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Edited by
Katherine McManus and
Kurt W. Gottschalk

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FOREWORD

This meeting was the 21st in a series of annual USDA Interagency Research Forums that are sponsored by the Forest Service, Animal and Plant Health Inspection Service, Agriculture Research Service, and National Institute for Food and Agriculture. 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 four 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
THE MOUNTAIN PINE BEETLE: CAUSES AND CONSEQUENCES OF AN UNPRECEDENTED OUTBREAK

Allan L. Carroll
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ABSTRACT

The mountain pine beetle (*Dendroctonus ponderosae*) is native to the pine forests of western North America where it normally exists at very low densities, infesting only weakened or damaged trees. Under conditions conducive to survival, populations may erupt and spread over extensive landscapes, killing large numbers of healthy trees. Although several significant eruptions by mountain pine beetle occurred during the past century in western North America, the ongoing epidemic is unprecedented in its size and severity.

For a mountain pine beetle outbreak to occur, there must be an abundance of large, mature pine trees (the beetle’s preferred resource) combined with several years of favorable weather for beetle survival. Due to fire suppression and selective harvesting (for species other than pine) during the latter half of the previous century, there was more than three times the amount of mature pine in western Canada at the start of the current outbreak than 100 years earlier. Furthermore, as a result of climate change, conditions relevant to mountain pine beetle survival have improved over much of its range during recent decades, allowing populations to invade formerly climatically unsuitable pine forests. Thus, both required conditions for an outbreak have coincided with sufficient magnitude to cause the largest outbreak in recorded history.

The current mountain pine beetle epidemic has had extensive direct ecological and economic impacts, affecting wildlife habitat, water quantity/quality, recreational values, and timber quantity/quality. Furthermore, the widespread tree mortality inflicted by the mountain pine beetle has also caused significant longer term impacts to regional carbon dynamics. The loss of carbon uptake and the increased emissions from decaying trees have converted the forests of western Canada from a small net carbon sink to a large net carbon source to the atmosphere. Also as a consequence of the unprecedented outbreak, large numbers of beetles have blown across the northern Rocky Mountains and established across the Alberta Plateau in close proximity to the boreal jack pine forests that extend across the continent. High host susceptibility, coupled with anticipated increases in climatic suitability as a result of global warming, suggests there is a significant risk of additional eastward expansion by the mountain pine beetle and eventual invasion of the boreal forest.
PROGRESS IN UNDERSTANDING THE ECOLOGY AND DETECTION OF SIREX NOCTILIO

Matthew P. Ayres¹, Jenna M. Sullivan¹, Tina Harrison¹, Kelley E. Zylstra², Kevin J. Dodds³, Jeffrey R. Garnas⁴, Victor C. Mastro⁵, and Maria J. Lombardero⁶

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ABSTRACT

We have been developing techniques for detecting the presence of Sirex noctilio and studying the ecology of Sirex noctilio in North America with the goal of anticipating the trajectory of the Sirex invasion. A challenge for detection efforts is the low efficiency of trapping. Fortunately, it has been possible to develop guidelines for identifying the presence of Sirex based on examination of emergence holes in host trees. The key diagnostic features are easy enough to use with little experience: (1) location of feeding galleries within the tree, (2) shape of emergence holes, (3) hole size, (4) patterning of holes within trees, and (5) form of feeding galleries and insect frass within the wood. An advantage of this identification technique is that the emergence holes remain easily detectable for many years. A weakness is that holes alone cannot distinguish between S. noctilio and native Sirex spp. S. noctilio is indicated by the presence of resin drops or drippings from infested trees, but the absence of detectable resin does not rule out S. noctilio. We are developing techniques for isolating and identifying the species of fungal associate (by sequencing of the ITS region of ribosomal DNA) and thereby identifying the species of woodwasp.

A key to anticipating the consequences of S. noctilio in North America is an understanding of why the species has perpetually low abundance in its native Europe while being capable of catastrophic outbreaks in the Southern Hemisphere. We suggest that the population dynamics of S. noctilio may be characterized by alternate attractors, which tends to regulate populations at low levels unless abundance exceeds some escape threshold that permits populations to grow to epidemic proportions. We have been testing for evidence of positive density dependence in North American populations, which would suggest the potential for outbreaks. There is enormous variation in adult fecundity and therefore potential population growth. Adult size seems to be largely unrelated to nutrition. S. noctilio grows as large or larger in red pine as in Scots pine. Adult size varied among sites in upstate New York but was unrelated to local abundance. S. noctilio progeny per trap varied among sites without obvious spatial structure. Sex ratios were highly male biased but unrelated to abundance. Emergence of S. noctilio was highly aggregated within individual trees, which suggests destabilized positive feedback in population dynamics. Parasitism by Ibalia leucospoides decreased
with local abundance, which indicates destabilizing positive feedback (numerical escape from an important natural enemy). The presence of blue-staining Ophiostomatoid fungi was negatively correlated with the success of *S. noctilio* within and among trees, perhaps because the bluestain fungi interfere with the establishment of *Amylostereum areolatum*, the mutualistic symbiont of *S. noctilio*. Our studies of *Sirex* nutritional ecology indicate that *Sirex* assimilate about 31 percent of the cellulose they consume, which is presumably what permits their development within a nutritionally challenging environment (pine xylem) and is possible only because of their fungal symbiont.

Continuing work will include studies of insect-mediated interactions between native Ophiostomatoid fungi and *A. areolatum* (in collaboration with Daniel Gruner, University of Maryland) and studies of attack rates in suppressed and vigorous trees, including comparisons of Scots vs. red pine in the U.S., and pines in the U.S. vs. Spain (in collaboration with Maria Lombardero, Universidad de Santiago, Spain).
MOLECULAR GENETIC PATHWAY ANALYSIS
OF ASIAN LONGHORNED BEETLE

Evan Braswell
USDA APHIS CPHST, Moore Air Base, Edinburg, TX 78541

ABSTRACT

The Asian longhorned beetle, *Anoplophora glabripennis*, is a destructive pest of hardwood trees. Historically, *A. glabripennis* was geographically restricted within China and Korea and not of economic importance. However, as a result of massive reforestation programs designed to combat desertification, the species emerged as a pest and rapidly spread throughout China.

The presence of multiple outbreak populations of *A. glabripennis* within the U.S. raises questions about its movement. Did the species invade the U.S. once, subsequently spawning additional populations, or did multiple invasions occur undetected until populations established? In either case, can we trace the pathways of invasion and thereby limit future threats?

We set about to address these questions by amplifying and sequencing 1,300 bases of the Cytochrome Oxidase I gene in the mitochondrial genome. The resulting 43 unique DNA sequences (haplotypes) indicate that a minimum of 33 female beetles have invaded the U.S. (excluding foreign haplotypes). Most of these haplotypes are rare, having been sampled from a single beetle each, indicating a need for further sampling.

Using statistical parsimony, we traced the ancestry and inferred patterns of relationship among the observed haplotypes. This analysis found that the sole post-eradication beetle found in Chicago, after 5 beetle-free years, was genetically divergent from pre-eradication samples taken from the Chicago infestation. Conversely, the recently discovered Worchester, MA, population shares most haplotypes with New York beetles. Jersey City beetles are genetically identical to New York beetles, suggesting the population originated from New York migrants.

Confirming movement within the U.S., as well as detecting the direction and frequency of movement, will require additional samples to assess the genetic variation present as well as additional genetic markers to make the most use of that genetic variation.
Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a devastating invasive pest of North American ash trees (*Fraxinus* spp.) that was first discovered outside of its native range of northeastern Asia in 2002 (Haack et al. 2002). With unintended assistance from human movement of infested ash material, EAB spread swiftly from its initial zone(s) of discovery in the Detroit, MI/Windsor, ON, metropolitan area and now can be found in 13 states in the United States and 2 provinces in Canada. Characterizing the structure of EAB populations could provide valuable information on EAB geographic origin (and thus the location of possible effective biological control agents) and host range potential, and it could provide evidence of the main mode of spread. This study thus had two main goals: (1) to obtain samples from throughout the native and introduced ranges of EAB and (2) to characterize the genetic population structure of EAB in its native and introduced range using mitochondrial partial gene sequencing and DNA fingerprinting using amplified fragment length polymorphisms. To accomplish the first goal, a network of collaborators was established to obtain samples from the native range of EAB in China, South Korea, and Japan, and its introduced ranges in the United States, Canada, and western Russia. This effort yielded a collection of 1,799 EAB specimens from 7 states in the United States, 114 specimens from Ontario, Canada, 12 from Moscow, Russia, 274 from China, 17 from South Korea, and 3 from Japan. To accomplish the second goal, eight Asian and seven North American populations were characterized with partial mtDNA cytochrome oxidase subunit I sequence (481 bp) and four AFLP primer pair combinations (108 loci). Analysis of COI sequences revealed one common haplotype in China, South Korea, and all samples from N. America, as well as three unique haplotypes in China and four haplotypes from South Korea, which differed from the common haplotype by 1 to 2 nucleotides. A haplotype from a single EAB individual from Japan differed by 22 nucleotide changes (3.7 percent). Very weak genetic structure was detected, and most of the AFLP genetic variability was within populations (87 percent) and not among populations (13 percent). Average pairwise Φpt between North American EAB (considered as a single population) and Asian EAB populations revealed the lowest population differentiation between North America and Dagong and Tangshan, China (Φpt = 0.0877 and 0.0848, respectively). Further, population assignment tests assigned more than 67 percent of the individual beetles from North America to either Dagong or Tangshan, China. AFLP analysis also revealed local isolation by distance but did not reveal long-range isolation by geographic distance. This suggests that EAB, while dispersing at a local scale, is moving longer distances primarily due to human-mediated transport.

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DEMONSTRATING THE BENEFITS OF PHYTOSANITARY REGULATIONS: THE CASE OF ISPM 15

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ABSTRACT

Invasions of non-indigenous insects and pathogens threaten trees and forest ecosystems worldwide. For example, the arrival and spread of the pathogens causing chestnut blight and Dutch elm disease, along with the bark beetles vectoring the latter, had dramatic effects on North American forests. Despite our improved awareness of the risks associated with biological invasions, globalization and an increase in international trade have facilitated the continued arrival and establishment of non-indigenous forest pests and diseases. Several recent invaders, including the Asian longhorned beetle (*Anoplophora glabripennis*) and emerald ash borer (*Agrilus planipennis*), are particularly worrying because they infest and kill apparently healthy host trees. The overall economic impacts of invasive forest pests, including damage to trees, management and control actions, loss in amenity value, trade restrictions, and various other costs are expected to amount to many billions of dollars.

The use of wood packaging materials in international trade has been identified as an important pathway for the introduction of insects and pathogens associated with wood and timber. In recognition of the risk of further invasions occurring as a result of the use of untreated wood packaging materials (such as pallets, cases, drums, skids, and dunnage), the International Plant Protection Convention (IPPC) developed the International Standards for Phytosanitary Measures (ISPM) No. 15 (Guidelines for regulating wood packaging material in international trade). ISPM 15 prescribes the use of heat treatment or methyl bromide fumigation to kill any life stages of insects and pathogens associated with wood packaging materials before export and thereby mitigate pest risks. ISPM
15 has been widely adopted since its endorsement by the Interim Commission on Phytosanitary Measures in March 2002; as of 2010, it has been implemented by more than 50 countries. In the United States, all wood packaging material entering the country has had to comply with ISPM 15 since February 1, 2006.

The Nature Conservancy and the National Center for Ecological Analysis and Synthesis (NCEAS) at the University of California, Santa Barbara, have collaborated to establish a working group for increasing our understanding of the effects of phytosanitary policy in preventing the establishment and spread of invasive forest pests. The specific objectives of the NCEAS working group are to (1) estimate the costs and benefits of phytosanitary policy, (2) develop a conceptual model incorporating both ecological and economic elements to capture these costs and benefits, and (3) fully integrate and parameterize these models for a formal cost-benefit analysis of ISPM 15 as a case study. Our ultimate aim is to calculate the net benefits or costs of this policy, thereby providing an analytical framework for the development of further phytosanitary policies designed to mitigate other high-risk pathways, such as live plants (i.e., Plants for Planting).

ISPM 15 was chosen for a case study because its implementation in 2006 enabled a before and after analysis by comparing real-world data for the pre-ISPM 15 and post-ISPM 15 periods. Various data sources and a trade model are being used to estimate the costs of ISPM 15 compliant treatments and lost benefits from trade caused by the increased cost of transporting commodities. The benefits of the policy are being estimated as the averted damages, compared to a no-policy situation. These averted damages are estimated from the reduction in pest arrival rate (i.e., reduced propagule pressure) and the resulting decrease in the rate of pest establishments. USDA APHIS records of pest interceptions at U.S. ports-of-entry (AQIM and PestID) are being used to estimate changes in pest arrival rate due to ISPM 15, and the relationship between the interception rate of particular pest species and their establishment. Interceptions serve as a proxy of arrival rate because there is no information on the actual rate of pest arrival.

Preliminary results indicate that ISPM 15 has not completely closed the pathway associated with wood packaging materials because several post-ISPM 15 interceptions have been recorded, although there appears to be a downward trend. We have identified a number of confounding factors that could either be masking the effects of ISPM 15 or impeding its desired effects. Our analyses and economic and ecological models are being finalized, and we plan to release the main findings in late 2010. Another output will be advice for policymakers on improved data collection that would greatly assist with future efforts in demonstrating the effects of other phytosanitary measures, such as Plants for Planting, which is currently under development.
**IS IPS GRANDICOLLIS DISRUPTING THE BIOLOGICAL CONTROL OF SIREX NOCTILIO IN AUSTRALIA?**

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**ABSTRACT**

*Sirex* woodwasp (*Sirex noctilio*) is considered one of the most serious threats to exotic *Pinus radiata* plantations in Australia. This exotic wasp has been established in Australia for more than six decades. The most significant outbreak occurred in the Green Triangle region of southeastern South Australia-western Victoria in the late 1980s, where more than 5 million trees were killed (Haugen 1990). This outbreak highlighted the need for a national strategy for *Sirex* control and led to the development of the National Strategy for Control of *Sirex noctilio* in Australia (Haugen 1990). The implementation of this strategy during the 1990s resulted in a significant reduction in outbreaks of *Sirex* in Australia (Carnegie et al. 2005, Collett and Elms 2009).

Over recent years, an inconsistency, and in some cases gradual decline, in nematode parasitism rates of *Sirex* has been emerging from inoculated trap trees in several regions in Australia. In parallel with this has been an increase in the numbers of *Ips grandicollis* bark beetles in several pine-growing regions. Like *Sirex*, *I. grandicollis* is also attracted to stressed trees (Erbilgin et al. 2002); and in New South Wales (NSW) and South Australia, it has been attacking trap trees poisoned for the *Sirex* biological control program. This is likely to create a problem for *Sirex* control. *Ips grandicollis* populations peaked in NSW in 2006-2007, resulting in large areas (more than 17,000 ha) of tree mortality associated with a corresponding drought and bark beetle attack (Carnegie 2008). It is believed that milder winter temperatures enabled more generations to overwinter than in more normal years with colder winters. In parallel with the increase in *I. grandicollis* numbers, surveys have revealed that the majority of trap trees (established for the *Sirex* biological control program) in Hume Region (NSW) have been attacked by *I. grandicollis* over the past few years.

With the decrease in nematode parasitism and increase in *I. grandicollis* numbers, is *I. grandicollis* (plus *Ophiostoma ips* and/or *Ips*-nematodes) disrupting the biological control of *Sirex*? Several research projects have been initiated in Australia to investigate this problem.

The first project aimed at quantifying the efficacy of anti-aggregation pheromones (deterrents) in reducing *I. grandicollis* numbers and damage to *Sirex* trap trees. It was thought that *I. grandicollis* attack on trap trees may be reducing the attractiveness of trees to *Sirex* and/or the efficacy of the biological control agents. Five paired trap-tree plots were established in mid-November 2007 in *P. radiata* plantations in each of two State Forests in Hume Region, NSW (a total of 200 trees). North American colleagues recommended a push-pull strategy for *Ips* control (K. Dodds and D. Miller, U.S. Forest Service). The “push” part of the strategy involved putative anti-aggregation pheromones (verbenone pouches and *Ips* Dienol caps) placed on trees in treatment plots, two of each per tree per month. Control trees were left untreated. Early field observations indicated that *Ips* Dienol was not an effective anti-aggregant for *I. grandicollis* (and in fact may be an aggregant/attractant), so its use was discontinued after 1 month. Several researchers also showed *Ips* Dienol to be an aggregant (Dodds and
Miller, unpublished) while others showed it to be an anti-aggregant (Wilson 1995). The “pull” part involved intercept panel traps, baited with *Ips* enol and alphapinene, placed near the plots. Monthly assessments were made of *I. grandicollis* and *Sirex* attack and the rate of tree morbidity (i.e., how quickly poisoned trees were dying), as well as emergence data (in 2008/2009).

The main finding was that the push-pull strategy did not work; *I. grandicollis* attacked treated and untreated (control) trees similarly. All but a few trees had been attacked by *I. grandicollis* by May 2008 (Fig. 1). *Ips* grandicollis appeared to attack trees once the foliage changed to less than 50 percent green and greater than 50 percent red (Figs. 1-2), which occurred 2 months after trees were poisoned. There may be several reasons for this failure; perhaps levels of *I. grandicollis* were too high (i.e., too much bark beetle pressure) or poisoned trees emitted a higher level of attractants than the deterrents we applied.

Results also showed that 50 percent of trees were also attacked by *Sirex*. Observations (and evidence) of female *Sirex* ovipositing indicated they preferred

![Figure 1](image1.png)

Figure 1—Observed *Ips grandicoli* attack on paired trap-tree plots (treated and untreated) in Buccleuch State Forests.

![Figure 2](image2.png)

Figure 2—Rate of tree morbidity of trap trees in Buccleuch State Forest following poisoning in November 2007.
Ips-free trees and trees with some green foliage. Observations of Sirex emergence holes on billets from selected trees showed that wasps emerged not only in areas (on billets) where there was no I. grandicollis attack, but also in areas of high levels of attack. Importantly, nematode parasitism of Sirex emerging from Ips-attacked trees was variable and inconsistent, but low (7 to 44 percent). Similarly, parasitism by Iballa leucospoides was variable (15 to 90 percent, mean 50 percent).

In summary, it appears that I. grandicollis attack does not stop Sirex or I. leucospoides developing in trees, but may affect the length of time (window of opportunity) that Sirex can oviposit in trees and may affect nematode parasitism. Research on this is continuing: further investigations are planned on interactions between Sirex and I. grandicollis, and associated fungi and nematodes, and on optimization of trap tree establishment techniques.

**Literature Cited**


Extensive, unprecedented wilt and mortality of the highly invasive, exotic tree-of-heaven (*Ailanthus altissima*) occurred recently within mixed hardwood forests in south-central Pennsylvania. Until this study, the cause of the epidemic was unknown. *Verticillium albo-atrum* was consistently isolated from symptomatic *Ailanthus* seedlings and trees in areas having high levels of mortality, whereas *V. dahliae* was isolated from small scattered patches of diseased *Ailanthus*. Inoculations of potted *Ailanthus* seedlings in the greenhouse, as well as canopy trees in the field, revealed that both *V. albo-atrum* and *V. dahliae* were capable of infecting *Ailanthus*. However, *V. albo-atrum* was much more pathogenic. *Ailanthus* seedlings and canopy trees inoculated with *V. albo-atrum* usually died within 3 months. In contrast, *Ailanthus* seedlings and canopy trees inoculated with *V. dahliae* became symptomatic, but most were still living 1 or more years following inoculation. We conclude that the major cause of *Ailanthus* wilt and mortality within forests of south-central Pennsylvania is *V. albo-atrum*.

Regarding the host range of our isolate of *V. albo-atrum*, stem inoculation of potted *Ailanthus* seedlings in the greenhouse and canopy *Ailanthus* trees in the field with *V. albo-atrum* resulted in 100 percent mortality. In these same studies, stem inoculation of understory striped maple saplings in the field also resulted in 100 percent mortality. However, the high susceptibility of striped maple was not observed in naturally infected stands, where only 1 percent of striped maple saplings exhibited *Verticillium* wilt. Inoculation of chestnut oak, northern red oak, red maple, sugar maple, white ash, and yellow-poplar seedlings and/or canopy trees with *V. albo-atrum* did not result in *Verticillium* wilt symptoms. Non-*Ailanthus* tree species growing adjacent to dead and dying *Ailanthus* in the field were asymptomatic.

Current studies at Penn State include conducting a greatly expanded host range study, as well as dissemination studies involving transmission of *V. albo-atrum* by root grafts, infected wind-blown leaflets, infested ambrosia beetles, and infected or infested seeds. Various isolates of *V. albo-atrum* are being identified molecularly using PCR and DNA sequencing. Research results to date indicate that *V. albo-atrum* should receive serious consideration as a potential biocontrol agent for the invasive *Ailanthus altissima*. 
PROTECTING AMERICA’S ECONOMY, ENVIRONMENT, HEALTH, AND SECURITY AGAINST INVASIVE SPECIES REQUIRES A STRONG FEDERAL PROGRAM IN SYSTEMATIC BIOLOGY

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ABSTRACT

Systematics is the science that identifies and groups organisms by understanding their origins, relationships, and distributions. It is fundamental to understanding life on earth, our crops, wildlife, and diseases, and it provides the scientific foundation to recognize and manage invasive species. Protecting America’s economy, environment, health, and security against invasive species requires a strong Federal program in systematic biology.

Systematics is in crisis. As systematists retire, they are not replaced, and universities train too few professionals in systematics. Furthermore, the biological collections needed to support systematics languish in substandard facilities lacking adequate staffing, technology, and coordination. As a result of this inadequate support, the United States cannot effectively manage the threat posed by invasive species.

The purpose of this report is to increase awareness of the crisis in systematics and to advocate the need for a permanent, viable, and coordinated Federal systematics program. Systematics expertise and use is distributed across the Federal sector, so participation will be inclusive; no single agency can serve as the steward for the proposed program. The proposed systematics program requires four components: research, specimen-based collections, an informatics network, and the education of future systematists. These are collectively designed to provide the means to detect, identify, and predict the behavior and consequences of invasive species.

In working toward its mandate to limit damages from invasive species, the Systematics Subcommittee (SSC) of the Federal Interagency Committee on Invasive Terrestrial Animals and Pathogens (ITAP) has a 20-year vision: “Strengthen national and global systematics to predict, prevent, and manage invasive species to ensure biosecurity; public health; economic, environmental, and agricultural security; and sustainability.”

When this vision is achieved, the U.S. will have:

- Systematics expertise covering all groups of organisms
- An effective communication network linking Federal, academic, and international taxonomic resources
- A Web-based information system that integrates organismal biology, geography, and taxonomy with diagnostic keys and specimen data
- Adequate human and physical resources to manage Federal systematics collections
- A re-invigorated capacity and commitment by universities to prepare professionals in systematics
- A culture that values systematics and sustains its systematics resources
The SSC will conduct a comprehensive survey of Federal agencies to determine the agencies’ present and future needs as well as their capacity to promote research, collections, and information resources. Based on these findings, the committee will develop a 10-year plan for an enhanced, integrated systematics program. Phased in over 10 years, an enhanced Federal systematics program will better counter national security threats posed by invasive species, foster a new generation of systematic biologists, and establish contingencies for continuing operations in case of emergency or catastrophic loss. An interagency body will monitor the program’s progress.

The full report is available at http://itap.gov/nal_web/itap/docs/itap_report_mar23.pdf. It was written by the Invasive Terrestrial Animals and Pathogens (ITAP) Systematics Subcommittee, which includes David Chitwood, USDA Agricultural Research Service (ARS); Hilda Diaz-Soltero, USDA; Eric Hoberg, USDA ARS; Scott Miller, Smithsonian Institution (SI); Robert Reynolds, Department of the Interior, U.S. Geological Survey; Benjamin Rosenthal, USDA ARS; Amy Rossman, USDA ARS; Marsha Sitnik, SI; and Alma Solis, USDA ARS.
COMMUNITY AND ECOSYSTEM CONSEQUENCES
OF MICROSTEGIUM VIMINEUM
INVASIONS IN EASTERN FORESTS

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ABSTRACT

Over the past two decades, biological invasions have come to the forefront as a major factor driving global environmental change. Introduced species can reduce biodiversity, inhibit the natural process of succession, and alter ecosystem functions such as nutrient and carbon cycling. There is an urgent need to understand the effects of invasions on native systems in order to prioritize species for research and management, to motivate local citizens and governments to take action, and to inform policymakers and land managers. In addition, because invaders are unique species being introduced into a novel environment, the study of biological invasions can provide important opportunities for understanding basic ecological and evolutionary processes. Much of the work on the effects of invasions has been observational, which makes it difficult to determine if the invaders are actually causing differences in invaded communities or if native and invasive species are simply responding differentially to changing environmental conditions. To properly quantify the effects of invaders, experimental methods such as removal or addition of invasive species are needed.

