzed separately by both UHLC/MS/MS and ELISA techniques using established methods to detect presence and concentration

of the insecticides. In assessing the consistency between the two analytical methods used to determine insecticide residue in hemlock foliage and twigs, ELISA determinations compared well to UHLC/MS/MS (sum of imidacloprid parent compound and the metabolite olefin) and there were strong linear relationships for twigs ($r^2 = 0.86$; $P<0.001$) and needles ($r^2 = 0.68$; $P<0.001$).

Trees were grouped by the number of months between insecticide treatment and our sampling for several analyses. Groupings were 4-9, 18-22, 31-34 and 40-42 months post-treatment. Primary comparisons beside treatment timing included treatment method (trunk injection vs basal trunk sprays), plant tissue analyzed (twigs vs needles) and aspects of imidacloprid and dinotefuran degradation and longevity. Estimates from previously conducted field or laboratory studies indicated LC_{50} values for HWA are approximately 0.12 ppm for imidacloprid and 0.06 ppm for olefin, a secondary imidacloprid metabolite. To our knowledge, HWA LC_{50} values for dinotefuran have not been determined or published. Residue levels in twigs and needles from nearly all of the treated hemlocks exceeded this LC_{50} value for all samples except in the 4-9 month group treated with basal bark sprays, which were mostly below the LC_{50}.

There was a significant and positive correlation in imidacloprid levels in foliage and twigs from the same trees, which became more variable when residue values were highest ($r^2=0.70$; $P<0.0001$). Twigs had significantly higher residues than foliage across all time periods and application methods ($F=11.04$; df = 1, 408; $P<0.05$) and trees receiving a trunk injection of imidacloprid had higher residue values compared to those treated by basal bark sprays ($F=15.36$; df = 1, 408; $P<0.001$). Imidacloprid residues differed among groups of trees treated in different time intervals ($F=48.15$; df=2, 408; $P<0.001$). Residues were highest in trees sampled 18-22 months post-treatment regardless of treatment method or plant tissue analyzed, reflecting the relatively slow translocation of imidacloprid into shoots and foliage and its subsequent degradation as time post-treatment increased.

In contrast to imidacloprid residues, dinotefuran residues were substantially higher in needles than in twigs, while the highest residue values were 18-22 months post-treatment. Samples were not available for the shortest time interval of 4-9 months. Trees in this group were treated with a basal trunk spray application using the lowest labeled rate and dinotefuran degradation in the samples was evident in the 31-34 month sample, while the lowest residues were found in the twig tissues. Dinotefuran is known to degrade more rapidly than imidacloprid, which is consistent with our data. Results indicate that residues remain high enough to control HWA for at least 22 months (presumably two years), but trees may then require retreatment.

Average residues of imidacloprid parent material and the imi-olefin metabolite, with a reported LC_{50} for HWA of 0.06 ppm for olefin were also examined. We expected concentrations of imi-olefin in needles to increase over time as the imidacloprid parent material broke down within tree tissues. Olefin is more toxic to HWA than imidacloprid parent material and is frequently credited for extending the efficacy of imidacloprid applications for HWA. However, olefin concentrations mirrored those of parent material; trees sampled 18-22 months after treatment had the highest levels of both compounds while the trees treated only 4-9 months before sampling had the lowest levels of both compounds. Levels of olefin in twigs was quite low for all samples.

Additional imidacloprid metabolites were also analyzed but all had residues substantially lower than olefin or the imidacloprid parent material while following a similar pattern. These compounds are likely less toxic and their relatively low concentrations in hemlock tissues suggests their role in protecting trees from HWA may be minimal.

This work highlights that basal trunk sprays of imidacloprid can effectively reduce HWA densities, but higher residues achieved in trunk injected trees (across all post-treatment time intervals) suggest this application method is likely to provide a longer period of efficacious HWA control. Data analysis for both chemicals including the influence of tree size (DBH) on insecticide residues is ongoing.

