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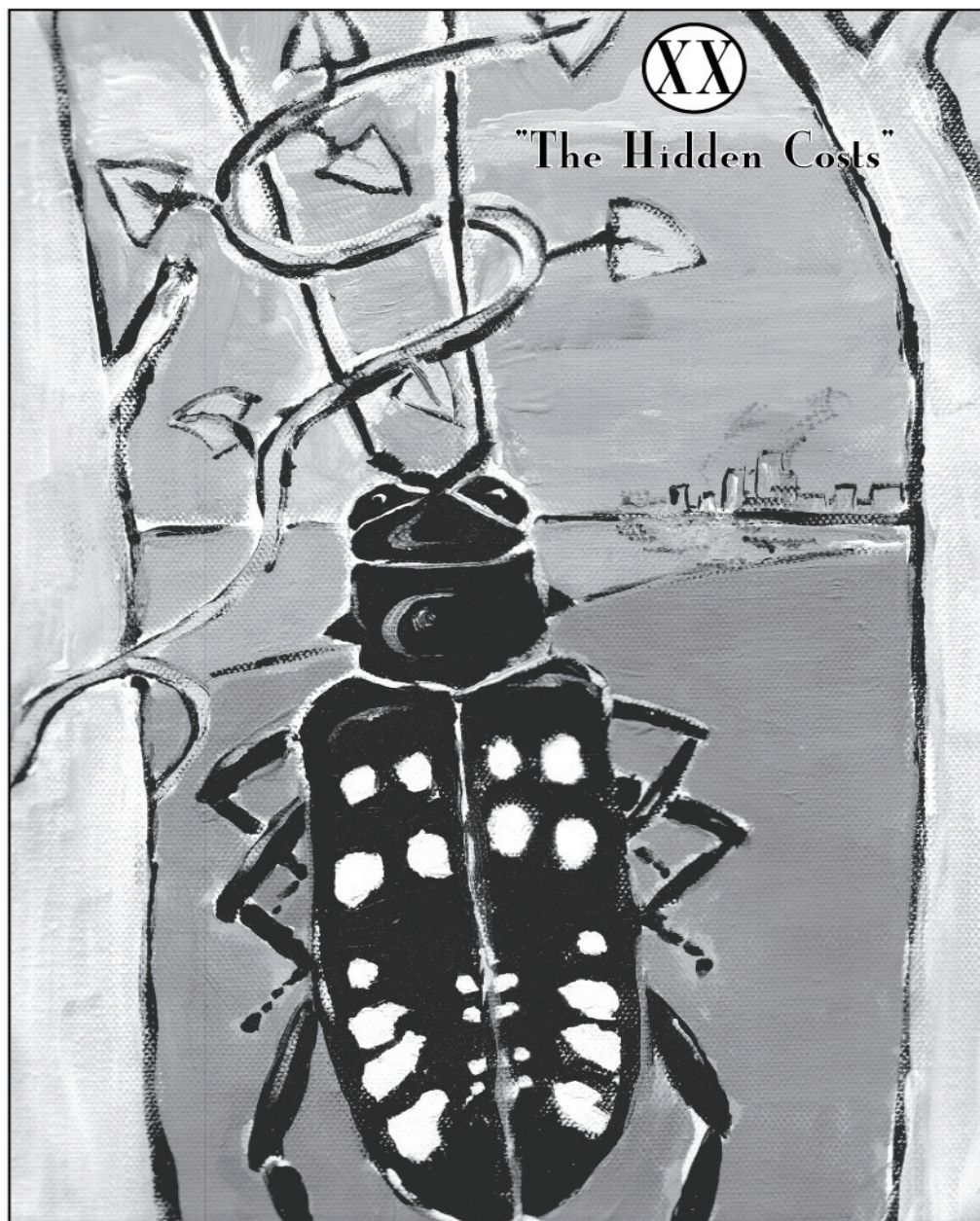
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USDA RESEARCH FORUM
on INVASIVE SPECIES

January 13-16, 2009
Annapolis, Maryland

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20th U.S. Department of Agriculture Interagency Research Forum on Gypsy Moth and Other Invasive Species, 2009



January 13-16, 2009
Loews Annapolis Hotel
Annapolis, Maryland

Edited by
Katherine McManus and
Kurt W. Gottschalk



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FOREWORD

This meeting was the 20th in a series of annual USDA Interagency Research Forums that are sponsored by the Forest Service, Animal and Plant Health Inspection Service, and Agriculture Research Service. The group's original goal of fostering communication and providing a forum for the overview of ongoing research among the agencies and their cooperators is being realized and facilitated through this meeting.

The 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 three USDA agencies and the management and staff of the Loews Annapolis Hotel for their continued support of this meeting.

Program Committee

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

Local Arrangements

Katherine McManus

Proceedings Publication

Katherine McManus, Kurt Gottschalk

EVOLUTION OF A MEETING: 20+ YEARS OF RESEARCH COMMUNICATION AND COORDINATION

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The genesis of the USDA Interagency Research Forum on Invasive Species can be traced back to a special appropriation from Congress in 1983 to initiate a Gypsy Moth Research and Development Program in response to the massive gypsy moth outbreak of 1979-1982. Over 4 million hectares of forest land were defoliated in 1981. Though the Northeastern Research Station was the lead agency in this initiative, the U.S. Congress directed that the majority of the funding should be facilitated through state and university cooperators. In 1986, the Northeastern Research Station scheduled a review in Morgantown, WV, to review accomplishments of the research funded to date, reassess priorities, and identify research needs.

In July 1989, representatives of the Forest Service (FS), Animal and Plant Health Inspection Service (APHIS), and Agricultural Research Service (ARS) initiated regular meetings to discuss opportunities to improve cooperation and maximize resources among those agencies conducting research on the gypsy moth. Representatives from the FS, State and Private Forestry, and the Cooperative States Research Service were added to the group which was called the “USDA Gypsy Moth Research and Development Coordinating Group” and was chaired by Max McFadden (FS, retired). The group determined that a combined interagency review of all gypsy moth R&D activities, including those of cooperators, would add immeasurably to better communication, and also agreed that proceedings should be published following the meeting. The initial meeting was held January 22-25, 1990, in East Windsor, CT, and was referred to as the USDA Interagency Gypsy Moth Review (Gottschalk et al. 1991). The objectives of the inaugural and subsequent meetings was to coordinate research on the European and Asian gypsy moth among USDA scientists and their extramural cooperators by facilitating the open exchange of information and data, and to encourage collaboration among the many scientists involved.

Beginning in January 1991, this meeting was held at the Loews Annapolis Hotel in Annapolis, MD. The meeting gained added stature when scientists from Asia, Europe, Australia, and elsewhere in North America were invited to attend and participate. The involvement of foreign scientists from countries where the gypsy moth and related species of Lymantriids are endemic added a global perspective to the meeting and enhanced international cooperation, particularly in the development and use of biologically based technologies. The introduction of the Asian gypsy moth to ports in Washington, Oregon, and Vancouver in 1991, and to Wilmington, NC, in 1993 provided increased relevance and significance to the meeting.

The program for the 1996 meeting was broadened to include topics related to the potential impacts of nonnative invasive species, prompted by introductions into North America of several exotic bark beetles and emerging concern over exotic weeds. The change in direction was especially timely since the Asian longhorned beetle (ALB) was detected in several locations in the greater New York metropolitan area in the fall of 1996 and in Chicago, IL, in 1998. To reflect the increase in emphasis by federal agencies to address the emerging ALB problem and to develop eradication strategies, the title of the 1999 meeting was modified to “USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species.”

In recent years, a complex of nonnative invasive species including emerald ash borer, citrus longhorned beetle, the European wood wasp, and several species of ambrosia and Scolytid bark beetles have been introduced into North America and collectively threaten forest and urban ecosystems; other introduced species such as the hemlock woolly adelgid and the gypsy moth continue to extend their range further south and west. Additionally, pathogens that cause sudden oak death and butternut

canker, and a plethora of invasive plants contribute to our management problems and threaten native ecosystems. All of the aforementioned invasive species have been addressed at the annual meetings in individual sessions or through oral and poster presentations.

The European community has become more actively involved and concerned about the threat of invasive species since many of the species mentioned have recently been detected in several western European countries with the emerald ash borer being discovered in Moscow in 2007.

Finally, in 2008, the title of the meeting was again modified to “USDA Research Forum on Invasive Species” to better reflect the emphasis and content of the meeting. The objectives of the Research Forum are as relevant in 2009 as they were at the meeting’s inception in 1990: to coordinate research among USDA scientists and

extramural cooperators by facilitating an open exchange of information and data and to encourage collaboration. However, the scope of the collaboration now covers a multitude of invasive species including the Asian and European gypsy moths that started the evolution.

The series of meetings has produced a parallel series of proceedings. Early on, the decision was made to focus primarily on abstracts of the presentations with a few longer papers to summarize key findings. The extended abstract approach provided two valuable benefits—it did not preclude the researchers from publishing their findings in peer-reviewed journals and it allowed for rapid publication of findings in an abbreviated form but that could be cited. The series of proceedings publications (Appendix A) provide a “trail or roadmap” of how USDA research has responded to the introduction of a number of important forest invasive species over the years.

APPENDIX A

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ASIAN LONGHORNED BEETLE SUCCESSES AND CHALLENGES IN 2008

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Eradication Successes

In 2008, the Asian longhorned beetle (ALB) (*Anoplophora glabripennis*) program announced the eradication ALB in two previously infested areas—Hudson County, NJ, on April 7 and Chicago, IL, on April 17. Hudson County was a small infested site just west of Manhattan, NY. The first and last ALB detection was in October 2002. One hundred thirteen infested and 348 high risk host trees were removed. Four years of survey and 3 years of chemical treatment were completed to ensure ALB was eradicated from the area. ALB was first detected in Chicago in July 1998. At its peak, 35 square miles were quarantined. The program removed 1,551 infested and 220 high risk host trees from the area. In addition, host trees in a 61 square mile area were surveyed for 4 years and trees in the core infested area were treated a minimum of 3 years. The last detection in Chicago was in November 2003. Early cooperation among Federal, State, and local agencies made the program a success.

Illinois – Deerfield

On August 5, one adult ALB collected in Deerfield, IL was confirmed by the USDA Systematic Entomological Laboratory. The beetle was found by an alert individual in a Deerfield parking lot located about 12 miles north of the northern boundary of the previously regulated area of Chicago. Surveyors examined 9,511 primary host trees. APHIS officials investigated companies within the surrounding area who imported cargo from Asia as a potential source of introduction. In addition, information was sent to 343 green industry contacts asking for assistance in locating the source of the beetle. No infested trees were identified in Deerfield, Northbrook, Northfield Township, or Highland Park. A public outreach campaign will be executed in 2009 to assist the program in possibly locating the source of the beetle.

New York

In New York, eradication activities are ongoing. There is a 140 square mile quarantine in effect. Ground surveys continue in all program areas with climbing surveys focused in Islip to confirm that there are no remaining ALB infested trees in the area. These surveys will continue through 2009 in Islip and Staten Island to delimit the infestation.

In 2008, 52 infested trees were discovered in infested areas of New York: 49 in Brooklyn, one in Queens, and two in Central Long Island. Additionally, on December 31, 2008, 12 infested trees were found in the Staten Island quarantined area as a result of the delimitation surveys being conducted in response to the 2007 detections on Prall's and Staten Islands. Initial aging suggests these trees were first infested in 2005. Scientists are proposing that these infested trees could have been the result of dispersal from the nearby infestation detected in 2007. The infested trees will be removed as well as 25 high risk host trees in the immediate area. The regulated area will expand 2 square miles to the east in response to this detection and an additional 8,200 trees will be treated in spring 2009. Survey activities will also expand.

In 2008, chemical treatments were applied to 49,404 ALB host trees in sections of New York: 14,670 in Queens, 34,734 in Brooklyn, and 17,927 in Staten Island. In 2009, approximately 40,000 trees are anticipated to be treated in sections of Brooklyn and Queens; 26,100 trees will be treated in Staten Island.

New Jersey – Middlesex/Union Counties

In May 2008, the delimitation survey (utilizing climbing, bucket trucks, and ground surveyors) of the 25 square

mile New Jersey quarantine was completed. No infested trees have been detected since 2006. With the completion of the delimitation survey in New Jersey, resources were moved to Staten Island to work on the delimitation of that area.

In 2008, chemical treatments were applied to 12,370 ALB host trees in Carteret, Linden, and Roselle. Treatments were reduced by approximately 58 percent since 3 years of chemical treatment were realized in 2007 within the infested areas of Avenel, Rahway, the majority of Carteret, and sections of Linden and Roselle. In the spring of 2008, New Jersey State Forestry planted 749 ALB nonhost trees.

Massachusetts

On August 1, 2008, an ALB infestation was detected in Worcester, MA. This was the first find in the State and was most likely a separate introduction from other ALB detections, since the core infested area is in a light industrial area consisting of companies that import or have imported products from Asia. Personnel were deployed by APHIS, U.S. Forest Service, Massachusetts Department of Conservation and Recreation, and the city of Worcester to survey the area.

In 2008 a total of 6,431 infested trees were detected, three in West Boylston and the remainder in Worcester. Sixty-four square miles are currently regulated by APHIS and the state of Massachusetts, including the entire city of Worcester and portions of the towns of Holden, Boylston, West Boylston, and Shrewsbury. In order to control the disposal of wood

from the regulated area, a centralized disposal site has been established for use by the regulated municipalities and companies working within the regulated area.

On December 11, 2008, a severe ice storm hit New England. Significant tree damage and limb loss occurred within a 16-square-mile area covering the core of the ALB infestation. The ice storm created a very large volume of woody debris in the core infested area that required proper disposal to prevent spread of the infestation. Compliance trainings were offered on a daily basis to accommodate the influx of contractors working on debris removal in the ALB regulated area, and the state issued emergency debris removal contracts to assist the infested municipalities in clean-up operations. On January 5, 2009, a federal disaster declaration for public assistance was issued. APHIS received a technical assistance mission assignment from FEMA to provide guidance in the clean-up efforts.

Host removals began on January 5, 2009. Both infested and high risk host trees are being removed within the 2 square mile heavily infested core area. Ninety-two percent of private property owners within this 2-square-mile area are allowing complete host removal from their properties. Approximately 25,000 host trees are projected for removal in 2009. Forty percent of the trees (approximately 10,000), are less than 5 inches d.b.h..

For further information, refer to the APHIS ALB web site at http://www.aphis.usda.gov/newsroom/hot_issues/alb/alb.shtml

EMERALD ASH BORER BIOLOGICAL CONTROL: REARING, RELEASING, ESTABLISHMENT, AND EFFICACY OF PARASITOIDS

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ABSTRACT

The emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) is an invasive buprestid native to Asia that has killed millions of ash (*Fraxinus* spp.) trees in North America. It was first discovered in 2002 in areas of southern Michigan and Ontario, and infestations have since been found in Ohio, Indiana, Illinois, Maryland, Virginia, Pennsylvania, West Virginia, Wisconsin, Missouri, and Quebec. Due to extent of the outbreak and the challenges of locating and eradicating new infestations, regulatory agencies are now seeking methods for managing this destructive pest throughout North America. We began our search for EAB natural enemies in Michigan and in China in 2002. After finding only incidental parasitism of EAB in Michigan, we focused our research efforts on classical biological control of EAB using two EAB parasitoid species discovered in China. We are also collaborating with USDA APHIS (J. Gould) on a third EAB parasitoid species from China, as well as with scientists at Michigan State University (D. McCullough, D. Cappaert) to investigate the possible use of other EAB natural enemies in North America.

In southeastern Michigan from 2002-2004, no egg parasitoids were found and less than 1 percent of EAB larvae were attacked by parasitoids of other wood-boring insects. Through laboratory rearing, the following hymenopterans have been confirmed to parasitize EAB: three braconids *Atanycolus hicoriae*, *A. simplex*, *Spathius floridanus*=*simillimus*; one chalcid *Phasgonophora sulcata*, and native and exotic eupelmids, *Eupelmus pini* and *Balcha indica*, respectively (Liu and Bauer, unpublished data). Except for the two eupelmid species, these are known parasitoids of other *Agrilus* spp. in

North America. All are ectoparasitoids except *P. sulcata*. Another possible parasitoid of EAB reared from infested ash is the ichneuemonid *Dolichomitus dolichosoma*, however, parasitism of EAB is unconfirmed.

Although the diversity of parasitoids attacking immature EAB in Michigan was relatively high, prevalence of parasitism was lower than determined for EAB in China and for our native *Agrilus* spp. However, the recent discovery of 15 to 56 percent parasitism by *A. hicoriae* at two sites in southeastern Michigan from 2007 to 2008 has renewed interest in the potential of native parasitoids as EAB natural enemies (Cappaert and McCullough, unpublished data). They found this parasitoid also attacks several species of native *Agrilus* in Michigan, and it is likely that some parasitoids of wood-boring insects are more specific to niche than to host species. We developed a laboratory-rearing method for *A. hicoriae*, which can be used to elucidate its biology, behavior, host range, and potential for augmentative release against EAB.

In China, we found three hymenopteran parasitoids for use as EAB biocontrol agents in North America. These included: a gregarious larval endoparasitoid *Tetrastichus planipennisi* (Eulophidae), a gregarious larval ectoparasitoid *Spathius agrili* (Braconidae), and a solitary, parthenogenic egg parasitoid *Oobius agrili* (Encyrtidae). In our laboratory and in China, we studied *O. agrili* and *T. planipennisi* biology, developed rearing methods, quantified their effects on EAB and ash health in China, and evaluated their host specificities (Liu and Bauer, unpublished data). Similar research was completed for *S. agrili* by J. Gould and scientists in China.

In January 2007, we submitted permit requests and risk benefit analyses to APHIS to release the EAB parasitoids in Lower Michigan. This information was compiled into an environmental assessment and posted on the Federal Register for public comment. After review by researchers, land managers, and the public, APHIS issued a “Finding of No Significant Impact”, and granted release permits in July 2007. *O. agrili* and *T. planipennisi* were each released at two different sites in central Lower Michigan. The next year, *O. agrili* was recovered at both sites confirming successful reproduction and overwintering at these sites. *S. agrili* was released at three Michigan sites and later recovered at one.

In 2008, parasitoid release permits were requested and approved for release of EAB biocontrol agents in Indiana and Ohio. In collaboration with C. Sadoff (Purdue University), *O. agrili* was released at two sites in Indiana. In Ohio, *O. agrili* and *S. agrili* were released at two sites. In Lower Michigan, we established additional release sites at different EAB-population densities,

and in collaboration with J. Duan, J. Gould, and R. Van Driesche (University of Massachusetts), we started an EAB life table study to determine stage-specific parasitism by each parasitoid.

In addition, APHIS and Forest Service developed an EAB Biological Control Program designed to facilitate rearing, releasing, and evaluating establishment and efficacy of the three parasitoids species from China to reduce ash mortality in the United States. To help accomplish this, a parasitoid mass-rearing laboratory was built in Brighton, MI, and became operational in January 2009. Despite the challenges associated with rearing these parasitoids, we anticipate production scale-up and greater parasitoid availability over the next few years. To evaluate the efficacy of these EAB biocontrol measures, we are collecting field data, including ash health and EAB population densities at both release and control sites. This information will allow us to evaluate and optimize future EAB parasitoid releases.

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CHEMICAL AND PROTEOMIC APPROACHES TO DISSECTING ASH RESISTANCE TO THE EMERALD ASH BORER

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ABSTRACT

The literature contains relatively few studies addressing mechanisms of resistance of deciduous trees to wood-boring beetles. Several studies have hypothesized that both constitutive traits and feeding-induced responses in phloem tissue are key to resistance. Defense traits associated with resistance to herbivory fall into two major categories: 1) the presence of low and high-molecular weight anti-feedant/recalcitrant/deterrent/toxic metabolites, including defense-related proteins; and 2) physical barriers (e.g. lignified tissues) inhibiting spread of the invader. We are using a comparative approach to characterize the biochemical and molecular, constitutive and induced, phloem differences of susceptible and resistant ash species in order to identify key resistance mechanisms. Protein profiles will be compared using

differential in-gel electrophoresis (DIGE) and differentially expressed proteins will be sequenced and annotated (where possible). Homologous genes in model plants for which sequenced genomes are available (*Arabidopsis*, poplar) will be used to design primers to determine gene frequencies and expression levels. Phenolics and other secondary metabolites will be identified using HPLC-mass spectrometry and correlated with resistance. The results of this study may lead to the development of molecular markers in North American ash or North American/Asian ash hybrids that could accelerate selection of resistant individuals for use in ecosystem restoration, urban forestry, and commercial exploitation.

VOLATILE PROFILES AND TRAP CATCHES OF TWO PINE-HOST SPECIES OF *SIREX NOCTILIO*

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ABSTRACT

The woodwasp *Sirex noctilio*, Fabricius (Hymenoptera: Siricidae) is a pest of pine species first detected in the U.S. in New York state in 2004. Females inject mucus and the spores of the symbiotic fungus *Amylostereum aerolatum* when ovipositing or probing through the bark, which may eventually lead to the death of the tree. In North America the major host species are Scots pine (*Pinus sylvestris*), red pine (*P. resinosa*), and white pine (*P. strobus*). The use of herbicide treated trees for trapping is the most efficient way to monitor the insect; however this procedure causes death of the trap trees. Our goal is to develop a lure that is efficient enough to replace the herbicide treatment of trees.

We learned from studies conducted in 2006 by USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine (APHIS PPQ), in New York state, that Scots pine is the most attractive to the wasp among the above mentioned pine species. Moreover, it is widely accepted that volatile terpenes emitted by the trunk section of stressed trees play an important role in attracting the woodwasp to its host. Therefore we designed an experiment to investigate whether differences between the volatile production of Scots pine and white pine could possibly account for the difference in the observed preference of *S. noctilio*. We monitored the

volatile emission of untreated and herbicide treated Scots pines and white pines equipped with funnel traps in parallel with trap catches from early June through August 2008. A nondestructive volatile collection system developed at Penn State allowed us to collect samples multiple times from the same trees used in the experiment without causing additional damage. Herbicide treated Scots pines emitted significantly larger amounts of terpenes than herbicide treated white pines throughout the season, which correlates well with the significantly higher catches of the Scots pine trap trees. Furthermore, the percentage amount of a few terpenes, such as sabinene, β -myrcene, and Δ -3-carene, was significantly larger in the volatile blend emitted by herbicide treated Scots pines compared to herbicide treated white pines.

In our study in 2008 we were able to demonstrate that stress caused by injection of an herbicide affects the volatile production of Scots pine and white pine differently. Assuming that terpenes emitted through the trunk section of the trees attract *S. noctilio* to its host, the qualitative and quantitative differences observed between the volatile profiles of the two host species may explain the wasp's preference to Scots pine. We are planning to test this hypothesis in behavioral assays in the future.

BIOSURVEILLANCE: USING NATIVE WASPS TO FIND EMERALD ASH BORERS

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ABSTRACT

Successful management and mitigation of any invasive pest species depends on early detection tools. The emerald ash borer (EAB) (*Argilus planipennis* Fairmaire) (Coleoptera: Buprestidae) has already gained a substantial foothold in eastern North America and is having a profound effect on the region's ash-dominated forests. A native ground-nesting wasp, *Cerceris fumipennis* Say (Hymenoptera: Crabronidae), could prove to be a practical solution for this EAB detection problem. The wasp preys on the adult EAB, as well as related native beetle species. The wasp carries the paralyzed beetle back to its ground nest to feed to its larva.

Monitoring for the EAB involves watching the wasps as they return to their nests with prey. It has been shown that by simply observing the ground-nesting wasps provision their nests it is possible to quickly identify the presence of an EAB infestation.

Since 2006 we have worked to assess the biosurveillance potential of *C. fumipennis* as an EAB monitoring tool. Our field work has led us to the conclusion that this native wasp can be successfully used as such a tool.

Cerceris fumipennis effectively locates often cryptic buprestid beetles, including the EAB. The wasps not only discover beetles in often inconspicuous locations but they also proceed to carry the paralyzed prey back to a conspicuous wasp nest. Often found in large aggregations of independent burrows (a colony), the active wasps are capable of presenting the human observer with many buprestid beetles in a single day. Wasp colonies are frequently found in areas disturbed by human activity and are easily accessed for surveys. The wasp's foraging behavior is neither negatively influenced by close human scrutiny nor by the disruptive

process of 'prey inspection.' *Cerceris fumipennis* show no inclination to sting humans (even when roughly handled). The foraging of this wasp overlaps with EAB's flight season and may extend for a 2-month period, weather permitting. Taking advantage of the wasp's foraging activity for biosurveillance is a simple matter: at a naturally established wasp colony, plastic cups and a stopwatch are all the equipment needed to monitor for EAB. If naturally established *C. fumipennis* colonies cannot be found where needed the species also lends itself well to nest extraction and relocation.

Our primary research objective in 2009 is to assess the practicality of using mobile *C. fumipennis* nests to monitor for EAB. The conventional monitoring tools (trap trees and prism traps) will be used concurrently with mobile wasp nests thereby comparing the wasp's efficacy to the existing monitoring tools.

In addition to the comparison trial work, we will begin utilizing eastern North America's naturally established wasp colonies. These wasps have proven themselves unique in their ability to find EAB infestations but most colonies remain undiscovered or underutilized. They represent a pre-existing, currently operating, survey tool that simply needed to be found and observed. Mimicking a successful program from the state of Maine, we will foster and support a 'Cerceris Outreach Program'. The outreach program will educate and train public employees and volunteers as wasp watchers.

Individuals interested in learning more about *C. fumipennis*, searching for their own colonies or participating as a wasp watcher are encouraged to visit www.cerceris.info and email Philip Careless at pcarles@uoguelph.ca.

STRIKING GOLD IN SOUTHERN CALIFORNIA: DISCOVERY OF THE GOLDSPOTTED OAK BORER AND ITS CENTRAL ROLE IN OAK MORTALITY

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Since 2002, aerial survey data have revealed extensive oak mortality on Federal, State, tribal, and private lands in San Diego County, California. About 17,000 coast live oaks (*Quercus agrifolia*), California black oaks (*Q. kelloggii*), and canyon live oaks (*Q. chrysolepis*) have died in a 1,200 km² area centered on the Descanso Ranger District of Cleveland National Forest, and Cuyamaca Rancho State Park. Drought was considered the principal cause of this tree mortality for many years, and various pathogens have been suspected but never confirmed. In June 2008, the goldspotted oak borer, *Agrilus coxalis* Waterhouse (Coleoptera: Buprestidae), was identified as the primary cause of this oak mortality. An investigation of the collection history of *A. coxalis* (58 specimens or records from 25 collections) revealed that it was first recorded in the 1880s in Guatemala and southern Mexico, and then later in the early 1900s in southeastern Arizona (Table 1), suggesting that *A. coxalis* is native to Central and North America. It was first collected in southern California in 2004 through Department of Food and Agriculture survey traps. We hypothesize that *A. coxalis* arrived in southern California during the last 10-15 years as a consequence of either a continuous range expansion from adjacent Arizona or Mexico, or an introduction on oak firewood. The latter hypothesis is highly likely because firewood has been imported into southern San Diego County from Mexico for the last 20 years and the oak forests of southeastern Arizona are a relatively short auto trip from the southern California zone of mortality. Because the zone of mortality is isolated by desert to the east and by a band of healthy host type to the south and southeast, we conclude that the hypothesis of continuous range expansion is unlikely.

Ground surveys in 2008 established that the distribution of *A. coxalis* in southern California is contiguous with the zone of tree mortality. Observations documented through these surveys provide the first record of larval habits, host association, damage, and mortality associated with *A. coxalis* (Coleman and Seybold, 2008a, b), and firmly establish that the recent oak mortality in southern California, known colloquially as “oak croak,” can be explained logically and entirely by the feeding activity of this aggressive buprestid beetle. Early symptoms of infestation are dark-colored stains on the bark surface, D-shaped adult exit holes, and thinning crowns. Bark removed by foraging woodpeckers is also a common sign on *Q. agrifolia*. *Agrilus coxalis* attacks oaks aggressively along the main stem and largest branches. No additional insect species are associated with early *A. coxalis* injury. Larval galleries of *A. coxalis* are abundant on the wood surface, patches of cambium are killed, branches die back, and eventually trees die after several years of continuous infestation.