Across much of the eastern U.S., forests are rapidly being invaded by the non-native grass Microstegium vimineum (Japanese stiltgrass). This grass was unintentionally introduced in the early 1900s, but has only recently been recognized as a widespread invader. It colonizes roadsides, trails, and disturbed areas, but can also invade intact forests. I have conducted two experiments to test for community and ecosystem effects of Microstegium invasions. In the first experiment, I identified eight sites in southern Indiana with widespread and dense Microstegium invasions. I then established 40 plots at each site and randomly applied one of the following four treatments to each plot: (1) reference plots; (2) hand-weeding (HW); (3) grass-specific post-emergent herbicide (POST); or (4) grass-specific post-emergent herbicide plus pre-emergent herbicide (POST + PRE). After 2 years, we saw an overall positive response in native community biomass in removal plots compared to reference plots, regardless of the treatment used to remove Microstegium. However, there were significant differences in native herbaceous diversity and tree regeneration among the removal treatments. Removal of Microstegium using hand-weeding or POST resulted in positive responses in diversity, whereas POST + PRE did not allow for increased native diversity. Similarly, more than twice as many tree seedlings colonized the POST plots than the reference plots. There was no increase in tree regeneration under the HW or POST + PRE removal treatments. These results suggest that use of a post-emergent grass-specific herbicide is an effective tool for managing Microstegium invasions. Furthermore, the positive responses in native species biomass and diversity and in tree regeneration when Microstegium was removed suggest that invasions were suppressing native species.

In the second experiment, we added Microstegium to individual field plots and compared native herbaceous species, tree species, and arthropod responses between invaded and control plots. We established 32 plots (5.25 m x 5.25 m) and planted 9 species of native
tree saplings in half of the plots. To test if the effects of invasion depended on tree life history stage, we planted the same 9 species of trees as seed in the other 16 plots. We also added 12 native herbaceous species to all plots. After the plots were established, we randomly seeded half of the plots with Microstegium. After 2 years, invaded plots had dramatically reduced native herbaceous biomass and diversity. Invasion also reduced the success of small-seeded tree species such as box elder and sweetgum, but did not affect large-seeded tree species such as black walnut and shellbark hickory. Microstegium invasion also caused declines in the abundance and diversity of arthropods and reduced the survival of two tick species. Ongoing work also suggests invasions are altering nitrogen cycling and decomposition processes. In sum, this experiment has demonstrated that Microstegium can have remarkable consequences for native biodiversity, tree regeneration, and ecosystem processes.

Additional ongoing work on Microstegium includes investigations of the interaction between fire and invasions and the process of pathogen accumulation on invasive populations. Initial results have shown that fire intensity is significantly increased in invaded areas with greater peak fire temperatures, taller flame heights, and a greater percentage of the area burned, possibly due to reduced green fuel loads. Further research on fire and Microstegium will determine how invasion dynamics and native species, including tree regeneration, are affected by fires and the timing of fires. In separate work, we recently found a previously undescribed fungal pathogen on invading Microstegium and identified it as a Bipolaris sp. We have also isolated more than a dozen other microbes that are possible pathogens on Microstegium. The accumulation of pathogens on Microstegium would suggest that the release of Microstegium from natural enemies may be temporary.
MICROBIAL ACROBATS: TRACKING THE WHEREABOUTS OF FOREST \textit{PHYTOPHTHORA} SPECIES

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ABSTRACT

Over the past few years, significant new findings have jolted the forest \textit{Phytophthora} research community; the following is a synopsis of significant developments in our understanding of these adroit and often surprising organisms. Surveys and investigations demonstrate that \textit{Phytophthora} species continue to be introduced into new areas and persist in soil and water. Areas at highest risk to damage from these plant pathogens are urban-wildland interface areas, typically where nursery stock exposes woodland vegetation to new diseases. Once they become established, \textit{Phytophthora} species can be difficult to eradicate so our society would benefit ecologically and economically from prevention of \textit{Phytophthora} introductions.

Despite Federal quarantines, \textit{Phytophthora ramorum} Werres, de Cock & Man in’t Veld, cause of sudden oak death and ramorum blight, was detected in 2009 on 10 sites in seven states, adjacent to infested nurseries. The pathogen moved off nursery property in contaminated runoff and was recovered in adjacent waterways. In Pierce County, WA, in summer 2009, regulatory officials reported the first documented case of the pathogen becoming established on native forest vegetation adjacent to a contaminated stream, with the NA2 strain on salal (\textit{Gaultheria shallon} Pursh). This was also the first detection of the NA2 strain in a North American forest; previously this strain had been detected only in a few nurseries in Washington and California.

In the U.K., \textit{P. ramorum} erupted on several new hosts: Japanese larch (\textit{Larix kaempferi} (Lam.) Carrière), western hemlock (\textit{Tsuga heterophylla} (Raf.) Sarg.), and birch (\textit{Betula pendula} Roth). Several years of wet weather provided ideal conditions for disease development on shoots and foliage in mature larch plantations. Large areas of larch are being cut to prevent pathogen spread. Understory Douglas-fir (\textit{Pseudotsuga menziesii} (Mirb.) Franco) and western hemlock have been found with bole cankers. Many confirmed sites have no rhododendron present. Before these findings, tree infection in the U.K. had been identified only in relatively close proximity to infected rhododendron.

In France, \textit{Phytophthora lateralis} Tucker & Milbrath has been identified killing Port Orford cedar (\textit{Chamaecyparis lawsoniana} (A. Murray) Parl.) hedges in the Bretagne region. Previously, \textit{P. lateralis} had only been isolated (in 1996 and 1998) related to an infestation of potted trees in a commercial nursery, itself the result of an introduction from North America. Disease progression in France is similar to our western U.S. infestation, which was first detected associated with a nursery in Washington State in the 1920s; it then moved through landscape hedges along Oregon’s Willamette Valley, eventually becoming established in native forests near Coos Bay, OR, in the 1950s. The origin of \textit{P. lateralis} is not known, but Clive Brasier and colleagues reported the detection of \textit{P. lateralis} in an old growth \textit{Chamaecyparis} forest in Taiwan, suggesting that the pathogen may be native there.

In Alaska, \textit{Phytophthora alni} ssp. \textit{uniformis} Brasier (PAU) was detected for the first time in North America.
PAU is one parent of a highly damaging hybrid *P. alni* strain (*P. alni* ssp. *alni* Brasier (PAA)) that has killed thousands of riparian alder trees in Europe. The Alaska detection was in an area with unprecedented dieback and mortality of thinleaf alder (*Alnus incana* (L.) Moench ssp. *tenuifolia* (Nutt.) Breitung), but the *Phytophthora* has not been isolated from symptomatic plants, only from soils, and is not thought to be the cause of the Alaskan alder die-off. PAU was widely distributed, suggesting it may be native to Alaska.

*Phytophthora pinifolia* Durán, Gryzenh, and M.J. Wingf. was discovered associated with a new needle blight on *Pinus radiata* D. Don in Chile. The disease, Daño Foliar del Pino, defoliated plantation trees over about 60,000 ha in 2006. Research is underway to fully understand the impact of this new species and to develop management strategies. In 2008 and 2009, weather conditions were less conducive to disease development and the extent of damage decreased.

For more recent developments on *P. ramorum*, sudden oak death, and other forest *Phytophthora* species, go to the California Oak Mortality Task Force Web site at www.suddenoakdeath.org.
ABSTRACT

The Eurasian woodwasp, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), is an introduced invasive pest in North America. This siricid woodwasp is native to Europe, Asia, and Africa where it is considered to a secondary colonizer of conifer trees. However, it is a primary colonizer of conifer trees in its non-native zone in the Southern Hemisphere. *Sirex noctilio* is a polyphagous woodwasp with a wide host range, mainly in commercially important pine species and occasionally on larch, fir, and spruce. *Sirex noctilio* kills trees through a combination of female oviposition activity, introduction of phytotoxic mucus, and fungal spores for larval feeding, and it can cause up to 80 percent tree mortality. The extensive conifer forests of North America may be at high risk because native trees lack the necessary host resistance mechanisms to defend themselves, as has been found for the exotic emerald ash borer on North American ash trees.

In North America, *S. noctilio* has been found in the Great Lakes region, specifically in New York, Pennsylvania, Michigan, Ontario, and Vermont, but it may yet be found in other states through a greater detection effort. It is expected that if *S. noctilio* is not contained or eradicated, then populations of *S. noctilio* may become established in other parts of the U.S. The risk of introductions into the Southeast is relatively high due to the high volumes of international commodities moving through this region. Forests in the southeastern U.S. are dominated by southern pine species that are known to be highly susceptible to colonization by *S. noctilio*. Further, the presence of many plantations in the Southeast indicates that this region has a high potential for invasion and economic impacts by *S. noctilio*.

We have two projects related to the potential invasion of *S. noctilio* in the southeastern U.S. The first project is to develop spatially referenced risk models of *S. noctilio* that incorporate host preference information on 8 to 10 southern conifer tree species. The susceptibility of southern conifer tree species to colonization by *S. noctilio* will be assessed using feeding bioassays. The second project is to compare the species complex of native siricids and their hymenopteran parasitoids in the Appalachian, Piedmont, and Coastal Plain regions of the Southeast. We also intend to assess the host-specificity and parasitism rates of siricid wasps by hymenopteran parasitoids in various species of southern pines.
rich complex of potential competitors and established parasitoids in the southern pine stands may hinder the establishment and spread of the populations of *S. noctilio*, if introduced in this region.

We determined the oviposition and colonization preferences of *S. noctilio* on loblolly (*Pinus taeda* L.), Virginia (*Pinus virginiana* Mill.), and Scots (*Pinus sylvestris* L.) (control) pines in June 2009. For the host choice experiment, four logs each of the three pine species (loblolly, Virginia, and Scots) in two diameter classes (small and large) were placed in random locations within an arena. Males and females of *S. noctilio* were released within the arena. The activities of adult *S. noctilio* on logs (e.g., ovipositing, sitting, or walking) were observed. Logs were then individually enclosed in mesh sleeves for storage. For the host no-choice study, two large diameter class logs of each of the three pine species were individually enclosed in mesh sleeves. Male and female *S. noctilio* were introduced into each sleeve. Observations similar to those in the host choice experiments were taken. Once the wasps finished emerging in fall 2009, we dissected the logs to record data on host preference. Preliminary results indicate that in the host choice experiment, significantly more males and females of *S. noctilio* were observed on Virginia than on Scots or loblolly pines. Female *S. noctilio* were observed drilling with their ovipositor in the southern pine logs, especially on Virginia pine. In general, more adults of *S. noctilio* were found and emerged from larger than smaller diameter Virginia pines, indicating a host preference.

In 2010, we will conduct host choice and no-choice experiments with 8 to 10 southern pine species, assess antennal responses of female *S. noctilio* to bark volatiles, and determine differences in wood and resin quality of southern pines to explain the above patterns.

To sample native siricids and their hymenopteran parasitoids, we established sampling plots in three states: Georgia (Piedmont region), and Louisiana and Virginia (Coastal regions) in fall 2009. These sampling plots had experienced recent disturbance such as thinning and/or clearcutting activities that may attract more native siricids and their parasitoids.

The timing of sampling varied across the regions to allow us to trap during the maximum activity of siricid wasps during the year. To assess the species complex of native woodwasps and their parasitoids in each state, we established 30 intercept panel traps that are widely used for monitoring *S. noctilio*. Each intercept trap was (1) unbaited, (2) baited with commercially available *Sirex* lure (alpha- and beta-pinenes), or (3) baited with *Sirex* lure and high release ethanol. We are also assessing the viability of these lures in catching siricids across the southeastern U.S. In Georgia, we are further testing whether funnel or intercept traps were more efficient in catching native siricids. Another 30 funnel traps with the identical baits were placed in the same locations in Georgia. Hymenopteran parasitoids were sampled using 10 Sante traps (similar to Malaise traps) placed high in the canopy. Traps were emptied every 14 days.

To further compare the species complex of these two taxa in different pine species, we created trap logs in each state. In late August 2009, we cut down two trees each of loblolly and shortleaf (*Pinus echinata* Mill.) pines in Virginia. In late September 2009, we cut down three trees of slash pine (*Pinus elliottii* Engelm.) and two of longleaf pine (*Pinus palustris* Mill.) in Georgia. In Louisiana, we are also testing the effects of different methods of inducing tree decline and mortality and the effects of different times of the year on the colonization activity of woodwasps. In treatment 1, we girdled and applied herbicide (dicamba) to four loblolly pine trees between September 1 and October 1, 2009. In treatment 2, we cut down four loblolly pine trees during the week of November 2, 2009. In treatment 3, we cut down four loblolly pine trees during the week of November 16. A panel intercept trap was attached to each of the trees, and the traps are being emptied every 14 days. In fall 2010, we will cut these trees into logs (34 trees across states) and place them in emergence cages to rear out woodwasps and their parasitoid species.

Preliminary results from the trapping experiments indicate that we have caught a total of 70 woodwasps, with the greatest numbers in Virginia, followed by
Louisiana and Georgia. Five species of woodwasps have been indentified; all these species are known to be present in the Southeast. *Sirex nigricornis* Fabricius was the most abundant woodwasp species, followed by *Sirex edwardsii* Brullé, *Ériotremex formosanus* (Matsumura) (exotic), *Urocerus cressoni* Norton, and *Tremex columba* (Linnaeus). About equal numbers of woodwasps were caught in the traps baited with *Sirex* lure and those with *Sirex* lure with ethanol, indicating that either of these lures could be used to monitor native woodwasps in these areas. In 2010, we will resample these sites for wasp communities, add North Carolina and Florida to the sampling, rear out all parasitoids to assess specificity, and further explore the individual effects of monoterpenes on wasp catches and diversity.

Overall, results from the two projects will assist in evaluating which southern pine species may be better able to withstand invasion by *S. noctilio*. We intend to geospatially reference the host preference data with existing host density, type, and condition maps to provide recommendations for relative susceptibility of southern pines to invasion by *S. noctilio*. Further, our projects will provide important biological information about the existing communities of native siricid and parasitoid wasps in southern pine species for biocontrol management options in the future. Since the southeastern U.S. is currently ahead of the invasion curve of *S. noctilio* in North America, it provides us with a unique opportunity to assess the host preference and complex of potential native competitors and parasites of *S. noctilio* before this exotic species arrives in the southern pine stands.
HITCHIKERS ON INTERNATIONAL TRADE ROUTES:
ESTIMATING THE PROBABILITIES OF INTRODUCTION
AND ESTABLISHMENT OF ASIAN
GYPSY MOTH WITH GLS-2D

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ABSTRACT

As global trade rises so too does the probability of introduction of alien species to new locations. Estimating the probability of an alien species introduction \( p(I) \), and establishment following introduction \( p(E|I) \), is a necessary step in estimating risk (probability of an event times the consequences, in the currency of choice, of the event should it occur); and risk estimation is a valuable tool for reducing the risk of biological invasion with limited resources. The Gypsy Moth Life Stage (aka the Gray-Logan-Sheehan) model (GLS) is an accurate and geographically robust model of insect phenology that has been used to estimate \( p(E|I) \) of the gypsy moth in western Canada (Régnière and Nealis 2002), Utah (Logan et al. 2006), North America (Gray 2004), and New Zealand (Pitt et al. 2007).

Despite the importance of long-range trade routes in the introduction of gypsy moth, the spatio-temporal variability in weather and its effect on gypsy moth phenology has never been a consideration in estimating either \( p(I) \) or \( p(E|I) \). That is to say: the degree to which pre-departure phenological development at the source, plus phenological development en route, plus post-arrival development at the destination, influence \( p(I) \) and/or \( p(E|I) \) has not been considered in the invasion risk calculations. GLS-2d is a two-dimensional phenology model that intersects ship arrival/departure with simulated phenological development at the source and then simulates subsequent development on the route to the destination. GLS-2d estimates: (1) the probability of introduction from the proportion of the source population that would achieve the next developmental stage at the destination, and (2) the probability of establishment from the proportion of the introduced population that survives until a stable life cycle is reached at the destination.

GLS-2d was demonstrated using three common marine trade routes (to Auckland, New Zealand from Kobe, Japan and to Vancouver, Canada from Kobe and from Vladivostok, Russia). The effect of shipping schedule on the probabilities of introduction and establishment was examined by varying the departure date from January 1 to December 25 by weekly increments.

For the work described here, GLS-2d ran 25 independent simulations with randomly chosen years for each weekly departure date. For each simulation, GLS-2d estimated oviposition at the port of origin using its “stabilization” option wherein the model determines the location-specific oviposition pattern that is most likely (see Gray 2010 for a complete description). The simulated oviposition pattern of this \( F_0 \) generation was intersected temporally with the arrival/departure of a target ship by GLS-2d to estimate the infection level of the departing ship and its containers. Daily phenological development was simulated during transit using the weather records of the location in the GLS-2d database nearest the estimated location on the prescribed route and thereafter using the weather records from the destination. The proportion of the \( F_0 \) eggs oviposited at the origin that subsequently hatched at the destination was calculated for each of the 25 simulations, and \( p(I) \) for the departure date was calculated as the average of the 25 proportions.
When egg hatch was successful at the destination, the simulation continued for seven additional generations (hatching eggs to hatching eggs), and the geometric average of inter-generational survival \( \left( \prod_{i=1}^{7} \frac{y_{i}}{y_{i-1}} \right)^{\frac{1}{7}} \) was calculated for each simulation. The \( p(E|I) \) for the departure date was calculated as the average of the 25 geometric averages. Seven generations were used for the calculation of \( p(E|I) \) because a stabilized life-cycle pattern is usually achieved within seven generations (Gray 2004).

Successful introduction of Asian gypsy moth requires two things. First, containers and/or ships must be present at the port of origin during egg oviposition. The \( p(I) \) increases as the temporal intersection between oviposition period and presence of containers and ship increases—ships depart with a greater abundance of egg masses. Second, the combined weather conditions of the origin, route, and destination must satisfy life-cycle requirements—eggs must hatch at the destination. GLS-2d is able to estimate these factors. Introduction to Vancouver from Vladivostok is less likely than from Kobe in part because the more prolonged oviposition period in Vladivostok means fewer egg masses are laid per day there, which results in a generally lower infestation on containers and ships that spend a short time in port. The \( p(E|I) \) depends on transit route, departure date, and the climate at the destination. GLS-2d simulations indicate that the large latitudinal difference between Kobe and Auckland, and the reversal of the seasons that occurs en route, will partially disrupt phenological development and reduce inter-generational survival until stable seasonality is achieved.

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PUTATIVE SOURCE OF THE INVASIVE SIREX NOCTILIO FUNGAL SYMBIONT, AMYLOSTEREUM AREOLATUM, IN THE EASTERN UNITED STATES AND ITS ASSOCIATION WITH NATIVE SIRICID WOODWASPS

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ABSTRACT

The white rot basidiomycete fungus Amylostereum areolatum is carried by females of Sirex noctilio in mycangia at the base of their ovipositors and inserted into pine trees when eggs are deposited. S. noctilio and A. areolatum are native to Europe but have been introduced throughout many areas in the Southern Hemisphere where pines have been planted (pines are not native to the Southern Hemisphere). S. noctilio was first trapped in North America in 2004 in New York State, near Lake Ontario. This constitutes the first time that S. noctilio and A. areolatum have become established in an area where pines are native and where there are native siricids. In Europe, there are four species of Sirex, while in northeastern North America, there are three species of Sirex, based on a recent revision of this genus by Dr. Henri Goulet. Before S. noctilio arrived in North America, it was thought that A. areolatum did not occur here; thus, all North American Sirex species were thought to be associated with A. chailletii, which also occurs in Europe in association with some Sirex species.

We collected Sirex noctilio and native Sirex species from New York State, Pennsylvania, and Maine. We isolated Amylostereum from female mycangia for DNA extraction or extracted fungal DNA directly from female mycangia. We compared the sequences of the intergenic spacer regions (IGS) of the fungal DNA from our samples with IGS sequences from samples from both Europe and the Southern Hemisphere (Slippers et al. 2002). The IGS region demonstrates that there is variability among isolates of A. areolatum both in Europe and North America (Nielsen et al. 2009). However, only one IGS strain (AB) has been identified from the Southern Hemisphere. The AB strain was also found in Denmark but was not found in North America, which suggests that the S. noctilio and A. areolatum that have become established in northeastern North America probably did not originate from the Southern Hemisphere.

From our North American samples, we found that two strains of A. areolatum are present in northeastern North America (Nielsen et al. 2009). We cannot say whether this means that there were one or two introductions, but we know that two strains of A. areolatum can occur in the same log. Therefore, it is possible that although there are two strains, only one introduction resulted in the present infestation. One of the strains we identified in North America was previously found in Germany (D). We found that two individuals of the North American native Sirex edwardsii that emerged from the same piece of a pine tree as S. noctilio carried the same fungal strain as that S. noctilio. Before this, it was assumed that S. edwardsii carried A. chailletii. However, there are some questions in the older literature about just how specific siricid species are about the fungal species they will carry (A. areolatum versus A. chailletii). We also found that the native North American S. nitidus from Maine (where S. noctilio does not occur) carried a new IGS strain of A. areolatum (BE). This also refutes
previous assumptions that North American native *Sirex* species all carry *A. chailletii*. We assume the BE strain is native to North America.

Our studies of vegetative compatibility groups of these fungi mostly agreed with molecular work on the strains of *A. areolatum*. All different IGS strains of *A. areolatum* were incompatible except the strain that is used to mass produce the *S. noctilio*-parasitic nematode (*Deladenus siricidicola*) for biological control in Australia (BDF).

**Literature Cited**


HEMLOCK WOOLLY ADELGID BIOLOGICAL CONTROL: MOLECULAR METHODS TO DISTINGUISH LARICOBIIUS NIGRINUS, L. RUBIDUS, AND THEIR HYBRIDS

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ABSTRACT

Molecular diagnostics use DNA-based methods to assign unknown organisms to species. As such, they rely on a priori species designation by taxonomists and require validation with enough samples to capture the variation within species for accurately selecting diagnostic characters. Molecular diagnostics are increasingly recognized as powerful tools to facilitate biological control programs. They can provide reliable and inexpensive identification of natural enemies, track their population dynamics, and monitor impacts on target and non-target organisms. We report preliminary results using DNA-based methods to distinguish Laricobius nigrinus (Coleoptera: Derodontidae) from L. rubidus, which is native to eastern North America where it is commonly associated with pine bark adelgid, Pineus strobi (Hemiptera: Adelgidae). Laricobius rubidus also feeds on the introduced hemlock woolly adelgid, Adelges tsugae, but has not been shown to impact its populations. Laricobius nigrinus (Coleoptera: Derodontidae) from L. rubidus, which is native to eastern North America where it is commonly associated with pine bark adelgid, Pineus strobi (Hemiptera: Adelgidae). Laricobius rubidus also feeds on the introduced hemlock woolly adelgid, Adelges tsugae, but has not been shown to impact its populations. Laricobius nigrinus (Coleoptera: Derodontidae) from L. rubidus, which is native to eastern North America where it is commonly associated with pine bark adelgid, Pineus strobi (Hemiptera: Adelgidae). Laricobius rubidus also feeds on the introduced hemlock woolly adelgid, Adelges tsugae, but has not been shown to impact its populations. Laricobius nigrinus (Coleoptera: Derodontidae) from L. rubidus, which is native to eastern North America where it is commonly associated with pine bark adelgid, Pineus strobi (Hemiptera: Adelgidae). Laricobius rubidus also feeds on the introduced hemlock woolly adelgid, Adelges tsugae, but has not been shown to impact its populations. Laricobius nigrinus is native to western North America and has been released as a biological control agent of A. tsugae in multiple locations in eastern North America where L. rubidus occurs. The two species can be distinguished as adults by color and by differences in male genitalia. Laricobius larvae cannot be readily distinguished using morphology; rearing them to the adult stage for identification is labor intensive, and many beetles die in the process. In addition, L. nigrinus and L. rubidus have been observed mating in the field (Mausel et al. 2008) and were found to be closely related sister species (Montgomery et al. in prep.), which has drawn attention to the possibility of successful hybridization.