EFFECT OF SILVICULTURAL CANOPY GAPS ON HEMLOCK WOOLLY ADELGID DENSITY AND EASTERN HEMLOCK HEALTH IN THE SOUTHERN APPALACHIANS

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ABSTRACT

A common goal in the use of silviculture is the creation of forest stands with composition and structure that provides resilience against environmental stressors, such as forest pests. Recent research (1-4) has demonstrated that the shade-tolerant foundation species, eastern hemlock (*Tsuga canadensis* (L.) Carrière), benefits from elevated sunlight levels when infested with the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand), an invasive insect that threatens the sustainability of hemlock in eastern North America. In this study, small silvicultural gaps were cut in the canopy above individual understory eastern hemlocks in mixed hardwood-conifer stands of the southern Appalachian Mountains to increase the amount of sunlight reaching each target hemlock.

Treatments included the combination of two gap sizes (Small or Large, averaging 0.02 ha and 0.06 ha, respectively) and two gap-creation methods (Fell or Girdle of competing trees), plus an untreated Control. A competition index was calculated post-treatment to reflect the amount of competition removed around each hemlock. Gap treatment effects on HWA densities, hemlock crown condition (foliage transparency and dead shoot tips) and hemlock growth (shoot and basal area) were examined. Results were analyzed separately for three latitude groups: South (34.7°N - 35.7°N, in GA, TN, and NC), Central (36.7°N - 37.7°N, in VA) and North (38.7°N - 39.7°N, in MD).

In all three latitude groups, the treatment that removed the most competition (Large Fell) increased the production of new shoots, reduced the percentage of dead shoots, reduced foliage transparency, and increased basal area increment of target hemlocks relative to unreleased Control trees. Furthermore, small gaps and those that removed fewer competing live trees (Girdle treatments) also improved hemlock health, particularly in the South latitude group. These positive effects of canopy gaps on hemlock crown condition and growth occurred despite the fact that trees in Large Fell gaps generally sustained similar or higher HWA densities than Control trees. Regression analysis revealed that changes in crown transparency and basal area growth were strongly related to the competition index of live trees remaining post-cut. In the South latitude group, the stem density of woody species regeneration (stems ≥ 1 m tall and < 7.6 cm dbh) increased significantly in the three years following gap creation and were highest in the Large Fell treatment.

Our results support the previously proposed (2,4) hypothesis that the increased availability of sunlight (and possibly other limited resources) resulting from canopy gaps functions to improve the physiological tolerance of eastern hemlock to HWA infestation. Additionally, the results suggest that silvicultural release of infested eastern hemlock may favor more temporally consistent adelgid populations through maintenance of better host quality and availability of feeding sites. Thus, silvicultural manipulations that increase sunlight on hemlock could compliment biological

control efforts by promoting a more consistent source of prey for predators while simultaneously improving crown health. Because increases in sunlight stimulate more regeneration of faster-growing hardwood species, silvicultural approaches using larger gaps may need to consider vegetation control methods to minimize future shading of hemlock.

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OOBIUS AGRILI: ESTABLISHMENT, PERSISTENCE, AND EMERALD ASH BORER EGG PARASITISM

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ABSTRACT

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a solitary egg parasitoid that is native to Northeast China (Liu et al. 2007, Wang et al. 2010, Zhang et al. 2005). In 2007, *O. agrili* was approved for release in the U.S. for biocontrol of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a highly destructive pest of ash trees (Bauer et al. 2015, Duan et al. 2018). To date, over 2.5 million *O. agrili* have been released in North America, encompassing much of EAB's current invaded range. However, few studies have investigated its impacts on EAB populations. This can be partially attributed to the difficulty of surveying for *O. agrili* given that it is only 1 mm long, and the EAB eggs it attacks are hidden in bark cracks and crevices. We evaluated its establishment success, persistence, and EAB egg parasitism rates in two geographic areas where *O. agrili* was released at different times following the initial EAB invasion. One area was in south-central Michigan, where *O. agrili* was released during 2007-2010, and established for several years; and the second in three Northeastern U.S. states, where *O. agrili* was released in 2015-2016 (MapBioControl 2020). To sample *O. agrili*, we used a draw knife to scrape the outer corky bark layer from EAB-infested ash trees, and then sifted and sorted the bark in the laboratory for EAB eggs, following methods described in Abell et al. (2014). Each recovered EAB egg was closely examined to determine if it had hatched, was parasitized by *O. agrili*, or died from other causes. These data were used to determine *O. agrili* presence and percentage of EAB eggs attacked at each study site.