Our preliminary observations in CA suggest that *A. coxalis* tends to prefer *Q. agrifolia* and *Q. kelloggii* (both “red” oaks, subgenus *Erythrobalanus*) more than *Q. chrysolepis* (an intermediate oak species, i.e., neither a red nor a white oak, subgenus *Lepidobalanus*). There have been no observations of injury by *A. coxalis* to Engelmann oak, *Q. engelmannii*, a white oak species that occurs in San Diego Co. During a detection survey in the Huachuca and Santa Rita Mountains of southeastern Arizona (Cochise, Pima, and Santa Cruz Counties), we observed D-shaped exit holes, meandering dark-colored larval galleries on the sapwood, and pupal cells in the outer bark of silverleaf oak, *Q. hypoleucoides* (a thick-barked red oak). Similar

injury symptoms were noted and mature *Agrilus* sp. larvae were collected from the outer bark of a dying Emory oak, *Q. emoryi* (another red oak), at a location in the Santa Rita Mountains (Pima County). We suspect that these two native Arizona *Quercus* spp. are hosts of *A. coxalis*, whereas we found no evidence that the two native Arizona white oaks, Arizona white oak, *Q. arizonica*, and Gray oak, *Q. grisea*, had injury symptoms from *A. coxalis* or any woodboring Buprestidae. We hypothesize that phloem thickness, bark structure, and host chemistry may influence susceptibility to *A. coxalis*. White oaks commonly have fibrous, furrowed bark and thin phloem, whereas red oaks have thick phloem. Additional observations and host susceptibility tests are needed to test this hypothesis. Trapping studies were initiated in 2008 in two stands of *Q. agrifolia* in California to assess trap efficacy and flight periodicity of *A. coxalis*. Lindgren funnel traps (hung at 1.5 m), window traps (1.5 m), and purple prism flight-intercept sticky panel traps (3 m) were assessed for trap efficacy. Traps were baited with a high release rate ethanol attractant. Four traps were assessed for each trap type at each site and monitored weekly. Trap catches were 0 for funnel traps, 1 ± 0.4 for window traps, and 50.4 ± 12.4 for purple prism traps (mean \pm SE/trap/6 mo). Purple prism traps were significantly more effective at attracting *A. coxalis* than both other trap types ($F_{2,21}=15.1$, $P<0.001$).

Long-term plots were established in 2008 in stands of *Q. agrifolia* and *Q. kelloggii*/Jeffrey pine (*Pinus jeffreyi*) in California to assess oak susceptibility, forest stand mortality, regeneration, and to gather additional insect life history information. On these plots, 76 percent of the trees were *Quercus* spp., and 67 percent of these *Quercus* spp. had evidence of injury from *A. coxalis*, but no trees <15 cm at breast height had this injury. Oak mortality attributed to *A. coxalis* currently represents 13 percent of the total density of these stands.

The native distributions of the three California hosts of *A. coxalis* extend north through most of the state along the coastal foothills and along the Sierra Nevada Mountains (Fig. 1). The buprestid is currently injuring and killing *Quercus* spp. between 380 to 1830 m in southern California (Fig. 2). Previous collection records in its native region extend to 2,195 m (Table 1). Thus, this new pest to oaks has the potential to impact more northern regions in California. Firewood movement represents a significant pathway for introducing this species into these regions. Future studies will attempt to more clearly define the distribution of *A. coxalis* in southern California with prism flight-intercept sticky panel traps. Additional projects will investigate the potential for oak-specific kairomonal attractants and assess the efficacy of purple and lime-green prism traps at three heights and with three baits. Emergence and management of *A. coxalis* from oak firewood will also be examined.

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Table 1. Historical collection records for the goldspotted oak borer, *Agrilus coxalis* Waterhouse (Coleoptera: Buprestidae)

Date	Locality	Collection	Site Description	Notes/Comments
undated	Juquila, Mexico	BMNH		From Waterhouse (1889); Hespenheide (1979), lectotype
undated	Cordova, Mexico	BMNH		From Waterhouse (1889); Hespenheide (1979), paratype
undated	Capetillo, Guatemala	BMNH		From Waterhouse (1889); Hespenheide (1979), paratype
undated	S. Geronimo, Guatemala	BMNH		
undated	AZ	HESP	Miller Canyon, Huachuca Mts.	From Waterhouse (1889); Hespenheide (1979), paratype
undated	AZ	FMNH	Huachuca Mts.	From Hespenheide
				From J.N. Knull personal collection†, 2 specimens,
				Chas. Schaeffer co-types
VI-16	Cochise Co., AZ	FMNH	Palmerlee, Huachuca Mts.	From J.N. Knull personal collection†, determined by C. Schaeffer
VII-26	AZ	CAS	Santa Rita Mts.	From Chamberlain personal collection†; Also in Fisher (1928)
VII-26	Cochise Co., AZ	CAS	Palmerly	From Chamberlain personal collection†; catalog #231, paratype
VIII-4	Cochise Co., AZ		Rams (Ramsey) Cn., Huachuca Mts.	From Fisher (1928); C. Schaeffer personal collection
VIII-15	Cochise Co., AZ		Palmerlee, Miller Cn., Huachuca Mts.	From Fisher (1928); C. Schaeffer personal collection
IX-14	AZ	FMNH	Chiricahua Mts.	From J.N. Knull personal collection†
VIII-2-1905	AZ	AMNH	Huachuca Mts.	“R.C.”
VIII-4-1905	Cochise Co., AZ	USNM	Palmerlee, Miller Cn., Huachuca Mts.	From C. Schaeffer personal collection, 3 specimens, including 1 cotype†, Also in Schaeffer (1905), beating black oak branches ^s
VIII-10-1908	AZ	CAS	Chiricahua Mts.	From Van Dyke personal collection†
III-21-1939	Tamaulipas, Mexico	FMNH	Santa Engracia	From J.N. Knull personal collection†
VIII-15-1940	Pima Co., AZ	CAS	Madera Cn. Foothills, Santa Rita Mts.	Donated 1962†
VII-12-1950	AZ	FMNH	Huachuca Mts.	From J.N. Knull personal collection†, 3 specimens, including 1 collected on VII-19-1950
VII-15-1953	AZ	FMNH	Chiricahua Mts.	From J.N. Knull personal collection†, 2 specimens
VII-5-1956	Chiapas, Mexico	EMEC	5 mi SE San Cristobal de las Casas	From GHNC
VII-5-1956	Chiapas, Mexico	FSCA	5 mi SE San Cristobal de las Casas	From Hespenheide
VII-17-1957	AZ	FMNH	Chiricahua Mts.	From J.N. Knull personal collection†, 4 specimens
VI-17-1963	Santa Cruz Co., AZ	CIDA	Santa Rita Mountains, Madera Canyon, 1706 m	†
VII-24-1965	Pima Co., AZ	UAIC	Upper Bear Canyon, Santa Catalina Mts.	
VII-13-1965	Chiapas, Mexico	GHNC	2 mi NW Pueblo Nuevo, LLU Bio. Station	
V-11-12-1969	Chiapas, Mexico	CMNC	10 mi E Teopisca	From Hespenheide
V-11-12-1969	Chiapas, Mexico	HESP	10 mi E Teopisca	From Hespenheide
V-14-1969	Chiapas, Mexico	CMNC	16 mi E Teopisca	From Hespenheide
V-17-1969	Chiapas, Mexico	CMNC	8 mi NE San Cristobal de las Casas	From Hespenheide
V-26-1969	Chiapas, Mexico	CNCI	4 mi SE San Cristobal	From Hespenheide
VI-8-1969	Chiapas, Mexico	CNCI	3 mi NE San Cristobal	From Hespenheide
V-30-1969	Chiapas, Mexico	CNCI	Laguna Montebello Parq. Nat., 1524 m elev.	From Hespenheide
VI-5-1974	Chiapas, Mexico	CLBC	7 mi SE Teopisca	From Hespenheide
VIII-30-31-1977	Baja California Sur,	RLWE	Sa. Victoria, Sierra de La Laguna, trail W of La Laguna, 1830 m elev.	Beating <i>Quercus</i> sp.; Also in Westcott (2005)

VII-19-1981	Veracruz, Mexico	EMEC	Jalapa, Veracruz	
V-25-1987	Chiapas, Mexico	CLBC	11 km NE San Cristobal d. I. Casas	From Hespenheide
IX-28-1989	Chiapas, Mexico	CSCA	10 km E San Cristobal de las Casas	From Hespenheide
IX-1-1990	Chiapas, Mexico	HESP	16 km SO Ocosingo	From Hespenheide
VI-20-1990	Chiapas, Mexico	RHTC	30 km W Comitán	From Hespenheide
VII-25-30-1990	Chiapas, Mexico	TAMU	Municipio San Cristobal San Felipe, 2194 m elev.	Malaise Trap, From Hespenheide
VI-21-1990	Chiapas, Mexico	FSCA	Laguna Montebello Parq. Nat., 1524 m elev.	From Hespenheide
V-24-1991	Baja Vera Paz, Guatemala	CMNC	7.8 km W Chilasco, 1700 m elev.	From Hespenheide
V-10-1994	Tamaulipas, Mexico	USNM	10 m E Tula, 1189 m elev.	Oak Forest; J.E. Wappes
VII-5-2001	Oaxaca, Mexico	RLWE	10 km E Mitla, 1890 m elev.	Beating oak
VII-16-30-2004	San Diego Co., CA	CSCA	Paco Picacho Campground, Cuyamaca State Park	Also in Westcott (2005), 2 specimens
VI-18-2004	San Diego Co., CA	CSCA	Chamber Park, Cuyamaca State Park	Also in Westcott (2005)
VII-24-2006	San Diego Co., CA	CSCA	Julian, 4945 Heist Park Road	Funnel trap catch with exotic Ips lure
VI-27-2008	San Diego Co., CA	CAS	Noble Canyon Trailhead, Cleveland NF	Purple flight intercept traps near <i>Quercus agrifolia</i>
	No holdings	BPBM		Surveyed March 2009
	No holdings	BYU		Surveyed March 2009
	No holdings	LACM		Surveyed Dec. 2008
	No holdings	SBNM		Surveyed Jan. 2009
	No holdings	SDMC		Surveyed Dec. 2008
	No holdings	UCDC		Surveyed Nov. 2008
	No holdings	UCR		Surveyed Dec. 2008

Museum acronyms (all in the United States unless otherwise indicated)

AMNH—American Museum of Natural History, New York, NY
BMNH—The Natural History Museum, London, UK
BPBM—Bernice P. Bishop Museum, Honolulu, HI
BYU—Provo, Brigham Young Univ., Monte Bean Life Science Museum, Provo, UT
CAS—California Academy of Sciences, San Francisco, CA
CIDA—College of Idaho, Orma J. Smith Museum of Natural History, Caldwell, ID
CLBC—Charles L. Bellamy—affiliated with CSCA
CMNC—Canadian Museum of Nature, Ottawa, Ontario, Canada
CNCI=CNC—Canadian National Collection of Insects, Ottawa, Ontario, Canada
CSCA—California State Collection of Arthropods, Sacramento, CA
EMEC—University of California, Essig Museum of Entomology, Berkeley, CA
FMNH—Field Museum of Natural History, Chicago, IL
FSCA—Florida State Collection of Arthropods, Gainesville, FL
HESP—Henry A. Hespenheide, personal collection, Los Angeles, CA
LACM—Los Angeles County Museum of Natural History, Los Angeles, CA
MCZ—Museum of Comparative Zoology, Harvard University, Cambridge, MA
RHTC—Robert H. Turnbow, personal collection
RLWE—Richard L. Westcott, personal collection
SBNM—Santa Barbara Museum of Natural History, Santa Barbara, CA
SDMC—San Diego Natural History Museum, San Diego, CA
TAMU—Texas A & M University, College Station, TX
UAIC—University of Arizona Insect Collection, Tucson, AZ
UCDC—University of California, The Bohart Museum of Entomology, Davis, CA
UCR—University of California Riverside, Riverside, CA
USNM—National Museum of Natural History, Washington, DC

†These specimens were accessioned under the previous synonym, *Agrilus auroguttatus*. §Another specimen from this series is accessioned in the MCZ.

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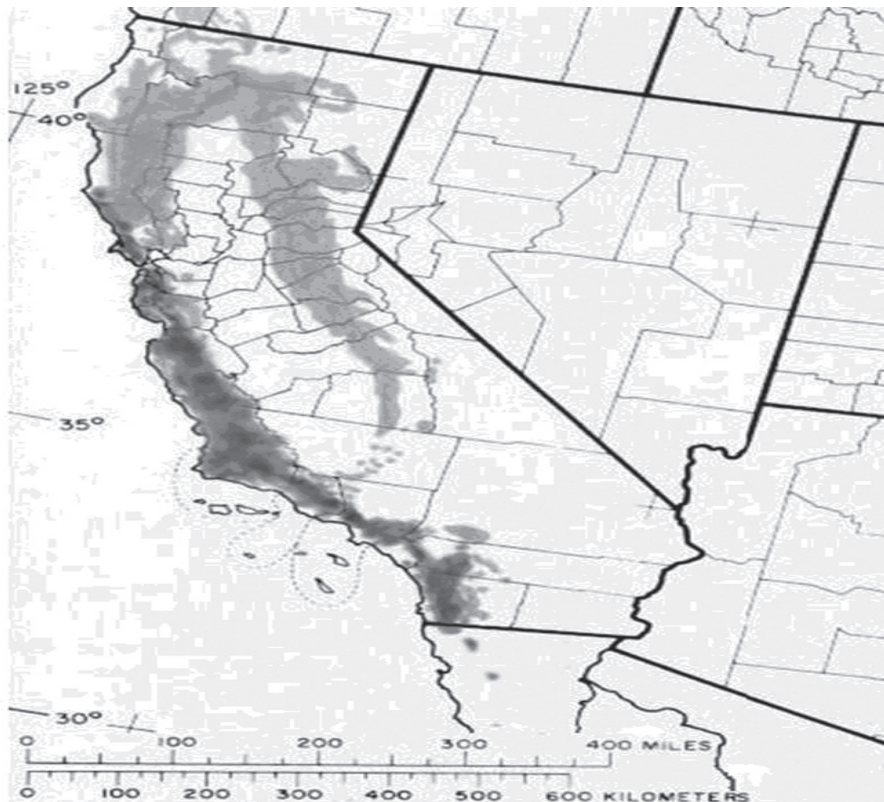


Figure 1. Native distribution of coast live oak (*Quercus agrifolia*) (dark grey) and California black oak (*Q. kelloggii*) (light grey) in California, both are confirmed hosts of the goldspotted oak borer (*Agrilus coxalis*).

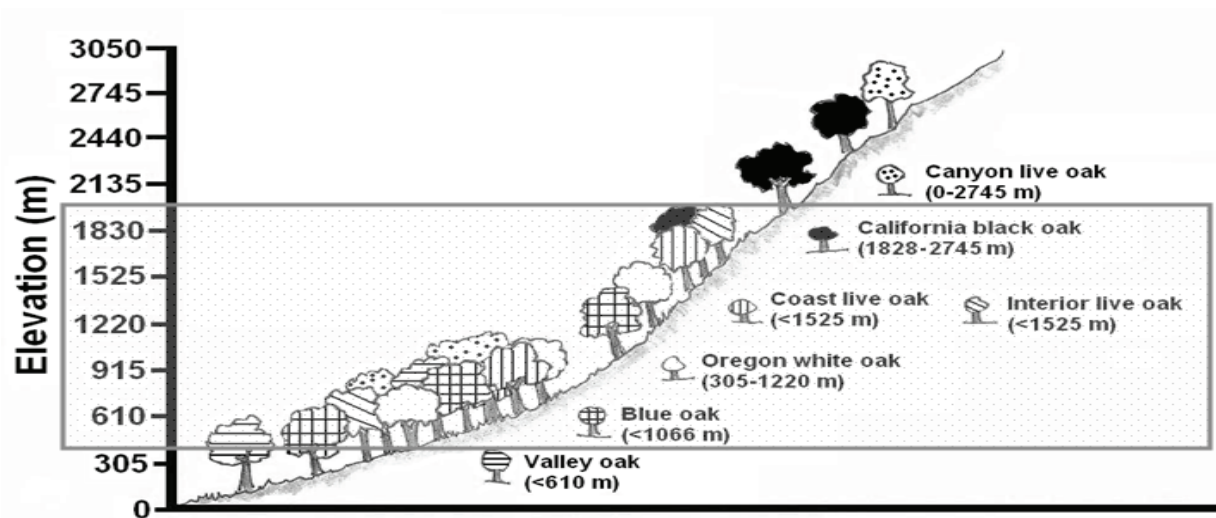


Figure 2. Elevational range of native oaks (*Quercus* spp.) and current distribution of goldspotted oak borer (*Agrilus coxalis*)—caused mortality observed in California (shaded area).

ACTIVITY AND PERSISTENCE OF SYSTEMIC INSECTICIDES FOR MANAGING HEMLOCK WOOLLY ADELGIDS

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ABSTRACT

Systemic insecticides have protective and therapeutic value for managing hemlock woolly adelgid in forests (Webb et al. 2003, Cowles et al. 2006, Cowles 2009). Although use of insecticides in forests for managing this pest cannot be considered a sustainable long-term strategy, systemic insecticides are an effective option for maintaining the health of hemlock trees so that they can continue to provide ecosystem and aesthetic functions until such time that biocontrol or plant resistance become established.

Two systemic neonicotinoid insecticides, imidacloprid and dinotefuran, are effective for managing hemlock woolly adelgids. Imidacloprid has been available for use in landscapes since about 1993 and has been extensively studied. Ever since it was first evaluated in the landscape against various hemipteran pests, multiple-year benefits have been observed (Cowles and Cheah 2002, Cowles et al. 2006, Cowles 2009). It remains unknown if the multiple-year benefit has been due to extreme efficacy within the first year after application, resulting in defaunation within a tree and slow decolonization, or if it is due to the continued presence of effective titers of insecticide. Because imidacloprid is a semi-polar compound with complex metabolism within trees, addressing this question has had to wait until analytical equipment of sufficient sensitivity became available. High performance liquid chromatography used with tandem mass spectrometry (LC/MS/MS) is well suited for this task because the parent compound and metabolites can be selectively analyzed and sensitively quantified at part per billion (ppb) concentrations. Although ELISA methods have been useful in the past to obtain rough estimates of imidacloprid concentrations in tissues, these results were semiquantitative because imidacloprid metabolites

could contribute to falsely elevated imidacloprid readings and the relative role of parent compound and metabolites remained unknown (Cowles et al. 2006).

Multiple years of field tests using standard application practices for imidacloprid provided an opportunity to explore the long-term metabolism of imidacloprid within hemlocks. Trees treated with soil injections of imidacloprid in 1999, and consecutive years from 2002 to 2006 in Connecticut and Pennsylvania, were sampled during the autumn of 2007. Foliage from new growth was dried, pulverized, extracted with acetonitrile, and analyzed by LC/MS/MS. Other samples taken within the first year of soil or trunk injection were provided by Rick Turcotte, U.S. Forest Service, Morgantown, WV. Analyses revealed that imidacloprid titers peaked approximately 2-6 months after trunk injection and 18-20 months after soil injection. Titrers fell below ~100 ppb in about 3 years, but remained detectable 8 years after treatment. Imidacloprid metabolized readily to its olefin, known to be 10-16× as active as the parent compound (Nauen et al. 1998, Nauen et al. 1999). The concentrations of this metabolite parallel and are equivalent to those of imidacloprid until about 5 years after treatment, at which time the imidacloprid residues decreased while the olefin metabolite remained stable. Therefore, we conclude that the high degree of adelgid suppression is due to the continued presence of imidacloprid and imidacloprid olefin, which continued to translocate to new growth over several years. Mortality is compounded over multiple generations, so that a single soil application can provide 5-7 years of protection. Trunk injected trees have average insecticide and metabolite titers similar to trees treated through soil injection. Therefore, poorer efficacy resulting from trunk injection methods is due to uneven distribution uniformity.

Dinotefuran is a highly water soluble and extremely upwardly systemically mobile compound compared to imidacloprid (EPA 2004). It was first registered for use in the landscape in 2005. A test of this product for hemlock woolly adelgid in Connecticut compared application of 0.75 g active ingredient per inch d.b.h., applied either through shallow subsurface soil injection or applied with the organosilicone surfactant PentraBark® as a spray to the lower 1.5 m of the trunk. Application in early October, 2007, resulted in adelgids dying at branch tips on new growth within 2 weeks. Those adelgids developing on 1-year-old growth required a longer time to die, as evident from a greater amount of wool. Mortality of sistens in 2007 was approximately 80 percent with each of these two application methods. One year later, the populations of sistens were reduced by 100 and 96 percent, respectively, for soil vs. trunk spray application methods for Safari when compared with untreated controls; the results from these application methods do not significantly differ. Although residue data for dinotefuran in hemlock tissue has not been completed, it is expected from metabolism studies in other plants that it may not provide adelgid control beyond the second year following application.

The differences in systemic mobility and residual activity between imidacloprid and dinotefuran reveal complementary temporal properties. Imidacloprid is probably best suited for protection of trees at the infestation front, before decline in tree health occurs, or in areas where populations of adelgids have been reduced by low winter temperatures. It is mobilized so slowly through trees that the full effects of soil treatment are not observed for at least 1 year following application. Effects are even slower when using the slow-release tablet formulation, CoreTect®, which can take 1 year longer than the wettable powder or flowable formulations to achieve adequate control. In the southern Appalachian mountains, where the largest trees may succumb about 3 years after initial infestation (Will Blozan, personal communication), treating already stressed trees with imidacloprid may not provide the rapid adelgid reduction needed to preserve a tree. Dinotefuran can quickly reduce populations of adelgids, a property that is especially important for stressed

trees. The availability of a trunk spray application method should mitigate environmental contamination with this insecticide. Although held less tightly by organic matter than imidacloprid, any residues leaching from bark with subsequent rainfall would be expected to bind to soil organic matter or be degraded by light on the soil surface. Where labor costs due to difficult access to trees is an overriding component to the cost of chemical treatment, combining the rapid effect of dinotefuran with the slow-acting but longer-lasting effects of imidacloprid should be considered, especially where exceptionally valuable trees are already experiencing stress from adelgid feeding.

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UPDATE ON WINTER MOTH IN NEW ENGLAND

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ABSTRACT

The winter moth, *Operophtera brumata*, a leaf-feeding geometrid native to Europe, has recently invaded eastern New England and is causing widespread defoliation. Previous invasions by this species in Nova Scotia and British Columbia have been suppressed by the introduction of two parasitoids from Europe, the tachinid *Cyzenis albicans* and the ichneumonid, *Agrypon flaveolatum*. As a result of these introductions, low-density populations of winter moth now persist indefinitely in these regions similar to those that exist in Europe. Over the past 4 years we have introduced *C. albicans* at six locations in Massachusetts and in 2007 we recovered the first parasitized larvae at our release site in Falmouth, MA. With the help of colleagues at the USDA APHIS laboratory at Otis Air Base, we are developing a mass rearing program for this tachinid and its winter moth host on artificial diet so that we can release large numbers of this parasitoid at many locations in the future. We focus our efforts on *C. albicans* because it specializes on winter moth and it is thought to be the agent primarily responsible for the decline of winter moth densities in Canada. We have established long-term monitoring plots where we will quantify densities of winter moth life stages and document parasitism before and after establishment of *C. albicans*.

We conducted a survey for winter moth across southern and eastern New England with pheromone-baited sticky traps beginning in November 2005. We expanded this

survey in 2006 and 2007 to include the entire Northeast from Pennsylvania to Nova Scotia. The traps attracted both winter moth and the North American congener of winter moth, Bruce spanworm, *Operophtera bruceata*. We used dissection of male genitalia to distinguish between these two species. In New England, we recovered winter moths at sites that stretched from eastern Long Island, southeastern Connecticut, all of Rhode Island, eastern Massachusetts, coastal New Hampshire, and southern coastal Maine. We caught winter moths in areas that were at least 100 km from any areas known to be defoliated by winter moths. Traps further west and north and south caught exclusively Bruce spanworm. We confirmed these identifications by sequencing the CO1 mitochondrial gene of specimens of these two species. This technique does not distinguish between possible hybrids of these two species. To accomplish this purpose we sequenced the nuclear gene G6PD. We have confirmed the presence of hybrids between these two species although they are not very abundant. The survey in 2007 showed that winter moth occurs in Nova Scotia but not interior areas of Maine or New Brunswick. We suspect that winter temperatures may prevent winter moth from invading these regions. Winter temperatures in Nova Scotia are very similar to those in southern New England. In 2008 we deployed pheromone traps in southern New England in the same locations we had sampled in 2005. Our purpose was to measure the rate of spread of winter moth over the 3-year period.

GREEN ARMOR AND CUL DE SAC CORRIDORS

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ABSTRACT

Water quality is a critical conservation issue worldwide and in the United States. Nutrient loading as a result of nonpoint source not only degrades freshwater habitats due to eutrophication, but is causing dead zones in such important commercial fishing grounds as the Gulf of Mexico and the Chesapeake Bay. One technique for mitigation of nonpoint source pollution is the establishment of forested riparian buffer strips, which have long been used to prevent erosion and runoff in agricultural landscapes. Given the recent boom in suburban and urban sprawl, however, it is also important to consider a forested buffer's ability to protect surface waters as the surrounding landscape changes from agricultural to residential.

In highly managed agricultural and residential landscapes, these forested corridors along streams may represent a majority of the forested land cover, and indeed may be the only "natural" habitats present. If we are interested in maximizing native biodiversity in all of our environments, then understanding how best to protect it in these fragments and corridors is imperative. Invasive exotic plants displace native plants and disrupt local food chains, thus negatively impacting local native biodiversity. Because most invasive plants are edge specialist or generalist species that thrive in disturbed habitats, we wished to investigate how far into a forested riparian corridor invasive plants can remain dominant, as these habitats suffer from high rates of disturbance and an abundance of edge area.

In 2007 and 2008, we surveyed 39 corridors of varying widths throughout the White Clay Creek watershed of Pennsylvania and Delaware. Using three transects at each site, the relative abundances of invasive and native

plants were measured as percentages of ground cover through the corridor from the surrounding landuse inward to the stream bank. Linear regression of data collected in 2007 shows a strong negative trend in invasive plant cover with increasing distance from the corridor edge ($R^2 = 0.333$, $P < 0.001$). Data from 2008 are still being processed, but preliminary analyses support the findings from 2007 showing that invasive plant density is strongly curtailed by 60 m from the buffer edge and even more so by 100 m.

At the same time we wished to measure the efficacy of these corridors as buffers against phosphorus loading. Analyzing soil cores from across the same transects used for measuring invasive plant cover, most of the P migrating from the surrounding land use appears to be sequestered quickly, but a more refined, fine-scale sampling protocol is required to adequately determine the relationship between corridor width and P buffering. This is currently under way.

In addition to their capacity to protect surface waters from nutrient pollution and to act as refugia for native species in highly managed ecosystems, forested riparian corridors also have intrinsic cultural value as greenspace. Traditional greenspaces in planned residential communities tend to lack native species and provide little habitat for wildlife. While many of the species capable of persisting within the patches and corridors provided by leaving forests along waterways may be common and the resulting ecosystems are far from pristine, they are still valuable within our communities capable of increasing society's familiarity with nature and their likelihood to act in its favor.

OBSERVER BIAS AND THE DETECTION OF LOW-DENSITY INFESTATIONS: A CASE STUDY WITH THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

Monitoring programs, often comprised of volunteers, increasingly are used to document the spread of forest pests in the hope of detecting and eradicating low-density infestations before they become established. However, interobserver variation in the detection and correct identification of low-density populations of forest pests remains largely unexplored. In this study, we compared the abilities of novice observers and experienced individuals to detect low-density populations of the hemlock woolly adelgid (HWA) and we explore how interobserver variation can bias estimates of the proportion of site infested derived from models. We found that, compared to experienced individuals, novice observers detected HWA infestations at smaller proportion of sites and, on average, failed to detect low-density infestations. In contrast, models suggested that experienced observers had a higher probability of falsely detecting HWA as present than did novice individuals. This latter, unexpected finding can be explained by invoking heterogeneity in detection probabilities associated with variation in population abundance and differences in the ability of observers to detect low-density infestations. Our findings highlight some of the difficulties in sampling for low-density infestations of forest pests in general and for HWA in particular. More broadly, our results caution against the use of different sampling protocols in the same survey and suggest that models that estimate infestation rates should include survey-specific covariates that account for biases in detection probabilities introduced by interobserver variation or survey methods.

INTRODUCTION

The growing threat posed by invasive species has focused increased attention on the importance of documenting the distribution and spread of introduced organisms. Monitoring programs aimed at detecting low-density ‘founder’ populations can play a critical role in slowing or even stopping the spread of harmful invasives by identifying recently established populations that can be targeted for control and/or eradication (e.g., gypsy moth ‘Slow the Spread’ program). These efforts have proven remarkably successful against actively dispersing species, but founding populations of species that disperse passively by means of wind, water, or phoresy often prove far more difficult to locate. Without the ability to attract the organisms to a trapping location, researchers face the often daunting task of repeatedly searching potential habitats for low-density populations of the invading species.

The surveying problems posed by passively dispersing species are exemplified by the hemlock woolly adelgid, an invasive pest of eastern hemlock and Carolina hemlock in the eastern United States. HWA is a minuscule (<1-mm long adult), flightless insect that in the United States is both obligately parthenogenetic and exclusively passively dispersed. The parthenogenetic nature of HWA means that even a single colonizing individual can start a new infestation, producing an initially low-density population that only can be detected

by costly and time-consuming surveys. Such surveys are increasingly being met in part by volunteer-based or 'citizen science' monitoring programs (e.g., CitSci.org). Although the educational and scientific benefits of volunteer-based invasive species monitoring programs are clear, the reliability of data collected by novice individuals has sometimes been questioned. However, these concerns stem mostly from the lack of studies comparing the quality of volunteer collected data versus professionally collected data rather than from studies demonstrating that volunteers collect unreliable data.