We used three molecular approaches to address these identification and hybridization issues. The first method we used to monitor post-release establishment and spread of L. nigrinus was polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP). This method uses restriction enzymes to cleave DNA at sites with specific short nucleotide sequences. Restriction enzymes were chosen that target four diagnostic nucleotide positions in the mitochondrial cytochrome oxidase I (COI) gene that reliably differ between the species. Following PCR, restriction enzymes are used to cut the DNA into different sized fragments. This results in different banding patterns for each species when resolved by gel electrophoresis (Fig. 1). It should be noted that because COI is a mitochondrial gene, this assay identifies only an individual’s maternal lineage and therefore cannot detect hybrids.

The second method we developed is a real-time PCR assay that amplifies a 117-base pair portion of COI and uses unique 20-nucleotide TaqMan® probes (Applied Biosystems). Separate probes were designed to bind specifically to L. nigrinus or L. rubidus and were tagged with different colored dyes. During amplification, the dye associated with the
beetle species’ DNA that is present is released and detected (Fig. 2). To our knowledge, the application of TaqMan® real-time PCR to distinguish cryptic species is novel to biological control. The per reaction cost of real-time PCR reagents is greater than PCR-RFLP, but the second method is less time consuming.

Finally, we developed microsatellite markers to address whether L. nigrinus and L. rubidus have successfully hybridized in the eastern United States. Microsatellites are nuclear, co-dominant population genetic markers that, in contrast to mitochondrial DNA, provide information about both parental lines. Ten polymorphic
dinucleotide microsatellite markers were isolated and characterized for both beetle species (Klein et al. in press). Six of these were used to genotype Laricobius larvae collected from sites with known L. nigrinus release dates. To date, we have genotyped larvae from sites in Tennessee and Pennsylvania (L. nigrinus released in 2004) and in North Carolina (L. nigrinus released in 2005). We found clear evidence of hybridization at all three sites, with backcrosses in both directions (example from Tennessee is shown in Figure 3). The consequences of hybridization between L. nigrinus and L. rubidus for adelgid biological control are not known. Hybrid offspring could be more fit than their parents (hybrid vigor), which could enhance biological control but result in homogenization of the two species; or, hybrids could be less fit (outbreeding depression or hybrid sterility), which could ultimately maintain species integrity but could impair control efforts. Introgression of genes between species could also change their host preferences. Future work will evaluate the impacts of hybridization on the efficacy of adelgid biological control.

Accurate species determination is vital to biological control efforts. PCR-based molecular diagnostics can provide rapid and inexpensive identification when morphological characters are not sufficient. The combination of PCR-RFLP, real-time PCR, and microsatellites has proven to be very useful for understanding the establishment and spread of L. nigrinus.

Figure 3—The chart shows the probability of assignment (q; y-axis) to L rubidus (dark gray) or L. nigrinus (light gray) for individual beetles (x-axis). Data are from six microsatellite loci and are analyzed using the software Structure (Pritchard et al. 2000). Reference samples of 248 L. nigrinus from Washington, Oregon, Idaho, and British Columbia are on the right, and 164 L. rubidus from non-release sites in Connecticut, Massachusetts, Kentucky, Pennsylvania, and Minnesota are on the left. Laricobius larvae collected over 3 years from a site in the Great Smoky Mountains National Park in Tennessee where L. nigrinus was released in 2004 are in the center. For the release site, an increase in the mean probability of assignment to L. nigrinus (light gray) over time indicates that the proportion of pure nigrinus and its hybrids is increasing (2007: q=0.01, 2008: q=0.29, 2009a: q=0.41).
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Establishment and spread of *Microstegium vimineum*, an invasive exotic grass, in closed-canopy U.S. eastern forests were evaluated across a local (roadside to forest interior) and regional (across two geographic provinces) environmental gradient. Seed dispersal distances from roadside populations into forest interiors based on seed rain and soil seed bank data were determined. Biotic, abiotic, and disturbance variables were measured in plots with and without *M. vimineum* and were compared using a generalized linear model and logistic regression. Colonization and extinction of *M. vimineum* patches were followed over 3 years, and spread rate was estimated using a reaction-diffusion model.

Direct seed dispersal from the roadside populations occurred primarily adjacent to the maternal plants, indicating that the disjunct *M. vimineum* patches within the forest interiors occurred via secondary seed dispersal over longer distances. The decreasing stem height and reduced reproductive capacity of the forest interior *M. vimineum* compared to roadside populations were confirmed over the local environmental gradient.

Patches of *M. vimineum* in the forest interiors across the regional gradient were best defined by high native plant richness and diversity. Greater canopy opening, more moss, and shallower litter depths were positively and significantly associated with *M. vimineum* presence but only during the driest year.

Colonization rates of the forest interiors were significantly higher for the more mesic sites than the more xeric sites. The same trend was noted for the spread rate. These results support the possibility of accelerating spread rates weighted by a reduction in *M. vimineum* fitness in shaded environments.
APPLICATION OF DNA BARCODING IN FOREST BIOSECURITY SURVEILLANCE PROGRAMS

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ABSTRACT

The ability to distinguish non-indigenous species from the background diversity of native taxa is critical to the success of surveillance programs for detecting new introductions. Surveillance programs for alien taxa rely on the precise diagnosis of species, which can be complicated by sizable trap samples, damaged specimens, immature life stages, and incomplete taxonomy. The recent advent of DNA barcoding shows promise to circumvent some of the obstacles of pest monitoring and surveillance. We review the requirements for integrating DNA barcoding into monitoring and detection programs, and we note its relevance to the International Standards for Phytosanitary Measures #27 (Diagnostic protocols for regulated pests). We highlight the utility of COI for detection with examples in tussock moths (\textit{Lymantria} spp.) and the poplar shoot borer (\textit{Gypsonoma aceriana}). Secondly, we present the results of a faunal survey of nocturnal Lepidoptera in North America’s largest urban parks. We reveal how DNA barcoding facilitated the inventory by minimizing specialist time and increasing the sensitivity of detecting species at low density, including four newly introduced species. And lastly, we provide an update of the progress in developing barcode libraries for bark and wood boring Coleoptera.
AN UPDATE ON THE STATUS AND MANAGEMENT OF
THE SIREX WOODWASP IN SOUTH AFRICA

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ABSTRACT

The woodwasp *Sirex noctilio* was first detected in South Africa in 1994. By 2009, it had spread to most of the pine-growing provinces in the country, and it continues to move northwards to the remaining pine areas and toward Zimbabwe. In the summer rainfall area of South Africa, *S. noctilio* has caused serious losses. The management approach for this pest has included monitoring, reducing stress on trees via silviculture, and introducing biological control agents.

The primary biological control agent introduced into South Africa has been the parasitic nematode *Deladenus (= Beddingia) siricidicola*. This nematode was reported to be successful in reducing the impact of *S. noctilio* in other Southern Hemisphere countries. However, parasitism results from the first 2 years of inoculations in the summer rainfall area of South Africa were very low (below 10 percent). Parasitism rates have increased in subsequent years, but this has been primarily due to the natural spread of the nematode. Parasitism obtained directly from inoculations (inoculation success) has remained low, implying a reduced ability to suppress new infestations of *S. noctilio*.

Progress has been made toward understanding the barriers to inoculation success in the summer rainfall areas of South Africa. Moisture content of the wood has been shown to be one of the factors limiting success, but other factors are also believed to be involved. These include a possible reduced survival of the nematode on the South African fungal symbiont of *S. noctilio*, *A. areolatum*, compared to the Australian strain of the nematode that is used to rear the nematodes. Another possible factor contributing to the poor biological control results that is being investigated is the competition of *A. areolatum* with sapstain fungi such as *Diplodia pinea* and *Ophiostoma ips*, which develop rapidly in trees more or less at the same time as *S. noctilio* is developing in the wood. In this regard, sapstain fungi could outcompete *A. areolatum* and thus reduce the food source and survival of the nematode.

Efforts are underway to collect new and potentially more effective strains of *D. siricidicola* in various parts of the world. These strains will be tested for their compatibility with the South African strain of *A. areolatum* and for their survival in wood of low moisture content. Molecular markers have been developed to distinguish between nematode strains. These markers have revealed that there is no diversity within the Kamona strain of the nematode introduced into the Southern Hemisphere.

The introduction of biological control agents, specifically *D. siricidicola*, into South Africa to control *S. noctilio* has clearly shown that local adaptations of control strategies can be required as a pest moves into new areas.
SIREX RESEARCH AND MANAGEMENT IN SOUTH AMERICA

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ABSTRACT

The European woodwasp, Sirex noctilio Fabricius, 1793 (Hymenoptera: Siricidae) is being monitoring and/or controlled on about 4.1 million hectares Pinus spp. plantations in South America’s Southern Cone. Most of these stands consist of a small number of species that were planted at high density and have not received adequate forest management. These areas serve to enhance the natural spread potential of the pest, mainly in the Southern Cone where 75 percent of the species of Pinus (P. taeda, P. radiata, and P. elliottii) are susceptible to attack by Sirex. The other 25 percent are tropical pine species, which are also susceptible to attack by S. noctilio. When tropical species such as P. kesiya, P. caribaea caribaea, P. caribaea hondurensis, P. caribaea bahamensis, P. oocarpa, and P. strobus chiapensis were artificially exposed to attack by S. noctilio in Brazil, the insect demonstrated that it was able to attack successfully and complete its development in these hosts (DURAFLORA 1993).

In South America the insect was first recorded in Pinus taeda and P. elliottii stands at the Department of Cerro Largo in Uruguay in 1980. In 1985, it was detected in P. taeda stands in the Province of Entre Rios, Argentina and eventually dispersed to Corrientes (1993), Jujuy (1994), Misiones (1995), and Cordoba (1995) Provinces. In 1991, it was first detected in the Argentinian Patagonian Andes threatening stands of little economic significance but of strategic ecological importance. In Brazil, it was introduced in Rio Grande do Sul (1988), Santa Catarina (1989), Paraná (1996), São Paulo and Minas Gerais States (2005), and currently infests around 450,000 ha, most of it consisting of P. taeda. In Chile, the insect was first recorded in 2001.

In Brazil, Sirex life cycle is usually about 1 year in duration, but there is a short period or summer cycle that is completed in 3 to 4 months. This occurred in about 2 to 3 percent of the Sirex population (Reis Filho et al. 1998). The pattern of adult emergence varies considerably under different climatic conditions. In Brazil, adults emerge and attack trees between mid spring (October) and early autumn (April). The peak of emergence occurs between November and December, and the short life cycle between March and April (Carvalho et al. 1993, Iede et al. 1998). Longevity rate of adults varies from 5 to 12 days for males and from 4 to 5 days for females in the summer (Iede et al. 1988, Carvalho et al. 1993). Females lay around 212 eggs (Carvalho et al. 1993). The woodwasp population is found between 30 and 80 percent of stem length, in the medium third and in the lower half of the upper third of the stem (Penteado et al. 2000).

Monitoring and early detection are among the main preventive measures that have been adopted by all countries including Paraguay, where the pest is not present. Monitoring is conducted by using trap trees that have been stressed with herbicides and increase their attractiveness to the pest.

In the countries where S. noctilio is present, control measures are similar but enforced with different intensity. These measures include monitoring for early detection and dispersal of the pest, using trap trees; adopting preventive strategies such as silvicultural practices, i.e., thinning of overstocked stands, to improve their phytosanitary condition; adopting quarantine strategies to control and slow down dispersal; and introducing nematodes and parasitoids to increase the range of natural enemies.
Applications of the nematode *Deladenus siricidicola* have been conducted in 1988 in Uruguay, in 1989 in Brazil, in 1995 in Argentina (Iede et al. 2000a), and in 2001 in Chile. The nematode was obtained from the Forest Research Institute, New Zealand, in October 1987 for the introduction in Uruguay. The release was conducted in a 3,000-ha *Sirex*-infested pine plantation in Paysandu Department (State), and the parasitism level reached 35 percent in the first year. Another evaluation was conducted in 1994/95, in Soriano and Tacuarembo Departments, where the nematode had not been previously released; the parasitism level reached 67 percent in the Soriano Department and 53 percent in Tacuarembo. *Ibalia leucospoides* parasitism was 20 percent at Soriano and 18 percent at Tacuarembo (COSAVE 2001).

In Brazil the first introduction of the nematode occurred in 1989-1990, using material obtained from CSIRO, Australia, but the strain that was introduced became defective. Two strategies were used to solve the problem: (1) buying and importing the Kamona strain re-isolated in Tasmania from CSIRO, Australia and released in 1995; (2). new strains re-isolated from the field in Brazil, where the nematode demonstrated good efficiency (mainly the Encruzilhada strain), probably due to adaptation to local conditions. New strains were re-isolated and released every year beginning in 1995. Every year the level of nematode parasitism is being evaluated and during 1999-2000, this occurred in seven monitoring localities. Parasitism varied from 17 to 65 percent, in four of the sites and parasitism levels were more than 92 percent in another three localities. In 2000-2001 in nine localities, the level of parasitism recorded varied from 65 to 80.5 percent in three locations and exceeded 91 percent at the other six sites. During this same period, at 68 company-owned farms, the average parasitism was 77 percent; 3,036 insects were evaluated in this study. In Argentina, the monitoring and control program started in 1993 when the insect was detected in San Carlos de Bariloche, Patagonian Region. Initially, attempts were made to eradicate the pest, with trap trees and burning of *Sirex* attacked trees, but this was not successful. After the pest was detected in pine plantations from the Provinces (States) of Entre Rios, Corrientes, Misiones, Cordoba, and Jujuy, the nematode *Encruzihada* strain was imported from Brazil and released in 1995-1996. The nematode mass rearing laboratory is located in INTA (Instituto Nacional de Tecnologia Agricola) Montecarlo, Province of Misiones (COSAVE 2001).

*Ibalia leucospoides* (Hym.: Iballidae), a parasitoid of the woodwasp eggs and young larvae, was accidentally introduced together with the pest and is present throughout the infested area. It has an average parasitism of 23 percent (ranging from 4 to 45 percent) and a high capacity of establishment. In South America, *I. leucospoides* was first reported in Uruguay in 1984, attacking on average 24 percent of the *S. noctilio* population (Rebuffo 1988); according to Klasmer et al. (1998), in 1993-1994 the parasitism level reached 20 percent in Argentina. In Brazil, the parasitoid was detected in 1990, attacking up to 29.05 percent of the pest population (Carvalho 1993). *Ibalia leucospoides* was not released at any of these locations. However, in some cases, the companies did mass rear the parasitoid in the laboratory and later released them in the field with the objectives of introducing the parasitoid into areas where it was not yet present or enhancing parasitism rates.

In Brazil, the program is being complemented by the introduction of *Megarhyssa nortoni* and *Rhyssa persuasoria* (Hym.: Ichneumonidae), both imported from Tasmania, Australia. The introductions were made in 1996, 1997, and 2003, through a cooperative project of Embrapa Forestry, CSIRO, the International Institute of Biological Control (CABI-Bioscience), and the USDA Forest Service. At the moment the establishment of these species in the release areas has not been confirmed.
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PREDICTING THE FEMALE FLIGHT CAPABILITY OF GYPSY MOTHS BY USING DNA MARKERS

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ABSTRACT

Gypsy moths (Lymantria dispar L.) from different geographic origins have different biological and behavioral traits that can affect the risk of establishment and spread in new areas. One behavioral trait of major concern is the capacity of females from some geographic origins to fly, thus increasing the potential rate of spread and making detection and delimitation more difficult. Gypsy moths from some areas where females are capable of flight also possess traits that make them more threatening to North American forests than the established Western European strain: the ability of larvae to use hosts that are only marginally acceptable to gypsy moths from other areas, shortened egg chill requirements, and reduced susceptibility to some available biopesticides, all of which could influence the effectiveness of eradication or control programs. The flight-capable females are attracted to lights and will lay their egg masses on nearby vehicles or cargo, giving them an easy pathway around the world. Multiple introductions into North America of gypsy moth strains with flight-capable females have occurred and prompted major eradication programs, the largest occurring in 1992 and 1994. For regulatory purposes, USDA APHIS refers to any biotype of Lymantria dispar possessing female flight capability as the Asian gypsy moth. Individuals from populations with and without flight-capable females cannot reliably be distinguished morphologically so molecular techniques were developed to determine the origin of males caught in pheromone traps. There had been no previous attempt to compare the world variation in all of these markers, and they had never been used on the same populations in which female flight capability had been specifically determined. Understanding the relationship between marker results for intercepted males and female flight capability of different world populations would help in assessing the risk that an introduction contains flight-capable females.

We documented the female flight capability and the traits that affect it (wing length and muscle strength) from 46 strains of gypsy moth from throughout its range. For 31 of the strains, we determined the mtDNA haplotype based on two polymorphic COI mtDNA restriction sites, the nuclear FS1 genotype and four microsatellite loci for males from the same generation as the flight-tested females. We discuss the relationship between the marker results and female flight capability as well as implications for management programs.
Females capable of strong directed flight were found in strains that originated from Asia, Siberia, and northeastern Europe, but flight capability was not fixed in most strains (Fig. 1). One strain from Siberia (RS) was the only one in which 100 percent of the females flew. Far Eastern strains exhibited >64 percent female flight while European strains exhibited lower percentages. No flight-capable females were found in strains from the United States or Europe south of the Carpathian Mountains and Alps, most of France, or further west in northern Europe. Wing size and musculature were shown to correlate with flight capability and could potentially be used in predicting female flight capability.

The mtDNA haplotypes broadly separated the gypsy moth strains into three groups: North American, European/Siberian, and Asian. All Far East strains (except for JS) had the N+B+ mtDNA haplotype, the strains from Siberia and Europe all had the N+B- haplotype, and the strains from the United States had the N-B- haplotype. The AA FS1 nuclear DNA genotype occurred in 100 percent of the males sampled from the Asian and Siberian strains, except for the CS and RB strains. Males from three of the four United States strains and the PP strain all had the NN genotype. Across Europe, both alleles are present and the percentage of the A allele tended to decrease from east to west. Up to 70 percent of the males from strains

![Figure 1.—World variation in female flight capability.](image)
with no flight-capable females had the AA genotype. Specific microsatellite or FS1 alleles were fixed only in a few strains, and there was a gradual increase in the frequency of alleles dominant in Asia at both the nuclear and microsatellite loci moving geographically from west to east. The U.S. strains had only two to three alleles per microsatellite locus, and in general these were the most common worldwide. There were only nine private (i.e., occurred in only one strain) alleles, seven of which were found only in single Far East Asian strains and none were found in U.S. strains. An additional seven alleles were found only in Asian strains (Fig. 2). The average number of total alleles was highest in the Asian strains (18.3), intermediate in the European/Siberian strains (14.8), and lowest in the U.S. strains (9.0). When all the genetic marker information was used, 94 percent of the individuals were accurately assigned to their broad geographic group of origin (North American, European, Siberian, and Asian), but female flight capability could not be predicted accurately. This suggests that gene flow or barriers to it are important in determining the current distribution of flight-capable females and demonstrates the need for added markers when trying to predict female flight capability in introduced populations, especially when a European origin is suspected. For a complete discussion of these data, see Keena et al. (2008).

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DEVELOPMENT OF MOLECULAR TOOLS FOR USE IN BEECH BARK DISEASE MANAGEMENT

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ABSTRACT

Beech bark disease (BBD) has been killing American beech trees in eastern North America since the late 1890s. The disease is initiated by feeding of the beech scale insect, Cryptococcus fagisuga, which leads to the development of small fissures in the bark. Over time, as the population of scale insects builds on the bark, the small wounds provide entryway for fungal infection by one of the species of Neonectria. As the fungus invades, it kills the inner bark tissue and may completely girdle the tree, leading to death. Cankers may form as the tree attempts to stop the infection from spreading, resulting in wood defects. Often trees are weakened to the point that they are susceptible to splitting or snap during windy conditions. Large numbers of severely deformed American beech persist in long-affected stands, and their propensity for root-sprouting can result in the dense beech “thickets” that prevent other species from establishing, while offering little economic or ecological value. As a result, BBD has the potential to severely degrade and alter the species composition of the forests it occupies.

BBD will continue to spread throughout the natural range of American beech in the United States. Current forest inventory data suggest that BBD has already invaded most of the area with relatively high densities of beech, but has yet to invade the bulk of the range of beech (Morin et al. 2007). This means there is still time to develop management strategies aimed at minimizing the impacts of the disease ahead of the disease front, in addition to developing strategies for restoration of aftermath forests.

Fortunately, some beech trees remain disease-free even in heavily affected areas. Testing has shown that they are resistant to the insect portion of the disease complex (Houston 1983), which we have confirmed in recent genetic experiments. In these studies, families of beech seedlings were tested for resistance to the scale insect by artificially applying insect eggs to their stems (Koch and Carey 2005). One family that was tested came from a resistant tree, ME(R), located in a stand in Sebois County, ME, that had been managed for BBD through the removal of all diseased American beech trees in 1991 (Farrar and Ostrofsky 2006). The only possible paternal parents (i.e., pollen donors) were the remaining resistant trees, so this family can be considered as having two
resistant parents. The families that had the highest proportion of resistant seedlings were those with two resistant parents, including the open-pollinated family from ME(R), providing evidence that management directed at the removal of diseased trees can lead to stand improvement. Comparisons between the different families in this study demonstrated that the degree of genetic influence (versus environmental influence) on resistance to the beech scale insect is sufficient to realize genetic gain (Koch et al. in press).

Genetic improvement of stands can be realized either through traditional tree improvement programs (seedling development and planting) or through silvicultural methods designed to manipulate stand genetic composition by favoring resistant trees (and their natural regeneration), or a combination of both. The deployment of any of these strategies could be expedited through the application of molecular technologies to identify marker(s) for resistance to BBD. We have initiated a framework for the development of such molecular tools through the establishment of a linkage map in American beech. Two full-sib families were used to construct four linkage maps and perform QTL analysis of beech scale resistance/susceptibility. Fifty AFLP primer pairs generated 550 markers heterozygous in both parents segregating in a 3:1 ratio and 285 markers heterozygous in one parent segregating in a 1:1 ratio for a total of 1,130 markers for each family on average. The linkage maps ranged from 12 to 16 linkage groups spanning 547 to 750 centimorgans (cM) with an average of 210 markers mapped for each map. Two loci were identified that were linked to beech scale resistance, and two markers closely associated with one of these loci predict the correct phenotype of individuals within the families 86 percent of the time. Our current goal is to increase the map density to identify markers that are more closely linked to the resistance phenotype so that they may be used for marker-aided selection (MAS). Such an approach would replace the current labor intensive method of screening for resistant seedlings, which relies on artificially infesting the seedlings with eggs from the beech scale insect.

An American beech genomics project has been initiated using ultra high-throughput DNA sequencing of the American beech transcriptome. The resulting sequences (more than 150 million bases) will be aligned and analyzed with bioinformatics software to identify SNPs (single nucleotide polymorphism), a type of DNA-based marker. The goal is to identify as many markers as possible, so that a large enough portion of the genome is covered to maximize the probability of identifying a marker that is very closely linked to disease resistance. Priority will be given to SNPs that are identified in sequences homologous to known defense response genes and in genes differentially expressed between resistant and susceptible American beech. The selected SNPs will be used to prepare a high-throughput genotyping assay for beech. The beech mapping population will be genotyped using this assay, and a minimum of 200 SNPs will be added to the beech map, greatly increasing the likelihood of identifying marker(s) that are sufficiently closely associated with the resistance phenotype to be useful for MAS.

The selected SNPs will also be used to genotype 250 resistant beech and 250 susceptible beech that have been collected from five stands in Maine, West Virginia, Massachusetts, Michigan, and Nova Scotia (Houston and Houston 1994, 2001). Genetic association tests will be performed to determine which of the many SNPs are most predictive for BBD resistance in natural populations. Such a marker will be a useful tool for carrying out silvicultural management strategies ahead of the disease front by allowing forest managers to identify and retain BBD-resistant American beech trees while removing the susceptible beech trees.
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ERADICATION OF AN EXOTIC AMBROSIA BEETLE, 
XYLOSANDRUS CRASSIUSCULUS 
(MOTSCHULSKY), IN OREGON

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ABSTRACT

The Oregon Department of Agriculture (ODA) has
been monitoring an industrial plant in The Dalles,
OR, for exotic wood boring insects since 1998. This
plant receives raw railroad ties from British Columbia
and several localities in the United States, including
Arkansas, Missouri, and Texas. On several occasions,
ties were imported from Mexico and Russia. Upon
receipt, the ties are left outside to dry in the local arid
climate, sometimes for more than a year. During this
period, the ties are often stored adjacent to potential
hosts for wood boring insects. When dry, the ties are
-treated with creosote and used in the construction of
rail lines.