In Michigan, we sampled 5–10 trees each year at six *O. agrili* release sites during 2016–2018 and 2020. Each site included a plot where *O. agrili* were released, and a control plot located 0.6-9.1 km away where parasitoids were not released. We found that *O. agrili* was persisting at five of the six original release plots. *Oobius agrili* had also spread to all control plots, including the control plot paired with the release site where *O. agrili* was not established. It is likely that *O. agrili* had spread to our plot from another parasitoid release plot not associated with our study that was located 10.2 km away. Although, EAB egg parasitism varied significantly among individual study plots, regardless of treatment, the overall EAB egg parasitism did not vary significantly between release (23.5 ± 3.9 percent) and control plots (19.7 ± 3.2 percent).

In the Northeastern U.S., we sampled five trees each year at five *O. agrili* release sites during 2018-2020: three in Connecticut, one in Massachusetts, and two in New York. We found evidence of *O. agrili* establishment at all sites, but parasitism rates varied significantly among sites. EAB egg parasitism across all sites averaged 3.2 ± 2.7 percent in 2018 (2-3 years after initial release) and increased to 26.2 ± 6.1 percent and 21.2 ± 5.5 percent in 2019 and 2020, respectively.

Our results confirm that *O. agrili* is establishing and persisting in northern regions of the U.S. and is spreading several km from release sites. Overall EAB egg parasitism rates were not as high as some reports for the introduced EAB larval parasitoids, *Tetrastichus planipennisi* Yang

(Hymenoptera: Eulophidae) and *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) (Duan et al. 2017, 2019, 2021, 2023; Gould et al. 2022). However, *O. agrili* does have a unique advantage over larval parasitoids in that it kills the EAB host before larval feeding damages the tree's vascular system. Given that *O. agrili* is currently the only EAB egg parasitoid approved for release in the U.S., this species remains an important component of the EAB biocontrol program.

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MONITORING THE OCCURRENCE OF L. *CRENATAE MCCANNII*; THE CAUSATIVE AGENT OF BEECH LEAF DISEASE ACROSS OHIO

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ABSTRACT

The foliage nematode *Litylenchus crenatae* ssp. *mccannii*. (LCM), is a nonnative pest in North America and Canada, and is the cause of Beech Leaf Disease (BLD). BLD can be characterized as interveinal leaf darkening, gal formation, and thickening of leaf tissue on *Fagus grandifolia* (American beech). Original detection was in Lake County, Ohio in 2012 and has since been found across the northeastern United States and into Canada. This nematode impacts tree health and damages leaf tissue prior to bud break.

In partnership with Ohio Department of Natural Resources, the goal of this monitoring project is early detection of LCM across 24 counties in Ohio. To obtain the greatest concentration of nematodes, beech buds were collected in the spring before bud break. Molecular techniques were used to extract environmental DNA (eDNA) from buds followed by Polymerase Chain Reaction (PCR). PCR provides positive or negative results of eDNA and is used to amplify the ribosomal RNA region with primers specific to LCM (33F, 234R) and is followed by Sanger Sequencing of positive samples to confirm the accuracy of the PCR primers.

Results show that counties with known BLD symptoms had high levels of LCM detection in buds in 2021 and 2022. We found several asymptomatic counties that were negative in 2021 but positive in 2022. This suggests that nematode eDNA may be transitory or just arrived (early detection) in many locations; however, additional monitoring is needed. There were three asymptomatic counties consistently positive for LCM eDNA in both years. A follow-up survey in 2023 is needed to determine if eDNA is transitory or indicative of live nematodes bud tissue. One of our sites had low detection of LCM eDNA for both years, and in the second year we found symptomatic leaves near the plot. This suggests consistent positive LCM could indicate infestation even in the absent of visible symptoms. This work is significant in developing an early warning system for mass testing of A. beech in the detection of LCM.

INTERACTIONS BETWEEN EXOTIC EARTHWORMS AND THE INVASIVE PLANT PALE SWALLOW-WORT

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ABSTRACT

The invasion of northeastern forests by exotic earthworms has had profound effects on soil structure, hydrology, and biogeochemical processes. Long-established European earthworms are now competing with a new wave of Asian species, colloquially referred to as jumping worms.