In this study, we first compare the abilities of inexperienced volunteers and experienced observers to detect low-density populations of an actively spreading forest pest, the hemlock woolly adelgid. We then use these data to explore the general question of how interobserver variation can bias estimates of the proportion of sites infested derived from models. We hypothesized that relative to experienced observers, novice individuals should be less likely to detect low-density populations and would be more prone to misidentification of the study species. To explore these hypotheses, we use maximum likelihood methods to select among models that consider differences in the ability of observers to both detect and correctly identify HWA. We parameterize these models using data from a 420-tree survey conducted by nine volunteers and three experienced individuals at Cadwell Memorial Forest in Pelham, MA. Our results support the notion that novice volunteers and experienced observers differ in their ability to detect low-density populations and that such differences in observer ability can bias estimates of the proportion of sites occupied. However, this bias manifests itself in unexpected ways.

METHODS

Twelve observers participated in the sampling effort: three experienced individuals who perform field research on HWA and nine volunteers who had no prior experience sampling for HWA populations. Prior to the sampling, the volunteers were trained for 15 minutes on the sampling methodology (see below) and on identifying HWA infestations. Each person was then assigned to one of four groups ($n=3$ persons per group). Two of the groups

entirely were comprised of volunteers (hereafter referred to as 'volunteer-only'). The remaining two groups contained one experienced and two volunteer individuals and two experienced and one volunteer individual (hereafter referred to as 'volunteer/experienced').

Observers searched all accessible branches for evidence of white woolly masses characteristic of the HWA sistens generation. Each search continued until either HWA was detected or a 2-minute sampling period had expired. To ensure that sampling was independent, no two observers sampled a tree at the same time and observers were instructed not to communicate the infestation status of trees to the other observers. To examine whether there were differences between volunteers and experienced individuals in terms of the density of infestations detected by each type of observer, two experienced individuals returned to all trees where HWA was detected, thoroughly searched all accessible branches, and counted the number of white woolly masses observed on the tree. This count provided an estimate of the number of detectable individuals on the tree. We used a t-test on log-transformed HWA abundance to compare the mean abundance of HWA infestations that were and were not detected by volunteers.

We used differences in detection abilities between volunteer and experienced observers to determine how such differences influence estimates of the proportion of infested hemlock trees. Our models incorporated three parameters: ψ , the proportion of infested hemlock trees, $p11$, the 'detection probability', the probability of detecting the species, given that the species is actually present at the site, and $p10$, the 'misclassification probability', the probability of falsely detecting the species at an unoccupied site. We considered four models that make different assumptions regarding $p11$ and $p10$. The simplest model assumes false positives are not possible ($p10 = 0$) and that detection probabilities are constant across observers. The second model again assumes that false positives were not possible, but allows observers to differ in their probability of detecting HWA. The final two models both incorporate the possibility of misclassification ($p10 > 0$), with the simpler of the two assuming that observers do not differ in their probability of detecting

or misclassifying HWA. The more complex of these two models assumes that observers can differ in their probability of detecting and misclassifying HWA.

RESULTS

We found that relative to volunteers, experienced observers (1) detected infestations at a greater proportion of trees; (2) had a higher probability of detecting infestations; and (3) detected smaller infestations. Surprisingly, when compared to volunteers in their group, experienced observers had a higher probability of misclassifying other organisms as HWA. The form of the best-supported model also differed between volunteer-only groups and volunteer/experienced groups. For volunteer-only groups, models where the probability of misidentifying HWA was 0 ($p_{10} = 0$) were best supported by the data. In contrast, the best supported model for volunteer/experienced groups assumed misclassification probabilities were greater than 0 and both detection and misclassification probabilities differed between observers. There was little support for models that assumed that experienced and volunteer observers had equal probabilities of detecting HWA infestations.

CONCLUSIONS

We were initially surprised by the apparent result that experienced observers were more likely to misclassify HWA than volunteers. However, when we inspected detection histories we found that, for the team with one experienced and two volunteers, the two inexperienced

observers detected HWA on only 1/125 trees when the experienced observer did not. In contrast, the experienced individual detected HWA 23 times when the two volunteers did not. These results suggest a failure by inexperienced observers to detect low-density infestations rather than misidentification by experienced observers. These results also reveal an issue regarding the absence of statistical weighting in the model. When a low-density infestation is detected by one observer, but missed by the remaining two individuals, statistical support tips in favor of misclassification. This finding cautions against the use of different survey protocols (or observers of differing levels of experience) in the same survey and suggest the need to include in models survey-specific covariates that account for biases in detection probabilities introduced by differences in observers or survey methods.

What do our results say about the adequacy of data on the distribution of low-density populations collected by volunteers? We suggest that the answer to this question depends on the ultimate use of the data and on the system under study. HWA, though easy to identify to the trained eye, can be extremely difficult to detect when occurring at low densities; our results suggest field experience can improve the ability to detect such infestations. Taken together, our results underscore the importance of adequate training for novice individuals taking part in monitoring programs and the need to document and account for interobserver variation in analytical estimates of infestation rates.

CREATING TRAP TREES FOR DETECTION AND CONTROL OF *SIREX NOCTILIO*

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ABSTRACT

The ongoing goals of this research is to develop trap trees for *Sirex noctilio* (Hymenoptera: Siricidae), an invasive Eurasian pest of pine, recently discovered in New York, Pennsylvania, Vermont, Michigan, and Ontario, Canada. Trap trees for this pest can be used for two purposes: detection (in conjunction with traps and trapping devices), and control (as reservoirs of host material for the nematode, *Deladenus siricidicola*). Our current research efforts have been conducted in the central New York counties of Onodaga, Oswego, Cayuga and Madison.

A study was conducted to determine the optimal time to chemically treat trees with the herbicide Banvel (dicamba). Trees were treated with herbicide at three time periods: 3 months (April) prior to, 1 month (June) prior, and at the time of anticipated flight (July). Traps on trees treated with dicamba one month prior to anticipated flight caught more *S. noctilio* than traps on untreated control trees or trees treated 3 months prior to flight. However, more *S. noctilio* emerged from trees treated at the time of anticipated flight than untreated controls, trees treated three months prior to flight and trees treated 1 month prior to flight.

A second study was conducted in 2007 comparing the herbicide triclopyr (Garlon 3A) with dicamba as well as a mechanical (phloem and xylem removal) girdle. While both dicamba and triclopyr are auxin mimics and researchers in Australia have found success with dicamba, triclopyr is recommended for control of Scots pine (*Pinus sylvestris*), a preferred host of *S. noctilio* in central New

York. Traps on dicamba-treated trees caught more *S. noctilio* than control or mechanically girdled trees, but there were no differences between triclopyr-treated trees and any of the other treatments.

Trees treated with dicamba in 2006 and 2007 received a dose of 1.0 ml A.I. per 10 cm of tree circumference. In 2007, trees treated with triclopyr received approximately 0.25 ml A.I. per 10 cm (the label rate). Even though the two herbicides did not produce significant differences in trap catch, triclopyr-treated trees took longer to show visible stress symptoms which may have led to a lower catch. In 2008, we compared catch in traps on treated with three concentrations of triclopyr [0.24 ml (1x), 0.48 ml (2x) and 0.96 ml (4x)] and the standard concentration of dicamba used in previous studies. There were no significant differences in trap catch among the triclopyr or dicamba treatments, but the 2x dose of triclopyr caught significantly more *S. noctilio* than untreated control trees. Treated and control trees were felled and subsampled in November and December of 2008, and emergence data from logs will be collected in Spring 2009.

Trees that were mechanically girdled in early June showed lessened stress symptoms when compared with trees chemically treated (at the same time) with herbicides in 2007. While chemically treated trees were had yellow and brown crowns 3 months following treatment, many mechanically girdled trees still had green crowns. In 2008, a study was performed to compare trees that had been mechanically girdled four times throughout the

spring and summer: (a) 6 weeks prior to flight; (b) 3 weeks prior to flight; (c) at time of anticipated flight; and (d) 2 weeks following the start of flight. Trees girdled 6 weeks prior to flight caught significantly more *S. noctilio* than trees girdled during any other period. If trees are to be mechanically girdled, they should be treated at least weeks in advance of the anticipated flight for *S. noctilio*, if not earlier. Treated and control trees were felled and subsampled in November and December of 2008, and emergence data from logs will be collected in Spring 2009.

Catch in most of the *S. noctilio* trapping studies mentioned above has been relatively low (~3-4 adults being caught per trap). A field assay was conducted in 2008 to compare three trap designs, a multi-funnel trap (wet cup), a Tanglefoot®-coated crossvane trap (0.46 m x 1.22 m x 4 sides) and a Tanglefoot®-coated plastic hardware cloth panel trap (0.50 m x 1.00 m x 2 sides). Traps were compared together in clumps of dicamba-treated trees. There were no significant differences between either of the glue-coated traps and the funnel trap. A better receptacle to retain *S. noctilio* falling off, may increase glue-coated trap efficiency, funnel traps remain the most convenient trap available for *S. noctilio*.

BIRCH LEAFMINER: A SUCCESS

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ABSTRACT

Introduction

An account is provided of the classical biological control program directed against the birch leafminer, *Fenusa pusilla* (Lepeletier) (Hymenoptera: Tenthredinidae), in North America during the period 1974-2007. Emphasis is placed on the overseas exploration for importation, release, recovery, and evaluation of natural enemies in the northeastern United States.

First reported from Connecticut in 1923 (Friend 1933), *F. pusilla* dispersed throughout most of the northern U.S. and much of Canada, where it became an important defoliator of white barked birches (*Betula* spp.). This species has up to four generations per year and overwinters in a cocoon in the soil. The adults emerge and begin ovipositing in late April-early May, depending on the latitude. Damage is caused by the larvae, which mine through the palisade parenchyma of the leaves; trees were frequently attacked to the point at which the foliage of the entire crown turned brown, adversely affecting the trees normal development, aesthetic appearance, and resistance to other pests.

Though native parasitoids, primarily polyphagous chalcidoids, had been observed attacking the pest in Quebec (Cheng and Leroux 1969), Connecticut (Friend 1933), and the Middle Atlantic states (Fuester and Taylor, unpublished data), these studies indicated that the natural enemy complex consisted primarily of polyphagous chalcidoid wasps and that parasitism was very low, having little impact on populations of *F. pusilla*. Observations by Eichhorn and Pschorn-Walcher

(1973) in Europe, where the pest originated, indicated that there was a rich complex of parasitoids (17 species) attacking the pest and that total annual parasitism was 38-47 percent.

Importation and Release of Natural Enemies

Because parasitism of *F. pusilla* was much higher in Europe, a classical biological control program was mounted against the pest in eastern North America during the 1970s. The initial releases of natural enemies were made in 1974 in eastern Canada (Raske and Jones 1975, Guèvremont and Quednau 1977), followed by others in the Middle Atlantic states (Fuester *et al.* 1984) and New England (Van Driesche *et al.* 1997). Brief notes on the four species of parasitoids imported and released in North America follow:

Lathrolestes nigricollis Thomson (Hymenoptera: Ichneumonidae) is the dominant parasitoid of *F. pusilla* in Europe. This multivoltine endoparasitoid attacks intermediate to late stage larvae and exhibits a high degree of host specificity. Although a high proportion of its eggs are melanized and encapsulated by the host, the larvae frequently overcome host defenses and complete their development, which is not completed until the host spins its cocoon. A total of 9,670 adults were released in the Middle Atlantic states during 1976-1982.

Grypocentrus albipes Ruthe (Hymenoptera: Ichneumonidae) is second in importance to *L. nigricollis* in Europe, but is similar in being highly host specific, having several generations per year, and attacking intermediate- to late-stage larvae. It differs, however, in

developing as an ectoparasitoid, the kidney-shaped egg being attached to the host by an anchor. The egg does not hatch until the host spins its cocoon. A total of 2,582 adults were released in the Middle Atlantic states during 1977-1982.

Phanomeris probably *catenator* Haliday (Hymenoptera: Braconidae) is a multivoltine ectoparasitoid of small and intermediate sized larvae. The host range of this species is poorly known and it has a somewhat restricted distribution in Europe. Multiparasitism with the aforementioned ichneumonids is infrequent, but *Phanomeris* usually wins, probably because it does not have to wait until the host spins its cocoon before completing its development. A single release of 47 adults was made in Pennsylvania during 1979.

Chrysocharis nitetis Walker (Hymenoptera: Eulophidae) is the most abundant chalcidoid parasitoid of birch leafminer in Europe. It develops endoparasitically within 1st and 2nd stage leafminer larvae. Parasitization by this species is sometimes high locally, approaching 100 percent. A total of 557 adults were released in Pennsylvania during 1981-1983.

Monitoring and Evaluation

Of the four parasitoids released, only one—the ichneumonid *L. nigricollis*—became established and spread widely. Studies of its preliminary impacts were made at several locations in the 1980s and 1990s, but full impact of the parasitoid on host density was not yet achieved in that period. Therefore, we conducted surveys in seven states (MA, CT, RI, NY, PA, NJ, DE) in 2007 documenting the current birch leaf miner levels (as percentage of leaves mined in spring) and parasitism. Birch leaf miner populations have come under complete biological control in all areas surveyed above 40° N latitude (central New Jersey). Mined leaves were extremely difficult to find in MA, CT, RI, PA, and NY (Long Island). In these areas, birch leafminer larvae could not be recovered in large enough numbers to estimate rates of parasitism. In New Jersey, the pest's density has been suppressed at most sites north of 40° N, but south of this latitude, pest densities remain high. Parasitism at New Jersey sites above 40° N averaged 58 percent, about twice the 28 percent rate found in southern New Jersey. Infestations of birch leafminer at

Newark, DE, were highly variable, with the incidence of mined leaves ranging from 0 to 57 percent on different trees and averaging 10.4 percent, intermediate between New Jersey and the other states. Parasitism was low (1.2 percent), but samples were taken when most of the mines were small. Because *Lathrolestes nigricollis* females prefer to attack semi-mature and mature larvae (Eichhorn and Pschorn-Walcher 1973), sample timing may have caused parasitism to be underestimated.

In summary, results show that the pest has declined dramatically to barely detectable levels in five states (MA, CT, RI, NY, PA) but that in Delaware and southern New Jersey, the pest remains fairly abundant (up to 50 percent leaves mined) despite significant parasitism levels. Survey results, in context with previous evaluations made when populations were still declining, show that the project has been completely successful in much of the northeastern United States, but that there is a limit to efficacy along the pest's southern distribution. Possible reasons for lack of control in this area, in contrast to high levels of control elsewhere, include the following:

1. Population dynamics: In southern New England, birch leafminer is largely univoltine and about 75 percent of the first generation *L. nigricollis* diapause. In New Jersey and Delaware, birch leafminer has multiple generations which might present synchrony problems to the parasitoid.
2. Tree stress: Birches are primarily boreal trees, and most of the species preferred by birch leafminer do not occur naturally south of 40° N, except at high elevations in the Appalachians. Generally, they exist only as planted landscape trees which are often in stressed condition due to climate. Thus, they might be less resistant to leafminer infestations.
3. Difficulty in parasitoid adaptation: The birch leafminer and its parasitoids have a northern distribution in Europe, which characteristically has cool summers. The area in North America where biological control of birch leafminer is good corresponds with the Canadian and Transition Life Zones, whereas the area where biological control of birch leafminer is poor corresponds with the Upper Austral Life Zone, which is characterized by mean summer temperatures over 72 °F.

Conclusions

Classical biological control of birch leafminer by the introduced parasitoid *L. nigricollis* has been highly successful throughout all but a small part of the pest's range in North America. The results of our surveys illustrate the importance of continued monitoring of a classical biological control project for an extended period, in this case 34 years since the first releases. Although this case might be a bit extreme, Pschorn-Walcher (1977) has pointed out that monitoring in some classical biological control projects on forest pests has been discontinued too early. We concur with this idea, because evaluations on short time scales (3-6 years), typical of many research projects, may be premature and underestimate ultimate impacts of new biocontrol agents, especially when monitoring is done over very wide geographic areas.

Acknowledgments

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UPDATE ON EAB BIOCONTROL: RELEASE AND RECOVERY OF *SPATHIUS AGRILI* AND DISCOVERY OF NEW POTENTIAL BIOCONTROL AGENTS IN ASIA

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ABSTRACT

Spathius agrili is a gregarious, idiobiont parasitoid that was discovered attacking the emerald ash borer (EAB) in Tianjin City, China. Parasitism rates ranged from 30 to 90 percent on velvet ash, which is native to the southwestern United States. *Spathius agrili* females find their hosts by listening for the sound of feeding larvae. They insert their ovipositor through the bark, paralyze the EAB larva, and deposit up to 20 eggs. The parasitoid larvae develop outside the EAB larva, spin cocoons, and emerging adults chew through the bark to emerge. This parasitoid can develop through three generations per year compared to one for the EAB host. *Spathius agrili* is well synchronized with the occurrence of its favored host stage, late instar larvae. Adult *S. agrili* emerge 1 ½ to 2 months after emergence of EAB adults, allowing time for EAB to develop to the appropriate stage.

Rearing *S. agrili* presents a challenge because the larvae must be hidden and feeding or they will not be attacked. We solved this problem by drilling a chamber in the outside of an ash stick, inserting an EAB larva, and wrapping the stick with floral tape. Host specificity testing discovered that *S. agrili* attacked significantly more EAB than nontarget hosts in no-choice tests. In olfactometer tests, *S. agrili* was attracted to ash and to a lesser extent willow and Chinese prickly ash, but not to any other plants tested. In the laboratory, *S. agrili* lays significantly more eggs when the chamber is filled with ash foliage to stimulate oviposition. These and other data were submitted for public comment, and in July of 2007 a permit was issued for field release of *S. agrili*.

More than 300 female parasitoids were released at three sites in Michigan starting in August 2007. In March 2008, four trees were cut at the release location at all three sites. One-half of each tree was peeled and the other half was placed in paper tubes to collect emerging parasitoids. We found that 18 percent of the EAB at the Oakland, MI, site

were parasitized by a gregarious braconid, which upon adult emergence was determined to be *S. agrili*. From this we can conclude that *S. agrili* was able to reproduce and overwinter in southern Michigan. In September 2008 we cut down 10 trees at each of 30, 60, 90, and 120 m from the release point to determine if *S. agrili* had continued to reproduce at that site. Half of each tree was peeled and half were put in emergence tubes. Unfortunately, we saw no sign of *S. agrili*—not even old galleries from 2007.

In 2008, 300 female *S. agrili* were released at seven sites in Michigan and Ohio. Establishment and impact evaluation protocols were standardized with U.S. Forest Service and Agricultural Research Service scientists so that comparisons can be made among the three parasitoid species being released. Four trees were cut at each release site in December 2008; it is too early to determine if *S. agrili* will emerge. A rearing facility dedicated to rearing EAB parasitoids is up and running in Brighton, MI. The goal is to rear large numbers of parasitoids in this facility for release in 2009 and beyond.

Foreign exploration is continuing with an emphasis on finding natural enemies that attack EAB in colder climates and where the host is found at low population density. In China, trees were girdled at 10 locations where EAB is present but rare. In addition to *Tetrastichus planipennis*, two insects were found associated with EAB overwintering galleries. One is probably a clerid beetle, and we are rearing the other species to the adult stage for positive identification. In Korea, several new species were discovered: *Teneroides maculicollis* (Cleridae), *Tetrastichus telon* (Eulophidae), and an unidentified *Spathius* that is not *S. agrili*. In Russia, cooperators recovered *Spathius depressithorax* and an as yet unidentified *Tetrastichus*. The goal for 2009 is to start colonies of many of these insects and study their biology and host interactions to evaluate them for release.

BIOLOGICAL CONTROL FOR KNOTWEEDS IN NORTH AMERICA

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ABSTRACT

Knotweeds are a complex of closely related invasive plants in the genus *Fallopia* in the family Polygonaceae. Introduced into North America from Japan, these large herbaceous perennials form dense thickets that crowd out native plants, impede recreation, increase erosion, and reduce the quality of habitat for wildlife. They are particularly aggressive invaders of stream banks and flood plains. Our team is carrying out research to develop biological control program for knotweeds that would employ natural enemies introduced from the native range of the weeds (Japan). Two insect species, a sap-feeding psyllid, *Aphalara itadori*, and a leaf and stem-feeding moth, *Ostrinia ovalipennis*, are being tested at the Oregon State University quarantine facility to determine if they are sufficiently host-specific for

release into North America. Additionally, a distinct southern strain of the psyllid is also being tested by CABI-Bioscience in the United Kingdom. Seventy native and economically important plants were selected for testing. The primary test procedure involves caging adult insects onto individual test plants and measuring any oviposition and subsequent development that occurs. Testing of the psyllid is near completion, while testing of the moth has just begun. The psyllid has demonstrated a high level of host specificity with only very marginal development on three of the nontarget hosts. These results will be submitted for review by the regulatory authorities for a possible release as early as 2010. Testing of the moth will take at least 2 more years for completion.

AN ECONOMIC IMPACT ASSESSMENT FOR OAK WILT IN ANOKA COUNTY, MINNESOTA

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ABSTRACT

Sound economic assessments of damages caused by exotic invasive species provide a basis to determine whether management programs should be established, modified, or discontinued. Few analyses have attempted to carefully quantify those damages, especially for forest pests. Oak wilt is the most significant disease of oaks (*Quercus spp*) in the north central United States and is caused by a nonnative fungus, *Ceratocystis fagacearum*. Red oaks (section *Lobatae*) are more susceptible than white oaks (section *Quercus*) and can die within weeks after infection. Local spread occurs through root grafts and overland spread occurs by sap beetles (Family: *Nitidulidae*), thus, management typically relies on severing root grafts and removing infected or potentially infected trees.

We developed a measure of the economic impact of oak wilt in Anoka County, MN, over the next 20 years in the absence of management. The county was divided into grids of 1-km² cell. Each grid cell contained information on soil type, oak density, oak size, and the number of active infection centers. We assumed that the number of infected trees within each grid cell increased over

time following a logistic function. We also assumed that each infected tree died and was removed at an average cost of \$314/tree. In the model, tree removals did not affect disease dynamics. A discount rate of 0.05 was applied to express losses in current dollars.

Anoka County had nearly 3 million oak trees and 990 active infection centers in 2008. If oak wilt is not managed, our model predicts that 21,000-29,000 trees would die each year and approximately 20 percent of all oaks would be killed over the next 20 years. If all dead oaks are removed, we predict discounted damages of at least \$88.8 million in 5 years, \$111.1 million in 10 years, and \$143 million in 20 years. These damages do not include losses from other services that oaks may provide, such as carbon sequestration, energy conservation, or wildlife habitat. The value of these services is difficult to quantify. Removal costs require fewer assumptions and provide a reasonable, though incomplete, metric of the damages caused by invasive pests. By this single metric, projected damages can be severe. This metric may be adequate to inform decisions by policymakers and managers.

ECONOMIC ASSESSMENT OF POTENTIAL EMERALD ASH BORER DAMAGE IN URBAN AREAS IN THE UNITED STATES

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ABSTRACT

Emerald ash borer (*Agilus planipennis*), a beetle native to Asia, was discovered in southeastern Michigan near Detroit in the summer of 2002 and by the end of 2008 it had been found in locations in 10 states. Emerald ash borer (EAB) has the potential to spread and kill native ash trees (*Fraxinus sp.*) throughout the United States. While EAB infestations may initially spread relatively slowly, humans spread the insect much farther by moving infested ash logs, firewood, or nursery stock. State and Federal agencies have responded with quarantines on the movement of ash material and surveys to detect new infestations. These programs are expensive, yet there is very little scientific literature on the number of ash trees and the aggregate cost of treating trees to prevent infestation and removing infested trees, especially in urban areas. Assessing the potential economic impacts of EAB is important to evaluate the benefits of slow-the-spread efforts, as well as investments in research on EAB biology and management. We estimate the discounted cost of ash treatment and removal in urban areas in a 16-state study area centered on Detroit under one scenario of EAB infestation over the next decade (2009-2018).

Collectively, city boundaries enclose more than 11 million hectares (ha) within the study area, including both developed and undeveloped land. We estimate the number of ash trees in the developed portion of urban land because these are the trees that will most likely be treated or removed in response to EAB infestation. A little more than half of the urban area (6 million ha) is developed, and canopy cover is about 10 percent of developed land (0.6 million ha).

We obtained urban forest inventory information for 11 cities from web sites, publications, and personal communication with city foresters. Each source includes an estimate of the total number of ash trees within a city boundary, including trees on streets, parks, and private lands. From this inventory information, we compute the number of ash trees per hectare of tree cover for each city. Average ash density is 53 trees per ha of tree cover with a range of 289 trees per ha in Chicago to 3 trees per ha in Washington, D.C.

The urban forest inventory results are the basis for estimating number of ash trees in the developed portion of urban areas. First, we divide the study area into 16 mapping zones that represent relative homogeneity of landform, soil, and vegetation. Next, we assign each city with an urban forest inventory to a mapping zone and compute average ash density (trees per ha cover) for each zone. Then, we multiply the average ash density times the developed area of tree cover to estimate number of ash trees in the mapping zone. Adding up the number of ash trees across mapping zones, we estimate about 51 million urban ash trees in the study area.

We created a scenario for the expansion of EAB infestations from their known locations in December 2008. As EAB infestations expand, we predict the number of trees that are treated and removed and sum the discounted treatment costs and removal and replacement costs using a 5 percent discount rate. Total discounted cost is \$21.16 billion, with a majority of that cost occurring in commercial districts, industrial land, and parks with the removal of relatively small ash trees. If all ash trees are treated or removed

at once, a common assumption, the total cost is \$33.94 billion, 60 percent higher.

Our estimate of total discounted cost of treatment and removal activities over a 10-year horizon, \$21.16 billion, suggests that a substantial investment can be efficiently spent to slow the spread of EAB and postpone treatment and removal costs. These investments could include enforcement of quarantines on the movement of ash material, detection of new infestations, and destruction of infested trees. Investments also include research on effective chemical and biological control agents.

While estimates of ash numbers in cities for which we have inventory information are statistically sound, expanding those numbers to places without tree inventories should be viewed with caution because we have relatively few city-level inventories and those we have do not represent a random sample of urban areas. A systematic sample of the urban forest throughout the study area is needed to obtain statistically sound estimates of urban ash.

Our estimate of discounted cost of treatment and removal activities represents an income transfer from homeowners and municipalities to the tree-care industry and does not represent a net loss to society. Further work is needed to estimate nonmarket values of ash trees that are lost because of EAB infestation. Estimates of the reduction in nonmarket values represent a loss to society.

A SPACE-TIME ODYSSEY: MOVEMENT OF GYPSY MOTH AND ITS PATHOGENS

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ABSTRACT

Gypsy moth (*Lymantria dispar* (L.)) populations in the United States are constantly spreading to the west and south, although spread is slowed significantly due to the activity of the Slow the Spread Program (Tobin & Blackburn 2007). As gypsy moth spreads, newly established populations can increase quickly. We investigated the period of time required for the entomopathogens and parasitoids infecting gypsy moth to catch up with newly established gypsy moth populations. The specific entomopathogens we investigated were the gypsy moth fungus (*Entomophaga maimaiga*) and the gypsy moth nucleopolyhedrovirus (*LdMNPV*) and the parasitoids we investigated were tachinids. We identified sites where both pathogens had been released in the past. Studies were conducted in sites with low densities of gypsy moth (quantified using male moth pheromone catches) in south-central Wisconsin from 2005-2007. During 2005 and 2006, studies included: 1) caging larvae at bases of trees; 2) caging larvae in the lower canopy; 3) caging larvae on soil samples in the lab; 4) collecting cadavers in the field; and 5) collecting living larvae in the field and rearing them. In cases when gypsy moth densities were so low that we could not find larvae in the field, we still did not detect any levels of infection in larvae when deployed in the field in cages, or when exposed to field-collected soil under laboratory conditions. Comparing sampling methods from 2005 and 2006 demonstrated that we detected higher levels of infection when rearing field-collected larvae so during 2007 we only employed this latter method.

During 2007, *E. maimaiga* was detected at 38.7 percent of the 31 sites, yielding an average of 12.4 ± 4.8 percent infection at sites where it was present. Across all years, levels of infection were not associated with distances from release sites although infection was positively associated

with male moth density the previous year and negatively associated with male moth density later that same year. *E. maimaiga* infection was also associated with rainfall during the first 3 (2006) and 2 (2007) weeks of April. During 2007, *LdMNPV* was detected at 20.8 percent of sites, yielding 2.0 ± 0.5 percent infection at sites where it was present. The male moth density the previous year was positively associated with infection but male moth density that same year was not significant. We estimated that fungal and viral presence were more likely to be detected at moth densities of at least 68 and 158 gypsy moth males/trap in the prior year, respectively. During 2007, 85.7 percent of tachinid parasitism was due to *Compsilura concinnata*, with tachinids present at 34.4 percent of sites, although parasitism averaged only 1.3 ± 2.0 percent. More gypsy moth larvae were parasitized with higher male moth densities later that year but there was no association of parasitism with the previous year's gypsy moth density.