ODA used Lindgren funnel traps baited with alpha-
-pine and ethanol, ethanol alone, and exotic ips
-lures to survey for target insects. Wood boring
-insects known from the southeastern U.S., but
-not from the West, were detected almost from the
outset of trapping. Through 2003, these included
Monochamus carolinensis (Olivier), Tetropium
castaneum (Linnaeus), Xylotrechus sagittatus (Germar)
(Cerambycidae), Gnathotrichus materiarius (Fitch),
and Xylosandrus crassiusculus (Motschulsky)
(Curculionidae: Scolytinae). Two species were exotic
to North America: T. castaneum, a European species
never before trapped in the U.S., and X. crassiusculus,
an Asian species exotic to North America but known
to have been established in the Southeast since 1974.
Other than X. crassiusculus, these species are known
only to attack coniferous hosts. This made sense, since
the bulk of the ties received at the plant were from
conifers. Trapping and other methods (e.g., bait logs)
subsequently determined that neither of the latter two
species had become established at The Dalles.

In 2004, there was a puzzling change. Additional
species known from the Southeast, but not the West,
were detected, including Euplatypus compositus
(Say), Monarthrum fasciatum (Say), M. mali (Fitch),
and Oxoplatypus quadridentatus (Olivier). Unlike
the previous detections, these species were, for the
most part, associated with broad-leaved hosts. Most
alarming were the high numbers of X. crassiusculus.
Tie plant staff verified that the source and nature of
ties received at the plant had changed to predominantly
hardwood ties, mainly oak and hickory, from the
Southeast. Seven additional Lindgren traps were placed
around the immediate outskirts of the tie plant. By
mid-November of 2004, when trapping was terminated,
156 X. crassiusculus individuals had been captured.
The pattern of catch suggested not only an established
population of X. crassiusculus, but also a very
localized infestation.

ODA was very concerned about the possibility of
this exotic pest becoming permanently established in
Oregon. This ambrosia beetle is becoming a significant
pest of nurseries, ornamental plantings, and orchards in
the Southeast. It is known to attack and kill apparently
healthy hosts and has been documented from more
than 200 species of woody plants, including cherries,
grapes, oaks, peaches, pines, poplars, and many
important nursery plants. It could pose an immediate
threat to Oregon agriculture because The Dalles is a
major production area for cherries and other fruits.
The Dalles is also only a few miles east of the heart
of Oregon’s orchard country. Because Oregon nursery
and greenhouse sales were almost $1 billion in 2007
(the most recent year for which this information was
available), any threat to this industry must also be
taken seriously. Consequently, if *X. crassiusculus* was established in The Dalles, ODA felt compelled to consider eradicating this pest.

Although the 2004 trapping suggested the *X. crassiusculus* infestation was sufficiently restricted to allow possible eradication, this was not certain and could only be ascertained through a delimitation survey. Such a survey was initiated in 2005, using 12-funnel Lindgren traps baited with four ultra-high release ethanol lures per trap. Based on the delimitation schemes ODA used for gypsy moth infestations, the traps were deployed at 49/mi² in a core area centered around positive 2004 trap sites, at 25/mi² for a half-mile buffer around the core area, and at 5/mi² beyond the buffer boundaries, for a total of more than 200 traps. All traps were placed by April, before any overwintering beetles were expected to fly. Although traps remained active until mid-November, by mid-July it was apparent that emergence and flight of *X. crassiusculus* had peaked in mid-June. The delimitation trap data to that point, along with the 2004 data, indicated the bulk of the infestation extended over an area of about 394 acres, and all of the catches could be encompassed within 860 acres. ODA concluded that eradication was possible.

Preparations for a potential eradication program in 2005 began late in 2004. There were many challenges to consider. As best we knew, no such program targeting an ambrosia beetle had been conducted anywhere, so there were no established protocols. In addition, no insecticide was known or registered for the eradication of ambrosia beetles. Because *X. crassiusculus* is facultatively parthenogenetic, 100 percent mortality was necessary for successful eradication. Sited in the western end of the Columbia Gorge, The Dalles is one of the wind-surfing capitals of the world, with almost constant high winds during the day. It is also the junction of a major interstate freeway and several state highways, with much human traffic and activity during the day. The proposed treatment blocks were bounded to the north by the Columbia River and included several streams and other bodies of water. The pesticide ODA selected could not be applied over water. The broad known host range of *X. crassiusculus* suggested that virtually any woody plant could be a host. Access to some hosts was difficult because of their size or location in the often rough and steep terrain. Finally, the volume of material to be treated at the tie plant was daunting – almost 1/2 million ties.

Eradication efforts took place in 2005 and 2006. The first eradication action was to request that the plant immediately cease further importation of all rail ties from the Southeast. Any subgrade ties and pieces of wood used to separate ties during shipping were to be burned. All ties on the plant premises were to be heat treated and creosoted as soon as possible, which was completed by October 13, 2005. Before that treatment, all ties on the premises were sprayed with a permethrin product, Perm-up 3.2EC. This pesticide was selected because it binds to cellulose and thus has long residual activity, up to 6 months. At least some potential host material was removed from the tie plant premises and chipped or burned, along with recently cut wood and limbs at a nearby park.

Three ground sprays of all shrubs and trees in treatment blocks, using Masterline Permithrin Plus-C, were conducted. Like Perm-Up, this formulation binds to cellulose and has long residual activity. Unlike Perm-up, it is registered for use on live plants. Because of the high winds and extensive human traffic in the area during the day, applications were primarily made at night. Permithrin cannot be applied over water, so host plant treatments had to be performed as ground applications. This required obtaining permission to spray from all private landowners. A day crew acquired permissions and a night crew conducted applications. Because some potential hosts were very tall or difficult to access, “bucket” trucks were often necessary. The first treatment was conducted in early July 2005 over the large block of 860 acres. The second treatment, targeting any *X. crassiusculus* that may have emerged from untreated ties, took place in the core block of 394 acres. A final application over the large block was
performed in April 2006, before any surviving and overwintering beetles would become active.

Post-treatment surveillance (with more than 157,000 wood boring insects trapped and examined) since 2005 has detected no further *X. crassiusculus* or other wood boring species of concern. The rail tie plant is now under a compliance agreement with ODA and can bring in ties from the Southeast only from December through February, when the risk of tie infestation and subsequent emergence of *X. crassiusculus* in Oregon is lowest. This is the first instance of the eradication of an exotic ambrosia beetle in North America.
Non-native forest insects and pathogens affect a variety of forest and urban settings across the U.S., and introductions are likely to continue as global trade and travel expand. Past efforts to assess economic impacts of invasive forest pests have been useful for bringing attention to the issue, but a broad, rigorous cost analysis is critically needed by policymakers. A sound estimate of the costs associated with non-native forest pests would provide a basis for decisions related to trade policies, regulations, and allocation of scarce resources to detect, eradicate, or manage invasive forest pests. The Nature Conservancy organized two working groups at the National Center for Ecological Analysis and Synthesis in Santa Barbara, CA, in 2007. Working Group 1, comprised of 13 scientists with expertise in forest entomology, pathology, ecology, or economics, was asked to address the economic impacts of invasive forest pests.

A list of the non-native forest insects and pathogens known to be established in the U.S. was developed, and a subset of those species that have caused detectable damage was identified. We recorded taxonomic information, year of detection, and major host species for each organism and assigned the insects to feeding guilds. For each “high impact” species, we also determined the spatial distribution of the organism. More than 450 non-native insect species that feed on forest trees are established in the U.S., and the accumulation rate was relatively steady from 1860 to 2006. Slightly less than 15 percent of the insect species, along with 16 pathogens, have caused reportable damage. Sap feeding insects such as scales, aphids, and adelgids dominated the complete list of non-native insects, while foliage feeders were most abundant in the list of damaging pests. Most notable was the dramatic increase in the number of non-native insects that bore into phloem or wood since the 1980s. A map of the spatial distribution of the pests on the high impact list clearly demonstrates that damaging, non-native forest pests are notably more abundant in the northeastern U.S. than in other regions of the country.

A cost curve was developed for each insect feeding guild by plotting the number of insect species against the economic cost associated with that guild. Two points were defined on the curve reflecting the costs associated with (1) a very damaging “poster pest” for each guild and (2) the lowest cost of a species assigned to the shorter list of damaging insects. Intensive analyses for each poster pest were conducted to derive estimates for each of five cost categories over a 10-year period. Cost categories included expenditures by Federal and local governments and households, decreases in property value, and losses associated with marketable products (e.g., timber). Using Bayesian averaging, the area under the curve developed for each feeding guild could be integrated for each distinct cost category. These methods enabled us to avoid double-counting costs or underinflating or overinflating economic impacts. Costs associated with each of the three poster pests and average costs associated with each feeding guild were determined for the five categories.

Extent of costs reflected the type of damage associated with each guild, the value of affected trees, human population density in the area affected by the pests, and the stage and rate of invasion. Results showed that the
economic impacts of non-native forest insects exceed previous estimates (based largely on timber losses) by orders of magnitude. Phloem or wood boring insects were the most costly guild, largely because several of these species kill their hosts, including landscape and high value urban trees. This result is particularly alarming given the recent upsurge in borer detection and establishment.

Costs associated with non-native forest insects were not uniformly allocated among the cost categories. Timber and related market losses made up a relatively small proportion of the costs associated with any of the insect feeding guilds. Overall, results showed that homeowners and municipal governments bear the greatest proportion of the costs of non-native forest insects.
EMERALD ASH BORER: CHEMICAL AND BIOLOGICAL CONTROL

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ABSTRACT

Emerald ash borer (EAB) (Agrilus planipennis), an invasive buprestid native to northeast Asia, has killed tens of millions of ash (Fraxinus) trees in infested areas of eastern North America. EAB apparently arrived in infested solid wood packaging materials from China in the early 1990s near Detroit, MI, but was not identified as the cause of local ash mortality until 2002. At that time, very little was known about its biology, and there was no information on how to manage it. Since then, much progress has been made in evaluating control tactics for EAB including the use of insecticides and biological control.

Insecticides. Many research groups are involved in conducting studies to evaluate various insecticide products and application techniques for controlling EAB including cover sprays, soil applications, bark-penetrating trunk sprays, and stem injection with systemic insecticides. Several recent studies were highlighted. Overall, the studies found that emamectin benzoate (TREE-äge™) provided excellent control of EAB for at least 2 to 3 years, and neonicotinoid insecticides (imidacloprid and dinotefuran) could protect most ash trees but must be applied annually. In one study, mortality of EAB adults fed on leaves from trees treated with emamectin benzoate (0.4 g ai/ inch d.b.h.) was 100 percent in the first year and more than 90 percent in the second year following a single treatment. Larval density in emamectin benzoate-treated trees was reduced by >99 percent in both the first and second year after treatment compared to larval density in untreated control trees (McCullough et al. in press). In another study, trees were treated with four different doses (0.1, 0.2, 0.4, or 0.8 g ai/inch d.b.h.) of emamectin benzoate in 2006, and canopy decline and exit-hole density were monitored for 4 years. The three lowest doses provided excellent control for 3 years (<10 percent canopy decline and <2 exit holes per m² compared to 53 percent canopy decline and ~20 exit holes per m² on untreated control trees). The highest dose provided excellent control for 4 years (5 percent canopy decline and 0.5 exit holes per m² compared to 96 percent canopy decline and ~25 exit holes per m² on untreated control trees) (Herms et al. 2010). For the neonicotinoid insecticides, imidacloprid (Merit 2FTM 1.4 g ai/inch d.b.h. or Xytect™ 1.4 or 2.8 g ai/inch d.b.h.) soil drenches applied annually in spring or fall from 2006 to 2009 provided good protection of large trees (~10 percent canopy decline in trees treated with the high dose of Xytect™, 20 to 40 percent decline in trees treated with the low dose of Merit 2FTM or Xytect™, and ~90 percent decline in untreated control trees) even under high EAB pressure, particularly when applied at the high rate (Herms et al. in press). Dinotefuran (Safari™ 1.7 g ai/inch d.b.h.) and imidacloprid (Macho™ 1.7 g ai/inch d.b.h.) applied with or without PentraBark™ as bark-penetrating trunk sprays and imidacloprid (Imicide™ 0.15 g ai/ inch d.b.h.) applied with Mauget capsules as a trunk injection, reduced EAB density by 30 to 60 percent.
compared to untreated controls after two consecutive years of treatment. There was no reduction in larval density in the second year after a single treatment (McCullough et al. in press).

**Biological Control.** Since 2002, much progress has been made toward developing a biological control program for EAB. Explorations in China yielded three parasitoid species: *Tetrastichus planipennisi* (gregarious larval endoparasitoid, Eulophidae), *Spathius agrili* (gregarious larval ectoparasitoid, Braconidae), and *Oobius agrili* (solitary egg parasitoid, Encyrtidae) (Liu et al. 2003, Yang et al. 2005, Zhang et al. 2005). By 2007, research findings on parasitoid biology (Liu et al. 2007), laboratory rearing, host specificity (Yang et al. 2008), and risk assessment were completed and submitted to USDA APHIS, along with requests for permits to release the three EAB parasitoids from China in Michigan. An environmental assessment was compiled and posted on the Federal Register by APHIS for public comment (Federal Register 2007). After review by researchers, land managers, and the public, APHIS issued a finding of no significant impact (Federal Register 2007) and granted release permits for the three parasitoid species in Michigan in late July 2007 (Bauer et al. 2008).

This allowed researchers to begin studying the three EAB biocontrol agents at field sites in Lower Michigan in 2007. The objectives were to assess parasitoid reproduction and overwintering. That summer and fall, *O. agrili* and *T. planipennisi* were each released at two different sites in Ingham County, and *S. agrili* was released at one site each in Gratiot, Oakland, and Saginaw Counties. During the winter and early spring of 2008, *O. agrili* was recovered from one Ingham County site and *S. agrili* was recovered from the Oakland County site, confirming field reproduction and overwintering in Michigan. In 2008, additional research sites were established in Michigan, Indiana, and Ohio for long-term monitoring of parasitoid establishment and efficacy. Also in 2008, a replicated multi-year cohort life table study in Ingham County was initiated to determine stage-specific mortality of EAB by each parasitoid species and other factors. In 2008 and 2009, *O. agrili* was recovered at low prevalence (<1 percent parasitism) from EAB eggs sampled at the three replicated release plots; none were detected in the non-release control plots. In 2009, *T. planipennisi* was recovered from ~10 percent of EAB larvae at the three life table release sites. At two of those sites, *T. planipennisi* was also recovered ~ 800 m from the release epicenters (Bauer et al. in press).

In 2009, new EAB biocontrol study sites were established in Michigan, Illinois, and Maryland. At these and previously established sites, parasitoid reproduction, phenology, overwintering, establishment, spread, and prevalence were monitored. This monitoring was done mainly by sampling and dissecting ash trees, although emergence traps stapled to tree trunks, and sentinel logs were also tested as detection tools. At selected sites, data were collected to evaluate the impact or efficacy of biocontrol by comparing changes in ash condition and EAB densities in parasitoid-release vs. non-release control plots (Bauer et al. in press).

Recent advances have also been made in rearing methods for the three EAB biological control agents. Laboratory conditions for diapause induction in *O. agrili* were determined. When in diapause, *O. agrili* can be stored in the refrigerator for more than 10 months. This allows for mass production and year-round stockpiling of *O. agrili* until needed for the relatively narrow release window during EAB’s egg-laying period. For *S. agrili*, parasitism and progeny production were enhanced by allowing group mating vs. single pair mating and by the presence of ash foliage (with or without EAB feeding) (Gould et al. in press). For *T. planipennisi*, parasitism of EAB larvae was enhanced in ash logs vs. ash sticks (Ulyshen et al. 2010, Duan et al. in press). The advances in rearing techniques, along with the completion in 2009 of the EAB Biological Control Production Facility in Brighton, MI, will provide EAB parasitoids for implementation of the USDA Emerald Ash Borer Biological Control Program (USDA 2010).
Development of this classical biological control for EAB was supported by research showing that parasitism by native natural enemies did not exceed 1 percent (Bauer et al. 2005). In 2007 and 2008 at two sites in Oakland County, however, an unknown Atanycolus sp. (Hymenoptera: Braconidae) was discovered parasitizing 9 to 71 percent of EAB larvae (Cappaert et al. 2009). Atanycolus are solitary larval ectoparasitoids reported from many Agrilus species. This Atanycolus species was recently described as A. cappaerti Marsh and Strazanac (Marsh et al. 2009), and its biology and parasitism of EAB and native Agrilus hosts were investigated in the field (Cappaert et al. 2009, Tluczek et al. in press). In the future, this species may prove useful as a fourth parasitoid species for EAB biocontrol.

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Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control. 42: 61-71.


STUDYING THE EFFECTS OF MANAGEMENT PRACTICES ON AILANTHUS POPULATIONS IN OHIO FORESTS: A RESEARCH UPDATE

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ABSTRACT

Although commonly perceived as an urban and roadside problem, Ailanthus altissima is a highly invasive non-native tree that is present in many forested landscapes in the eastern U.S. It has been a persistent feature of eastern U.S. landscapes since its introduction to North America as an ornamental shade tree in Philadelphia in 1784. Ailanthus possesses numerous characteristics often associated with highly invasive species. It is extremely fast growing, reaching heights of 25 to 30 m (80 to 100 ft). It is dioecious and is a prolific seeder, producing up to 350,000 seeds per tree in a single growing season (Pannell 2002). Seeds develop in the summer, mature in early fall, and typically persist until March. Wind-dispersed seeds can travel more than 100 m (Landenberger et al. 2007). Persistence of seeds in soil seed banks is underreported. It appears that seeds are short-lived, typically 1 to 2 years, but germination rates are very high (80 to 100 percent) in disturbed stands. In addition, Ailanthus is capable of aggressive clonal spread, often creating dense thickets that can outcompete native trees. While considered shade intolerant, clonal sprouts attached to a parent tree can persist in a shaded forest understory for up to 20 years (Kowarik 1995). Vigorous sprouts can develop 15 to 30 m (50 to 90 ft) from a parent tree (Illick and Brouse 1923). Although the long-term effects of Ailanthus on native tree regeneration are not known, it likely has a negative impact because of its highly competitive traits and production of the allelopathic compound ailanthone (Hiesey 1996).

Both natural (e.g., tornadoes, wind or ice storms, insect and disease outbreaks) and human made (e.g., prescribed fire, timber harvesting, skid trails, roads, rights-of-way) disturbances have the potential to facilitate Ailanthus establishment and spread within forested landscapes. These disturbances can promote Ailanthus populations through increases in forest-floor light levels, soil disturbance, and propagule movement by heavy equipment; as well as enhanced germination by reduced leaf litter and reduced plant competition. While studying the effects of prescribed fire and thinning on oak regeneration, we became aware of the potential increased risk of non-native invasive plant expansion. The use of prescribed fire as a viable management tool in Ohio’s public forests has increased rapidly in the last decade (Bowden 2009). However, very little is known about the direct and immediate effects of fire on Ailanthus. Some forest managers have reported observing increases in Ailanthus via seed germination immediately following fires. Saplings are easily topkilled by fire, but resprouting can be prolific (Lewis 2007). It is unknown if post-burn Ailanthus expansion occurs when propagule pressure is high.

Our research has expanded to include the impacts of prescribed fire and timber harvesting on Ailanthus populations within mixed oak forests. The primary
objectives are to (1) determine how the distribution and abundance of *Ailanthus* is related to recent prescribed fires, other management activities, and landscape features; and (2) document the direct effects of prescribed fire on the demography of *Ailanthus* populations and explore the use of pre-burn herbicide application to mitigate the risk of *Ailanthus* expansion after fire. The study area is within the Tar Hollow State Forest (16,354 acres), located within the Southern Unglaciated Allegheny Plateau of southeastern Ohio. The topography is highly dissected, consisting of sharp ridges, steep slopes, and narrow valleys. The forest has active timber management and prescribed fire programs. Fourteen prescribed burns, covering more than 2,600 acres, were carried out between 2001 and 2008.

Geo-referenced digital aerial sketch mapping technology in a low-flying helicopter was used to identify seed-producing female trees and patches of *Ailanthus* in winter 2008, when persistent seeds were highly visible. During a 2-hour flight, 98 seed-bearing females and 42 patches, ranging in size from 0.18 to 13.4 ha, were identified within a 3,885-ha (9,600-acre) area. Aerially identified females were ground-truthed (95.7 percent accuracy) using hand-held GPS units. We conclude that the method is an effective and efficient way to survey for seed-producing *Ailanthus* across a forested landscape. In summer 2009, sampling of individual female trees as well as a systematic grid (N=267) was initiated to model *Ailanthus* abundance, demography, and spread in relation to landscape and stand attributes and management practices such as harvesting and prescribed fires. Research plots were also installed to study the direct effects of prescribed fire and herbicide treatments on *Ailanthus* demography and spread. Herbicide stem injections (hack-n-squirt with imazapyr) of these geo-referenced trees were completed in autumn 2009, and prescribed burns will be completed in 2010. Treatment effectiveness and subsequent woody plant regeneration will be monitored over time.

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ASIAN LONGHORNED BEETLE, OVER THE RIVER AND THROUGH THE WOODS:
HABITAT-DEPENDENT POPULATION SPREAD

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ABSTRACT

The Asian longhorned beetle (ALB), Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), is an introduced pest of hardwood trees in North America. This paper addresses population spread in open landscapes and wooded areas, with emphasis on recent findings from Staten Island, NY, and Worcester, MA. ALB was first discovered in New York City and on Long Island, NY, in 1996; in Chicago, IL, in 1998; in Jersey City, NJ, in 2002; in Toronto, ON, in 2003, and in Carteret, NJ, in 2004. The landscape in all of these locations was primarily urban/suburban, in which ALB was typically found inhabiting such land use categories as urban centers, residential neighborhoods, commercial and industrial properties, institutional and municipal grounds, parks, cemeteries, golf courses, and greenways along streets and highways. Tree density in these habitats varies from moderate to high, with a high percentage of host species and high host diversity (a variety of maples, elms, birches, willows, horsechestnuts, poplars, London plane trees, and other species). In these settings infested trees are generally quite accessible for survey, treatment, and removal operations, and damage caused by ALB is readily apparent from the ground to experienced surveyors after the first year or two of infestation.

Research has shown that in such habitats populations of ALB tend to spread slowly, developing in the initial years on just a few trees near the point of origin. For example, in residential areas in Chicago, the majority of trees with ALB egg sites were found very near trees bearing exit holes, showing that when hosts were readily available nearby, females did not travel far after emerging as adults before laying eggs. The cumulative distribution function for ~ 1,000 trees in Chicago was P = 1 - 0.734*EXP(-0.014*D), where P is the cumulative proportion of trees and D is the distance (m) from a tree with one or more egg sites to the nearest tree bearing one or more exit holes (Fig. 1). Key points on the distribution curve were 90 percent ~ 140 m, 95 percent ~ 200 m, and 99 percent ~ 300 m. Likewise, in a detailed study of population development and spread in Carteret, NJ, an ALB infestation remained confined to just a few trees within 260 m of the point of origin for 5 years, although hundreds of adults had emerged from some trees by that time.

In 2006, for the first time, an ALB population was discovered inhabiting what was primarily an open landscape. The infestation in Linden, NJ, east of the turnpike (I-95), was centered in an industrial wasteland that had sparse stands of red maple, gray birch, and poplar (quaking aspen and eastern cottonwood). The site was surrounded by freshwater and tidal marshes, open fields, vacant land, industrial plants, chemical tank farms, parking lots, highways, railroad tracks, and open water. The infestation originated in ca. 2000 from an unknown source. Although the population was genetically indistinguishable from
beetles from the Carteret infestation, temporal and spatial discontinuities suggest an independent origin. Infested trees found up to 1.5 miles to the west in 2005 and 2006 could be tied to Linden as a source, but not to Carteret. In 2007 and 2008, additional sites were discovered on Prall’s Island and Staten Island, up to 2 miles to the east. Again, these could be tied circumstantially to Linden as the likely source. The open landscape was characterized by a low overall density of trees, moderate to high percentage of host species, and low host diversity. Damage was readily apparent because of the generally small tree size and open habitat, but accessibility to the sites for survey, treatment, and host removal was variable and often poor. DNA evidence from beetles collected in Linden and on Prall’s Island and Staten Island, along with temporal, spatial, and numerical population analyses, strongly suggests that these groups were related and all derived from the Linden focal point. That initial introduction had spread approximately 2 miles to both the east and the west in 5 years, in marked contrast to the much slower spread of the Carteret population. The key difference appears to have been the open nature of the landscape in Linden, which raised few impediments to population spread and offered only sparse resources to arrest dispersing beetles. We now know that ALB populations can spread rapidly across open landscapes and beetles are able to locate distant hosts, although behavioral mechanisms and the probability of doing so are unclear. In light of the knowledge gained by studying population spread in this new landscape, the ALB Eradication Program expanded the customary boundaries for host removal, chemical treatment, and survey on Staten Island. The small pocket of infested trees found on Staten Island in December 2008 was in a location that analysis had suggested might be reached by beetles dispersing from Linden or Prall’s Island, in a broad greenbelt growing on the far side of an open expanse of marshes and industrial wasteland. In fact, the locations in Linden and on Prall’s and

Figure 1.—Distance from trees with oviposition sites to nearest tree with ≥ 1 exit hole. Based on approximately 1,000 trees in Chicago, IL, 1998-2003. 

\[ P = 1 - 0.734e^{-0.014D} \]
Staten Islands were all discovered by surveying areas specifically suggested by an improved understanding of landscape-dependent spatial dynamics.