These forests have also been extensively altered by invasive plants, including pale swallow-wort (*Vincetoxicum rossicum*), a perennial vine that can dominate deciduous forest understories.

Understanding the potential for synergistic or antagonistic interactions between these concomitant invasions is important for predicting outcomes and developing management strategies. Beneficial interactions among invasive plants and exotic earthworms have been identified and these positive feedback loops raise concerns about further spread and proliferation.

The overarching goal of this research was to quantify the interactions between pale swallow-wort and earthworms to determine if they have synergistic or antagonistic relationships. Field surveys were conducted throughout Central New York to determine earthworm abundance and species composition in quadrats with varying amounts of pale swallow-wort cover. Earthworm species diversity was lowest in quadrats with the most swallow-wort. Earthworm species composition varied with soil pH, September soil temperature, and July soil moisture. These data have not provided evidence of a relationship between pale swallow-wort and earthworms thus far. Further research and statistical analyses will expand upon these preliminary findings.

PHYLOGENETIC STUDIES AND DNA BARCODING OF THE INVASIVE CHESTNUT GALLWASP *DRYOCOSMUS KURIPHILUS* (HYMENOPTERA: CYNIPIDAE: CYNIPINI) IN THE EASTERN UNITED STATES

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ABSTRACT

The chestnut gallwasp (CGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), is a globally invasive pest threatening the global chestnut industry. In the US, CGW could have a profound effect on American chestnut restoration efforts by the USFS. Originating in China, CGW has been widely spread in the Eastern United States. Recent discovery of *D. zhuili* Liu and Zhu, which is attacking another chestnut species, *Castanea henryi*, in China, has complicated our understanding of which gallwasp species are affecting American chestnut restoration in the Eastern United States. We reconstructed phylogenies for CGW and its global congeners to improve our knowledge of this pest. Both the four-gene and UCE-based phylogenies revealed *Dryocosmus* is polyphyletic, and CGW is distantly related to congeners in North America, and the four-gene data supported CGW as closely related to congeners that gall *Castanopsis* in Asia. DNA barcoding from four states revealed CGW is the single *Dryocosmus* pest species currently affecting chestnuts in the Eastern United States. Our results suggest North American *Dryocosmus* species are less pestiferous to chestnuts. Lastly, our phylogeny will serve as a backbone for a complete reclassification of the species currently placed in *Dryocosmus*.

56/30 WAS MADE TO FAIL: THE STORY OF AN INADEQUATE PHYTOSANITARY MEASURE

Joshua Vlach

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ABSTRACT

Exotic woodboring pests continue to be intercepted at North American borders and found within North America, despite ISPM15 having been in place for 16 years. One question is whether the heat treatment requirement of 56 degrees C for 30 minutes (56/30), measured at the core of the wood piece, is adequate to act as a phytosanitary measure.

International Standards for Phytosanitary Measures Number 15 (ISPM15) was developed under the International Plant Protection Convention (IPPC) to regulate treatment of wood used in transporting goods (solid wood packing material or SWPM). Countries began adopting these standards in 2003. It was thought that appropriate treatment could effectively eliminate the movement of tree attacking pests.

The available data on heat treatment efficacy is based on 11 out of nearly 60,000 species in important woodborer Families. Particularly notable is a lack of data for ambrosia beetles (Scolytinae) and woodwasps (Siricidae). There are numerous indications of the inadequacy of 56/30. Several of the heat treatment studies found less than 100 percent control. Woodborers are routinely intercepted at international ports. For the US, studies have found that woodborer interceptions have reduced somewhere in the range of 36 to 61 percent from pre-ISPM15, depending on the timeframes and commodities compared. Woodborers are still found establishing on new continents. Importers are experiencing rejections due to detections of infested SWPM, to the point where some are trying to improve wood quality on their own.

Unfortunately, the standard of 56/30 sets the IPSM15 program up for significant failure. If shipping companies are working to meet 56/30 and still experiencing rejections, this is no surprise. Eliminating port rejections for rule abiding importers will be difficult until an adequate temperature regime is adopted. Any increase in time and temperature is desirable, but 71/60 is the ideal and would leave only incompetence and fraud as the cause of woodborer detections. 60/60 would be a significant improvement, and while we might expect occasional survival, we would expect a significant reduction from current detections. Changes can still be made to prevent further movement of wood attacking pests!