In summary, *E. maimaiga* and *LdMNPV* dispersed and arrived fairly quickly at newly colonized gypsy moth populations. Presence of both of these entomopathogens was associated with the prior year gypsy moth density while only *E. maimaiga* was associated with decreased gypsy moth density later that same year. Tachinid parasitoids also responded to these low density gypsy moth populations, being found at over one-third of the sites.

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CURRENT STATUS OF *ANOPLOPHORA* SPP. IN EUROPE AND AN UPDATE ON SUPPRESSION EFFORTS

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ABSTRACT

The Asian longhorned beetle (ALB) *Anoplophora glabripennis* (Motschulsky), and the citrus longhorned beetle (CLB) *Anoplophora chinensis* (Forster) (Coleoptera, Cerambycidae) have been accidentally introduced into 15 urban sites in Europe where they are considered as serious threats to urban and natural forests and are subject to eradication efforts. In their native area, both pests cause serious damage to many deciduous trees, mainly the genera *Populus*, *Acer* and *Salix*. CLB is also a major pest of citrus in Japan and China. At European ports of entry, only four interceptions of live adults of ALB or CLB were made in 28 years (1980-2008). In Europe, most ALB individuals imported in wood packing material from China, and most CLB individuals imported in bonsais or in plants for planting from China and Japan, remained undetected until the goods reached the final customer's place. In most cases, they were intercepted without establishment of an infestation: ALB was intercepted 48 times between 1994 and 2008, and CLB was intercepted 45 times between 1980 and 2008. On the other hand, some introductions remained undetected until an established infestation was discovered. As of December 2008, breeding populations of ALB had been found in several sites in four European countries: Austria (2001); France (2003, 2004 and 2008); Germany (2004 and 2005); and Italy (2007). As of December 2008, breeding populations of CLB had been found in multiple sites in three European countries: Italy (2000, 2006, 2007 and 2008); France (2003); and The Netherlands (2007). In Europe, the top five host tree genera of ALB are *Acer*, *Betula*, *Salix*, *Aesculus*, *Carpinus*, and those of CLB are *Acer*, *Betula*, *Corylus*, *Carpinus*, *Alnus*.

Eradication is the goal for all infestations. By the end of 2008, the numbers of CLB- or ALB-infested trees detected and destroyed in some European countries were: 192 in Austria, 175 in France, 106 in Germany, and 7 in The Netherlands. By the end of 2007, 3,009 CLB-infested trees had been destroyed in Italy. In that country, four ALB-infested trees were destroyed during 2008. Preventive destruction of high-risk host trees of ALB were occasionally performed around the core of some infestations to stop the pests from spreading: in Austria, 900 maple trees were cut during the winter of 2001-2002, and a small urban forest was destroyed in the spring of 2007; in Italy, 309 trees were destroyed during spring, 2008. Occasionally, when one or a few CLB beetles were intercepted at a tree importer's premises, high numbers of plants or the whole consignment were destructed, as was done in the United Kingdom, The Netherlands, France, Switzerland, Belgium, and Croatia. As of December 2008, ALB and CLB control costs had exceeded €3.3 million in Europe. In France and Germany, ALB populations decreased considerably and eradication still seems possible. In Soyons, France, no CLB symptoms have been detected since 2003 and thus the infestation was declared eradicated there. In Austria, despite eradication efforts, the breeding population is still very active, thus a new reinforced project with €472,500 budget was implemented in July 2008. In Italy, despite some eradication efforts, the CLB situation worsened. New heavy infestations were discovered in the past 3 years. In 2008, the European Commission gave the Lombardy Region a €10 million budget to improve CLB control in north of Italy.

ECOLOGICAL IMPACTS OF EMERALD ASH BORER IN FORESTS OF SOUTHEAST MICHIGAN

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ABSTRACT

Emerald ash borer (EAB), *Agilus planipennis*, has killed millions of ash (*Fraxinus* spp.) trees since its accidental importation from Asia. Congeneric relatives endemic to North America only colonize stressed trees, apparently as does EAB in Asia. However, EAB is killing healthy trees on high quality sites in North America, creating a wood-borer outbreak of unprecedented intensity.

We have established 38 transects containing 114 plots (0.1 ha) in forests of the Huron River watershed in southeast Michigan to quantify effects of emerald ash borer on (1) patterns and rates of ash mortality in relation to community composition; (2) successional responses to gap formation including establishment and spread of invasive plants; (3) ash seed bank and seedling regeneration dynamics; (4) ground beetle (Coleoptera: Carabidae) assemblages; and (5) dynamics of coarse woody debris accumulation.

Once trees began to die in infested stands, ash mortality increased 30 percent per year and now stands at 99.2 percent when averaged across all plots, with the majority of the few surviving trees clustered in the 1-2 inch d.b.h. size class. Rate of black ash (*F. nigra*) decline and mortality was advanced about 1 year relative to that of white (*F. americana*) and green ash (*F. pennsylvanica*). There was no relationship between ash mortality and

ash density, ash basal area, ash importance, total stand density, total stand basal area, or any measure of biodiversity. From 2004 to 2006, there was a highly significant negative relationship between percent ash tree mortality and distance from the putative epicenter of the infestation in Canton Township, with mortality decreasing 2 percent with each kilometer away from the epicenter. However, this relationship was not significant in 2007, as ash mortality is nearly 100 percent in all plots.

Besides ash, red maple (*Acer rubrum*) and elm (*Ulmus* spp.) are the most common species in the understory and seedling strata, and appear poised to exploit gaps created by ash mortality. Ash species are the most common species in the seedling layer, which could facilitate ash regeneration, or provide continued host material that prolongs the EAB outbreak. Four years of intensive sampling has revealed no ash seed bank. Invasive woody plant species are present in low numbers in almost all plots, and appear poised to exploit gaps formed by ash mortality.

Trap catches and species richness of ground beetles were higher in stands with small gaps. There were significant negative correlations between numbers of ground beetles captured in pit-fall traps and size of canopy gaps, as well as percent ash tree mortality. Cluster analysis revealed

that ash mortality and gap formation had larger effects on ground beetle species composition in black ash stands than in white or green ash stands.

In 2008, we assessed the effects of EAB-induced ash mortality on patterns of downed coarse woody debris (DCWD). Volume of ash DCWD averaged $96 \pm 27 \text{ m}^3/\text{ha}$, and did not differ significantly among species averaging about or just over $30 \text{ m}^3/\text{ha}$ for black, green, and white ash, respectively. Total biomass of ash DCWD was $1.3 \pm 0.4 \text{ Mg/ha}$ with carbon stock equal to $0.6 \pm 0.2 \text{ Mg/ha}$. There were no differences among ash species in total biomass or carbon stock of DCWD. There was no relationship between distance from the epicenter of the infestation and volume of DCWD. Manner of tree-fall was also assessed, with a higher proportion of all three ash species snapping at the main stem than uprooting. Rapid accumulation of DCWD following ash mortality suggests that EAB may have substantial impacts on patterns of nutrient cycling.

Complete mortality of white, green, and black ash regardless of density and community composition suggests little potential for silvicultural management of EAB. Initial studies on invasive plants, native ground beetles, and coarse woody debris suggests widespread gap formation resulting from ash mortality will have pervasive ecological impacts. The lack of an ash seed bank coupled with mortality of ash saplings before reproductive maturity suggests that long-term perpetuation of ash is precarious. As EAB continues to spread, it clearly has the potential to decimate ash throughout North America with ecological impacts reminiscent of chestnut blight and Dutch elm disease.

STATUS REPORT: MILE-A-MINUTE WEED

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ABSTRACT

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross, is an invasive annual vine of Asian origin that has developed extensive monocultures, especially in disturbed open areas throughout the Mid-Atlantic region of the United States. A host-specific Asian weevil, *Rhinoncomimus latipes* Korotyaev, was approved in 2004 for release in North America. Weevils were released in three replicated arrays in southeastern Pennsylvania in 2005 and have been intensively monitored since then. Mile-a-minute weed populations decreased and weevil numbers increased at all three sites, but weevil populations were especially large at the site that initially had the most dense mile-a-minute weed population. At least three to four overlapping generations of weevils were produced between May and October each year. Studies of *P. persicaria* plants in field cages with and without weevils confirmed that weevil feeding delays and suppresses seed production and can cause plant mortality in the presence of competing plants.

R. latipes have been reared at the New Jersey Department of Agriculture Beneficial Insects Laboratory in Trenton since 2004. By the end of 2008, more than 137,000 weevils had been reared and released, mostly in

New Jersey but also at sites in Delaware, Maryland, Pennsylvania, and West Virginia. The beetles overwintered and established populations at 54 out of 56 (96.4 percent) of sites where they were released between 2004 and 2007. Standardized monitoring of fixed quadrats was conducted for at least 3 years in paired release and control sites in New Jersey (two sites), Delaware (two sites), Maryland (one site), and West Virginia (three sites). However, only one control site in New Jersey remained “weevil free” for 3 years; this site had significantly higher mile-a-minute weed populations compared with its paired release site. All other control sites were colonized by the weevils within 1 or 2 years, indicating excellent dispersal and host-finding ability by *R. latipes*. Monitored release sites in Delaware and New Jersey showed large increases in weevil populations and reductions in mile-a-minute weed populations over time, while monitored quadrats in West Virginia and Maryland sites experienced reductions in mile-a-minute weed populations at all sites without weevil population outbreaks, probably due at least in part to environmental factors.

GENETICS AND CONSERVATION OF HEMLOCK SPECIES THREATENED BY THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

Camcore is a forestry research cooperative program housed in the Department of Forestry and Environmental Resources at North Carolina State University, specializing in the applied conservation and domestication of forest genetic resources utilizing an *ex situ* approach. We make genetically representative seed collections from populations of threatened and endangered tree species that cannot be reliably conserved in their natural environment and send those seeds to regions of the world with suitable climates where trees are grown in protected plantations. Once threats in the area of origin are mitigated, seeds or other propagules are collected from these conservation plantings and utilized to restore the species to its native environment. In our 29-year history, Camcore and its member organizations have utilized this approach to help conserve more than 40 tree species worldwide, working in the United States, Central and South American, South Africa, Kenya, Tanzania, Uganda, and Indonesia with numerous genera including *Pinus*, *Eucalyptus*, *Gmelina*, *Tectona*, and *Tsuga*.

The USDA Forest Service Forest Health Protection, in 2003, entered into a three-phase cooperative agreement with Camcore to preserve seeds of eastern (*Tsuga canadensis* Carr.) and Carolina (*T. caroliniana* Engelm.) hemlocks threatened by the hemlock woolly adelgid (*Adelges tsugae* Annand) in the eastern United States. The objectives of the agreement are to: 1) develop a framework plan for hemlock gene conservation; 2) describe patterns of hemlock genetic diversity across the geographic range of both species; 3) collect seeds from hemlock populations distributed across the geographic range of both species; 4) place hemlock seeds into long-term cold storage at Camcore facilities and those maintained by the National Germplasm Repository; and 5) establish national and international conservation seed orchards for both hemlock

species. The first phase of this effort began in 2003 with seed collections from populations of Carolina hemlock across its Southern Appalachian range. The second phase began in 2005 and is focused on seed collections from eastern hemlock populations distributed throughout the southeastern U.S. portion of the species' geographic range. The third and final phase will follow in late 2009 with a 4-year effort to make seed collections from portions of the eastern hemlock range in the northeastern and Midwestern regions of the United States. The overall goal of this cooperative program between Camcore and the U.S. Forest Service is to secure genetically diverse seed reserves and maintain viable populations of eastern and Carolina hemlock in perpetuity so that genetic material will be available for restoration efforts in areas where the hemlock woolly adelgid eliminates hemlock from the forest. The following is a brief synopsis of project accomplishments to date.

Hemlock Genetic Diversity: Genetic diversity studies with amplified fragment length polymorphisms (AFLPs) indicated that Carolina hemlock has moderate levels of diversity compared with other conifer species, and that there is a general trend of decreasing diversity moving from south to north across the species geographic range (Jetton et al. 2008). Eastern hemlock was found to have overall low diversity in the southeastern U.S. portion of its range in a study using isozyme markers, with diversity decreasing from east to west (Potter et al. 2008). Currently, Camcore and the U.S. Forest Service are cooperating on a microsatellite (SSR) marker study to describe patterns of genetic diversity within and among populations of eastern hemlock throughout its entire geographic range in the United States and final results are expected in late 2009.

Hemlock Seed Collection: The genetic studies serve as important guidelines for the conservation effort, allowing targeted seed collections in portions of the geographic range that will result in capture of maximum levels of diversity for both species. Using this data, steady progress has been made from 2003-2009 with seed collections from surviving hemlock stands in the southeastern U.S. Seeds have been acquired from a total of 97 mother trees in 13 populations of Carolina hemlock in Georgia, Tennessee, Virginia, and North and South Carolina. Eastern hemlock collections represent 195 mother trees from 26 populations in Georgia, Kentucky, Tennessee, Virginia and North and South Carolina. In 2010, we hope to reach our seed conservation goals for eastern and Carolina hemlock in the southeast and plan to initiate eastern hemlock collections in the northeast and Midwest.

Our seed collections to date are in support of the *ex situ* conservation effort, and the Camcore seed bank in Raleigh, NC, serves as the primary repository. Utilizing recommendations from the Woody Plant Seed Manual (Bonner and Karrfelt 2008) and results of ongoing studies at Camcore, we are currently developing optimal seed storage protocols for both species. Small amounts of Carolina hemlock seed have also been submitted to the U.S. Forest Service National Tree Seed Lab for accession in the National Germplasm Repository, and we will do the same with eastern hemlock in the future.

Hemlock Conservation Seed Orchards: Camcore members Arauco-Bioforest and Forestal Mininco in Chile and Rigesa-MeadWestvaco and Klabin SA in Brazil are working with Camcore and the U.S. Forest Service to establish hemlock *ex situ* conservation plantings in South America. In the United States, the University of Arkansas and the U.S. Forest Service are working together to establish similar plantings in the Ozark Mountains. Currently, one operational Carolina hemlock conservation bank, containing approximately 1,400 seedlings representing 64 mother trees and nine populations has been planted by Arauco-Bioforest in Chile. Additional conservation plantings are planned for 2010 establishment utilizing eastern and Carolina hemlock seedlings under cultivation at forest nurseries in Brazil and Carolina hemlock seedlings being grown at the University of Arkansas. In 2009, all cooperators in Chile, Brazil, and the U.S. will receive additional seeds of both species for future conservation bank expansion.

In addition to these plantings outside of the hemlock range, Camcore is also working to establish small hemlock seed orchards within the species' native ranges. In 2007, the first of these, containing 400 seedlings, was established for Carolina hemlock in Ashe County, NC and we plan to expand this to include eastern hemlock in the near future. Although such plantings will eventually require insecticide protection from the hemlock woolly adelgid, they are important tools for research on hemlock establishment requirements in seed orchard settings and breeding strategies that will be needed for restoration efforts.

Acknowledgments

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THE SOD FACTS OF LIFE: PROPERTY VALUE LOSSES FROM SUDDEN OAK DEATH IN MARIN COUNTY

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ABSTRACT

A difference-in-difference (DID) hedonic property price model examines the property value damage from the pathogen *P. ramorum* in Marin County, California. The mortality of tanoaks and coast live oaks in Marin County was first observed in late 1998, and the mortality continues throughout the central and north coast of California to this day. The pathogen's growth on the foliage and branches of a variety of tree and shrub species, the ability to spread aurally, and the broad geographic range of the host species makes this disease a serious threat to many forest ecosystems. We determine the property value damage in the geographically diverse and affluent Marin County from coast live oak mortality with a spatial DID model of parcel transactions from 1983-2008, combining knowledge of the year of invasion and several indicators of sudden oak death damage. This study is the first to make use of the DID model to look at the damages of an invasion that spans a decade, with findings for each year of the invasion, with the hedonic property price model.

There are several indicators of sudden oak death damages that include: 1) proximity to coast live oak woodlands; 2) confirmed infections of coast live oaks; 3) aerial observed oak mortality; and 4) arborist reported neighborhoods of oak mortality. Properties close to confirmed infections, aerial observed, or arborist reported neighborhoods of oak mortality have property value losses between 5 and 8 percent before the infected trees are removed. After the removal of the infected trees, property values return to the level typical for that area of the county. There is evidence of sustained property values losses between 2 and 5 percent for properties beside coast live oak woodlands. We believe the sustained property value losses are

where there is a high likelihood of oak mortality around the properties (even if mortality is currently not in the area.)

The results of an initial cross section analysis do not account for the potential feedback of the location of the invasion and the probability that the location is invaded. For instance, some locations may have unobservable characteristics, for instance more recreation activity, that transmits the pathogen to the location more frequently. To overcome this potential problem, we use a fixed-effects difference-in-difference model. This is possible because there are parcel transactions available from 1983 to 1998, before the invasion, in addition to parcel transactions from 1999-2008 during the invasion. The fixed-effects estimator is useful because we identify 53 distinct communities in Marin County for grouping parcel transactions. This way, any time-invariant unobservable variables, in the community, potentially correlated with the regressors will not influence the coefficients.

The County of Marin publicly provides a rich set of geographic information system (GIS) data to fully specify the hedonic property price function. A variety of location, environmental, transportation, topographic, geographic, and neighborhood characteristics for every property are calculated from this GIS data. GIS county wide data of the tree species affected by SOD, the locations of *P. ramorum* confirmations, and arborist reported neighborhoods of oak mortality were obtained from the Marin County Cooperative Extension and the UC Berkeley, Kelly research and outreach lab. GIS county data of oak mortality for the years of 2001-2002 and 2005-2007 were obtained from Pacific Southwest Region, U.S. Forest Service.

JAPANESE *LARICOBIOUS* SP. N., A PROMISING BIOLOGICAL CONTROL CANDIDATE FOR HEMLOCK WOOLLY ADELGID

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ABSTRACT

The U.S. Forest Service foreign exploration effort targeting HWA predators in Asia produced a new *Laricobius* species from Japan. The purpose of this project is to evaluate the newly imported biological control agent, *Laricobius* sp. n., in quarantine and in its native range in Japan. Preliminary host range and basic biology studies show this species has a preference for adelgids and can perhaps inhabit a wide geographic range. Phenological studies of *Laricobius* sp. n., other predators, and their host, *Adelges tsugae* (HWA), are underway in Japan. The presence of each life stage of HWA and its predators have been recorded over a full year. Results indicate the phenology of HWA in Japan is similar to that observed in the southern range of HWA in the eastern US. *Laricobius* sp. n. is present from November to early May, whereas *Sasajiscymus tsuga* and generalist predators are present from late April to early July. Weekly observations of foliage samples indicate the overall impact of natural enemies, on the sistentes and progredientes generations, is significant.

A predator exclusion experiment was conducted from December to the end of April. *Laricobius* sp. n. was the only predator observed during this period. Samples from 20 caged and 20 uncaged branches were removed and the number of HWA counted. The number of HWA surviving within the cages was 42 percent compared to only 5 percent on branches without cages, indicating that *Laricobius* sp. n. is important in the regulation of the sistentes generation.

Results from these studies support the effort to receive permission for *Laricobius* sp. n. to be removed from quarantine in the United States. Furthermore, these results indicate that predators play an important role in HWA regulation in their native range of Japan.

COLD TOLERANCE AND HOST RANGE COMPARISONS BETWEEN COASTAL AND INLAND WESTERN UNITED STATES POPULATIONS OF *LARICOBIOUS NIGRINUS*

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ABSTRACT

A fundamental difficulty in classical biological control is to ensure that natural enemy releases have appropriate genetic quality, which, if lacking, may reduce the species' adaptability to the new environment resulting in lowered impact or even failure. "Biotypes" of *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of *Adelges tsugae* (Hemiptera: Adelgidae), may provide an example of this phenomenon. This predator, first collected for biological control use from the coastal city of Victoria, British Columbia, Canada, established at high rates in warmer parts of the invaded range of *A. tsugae* but consistently failed to establish in cold areas (e.g. northeastern United States), making a cold tolerant biotype desirable. Exploration in the northern Rocky Mountains confirmed the presence there of a geographically isolated *L. nigrinus* population adapted to a climate much colder than that experienced by the coastal population of this predator. To support planned releases in the northeastern United States of this rediscovered inland population, we compared the host range and cold tolerance of inland (northern Idaho) and coastal (Seattle, WA) populations of this species. Host acceptance and suitability tests with *A. tsugae*

and a nontarget adelgid, *Pineus strobi*, indicated that inland *L. nigrinus* also has a narrow host range, as was previously documented for the coastal population. The inland population of *L. nigrinus* was found to be more cold tolerant than the coastal population, based on the super-cooling points of adult *L. nigrinus* from Seattle, WA (-16.9 °C), Coeur d'Alene, ID (-19.2 °C), and Moscow, ID (-18.6 °C) and differential survival rates among populations in a 1 month field cage study during winter in Massachusetts [Seattle, WA (49 percent survival), Coeur d'Alene, ID (90 percent), and Moscow, ID (90 percent)]. Last, comparisons of Seattle, WA and Coeur d'Alene, ID collection locations via CLIMEX v.2 confirmed that the optimal release locations in the eastern United States for the coastal *L. nigrinus* population is the mid- to southern Appalachians and warm coastal areas, while that of the inland population would be the northeastern and Great Lake region (an area of expected *A. tsugae* spread). As such, we recommend release of the coastal *L. nigrinus* population in USDA plant hardiness zones 6a,b and higher; while in zones 5a,b and lower, the inland *L. nigrinus* is preferred.

SLOWING ASH MORTALITY: A POTENTIAL STRATEGY TO SLAM EMERALD ASH BORER IN OUTLIER SITES

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ABSTRACT

Several isolated outlier populations of emerald ash borer (*Agrilus planipennis* Fairmaire) were discovered in 2008 and additional outliers will likely be found as detection surveys and public outreach activities continue. In past years, regulatory officials attempted to eradicate selected outlier populations by removing and destroying ash trees within 400 to 800 m radius of known infested trees. These efforts were expensive, difficult to implement and often alienated affected landowners. Most eradication projects were unsuccessful, largely because of the difficulty of identifying infested but nonsymptomatic trees.

Currently, when a new outlier site is discovered, the township or county is quarantined. Efforts may be made to delimit the extent of the infestation, but no additional action is taken to control or contain the emerald ash borer (EAB) population. This is effectively a “do-nothing” approach that ensures the EAB populations in these sites will build and expand. Results of studies in southeast Michigan, where the EAB infestation in North America originated, indicate that as outlier populations build and coalesce, the rate of spread increases substantially. Additional research shows that once EAB infests a stand, ash (*Fraxinus* sp.) mortality approaches 100 percent, regardless of site or stand variables. If this situation continues, more than 8 billion ash trees representing at least 15 species in U.S. forests may be at risk.

We have learned a considerable amount about EAB since it was discovered in North America in 2002. Integrating the tools currently available to slow the growth and spread of EAB populations in outlier sites could provide a means to slow the expansion of this destructive pest across North America. This would offer communities, resource managers, and landowners with more time for planning. It may also buy time for researchers working with biological control organisms, microbial insecticides, or other potential controls. The gypsy moth Slow-the-Spread

(STS) program is an example of what can be achieved by implementing a similar strategy. In the STS program, detection of a new infestation in the action area is followed by intensive surveys to define the extent of the population. Subsequent applications of pheromone flakes or *Bacillus thuringiensis* (Bt) typically follow. Although these activities occur in an action zone, they serve to protect areas well in advance of the action zone from gypsy moth infestation.

We have begun to develop, implement, and evaluate a strategy to delay the onset and progression of ash mortality caused by EAB. This strategy, termed SLAM (SLow A.sh M.ortality), integrates management tools or options that are appropriate for a specific site. The overall goal of SLAM is to slow the progression of ash mortality, which requires that we reduce the rate at which EAB populations build and expand. Several tools, including girdled ash trees, insecticides, and ash utilization, can be applied in an integrated manner appropriate for conditions at a specific site.

Girdled ash trees are highly attractive to adult EAB, particularly where EAB densities are at low to moderate levels. Girdled trees can serve many purposes in a SLAM site. Trees that are girdled in spring, then felled and debarked in fall provide information about EAB density, distribution and larval development. Girdled trees can function as a “sink” for EAB larvae; when female beetles preferentially oviposit on girdled trees that are subsequently removed or destroyed, a substantial portion of the next generation of beetles is eliminated. At low EAB densities, girdled trees may even influence beetle dispersal, providing opportunities to draw beetles away from an area of concern. Removing girdled trees also reduces the ash phloem available to future generations of EAB larvae.

Insecticides provide another option for slowing the rate of EAB population growth. Our data from a 2007-2008 study show that injecting ash trees with emamectin benzoate, a recently developed product sold as Tree-äge, provided nearly 100 percent control of EAB for at least 2 years post-treatment. Tree-äge currently has a special registration in 10 states and full EPA registration has been requested. This product not only alters the economics of treating ash trees in landscape settings, it may also have a role in slowing EAB population growth in outlier sites.

Utilizing ash trees for timber or firewood may provide some value to landowners, as well as reducing the potential number of EAB that can be produced in a given SLAM site. Research we published in 2007 showed that on average, about 100 EAB adults can develop per m² of ash phloem. Complete ash inventories collected in five different sites have shown that only 5 to 6 percent of the ash trees in a site are ≥25 cm in d.b.h. (10 inches). These large trees, however, contain at least 50 percent of the ash phloem in the site. Selectively harvesting only the large ash trees, which make up only 5 to 6 percent of all the ash stems, could reduce the potential EAB production in a site by at least 50 percent.

Other tools that could eventually be incorporated into a SLAM effort may include biological control. Asian parasitoids of EAB eggs or larvae were introduced into several sites in 2007 and 2008; their ability to establish and exert some effect on EAB populations will be evaluated over the next few years. Similarly, native parasitoids such as *Atanycolus hicoriae*, may have potential for augmentative biocontrol. Woodpeckers remain the single most important cause of EAB larval mortality, but we still know relatively little about whether woodpecker predation of EAB could be enhanced.

If a SLAM strategy is implemented at an EAB outlier site, evaluation will become important. We are developing a simulation model that can use spatially explicit data to estimate how EAB populations will build and spread and how ash mortality will advance if no action is taken in an outlier site. We can also compare the rate at which ash mortality progresses in a SLAM site that observed in southeast Michigan, where an extensive dendrochronological analysis was completed. An

economic analysis of costs and benefits associated with a SLAM project will obviously be important.

Several cooperators are currently working to implement a SLAM pilot project in the eastern Upper Peninsula of Michigan near the towns of Moran and St. Ignace. This EAB infestation was discovered in fall 2007 when a girdled detection tree was found to be infested. The Michigan Department of Agriculture felled several additional ash trees in the area in winter 2007 and a total of 13 infested trees were found. The infestation appears to be relatively recent in origin and to date, no ash trees exhibit any external symptoms of infestation.

In 2008, the area was intensively surveyed to better define the extent and distribution of the infestation. Using a grid pattern, girdled ash detection trees were established at densities of 16, 4 or 1 trap tree per mi² within a 1, 1-2 and 2-3 mile radius, respectively, of the trees determined to be infested in 2007. More than 500 trap trees, typically 4-6 inches d.b.h., were girdled in June, then felled and debarked in September and October. Survey crews recorded number and stage of larvae on positive trees. If there was no suitable ash tree for girdling in a grid cell, an APHIS program trap (purple canopy trap baited with Manuka oil) was installed. In addition, APHIS officials required that a purple canopy trap be placed in every grid cell, in addition to the girdled tree.

Results from the intensive sampling in 2008 showed that in the area centered around Moran, there were 24 girdled trees that had EAB larvae, with an overall average density of approximately eight larvae per m². Several trees had only small larvae that would likely have fed again in 2009 and emerged as adults in 2010. Nine of the panel traps captured at least one EAB adult; seven of those traps, however, were attached to girdled trees that had larvae. Two additional positive trap trees were located in grid cells near St. Ignace.

Density and development stage of EAB larvae (1-year or 2-year larvae) on each positive girdled tree were imported into a GIS. Maps revealed that a core infestation and four distinct satellite populations could be defined. Cooperators drafted a plan of action for 2009 that includes establishing clusters of 2 to 3 girdled ash trees within the core infestation centered on Moran. The goal of

these girdled trees would be to attract and contain adult female EAB and to reduce the likelihood of oviposition on trees outside that core area. The trees will be removed in fall 2009. An additional 50 ash trees, located 400-800 m around the perimeter of the core infestation, would be treated with a trunk injection of Tree-äge insecticide. Ideally, this would establish a “buffer” of trees that would be toxic to any EAB that dispersed beyond the existing core. In the satellites, up to four girdled trees would be established within 150 m of the original positive tree and at least four additional trees located 150-400 m around the perimeter would be treated with Tree-äge.