Until 2008, only small wooded areas in New York, Illinois, New Jersey, and Toronto had been found to be infested by ALB. However, in August of that year, a well-established infestation was discovered in Worcester, MA. Because of the close proximity of affected areas to the extensive northeastern deciduous forests, alarm was raised about the potential impact of the ALB on this valuable resource and the greater challenges this presented to the ongoing eradication effort. Due to our lack of prior experience, little or nothing was known about how populations of ALB will behave in forested areas. Surveyors soon found hundreds of infested red and sugar maple trees in the densely wooded, 50-ha (120-acre) Bovenzi Park on the city’s northwest side. The earliest damage, dating to around 1999, was located about 100 m in from the southeast corner of the woodlot, in the direction of the apparent epicenter of Worcester’s ALB infestation. In general, the heaviest and oldest damage was concentrated along the eastern edge of the woods, strongly suggesting an edge effect during invasion. Still, by 2008 the population had permeated the wooded tract. One tree with exit holes was found more than 200 m into the woods, about halfway to the center. In the interior, infested trees (most bearing egg sites only) were concentrated in, although not limited to, riparian areas; this may simply reflect the distribution of the principal host species, red maple. Given the early date of invasion, it is surprising that damage levels on individual trees were not higher (45 exit holes on the most heavily infested tree). It appears that either the rate of increase of the population was lower in the woods than we’ve seen elsewhere, or insects were distributed over a greater number of trees because of their close proximity and the ease of moving from one to another.

The nature of the northeastern deciduous forest varies by latitude, elevation, soil class, and moisture regime, but in general is characterized by a high density of trees, a high percentage of ALB hosts, moderate host diversity including primarily red and sugar maples; paper, gray, river, yellow, and black birches; and poplars (eastern cottonwood and quaking and big-tooth aspen). ALB damage will not be readily apparent in this habitat because of the density of trees, tree size, and poorer lighting than in more open landscapes, so early detection is problematic. Accessibility for survey, treatment, and host removal is poor, and these operations will be difficult and costly. Modified sampling-based survey techniques (rather than complete inspection of all hosts) may be necessary. Chemical treatment plans for high host density situations are still under development. Many control options will be controversial, and eradication may be unfeasible should ALB become established in extensive wooded areas.

Through the study of ALB populations inhabiting a variety of landscapes, our understanding of the beetle’s infestation dynamics has improved greatly in recent years. As a result, strategies for combating it have evolved as well. In both open landscapes and forested areas, detection is less likely because of the large areas involved and accessibility and apparency issues. Survey and control operations are more costly and problematical. We now realize that flexibility in operations and case-specific approaches to eradication must be employed. Making the case to the public for such flexibility is a new challenge faced by program managers.
The walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae) (*sensu* Wood 2007), is a native North American bark beetle that has been recently implicated as the vector of thousand cankers disease of walnut trees in the western U.S. (Tisserat et al. 2009, Utley et al. 2009, Seybold et al. 2010). The disease, caused by a pathogenic fungus in the poorly studied genus *Geosmithia*, is widespread on *Juglans* in California and evident in urban plantings, trees along rural highways and agricultural lands, and collections of trees in parks and germplasm reserves (Graves et al. 2009, 2010). Disease symptoms and tree mortality in California have also been observed on native walnut trees in the Los Padres National Forest (*J. californica*) and in riparian areas of the lower Sacramento River Valley (*J. hindsii*). The fungus invades the phloem of walnut branches and stems after the beetle has introduced it through its subcortical feeding and reproductive behavior. Large numbers of cankers coalesce and girdle the branches and stem, likely hindering the movement of carbohydrate in the tree. The disease has been observed principally on black walnuts in the section *Rhysocaryon*, but has also been found in several instances on English walnut (*J. regia*, section *Juglans*), as well as on the Paradox rootstock (*J. hindsii* × *regia*) used in commercial culture of *J. regia* in the Sacramento, San Joaquin, and peripheral valleys of the state. Branches and stem sections from dying walnut trees in California have been placed in rearing cages (Browne 1972) in our laboratory, and other subcortical insects such as herbivorous beetles in the families Bostrichidae, Cerambycidae, and Scolytidae [e.g., the ambrosia beetle, *Xyleborinus saxeseni* (Ratzeburg)], and predaceous beetles in the families Laemophloeidae, Monotomidae, and Trogossitidae have emerged synchronously with *P. juglandis*. Parasitic Hymenoptera in the families Bethylidae and Pteromalidae have also been observed to accompany emerging *P. juglandis*. Some of the associated species of Coleoptera have been captured on yellow sticky card traps in conjunction with the flight of *P. juglandis*, further underscoring their association with the twig beetle.

The origins of the fungal pathogen are not known, but *P. juglandis* is likely native to Arizona, California, New Mexico, and Mexico. In the last 10 to 20 years, apparently aided by the pathogen, *P. juglandis* has expanded its distribution into Colorado, Idaho, Oregon, Utah, and Washington. The hypothesis that *P. juglandis* is native to California is supported by:

(1) An analysis of the North American *P. juglandis* collection history (Bright 1981, Wood and Bright 1992, Seybold et al. in prep), which revealed only eight records from Arizona and New Mexico before the first collection in California in 1959.
(2) The broad current distribution of *P. juglandis* in California

(3) The presence of *P. juglandis* at a remote California location (Lassen County) in 1974 before the relatively recent and noticeable increase in trees with symptoms of thousand cankers disease

(4) A relatively rich subcortical insect community, including natural enemies, associated with *P. juglandis*

(5) The presence of two native endemic species of *Juglans* in California

*Pityophthorus juglandis* appears to be restricted in host range to the genus *Juglans*, but is relatively polyphagous, colonizing and completing development in more than 10 species of *Juglans* or their hybrids in California. The beetle prefers to colonize branches that are greater than 1.5 cm in diameter and will even colonize the main stem, making the common name “twig” beetle something of a misnomer.

In research on the pheromone biology of *P. juglandis*, we have found that the aggregation pheromone is comprised of components contributed by both sexes and that the sex-specific components act in synergy to attract both sexes of the beetle in flight. In California, flight occurs primarily in the early evening, i.e., the species is crepuscular, and more females than males are attracted to the pheromone baits. Research in our lab, and in those of cooperators, is progressing on the production and analysis (GC-EAD and GC-MS) of extracts of volatiles associated with male and female *P. juglandis* feeding in small cut branches of *J. hindsii*. The goal of this effort is to develop a pheromone-baited survey trap to facilitate the early detection of founder populations of *P. juglandis* should they be introduced east of the Great Plains where eastern black walnut, *J. nigra*, is a highly prized timber species.

In summary, preliminary surveys of California for thousand cankers disease suggest that both the beetle and the pathogen have been in California for a long time. The beetle is likely native and co-evolved with *J. californica* and *J. hindsii*; the beetle-pathogen complex has invaded several remote locations. The numerous cankers, even sometimes in association with extremely short attempted feeding galleries, imply that *P. juglandis* appears to be a highly efficient vector of the pathogen. Finally, the aggregation pheromone of *P. juglandis* is a synergistic combination of volatile chemical components from both sexes. When fully developed and commercialized, the pheromone may be a useful survey tool for the eastern U.S. and for California walnut orchards

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ADAPTING NEMATODE-BASED BIOLOGICAL CONTROL SYSTEMS TO NORTH AMERICAN POPULATIONS OF SIREX NOCTILIO

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ABSTRACT

The European woodwasp, Sirex noctilio, was found in Oswego County, NY, in 2004. During the past 6 years, surveys have revealed that it is distributed over a wide area of eastern North America. Because S. noctilio kills relatively healthy trees, it poses a serious threat to pine forests and plantations in the United States and Canada if it is not controlled. The parasitic nematode, Beddingia (Deladenus) siricidicola, is its most effective natural enemy. This nematode has been used as an agent in Sirex biological control programs throughout the Southern Hemisphere. Its use as a management tool is facilitated by its unique life history. The nematode can develop into either of two forms dependent upon physical conditions in a tree. The mycetophagous form feeds on the Sirex symbiotic fungus, Amylostereum areolatum, as it builds populations inside a tree. The parasitic form attacks S. noctilio larvae and ultimately sterilizes emerging woodwasp females. The following provides an update on activities in the past year to use the nematode in the USDA biological control program for S. noctilio.

The developing biological control program in USDA uses a highly infective strain of B. siricidicola from Australia called the Kamona strain. However, a North American nematode was discovered attacking S. noctilio in 2006 in New York and Ontario. The species was examined morphologically and identified as a strain of B. siricidicola. Molecular analysis has confirmed that it is B. siricidicola. The nematode is presumed to have entered North America with an invasion of S. noctilio, and it is well established in central New York State. The presence of a pre-existing strain of B. siricidicola complicates the development of a nematode biological control program because the North American and Kamona strains are distinguishable only by molecular techniques. Thus, expensive and time-consuming analysis will be needed to discriminate between the two strains and evaluate the effectiveness of the Kamona strain in field studies. Another potential problem is that the two strains are likely to hybridize in the field. Hybrids will not be recognizable by the molecular techniques, and hybridization may dilute the high infectivity rate of the Kamona strain.

A useful characteristic of the Kamona strain in Australia is that juvenile nematodes enter the eggs of the Sirex female and sterilize them. However, other nematode strains do not sterilize eggs. Juveniles remain in the body cavity or along the egg sheaths but do not enter the egg. This phenomenon of “nonsterilization” has been observed in the North American strain in both the United States and Canada. It is not a desirable trait for a biological control program because the Sirex egg is not killed and the transmission of a few nematodes stuck to the outer surface of an egg does not efficiently disperse juveniles into new trees. It is currently not known whether the Kamona strain will also be nonsterilizing with the Sirex strain in North America.

Because of lingering concerns about possible effects of the Kamona strain on non-target siricid species, nematode releases over the past four seasons have been “controlled”: infested pine trees are inoculated with nematodes in the fall, sample billets are removed for rearing in late winter, and the remains of the trees are chipped before insect emergence in the spring. The general objectives of the four releases have been to test the inoculation method developed in Australia, assess the establishment of nematodes in American pine species, and evaluate overwintering survival of the Australian nematodes under winter conditions in North
America. In addition, the 2008 release was designed to investigate the nonsterilization problem, whereas the 2009 release was set up to compare three system components, including the infectivity of the Kamona and North American nematode strains in Scots pine, the Kamona strain infection rates in red and white pines, and the infection rates of the Kamona strain when grown on different fungus strains.

The 20 species and subspecies of Siricinae in North America have the greatest potential for impact by the biological control program and make up three genera: *Sirex*, *Urocerus*, and *Xeris*. All species attack conifers, and all *Sirex* species, as well as four *Urocerus* species, attack pines. The primary factor affecting exposure of a siricid species to *B. siricidicola* is its fungal symbiont. The two most common symbionts of siricids worldwide are *A. areolatum*, which apparently originated in Eurasia, and *A. chailletii*, which apparently originated in North America. *B. siricidicola* feeds only on *A. areolatum*. Thus, siricid species that feed on *A. chailletii*, including most of those in North America, effectively have a refuge from *B. siricidicola*.

Although the fungal associate of a siricid species appears to be the key to its susceptibility to *B. siricidicola*, the faithfulness of the association between a woodwasp and a fungus is still a matter of debate. Recent work by Nielsen et al. (also reported by Hajek in this volume) provides some information. Contrary to expectations, they found that the North American species, *S. edwardsii*, emerged from the same tree as *S. noctilio*, carrying the same strain of *A. areolatum* as that species. Moreover, they reported that another North American species, *S. nigricornis*, emerged from the same part of the same tree but carrying *A. chailletii*. They also reported that the native species *S. nitidus* emerged from a spruce tree in Maine carrying *A. areolatum* and noted that this find was outside the currently known range of *S. noctilio*. The implications of this work are that the associations of North American siricids and their fungal symbionts are less clearcut than they appeared previously. Only a few other North American siricid species potentially may feed on wood colonized by *A. areolatum*. In particular, *Xeris* species are not associated with a fungus symbiont, but instead, oviposit in trees already attacked by another siricid species and inoculated with its fungus. In summary, according to the best available information, two native *Sirex* species and three *Xeris* subspecies that are associated with *A. areolatum* potentially may be exposed to *B. siricidicola* when it is released as a biological control agent.
CHARACTERIZING PATHWAYS OF INVASION USING 
STERNORRHYNCHA ON IMPORTED PLANT 
MATERIAL IN CARGO

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ABSTRACT

Non-indigenous Homoptera, mainly scales, aphids, and mealy bugs, intercepted on plants destined for cultivation represent an elevated risk for the establishment of invasive insects in North America. These insects [grouped as the suborder Sternorrhyncha] are often parthenogenetic and are imported on viable host plants. This may allow these species to effectively bypass major biological obstacles to establishment in North America. Here I examine five factors often attributed to elevated risk within pathways of introduction for these organisms: (1) number of points of interception, (2) number of countries of origin, (3) number of host plants, (4) total number of reported hosts plants worldwide, and finally (5) trade volume as expressed as value of imported plants destined for cultivation.

My analysis is based on interceptions recorded in the PestID database maintained by USDA APHIS between the years 1984 and 2000. These data included 4,681 recorded interceptions of Sternorrhyncha insects associated with imported plant products in cargo. These products included those related to nursery stock and ornamental plants but excluded fruits specifically (as hand baggage remains a dominant pathway of introduction for insects associated with fruit). I compared factors related to elevated risk within pathways using log-log linear regressions.

Armored and soft-shell scale insects as well as mealy bugs were the most abundant insects intercepted on plants destined for cultivation. While abundant, soft-shelled scale insects were less numerous in terms of species richness in comparison to either armored scales or mealy bugs. Overall patterns of interceptions varied extensively over the 16 years examined. For example, I observed a major peak in interceptions mainly attributed to three species (Aleuroplatus cococolus, Asterolecanium inlabefatum, and Dinaspis aculeate) primarily from Mexico and Guatemala into El Paso, Laredo, Miami, and to a lesser extent Chicago. During this peak, accumulation of overall number of species was three times higher than compared to the rest of the 16-year period. Nearly all of these interceptions were recorded on Chamaedorea palms. In contrast to this pulse of interceptions, I also observed highly regular, annual patterns of interceptions for species such as Parlatoria blanchardii on Phoenix, which showed increased interceptions every September for the 16 years examined.

When comparing factors related to pathways of introduction, I observed numerous strong relations between (a) number of ports and number of origins ($r^2=0.93, P<0.001$), (b) number of origins and number of host plants ($r^2=0.88, P<0.001$) and (c) number of hosts and number of reported hosts worldwide ($r^2=0.91, P<0.001$). However, none of these variables were strongly linked to increased numbers of interceptions nor was there a steadily increasing trend in the value of plant commodities imported over this period. The first trend seems logically related to shipping practices: commodities originating from numerous origins should arrive at numerous locations...
thus minimizing shipping distances. The second and third trends suggest that more cosmopolitan species are likewise associated with a greater number of imported plant species and a larger range of plant hosts in general. I suggest that insects that are intercepted frequently but that depart from the log-log models may be those species that are easily targeted for increased inspections or actions on the part of APHIS. For example, species such as *A. inflabefactum*, a pitscale that arrives at numerous port locations, originates from only two countries. Foreign inspection stations in these countries may thus limit potential introductions. Similarly, species such as *A. hardii* (yam scale) arrive from numerous origins but on a single host plant. Thus, inspection efforts for this species could be increased based on host plant preferences. These species serve as examples that could be expanded to other insect and host plant species. One plant genera, *Tillandsia*, for example, merits further attention because it has both frequent interceptions and harbors a diverse community of Homoptera.
ASH PHLOEM REDUCTION MODELS VARY
AMONG SPECIES AND GROWING CONDITIONS

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ABSTRACT

The exotic insect, emerald ash borer (Agrilus planipennis), is responsible for the death of millions of ash trees. Removal of ash from areas in close proximity to outlier populations will reduce the potential population density of emerald ash borer (EAB). EAB larvae develop in the phloem of ash trees in stems and branches above approximately 2.5 cm (1 in.) in diameter. We can estimate the amount of phloem available to the insect in a stand containing ash and develop models of the amount of ash tree removal necessary to reduce the breeding substrate by a target percentage. Other research studies on bark and wood boring beetles indicate they may be selecting trees based on growth rate, vigor, or phloem thickness. Relationships between surface area of ash and diameter have been reported and used in estimating population potential of EAB. We have characterized significant quadratic relationships between diameter at breast height (d.b.h.) and surface area, phloem volume, and phloem basal area for white, green, and black ash in both open grown and forested settings across Michigan and northern Wisconsin.

The surface area and phloem thickness of more than 500 ash trees were measured using standing trees as well as cut trees throughout the Lower and Upper Peninsulas of Michigan and northern Wisconsin. White, green, and black ash trees in open grown and forested settings were all represented. There are strong quadratic relationships between diameter at breast height and calculated surface area of the tree, but these quadratic relationships differ significantly between open grown and forest grown trees when the different ash species are considered. Multiple models have been developed for use in management prescriptions to reduce the amount of ash available to emerald ash borer. These models are based on ash species and crown light exposure. Information on ash species and the light exposure for most of the trees in a stand (i.e., forested or open grown trees) may allow managers to use a more specific model to fit their stands.

These models may be used with stand and stock tables to determine diameter limits for cutting to meet prescribed ash phloem reduction targets. By reducing emerald ash borer populations through phloem reduction, and decreasing the removal of the smaller trees in a stand, this model will enable the genetic diversity of ash to be optimized during ash reduction efforts. Similar models are available for use when the management goal is to retain large trees within a stand. Forest resource managers are able to access the models online at www.ashmodel.org and determine the diameter limit for removal of ash to achieve the phloem reduction target within the context of other forest management goals.
A NEEDLE IN A HAYSTACK: THE EFFECT OF ALIEN PLANTS ON HOST FINDING

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ABSTRACT

This study was designed to test several possible hypotheses concerning what might happen to the insect community on a native plant when it is surrounded by a non-native plant. We established four randomized complete blocks on land at Tyler Arboretum in Media, PA. Each block was comprised of four 8-m-diameter treatment plots consisting of a cluster of at least three focal plants, milkweed (*Asclepias syriaca* L.) surrounded by a matrix of one of four possible treatments. The matrix was mowed (“empty”), planted with a non-native plant (yarrow, *Achillea millefolium* L.), planted with a native plant (goldenrod, *Solidago rugosa* Mill.), or planted with milkweed (“conspecifics”). Weekly beginning July 6, we sampled the insect community on three randomly selected focal plants. We counted the number of leaves on each plant and counted and identified the insects on each plant. For each plot on each sample date, the number of herbivores and total insects per leaf were calculated. The maximum value for each plot among all 6 sample dates was used in the statistical analyses. The log transformed values were analyzed using Proc GLM in SAS, and post-hoc multiple comparisons were made using Tukey’s test.

These results are a preliminary analysis of the data collected from one summer. The mean number of herbivores per leaf for each treatment is as follows: conspecifics, 0.6647; natives, 0.3095; empty, 0.1457; and non-native, 0.1331. The GLM reported significant differences between these treatments with a p <0.05. Tukey’s test showed that the number of insects per leaf on conspecifics was significantly different from the empty and alien treatments, and the native treatment was significantly different from non-native treatments. These results suggest that large patches of milkweed attract a greater number of insects per leaf and that native plants surrounding milkweed are less likely to obscure the host than milkweed surrounded by non-natives. However, given this first year of data, there is insufficient support for the notion that non-native plants are obscuring the host by means of associational resistance, because there was no difference between that treatment and focal plants surrounded by mowed ground. In the following year, we may find a different trend, because the yarrow plants in this year were very small due to vertebrate herbivory and perhaps not sufficiently different from the empty plots.
BARK BEETLE *POLYGRAPHUS PROXIMUS*: A NEW AGGRESSIVE FAR EASTERN INVADER ON *ABIES* SPECIES IN SIBERIA AND EUROPEAN RUSSIA

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ABSTRACT

*Polygraphus proximus* Brandford (Coleoptera: Scolytidae) is a common feeder on Far Eastern firs: *Abies nephrolepis*, *A. hollophyll*, and *A. sachalinensis*. Its native range occupies northeastern China, Korea, Japan, Kurile and Sakhalin Islands, and the southern part of the Russian Far East (Primorskiy and Khabarovskiy Kray). The beetle attacks fresh logs and trees, weakened by fires, pathogens, or defoliation.

In 1999, *P. proximus* was found on spruce on the western Russian border, on the Baltic Sea coast near St. Petersburg (Mandelshtam and Popovichev 2000). This finding was evaluated as a small incidental introduction. It was never repeated and eventually forgotten. After 10 years, the species was found in five distantly located places around Moscow (Chilahsayeva 2008). Beetles infested many trees of *Abies sibirica* and *A. balsamea* planted along highways. They also were found under the bark of fallen trees of spruce (*Picea abies*) in forest stands.

*Polygraphus proximus* differs from other co-occurring *Polygraphus* species on conifers by its six-segment antenna. Other species have only five segments.

In spring 2009, *P. proximus* was found in two locations in Krasnoyarsk Kray (Southern Siberia) in the Bogotolskiy and Kozulskiy regions. There were two outbreak areas of about 3,000 ha each in a pure Siberian fir taiga forest stand. Outbreak foci were at least 3 years old with a lot of freshly infested fir trees at the periphery of the foci. Trees crowns were visually healthy, but stems were fully covered by drops and streams of resin exuded from beetle entrance holes. In autumn, all infested trees were dead with yellow crowns. Each nest consisted of two to three female galleries up to 8 cm long, horizontally oriented on surviving trees. Larval galleries were always oriented along the tree stem and reached 7 cm in length. Adults prefer to overwinter somewhere out of stems: there were only dead beetles under the bark of freshly killed firs.

In June 2009, several *P. proximus* adults were found in pheromone traps not far from the city of Tomsk (Western Siberia). Traps were located in Siberian pine (*Pinus sibirica*) stands to monitor pine bark beetle *Ips sexdentatus* populations. *P. proximus* was found in traps only in one place with a low concentration of firs in the stand.

Before 2009, there was no information on *P. proximus* from Siberia and it was generally believed that *P. proximus* could not develop on *Abies sibirica*.

Now, *P. proximus* is considered to be the most aggressive bark beetle ever found on firs in Siberia. Previously, only *Monochamus urussovi* Fish., a cerambid species, was known to be able to attack and kill healthy firs. For a successful attack and rapid weakening of the host tree, *M. urussovi* used spores of blue-stained fungi. In Japan *P. proximus* is also known
to infest *Abies* with spores of a few aggressive phytopathogenic *Ophiostoma* fungi (Yamaoka et al. 2004).

Special studies should be planned to study distribution and ecology of *P. proximus*, a new aggressive invader and a possible threat for both Asian and European conifer forests.

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**Literature Cited**


EMERALD ASH BORER IN RUSSIA: 2009 SITUATION UPDATE

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is a beetle native to East Asia and the Russian Far East where it is considered a minor pest, attacking weakened or dying ash trees. In 2006, EAB was found to be responsible for enormous damage of ash species in Moscow, which causes serious concern for Europe. Recently we reviewed the EAB situation in Russia and made recommendations for research and management strategies in Europe (Baranchikov et al. 2008). Here we report the results of a 2008-2009 survey of further EAB distribution in the Russian Federation.

*Agrilus planipennis* in the Asian Part of Russia

In the eastern part of Russia, the EAB is distributed through nearly all regions occupied by native ash species *F. chinensis* and *F. mandshurica*. It was found from Vladivostok in the South to the village of Dzhonka on the Amur River in Khabarovskiy Kray in the North. There were no signs of EAB at South Sakhalin Island, although there are native stands of *F. mandshurica* there.