ADULT EMERGENCE OF THE EMERALD ASH BORER IN ASH AND FRINGE TREES: IMPLICATIONS FOR HOST RANGE EXPANSION

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ABSTRACT

Emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) is an invasive wood-boring beetle native to Asia. Since it was first detected in the United States in 2002, EAB has killed hundreds of millions of ash trees (*Fraxinus* spp.) and is the costliest forest pest to ever invade North America. Though EAB was long considered an ash specialist, it has recently been recorded developing on white fringe tree (*Chionanthus virginicus* L.), a member of the olive family closely related to ash. The extent to which EAB attacks and develops on white fringe tree in the field and, thus, the potential for white fringe tree to become an EAB reservoir, is uncertain. In spring of 2021, eighteen healthy potted green ash trees (*Fraxinus pennsylvanica* Marsh) and white fringe trees were bought from a nursery, randomly paired, and exposed to natural EAB populations in Newark, DE for one year. The trees were inspected for signs of EAB attack in summer of 2022. The same summer, eighteen white fringe trees and twelve green ash, black ash (*Fraxinus nigra* Marsh), and white ash trees (*Fraxinus americana* L.) growing in Newark, DE were inspected for EAB damage. The proportions of ash trees versus fringe trees exhibiting EAB exit holes were compared in both the potted and field populations. After a year, a significantly higher proportion of potted green ash trees exhibited EAB exit holes than potted white fringe trees of the same size and age. Two out of eighteen potted white fringe trees (11 percent) had exit holes, compared with 61 percent of potted green ash trees. Similarly, a greater proportion of ash trees in the field survey exhibited EAB exit holes than white fringe trees. Five out of twelve wild ash trees (42 percent) exhibited exit holes, whereas none of the eighteen field-grown white fringe trees showed signs of EAB emergence. Overall, successful EAB development is lower in white fringe tree than in ash. In this study, EAB only emerged from potted fringe trees, which were likely already stressed. Naturally growing white fringe trees in the same area did not exhibit any signs of adult emergence. It is unclear whether EAB oviposit less frequently on white fringe tree, or if fringe tree defenses impede EAB's successful development to adulthood, making exit holes less common. Though stressed white fringe tree has the capacity to support successful EAB development, it may act more as a sink rather than a reservoir, preventing complete development in most cases. If so, the role of white fringe tree as a reservoir for EAB will be limited.

IN MEMORIAM

Leland (Lee) Humble, 1951-2020

Lee Humble was an eminent forest entomologist working at the Canadian Forest Service Pacific Forestry Centre in Victoria BC for 35 years. During his career, Lee made many important contributons, but much of his work focused on the biology and ecology of non-natve forest pests in North America. He was one of the first scientsts to use chemical atractants to survey for ambrosia beetles and discovered several non-natve species that were previously not known to be established in North America but his work showed that they were widely established and sometmes displacing natve species. He was a regular presenter at the Interagency Research Forum where he shared many of his important research results and generously contributed advice to other scientsts.

Max W. McFadden, Jr., 1936-2022

Max McFadden worked in the US Forest Service Research & Development for 25 years under various leadership capacites. From 1974-1984, he served as program manager for the Douglas-fir Tussock Moth and Western Spruce Budworm research programs in the Pacific Northwest Research Staton. He later joined the Northeastern Forest Experiment Staton where he served as Program Manager for the Expanded Gypsy Moth Research Program (now ‘spongy moth’), Project Director for Acid Rain research and Assistant Staton Director for research units on wildlife, entomology, harvestng, watershed hydrology, sociology, fire and forest management. It was under his leadership that the current Research Forum on Invasive Species was initally conceived as the Annual U.S. Department of Agriculture Interagency Gypsy Moth Research Forum.