Additional activities in the SLAM outlier site also were initiated in 2008. A consulting forester worked with private landowners in the area and arranged a timber sale, which will reduce ash phloem and provide some financial reward for the landowners. On national forest land, several large ash trees were identified in an area accessible by foot only during winter. These trees, which were probably not

yet infested, could not be harvested or otherwise treated, so they were felled, bucked into sections and left on site, simply reducing the potential production of adult EAB in that area. A street tree inventory was completed for St. Ignace to identify the abundance and size of ash trees that may require insecticide treatment or replacement. Outreach activities were initiated with residents and property owners in the area to inform them about SLAM and potential options for treating their landscape trees. Efforts are underway to quantify the ash resource within and around the SLAM site.

It seems likely that the SLAM strategy developed for this outlier site should be successful, assuming that adequate funding is available. A SLAM strategy needs to be tested in additional pilot sites, however, where conditions and management options are different. Knowledge gained from additional pilot sites, as well as ongoing EAB research, will be needed to develop an effective approach to dealing with this devastating pest.

COMPARISON OF EMERALD ASH BORER PREFERENCE FOR ASH OF DIFFERENT SPECIES, SUN EXPOSURE, AGE, AND STRESS TREATMENTS IN RELATION TO FOLIAR VOLATILES AND NUTRITION

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ABSTRACT

We investigated the host selection behavior and feeding preference of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) on six different species of ash including Manchurian ash (*F. mandshurica* Rupr.), green ash (*F. pennsylvanica* Marsh), white ash (*F. americana* L.), black ash (*F. nigra* Marsh), blue ash (*F. quadrangulata* Michx.), and European ash (*F. excelsior* L.). Manchurian ash is native to Asia whereas the other species represent novel hosts for emerald ash borer. We released ~5-day-old beetles into metal screen cages (60 × 60 × 60 cm) containing one leaf from each of the six ash species. Foliage was cut from 2- to 4-year-old greenhouse saplings. Fifteen saplings of each species were used for the study and care was taken to see that leaves from the same plant were not used more than once. Leaves were placed individually in glass vials containing water and arranged in a row across the center of the cage in random order. Thirty beetles were released into each cage, and their distribution on the leaves of the different ash species was observed every 2 hours during the day for 48 hours. Leaves were scanned before and after the experiment to determine the amount of each leaf consumed. This six-choice feeding bioassay was replicated 14 times for each sex. We found beetles landed on and consumed significantly more of the leaves of green, black, and white ash than of blue, European, and Manchurian ash. While beetles fed on leaves from each ash species offered, Manchurian ash and blue ash were least preferred in the six-choice feeding bioassays. When fed on green and Manchurian ash foliage from adult emergence in a no-choice assay, beetles on green ash consumed significantly

more based on leaf area compared to those fed on Manchurian ash. However, neither longevity nor beetle body weight differed in either case, suggesting that Manchurian ash might have greater nutritive value or resistance than green ash.

We then compared the leaf volatiles from intact and girdled (stressed) ash trees for quantitative variation in 12 compounds, which had elicited antennal activity in an earlier study. We found that the overall volatile profiles of the six ash species differed significantly in their relative amounts of these 12 compounds. Green ash had lower relative amounts of volatiles compared to Manchurian ash, which might render it more attractive and less resistant to emerald ash borer. Relative volatile amounts of the other four species were intermediate between levels in green and Manchurian ash.

Finally, we evaluated the performance of emerald ash borer adults fed green ash leaves in relation to nutrition and defensive compounds. The effects of leaf age, sunlight exposure, and tree girdling were compared. Nutrition was quantified by measuring total amino acid content, total soluble protein (P), and nonstructural carbohydrate (C). Defensive compounds measured included trypsin and chymotrypsin inhibitors and total phenolics. We found mature leaves were significantly more nutritious than young leaves, with the highest nutrition in leaves were detected in trees grown in the shade. When compared to young leaves, the mature leaves also had significantly lower levels of both protease inhibitors and total phenolics. Leaves grown

in the sun had reduced amino acids and a lower P:C ratio, irrespective of leaf age and girdling. Sunlight also dramatically increased all defensive compounds of young leaves, but not old leaves. Overall, girdling reduced green ash foliar nutrition but had no effect on the protease inhibitors. Emerald ash borer survival and longevity were greater on mature than on young leaves but did not differ among sunlight treatments, nor between girdled and control treatments. One explanation is that beetles grown on lower quality food compensated by increasing consumption rate. Lower defense of mature leaves alone, or along with higher nutritional quality, may lead to better survivorship and greater longevity of emerald ash borer feeding on mature leaves.

A PROACTIVE APPROACH TO PREVENT INVASIVE PLANT PATHOGENS

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ABSTRACT

This paper describes proactive work by federal agencies to prevent new introductions of forest pathogens.

Executive Order 13112 requires federal agencies to work together to enhance our abilities to prevent, eradicate, and control invasive species, and to restore the structure and function of invaded ecosystems. This federal order established the National Invasive Species Council (NISC), and entomologists and pathologists from all the federal agencies in Washington, DC work together in subcommittees of the Invasive Terrestrial Animals and Pathogens (ITAP) group that formed under NISC. Each of these subcommittees has developed a strategic plan to determine how working together could improve our collective abilities to predict, prevent, eradicate, and control invasive species.

The Plant Pathology subcommittee of ITAP has members in the Agricultural Research Service (ARS), Animal and Plant Health Inspection Service (APHIS), Cooperative States Research, Education, & Extension Service, Department of Homeland Security, Economics Research Service, Forest Service, National Park Service, Natural Resources Conservation Service, and the U.S. State Department.

Our strategy has goals that support the “human elements” of invasive pathogen science, such as completing and sharing an inventory of personnel, with information about their areas of expertise. We support the need for improved capacity in the field of systematics in which the number of trained personnel has declined. Systematics is essential for preventing and detecting new forest pathogens in the United States. A report on this crisis, which is certainly not limited to plant pathology, is available at www.itap.gov. We also support the need for, and are contributing to, economic assessments of invasive plant pathogen impacts, as economic data are sorely needed to demonstrate

the value of plant pathology research and proactive management strategies.

Another goal is to develop lists of the most important plant pathogens, with fact sheets that illustrate their diagnostic features, biology and pathways. Rossman et al. have nearly completed the list of pathogenic fungi, and the fact sheets are about halfway done. Some are available at <http://nt.ars-grin.gov/sbmlweb/fungi/diagnosticfactsheets.cfm>.

ITAP offers the opportunity to improve prevention through projects that piece together data collected by different agencies. For example, our subcommittee reviewed and evaluated APHIS’ regulated plant pathogens list (Rossman et al. 2006). We also have reviewed the list of invasive forest pathogens and their likely pathway of entry. Of 17 pathogens, three likely entered the United States on logs or wood products (*Ophiostoma novo-ulmi*, *O. ulmi*, and *Raffaelea lauricola*), and nine (*Cronartium ribicola*, *Cryphonectria parasitica*, *Cryptodiaporthe populea*, *Discula destructiva*, *Lachnellula willkommii*, *Melampsora laricis-populina*, *Phytophthora lateralis*, *Phytophthora cinnamomi*, *P. ramorum*) are considered to have entered on nursery stock. Since this is such a critical pathway, the pathology subcommittee strives to support the revision of “Q-37”, the regulations for plants for planting. One way to do this is to provide information on pathogens that exist in other countries, but not yet in the U.S. Plant host genera of concern will be considered for potential listing in the proposed new category of plants for planting called NAPPRA (Not Authorized Pending a Pest Risk Assessment).

Another effort that ITAP pathologists and entomologists are supporting is the development of a Sentinel Plant Network. Botanic gardens and arboreta are being

asked to monitor their collections and report any new or unusual pest activity to the Cooperative Agricultural Extension Service in their state, university plant pathology laboratories that are linked in a National Plant Diagnostic Network (NPDN). These labs can provide advice on control measures if the pest is known already to occur in the U.S. If the pest is exotic, the NPDN labs report it to APHIS, who will notify counterparts overseas. If the pest arrived with the plant, experts overseas can help mitigate the problem. If the problem is a local pest that the host plant has never seen before, APHIS counterparts overseas will be grateful for an early warning that such a pest exists. If hosts are moving in trade, the relevant National Plant Protection Organizations will take steps to ensure that such exchanges are from clean stock only. You can read more about this project at www.itap.gov/SentinelPlantNetwork/ Lastly, the ITAP Plant Pathology subcommittee has developed a website on invasive pathogens with the help of professionals at the ARS National Agricultural

Library. We hope this website will provide you with useful information such as data sources, species profiles, economic impact references, and management plans as these are developed. All this and more is available at www.itap.gov.

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DEVELOPMENT OF A WEB-BASED TOOL FOR PROJECTING COSTS OF MANAGING EMERALD ASH BORER IN MUNICIPAL FORESTS

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ABSTRACT

City managers faced with the invasion of emerald ash borer into their urban forests need to plan for the invasion in order to obtain the resources they need to protect the public from harm caused by dying ash trees. Currently, city foresters can avoid harm from falling trees by removing, replacing, or treating them with insecticides. Costs for these activities vary widely between municipalities depending on local real estate values, labor costs, and the type and frequency of insecticide treatment. The particular combinations of these tactics that work best for a particular city depend on forest composition, local needs and budgets.

The emerald ash borer cost calculator is a web-based tool that allows municipal arborists use local information to develop a set of management strategies, calculate their projected costs over a 25-year period, and make comparisons. To run the web tool, forest managers need a current inventory of ash trees that details the number present in a user defined set of size classes measured as diameter breast height. They then need to obtain insecticide treatment costs, tree removal and tree replacement costs from in-house cost schedules or as bid from subcontractors. The calculator then uses these costs to estimate expenses of a particular management strategy for a 25-year period. Cost projections are based

on a growth model developed for Indianapolis ash trees during a municipal forest tree resource assessment (Peper et al. 2008). The calculator allows the user to define management strategies by assigning proportions of tree for treatment with insecticides, for removal and replacement, or removal only. Up to three management strategies can be compared at a particular time. Users can specify a discount rate of their choosing to account for the time value of money.

A mock analysis using actual tree inventory data from the city of Indianapolis and maintenance costs was reviewed to demonstrate the potential of the calculator to explore costs of management scenarios over a 25-year period. The calculator is available at the following website <<http://extension.entm.purdue.edu/treecomputer/index.php>>.

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***AILANTHUS*, TREE-OF-HEAVEN UPDATE, A NORTHEAST REGIONAL BIOLOGICAL CONTROL PROJECT**

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ABSTRACT

The tree-of-heaven (TOH), *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), is an invasive weed tree distributed throughout most of the continental United States. It is a hardy pioneer species that colonizes disturbed sites, such as road medians, rights-of-way, and forest settings. It produces allelopathic chemicals, millions of seeds, and sprouts back when cut, making it an extraordinarily good competitor, resulting in the displacement of native plant species. Herbicide treatments can provide short-term relief, but are expensive and unsustainable. In 2002, Ding Jianqing, Biological Control Institute of China, identified two weevil herbivores from China as potential biological control agents for TOH. They are the closely related *Euchryptorrhynchus brandti* (Harold) and *E. chinensis* (Olivier) (Coleoptera: Curculionidae). Both species develop under the bark of the main stem of the tree and are considered tree killers and major pests of TOH in China. Additionally, both species are not known to feed on other plants in their native habitat. Due to this recommendation, Virginia Tech began importing both weevil species to their Beneficial Insect Quarantine Laboratory in 2004 to initiate quarantine studies of both species. *E. brandti*, the smaller of the two species, has been easier to maintain and study and has been the subject of most of our testing. Since its arrival, we have studied the insect's biology, developed laboratory-based rearing procedures, and carried out the full suite of host-range studies. All studies are nearing completion and we hope to petition USDA APHIS for release of this insect in 2010.

In an effort to learn more about *E. brandti* in its native habitat, we initiated a new study in 2008 that examined the life cycle of the weevil at TOH sites in eastern China (Shandong Province). More specifically, we examined seasonal activity, feeding and oviposition behavior, and colonization of trees as a function of tree health. After 1 year of sampling, we found that *E. brandti* often co-occur with *E. chinensis* on the lower boles of trees. They were commonly found mating and active on trees throughout the spring, summer, and fall, and were rarely found in the canopy and/or on foliage. Oviposition could only be sampled by cutting bark sections off the tree. It was found that eggs were present in the phloem tissue from spring to fall, and in most cases, development on any single tree never went beyond the first instar. Emergence was observed only from trees that were dead. Our hypothesis is that their development cannot be completed in healthy trees, only weakened, dying trees. We were not able to observe trees in decline, therefore more work is planned in 2009 to try to better understand this relationship between the herbivore and its host.

In south-central Pennsylvania, TOH invaded an oak-dominated forest that had been logged and left with large openings. In 2000, the TOH within the stand started to show signs of wilting. Fungi isolated from these trees included *Verticillium albo-atrum* and *V. dahlia*. Pathogenicity tests showed that *V. albo-atrum* was capable of killing inoculated seedlings in the lab and inoculated trees in the field. The spread of the

pathogen was tracked annually, and by 2007, 7,000 trees were dead. Preliminary host-range testing shows that northern red oak, chestnut oak, red maple, sugar maple, yellow-poplar, and white ash are not susceptible to the pathogen. However, striped maple does show some signs of susceptibility.

Future work in biological control will consider testing whether *E. brandti* can serve as a vector for the pathogen and enhance its spread. The insect attacks and feeds on live trees in the lower boles. In a preliminary test in the lab, weevils emerging from TOH bolts infected with the fungus did carry the pathogen. Studies are underway to determine the extent to which *E. brandti* and *V. albo-atrum* can be used together as biological control agents for TOH.

MANIPULATING SPREAD AND PREDICTING DISPERSAL OF ISOLATED EMERALD ASH BORER POPULATIONS

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ABSTRACT

The ability to manipulate the spread of an invasive species could potentially be integrated into an effective management strategy to delay dispersal to uninfested areas while concentrating the population in an area where suppression activities could be applied. Here we examined the influence of clusters of girdled ash trees on the spread of isolated emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), populations in forested areas. Two 16-ha (40 ac) forested sites with low-density emerald ash borer populations were selected for this study. Each site was divided into sixty-four 50×50 m cells and complete ash inventories by diameter class were conducted. The amount of ash phloem in each 50×50 m cell was calculated as per McCullough and Siegert (2007) and two 4-ha (10 ac) blocks with similar ash density and distribution of total ash phloem were selected per site. At each site, four clusters of girdled ash trees (three trees per cluster; n = 12 total girdled trees per site) were established in one of the randomly selected 4-ha blocks. Beetles were released from infested logs placed between the two blocks. Girdled and nongirdled ash trees (n = 230 trees) were sampled the following winter to assess spread of the populations. Preliminary results indicate that clusters of girdled trees strongly influenced the spread of the low-density emerald ash borer populations at both sites. Overall, emerald ash borer densities were 4.6× and 7.0× greater on the 4 ha with clusters of girdled trees compared to the 4 ha without girdled trees at the two sites, respectively. Girdled trees had densities that were 9.0× and 14.6× higher than emerald ash borer densities on nongirdled trees in the control blocks at the two sites.

Once an invasive species has become established and eradication is unlikely (or too costly), a successful strategy may be to stop or slow the spread of its population. This strategy has been proven to successfully reduce the negative impacts of numerous invasive species (Liebhold & Tobin 2008), most notably the Slow The Spread program implemented to manage the gypsy moth, *Lymantria dispar* (Tobin & Blackburn 2007). For this strategy to be effective, the ability to predict the potential effect of management options on the spread of the invasive species is essential. Here we incorporated components that allow the simulation of three potential management strategies to a model of the local spread of emerald ash borer (see Mercader et al., this volume). The potential management options simulated were the use of (1) a grid of trap trees; (2) phloem reduction; and (3) insecticide use. Within a homogeneous environment insecticide treatments applied to 50 percent of the phloem (~6 percent of stems if large trees are chosen) were more effective at slowing the spread of isolated populations of emerald ash borer than the use of a single trap tree per year per 50×50 m cell, which in turn was more effective than 50 percent phloem reduction. Results from simulations performed in environments varying in resource distribution indicated that the distribution of ash trees in an area strongly influenced the spread of emerald ash borer and the efficiency of the different management options. Overall these three management options did significantly reduce the local population size of emerald ash borer, which has the potential to slow the spread of emerald ash borer as the population moves out of the management area. However,

within the management area, only the use of trap trees and insecticide treatments lead to a decrease in the local spread rate of emerald ash borer.

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RESEARCH UPDATE ON THE BROWN SPRUCE LONGHORN BEETLE, *TETROPIUM FUSCUM* (FABR.)

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ABSTRACT

A 3-year research project, funded by the Canadian Forest Service and the Canadian Food Inspection Agency, was initiated in the spring of 2007 to address key issues of provincial and forest industry stakeholders concerning the brown spruce longhorn beetle, *Tetropium fuscum* (Fabr.) (Coleoptera: Cerambycidae). *Tetropium fuscum* is an invasive wood boring beetle from Europe that has been established in Halifax, Nova Scotia since at least 1990. The project is focused on the development of practical tools and knowledge for risk mitigation, risk analysis, estimating rate of spread and pheromone-based methods of population suppression. In 2008-09 we began ecological studies on the effect of host condition, natural enemies, and competition on *T. fuscum* fitness. Here, we briefly highlight our progress to date.

Spruce logs infested with prepupae, pupae, and emerging adult *T. fuscum* and *Tetropium cinnamopterum* (Kirby) were processed by sawmill debarkers and hog machines to determine the relative risk of moving live *T. fuscum* in round wood, debarked logs, bark, and hog fuel. Significantly more *Tetropium* adults emerged per square-meter surface area from untreated logs and debarked logs than from bark. Bark from debarkers produced less than 0.15 percent of the *Tetropium* spp. of untreated logs. Processing the bark through a hog machine, a common practice at most Maritime sawmills, reduced the risk further still. No *Tetropium* adults emerged from an infested bark sample fed through a sawmill hog machine compared to 84 percent survival and emergence of *Tetropium* adults from an untreated control sample. Results indicate that bark and hog fuel have much lower risks of carrying *T. fuscum* than round wood.

In collaboration with Therese Poland (USDA Forest Service) and Robin Taylor (Ohio State University), we've been studying the flight behavior of *T. fuscum* on laboratory flight mills. Preliminary data indicate that most beetles make short flights but on average both sexes flew a total of >1 km in 24 h with some individuals flying >9 km and others not flying at all. Most flight occurred during the day (16 hr photophase) but the most flights per hour occurred at dusk and dawn. More flight mill data are being gathered to compare the effect of sex, size, mating status, and rest (24 hr on, 24 off) on mean distance flown per 24 h and per adult lifespan. Flight behavior and dispersal behavior of individual *T. fuscum* in the field will be measured in 2009 using a back pack harmonic radar unit and tagged *T. fuscum*, in collaboration with Gilles Boiteau (AAFC, Fredericton) and Bruce Colpitts (UNB, Fredericton). Laboratory trials with tagged beetles (with a copper-coated steel wire attached to the pronotum with crazy glue) indicate that tags did not significantly affect *T. fuscum* flight and mean flight angle after take off did not differ between tagged and untagged beetles.

Deepa Pureswaran is developing a population growth model for *T. fuscum* based on empirical studies of the community of organisms associated with *T. fuscum*, such as its fungal associates *Ophiostoma tetropii* and *O. piceae*, mites (phoretic or parasitic), parasitoids, predators, and native bark and wood borers that may act as competitors or synergists, such as the native spruce beetle, *Dendroctonus rufipennis* (Kirby). Leah Flaherty (Ph.D. candidate) is studying the effects of host tree condition, larval density, co-infesting phloem feeders, and two native endoparasitoids of *T. fuscum* (*Rhimphoctona*

macrocephala [Ichneumonidae] and *Wroughtonia occidentalis* [Braconidae]) on colonization success, stage specific mortality and overall fitness of *T. fuscum* (see Flaherty et. al., poster abstract in these Proceedings)

Male *T. fuscum* emit a pheromone (fuscumol) that in combination with host volatile lures synergizes attraction of male and female *T. fuscum* (Silk et al., 2007). We tested two methods for suppressing *T. fuscum* populations in 2008: 1) ground application of 10 percent fuscumol in Hercon flakes to disrupt mating; and 2) mass trapping of male and female *T. fuscum* in traps baited with fuscumol + host volatiles (100 traps per ha). For both methods we predicted a significant reduction in both mean catch of *T. fuscum* in traps baited with fuscumol + host volatiles and infestation in bait logs. Ground applications of Hercon flakes did not reduce attraction of *T. fuscum* to traps baited with fuscumol + host volatiles and had mixed results in terms of *T. fuscum* infestation in spruce bait logs. Mass trapping of *T. fuscum* reduced overall mean infestation of *T. fuscum* in bait logs and appeared to work better at low population densities. These data are preliminary and trials must be repeated in 2009.

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IMPLICATION OF GLOBAL CLIMATE CHANGE ON THE DISTRIBUTION AND ACTIVITY OF *PHYTOPHTHORA RAMORUM*

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ABSTRACT

Global climate change is predicted to alter the distribution and activity of several forest pathogens. Boland et al. (2004) suggested that climate change might affect pathogen establishment, rate of disease progress, and the duration of epidemics, each in a potentially different way. In some cases, climate changes may favor the onset of disease and potentially accelerate the displacement of a tree species from portions of its current geographic range. In other instances, climate changes may be detrimental to the development of disease. Boland et al. (2004) qualitatively predicted that climate change would have a strong positive net effect on four of 18 tree pathogens in Ontario, Canada and would have a negative net effect on another four pathogens.

Phytophthora ramorum is an alien invasive pathogen, likely present in the United States since the mid-1990s. The pathogen is the cause of Sudden Oak Death (SOD) and several other diseases. Infected tanoaks (*Lithocarpus* spp.) and oaks (*Quercus* spp.) are found in 14 western counties of California and one county in Oregon. Previous work has suggested that the distribution of the pathogen is affected by regional climate patterns.

The purpose of the study was to quantify the potential change in occurrence of climatically suitable habitat for *P. ramorum* under future climate scenarios.

All analyses were conducted with the ecological niche model, CLIMEX. Biological parameters describing the response of the pathogen to temperature and moisture were taken from Venette and Cohen (2006). Baseline and future climate projections, downscaled to a 10-minute resolution, were obtained from worldclim.org. Baseline data represented the period from 1961-1990. Climate projections were based on the Canadian General Circulation Model-1 (CGCM1) from the Canadian Centre

for Climate Modeling and Analysis under emissions scenario b2 (assumes slowed population growth and reduced greenhouse gas emissions). Climate projections were available for the years 2020, 2050, and 2080. For each year, CLIMEX provided several indices of climatic suitability for the presence of the species. The Ecoclimatic index provides a measure of overall habitat suitability.

The Ecoclimatic Index for the baseline climate data gave a qualitatively satisfactory fit to observed occurrences of the pathogen in California. The pathogen was observed more often in areas that were predicted to be favorable or very favorable than in areas predicted to be marginal or unsuitable. Because the model parameters were not estimated directly or indirectly from field observations, the field observations provide a completely independent validation of the model.

The baseline model predicts that climatically favorable or very favorable habitat in the contiguous US should currently extend along the west coast from approximately Monterey, CA to Puget Sound, WA. Large areas of climatically suitable habitat also occur in the eastern half of the United States. Based on the predictions from CGCM1, we predict that the area that is favorable or very favorable will decrease substantially in the eastern US, but will increase in WA, OR, and CA. By 2050, favorable habitat will extend from Los Angeles, CA to Puget Sound, WA. Inland progression of climatically favorable habitat, even by 2080, is predicted to be modest. In the eastern US, only fragmented pockets of favorable or very favorable habitat are predicted to occur in far western North Carolina, in the northeast quarter of West Virginia, and a small region from northern New Jersey to the southern half of Massachusetts.

This shift in distribution is not likely to be the result of cold stress acting on the pathogen. Cold stress consistently

diminishes from 2020 through 2080. Drought stress also does not seem to be driving the change in distribution. Although drought stress is predicted to increase in the western half of the United States, these changes appear to be occurring in areas that are already largely unsuitable for the pathogen.

The shift in distribution seems likely to be the result of changes in heat stress. Biological heat stress, based on the presumed tolerances of *P. ramorum*, is predicted to increase substantially across much of the contiguous US by 2080, with notable exceptions.

This preliminary model describes the direct effects of changes in temperature and moisture on the suitability of the climate for the pathogen. Climate change may also act indirectly on disease by causing tree stress and altering the susceptibility of trees to infection. More work is needed to relate CLIMEX indices to measures of disease incidence and severity.

Deductive ecological niche models such as CLIMEX are vitally important to integrate biological knowledge about a pathogen and its interaction with the environment. Model outputs provide essential information for quantitative assessments of future risks posed by the pathogen.

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STATUS REPORT: BIOLOGICAL CONTROL OF SWALLOW-WORTS

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ABSTRACT

Two swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*), originating from Europe, have become established in the eastern United States and Canada. Swallow-worts are herbaceous perennials that persist in a variety of habitat types in North America although their distribution in Europe is restricted. In North America, populations of *V. nigrum* grow in open habitats and along forest margins whereas *V. rossicum* penetrates dense forests. Their population expansion and aggressive growth threaten native biodiversity and disrupt ecological processes. Swallow-worts are becoming increasingly problematic in pastures and no till field crops. The lack of herbivore pressure in North America and the difficulty in controlling these weeds has spawned interest in a biological control program.

In 2006, we collected five insect species on swallow-worts in Central and Eastern Europe. In Europe, insect herbivores demonstrate strict habitat preferences—open fields or forests and seldom is a single species found in

both. More insect herbivores are found on *Vincetoxicum hirundinaria* in Europe than on *V. rossicum* and *V. nigrum*, the targets of biological control efforts in North America. Based on two seasons of host specificity studies, we tentatively conclude that the leaf-feeding larvae of the noctuids *Abrostola asclepiadis* and *Hypena opulenta* are specific to *Vincetoxicum*, while the larvae of chrysomelids *Chrysolina a. asclepiadis* (leaf-feeder) and *Eumolpus asclepiadeus* (root-feeder) can develop on additional plant genera. We are currently assessing adult beetle host preferences to determine their potential for nontarget effects. We recently confirmed acceptance and development of the seed predator *Euphranta connexa* on the target weeds. Further host specificity testing will determine whether North American plants are at risk from *E. connexa*. In 2008, we initiated impact studies with *A. asclepiadis*, *E. asclepiadeus*, and *H. opulenta*. Future studies will focus on host-specific herbivores and determining their impacts on swallow-wort population dynamics as individual species and in combinations.

BIOLOGICAL CONTROL OF *SIREX NOCTILIO* IN NORTH AMERICA BY *BEDDINGIA SIRICIDICOLA*: 2008 UPDATE

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ABSTRACT

The European woodwasp, *Sirex noctilio* F., was discovered in Oswego County, New York, in the autumn of 2004. The woodwasp is apparently not under effective natural control, is already distributed over a wide area in North America, and because it is a killer of relatively healthy trees, poses a serious threat to pine forests and plantations in the United States and Canada if not controlled. Its most effective natural enemy is a parasitic nematode, *Beddingia (Deladenus) siricidicola* (Bedding). This nematode has been used successfully as a biological control agent in management programs throughout the southern hemisphere where *Sirex* woodwasp has invaded. The nematode's unique life history facilitates its use as a management tool. Dependent upon physical conditions, it can develop into either of two forms. The mycophagous form feeds on the *Sirex* symbiotic fungus, *Amylostereum areolatum* (Fries) Boidin, as it builds populations inside a woodwasp-attacked tree. The parasitic form attacks *S. noctilio* larvae and ultimately sterilizes the emerging woodwasp females. We provide an update on our recent activities to utilize the nematode in a developing biological control program, including the identification of a native strain of *B. siricidicola*, results of controlled nematode releases during the past three seasons, and consideration of potential impacts of the biological control program on nontarget siricid species.

Our biological control program uses the highly pathogenic "Kamona strain" of *B. siricidicola* obtained from Ecogrow, the licensed nematode producer in Australia. However, as early as 2006, we found a nematode already infecting woodwasp larvae and adults in our study plots. This "native strain" was similar in appearance to the Kamona strain, and we speculated that it entered North America along with *S. noctilio*. As revealed by numerous trapping and rearing studies from 2006 to 2008, it is already present throughout an 80-km radius of Syracuse, NY. Our colleagues in the

Canadian Forest Service (CFS) reported a similar strain in 2008 and identified it as *B. siricidicola* using DNA analysis. With the help of a CFS scientist, we identified our native strain as a match to *B. siricidicola* and to the Canadian strain, but not to the Kamona strain. Clearly, the presence of a strain of the same species that we are releasing poses a challenge to our evaluation of the effects of the Kamona strain as a biological control agent and necessitates the development of powerful molecular tools for discriminating nematode strains.