In the Russian Far East, the beetle completes its life cycle in 1 (Southern Primoriye) or 2 years depending on the climate. Larvae are exclusively found in live cambium where they compete with bark beetles *Hylesinus eos*, *H. singulatus*, *H. laticollis*, and, in particular, *H. chlodkovskyi*. In the last case, the bark beetle was found to be about five times more abundant than *A. planipennis*. Two braconid species (*Spathius depressithorax* Belokobylskiy and *S. generousus* Wilkinson) and an undetermined eulophid (*Tetrastichus* sp.) were found parasitizing EAB in the Far East.

Surveys showed that the beetle is extremely rare on *F. chinensis* and *F. mandshurica* occurring in natural stands of various ages. It is more common, albeit not a serious pest, in sparse plantations near cities or villages or ornamental trees along streets or in parks. In mixed oak-ash forests, it was found infesting mature trees stressed by fire damage and infested by root rusts. No obvious difference was observed in the level of attack between the two native ash species, but EAB eliminated all trees of *F. pennsylvanica* planted in cities.

Current Situation in European Russia

In January 2007, EAB was officially registered in Moscow as a main source of tremendous ash mortality in the Russian capital. Hundreds of dead trees were cut as a result of the *A. planipennis* outbreak. In Moscow, ash trees play an important role in urban forestry. It is the sixth most abundant tree genus planted in the city. The introduced American species *F. pennsylvanica* largely dominates, but European ash *F. excelsior* is occasionally planted. Most infestations of *A. planipennis* were observed on *F. pennsylvanica*, but *F. excelsior* is obviously also very susceptible.

In Moscow, *A. planipennis* was often found infesting ash trees along with the bark beetle *Hylesinus fraxini*. No parasitoid was found associated with the buprestid beetle, and the woodpecker *Dendrocopus major* was the only observed predator of larvae. The diameter of infested ash stems ranged from 5 to 46 cm at breast height, and the number of trees with exit holes ranged from 35 to 100 percent. On the infested trees, the average density of exit holes at breast height ranged
from 1 to 2.4 per square decimeter. In Moscow, EAB completes its life cycle during 1 year.

In 2009, numerous site surveys were performed using visual inspection and dissection of infested trees within a ~150-km radius from Moscow. It was found that the EAB distribution area is limited by the cities of Mytiszhi in the North (20 km from Moscow); Bykovo in the East, 30 km from Moscow; Serpukhov in the South, 90 km from Moscow; and Mozhaisk in the West, 95 km from Moscow. If it is agreed that EAB was introduced to Moscow approximately 10 years before it was registered, the speed of its distribution was ≤4 km per year. Spread of the pest in firewood should be minimal in Russia because (a) wood for camping fires as a rule is prepared only near camping areas and (b) people do not use ash wood to heat houses, preferring conifers and birch.

In 2008 and 2009, surveys were also made on *F. pennsylvanica* stands in streets and parks of the South Siberian cities of Tomsk, Novosibirsk, Krasnoyarsk, Abakan, Ulan-Ude, and Yekaterinburg (Central Urals), but no sign of *A. planipennis* infestation was found.
EDDMAPS: A COLLABORATIVE, EASY-TO-USE, EARLY DETECTION AND DISTRIBUTION MAPPING SYSTEM

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ABSTRACT

EDDMaps – Early Detection and Distribution Mapping System (www.eddmaps.org) was designed to provide a more accurate picture of the distribution of invasive species. EDDMapS is being developed, implemented, and coordinated by the Center for Invasive Species and Ecosystem Health at the University of Georgia (www.bugwood.org).

EDDMapS allows land managers, agencies, and others to set priorities for early detection and rapid response (EDRR), as well as formulate overall invasive plant management action plans. It is a tool to develop more complete local, state, and regional level distribution data of invasive species, identify “leading edge” ranges of new invasive threats, provide a means of implementing EDRR, and help corroborate threats and refine invasive species lists and management priorities.

Benefits of EDDMapS:

- Fast and easy to use - no knowledge of GIS required
- Web-based mapping of invasive species distribution helps fill gaps and identify “leading edge” ranges
- Facilitates Early Detection and Rapid Response implementation with online data entry forms, e-mail alerts, and network of expert verifiers
- One database for both local and national data
- Data can be searched, queried, and downloaded in a variety of formats
- Cooperates with and aggregates data from other invasive species mapping projects
- Custom/hosted applications can be quickly and inexpensively developed

EDDMapS has been or is being implemented by the Southeast Exotic Pest Plant Council, Florida Exotic Pest Plant Council, Everglades Cooperative Invasive Species Management Area, Mid-Atlantic Exotic Pest Plant Council, and the Alaska Exotic Plant Information Clearinghouse. EDDMapS contains 610,000 county reports and 236,000 point reports on 1,728 species, and it is being employed by more than 1,100 users.
The Eurasian woodwasp, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae), is an introduced invasive pest in the Great Lakes region of North America. If *S. noctilio* is introduced to the southeastern U.S., it may cause severe economic and ecological impacts, especially in the extensive pine plantations. We are evaluating the species complex of native siricids and their hymenopteran parasitoids, as these two taxa may exert competitive and biocontrol pressure on *S. noctilio* in southern forests.

During fall 2009, plots were established in Georgia, Louisiana, and Virginia. Thirty intercept panel traps were placed in each of the three states. Traps were baited with *Sirex* lure alone, *Sirex* lure + ethanol, or left unbaited. In Georgia, 30 funnel traps were additionally used with identical lures, and four Sante traps were placed in the canopy with no lures. Trap logs were created in each state to attract native siricids and parasitoids: 5 trees were cut down in Georgia, 4 trees in Virginia, and 12 trees in Louisiana. All trap trees will be placed in emergence cages in summer 2010.

Preliminary results indicate that about 79 siricids were caught in the traps representing five species: *Sirex edwardsii* (Brullé), *Sirex nigricornis* (Fabricius), *Urocerus cressoni* (Norton), *Tremex colomba* (Linnaeus), and *Eriotremex formosanus* (Matsumura). The majority of siricids were caught in Virginia, which also had all the five siricid species. There was no significant difference in catches between *Sirex* lure alone and *Sirex* lure + UHR ethanol. More siricids were caught in the funnel than in the intercept traps in Georgia, but the difference was not significant.

Our results indicate that the Southeast has a healthy population of native siricids that may outcompete *S. noctilio* if it were to arrive in the region. Further, baiting only with *Sirex* lure will work just as well as including ethanol. Future studies will focus on re-sampling these study sites using similar trapping methods and will add North Carolina and Florida. Siricids and parasitoids will be reared out of trap logs, and the parasitism rate will be assessed.
EMERALD ASH BORER BIOLOGICAL CONTROL
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ABSTRACT

Emerald ash borer (EAB) (Agrilus planipennis), an invasive buprestid from northeast Asia, was identified in 2002 as the cause of ash (Fraxinus) tree mortality in southeast Michigan and adjacent areas of Ontario, Canada. This destructive beetle apparently arrived in North America via infested solid wood packaging materials from China in the early 1990s near Detroit, MI. This infestation has killed tens of millions of ash trees in Michigan, and infestations are now known in Illinois, Indiana, Kentucky, Maryland, Minnesota, Missouri, New York, Ohio, Pennsylvania, Virginia, West Virginia, and Wisconsin and in areas of Ontario and Quebec in Canada. Eradication of EAB is no longer possible, but management tactics to lessen its negative impact are being developed and evaluated for efficacy. Researchers studying populations of EAB since 2002 in the U.S. have determined natural enemies are scarce here, whereas in China, EAB control agents are prevalent and effective. After extensive research on the biology and host specificity of three EAB parasitoids from China, a USDA interagency classical biological control program is expected to be launched in 2010 (USDA 2010).

EAB natural enemies in the U.S. Research on EAB natural enemies started in 2003 throughout southeastern Michigan (Bauer et al. 2004). Less than 1 percent of EAB larvae were parasitized by five species of parasitic Hymenoptera, and no egg parasitoids were found (Bauer et al. 2008). The larval parasitoids included four native species [Atanycolus hicorae, A. simplex, Spathius simillimus (Braconidae), Phasgonophora sulcata (Chalcididae)] and one exotic species [Balcha indica (Eupelmidae)] (Bauer et al. 2008). In a more recent study of EAB in western Pennsylvania, two species of eupelmid, B. indica and Eupelmus pini; two braconids, Atanycolus nigropyga and Spathius laflammeii; and one ichneumonid, Dolichomitus viaticus, parasitized ~3.7 percent of EAB larvae (Duan et al. 2009). In an Ohio woodlot, ~2.8 percent EAB larvae were parasitized by Leluthia astigma, another native braconid species (Kula et al. in press). Except for the two eupelmid species (B. indica and E. pini), these wasps are reported in the literature as parasitoids of native Agrilus spp. Moreover, except for P. sulcata, which is an endoparasitoid, all are ectoparasitoids of late-instar larvae. Although the diversity of EAB parasitoids is relatively high and variable from site to site and from year to year, reports of prevalence remain substantially lower than for EAB in northeast China (Liu et al. 2007) and for native Agrilus spp. in North America. One exception, however, was the discovery in 2007-2008 of EAB larval parasitism ranging from 9 to 71 percent by Atanycolus cappaerti at two sites in southeast Michigan (Cappaert and McCullough 2009). This confirms that some native parasitoids have the potential to switch from native hosts to EAB.

EAB natural enemies in China. There are three important natural enemies of EAB in China: Tetrastichus planipennisi (Eulophidae), Spathius agrili (Braconidae), and Oobius agrili (Encyrtidae) (Liu et al. 2003, Zhang et al.
T. planipennisi, a gregarious koinobiont endoparasitoid of EAB larvae, overwinters as mature larvae in EAB galleries and produces an average of 68 adults per host larva. In areas north of Beijing, T. planipennisi is the most prevalent and widespread parasitoid of EAB. O. agrili is a parthenogenic, solitary parasitoid of EAB eggs up to ~13 days after they are laid; it diapauses as a mature larva inside EAB egg and likely completes two generations per year. At one field site in Jilin province, where the distribution of T. planipennisi and O. agrili overlap, parasitism suppressed EAB populations by ~75 percent on infested green ash trees (F. pennsylvanica) (Liu et al. 2007). S. agrili, a gregarious idiobiont ectoparasitoid of late-instar EAB larvae, produces an average of 8 adults per host larva and is found more commonly in ash trees south of Beijing.

EAB biological control starts in Michigan. By 2007, research findings on parasitoid biology, laboratory rearing, host specificity, and risk assessment were completed, and results were submitted to APHIS with requests for permits to release the three EAB parasitoids from China in Michigan. Our results were 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 (Federal Register 2007) and granted release permits for the three parasitoid species in Michigan in late July 2007.

Evaluating parasitoid reproduction and overwintering. The objective of the 2007 parasitoid releases was to assess reproduction and overwintering capability of these parasitoid species in central Lower Michigan. Parasitoid release sites with high ash densities were selected on state and township lands. The ash trees showed characteristics of building EAB populations (some canopy decline and woodpecker feeding on mid to upper trunks), but had low larval densities in the lower trunks based on sampling using bark windows. From Forest Service laboratory colonies, O. agrili (~700 females) were released in July and August and T. planipennisi (~600 mated females + some males) were released in July through September at two different sites in Ingham County. Reared by the APHIS Otis CPHST laboratory, S. agrili (~100 mated females + some males), were released in September at one site each in Gratiot, Oakland, and Saginaw Counties. The parasitoids were released as adults on GPS-marked epicenter trees at each site. In winter 2008, four release trees were felled and cut into logs for parasitoid recovery. For the two larval parasitoids, every other log section was peeled, and immature EAB and parasitoids were reared in the laboratory for parasitoid emergence. The other log section was placed in a cardboard rearing tube for emergence of adult EAB and parasitoids. For the egg parasitoid, O. agrili, release-tree logs were either (1) inspected for EAB eggs, which were then removed and held for parasitoid emergence in Petri dishes, or (2) placed in cardboard tubes for emergence. We successfully recovered O. agrili from one of the Ingham County sites and S. agrili from the Oakland County site, confirming field reproduction and overwintering in Michigan.

Establishing release sites to study parasitoid establishment, dispersal, and impact. In 2008, we selected EAB-infested sites in Michigan, Indiana, and Ohio where the three parasitoid species were released for long-term monitoring of parasitoid establishment and efficacy; three of the Michigan parasitoid-release sites are being monitored with cohort life tables and other methods. Due to successful research on O. agrili diapause induction, ~2,100 were available for release during EAB’s egg laying period; these were released in June at Indiana and Ohio sites and in July through August at Michigan sites. However, fewer than anticipated larval parasitoids were available for release because of unexpected rearing problems at the two laboratories; thus ~600 mated T. planipennisi females (+ some males) were released from June through October at Michigan sites, and ~300 mated S. agrili females (+ some males) were released in August at Michigan and Ohio sites. For recovery of larval parasitoids in 2008, trees were sampled and peeled during either the fall or winter after release;
egg parasitoid recovery was done by sampling EAB eggs from ash bark and rearing them in the laboratory. At the three life table parasitoid-release sites, trees were randomly selected and dissected in July 2009, resulting in the recovery of *O. agrili* and *T. planipennis*. We also recovered *T. planipennis* ~800 m away from the release epicenters at two of our release sites by dissection of heavily infested ash trees.

In 2009, we were able to increase the number of parasitoids released per site, due to better rearing methods, and construction and staffing of the new APHIS EAB Biocontrol Laboratory in Brighton, MI, completed in January. After technology transfer of the rearing methods, transfer of parasitoid stock cultures, periodic training, and trouble shooting, the personnel at the EAB Biocontrol Laboratory successfully reared ~7,000 *S. agrili* females (+some males) and ~1,000 *T. planipennis* females (+some males) for field release this summer. The combined total from the three laboratories resulted in release of ~8,000 mated *S. agrili* females (+some males), ~20,000 mated *T. planipennis* females (+some males), and ~5,200 *O. agrili* at research sites in Michigan, Ohio, Indiana, Illinois, and Maryland.

At release sites, we are monitoring for parasitoid reproduction, overwintering success, establishment, spread, and prevalence. This is done by dissecting infested trees for EAB and parasitoids; checking emergence traps stapled to tree trunks; setting out sentinel logs with EAB eggs or EAB larvae; and caging the larval parasitoids on tree trunks with EAB larval insertions. We are also collecting data to evaluate the impact or efficacy of biocontrol by comparing changes in ash condition and EAB densities in parasitoid-release vs. control (non-release) sites. Using hand-held computers with high resolution GPS, parasitoid-release and recovery data are being recorded for each tree. For 50 ash trees (<25 cm d.b.h.) at each release and control site in Michigan and Ohio, the following data were collected in 2008 and 2009: species, d.b.h., crown class, epicormic shoots, woodpecker feeding, and EAB exit holes.

**Life table study.** In 2008, we started cohort life table studies to determine stage-specific mortality of EAB by each parasitoid species. This 3-year study is being replicated at three parasitoid-release and control sites in Ingham County, Michigan. For the last 2 years, parasitoids were released on ash trees with EAB egg and larval cohorts. In 2008 and 2009, *O. agrili* was recovered at low prevalence (<1 percent parasitism) from eggs sampled at the three release sites; none were detected at control sites. To assess establishment of the larval parasitoids at our life table sites by 2009, trees were randomly selected and peeled, and larvae were reared to determine parasitoid prevalence. In fall 2009, *T. planipennis* was recovered from ~10 percent of EAB at the three release sites. At two of the sites, EAB larvae parasitized by *T. planipennis* were found about 800 m from their initial release points, suggesting good dispersal potential for this species.

In 2009, we also started field research to observe and detect parasitism in the field and determine overwintering survival of the two larval parasitoids, *T. planipennis* and *S. agrili*.

**Literature Cited**


EFFECTS OF *BACILLUS THURINGIENSIS* SDS-502 ON ADULT EMERALD ASH BORER

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ABSTRACT

Emerald ash borer (EAB), *Agrilus planipennis*, an intermittent pest of ash (*Fraxinus*) trees in northeastern Asia, was discovered in Michigan and Ontario in 2002. In North America, infestations of EAB are now known in 13 states and 2 provinces. This invasive buprestid attacks and kills ash trees and has caused the death of tens of millions of trees since its accidental introduction in the early 1990s. Tree mortality is caused by high population densities of EAB larvae feeding in the phloem. Although methods for controlling EAB in high value ash trees using systemic insecticides continue to be developed (Emeraldashborer.info 2010), management options are lacking for ash trees in forested and riparian ecosystems.

*Bacillus thuringiensis*. In environmentally sensitive habitats, the most widely used insecticides are made from strains of *Bacillus thuringiensis* (*Bt*), an insect-pathogenic bacterium. *Bt* is often used for control of invasive forest insects due to narrow host ranges, good safety records for human health and the environment, high public acceptance, and compatibility with other management strategies such as biological control. Generally, larvae are the target of *Bt* cover sprays; however, EAB larvae are inaccessible to topical sprays because they feed exclusively under tree bark. EAB adults, on the other hand, feed in the canopy on ash leaves and can be targeted by aerially applied foliar sprays. Our goal was to identify a *Bt* strain with sufficient virulence to control EAB adult populations below a tolerance threshold for ash survival (Bauer et al. 2006).

*Bt* toxicity in EAB adults. In EAB adults, we screened 23 *Bt* strains with toxicity against species of Coleoptera using a droplet imbiber bioassay method. We found *Bt* SDS-502 was the most toxic of the strains tested (Bauer and Londoño 2009). *Bt* SDS-502, which expressed the Cry8Da protein toxin, has known activity in some scarabs, but not Lepidoptera (Asano et al. 2000). Phyllom LLC (Mountain View, CA) retains the exclusive license agreement for this patented strain from S.D.S. Biotech K.K. We confirmed toxicity of *Bt* SDS-502 resulted from native Cry8Da crystals (130 kDa protein protoxin) in EAB adults. After ingestion, Cry8Da is then solubilized by midgut pH and cleaved by midgut proteases to a 65 kDa activated toxin. We found the Cry8Da native crystals, the protoxin, and the activated toxin were equally toxic; denatured Cry8Da was not toxic. The median lethal dose (LD₅₀) of the native crystal-spore complex ranged from 0.16 to 0.35 µg of toxin (Bauer and Londoño 2009).

*Bt* intoxication in EAB adults. During fermentation, *Bt* expresses one or more insecticidal crystal proteins or Cry toxins, which have varying levels of specificity to different insect groups. Upon ingestion by a susceptible insect, Cry toxins cause pores to form in the brush border membrane of midgut epithelial cells, resulting in cellular swelling and lysis; mortality by septicemia may result as bacteria invade the body cavity. To determine the mode of action of *Bt* SDS-502 in EAB, adult beetles were inoculated per os with 0.5 µL crystal-spore suspension containing 1µg Cry8Da toxin; control beetles were similarly dosed with water.
After 6 hours, the midguts from treated and control beetles were dissected, and the tissues fixed and embedded for transmission electron microscopy. We observed that Cry8Da caused similar ultrastructural damage in EAB adult midguts as observed for other Bt toxins in susceptible insect hosts, confirming a similar mode of action.

Lethal and sublethal effects of formulated Bt SDS-502 sprayed on leaves. Micron’s ULVA+ hand-held, low volume sprayer was used to simulate aerial application. A 1-ml aliquot of Bt formulation or blank formulation was injected into the spray nozzle with a pipettor, which facilitates reproducible transfer of a known quantity spray into the nozzle. A spray room (1 m²) was used to confine spray to the target area to allow comparisons between replicate spray assays and to compare formulations. Spray deposition on the excised ash leaves was measured using Teejet® water and oil sensitive spray paper (Spraying Systems Co, Wheaton, IL) placed at regular intervals between the leaves. The ash leaves were placed in a circle (8 to 10 leaves) along with four spray cards. The spray was applied 1 m above the leaves. After the leaves were sprayed, petioles were inserted into water vials, placed inside clear plastic boxes with three EAB adults per box, held in an incubator at 24ºC, and monitored daily for mortality for 5 to 7 days.

Bt SDS-502 paste was grown in a 200 L fermenter and spray-dried into technical powder; aliquots were formulated into flowable concentrates (FC) using paraffinic oils and other proprietary ingredients. Test formulations contained either 12 percent or 24 percent Bt SDS-502 technical powder. The mortality of EAB adults exposed to Bt SDS-502 occurred within 96 hours of feeding on sprayed leaves, and total mortality was similar for both Bt concentrations: 78 percent mortality for the 12 percent-Bt FC vs. 89 percent for the 24 percent-Bt FC. However, 39 percent of EAB adults died after 96 hours of exposure to the blank formulation, suggesting about half of the mortality resulted from the oil-based formulation alone. No significant sublethal effects of Bt SDS-502 on EAB adults surviving foliar sprays were found, although adult longevity and fecundity tended to be lower among the Bt-survivors than for controls.

This strain has much promise for controlling EAB using aerial applications; however, more research is needed on formulation and stabilization of a final product.

Literature Cited


GOING WITH YOUR GUT: INSIGHTS INTO NUTRITION AND DIGESTION IN SIREX NOCTILIO WOODWASPS AT EMERGENCE

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ABSTRACT

The threat to U.S. and North American forests posed by Sirex noctilio and its symbiotic fungal partner, Amylostereum areolatum, is expected to be significant in the coming decade. Feeding habits of insect pests present a reasonable target for managing them. Complete knowledge of S. noctilio larval nutrition and digestion is critical but perhaps underused information in controlling them. The following nutritional resources have been observed and described for Siricid woodwasps: mycelia of their fungal symbiont; wood cell contents; yolk remains from their eggs (first instar); wood cell walls digested by fungi; and frass recolonized by fungus (reverse feeding).

The larval gut is much simpler in S. gigas and S. cyaneus than in insects capable of digesting cellulose, a fact cited as indirect evidence for mycophagy. An early examination of the S. noctilio larval gut concluded that the gut is too simple morphologically to digest cellulose. The termite midgut by contrast is threefold longer than the midgut of phytophagous solid feeders that do not digest cellulose. Further, the termite midgut offers evidence of microorganismal symbiosis internal to the digestive system (cuticular spines). To date, no comparable morphological feature of the larval digestive tract suggestive of symbiosis appears in the literature. Our laboratory is examining the alimentary organs of newly emergent S. noctilio females for previously overlooked features suggestive of gut microflora.

Wood is an especially nitrogen-poor food source. If woodwasps derive significant nitrogen from prokaryotic nitrogen fixation in the gut, it is important to identify these organisms. The presence of previously unidentified participants in the pathosystem would offer additional targets for insect management, perhaps through nutritional means. The gut tissues of S. noctilio are fertile ground for immunocytochemical investigation of this question, and immunocytochemistry offers a viable means of identifying any such organisms.
IDENTIFICATION OF A NUCLEOPOLYHEDROVIRUS IN WINTER MOTH POPULATIONS FROM MASSACHUSETTS

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ABSTRACT

The winter moth, Operophtera brumata, originally from Europe, has recently invaded eastern Massachusetts. This insect has caused widespread defoliation of many deciduous tree species and severely damaged a variety of crop plants in the infested area including apple, strawberry, and especially blueberry. Using PCR with primers designed to amplify a 484 bp region of the baculovirus polyhedrin gene, we were able to identify O. brumata nucleopolyhedrovirus (OpbuNPV) infected winter moth larvae collected from field sites in Massachusetts. This represents the first report of OpbuNPV in winter moth populations in the U.S. An analysis of larvae from seven established winter moth populations in Massachusetts revealed the presence of the virus in two of these populations, with the prevalence of 40 percent in one population and 35 percent in the other. Subsequently, using this same approach, we were able to detect viral sequences in winter moth pupae that failed to emerge, suggesting that these insects died as a result of viral infection.
ARE NATIVE SONGBIRD POPULATIONS AFFECTED BY NON-NATIVE PLANT INVASION?

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ABSTRACT

Development into forested areas is occurring rapidly across the United States, and many of the remnant forests within suburban landscapes are being fragmented into smaller patches, impacting the quality of this habitat for avian species. An ecological effect linked to forest fragmentation is the invasion of non-native plants into the ecosystem. Thousands of non-native plants have been introduced into the United States since European settlement through landscaping and accidental release. Past studies have repeatedly shown a decrease in bird biodiversity with suburban development and the negative impacts of non-native plants on individual bird behavior. However, few studies have explicitly examined the link between the density of native plants and avian communities and habitat use.

The objective of this project is to estimate avian occupancy, abundance, and diversity as a function of non-native plant density and associated invertebrate abundance. I conducted 98 avian point counts three times between May 15 and August 15, 2009, in Delaware and Pennsylvania to quantify avian occupancy, abundance, and diversity within plots. Vegetative structure and composition was analyzed within a 500-m buffer surrounding each plot and within the plots by measuring understory coverage, canopy coverage, and proportion of stems that are native. Finally, invertebrate biomass (standardized by plant volume) was measured within each point by vacuum sampling to estimate the avian food supply. Avian surveys, vegetation surveys, and insect measurements will be repeated from May 15 to August 15, 2010.