Gary Lovett, 1953-2022

Gary Lovet was a forest ecologist at the Cary Insttute of Ecosystem Studies in Millbrook, NY. Gary did groundbreaking work documentng the vast ecosystem impacts of non-natve forest insects and disease on forests in eastern North America. In the later years of his life, recognizing the enormity of the ecological impacts of these organisms, Gary had a strong desire to improve the situaton in the future by advocatng for policies to improve exclusion of these species. As part of this work, he initated a program called Tree-SMART Trade, to educate the public and advocate for government policy on forest biosecurity in the US. This program lives on and serves as a tribute to Gary’s love of forest ecosystems.

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FORUM AGENDA

Tuesday Afternoon, January 10, 2023
Poster Session – Sheet Bend (Powerhouse, 3rd Floor)
3:00 – 6:00 PM

Wednesday Morning, January 11, 2023
Opening Session – Ballroom
8:15 A.M. – 12:00 P.M.

Moderator: Scott Pfister, USDA APHIS, Buzzards Bay, MA

Time	Session
8:15 a.m.	Welcome - Michael McManus, Emeritus Scientist, USDA Forest Service, Hamden, CT Opening Keynote Presentations
8:30 a.m.	Globalization and the accidental spread of invasive insects. Cleo Bertelsmeier, University of Lausanne, Lausanne, Switzerland
9:15 a.m.	Macrosystems invasion ecology: Patterns, processes, and prospects. Songlin Fei, Purdue University, West Lafayette, Indiana
10:00 – 10:30 a.m.	BREAK

Research Reports
10:30 a.m. – 12:00 p.m.

Moderator: Andreas Linde, University for Sustainable Development, Eberswalde, Germany

Time	Session
10:30 a.m.	Expanding the semiochemical toolbox for detection and monitoring of cerambycid beetles. Larry Hanks, University of Illinois, Urbana, IL
10:50 a.m.	Results of a worldwide trapping program using generic lures to detect cerambycid invaders at arrival on other continents. Alain Roques, INRA, Orléans, France
11:20 a.m.	The enigmatic return of a vanquished invader: Rise of the browntail moth. Dylan Parry, State University of New York, Syracuse, NY
11:40 a.m.	Spruce aphid population response to autumn temperature regimes in North American montane forests. Ann Lynch, USDA Forest Service, Tucson, AZ
12:00 – 1:00 p.m.	LUNCH

Wednesday Afternoon, January 11, 2023
Research Reports
1:00 – 3:00 P.M.

Moderator: Vince D'amico, USDA Forest Service, Newark, DE

Time	Session
1:00 p.m.	Using aerial imagery to detect and predict invasive plant spread using Callery pear as the model system. Jess Hartshorn, Clemson University, Clemson, SC
1:20 p.m.	Plant community dynamics following invasive species management depend on invasion intensity and forest site characteristics. Eric Moore, University of Delaware, Newark, DE
1:40 p.m.	Human-mediated dispersal drives the spread of the spotted lanternfly. Zachary Ladin, University of Delaware, Newark, DE
2:00 p.m.	Spotted lanternfly biological control prospects. Juli Gould, USDA APHIS, Buzzards Bay, MA
2:20 p.m.	Variation in spotted lanternfly response to temperature across the invaded range. Melody Keena, USDA Forest Service, Hamden, CT
2:40 p.m.	The ash forest project: A visual telling of the emerald ash borer story. Gabriel Popkin, Independent Writer and Leslie Brice, Photographer, Mount Rainier, MD
3:00 – 3:30 p.m.	BREAK

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FORUM AGENDA CONTINUED

Wednesday Afternoon, January 11, 2023
Research Reports
3:30 – 5:00 p.m.

Moderator: Melody Keena, USDA Forest Service, Hamden, CT

Time	Session
3:30 p.m.	Beech leaf disease: What's going on? And what does the future look like? Enrico Bonello, The Ohio State University, Columbus, OH
3:50 p.m.	Genetic and experimental evidence for complex patterns and feedbacks in the beech bark disease system. Jeff Garnas, University of New Hampshire, Durham, NH
4:10 p.m.	New pest detection across the US: the role of the public. Rebecca Epanchin-Neill, Resources for the Future, Washington, DC
4:30 p.m.	Elm zigzag sawfly (Aproceros leucopoda) invades the eastern US. Kelly Oten, North Carolina State University, Raleigh, NC
5:00 – 6:00 p.m.	POSTER SESSION
6:00 – 8:00 p.m.	MIXER

Thursday Morning, January 12, 2023
Emerald Ash Borer
8:00-9:50 a.m.