We have conducted three controlled releases in autumn during the 2006/7, 2007/8, and 2008/9 seasons. The goals of the studies were to test the Australian inoculation method, to assess the establishment of Australian nematodes in American pine species, and to evaluate overwintering survival of the exotic nematodes under North American conditions. The releases were "controlled" so that no nematodes could escape into the environment. Trees were inoculated in the fall, and billet samples were taken in winter for rearing of *Sirex* adults in the lab. All remaining tree materials were destroyed before insect emergence in the spring. Controlled releases were necessary for several reasons. First, the environmental assessment from APHIS Environmental Services that was needed for a full release was not finished by the first release date in November 2006. Second, we bought bulk nematodes from Ecogrow in 2006 for direct field release and did not want to risk releasing an aggressive strain of *A. areolatum* that was present with the nematode. Third, we were sensitive to concerns in the environmental community as to possible (albeit unlikely) impacts of the nematode on nontarget native siricids.

The controlled release in 2006 was carried out on 93 red and Scots pines, which were inoculated with Ecogrow nematodes in early November. It seems very likely

that the nematodes in this release did not establish in the trees because of cold temperatures late in the season. The subsequent two releases in 2007 and 2008 were made on red and Scots pines (95 and 85, respectively) with nematodes grown in the Otis Lab on a New York isolate of the fungus. Because they were made early, in early October and late September, respectively, it seems highly likely that the nematodes survived and established, although samples from the 2007 release are still being dissected and those from the 2008 release are still in the field.

Billet samples from the first release produced 2,224 *Sirex* adults, 810 *Ibalia leucospoides* (Hochenwarth) adults, and 56 *Rhyssa lineolata* L. adults. The overall rate of infection by the nematode was just 5.0 percent, whereas the overall rates of parasitism were 26.2 percent by *I. leucospoides* and 1.8 percent by *R. lineolata*. Releases were made in five sites in Onondaga, Oswego, and Madison Counties, NY, four of which contained Scots pines and one of which contained red pine. Rates of parasitism by *I. leucospoides* ranged from 23.7 percent to 32.9 percent across sites, whereas those by *R. lineolata* ranged from 0.0 percent to 12.0 percent. Rates of infection by nematodes ranged from 4.4 percent to 12.6 percent for the Scots pine sites, whereas the infection rate at the single red pine site was just 0.8 percent. This suggests real differences between the two pine species as hosts for *B. siricidicola*.

Controlled releases were carried out in 2007 in Oswego and Onondaga Counties, NY, in three sites containing Scots pine and one site containing red pine. To date, just 22 percent of the emerging *Sirex* adults have been dissected, but the preliminary results compared with those of 2006 suggest that nematodes established in 2007. Nematode infection rates ranged from 13.0 to 51.0 percent in the Scots pines, and the infection rate was 8.2 percent in the red pine site. Another small release was made in mid-October of 2007 in Macomb County, Michigan, near the first trap catch of *S. noctilio* in that state. In all, five naturally struck Scots pine trees were sampled, and they produced 15 *Sirex* adults, none of which were infected by nematodes.

Possible effects of the nematode biological control program on nontarget native borer species, especially siricids, continue to concern the environmental community.

Eastern North America has three native siricid species that use *Pinus* species as hosts: *Sirex edwardsii* Brullé, *S. nigricornis* F., and *Urocerus cressoni* Norton. The fungal symbiont of these species is the key to understanding their susceptibility to *B. siricidicola*. Two species of *Amylostereum* are commonly associated with siricids worldwide. *Amylostereum chailletii* (Pers. ex Fries) is native to North America, whereas *A. areolatum* is native to Europe. The nematode lives only on *A. areolatum*. Thus, siricids living on *A. chailletii* have a refuge from nematode parasitism. The three pine-feeding native siricids are likely to use *A. chailletii* because of their North American origin and not to be exposed to the nematode. Other than these, the primary species of nontarget concern among the siricids of eastern North America is *Xeris spectrum* (L.). It does not have a fungal symbiont and may feed on either *A. areolatum* or *A. chailletii*, thus rendering it susceptible to the nematode.

We suggest a strategy for evaluating the susceptibility of native siricids more rigorously. First, we should concentrate our efforts initially on the pine-feeding species, as they are closest taxonomically and in microhabitat to *S. noctilio*. Second, we should document the fungal symbionts of those species through field collections over a wide area. Third, we should verify experimentally that the Kamona strain does not develop and reproduce on *A. chailletii*. Although published literature suggests that it does not, this experimental work will be replicated at the Otis Lab. Fourth, if a siricid species is associated only with *A. chailletii* and *B. siricidicola* cannot live on *A. chailletii*, then we infer that the species is unlikely to be susceptible to the nematode. Finally, it is important to survey the native siricids widely for infections by the “native” nematode to investigate their more general susceptibility to nematode infection. Trapping studies in our lab during 2007 and 2008, despite capturing large numbers of infected *S. noctilio*, failed for the most part to find nematode infections among the native *Sirex* and *Urocerus* species that were trapped. The exceptions were two (of 13) specimens of *U. cressoni* in the 2008 collection that were infected by nematodes, whose identity is currently unknown. In addition to these studies, dissections of over 900 insects emerging in the 2007 controlled release study found no parasitism by *B. siricidicola*.

CLIMATE CONSTRAINS FOR SIBERIAN MOTH DISTRIBUTION IN EUROPE

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ABSTRACT

A simplistic bioclimatic model of the Siberian moth *Dendrolimus sibiricus* Tschtrk. (Lepidoptera: Lasiocampidae) is based on the moth's basic biological requirements, expressed through summer thermal conditions (growing-degree days above 5 °C, GDD5), moisture conditions (annual moisture index [AMI]) and the ratio of warm degree-days to annual precipitation. Siberian regional literature and moth inventory data allowed us to relate the Siberian moth distribution to climate. Climatic limits of moth ranges and outbreaks derived from these ordinations are as follow: the range limits 950-1350 °C of GDD5 and 1.3-3.0 of AMI; the outbreak limits are 1100-1250 °C of GDD5 and 2.0-2.5 of AMI.

To map the moth range and outbreak distributions, the bioclimatic model was coupled with climatic layers mapped across northern Eurasia. For the mapping, data of GDD5, NDD0 and annual precipitation from about 300 stations in Europe and 1000 weather stations in Siberia were assembled. Then, Hutchinson's (2000) thin plate splines were used to produce climate surfaces of these variables on the DEM at a resolution of 1 km. Climatic and topographic images were visualized using IDRISI32.

We found that our bioclimatic model of the Siberian moth range and outbreaks, when overlaid with the ranges of the host tree species (forest map of Russia, 1990), ideally coincide with current moth habitats and outbreak areas in Siberia and the Russian Far East. A dozen new areas with outbreak potential were found.

It was shown that European summer conditions of current climate may be considered suitable for Siberian moth north of latitudes 54-56°N within northwestern Russia, the Baltic countries, all of Finland, southern Sweden, the coasts of the North and Baltic Seas, and ranges in central Europe. In reality, the distribution of

the Siberian moth to the west is limited by the absence of forest stands dominated by pests' preferred food plants (species of *Larix* and *Abies*) and mild winter conditions. Overwintering larvae of the Siberian moth require continuous winters of a continental type with no autumn thaws which are fatal for the larvae (Rozhkov 1963, Kondakov 1974).

Thus, although our bioclimatic model of the Siberian moth distribution indicates that there are good possibilities for the moth to extend its range to northwestern Europe on the basis of summer temperatures, current mild winter conditions in the regions to the west of the Urals will not allow the larval stage of the pest to overwinter successfully. There is no reason to consider Siberian moth as a future threat for Europe.

We thank Marc Kenis (CABI Europe-Switzerland) for initiation of this research. The work was supported by EU FP6 project ALARM.

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FIELD PERSISTENCE AND EFFICACY OF THE FUNGUS *BEAUVERIA BASSIANA* AGAINST THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), was first discovered in 2002 near Detroit, MI, probably arriving in solid wood packing materials on cargo ships from Asia. Since then, the beetle has spread over much of northeastern North America. Within these areas more than 25 million ash trees (*Fraxinus* spp.) have been killed by EAB. Adult beetles feed only on foliage but the key damage is inflicted by larvae feeding on the inner bark of ash trees. Tunneling by a sufficiently high number of larvae effectively girdles the tree resulting in death.

As part of our multi-year study on the development and use of the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales) against the EAB, we are determining persistence of the fungus sprayed on ash trees and leached into soil. Fungal inocula present on ash bark

and leaves, collected at 30 min, 7 days and 14 days after spraying, were quantified by use of culture based (semi-selective medium) and molecular (real-time PCR assay) methods. In addition, we conducted bioassays using EAB adults to determine whether the level of inocula persisting in the field were sufficient to affect beetle survival on treated foliage or bark.

Our results showed that the fungus persisted for at least 2 weeks on both leaves and bark and caused mortality in beetles exposed to treated samples. Inocula remained on bark, however, at higher concentration than on leaves after 2 weeks and even increased after week 1, indicating that the fungus may have multiplied. These results suggest that pre-emergent sprays on ash trunks could be a practical means to target adults during emergence or oviposition.

NATURAL INFECTIONS OF *BEAUVERIA BASSIANA* IN *AGRILUS PLANIPENNIS* POPULATIONS IN MICHIGAN

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera, Buprestidae), is an invasive pest from Asia posing a serious threat to ash trees (*Fraxinus* spp.) in North America. Beetles have a 1- or 2-year life cycle completed entirely in association with ash trees. Since its discovery in 2002 near Detroit, MI, EAB has now spread over much of northeastern North America. Within these areas over 25 million trees have been killed.

The beetle is considered a minor and periodic pest in its native range, likely due to the presence of natural enemies and more resistant native ash species there. The potential of these natural enemies as biological control agents led to surveys in China, within the beetle's native range, and in Michigan, where it is most widespread in the United States. A survey of natural enemies conducted by Bauer and Liu from 2002 to 2006 in Michigan resulted in a collection of

fungal isolates including many of *Beauveria bassiana* (Ascomycota: Hypocreales) from late instar larvae and prepupae.

In this study we analyzed these EAB-derived isolates and compared them to ash bark- and soil-derived isolates to determine the sources of fungal inocula infecting EAB. Molecular analyses showed that most of the EAB-derived isolates clustered with soil and bark isolates from the same sites, indicating that beetles picked up indigenous inocula. Data also suggest that beetles carry the fungus from one tree to another. Furthermore, bioassay studies showed that representative isolates are pathogenic against EAB, and as virulent as a commercial strain. This suggests the potential of indigenous isolates as biocontrol agents.

FINE STRUCTURE OF ANTENNAL SENSILLA IN EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

The antennal sensilla of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), were examined using scanning and transmission electron microscopy. Male and female antennae have a scape, pedicel, and nine flagellomeres. Both male and female antennae share five sensillum types: sensilla chaetica (mechanoreceptors), three types of sensilla basiconica (olfactory), and uniporous gustatory/taste sensilla. Apical depressions containing large sensory fields of uniporous sensilla were seen on the eight most distal flagellomeres

of both sexes. Counts of sensillum types showed that males possessed significantly more uniporous sensilla than females. We hypothesize that antennal contact is important for mate recognition by male *A. planipennis*. The distal apices of the eight outer flagellomeres were seen to have “tufts” composed of two types of sensilla basiconica. A third type of sensilla basiconica was observed within the perimeter of the uniporous sensory fields. The structure and putative function of each sensillum type are discussed.

MULTIGENERATIONAL DISPERSAL OF AN INTRODUCED AND NATIVE *LARICOBIOUS* SPECIES WITHIN EIGHT HEMLOCK WOOLLY ADELGID INFESTED HEMLOCK STANDS

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ABSTRACT

Laricobius nigrinus (Fender), introduced from the Pacific Northwest, continues to show promise as a biological control agent for hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), in the eastern United States. A complicating factor for evaluating *L. nigrinus* is that a native predator of pine bark adelgid, *Laricobius rubidus* (LeConte), shows evidence of adaptation and exploitation of abundant HWA populations. An added challenge in studying the introduced agent is that the congener larvae are morphologically indistinguishable, necessitating identification of collected specimens through molecular diagnostic assays. For 2 years, HWA-infested trees have been sampled to assess dispersal of *L. nigrinus* following their release. This sampling has taken place at four sites confirmed to have established *L. nigrinus* populations. Larvae of both *Laricobius* spp. were recovered in spring 2007 and 2008, representing the F2-F3 and F3-F4 *L. nigrinus* generations, respectively. Sampling involved removing a cluster of branches from 16 eastern hemlock, (*Tsuga canadensis* L. (Carr.), trees located 50 to 900 m from the central release areas. In addition, four new *L. nigrinus* release sites were sampled (2 to 7 months post-release) in 2008, at 10, 30, 50, and 100 m from the release areas to monitor dispersal of the parent

generation. We found that the parent generation did not disperse much more than 10 m. The F2-F3 and F3-F4 generations were recovered at approximately 100 and 300 m, respectively, from the release areas. In stands that include eastern white pine (*Pinus strobus* L.), *L. rubidus* are commonly found on HWA infested hemlocks. In hemlock stands lacking white pine, *L. rubidus* appears to be less abundant. Our 2007 and 2008 data from the four established sites show that *Laricobius* population density per branch cluster (mean \pm SD, 20.6 ± 35.4 ; $n=131$) was positively correlated ($r=0.394$, $p=0.0001$) with HWA population density per branch cluster (mean \pm SD, 0.76 ± 0.79 ; $n=131$). This relationship suggests that the predator is able to find its prey and perhaps respond numerically to it. At this early stage it appears that adults do not move far away from the site of their release but that subsequent generations begin to disperse in earnest. For this biological control agent to have a large-scale impact, dispersal to new areas where releases have not taken place will be needed. The frequent recovery of *L. rubidus* on HWA infested hemlocks supports the importance of ongoing research of *Laricobius* spp. interactions.

PARASITOIDS ATTACKING EMERALD ASH BORERS IN WESTERN PENNSYLVANIA AND THEIR POTENTIAL USE IN BIOLOGICAL CONTROL

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ABSTRACT

Current biological control programs against the emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) have primarily focused on the introduction and releases of exotic parasitoids from China, home of the pest origin. However, recent field surveys in Michigan indicate that some North American native or extant parasitoids have become associated with EAB and play some role in suppressing the local populations of EAB. The objective of the present study is twofold: (1) to investigate if any extant parasitoid guilds have become associated with emerald ash borers in western Pennsylvania, where the pest was first discovered in 2007; and (2) to study the most abundant parasitoid for future development of augmentative biological control programs against EAB.

A total of 44 green (*Fraxinus pennsylvanica* Marshall) ash trees (average d.b.h. = 21.5 cm ranging from 10 to 45 cm) with obvious symptoms of EAB infestation (woodpecker pecks and thin canopy covers) were randomly located in Cranberry Township from 11 March to 23 October 2008, and sampled monthly for presence of various immature stages of emerald ash borers and associated

parasitoids. Several species of parasitic Hymenoptera were recovered and collected from these green ash trees infested with late instar EAB larvae, prepupae, and/or pupae, including the most abundant species, *Balcha indica* (Mani & Kaul), accounting for 82 percent of all the parasitoids recovered. These parasitoids together resulted in approximately 3.6 percent parasitism of EAB in the field. Laboratory assays further indicated that *B. indica* and another eupelmid wasp (*Eupelmus* sp.) are solitary ectoparasitoids of EAB larvae, prepupae and pupae. In addition, both *B. indica* and *Eupelmus* sp. reproduce thelytokously—i.e., virgin females reproducing daughters, and thus may be potentially complementary to the current classical biological control programs against EAB in North America. Studies are currently in progress in our laboratory on the reproductive and development biology and host finding and selection behavior of these two local parasitoids, and eventual development of mass rearing methods for their use in augmentative biological control programs against emerald ash borers in Pennsylvania and elsewhere.

CAN THE EXOTIC BROWN SPRUCE LONGHORN BEETLE, *TETROPIUM FUSCUM*, SUCCESSFULLY COLONIZE HEALTHY TREES IN CANADA?

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ABSTRACT

The exotic brown spruce longhorn beetle, *Tetropium fuscum* (Fabr.), (Coleoptera: Cerambycidae) was discovered emerging from red spruce, *Picea rubens* Sarg., in Halifax, Nova Scotia in 1999. It is likely that *T. fuscum* has been established in the Halifax area for more than 19 years and was introduced in infested wood packing material in shipping containers received at the Halifax port. *Tetropium fuscum* primarily infests weakened or recently cut Norway spruce, *Picea abies* (L.) Karst., in its native Europe, but in Canada has been reported to attack several species of apparently healthy spruce (*Picea* spp.). The mechanism that may allow *T. fuscum* to colonize healthy trees in Canada is unknown, however the potential

impact and risk associated with this invasion depends on whether this species acts as a primary or secondary colonizer. This research evaluates the effect of host tree condition on the colonization success and subsequent performance of *T. fuscum*. Performance was assessed with and without exposure to natural enemies. Preliminary results indicate that *T. fuscum* can colonize apparently healthy trees in Canada, but that their survival is reduced on these trees. Natural enemies causing mortality in exposed *T. fuscum* included two native endo-parasitoids, *Rhimphoctona macrocephala* (Provancher) and *Wroughtonia occidentalis* (Cresson), and woodpeckers.

OPTIMIZATION OF VISUAL TRAPPING METHODOLOGY FOR THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*

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ABSTRACT

As the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), spreads throughout the range of North American ash species, better tools are needed for the detection and delimitation of new infestations. Two field assays, comparing paints and plastics, were performed to determine the optimal color for EAB traps. A new custom corrugated plastic color based on a light green color previously found to be attractive to EAB was tested in both assays. Among traps of varying green wavelengths (500 to 570 nm) green traps ranging in wavelength from 525 to 540 nm caught significantly more adult *A. planipennis* than traps of other wavelengths. The highest ratio of males to females (2.8 :1) was recorded on traps painted with a green of peak reflectance at 525 nm. Among purple traps, a paint previously shown to be attractive to buprestids caught significantly more EAB adults than blue and red paints as well as purple plastic control, and was not significantly different from green plastic traps.

The current EAB prism trap is large and rather cumbersome. A field assay was also performed to determine if a smaller sized prism trap is as efficient as the current standard prism. While smaller “quarter-sized” traps were comparable to standard prism traps in terms of trap catch per surface area zero-catches were recorded on 20 percent of the quarter-size traps. All standard and double-length narrow traps caught at least one EAB.

We predicted percentage catch as a function of growing-degree days (base 50 °F) for each year in a 3-year period (2006 to 2008) using data from several local weather stations. From the estimated catch we used 5 percent, 50 percent and 95 percent to define the beginning (542.1 ± 3.4), peak (761.1 ± 8.4) and end (1068.7 ± 29.8) of the trap catching season, respectively. Similar results (572, 757, and 942, respectively) were found in the timing of EAB emergence from infested trees felled in West Virginia and held at the Otis laboratory.

POPULATION DYNAMICS OF THE FELTED BEECH SCALE AND ASSOCIATED *NEONECTRIA* SPECIES, CAUSAL AGENTS OF BEECH BARK DISEASE

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ABSTRACT

Biotic threats to tree growth, survival, or reproduction often arise from interactions among a suite of species, primarily insects and fungi, that function together to varying degrees to defeat host defenses, secure resources, and infect new hosts. Where two or more organisms interact, there is strong potential for positive or negative feedbacks that can have large effects on host population and disease dynamics. The strength and direction of such feedbacks are likely to vary spatially and temporally, and may depend in part on factors such as climate, host availability/quality, and forest history. We use a spatially replicated population time series for the two primary causal agents of beech bark disease (BBD) to test hypotheses concerning the potential for coupled dynamics between disease agents, and consider the relative importance of factors such as tree density and size, climate, forest and disease history in determining population dynamics, including the strength and form of density dependence.

BBD arises from the interaction between the felted beech scale (*Cryptococcus fagisuga* Lind.) and either of two ascomycete fungi of the genus *Neonectria* (*N. faginata* and *N. ditissima*). Analyzing population densities on individual trees from 29 sites from Maine to West Virginia sampled

annually from 1979 to 1992, we found strong evidence for negative density dependence for scale insects and fungi across all sites. Surprisingly, scale insect densities in year t and $t-1$ had very little effect on the population growth rate of *Neonectria*, despite the fact that *Neonectria* depends on insect feeding for initial access to phloem resources. Likewise, *Neonectria* density did not influence scale insect population growth to any great extent.

Density dependence for both insects and fungi was variable across sites, and model selection showed that climate variables such as winter temperatures and spring precipitation may influence population dynamics for each. However, the strongest and most general predictor was the duration of infection with BBD, suggesting that BBD may interact with the forest and host trees in ways that feedback to alter disease agent population dynamics over large spatio-temporal scales. This pattern is not adequately explained by other candidate variables that also vary with latitude. The exact nature of such feedbacks is unclear but may include changes in host susceptibility over time (via selection for resistance or patterns of susceptibility linked to forest structure) and likely involve human responses to BBD in the form of forest management.

WHY CAGE A TREE? USE OF WHOLE-TREE ENCLOSURES TO ASSESS INTRODUCED PREDATORS OF HEMLOCK WOOLLY ADELGID, *ADELGES TSUGAE*

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ABSTRACT

While commonly used approaches (petri dishes, small arenas, growth chambers, greenhouse studies, sleeve cages, etc.) for evaluation of natural enemies provide important information, does the small size of these arenas limit their usefulness when evaluating introduced natural enemies for release against pests of tree species? Can methods be improved to evaluate natural enemies of these pests? A project was developed to assess the use of large tree cages to enhance our understanding of the survival, colonization, and establishment of introduced biological control agents against the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, on eastern hemlock, and to assess the impact of these agents on population densities of this serious invasive pest and on tree health. This project focuses on the use of large (ca. 9 m [30 ft]) screened whole-tree canopy enclosures (cages) to assess the successful field application of three introduced biological control agents (*Laricobius nigrinus* [Ln] [Fender], *Sasajiscymnus tsugae* [St] Sasaji and McClure, and *Scymnus sinuanodulus* [Ss] Yu and Yao) of HWA. This study is being conducted at Blackberry Farm near the Great Smoky Mountains National Park in eastern Tennessee.

Trees (n=12) were caged from October to December 2007 and biological control agents were placed in the cages: Ln

adults (190/cage, 11 Jan. 2008), Ss adults (90/cage, 20 March 2008), and St adults (300/cage, 27 March 2008). Three caged trees without beetles and three noncaged trees served as control trees. Trees were sampled for beetles and HWA assessed every 2 to 3 months.

All three species of introduced natural enemies survived and reproduced within the cages. Larvae of each species were recovered in 2008. Adult Ln was found in March and November, adult Ss was found in April, June, and July, and adult St was found in April, May, June, July, and November. Adult Ln and St were recovered on 19-20 November 2008 (about 1 year after Ln was initially placed in the cages and about 8 months after St was placed in the cages). No Ss were found in the cages after 8 months (November 2008 sample). After 11 months, HWA densities in all beetle cages had declined. Of the release cages, the lowest decline in HWA densities was observed in the Ss cages, while the greatest decline was found in the Ln cages. Canopy enclosure cages are a new and innovative approach to assess natural enemies for release (single species or species complexes) against insect pests of trees. This research is expected to provide a better understanding of the role of natural enemies in suppressing HWA in forests.

BUILDING THE DATABASE FOR INTRODUCED PLANTS IN THE UNITED STATES

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ABSTRACT

More than 4000 nonnative plant species have been introduced to the U.S. and Canada. Identifying potentially invasive species is an important goal. Ecologists have generally agreed that there is no simple biological predictor of invasion success, but certain biological traits tend to be associated with invasion success more than others. For example, recent literature suggests that characteristics of successful invaders often included broad ecological tolerances, r-selected life strategies, associations with disturbed or anthropogenic habitats, and origins from large continents with diverse biotas. Life history/genetic information is thus critical for developing early warning/prevention systems, predictive simulation models, risk assessment, and management plans.

We are compiling data for biological traits including life cycle, growth form, woodiness, deciduousness, pollinating agent, fruit type, seed mass, dispersal agent,

and photosynthetic pathway. We collect data from a large body of literature and a variety of other sources including the Internet, herbarium specimens, and existing relevant databases. We also collect information regarding introduction pathways/vectors, introduction time and locations, and current distribution. We will ask the following questions: (1) What are the major life history characteristics and common traits of about 4,000 plant species introduced into the United States?; (2) What kinds of species are most invasive and is such invasiveness related to particular life history/genetic traits?; and (3) How do such biological traits as growth form, pollinating agent, dispersal agent, habitat distribution, and photosynthetic pathway influence the spread of introduced species naturalized in the United States and Canada?

IMPACT OF CHINESE PRIVET AND ITS REMOVAL ON POLLINATOR DIVERSITY AND ABUNDANCE

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ABSTRACT

Chinese privet (*Ligustrum sinense*) was introduced into the United States in 1852 as an ornamental shrub, and by 1932 was established throughout the Southeast. In the 1990s privet occurred on 2.9 million acres of forest in the Southeast. More specifically, it covered 59 percent of our study area, the Upper Oconee River floodplain in north Georgia in 1999. The objective of this research was to evaluate the effect of privet removal techniques on various components of the forest community including understory plants and insect pollinators (mainly bees).

Treatments were applied in autumn 2005 and consisted of: 1) an untreated control; 2) removal of privet with a rubberized track mounted chipper (gyrotrac) followed by herbicide treatment of the stumps; and 3) standard chainsaw felling followed by herbicide treatment of stumps. To sample pollinators we used pan traps (Solo™ brand bowls) in both blue and yellow since these colors have proven successful at attracting pollinators in previous studies. Ten pan traps (five trapping stations, each with one blue and one yellow trap) were placed randomly

throughout each 5-acre plot. We collected samples seven times from March-October 2006 and 2007. Traps were operated for 7 days each time we sampled. Bees were sorted and stored in ethanol until they could be mounted and identified.

Removal of Chinese privet resulted in an approximately tenfold increase in bee abundance and fourfold increase in bee richness. Each treatment saw significantly higher bee abundance and richness when compared to control plots in 2006 and 2007. The method of removal had no effect on pollinators. Abundance and richness were higher in both treatments the second year. Despite our encouraging results, these methods are not practical over large areas, so other controls are needed. There are no native *Ligustrum* spp. in the U.S., which makes Chinese privet an excellent target for biocontrol. We are currently evaluating a leaf beetle (Chrysomelidae) from China as a possible control agent. It will likely take an integrated approach in the future to control Chinese privet—a step definitely worth taking in order to restore native communities.

HEMLOCK WOOLLY ADELGID POPULATION GENETICS

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ABSTRACT

The hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), was first reported in eastern North America over 50 years ago. Efforts to control the impacts of the adelgids are focusing on classical biological control and tree resistance. The success of these strategies relies on knowledge of the ecological and evolutionary context uniting the pest, its host plants, and its natural enemies.

We report preliminary results of a project using 14 microsatellite markers to further understand the genetic structure of *A. tsugae*. Adelgid samples were collected from 27 sites throughout the range of hemlock in Japan, from Ullung Island, South Korea, and from eastern and western North America. A total of 553 individual adelgids have been genotyped to date. Principal component analysis of all genotypes resulted in three clusters. Samples from western North America show no evidence of recombination and are distinct from those in Japan and eastern North America. This agrees

with previous assertions of a separate, endemic lineage in western North America. There was also a clear distinction between samples collected from *T. diversifolia* and *T. sieboldii* in Japan. Samples collected from *Picea polita* grouped with those from *T. sieboldii* indicating that alternation between *T. sieboldii* and *P. polita* is common while alternation between *T. diversifolia* and *P. polita* is absent or rare. There appears to be a patchwork of entirely parthenogenetic and cyclically parthenogenetic populations in Japan with varying levels of recombination depending on the proximity of primary and secondary hosts.

The results of this project will be used to clarify hemlock adelgid taxonomy, to predict the adaptive potential of *A. tsugae* in its introduced range, to target exploration for biological controls, and to aid development of resistant hemlock genotypes.

CHARACTERIZATION OF MICROSATELLITE LOCI FOR *LARICOBIOUS NIGRINUS* AND *L. RUBIDUS*, PREDATORS OF ADELGIDS IN NORTH AMERICA

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ABSTRACT

Laricobius nigrinus (Coleoptera: Derodontidae) is native to western North America and has been released in eastern North America as a biological control of the hemlock woolly adelgid (HWA). In eastern North America there is a congeneric native species, *L. rubidus*, which feeds on white pine adelgid, *Pineus strobi*, and on HWA but not enough to control the pest. Phylogenetic analysis of the genus *Laricobius* using mitochondrial and nuclear DNA sequence data showed that *L. nigrinus* and *L. rubidus* are sister species and closely related. This has led us to question whether these species have diverged long enough to be reproductively isolated. Hybridization between the introduced *L. nigrinus* and native *L. rubidus* would have unknown consequences for biological control. We also found some evidence of population structure within the native range of *L. nigrinus* associated with geography and/or host association.