Preliminary data analysis has suggested that the occupancy of some surveyed bird species is related to native plant proportion within the survey plot. Analysis will continue with the selection of candidate species for which there are adequate data, and multiple linear regression will be used to find the relationship between bird density and patch and landscape vegetation measurements as well as insect biomass. Program PRESENCE will be used to model occupancy for candidate species while accounting for unequal detection probabilities and incorporating site and survey covariates. The models will be evaluated using Akaike Information Criterion (AIC) to determine the most parsimonious model.
LABORATORY OBSERVATIONS OF *SIREX NOCTILIO*: PURSUING AN EFFECTIVE BEHAVIORAL BIOASSAY

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ABSTRACT

Behavior and chemical ecology studies were initiated on the European woodwasp *Sirex noctilio*. Males caged together exhibited increased excitement when brought from a cool room to a warm room with full spectrum lighting and were observed to walk and fly with their genitalia everted. The everted structures, called the latimeres, are composed of the harpe (distal) and gonostipes (proximal). The harpe has a spongy surface used to grasp the female during copulation. It was hypothesized that in *S. noctilio* males, the everted genitalia may emit a sex pheromone or lekking pheromone. These structures were snipped from male *S. noctilio* and extracted in hexane. Headspace of excited males was also collected using SPME fibers. Both snip extracts and SPME samples, when examined using GC-MS, yielded similar chemical peaks. Further chemical and behavioral analysis is underway.
AERIAL APPLICATION OF THE INSECT-KILLING FUNGUS *LECANICILLIUM MUSCARIIUM* IN A MICROFACTORY FORMULATION FOR HEMLOCK WOOLLY ADELGID SUPPRESSION

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ABSTRACT

Forest populations of hemlock woolly adelgid (HWA) were reduced using an operational formulation of the insect-killing fungus *Lecanicillium muscarium* when it was supported by microfactory formulation technology. Mycotal is a commercial formulation of *L. muscarium* produced in The Netherlands by Koppert Biological Systems, Inc., and is registered overseas as a biological pesticide. Mycotal was tested alone and in combination with MycoMax, a formulation additive based on microfactory technology. The study design was a RCBD, and the site consisted of 12 plots of 1.25 acres on state land in Royal Blue, TN; four plots (5 total acres) were used for each experimental treatment. Co-dominant hemlock trees (10/plot) were surveyed after treatment by removing and examining foliage (5 samples/elevation) in the lower and upper (15 m) canopy and again from the lower canopy 5 weeks after treatment. Precision aerial treatments were applied via helicopter at a rate of 2.7 gallons/acre (1x10¹² sp/acre) (the sticker Hyperactive and ADDIT oil were added to both fungal treatments); identical applications were made in the evening and again the following morning. USDA PPQ Permits were secured for importation, transportation, and release of Mycotal for experimental treatment of ≤ 10 acres. Treatment occurred during egg hatch (late May), when an active crawler population was developing, and a preliminary examination of efficacy was made after crawlers were settled in preparation for summer aestivation. Spores of the insect-killing fungus were delivered throughout the hemlock canopy as indicated by recovery of fungal spores from capture plates stationed in the lower canopy. Temperature, humidity, rainfall, and leaf wetness were monitored at locations across the site and were found to be favorable for fungal germination and growth. Before treatment, there were no significant (α=0.05) differences (ANOVA-GLM) among the treatment plots in the populations of HWA woolly masses. When the newly aestivating sisten population was examined in July, it was found that the microfactory formulation (MycoMax) tended to improve the effectiveness of the fungus (Mycotal); populations dropped from 73 percent, with fungus alone, down to 43 percent of that in non-treated control plots when the microfactory additive was included (Fig. 1). Populations of HWA will be re-examined in
spring 2010 to further evaluate population levels in the upper and lower hemlock canopy. The pilot study is being conducted through the University of Vermont in cooperation with the U.S. Forest Service and state cooperators in Tennessee. Mycotal is currently being considered for a U.S. registration.

Mycotal contains the insect-killing fungus *Lecanicillium muscarium*, which was tested alone and in combination with MycoMax, a microfactory formulation additive designed to enhance establishment and persistence of insect-killing fungi.

Figure 1.—HWA sisten population 5 weeks post aerial treatment with insect-killing fungus.
MULTITROPHIC EFFECTS OF CALCIUM AVAILABILITY ON INVASIVE ALIEN PLANTS, BIRDS, AND BIRD PREY ITEMS

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ABSTRACT

Acid rain alters forest soil calcium concentrations in two ways: (1) hydrogen ions displace exchangeable calcium adsorbed to soil surfaces, and (2) aluminum is released to soil water by acid rain and displaces adsorbed calcium. This increases the absorption of aluminum by plant roots, and decreases the absorption of calcium, causing calcium to be more readily leached from the soil.

The effects of acid rain on soil calcium concentrations have been demonstrated in forest ecosystems in the U.S., including Hubbard Brook Experimental Forest in New Hampshire. Less is known about the response of invasive plant species to acidified soils and the effects of acidification on post invasion competition. Highly disturbed ecosystems are susceptible to invasion, and post invasion effects have been the focal point of several studies. This study is designed to evaluate the direct and indirect impacts of calcium availability on invasive plant success, snail abundance, and avian reproductive success in forest ecosystems in the Mid-Atlantic United States. We sampled soil, litter, and snails from three sub-sampling locations at each site to estimate soil chemistry, litter arthropod biomass, and gastropod abundance. We also recorded the vegetation cover type (non-native, native, open) immediately over the soil/litter collection site to determine if there were any patterns between vegetation cover and soil chemistry. We used Berlese Funnels to separate litter arthropods and dry the litter, then sifted the litter to collect the gastropods. A time-constrained search for snails was conducted in a 2.5-m-radius area centered on the soil samples. We sampled forest breeding birds by using three visits to 58 sites during the 2008-2009 breeding season (May-July).

We defined six avian guilds based on forest habitat requirements, nesting and foraging locations, and migration status. This consolidated the breeding community into functional groups. We used linear regression with pH and snail abundance to determine if there were patterns among these predictor variables and the six avian guilds. We found that more ground gleaner species were detected at sites with high pH. We also saw a significant relationship between ground cover types (native plant, non-native plant, and leaf litter) and mean pH; pH was highest under the non-native plant, Rosa multiflora. We did not detect a significant relationship between snail abundance and any avian guild, but did see a pattern between snail abundance and precipitation on the sampling day.
THE CENTER FOR INVASIVE SPECIES AND ECOSYSTEM HEALTH:
TOOLS AND INFORMATION AVAILABLE TO SUPPORT INVASIVE
SPECIES AND FOREST HEALTH EDUCATION

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ABSTRACT

The mission of the Center for Invasive Species and	● Serve as a clearinghouse for information, applied
Ecosystem Health at the University of Georgia research, and training
(www.bugwood.org) is to serve a lead role in	● Promote public awareness, education, and applied
developing, consolidating, and disseminating research in these areas
formation and programs focused on invasive species,
forest health, natural resource and agricultural
management through technology development,
program implementation, training, applied research,
and public awareness at the state, regional, national,
and international levels. The center was formalized in
2008 as an expansion of The Bugwood Network, which
is now the information technology component of the
center.

The goals of the center are to

● Become a pre-eminent national and international
public service and outreach center for invasive	

● Develop collaboration between the University
of Georgia and state, university, Federal, and	

● Integrate and develop information and programs

● Produce Web sites, publications, posters, and
texts presentations

● Serve as a clearinghouse for information, applied
research, and training

● Promote public awareness, education, and applied
research in these areas

This poster will introduce the center and provide
information about: (1) Bugwood Image Database
Systems (http://images.bugwood.org) including
ForestryImages and Invasive.org; (2) EDDMapS, The
Early Detection and Distribution Mapping System
www.eddmaps.org; (3) BugwoodWiki (http://wiki.
bloodwood.org); and (4) information about other center
projects.

Bugwood Web sites received 165 million hits and
were accessed by 9.1 million worldwide users during
2009. Through the Bugwood Image Database System,
110,000 images are available for educational uses.
EDDMapS is being implemented and used for invasive
plant, animal, and biological control projects across
the U.S. ranging from Florida, the Southeast, into the
Mid-Atlantic and upper Missouri River areas, and in
Alaska. EDDMapS contains 610,000 county reports
and 236,000 point reports on 1,728 species, and it is
being employed by more than 1,100 users.
DEVELOPING REARING METHODS FOR *TETRASTICHUS PLANIPENNISI* (HYMENOPTERA: EULOPHIDAE), A LARVAL ENDOPARASITOID OF THE EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE).

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**ABSTRACT**

*Tetrastichus planipennis* Yong, a gregarious koinobiont endoparasitoid, is one of three hymenopteran parasitoids being released in the U.S. for biological control of the emerald ash borer (*Agrilus planipennis* Fairmair, EAB), an invasive beetle from Asia causing mortality of the ash trees (*Fraxinus* spp.) in North America. One critical step in developing a successful biological control program is an efficient rearing method for each biological control agent. Here we report results from two experiments aimed at improving rearing methods for *T. planipennisi*. The first experiment sought to determine which developmental stages of EAB were most suitable or preferable to *T. planipennisi* using both naturally infested large ash logs and artificially infested small ash sticks. The second experiment sought to compare the fecundity of small vs. large *T. planipennisi* females. Findings from these experiments showed that *T. planipennisi* attacked significantly more third and fourth instars than J-shaped larvae of EAB and did not parasitize EAB pupae. More *T. planipennisi* offspring were produced from large hosts (fourth instars) than small hosts (third instars or younger). While *T. planipennisi* were capable of attacking parasitized J-shaped larvae and prepupae when artificially inserted beneath the bark in ash sticks, these were rarely parasitized in naturally infested logs, likely because these stages are too deep within the sapwood to be reached by ovipositing females. In addition, small and large *T. planipennisi* were equally capable of parasitizing EAB larvae inserted into ash sticks (i.e., exhibiting similar parasitism rates); small females produced fewer offspring than large females. However, the offspring of small females tended to be larger than those of large females. Consequently, small females are not without value to rearing programs.
HOURLY AND SEASONABLE VARIATION IN CATCH OF WINTER MOTHS AND BRUCE SPANWORM IN PHEROMONE-BAITED TRAPS

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ABSTRACT

Elkinton et al. recently completed a survey of northeastern North America for the newly invasive winter moth, Operophtera brumata L. The survey used traps baited with the winter moth pheromone, which, as far as it is known, consists of a single compound that is also used by Bruce spanworm, the North American congener of winter moth, O. bruceata (Hulst). We developed DNA techniques to complement dissection of male genitalia to distinguish between the two species. In the broad zone where the two species co-occur, we found that that only 3/158 (1.9 percent) were unmistakable hybrids, based on sequences of the nuclear gene G6PD. We and others have found that it is quite possible to mate the two species in the laboratory. The continued co-existence of the two species after 70 years in Nova Scotia and the rarity of F1 hybrids in our survey suggest that there must be one or more barriers to hybridization. In this study we explore the possibility of one such barrier: that the two species might fly and mate at different times of day or have a different seasonal flight period. We deployed pheromone-baited traps developed by Al Sawyer of USDA APHIS that recorded date and time of capture of each moth. We placed these traps on Staten Island, where we caught only Bruce spanworm, and in eastern Massachusetts, where we caught only winter moths.

We found that winter moths fly almost exclusively during the night. Peak flight typically occurs at dusk, but it will persist throughout the night as long as the temperature stays above freezing. Another peak often occurs at daybreak. On warmer nights there appeared to be three peaks of flight, at dawn, dusk, and near midnight. Not surprisingly, total number of males captured each night was positively correlated with nighttime temperatures (P= 0.02). In contrast, Bruce spanworm was captured in our trap on Staten Island exclusively during daylight hours. Peak flight occurred in early morning (7:00) and late afternoon (16:00) about an hour later and earlier, respectively, than peak flight for winter moth. These times represented the only periods of overlap between the two species.
DNA ANALYSIS OF THE ORIGINS OF WINTER MOTH IN NEW ENGLAND

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ABSTRACT

Elkinton et al recently completed a survey of northeastern North America for the newly invasive winter moth, *Operophtera brumata* L. The survey used traps baited with the winter moth pheromone, which, as far as it is known, consists of a single compound that is also used by Bruce spanworm, the North American congener of winter moth, *O. bruceata* (Hulst). In 2005 we concentrated our survey in the New England states. In 2006 and 2007, we extended the survey to Nova Scotia, New Brunswick, New York, Quebec, and Vermont. We also sent traps to many colleagues in Europe, Japan, Quebec, Ontario, Washington, Minnesota, Michigan, and British Columbia in hopes of using DNA analysis to identify the geographical origin of winter moths in New England.

Specimens collected were sent to the Elkinton Lab at the University of Massachusetts, Amherst. They were based on DNA analysis in Adam Porter’s lab. Total genomic DNA was extracted from the head and thorax. PCR was performed using the forward primer (5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’) and reverse primer (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’) to obtain a ~ 700 bp sequence of the mitochondrial COI gene. Sequencing was performed by Laragen Inc., Los Angeles, CA. Subsequent analysis amplified COII sequences. Here we focus on the COI results because the COII results added very little to our conclusions. We also sequenced the mitochondrial D-loop region. Sequences were edited using Sequencher V4.2. We developed a parsimony phylogram with Bayesian posterior probability values set on corresponding nodes; both analyses are based on an alignment of 597 bp of the mitochondrial gene COI. This phylogram is one of 728 most parsimonious trees as inferred using a heuristic search with default settings in PAUP 4.0b10.

DNA analyses showed that all 82 winter moths from North America (Nova Scotia, British Columbia, and New England) have an identical CO1 haplotype. This remained true when we included the COII sequences as well. In contrast, populations of winter moth in Europe and Bruce spanworm in North America both showed considerable sequence variation. These facts may indicate a founder effect and a single introduction of winter moth to North America, presumably to Nova Scotia that then spread to British Columbia and New England. Winter moth outbreaks began in Nova Scotia before 1950. However, the COI haplotype that is ubiquitous in North America is also both dominant and widespread in Europe, making up 60 percent of the 50 moths we sequenced from Sweden to Serbia. Thus, we cannot rule out multiple introductions of this haplotype to North America. When we looked at the mitochondrial D-loop, we saw that the New England clade was distinct from the moths collected from Nova Scotia. The latter were more similar to winter moths from British Columbia and various European sites. This may suggest a separate introduction of winter moths to New England rather than spread from Nova Scotia.
ECONOMIC ANALYSIS OF LIGHT BROWN APPLE MOTH USING GIS AND QUANTITATIVE MODELING

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ABSTRACT

We conducted an economic analysis of the light brown apple moth (LBAM), *Epiphyas postvittana* (Walker), whose presence in California has resulted in a regulatory program. Our objective was to quantitatively characterize the economic costs to apple, grape, orange, and pear crops that would result from LBAM’s introduction into the continental United States. This information can be used to inform regulatory policy and funding decisions on LBAM.

Our economic analysis had two components: (1) a geospatial analysis that identified areas at risk for LBAM establishment based on climate and hosts and (2) a quantitative analysis, using a probabilistic modeling approach, which estimated the economic losses LBAM could cause if introduced into these areas due to damage, control, quarantines, and research.

Our geospatial analysis estimated that LBAM could establish throughout most of the continental United States, including throughout most of the analyzed U.S. crop production areas. The mean total annual crop costs due to damage and control if LBAM were introduced in the at-risk areas was estimated at $104 million. The mean total economic costs including quarantines and research was $118 million.

Our analyses indicated that LBAM could cause substantial economic losses to U.S. apple, grape, orange, and pear crops if introduced into the continental United States. LBAM is polyphagous and would probably cause additional economic damage to other crops and sectors of the U.S. economy.
WHOLE-TREE CANOPY ENCLOSURES: WHY CAGE A TREE?

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ABSTRACT

The use of whole-tree canopy enclosures (i.e., cages) is not a typical approach to assessing biological parameters and interactions in a forest setting. However, the successful application of this technology may enable researchers to better understand certain types of tree/organismal interactions. One of these interactions that may be better evaluated using whole-tree canopy enclosures is the predator/prey relationship associated with the use of an introduced biological control agent against an invasive forest insect pest. Typical assessments of introduced biological control agents of invasive insect pests usually occur in the laboratory, either in petri dishes or small arenas, in larger predator/prey arenas established in a greenhouse, or in sleeve cages placed directly on the plant in the field. While these approaches provide important information, does the small size of these arenas limit their usefulness when evaluating introduced natural enemies of pests of tree species? Can whole-tree canopy enclosures improve our assessment of introduced natural enemies of pests of trees?

As part of this pilot project to assess the use of whole-tree canopy enclosures, research is underway to use these enclosures to evaluate introduced biological control agents against hemlock woolly adelgid, Adelges tsugae Annand, on hemlock trees. This research will enhance our understanding of the survival, colonization, and establishment of introduced biological control agents against this invasive insect pest, and it will allow us to assess the impact of these selected agents on population densities of this introduced insect pest and on tree health. This project focuses on the use of large (ca. 9 m [30 ft]) screened whole-tree canopy enclosures to assess the successful field application of three introduced biological control agents of hemlock woolly adelgid. This research includes the use of qualitative and quantitative measurements to assess and determine the colonization and impact of introduced biological control agents. This study is being conducted at Blackberry Farm near the Great Smoky Mountains National Park in eastern Tennessee.

Several needs must be considered in using whole-tree canopy enclosures in forest settings. These include the availability of a suitable location for 2+ years (the location must be easily available for use, generally secure, few activities in area, etc.), trees of appropriate height (20 to 30 feet tall) for canopy enclosures, trees that are healthy and consistently shaped, trees infested with appropriate densities of the targeted insect (a relatively new infestation is best), trees with new growth, trees that are easily accessible using a bucket truck or lift, trees that are “solitary” (none intermingled/side-by-side), a ground surface that is relatively flat or slightly slanted, and whole-tree canopy enclosures. Once these needs are satisfied and a design has been developed, canopy enclosures can be constructed and deployed into the field for use in biological assessments.

Once the enclosures are deployed, researchers must consider the potential advantages and disadvantages to the use of these enclosures. Whole-tree canopy enclosures have several advantages to their use. They provide a more realistic field assessment of introduced biological control agents (than previous methods);
they enable long-term monitoring of the impact of natural enemies on an invasive pest and on tree health, as well as predator performance and survival; they provide a way to assess single species or combination of species of natural enemies, and they provide a better understanding of actual predatory expectations in the field. However, there are several disadvantages or limitations to the use of whole-tree canopy enclosures. Enclosures provide an assessment of a “controlled” environment (how similar is “inside” to “outside”?) (in our cages, however, research has shown that temperature and humidity levels vary little between open and caged trees.); all stages of organisms cannot be removed from the trees before they are “caged”; and the environmental stresses to cages (e.g., high winds, snow, rain, hail, animals, humans) are difficult to control.

In summary, whole-tree canopy enclosure cages are a new and innovative approach to assessing natural enemies for release against invasive insect pests of trees. These types of enclosures could be used to assess single species or species complexes of natural enemies. This research is expected to enhance our knowledge of the establishment and effectiveness of introduced biological control agents and provide a better understanding of the role of natural enemies in suppressing invasive insect pests. Whole-tree canopy enclosures also could be used to assess other types of tree/organismal interactions.
THE EUROPEAN OAK BORER, *AGRILUS SULCICOLLIS* (COLEOPTERA: BUPRESTIDAE): NEW TO NORTH AMERICA

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**ABSTRACT**

The European oak borer, *Agrilus sulcicollis* Lacordaire, was first reported in North America in Ontario in 2008 and then in Michigan and New York in 2009 (Haack et al. 2009, Jendek and Grebennikov 2009). Subsequent examination of previously unidentified specimens in Canadian and U.S. museums and personal collections found *A. sulcicollis* individuals dating from 1995 in Canada and 2003 in the U.S. Specimens have been collected from English oak (*Quercus robur*) in the U.S. and red oak (*Q. rubra*) in Canada. *Agrilus sulcicollis* is native to most of Europe, where it infests primarily oak species, but occasionally it has been reared from species of *Carpinus*, *Castanea*, and *Fagus*. Adults are 5 to 9 mm long and various shades of metallic bronze, green, violet, and blue. Adults are similar in appearance to *A. cyanescens* (see Zablotny 2009). In Europe, *A. sulcicollis* usually has a 1-year life cycle, flies mostly in May-July, and infests stressed oak trees along with *A. biguttatus* and *A. angustulus*. We do not know what impact *A. sulcicollis* will have on oaks in North America, but so far no widespread oak mortality has been attributed to this new invader.

**Literature Cited**


DEVELOPMENT OF AN ARTIFICIAL DIET FOR WINTER MOTH, *OPEROPHTERA BRUMATA*

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ABSTRACT

The winter moth, *Operophtera brumata*, is an invasive pest that was introduced to North America in the 1930s. First identified in Nova Scotia, this small geometrid native to Europe has spread to New England. It has caused extensive defoliation of deciduous trees and shrubs. In Massachusetts, a biocontrol project is underway to reduce populations by introducing a parasitic fly, *Cyzenis albicans*. Development of a successful artificial diet for the winter moth is critical in generating mass populations of the tachinid fly for biocontrol release. We tested various diets and diet ingredients to arrive at one that gave the best performance in terms of larval survival, pupal weight, and adult emergence. We can now rear large numbers of winter moths from egg to adult stages on this diet. We have also investigated various ways to shorten the winter moth generation time in order to produce a year-round culture.

Hatch of winter moth eggs: Eggs were initially collected by banding trees in November. Bands were brought into the lab in January, and all eggs were chilled in a 3.5°C holding chamber. Eggs were pulled from chill at 14-day intervals and placed in one of three temperature treatments for incubation, 18.3, 19.4, and 25.5°C. Eggs were monitored daily to determine percent hatch in comparison to their incubation date.

Culturing: Once an optimal incubation time was determined, cohorts of eggs were pulled from chill and neonate larvae were infested onto four different diets. Larvae were maintained in environmental chambers controlled at 21°C ± 3°C with a 16:8 hour L: D photoperiod. All diets A-D were prepared in a commercial blender. Diet A, the gypsy moth diet, is composed of 66 percent wheat germ. Diet B, the pinto bean diet, is composed of 28 percent wheat germ and 26 percent ground pinto beans. Diet C, the modified gypsy moth diet, is composed of 63 percent wheat germ and 8 percent soy flour. Diet D, the general Lepidoptera diet, is composed of 50 percent soy flour (Bio-Serve).

Highest percent hatch results at various temperatures indicated that eggs need a 70- to 95-day chill period for best hatch. The eggs incubated at approximately 19°C had the greatest percent hatch.

Larvae infested on diets B and C experienced the highest percentage of mortality. Diet B was significantly drier than the other diets, and all larvae died within 14 days. Diet D also caused high larval mortality, and no insects were reared longer than 14 days. Diets B and D were eliminated after 21 days. Larvae reared on diets A and C were the most successful. On diet A, 39 percent of larvae infested onto the diet reached the pupal stage and 46 percent of larvae from diet C reached the pupal stage. Diet C is a modified version of the B4 gypsy moth diet. Female pupal weights from diet C were comparable to wild-collected pupae that fed on host plants. Diet C females also laid the most eggs after mating, much more than the wild-collected adults. Adult emergence was also greater from the larvae reared on diet C.
SCREENING FOR *PHYTOPHTHORA CINNAMOMI* IN RECLAIMED MINED LANDS TARGETED FOR AMERICAN CHESTNUT RESTORATION PROJECTS

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ABSTRACT

We are working toward restoring the American chestnut in southeastern Ohio, which was once part of the tree’s natural range. Some of these lands have been severely affected by excessive mining operations for several decades. Therefore, we are planning and testing use of ectomycorrhizal fungi in the restoration efforts. Mycorrhizal fungi may play a vital role in this, because they have been shown to be essential for the survival of seedlings by supporting growth under a variety of subnormal soil and other stress conditions. Our work has identified several species of ectomycorrhizal fungi that form associations with chestnut seedlings. We are testing their utility in restoration efforts.

Long before the chestnut blight fungus was introduced, other pathogens of the genus *Phytophthora* were introduced in the late 1700s or early 1800s. Among these, *Phytophthora cinnamomi* was responsible for the “ink disease” or “root-rot” that resulted in the widespread death of chestnut trees in the southern states. Its presence in the northeastern states, and especially in the Appalachian region, was recently noticed on chestnut seedlings. This could be detrimental to the planned reforestation and restoration efforts.