Moderators:
Juli Gould, USDA APHIS, Buzzards Bay, MA
Jian Duan, USDA ARS, Newark, DE

Time	Session
8:00 a.m.	Introduction to EAB Research, Jian Duan, USDA ARS, Newark, DE
8:05 a.m.	New detection of EAB in Oregon and implications for western US states. Max Ragozzino, Oregon Department of Agriculture, Salem, OR
8:20 a.m.	Proof of a genetic basis for resistance to EAB: Updates on breeding research. Jennifer Koch, USDA Forest Service, Delaware, OH
8:45 a.m.	EAB early detection in Europe: have we got the tools to find it quickly enough to contain it? Hugh Evans, Forest Research, Alice Holt Lodge, Farnham, UK.
9:00 a.m.	Introduction to classical biocontrol of EAB. Juli Gould, USDA-APHIS, Buzzards Bay, MA
9:05 a.m.	The USDA biocontrol program against emerald ash borer in the U.S.: parasitoid production, release, and recovery. Ben Slager, USDA APHIS, Brighton, MI
9:20 a.m.	Evaluating the factors affecting the establishment of EAB parasitoids. Rafael de Andrade, University of Maryland, College Park, MD
9:35 a.m.	Dispersal and spread of EAB parasitoids following release. Nicole Quinn, University of Florida, Fort Pierce, FL
9:50 a.m.	Impact of EAB invasion stage and post-release time on the persistence and impact of introduced EAB parasitoids. Claire Rutledge. CT Agricultural Experiment Station, New Haven, CT
10:05–10:20 a.m.	BREAK

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FORUM AGENDA CONTINUED

Thursday Morning, January 12, 2023
Emerald Ash Borer
10:20 a.m. – 12:00 p.m.

Moderators:
Juli Gould, USDA APHIS, Buzzards Bay, MA
Jian Duan, USDA ARS, Newark, DE

Time	Session
10:20 a.m.	Mortality of EAB and survival of sapling ash in forests following the collapse of EAB outbreaks. Juli Gould, USDA APHIS, Buzzards Bay, MA
10:40 a.m.	EAB population suppression by introduced natural enemies and impact on ash survival. Jian Duan, USDA ARS, Newark, DE
11:05 a.m.	Persistence of ash regeneration a decade after initial emerald ash borer infestation in New York State. Tim Morris, State University of New York, Syracuse, NY
11:25 a.m.	Integration of biocontrol with treatment of ash trees in urban and natural forests. Theresa Murphy, USDA APHIS, Buzzards Bay, MA
11:45 a.m.	Development of drone-based remote sensing technology with machine learning algorithms to assess the impact of EAB biocontrol program on ash growth and recovery. George Ni, University of Vermont, Burlington, VT
12:00 – 1:00 p.m.	LUNCH

Thursday Afternoon, January 12, 2023
Hemlock Woolly Adelgid
1:00 – 3:00 p.m.

Moderator: Joseph Elkinton, University of Massachusetts, Amherst, MA

Time	Session
1:00 p.m.	Prey-associated genetic differentiation in silver fly predators of hemlock woolly adelgid. Nathan Havill, USDA Forest Service, Hamden, CT
1:20 p.m.	Establishment of *Laricobius nigrinus* in the Northern US. Ryan Crandall, University of Massachusetts, Amherst, MA
1:40 p.m.	Utility of eDNA analysis for early detection and monitoring of HWA and biological control agents in eastern North America. Tonya Bittner, Cornell University, Ithaca, NY
2:00 p.m.	Evaluating efficacy and persistence of systemic insecticide applied with different methods for HWA control in Michigan. Phil Lewis, USDA APHIS, Buzzards Bay, MA
2:20 p.m.	HWA Biocontrol 2.0: Balancing operational and research activities between Laricobius beetles and Leucotaraxis silver flies. Scott Salom, Virginia Tech University, Blacksburg, VA
2:40 p.m.	Expanding the toolbox: Using silviculture in the integrated management of the hemlock woolly adelgid. Bud Mayfield, USDA Forest Service, Asheville, NC
3:00 – 3:30 p.m.	BREAK

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FORUM AGENDA CONTINUED

Thursday Afternoon, January 12, 2023
Research Reports
3:30 – 5:10 p.m.