As a first step toward obtaining a better understanding of the relationship between *L. nigrinus* and *L. rubidus* and of possible genetic structure associated with host or climate within *L. nigrinus*, we isolated and characterized

microsatellite markers for both species. An initial 14 loci were characterized using samples of *L. nigrinus* from Seattle, WA (n=25) and Portland, OR (n=17), and *L. rubidus* from Hamden, CT (N=28) where no *L. nigrinus* releases have been made. Nine loci were variable and amplified cleanly for both species. The number of alleles per locus varied from 4 to 16 (mean = 8.1) for *L. nigrinus* and 2 to 10 (mean = 4.9) for *L. rubidus*. Two loci displayed significant deviation from Hardy-Weinberg equilibrium in both species after controlling for multiple comparison false discovery rate. The F_{st} value (a measure of population differentiation) between *L. nigrinus* populations from Washington and Oregon was 0.02 while the F_{st} between *L. nigrinus* and *L. rubidus* was 0.34 indicating that these markers should provide adequate resolution to distinguish the two species and detect hybrids. We are currently working to add more loci to improve our ability to resolve *L. nigrinus* interspecific variation and are processing additional beetles to track diversity in laboratory colonies of *L. nigrinus* and to monitor the establishment and spread of different genotypes following release.

**FOLIAGE FEEDING TESTS OF *EUCRYPTORRHYNCHUS BRANDTI*
(HAROLD) (COLEOPTERA: CURCULIONIDAE),
A POTENTIAL BIOLOGICAL CONTROL AGENT
OF THE TREE-OF-HEAVEN, *AILANTHUS ALTISSIMA***

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ABSTRACT

Ailanthus altissima (Mill.) Swingle, tree-of-heaven, is a species native to China and North Vietnam. It was first introduced into the United States in the 1700s and is now distributed and invasive throughout much of North America where it out-competes native vegetation. The invasiveness of tree-of-heaven is primarily attributed to the lack of natural enemies in North America, its aggressive invasion of newly disturbed areas, tolerance of extreme conditions, and allelopathic properties. Mechanical and chemical controls are current tactics used for suppression, but implementation is costly, and can be as much as \$8,750/ha.

Biological control was initiated in 2004 as a potentially sustainable tactic for tree-of-heaven suppression. The weevil, *Eucryptorrhynchus brandti* (Harold) was identified in China and imported for quarantine testing as a possible biological control agent. We conducted adult choice and

no-choice feeding tests on foliage of tree-of-heaven in 2007 and 2008 to determine the host specificity of this weevil. Twenty-nine nontarget plant species from 14 families that are taxonomically, economically, and/or ecologically related to tree-of-heaven were tested. Results, to date, indicate that *E. brandti* feeds significantly more on foliage of tree-of-heaven when compared with all test plants. Mean range of feeding on North American *Ailanthus altissima* was 32.5 ± 22.2 to 106.5 ± 16.0 mm²/adult/day (\pm SD) in no choice tests, with significantly reduced feeding on *Simarouba glauca* DC, Paradise tree (7.7 ± 6.7 mm²/adult/day), and *Leitneria floridiana* Chapman, Corkwood (47.6 ± 20.8 mm²/adult/day). The mean range of feeding by *E. brandti* on all other test species was 0.0 to 3.3 ± 5.0 mm²/adult/day. These data are helping to guide us on which species to focus on for *E. brandti* developmental tests. At this time, *E. brandti* appears to be a host-specific herbivore.

BUILDING DNA BARCODE REFERENCE LIBRARIES FOR BARK AND WOODBORERS

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ABSTRACT

DNA barcoding is a molecular diagnostic tool that utilizes a standardized segment of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), for species identification. Attributes of the COI gene, including: the absence of introns; rarity of indels; ease of amplification by polymerase chain reaction; availability of universal primers; and ease of alignment after sequencing are of benefit for the development of diagnostic systems for arthropods. In order to identify unknown specimens, one must first create reference libraries of COI sequences from reliably identified voucher specimens.

In 2008 we focused on developing a library of reference sequences for bark- and wood-borers (Buprestidae, Cerambycidae, Scolytinae, and Siricidae). Single legs were removed from recently collected specimens and the associated collection and identification data was extracted from the specimen labels and data-based. All tissue samples were submitted for extraction, amplification and sequencing to the DNA analysis platforms at the Ontario Biodiversity Institute (University of Guelph, Guelph, ON). The resultant COI sequences, associated sequencer trace files and primer sequences were uploaded into the online BOLD and the BOLD-MAS platforms (<http://www.boldsystems.org/views/login.php>) used to analyze the resultant sequences and

generate neighbor-joining trees. Preliminary results for selected taxa are presented.

To date more than 2,300 specimens from the target taxa have been sampled: 1,800 samples have been processed and 1,370 sequences >500 bp in length have been generated. An example of a neighbor-joining tree for Scolytinae generated by the BOLD analysis platform is presented to illustrate previously unrecognized divergences within taxa. Large intraspecific sequence divergence within specimens identified as *Trypodendron lineatum* suggests that recent morphological observations of the existence of more than one species within the current concept of “*T. lineatum*” are correct. Similar large intraspecific sequence divergences in *Xyleborinus saxesenii* suggest that it also may also be a species complex.

DNA barcoding provides a powerful tool for the identification of unknowns and, because COI is present in all life stages, will be of great utility in identifying immature life stages of bark and wood-borers. The ability to reliably identify immature life stages from regulatory interceptions will be of great benefit in the analysis of introduction pathways for nonindigenous species.

ADDITIONS AND CORRECTIONS TO BARCODE LIBRARIES FOR *LYMANTRIA* SPP.

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ABSTRACT

The genus *Lymantria* includes a number of destructive forest pests such as the gypsy moth (*L. dispar*) and nun moth (*L. monacha*) in various regions across the northern hemisphere. A number of species have been transported beyond their native range, either intentionally or in conjunction with international trade. Egg masses of both *L. mathura* and *L. dispar asiatica* were recovered from vessels during quarantine inspections on the west coast of both Canada and the United States in the early 1990s. Populations of *L. dispar asiatica* that established in Vancouver, BC, Portland, OR and Seattle, WA, were subsequently eradicated at great expense. Populations of *L. dispar* and *L. mathura* are again increasing in Asia and viable egg masses of both species have been detected on vessels arriving in ports on the west coast of North America.

Multiple molecular studies have used the mitochondrial gene cytochrome c oxidase I (COI) to distinguish species or haplotypes of *Lymantria* species. Nomenclatural changes from a recent morphological revision have given rise to

errors in the species names associated with molecular data deposited in GenBank. We recently sampled additional specimens of *Lymantria dispar asiatica*, *L. mathura* and *L. monacha* from the Russian Far East, *Lymantria dispar* from regulatory interceptions in British Columbia, and additional specimens of *L. mathura* from Japan. The nearest-neighbor joining tree generated from COI data in GenBank in combination with the COI barcodes we generated provides genetic confirmation that *Lymantria mathura* from Japan is conspecific with populations from South Korea and the Russian Far East. The taxon previously reported as *L. mathura* from Japan (Okinawa) was recently described as a new species, *L. flavida*. The GenBank COI records [DQ116087 & DQ116088] attributed to *L. mathura* actually represent sequence data for *L. flavida*. Another five records attributed to *L. dispar* (or the subspecies *L. d. hokkaidensis*) in GenBank [DQ116179, DQ116180, DQ116181, DQ116182 and DQ116126] are now considered to be the species *L. umbrosa*. We also provide additional barcode sequences for *L. dispar* and *L. monacha* from the Russian Far East.

INTRASPECIFIC VARIATION IN *TSUGA* *CANADENSIS* FOLIAR CHEMISTRY

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ABSTRACT

Three groups of eastern hemlock (*Tsuga canadensis* Carr.) trees were analyzed to compare their chemical composition and the potential for naturally occurring resistance to hemlock woolly adelgid (*Adelges tsugae* Annand; HWA). Potentially resistant ‘parent’ trees located in southern Connecticut were compared with rooted propagules from those same trees and control trees located in northern Vermont (outside of the current range of HWA infestation). For trees in each of the three groups, the cations Ca, P, K, C and N were quantified

and terpenoid profiles were developed using SPME and GC/MS. There was no significant variation in terpenoid profiles between the three groups of hemlock trees. Propagules retained elevated levels of Ca and N from fertilization during propagation, suggesting that their chemical composition does not mirror the parent trees. The potentially resistant ‘parent’ trees had higher levels of K. This may impart some level of tolerance/resistance to HWA and explain their persistence in hemlock forests that have been decimated by HWA invasions.

FACTORS THAT INFLUENCE EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) ADULT LONGEVITY AND OVIPOSITION UNDER LABORATORY CONDITIONS

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis*, is a nonnative insect from Asia that threatens ash trees in the urban and natural forests of North America. Research on this invasive insect and rearing parasitoids for release rely heavily on the ability to artificially rear EAB. Methods to maintain EAB adults and obtain eggs exist, but result in inconsistent adult fecundity and longevity. Here, we evaluate the effects of host plant, temperature, humidity, and oviposition substrates on adult survival and fecundity. Each treatment used 21-40 pairs of adults that emerged from infested wood collected in Michigan. The pairs were held at 25 °C, 65 percent RH and 16:8 light:dark cycle except in the temperature and humidity studies.

EAB adults did not survive or reproduce well when fed *Fraxinus griffithii*, an evergreen ash of Asian origin, but did well when fed mature foliage of *F. uhdei*, an evergreen ash of North American origin. When adults were fed newly expanded foliage, fecundity, longevity, and the percentage of females that oviposited were all lower than when mature foliage of the same species was used.

The best oviposition substrate, of those tested, was a 2 x 20 cm bolt wrapped first with butcher paper and then with a 1.3 cm wide strip of purple curling ribbon (not overlapping). Using this substrate, 50 percent of the eggs were laid on the butcher paper which is a fairly clean substrate on which eggs hatch well. The eggs on butcher paper can also be placed on artificial diet and the larvae that hatch will burrow into the diet.

EAB adult longevity decreased as temperature increased from 20 to 30 °C. Only one female oviposited at 20 °C. Percentage females ovipositing and fecundity were higher at 25 °C than at either 20 °C or 30 °C. Percentage egg hatch was also highest at 25 °C. Female fecundity and survival were lower at 55 percent and 75 percent RH than at 65 percent RH. There was little effect of percent RH on male survival. Egg hatch was lower at 55 percent RH than in the higher percent RH treatments.

These results provide good methods for maintaining adults and obtaining eggs that hatch well. Further improvement may be possible by using different containers or group rearing.

EFFECTS OF EMERALD ASH BORER (*AGRILUS PLANIPENNIS*) ON FOREST ECOSYSTEMS

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ABSTRACT

The effects of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) on forest ecosystems are being studied through a collaborative research program involving U.S. Forest Service Northern Research Station (Research Work Unit 2) and Ohio State University. In more than 250 monitoring plots in forests in Ohio and Michigan representing a gradient of EAB infestation duration, we are monitoring the decline and mortality of thousands of ash (*Fraxinus* spp.) trees, the responses of both native and invasive plant species, changes in species composition and forest structure, and effects on other organisms and ecosystem processes. The plots are located in forest stands of different ages and in different habitats to include all five ash tree species native to the region (*F. americana* L., *F. pennsylvanica* Marshall, *F. nigra* Marshall, *F. profunda* Bush, and *F. quadrangulata* Michx.). Annual monitoring began in 2004 and continues to the present.

Our research has resulted in several key findings. Mortality of mature ash trees in a forest stand may reach 98 percent within 6 years of infestation by EAB and is not affected by the density, basal area, or species composition of ash trees. In areas that have been infested the longest, only 0.8 percent of mature ash trees remain alive while many ash seedlings and approximately one-third of ash saplings are alive. EAB persists at low densities in these stands. Invasive plant species are present in most plots, however, their percentage cover is generally low. These plants may increase in abundance due to canopy gaps that result from ash mortality. EAB may impact other species, including woodpeckers, carabid beetles, and native parasitoid wasps in these forest ecosystems.

MICROIMAGING OF *BACILLUS THURINGIENSIS* TOXIN-BINDING PROTEINS IN GYPSY MOTH LARVAL GUT USING CONFOCAL FLUORESCENCE MICROSCOPY

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ABSTRACT

After ingestion by susceptible insect larvae, *Bacillus thuringiensis* (Bt) insecticidal proteins bind to the brush border membranes of gut epithelial cells and disrupt the integrity of the plasma membrane by forming pores that lead to cell swelling and lysis. The presence or absence of specific Bt toxin-binding molecules on the brush border membrane of gut cells plays a critical role in determining the insecticidal activity of different Bt toxins. In gypsy moth, a membrane-anchored aminopeptidase (APN-1) and a 270 kDa glycoconjugate (BTR-270) have been identified as two major high-affinity binding proteins for Bt toxins. In other insects, cadherins (Cads) and an alkaline phosphatase (ALP) have also been

characterized as Bt toxin-binding proteins. In this study, immunohistochemical localization of gypsy moth APN-1, BTR-270 and cadherin (LdCad) was compared to Bt-toxin binding sites localized using AF546 fluorescently labeled Bt toxins produced by the HD-1 Bt strain using confocal laser scanning microscopy. Microvilli on the brush border membrane were found to be exclusively decorated with the antibodies directed towards APN and BTR-270 in the midgut and hindgut regions in both 3rd and 5th instar gypsy moth larval gut sections. The fluorescently labeled Cry1A toxin binding sites were found to be co-localized with the toxin-binding receptors identified in the gypsy moth.

CONTROL ACTIVITIES AND LONG-TERM MONITORING FOR THE WEST VIRGINIA EAB INFESTATION

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ABSTRACT

Project Background. An EAB population was discovered in late 2007 at a recreational area near Oak Hill in Fayette Co., West Virginia. A survey conducted within a half-mile radius of the original trap tree identified 312 ash trees (Fig. 1). Of these about half were observed with binoculars for signs of infestation; 21 trees were obviously infested (four heavily), 22 were possibly infested, and 110 showed no signs of infestation. A control and monitoring study was initiated in 2008 due to the following favorable factors: limited ash resource over a large area; isolated EAB population; EAB infestation was relatively light; infested ash trees were within a confined area. Ash trees were either cut down and disposed of (149 trees, mostly 3-inch d.b.h. or less) or treated with emamectin benzoate (Tree-äge, Arborjet, Inc.) as follows: 2-5 inches, 2.5 mL/d.b.h.-inch (34 trees, 0.1 g a.i.); 6-8 inches, 5 mL/d.b.h.-inch (45 trees, 0.2 g a.i.); 9+ inches, 10 mL/d.b.h.-inch (84 trees, 0.4 g a.i.). Treatment method used the Arborjet VIPER hydraulic device and a stainless steel tapered injection tip (no plugs, ~300 psi). Treatment times increased with increasing dosage, and overall averaged about 2 minutes per d.b.h.-inch.

Baseline monitoring. Foliar residue was monitored at 5 and 12 weeks post-treatment for about 20 percent of the trees, to encompass the range of size class and treatment rates applied. An ELISA analysis was used to determine emamectin content in leaf tissue, and this is being confirmed with an analytical method by the manufacturer (Syngenta). Sample trees were also assessed for D-shaped exit holes, woodpecker feeding, and canopy crown ratings. Binoculars were used to examine the main stem of the ash trees for emergence holes by standing in one place and slowly sweeping the view from a point 10 m above the ground down to the base of the tree. The orientation of the tree was noted in order to achieve consistency between

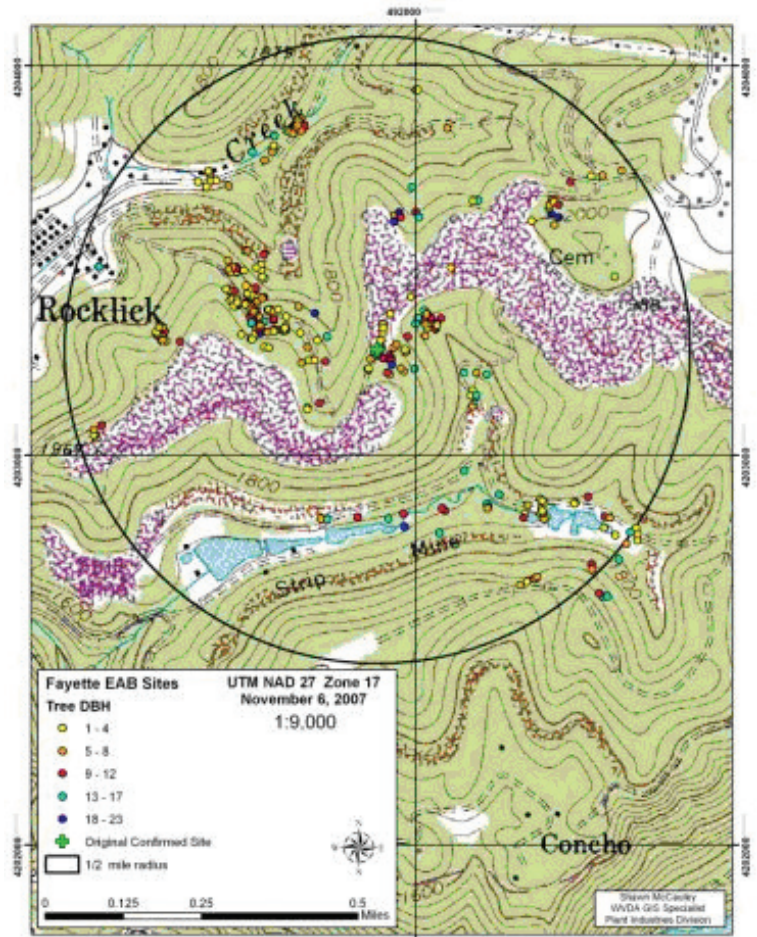


Figure 1.—Location of ash trees within the West Virginia EAB infestation.

observations over time. Emetectin residue was detected in significant quantities in all foliage samples for July and August, increasing by dosage applied. Residue levels did not change between months for the majority of samples (52 percent) and increased in 13 percent of them. Tree health data and presence of EAB exit holes in these study trees were collected in parallel with foliage collections. About 30 percent of the trees showed evidence of canopy dieback during the August observations, but this may be normal for ash trees growing in this area. There were very few woodpecker attacks on these trees, and only 6 of the 31 trees had two to seven putative EAB exit holes, evidence of a light infestation. Additional tree observations and foliage collections are planned for June and August of 2009 and 2010. Study trees will be felled

and examined for EAB infestation levels in the fall of 2010 for assessment of treatment impact.

Trapping. A trapping grid was set up to monitor the general EAB population in the area. All traps were EAB program panel traps, green in color and baited with Phoebe oil. The grid was 1 km, except for the core (blue ring), where it was ½ km. During the month of June, 95 traps were placed within the trapping grid and were checked once in July and again in August when traps were taken down. Only two adult beetles were captured on two of the 95 traps placed around the original infestation site (Fig. 2). The goal for 2009 is to increase the number of traps placed so that each 1 km quadrant has a trap and for additional traps to be placed near the two positive trap locations from 2008.

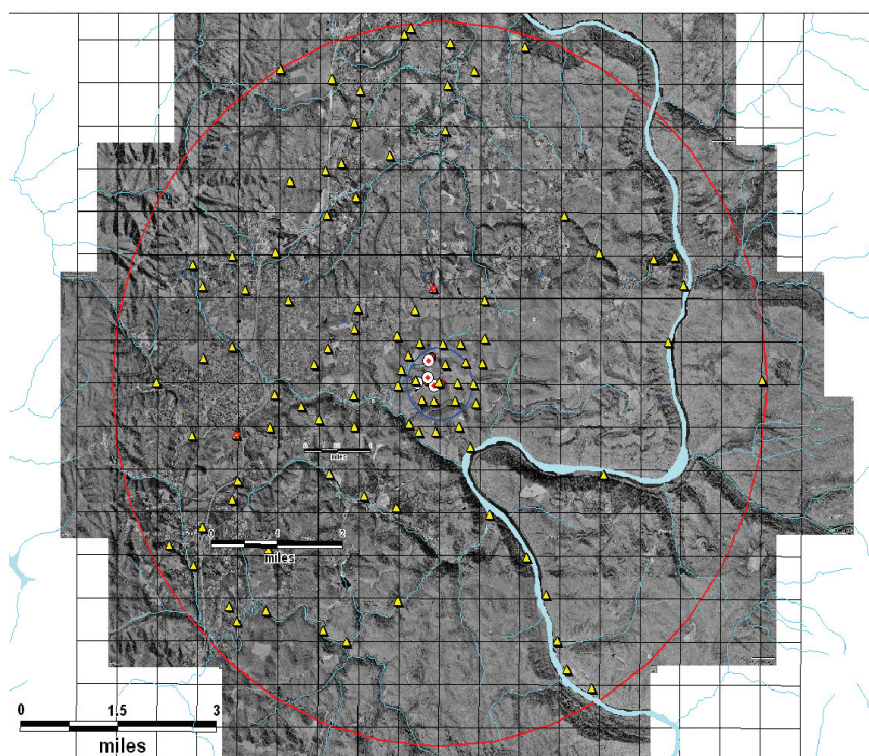


Figure 2—Trapping locations in West Virginia. White circles with a red bulls-eye, heavy infestation sites, red circles, positive traps.

A SURVEY OF PESTIFEROUS ANTS IN SUBURBAN LANDSCAPES OF MAINE AND NEW YORK

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ABSTRACT

Pestiferous ants in the suburban/urban exterior landscape are often overlooked in pest management programs but they can cause considerable problems for homeowners, businesses, and schools. This project aims to identify the most commonly reported species of ants in New York and Maine that cause problems for homeowners, businesses, and schools.

Approximately 500 mailers were distributed in each state to pesticide control operators, extension services, master gardeners, and the general public that have the potential of encountering pestiferous ants. Besides basic collection information, respondents were asked to answer questions about the nature of the complaint and the habitat. Some samples included in this analysis were collected after receiving complaints from the general public.

In Maine we received 90 outdoor pestiferous ant samples representing 15 of 16 Maine counties. Ninety-one percent of the samples originated from privately owned properties and businesses. The majority of the samples (67 percent) originated from coastal counties of Maine. Four genera were represented in the samples. The two most common species found in Maine complaint samples were *Formica*

exsectoides, the Alleghany mountain ant represented in 25 samples, and *Myrmica rubra*, the European fire ant represented in 16 samples.

In New York we received 98 outdoor pestiferous ant samples representing 20 of 62 New York counties. Eighty-eight percent of the samples originated from privately owned properties and businesses. The majority of the samples (49 percent) originated from central New York. Fourteen genera were represented in the samples. The most common pestiferous ant species found in New York were *Tetramorium caespitum* (21 records), *Camponotus pennsylvanicus* (seven records), *Lasius neoniger* and *Tapinoma sessile* (six records each).

The diversity of ants in the samples and the number of complaints was higher in New York, possibly reflecting higher human density and ant diversity. The need for educational materials on species specific ant biology and their true damage potential is evident in the disconnect between sample species and nature of the complaint. Our next step is to develop such materials for the northeast region.

A MODEL FOR THE SPREAD OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*, IN RECENTLY COLONIZED SITES

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ABSTRACT

We developed a flexible model to predict the spread of the EAB in recently colonized sites. This model is structured as a coupled map lattice built around an interchangeable “phloem grid”, which consists of a matrix of cells with estimates of the available phloem in each cell. Onto this lattice of ash phloem we coupled population processes of relevance to the spread of the EAB in three stages:

1- versus 2-year larval development

More than 200 trees across 18 different sites in Michigan were sampled to assess total density of EAB larvae per m² of surface area. The density of 2-year and 1-year larvae in each sample tree was also recorded. Using regression analysis, we analyzed the proportion of 2-year larvae by larval density. As in previous studies, we found a significant negative correlation between the proportion of larvae that require 2 years for development and larval density ($F_{1,17} = 60.83$, $P < 0.001$), and a reasonably good fit ($r^2 = 0.77$). This relationship was used in the model to estimate the proportion of beetles developing in 1 or 2 years.

Adult dispersal

Dispersal was determined using a negative exponential function estimated from data gathered from an infestation originating from a single point source in an isolated area in Fowlerville, MI. Sections of all ash trees on the site were debarked and the density of EAB larvae per m² of exposed phloem was recorded for each 10-m radius around the point source. We fit three likely models to this data using a maximum likelihood approach. (Ricker function

(RF), negative exponential function (NEF), inverse power function (IPF). The NEF fit the data best ($AIC_c = 949.5$, 117.7, and 321.5, respectively) and provided a very tight fit ($r^2 = 0.93$). To validate this model, we contrasted the model predictions to data from a second site located near Tipton, MI (Lenawee Co.). The infestation from this site, discovered in 2002, originated from a pile of infested wood near a wooded creek surrounded by agricultural fields, effectively creating a bidirectional corridor. As with the data above, we estimated the proportion of larvae per m² of exposed phloem, and contrasted the observed results with the predicted results. The predicted results fit the observed data fairly tightly ($r^2 = 0.6$).

Population growth and phloem consumption

After adult beetles disperse, the number of larvae expected to survive was determined based on the estimated growth rate from an outlier population in Ingham Co., MI. The quantity of phloem consumed by an individual larva was estimated from McCullough and Siegert (2007) to be 0.0113 m² of phloem per larvae. Population growth in the model is not allowed to exceed the maximum number of larvae that can develop from the available ash phloem resource.

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THE FOLIAR CHEMISTRY DYNAMIC IN EASTERN HEMLOCK AND HOW IT RELATES TO THE COMPETITIVE MECHANISM BETWEEN TWO INVASIVE HERBIVORES: *ADELGES TSUGAE* AND *FIORINIA EXTERNA*

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ABSTRACT

While invasive species themselves have been examined, little work has addressed the question of competition between two invasive specialists on a shared host. An example of this situation exists in the eastern United States, where eastern hemlock (*Tsuga canadensis*) forests are threatened by two invasive herbivores: the elongate hemlock scale *Fiorinia externa* (EHS) and the hemlock woolly adelgid *Adelges tsugae* (HWA). EHS was introduced into New York City in 1908 and began to spread into southern New England by the mid-1980s. EHS can reduce tree growth and cause premature needle drop in hemlocks. However, mortality is rare and occurs only in already stressed trees. Infestation of HWA is more severe. High HWA densities can kill mature hemlocks in 4 to 15 years. It has been hypothesized that HWA causes a hypersensitive response in eastern hemlocks; if correct, this may explain why HWA has a greater effect on plant growth and survival than otherwise expected. Prior research has shown competition between sap-feeding herbivores increases as plant nutritional quality decreases. We present the results of work assessing whether interspecific

competition occurs between HWA and EHS, and the effect of this interaction on host plant foliar chemistry.

In spring 2007, we inoculated previously uninfested hemlock saplings with HWA only, EHS only, both, or neither herbivore species. In October 2007, March 2008, and October 2008, we measured the impact of each herbivore on the population density of the other species as well as their individual and combined effects on foliar chemistry. Although EHS densities were lower in the presence of HWA, the presence of EHS did not significantly decrease HWA density. The HWA-only foliage was significantly lower in percent N and had a higher C:N ratio than all other treatments. The EHS-only, HWA&EHS, and control treatments did not differ in percent N, percent C or the C:N ratio. Interestingly, resource depletion (measured as percent N) in the HWA-only treatment was significantly higher than in the combined HWA&EHS treatment. Our findings suggest that the presence of multiple herbivore species in this system do not enhance detrimental impacts.

***SCYMNUS (PULLUS) CONIFERARUM* (COLEOPTERA: COCCINELLIDAE): AN ADELGID PREDATOR NATIVE TO THE WESTERN UNITED STATES**

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ABSTRACT

Scymnus (Pullus) coniferarum Crotch 1874, the conifer lady beetle (Coccinellidae), is generally distributed in the mountainous areas of the United States west of the Mississippi River, with many collections recorded in California. It is associated with conifers with the type material collected from “pine” (Crotch 1874) and large numbers from lodgepole and Monterey pines infested with adelgid in the genus *Pineus* (see Whitehead, in Gordon 1976).

Adults of *S. coniferarum* were encountered by us during field collection of *Laricobius nigrinus* from *Tsuga heterophylla* during February, April, May, and October in Seattle, WA. The extensive survey of Kohler (2007) in Oregon and Washington found only a single specimen of *S. coniferarum*. We have established a colony of *S. coniferarum* in the laboratory, where it has been reared from egg to adult on *Adelges tsugae* Annand. Currently, we are evaluating its biology and host range.

This species is very similar in appearance to *Scymnus (Pullus) suturalis* Thunberg, a native of Europe that is established in the eastern half of the United States, which is also reported as specific to conifers and has been reported to feed on both pine and hemlock adelgids (Montgomery and Lyon 1998). *S. coniferarum* can be distinguished from *S. suturalis* by its more elongate shape

(L: 1.75 mm, W: 1.15) and with punctures on the elytron, separated by less than the diameter of a puncture.

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USING ASIAN LONGHORNED BEETLE MALE-PRODUCED PHEROMONE AND HOST VOLATILES FOR MONITORING

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ABSTRACT

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae: Lamiinae), commonly known as the Asian longhorned beetle, is a wood-boring invasive species introduced from Asia to North America and Europe through solid wood packing material. *A. glabripennis* is a serious pest both in China and the United States. This research project was developed in response to the need for efficient monitoring traps to assess population density and dispersal in the field and to detect new introductions at ports of entry. The first stages of the project aimed at filling the gaps in our knowledge of the effect of semiochemicals on *A. glabripennis* adult behavior and exploring potential use of these chemicals for monitoring purposes. Semiochemicals studied were the male-produced putative volatile pheromone (blend of 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal) and plant volatiles.