Moderator: Therese Poland, USDA Forest Service, Lansing, MI

Time	Session
3:30 p.m.	The Palmetto State predicament: an update on the Asian longhorned beetle in South Carolina. David Coyle, Clemson University, Clemson, SC
3:50 p.m.	A programmatic update of ALB in South Carolina. Kimberly Dean, USDA APHIS, Hollywood, SC
4:10 p.m.	Tracking patterns of Asian longhorned beetle spread and eradication: Data synthesis and evolving needs. Talbot Trotter, USDA Forest Service, Hamden, CT
4:30 p.m.	Trends in woodborer detections in wood packaging materials since the U.S. fully implemented ISPM 15. Toby Petrice, USDA Forest Service, Lansing, MI
4:50 p.m.	Exploring pest mitigation research and management associated with the North American wood packaging supply chain: What and where are the weak links? Chris MacQuarrie, Canadian Forest Service, Sault Ste. Marie, ON

Friday Morning, January 13, 2023
Research Reports
8:00 a.m. – 11:00 a.m.

Moderator: Andrew Liebhold, USDA Forest Service, Morgantown, WV

Time	Session
8:00 a.m.	Synchrony of egg hatch with bud-burst drives population change of winter moth in eastern North America. Joe Elkinton, University of Massachusetts, Amherst, MA
8:20 a.m.	Change in the seasonal timing of flight of winter moth in eastern North America reinforces reproductive isolation in a clinal hybrid zone. Jeremy Andersen and Jennifer Chandler, University of Massachusetts, Amherst, MA
8:40 a.m.	Performance and impact of the invasive beech leaf-mining weevil, Orchestes fagi, on its native host (European beech) vs. new host, American beech. Jon Sweeney, Canadian Forest Service, Fredericton, NB
9:00 a.m.	Advances in biological control of the polyphagous shot hole borer. Christine Dodge, University of California, Riverside CA, and USDA APHIS, Buzzards Bay, MA
9:20 a.m.	Novel pathways for transport of fungal pathogens: Two case studies. Jason Smith, University of Florida, Gainesville, FL
9:40 a.m.	BREAK
10:00 a.m.	Biogeographic patterns of historical insect invasions. Andrew Liebhold, USDA Forest Service, Morgantown, WV
10:20 a.m.	Unintentional biological control is increasingly common for invasive insects. Don Weber, USDA ARS, Beltsville, MD
10:40 a.m.	Learning from the past: A retrospective of pests detected on sentinel North American trees abroad. Geoffrey Williams, USDA Forest Service, Washington, DC
11:00 a.m.	Concluding Remarks. Michael McManus, USDA Forest Service, Hamden, CT

CONFERENCE POSTERS

	Title	Speaker
1	OOBIUS AGRILI: ESTABLISHMENT, PERSISTENCE, AND EMERALD ASH BORER EGG PARASITISM	T.R. Petrice
2	"MONITORING THE OCCURRENCE OF L. CRENATAE MCCANNII; THE CAUSATIVE AGENT OF BEECH LEAF DISEASE ACROSS OHIO"	Mary C. Pitts
3	INTERACTIONS BETWEEN EXOTIC EARTHWORMS AND THE INVASIVE PLANT PALE SWALLOW-WORT	Laura K. Stark
4	PHYLOGENETIC STUDIES AND DNA BARCODING OF THE INVASIVE CHESTNUT GALLWASP DRYOCOSMUS KURIPHILUS (HYMENOPTERA: CYNIPIDAE: CYNIPINI) IN THE EASTERN UNITED STATES	Chang-Ti Tang
5	56/30 WAS MADE TO FAIL: THE STORY OF AN INADEQUATE PHYTOSANITARY MEASURE	Joshua Vlach
6	ADULT EMERGENCE OF THE EMERALD ASH BORER IN ASH AND FRINGE TREES: IMPLICATIONS FOR HOST RANGE	Heather L. Callahan

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