The first series of experiments were conducted using the male-produced blend, its two components and plant volatiles in choice bioassays against a hexane control. In Y-olfactometer and walking wind tunnel bioassays, virgin females were more attracted to the male-produced blend and its alcohol component than males. Virgin males were even repelled at higher doses. These results suggest that the male-produced pheromone plays a role in mate-finding. When plant volatiles were offered in the Y-olfactometer, males were more attracted than females. Out of 12 plant volatiles tested, (-)-linalool, cis-3-hexen-1-ol and linalool oxide were attractive to both genders, while 3-carene and trans-caryophyllene were only attractive to males. Combining the male pheromone

blend with (-)-linalool alone or with cis-3-hexen-1-ol attracted significantly more males than did the pheromone alone. Combinations of the pheromone and plant volatiles were also tested in the greenhouse, along with four trap designs, namely Intercept™ Panel, hand-made screen sleeve, plum curculio, and Lindgren funnel traps. The former two trap designs caught significantly more beetles than the latter two.

Subsequently, field trapping experiments were conducted in China in the summers of 2007 with Intercept™ panel traps hung on poplar trees and in 2008, with Intercept™ panel traps hung on poplar trees, screen sleeve traps wrapped around poplar trunks, and Intercept™ panel traps hung on bamboo poles 20 m away from host trees. Traps were baited with the *A. glabripennis* male-produced pheromone alone or in different combinations with plant volatiles. Traps baited with the male-produced pheromone alone caught significantly more females than control traps in both years. The addition of a mixture of (-)-linalool, cis-3-hexen-1-ol, linalool oxide, trans-caryophyllene and trans-pinocarveol to the pheromone significantly increased trap catches of virgin females. Screen sleeve traps baited with a combination of (-)-linalool and the pheromone caught the highest number of beetles overall in 2008, while traps placed on bamboo poles caught the lowest number. While the logistics for the most effective implementation of a trapping program using a mixture of the pheromone and plant volatiles require additional studies, these results indicate that this pheromone has considerable promise as a monitoring tool for *A. glabripennis* in the field.

GROWTH AND SURVIVAL OF THE HEMLOCK WOOLLY ADELGID (*ADELGES TSUGAE*)

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ABSTRACT

In order to characterize fluctuations in adelgid population size and their potential causes, we studied eight understory, 2- to 3-m hemlock trees at Mt. Tom Reservation in Holyoke, MA. Initial adelgid density (sistens generation) was measured on 10 randomly selected, 0.3-m branches per tree in November 2006. Overwintering mortality and sistens density were recorded each spring. In summer we recorded progrediens density, the proportion of branchlets producing new growth, and newly settled sistens density on new growth. Subsamples of new growth were marked and revisited in November to estimate of the amount of mortality occurring during aestivation. The population rate of increase, or λ , was calculated as N_{t+1}/N_t .

During the first 2 years of the study, we observed an increase and subsequent decline in adelgid densities similar to McClure (1991). The average initial density was 68.6 sistens per branch. Density peaked in mid-summer 2007 with 138.5 progrediens per branch, followed by a steady decline in adelgid density. In mid-summer 2008, the average number of adelgid per branch was only 4.7. As we continue to collect data, it will be interesting to see if we find another increase in adelgid on marked branches.

Although cold winter temperature is the most studied cause of adelgid mortality, the proportion of the population dying during aestivation and the progrediens

life stage was significantly greater than the proportion dying in the winters of 2007 and 2008. Possible reasons include exposure to harmful terpenoids when adelgid break aestivation in fall (Lagalante et. al. 2006) and poor branch quality during the progrediens stage, which settle on old growth already occupied by the sistens.

Another factor influencing adelgid survival and rate of increase (λ) was the amount of new growth produced in spring. Both survival and λ were significantly higher on branches that produced more new growth in the spring of 2007. In turn, the amount of new growth produced during the following spring of 2008 was negatively correlated with sistens 2007 overwintering density.

In addition to these factors we are also exploring the role of density dependence in population size fluctuation. We have preliminary evidence that survival of sistens and progrediens may partially depend on densities of adelgid at various times of the year.

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SPATIAL POPULATION DYNAMICS AND HETEROGENEITY OF AN INSECT/PATHOGEN INTERACTION

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ABSTRACT

Dispersal and habitat heterogeneity are known to play important roles in the dynamics of interactions between natural enemies and their hosts. The fungal pathogen *Entomophaga maimaiga* was first found in North America in 1989 and subsequently spread across the gypsy moth distribution, causing epizootics in gypsy moth populations. This pathogen originally was touted as the 'silver bullet' for control of gypsy moth, but recurring episodes of spatially patchy defoliation have recently been observed in several areas. In this study, we are

investigating spatial variability in the distribution and activation of *E. maimaiga*, and how *E. maimaiga* may be influenced by environmental factors. In both 2007 and 2008, we monitored *E. maimaiga* distribution by measuring infection of caged gypsy moth larvae. We found that soil moisture significantly predicted fungal infection across differing years and densities. We also observed that early season infections, which reflect the size and fitness of the fungal population, significantly predicted late season infections.

EMERALD ASH BORER DISPERSAL IN MARYLAND: GO FORTH YOUNG PEST!

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ABSTRACT

The emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire), an exotic invasive pest from Asia, was introduced into Maryland in April 2003 via infested nursery stock shipped from Michigan to a nursery in southern Prince George's County. A nursery inspector from the Maryland Department of Agriculture (MDA) noticed EAB infested trees at the site in August 2003. A half-mile buffer zone was established around the nursery, and all ash trees within the quarantine zone were destroyed in an effort to eradicate the pest. Between September 2003 and March 2004, about 1,100 ash trees were cut down and destroyed, including 119 of the original 121 nursery stock trees that were definitively recovered through trace backs.

From 2004 to 2006, sentinel trees were established and monitored, and surveys to locate ash trees in the area were initiated. EAB were detected again in August 2006. It was assumed the new detections dated from the initial infestation in 2003, so buffer zones were expanded 1.5 miles at that time to account for the potential movement of the pest over the previous 3 years. Between 2006 and 2008, thousands of new EAB detections were made and 25,000 trees were removed on over 14,000 acres in Prince George's County by 2007. On August 18, 2008, EABs were detected in Charles County, over 4.5 miles south of the original infestation site in Prince George's County from 5 years earlier.

Our objectives were twofold. First, we wanted to determine the rate of spread of EAB in Maryland and specifically address the question of whether, within an aggressively managed quarantine zone, the average annual movement of EAB exceeded a half-mile limit. Second, we examined the possibility that the primary

direction of dispersal of EAB in Maryland followed the direction of prevailing winds during the period of adult flight.

The Prince George's County nursery infested in 2003 provided the point of origin for the EAB infestation, and historical records from the MDA of EAB detections for 2006, 2007, and 2008 were used to determine the rate and direction of dispersal in Maryland. No detections were made in 2004 and 2005. Distinct detection points were selected in 2006, 2007, and 2008 and plotted onto a map of the quarantined area that was divided into 2.5-acre grids. The four most distinct detection points occurring furthest from the introduction site were then determined for each year using GIS survey data gathered by MDA. The maximum distances (one site in each of 3 years) and average distances of 12 subsamples (four sites in each of 3 years) were regressed against time to determine the average rate of spread for the 5-year period.

Using a quadrant graph, the four most distant points from 2006, 2007, and 2008 were plotted to determine direction of dispersal of EAB. Prevailing wind direction during the flight months of EAB (May – August) was determined by consulting records from the Department of Atmospheric and Oceanic Science at the University of Maryland. Prevailing winds during those months come from the southwest.

The results show that EAB had an average annual rate of spread in Maryland of 0.61 miles/year ($y = 0.61x - 0.21$, $P = 0.02$, $r^2 = 0.94$) and a maximum annual rate of spread of 0.90 miles/year ($y = 0.90x - 0.13$, $P = 0.02$, $r^2 = 0.95$) between 2003 and 2008. This exceeds the expected

annual distance traveled of 0.5 miles that has historically been used in EAB control programs. EAB has moved outward from the original introduction site into all four quadrants cardinal directions, with four detection points each in both the NW and SE quadrants, and two detection points each in both the SW and NE quadrants. EAB therefore does not appear to be moving preferentially in the direction of prevailing winds.

In an intensively managed quarantine site in Maryland, EAB is moving at an average rate of 0.6 miles/year and a maximum rate of 0.9 miles/year. These distances exceed the 0.5-mile buffer for the eradication zone established in 2003 when EAB was first detected in Maryland, but are in agreement with a dispersal distance of 0.93 miles (1500 m) reported by Haack and Petrice (2003). EAB is moving outward from its initial site of introduction in several directions, rather than in the direction of prevailing winds. We emphasize that this study does not exclude transportation or nursery stock, firewood, or vehicular traffic as facilitating the movement of this beetle. We also acknowledge that, in addition to natural dispersal

of beetles, movement of infested nursery stock early in the period of establishment of the beetle could exacerbate the rapidity of spread. The extent to which dispersal in Maryland is natural or anthropogenic is unknown. Further investigations will examine the importance of other physical features such as riparian areas, vegetation types, roadways, and land use on patterns of movement.

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Acknowledgments

Support for this project was provided by the USDA-APHIS-PPQ Center for Plant Health Science and Technology.

RESTORATION OF THE AMERICAN ELM ON THE CHIPPEWA NATIONAL FOREST THROUGH GENERATION OF DUTCH ELM DISEASE TOLERANT, COLD-HARDY, AND SITE-ADAPTED TREES

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ABSTRACT

The American elm component of hardwood forests and riparian ecosystems in forested landscapes has been greatly reduced or eliminated by Dutch elm disease (DED). The ecological significance of this reduced role of American elm in riparian ecosystems is likely to be more significant if the ash component is also lost due to emerald ash borer infestation. Efforts to restore the American elm within areas of its former range with unique climatological attributes pose a challenge. The Chippewa National Forest (CNF) and the Northern Research Station initiated a project in 2007 to restore the American elm to CNF's landscape through a unique approach. The goal of this effort is to generate trees that are DED tolerance, cold-hardy, and site-adapted. The approach used for this effort used pollen from large DED survivor American elms on the Chippewa to pollinate flowers on DED tolerant Valley Forge and R18-2 American elm selections to generate progeny trees. These trees, along with control trees will be planted at three sites on the Chippewa, and then challenged by direct

trunk inoculations of *Ophiostoma novo-ulmi*. The most vigorous, DED tolerant, and cold-hardy survivor trees will be clonally propagated and the trees used to restore the American elm on CNF. In the spring of 2008 pollen was collected from four American elm survivor trees on the Chippewa, and used in controlled pollinations on flowers in the laboratory and on Valley Forge and R18-2 trees in the Delaware, OH American elm seed orchard. Seeds were planted, and progeny trees were initially grown in a greenhouse and then a lath house until dormancy. Control trees were generated by vegetative cuttings. Six hundred seventeen trees ranging from 3-6 feet in height were shipped to the Chippewa National Forest after dormancy in November 2008, and will be planted in the spring of 2009. The remaining 463 trees needed for this effort will be generated in the spring of 2009 and will be planted in 2010. Partners on this project include State & Private Forestry, St. Paul, MN; Region 9 of the U.S. Forest Service, Milwaukee, WI; and Leech Lake Band of Ojibwe.

EMERALD ASH BORER INFESTATION RATES IN MICHIGAN, OHIO, AND INDIANA

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ABSTRACT

The goal of this study was to obtain an estimate of the infestation rate of ash trees with emerald ash borer (EAB) (*Agrilus planipennis*, Fairmaire; Coleoptera; Buprestidae), across its primary infestation zone of southern Michigan, northeast Indiana, and northwest Ohio. A two-stage, two-phase stratified statistical sample was performed on a 31-million-acre area, across two sampling zones; a more heavily sampled 12.7-million-acre core zone, primarily in southeastern Michigan (the primary quarantine zone at the time), and a more lightly sampled area northwest and south of the core area.

Based on this sample, in the core area 21 percent of the ash were estimated to be infested with EAB, about 8 percent were dead with signs of EAB, about 13 percent were alive with signs of EAB. In the outer area, 13 percent of the ash trees were estimated to be infested with EAB, about

2 percent were dead with signs of EAB attack, and about 11 percent were alive with signs of EAB. U.S. Forest Service Forest Inventory and Analysis (FIA) estimates for the forested portions of the sampled area are that there are about 287 million ash trees (d.b.h. ≥ 2 inches). Based on our survey estimates, there are about 30.8 million ash trees in nonforest conditions in the survey area. Using the area-wide estimate of a 16.89 percent infestation rate, about 53.6 million ash trees in both forest and nonforest conditions were estimated to be infested with EAB by 2007. The approximate estimate of sample standard deviation of the infestation percent is 4.3, about 25 percent of the 16.89 percent mean infestation rate. The 95 percent confidence interval for the number of EAB infested trees in the survey area is from 24.7 million to 82.7 million trees.

MICROSPORIDIAN PATHOGENS OF THE GYPSY MOTH: RESEARCH UPDATE

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ABSTRACT

Three genera of microsporidia, *Vairimorpha*, *Nosema* and *Endoreticulatus*, infect gypsy moth larval populations in Europe and have been documented to reduce the intensity and duration of outbreaks. Manipulation of these chronic pathogens involves knowledge of taxonomic relationships, host specificity, virulence, transmission, strain variability, interspecific competition and other aspects of host-pathogen interactions. Our ongoing research has addressed these issues and continues as we now conduct inoculative introductions in the U.S. and augmentative releases in Eastern Europe. Our research objectives include the following:

- Elucidate taxonomic relationships and variability between closely related isolates
- Study the mechanisms of microsporidian transmission
- Investigate basic host-pathogen interactions
- Release and monitor microsporidia in native populations of *Lymantria dispar*

We studied interspecific competition among the microsporidia *Nosema lymantriae*, *Vairimorpha disparis*, and *Endoreticulatus schubergi*, performing simultaneous and sequential inoculations of gypsy moth larvae.

Endoreticulatus, a gut pathogen was not influenced by competition with either *Nosema* or *Vairimorpha*, which both infect fat body tissues. Competition between *Nosema* and *Vairimorpha* altered successful establishment of infection; transmission and competition led to significant suppression of one species depending on timing and sequence of inoculation. The first inoculated species out-competed the second in sequential trials.

Nosema and *Vairimorpha* represent a closely related group with variable characteristics. One species, *Vairimorpha disparis*, was recently characterized and redescribed. Based on small subunit rDNA sequences, we consider the remaining isolates to be strains of *Nosema lymantriae*. The third genus is represented by the species

Endoreticulatus schubergi. Our studies using PCR-RAPDs, gene sequencing (rDNA, HSP70) and proteomics studies (2-D PAGE and DIGE) provide evidence of genetic variability of microsporidia in isolated *L. dispar* populations. The SSU-rDNA and HSP70 genes are nearly identical among *Nosema* isolates and differ only slightly for *V. disparis*. Soluble protein analyses suggest differences in gene expression, particularly between the octospore-producing *V. disparis* and the *N. lymantriae* isolates.

Results of field studies on the nontarget effects of ULV spray applications of *N. lymantriae* and *V. disparis* showed that *N. lymantriae* is ecologically host specific for the gypsy moth. *V. disparis* infected 10 of the 109 nontarget lepidopteran species collected (20 of 248 individuals of the susceptible species), but was not found infecting nontarget species in the same plots the following 2 years. Laboratory studies of *E. schubergi* suggest that it is a generalist pathogen and it is not being considered for release. Microsporidia recovered from nontarget species included generalists *Cystosporogenes operophterae* and *Orthosomella operophterae*. *N. lymantriae* [Bulgarian isolate] and *V. disparis* were released in Illinois in May 2008 and will be monitored over the next three seasons. Augmentative releases were also made in Bulgaria.

The three genera infecting *L. dispar* differ in virulence and in tissue specificity in the host. A series of laboratory experiments provided a good picture of the important pathways of horizontal and vertical transmission in the three genera. Laboratory experiments produced quantifiable data for input in mathematical simulation models of horizontal transmission. Horizontal transmission was also quantified in a field cage study. Acquisition of infection by test larvae increased with increasing ratio of initially infected larvae. At higher densities, percent infection in test larvae leveled off. The duration of the latent period, the time between acquiring infection and release of first spores, is important for horizontal transmission. Transmission of *N. lymantriae* began 11 d post-exposure of test larvae to inoculated larvae. We found the first infected test larvae at 20 dpi; transmission increased over time. Transmission of *V. disparis* also increased then levelled off (at 50 percent of larvae infected) with increasing ratio of larvae initially infected larvae. The correlation between probability of encounter of test larvae with dead, infected larvae and the number of test larvae developing infections is highly significant.

ROLE OF FECES AND EFFECTS OF SIMULATED RAIN ON HORIZONTAL TRANSMISSION OF THE MICROSPORIDIUM, *NOSEMA LYMANTRIAE*

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ABSTRACT

Two main routes of horizontal transmission have been identified for the microsporidium, *Nosema lymantriae* Weiser, a frequently occurring pathogen of *Lymantria dispar* in Europe. Spores are released from 1) feces of living, infectious larvae after a latent period of approximately 2 weeks; and 2) the cadaver after host death. Quantification of spore release from feces has been done in laboratory studies using a meridic diet. Under such circumstances, horizontal transmission of *N. lymantriae* is known to be extremely high. However, the quality of feces originating from meridic diet is substantially different to those from oak leaves.

We designed this experiment to prove the role of feces originating from oak leaves as food in horizontal transmission of *N. lymantriae*. Moreover, we tested the hypothesis that rainfall can aid transmission by increasing the attachment of the otherwise dry feces to leaf surfaces and better dissemination of spores. Therefore, we set up two 9.1-liter acrylic glass cylinders containing oak twigs and separated by steel mesh. Infectious *L. dispar* larvae that had been fed with foliage were put in the top cylinder; the mesh allowed feces to fall to the bottom

cylinder but prevented migration of larvae. After 24 h, the infectious larvae were removed and uninfected test larvae were put into each cylinder to measure transmission. A light intensity rain was simulated by sprinkling foliage with tap water from a watering can twice during the exposure period.

The inoculated larvae heavily contaminated their environment. Transmission to test larvae in the upper cylinders was often close to 100 percent. Furthermore, the experiment proved that fecal pellets are a source of spores for transmission, although at a rather low level under the tested, dry conditions. The mean percentage of *N. lymantriae* infection acquired by test larvae was 4.4 ± 2.4 percent in the dry, bottom cylinders. Longer exposure times or an experiment later in the infectious period would probably lead to higher transmission. Simulated rainfall significantly increased transmission in the bottom cylinders to 30.0 ± 8.2 percent. We conclude that light rain is likely to enhance transmission in the field by increasing the number of fecal pellets that stay on the foliage and by dissolving them, consequently spreading spores on leaf surfaces.

EFFECTS OF LURE COMPOSITION AND RELEASE RATE ON CATCH OF EDRR TARGET SPECIES AND OTHER FOREST COLEOPTERA

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ABSTRACT

The USDA Forest Service's Early Detection and Rapid Response Program (EDRR) is a nationally coordinated program that employs traps for detecting, delimiting, and monitoring newly introduced exotic bark and ambrosia beetles. The utility of the trapping portion depends upon effectiveness of lures—a difficult proposition because target species are varied and likely have specific host-finding behaviors. Our goal was to assess effects of release rates and compositions of host-based general attractants on catch of EDRR target species and other forest dwelling beetles.

Companion experiments were implemented in central Louisiana (LA) and northern California (CA) between April and August 2008. The first experiment addressed primarily the effects of monoterpene composition and release rate, while the second addressed ethanol (ETOH) dosage and interactions with monoterpenes. Commercial releasers were used along with homemade bottles (various wicks) to increase flexibility in release rates. Monoterpene treatments were α -pinene or mixtures based on pine monoterpene compositions in LA and CA.

Lure release rates were measured during the studies; in Louisiana, rates of monoterpene release ranged from 1.3 to 2.6 g/d and ETOH varied from 0.6 to 14 g/d.

Each experiment was conducted for 1 month. The first experiment consisted of six treatments with six replicates (36 traps), all having ETOH and either α -pinene or blended monoterpenes. The second experiment consisted of nine treatments (3 x 3 factorial) with six replicates (54 traps). Treatments in the second experiment were crossed from three monoterpene lures (Phero Tech UHR- α -pinene, Synergy LA blend, Synergy CA blend) and three ETOH lures (Synergy UHR-ETOH [x2], bottle ETOH with a pipe cleaner wick [x2], bottle ETOH with burner wick [x2]). This experiment was conducted in July, 1 to 2 months after the conclusion of the first experiment. Collections are still being processed, but to date, LA experiments have produced 26 EDRR target species (including one new North American record, *Xyleborus octiesdentatus*) and more than 50 additional beetle species.

CAN ALIEN PLANTS SUPPORT GENERALIST INSECT HERBIVORES?

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ABSTRACT

Rearing experiments were conducted to address two questions relevant to understanding how generalist lepidopteran herbivores interact with alien plants. We reared 10 yellow-striped armyworms (*Spodoptera ornithogalli*), luna moths (*Actias luna*), bagworms, (*Thyridopteryx ephemeraeformis*), and white-marked tussock moths (*Orgyia leucostigma*) from egg to 5th instar on the excised foliage of a variety of alien and native plant species in the laboratory, to determine the degree to which highly polyphagous lepidopteran herbivores are capable of surviving on the suite of alien plants naturalized near Delaware. These species were chosen because gravid females were easily collected, they represent four unrelated families, and they have exceptionally long host lists recorded in the literature. With few exceptions, all four generalists either quickly starved or grew at an ecologically hazardous rate on alien foliage. By the end of the experiment all 10 white-marked tussock moths died on 10 of the 20 alien plants tested; all bagworms died on 13 of the 16 alien plants tested; and

all luna moths died on 13 of the 15 alien plants tested. Armyworm performed slightly better, with only 2 of the 20 plants causing death of all the larvae; however on 18 of the 20 plants tested, biomass production was less than half of that obtained by the largest larvae. The data suggest that 1) alien plants are unlikely to produce as much generalist insect biomass as the native plants they replace; and 2) lepidoptera that qualify as generalists when host breadth is considered over their entire geographic range may express a far more specialized diet within local populations. Both of these conclusions support the hypothesis that alien plant invasions may seriously disrupt terrestrial food webs by reducing the insect biomass required by insectivores in higher trophic levels. The success of insectivorous vertebrates depends on heavily on the ability of insects to assimilate the energy produced by plants and turn it into insect biomass. Our results call into question the ability of even the most generalist of lepidopteran species to adequately perform that task on many alien plants.

HEMLOCK CANOPY ARTHROPODS: BIODIVERSITY ON A THREATENED HOST

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ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae*, Annand), a pest of hemlocks introduced from Japan in the first half of the last century, threatens the sustainability of both eastern and Carolina hemlock (*Tsuga canadensis* and *T. caroliniana*, respectively) in eastern North America. Hemlock represents a key ecological component in eastern forests and threats to these trees represent threats to birds, mammals, and fish, and impact forest vegetation composition and species richness. Hemlocks also support a diverse community of spiders, beetles, flies, and other predatory arthropod species which, although numerous, fail to exert control over hemlock woolly adelgid populations. Although past studies have sought to identify native predators that may impact the adelgid, little work has been done to document the entire arthropod community found on hemlocks, or to identify those factors which alter or dictate the composition and structure of hemlock arthropod communities. Here the initial findings of a series of studies designed to describe the A, B, and Γ diversity (using beat sheets and “branch bagging” to construct species accumulation curves, see Colwell et al. 2004) on hemlock, and evaluate the impacts of both the adelgid, and management efforts designed to control the adelgid, on the biodiversity and structure of this community are described. Briefly, these data show that:

- The α diversity estimate is high, with approximately 10 species of arthropods per branch.
- β diversity is also high, with an initial β estimate of nine (new) species/branch. There is very little $\delta\beta$ as represented by the change in slope, indicating that the accumulation curves are far from asymptotic.

- γ is likely high, however, because of the low $\delta\beta$, estimation of this value based on extrapolation methods yields inconsistent values, for example, the bootstrap estimate is 413 species, while the ICE estimate is 847.
- Diversity is high at multiple taxonomic levels, the 16 most abundant morphospecies include nine orders.
- Bagging 67 branches from 22 trees has produced more than 1,300 individual arthropods from approximately 300 morphospecies. Additional collections should move the accumulation figures closer to their asymptotes.
- The distribution of species abundances follows a typical pattern of exponential decrease, with a small number of highly abundant species, and a large number of rare species. Nearly half of the species appear as singletons.

These data clearly show that eastern hemlocks support a high diversity of arthropods. As this ecological resource is removed from forested systems through adelgid induced tree mortality it is likely to take with it a significant portion of the forest biodiversity. Although γ diversity estimates were highly variable (413-847 species), additional collections currently being processed should move the collection curve towards the asymptote, refining both estimates of diversity, and community structure in this threatened system.

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FIELD TRAPPING THE ASIAN LONGHORNED BEETLE (*ANOPLOPHORA GLABRIPENNIS*) IN NINGXIA, CHINA, USING HOST VOLATILES AND COMPOUNDS ISOLATED FROM VIRGIN FEMALE BEETLES

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ABSTRACT

Asian longhorned beetles (ALB), *Anoplophora glabripennis* (Motchulsky) have complex cuticular hydrocarbon profiles and the patterns vary consistently with sex, age, and mating status. Analysis of the cuticular hydrocarbon chemistry of virgin female ALB revealed many new additional compounds that were structurally related to the five monounsaturated compounds identified as the contact sex pheromone by Zhang et al. (2003) and are suspected to have dual roles in the hypothesized mating sequence of ALB as long-range attractants and mate finding, specifically 1) as precursors to volatile female pheromones; and 2) as trail/contact sex pheromones. These compounds are produced in maximum abundance by virgin females and then are “shut off” immediately after mating. These compounds are the basis for formulating lures for testing in China.

Lures containing female pheromone blends were tested in Ningxia, China during summers of 2006-2008 using flight-intercept panel traps. In July 2006, virgin female pheromone blends captured more beetles than mated female blends and single component lure, while in a second 2006 field experiment the full female blend plus plant volatiles and linalool oxide captured more beetles than controls. In July 2007, an experiment with more natural release rates, a three-component blend with plant volatiles and linalool oxide captured more beetles than controls and this treatment preferentially captured males. In July 2008, lures containing additional pheromone components (from virgin female extracts mentioned above, which are found in highest abundance in virgin females then decline precipitously after mating) plus plant volatiles and linalool oxide, again preferentially captured males. These results are the first field evidence of a female long-range pheromone in ALB, or any species of Lamiinae.

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SIMULATING THE IMPACTS OF ALTERED FIRE REGIMES AND LANDSCAPE STRUCTURE ON THE INVASION OF *PAULOWNIA TOMENTOSA* IN THE SOUTHERN APPALACHIANS

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ABSTRACT

The southern Appalachian forests have long been under multiple environmental threats, including periodic fires, insect outbreaks, and more recently, increased invasion by exotic invasive plants. Past studies suggested these multiple disturbances interact to shape the species-rich forest landscape, and hypothesized that the changed fire regimes, interacting with increasing landscape fragmentation, may insert complex influences on patterns and processes of the invasion. The long-term impacts of fires, landscape-scale interactions among the multiple influencing factors, and the sound forest management practices to reduce the damage, however, are still unclear. We developed a modeling approach to explore the synergistic effects of fires and fragmentation on spread of an exotic invasive plant, princess-tree (*Paulownia tomentosa* [Thunb.] Siebold & Zucc. ex Steud.). LANDIS-II, a spatial explicit forest succession model, was used to simulate vegetation dynamics and plant invasion in a hypothetical xeric landscape, which captures the predominant vegetation distribution in the southern Appalachian Mountains. We parameterized a pool of the 30 most dominant trees species using the

double-exponential seed dispersal algorithm. Changes of abundance of the invasive species were simulated over a 300-year period along a combined fire frequency and forest fragmentation (measured as edge density) gradient. We found that intermediate level of fire frequency (~10 years < fire spread age < 300 years) promoted spread of the princess-tree, while both low and high extremes of the fire frequency spectrum limited its invasion. Under the same level of fragmentation, higher proportion of initial forested patches resulted in an increase in abundance of the species in the landscape, in part due to greater seed availability. We also found that edge densities and abundance of princess-tree retained a positive log-linear relationship over time. Our study indicates that on more fragmented landscapes, intermediate level of fire frequency increased abundance of the invasive plant that resulted from a strong edge diffusion effect, and suggests that effective fire management combined with the harvest practices of creating less forest edges can help to reduce the frequency of the invasive plant in the landscape. This information may help forest managers in the region to develop effective strategies to manage the ongoing invasive plant problem.

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