We tested soil from several locations where we have planted and/or are planning to plant hybrid chestnut trees inoculated with mycorrhizal fungi. All these locations are coal mined areas that have been reclaimed. Soil was collected at a depth of 4 to 5 inches, placed in plastic zip lock bags, and stored at 22°C in the dark until used. A variation of soil dilution plating method was used to assay for *P. cinnamomi*. A positive control containing ~4 cfu/10 g soil was used in the analysis. After identifying the fungus morphologically, we used molecular methods (PCR using species-specific primers) to confirm that the fungus was indeed *P. cinnamomi*.

Our results showed that, at least in the locations we tested, *P. cinnamomi* was not present. It is possible that because most of these lands were only recently reclaimed, the fungus may not have established there yet. However, samples from locations that were reclaimed more than a decade ago also showed absence of this fungus. We will continue monitoring these and other locations marked for future chestnut plantings. It is noteworthy that the Chinese chestnut is resistant to *P. cinnamomi*, and recent findings have shown that hybrid chestnut trees (the ones we plant) are partially to reasonably resistant to the fungus.
IMPACT OF ENHANCIN GENES ON POTENCY OF LDNPV IN GYPSY MOTH

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ABSTRACT

*Lymantria dispar* nucleopolyhedrovirus (LdNPV) contains two enhancin genes (E1 and E2) encoding proteases that degrade key peritrophic matrix (PM) proteins, thereby promoting infection and mortality by the virus. In a previous study, gypsy moth larvae inoculated with LdNPV in which both E1 and E2 were deleted (double deletion virus) resulted in a non-additive decrease in viral potency compared to potencies of viruses with only 1 of the 2 genes deleted. Earlier studies on enhancins encoded by granuloviruses show there are specific binding sites on midgut cells for enhancins, suggesting enhancins may function to facilitate virus entry into midgut cells. We investigated the potency of viruses lacking E1 (E1cat), E2 (E1del) or both (E1delE2del) in larvae fed on artificial diet in the presence or absence of the PM using the fluorescent brightener M2R to degrade the PM. We hypothesized that if the enhancin genes have a function in addition to increasing permeability of the PM to virions, then the double deletion virus should be significantly less potent than the wildtype virus (A21) even in the absence of the PM. Removal of the PM as a barrier to baculovirus infection did not change the reduced potency of the double deletion virus in comparison with the wildtype virus. These data support our hypothesis that the enhancin genes of LdNPV have another function beyond increasing permeability of the PM to virions. We plan to determine the role of one or both of the enhancin genes of LdNPV (E1 and E2) in viral entry into midgut cells.
ASSESSMENT OF AN APPARENTLY ISOLATED POPULATION OF EMERALD ASH BORER IN UPPER MICHIGAN

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ABSTRACT

Emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) is an exotic forest pest of ash that is native to Asia. Since its discovery in North America in 2002, it has been found in 13 U.S. states and 2 Canadian provinces and has killed more than 50 million trees in Michigan, Ohio, and Indiana alone. The presence of EAB in Houghton County, MI, was confirmed in August of 2008. This was the northernmost find of EAB in North America at that time and more than 250 miles from the closest known population.

More than 90 ash trees have been destructively sampled to help determine how EAB has spread in Houghton County. Larval density and exit holes were quantified on all sample trees. Preliminary dendrochronological analysis indicates EAB has been present in Houghton County for at least 6 years.

Traditional EAB delimitation surveys involving grids of girdled trap trees and/or baited purple panel traps are costly and time consuming. A visual ground survey using a novel technique was initiated during the summer of 2009 to determine the extent of the ash resource and visual signs and symptoms of EAB around the Houghton County infestation. Transects were run in the four cardinal and four intercardinal directions for 3.2 km radiating outward from a known infested tree. Basal area by species was assessed with a 10 BAF prism every 161 m, and a 0.04-ha fixed-radius plot was established every 805 m. All trees larger than 5 cm d.b.h. were tallied, and any ash tree found was assessed for vigor, dieback, crown light exposure, canopy position, and signs and symptoms of EAB. Point quarter sampling was also performed at each prism point, and any ash trees within 20 m were assessed within each quadrant. Ground survey located two additional infestations 0.8 km south and 1.2 km southwest of the original EAB find. Another population was located by an earlier ground survey in October of 2008 0.8 km west of the initial find. Transects were run from each of these additional populations, but no new infestations were located.

Destructive sampling and dendrochronology work will continue during the winter of 2009-2010. The rapid assessment of EAB infestations and ash resources using the radiating transects will be further evaluated in 2010.
PISSODES CASTANEUS (DE GEER, 1775) (COLEOPTERA: CURCULIONIDAE), THE BARK PINE WEEVIL: A PEST OR A BIOLOGICAL INDICATOR?

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ABSTRACT

The risk of introduction of exotic forest pests is a global problem, evidenced by records of interceptions even in countries that have a quite effective system of plant protection. The banded pine weevil, *Pissodes castaneus*, is native to Europe and North Africa and was introduced into Argentina and Uruguay and recently into Brazil where it was first recorded in Rio Grande do Sul State in June 2001. Later it was recorded in Paraná and in 2002 in Santa Catarina States. The hosts of *P. castaneus* are in the conifer family Pinaceae: *Abies* spp., *Pinus* spp., and *Pseudotsuga menziesii*. The effects produced correspond to the damage caused by the adults feeding on the buds and young branches; however, the primary damage is caused by the larvae tunneling and feeding in the stems where they form galleries filled with excrement and thin fibers of wood.

*P. castaneus* has two generations per year, but in cold areas may produce only a single generation. Pest outbreaks may start in young plantations, usually in a few trees, but the population can grow rapidly. The increase of the population in the initial stage is associated with the existence of large numbers of susceptible trees. We analyzed six instances of *P. castaneus* outbreaks in *P. taeda* plantations in Southern Brazil, with the goal to make a diagnosis of the attack. The results indicate that the banded pine weevil is closely linked to the silvicultural condition of the planting, which may include the quality of the site, physical condition of the soil, planting technique, nutritional deficiencies, and occurrence of abiotic phenomena such as hailstorms, frosts, prolonged droughts, etc. Biotic factors such as prior attack by primary pests may also stress the plants and increase the emergence of plants attacked by *P. castaneus*.

In general, it can be stated that the presence of the pine weevil is an important indication that there is a silvicultural problem within the pine plantation. The weevil is an excellent biological indicator of the silvicultural quality of the pine crop and therefore is characterized as an opportunistic pest that can be enhanced by the occurrence of biotic and abiotic stress triggers within plantations. Thus, during the planning of a forest management program, it is necessary to examine all biotic and abiotic factors that may favor attack by the pest.
THE COOPERATIVE AGRICULTURAL PEST SURVEY PROGRAM (CAPS): SCIENTIFIC SUPPORT TO OPTIMIZE A NATIONAL PROGRAM

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ABSTRACT

The mission of the Cooperative Agricultural Pest Survey (CAPS) program is to provide a survey profile of exotic plant pests in the United States deemed to be of regulatory significance to USDA Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), State Departments of Agriculture, tribal governments, and cooperators by confirming the presence or absence of environmentally and/or economically harmful plant pests that impact agriculture or the environment and that have potential to be of phytosanitary significance; and by establishing and maintaining a comprehensive network of cooperators and stakeholders to facilitate our mission and to safeguard our American plant resources.

The Center for Plant Health Science and Technology (CPHST) supports PPQ regulatory decisions and operations through methods development work, scientific investigation, analyses, and technology.

For the CAPS program, CPHST develops tools for the survey and identification of CAPS target pests, including: (1) specific, effective attractants and traps, (2) molecular diagnostic and Lucid identification tools, (3) risk-based maps based on host, climate, and pathways of target pests, and (4) survey protocols based on commodities, habitat, and pathways.

Current challenges include (1) identifying the most appropriate survey method for early detection surveys versus methods used in monitoring surveys; (2) balancing the cost of survey equipment, efficiency of traps and lures, and identification resources; and (3) reporting negative data for targets without efficient attractants (i.e., wood borers and bark beetles, pathogens, nematodes, etc.).

For more information, contact Lisa Jackson at Lisa D. Jackson@aphis.usda.gov.
EMERALD ASH BORER AFTERMATH FORESTS:
THE FUTURE OF ASH ECOSYSTEMS

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ABSTRACT

The effects of emerald ash borer (EAB) (Agrilus planipennis) on forest ecosystems are being studied through a collaborative research program between the U.S. Forest Service and The Ohio State University. We are monitoring ash demographics, understory light availability, EAB population dynamics, native and non-native plants, and effects of ash mortality on other organisms and ecosystem processes in monitoring plots in forests in Ohio and Michigan.

In long-infested plots where 99.9 percent of ash trees have died, there are many established ash seedlings (191/ha) but very few new ash seedlings (<0.1/ha). Saplings too small for EAB to colonize are present (6/ha), but larger ash saplings and trees are very rare (<1/ha). The few large ash trees that have survived in these forests are being examined in collaboration with other FS and OSU researchers for possible resistance to EAB. In contrast, more recently infested plots with many live ash trees experienced a mast seed year in 2008, and the density of new ash seedlings averaged 35,000/ha in 2009. EAB population data from purple traps suggest that EAB populations peak, crash, and then persist at low densities in aftermath forests, probably surviving on ash saplings as they reach susceptible size.

As ash trees die in infested stands, understory light levels may increase gradually in stands without adequate mid-story or sapling trees of other tree species. Non-native plant species are generally present in ash ecosystems, with individual species distributions dependent on geography, habitat, and land use history. Invasive plant species may be facilitated by increased understory light in EAB aftermath forests.

The goal of the ALB program is to eradicate the pest in the United States to protect the hardwood forests of North America. To achieve this goal, the ALB program has developed and implemented eradication protocols, an area-wide, science-based strategy. Visual survey of host trees for signs of ALB: there are no traps or lures available to attract ALB; four negative surveys are required to declare eradication of ALB in an area; and surveys are conducted by climbers, ground crews, or bucket trucks. Tree removal: infested trees are removed; exposed host trees may be removed to further reduce populations. Removed trees must be chipped to become deregulated material. Chemical treatment: exposed host trees chemically treated for a minimum of 3 consecutive years to prevent infestation and reduce ALB populations. Regulatory activities to prevent the pest’s spread: enforce the quarantine to keep potentially infested materials from leaving the infested area; educate local tree care companies and other industries that work with regulated items about ALB regulations; issue compliance agreements to companies to ensure regulated materials are handled appropriately within the infested areas. Replant with non-host species to assist in the regeneration of the tree canopy. Outreach to educate the public and industry about the ALB: to obtain their assistance in looking for the beetle and to gain their cooperation while carrying out program activities. Besides standard program outreach to the infested areas, a large outreach initiative was carried out in nine northeastern states in 2009 to raise awareness of ALB. Other components of the eradication strategy include quality assurance to ensure survey, removals, and treatments are done correctly so that these actions are effective; and methods development to improve program effectiveness and delivery. Science-based eradication protocols: biology and population dynamics. New technologies for chemical control: basal soil injection; low-pressure trunk injection; Ecojet capsule injection—operational pilot. DNA analysis of populations: to assist in determining the origin of infestations; three confirmed ALB introductions into the United States—New York, Illinois, and New Jersey; DNA analysis is pending in Massachusetts. Aging studies of infested trees: to determine the first year of infestation; studies have shown that infestations remain localized for the first 4 years and then populations disperse.
PROSPECTS FOR LONG-TERM ASH SURVIVAL IN THE CORE EMERALD ASH BORER MORTALITY ZONE

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ABSTRACT

Attacking all North American ash species (*Fraxinus* spp.), emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) has caused significant mortality within its introduced range. For other forest pests, host bark plays an important role in infestation density and oviposition behavior. The objectives of this study were to (1) locate live ash trees in the core EAB-induced mortality zone in southeastern Michigan, (2) examine differences in live and neighboring dead trees in terms of d.b.h. and bark roughness, and (3) develop a deployable and simple ash mortality probability model.

An aerial helicopter survey was conducted in southeastern Michigan to digitally sketchmap locations of live ash trees. Live ash trees were identified to species and tagged within polygons mapped. Distance and d.b.h. of closest dead ash tree and distance to closest live tree regardless of species were measured for each live ash. Digital bark images were taken for each live tree and the closest dead ash tree centered at breast height. Images were converted to binary black and white. The percentage of black pixels in each image was used as a measure of bark roughness.

In the five Metroparks surveyed, 203 live ash trees were found. The majority of these trees exhibited signs and/or symptoms of EAB infestation. D.b.h. did not differ between live trees and dead trees. In addition, the live and dead trees followed a similar pattern in size class distribution. Live trees had significantly smoother bark; dead trees had a greater roughness value. The logistic regression between roughness value and tree mortality resulted in a significant relationship with the probability of an ash tree being dead increasing with each increase in roughness. The behavior of female EAB to lay eggs in bark crevices has been previously illustrated; trees with rough bark tend to have substantially more larval galleries than trees with smooth bark. Those trees with smooth bark may have survived because they provide fewer oviposition locations for female EAB. Trees with rough bark provide more oviposition locations. Bark roughness may help natural resource managers predict which ash trees will likely succumb to EAB attack.
MULTI-STATE COMPARISON OF TRAPPING TOOLS
AT SITES WITH LOW EMERALD ASH BORER DENSITY

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ABSTRACT

Developing tools for detecting emerald ash borer 
(EAB) (Agrilus planipennis Fairmaire) has been a 
major focus of research efforts in recent years as the 
search for an effective detection survey methodology 
continues. The objectives of this study were to (1) 
compare the effectiveness of EAB detection trapping 
tools at low density sites in Indiana, Michigan, 
Missouri, New York, Ohio, Pennsylvania, Virginia, 
and Wisconsin, (2) identify the most effective of 
available trapping techniques for capturing EAB at 
low density, and (3) develop monitoring and trapping 
recommendations for managers in locations with and 
without confirmed EAB populations.

Trapping tools were compared within 79 sites, 
including a girdled trap tree with a plastic wrap trap, 
green prism trap hung at 13 m, purple prism trap at 
1.5 m, purple prism trap at 6 m, and a double-decker 
purple prism trap in an opening 30 m from an ash tree. 
All prism traps had an 80: 20 manuka oil: phoebe oil 
lure. Traps were checked every 2 weeks and all adults 
were collected. Green prism traps at 13 m captured the greatest number 
of EAB adults, but this was not significantly different 
from the purple prism traps hung at 6 m. There was 
a significant positive relationship between tree vigor 
and percent dieback with EAB adults captured for the 
green prism traps. Because the green traps are hung 
at 13 m in the canopy, trees with higher vigor ratings 
(i.e., poor health and less canopy) and greater percent 
dieback have greater amounts of sunlight penetrating 
the canopy and reaching the traps.

The detection of EAB adults was not independent of 
the different trap type used. The purple prism trap at 6 
m and the double-decker trap had the highest rates of 
detection. It is important to note that whichever traps 
are incorporated into a survey program, none of these 
tested traps have 100-percent detection ability. The 
traps in this study were placed at sites where EAB had 
previously been detected and populations were known 
to exist. Even at sites where EAB is known to exist, 
one of the traps used in this study detected it at every 
site.
USE OF UNWOUNDED ASH TREES FOR THE DETECTION OF EMERALD ASH BORER ADULTS: EAB LANDING BEHAVIOR

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ABSTRACT

Incorporation of multiple trapping techniques and sites within a survey program is essential to adequately identify the range of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) infestation. Within natural forests, EAB lands on stick band traps wrapped around girdled ash trees at a rate similar to that on unwounded ash trees. The objective of this study was to identify characteristics of ash trees that allow for prediction of EAB landing behavior.

Ash trees were wrapped with plastic wrap traps in Burt Lake and Harrisville State Parks, MI, in 2008, and Farnsworth and Providence Metroparks, OH, in 2009. These data were used to create two separate multiple regression models for EAB/m² using the categorical variables crown light exposure and tree vigor. Two datasets from 2009 at Deford and Shiawassee State Game Areas, MI, were used to validate the 2008 and 2009 models and gauge the efficacy of each model to predict EAB/m². Paired t-tests were used to test for differences in the observed and predicted values at Shiawassee and Deford from the 2008 and 2009 models.

The 2008 model predicted the EAB/m² at Deford well: the predicted values did not differ from the observed values in a paired t-test. However, the 2008 model underpredicted EAB/m² at Shiawassee, resulting in a significant paired t-test. In contrast, the 2009 model overpredicted EAB/m² at Deford, but was successful at predicting EAB/m² at Shiawassee. When used to predict the EAB/m² values at sites with comparable EAB population size, each model performed well, predicting values that did not differ from the observed. Plotted as three-dimensional surfaces, the two models exhibited similar visual anomalies, which, although producing vastly different EAB/m² values, illustrate the overall similarities in EAB landing behavior. EAB population size variability makes it difficult to model the actual captures of EAB/m². While the models cannot effectively predict the actual capture abilities of a single tree, they did produce visually similar models that can be used to effectively select a tree that has the greatest probability of capturing EAB. A tree with a CLE of 4 and a vigor rating of 4 will most likely be effective at detecting EAB. Some natural resource managers and private landowners decline the request to girdle ash trees for trapping and detecting EAB. Because unwounded ash trees are just as effective in adult captures and have larval densities similar to girdled ash trees, unwounded trees may be a less expensive alternative to other trapping techniques and a simpler alternative to girdled trap trees.
RECOVERY OF BACILLUS THURINGIENSIS AND RELATED SPORE-FORMING BACTERIA FROM SOIL AFTER APPLICATION FOR GYPSY MOTH CONTROL

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ABSTRACT

*Bacillus thuringiensis* Berliner (*Bt*) has been applied for gypsy moth (*Lymantria dispar* L.) control in forests in the northeastern U.S. for many years. The subspecies of *Bt* that is used (*kurstaki*) is not common in U.S. soil. We attempted to recover *Bt* from soil that had been sprayed 2 years prior with *Bt* for gypsy moth control. By amplifying the bacteria found in the soil on bacterial agar and feeding this diverse microbial population to tobacco hornworm larvae, an insect that is very sensitive to *Bt*, we were able to further improve on traditional recovery methods. Out of 20 soil samples, 15 were found to have spore-forming bacteria that killed these larvae. These strains were identified to species by their 16S rDNA sequences. Most of the bacteria belonged to the *Bacillus cereus* Franklin and Franklin group (14/15) including 3 *Bt*. All the *Bt* strains and most of the *B. cereus* strains were closely related (<0.001 substitutions/site in their 16S rDNA) to the strain that was sprayed. The other two spore formers were identified as *B. weihenstephanensis* Lechner et al. and *Lysinibacillus fusiformis* (Meyer and Gottheil). All 15 strains were phenotypically distinguishable from one another, as well as from the *Bt* strain that was applied, by substrate utilization and antibiotic resistance, although seven strains differed from the *Bt* strain applied by 5 or less of the 24 traits tested. The toxicity to gypsy moth larvae of the three *Bt* strains isolated was similar to that of the applied *Bt* strain. Thus, amplification of bacteria present in soil, in combination with a sensitive insect, can recover insect toxic strains that are related to an applied strain.
SURVIVAL OF *BACILLUS THURINGIENSIS* STRAINS IN GYPSY MOTH (*LYMANTRIA DISPAR*) LARVAE IS CORRELATED WITH PRODUCTION OF UREASE

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ABSTRACT

We tested 50 lepidopteran-toxic *Bacillus thuringiensis* Berliner (*Bt*) strains with diverse phenotypes for the ability to survive repeated passages through larvae of the gypsy moth, *Lymantria dispar* (L.), without intervening growth on artificial media. These experiments have revealed a remarkable correlation between the production of urease (22 of 25) by the bacteria and its ability to survive repeated passages through larvae.
UPDATE ON EXOTIC ASH COLLECTION FOR HYBRID BREEDING AND SURVEY FOR EAB-RESISTANCE IN NATIVE NORTH AMERICAN SPECIES

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ABSTRACT

Contrary to the high levels of devastation observed on North American ash species infested with emerald ash borer (EAB) (\textit{Agrilus planipennis} Fairmaire), reports from Asia indicate that EAB-induced destruction of Asian ash species is limited to stressed trees. This indicates that Asian ash species have co-evolved resistance, or at least a high degree of tolerance, to this insect. We are investigating whether inter-species hybrids between Asian and North American ash species can be used to introgress EAB resistance into native ash species.

We have made progress toward addressing the following six pressing research needs:

1. Make accessions and propagate Asian ash trees
2. Confirm species identity, clonal ID, and relatedness of Asian ash accessions
3. Determine ploidy level of all species and of individual trees where ploidy varies within species
4. Develop methods to overcome obstacles to hybridization such as species differences in pollination system and phenology
5. Determine resistant Asian ash species and the level of phenotypic variability within species
6. Determine if “lingering ash” are EAB tolerant, and if so, what allows them to survive longer than other ash in the stand

The long-term goal of our research is to identify resistance to EAB in exotic ash species and/or select North American individuals (lingering ash) for use in establishing a breeding program. The use of exotic ash species to generate F1 hybrids may have immediate value to the nursery industry as street trees. F1 progeny as well as subsequent generations will also be valuable genetic resources for determining the heritability and molecular mechanisms of resistance through the use of genomics, transcriptomics, proteomics, and metabolomics. Backcross generations created through careful selection of parents for both North American characteristics and EAB resistance may someday provide the resources needed to re-establish EAB-resistant North American ash species in our forests. Gene conservation activities involving North American ash species are a critical component of such a breeding program so that genetic diversity across the native range is preserved for use in advance generations of breeding.
COMPARISON OF PROTEIN PROFILES OF BEECH BARK
DISEASE-RESISTANT OR BEECH BARK
DISEASE-SUSCEPTIBLE AMERICAN BEECH

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ABSTRACT

Proteomic analysis of beech bark proteins from trees resistant and susceptible to beech bark disease (BBD) was conducted. Sixteen trees from eight geographically isolated stands, 10 resistant (healthy) and 6 susceptible (diseased/infested) trees, were studied. The genetic complexity of the sample unit, the sampling across a wide geographic area, and the complexity of the BBD “treatment” all contribute to possible protein differences between trees in the study. This complexity required careful study design and more elaborate statistical considerations than many proteomics studies. Identification of up to 101 protein spots unique to an individual tree emphasizes the genetic diversity captured in our study. Despite the experimental complexity, 120 protein spots (22 percent of the matched spots) were identified as BBD significant, so the experiment was effective at finding proteins of interest.

Spots were selected for coring and sequencing based upon the significant BBD effect and the location of the spot in the gel. Sequenced spots have homology to known stress-, insect-, and pathogen- related proteins in other plants. These proteins can be separated into the following classes: reactive oxygen species-induced genes, pathogenesis-related proteins, proteins that control transcription or translation, genes of unknown function, and genes previously thought to have only a metabolic role but currently indentified as stress induced in plants. Taken together, the identification of genes generally identified in stress, insect, and pathogen attack in other plant systems and across several cellular systems indicates that beech trees have an active physiological response to BBD.

We have identified a small number of proteins broadly linked to the BBD disease state of the tree and having a stress-, insect-, or pathogen- related expression in other plant systems. Further study of these proteins should help us understand processes critical to resistance to BBD and to develop biomarkers for selection of BBD-resistant trees before or in early stages of BBD infection in stands.
ETHANOL AND (–)-α-PINENE FOR DETECTING AND MONITORING BARK AND AMBROSIA BEETLES IN THE SOUTHEASTERN USA

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ABSTRACT

Our objective was to verify the need for separate traps baited with ethanol or ethanol and (–)–α-pinene for bark and ambrosia beetles in pine stands of the southeastern U.S. Eight trapping experiments were conducted in stands of mature pine in Alabama, Florida, Georgia, North Carolina, and South Carolina. Sites contained various combinations of the following species of southern pines: Pinus echinata, P. elliottii, P. palustris, P. strobes, and P. taeda. In each experiment, 32 eight-unit multiple-funnel traps were grouped into 8 replicates of 4 traps per replicate with traps spaced 10 to 15 m within a replicate and replicates spaced 15 to 500 m apart. Each trap was suspended by rope between trees such that the bottom of each trap was 0.2 to 0.5 m above ground. The following treatments were randomly assigned to one of the four traps within each replicate: (1) unbaited control; (2) ethanol; (3) (–)–α-pinene; and (4) ethanol + (–)–α-pinene. The release rates for ethanol and (–)–α-pinene were about 1 to 5 g/day at 23 to 25 °C. Collection cups contained RV antifreeze. Collection periods were as follows: Alabama - April 28 to July 10, 2003; Florida (3 sites) - February 25 to May 25, 2002, February 26 to May 26, 2002, and March 30 to June 16, 2004; Georgia - June 12 to August 8, 2002; North Carolina (2 sites) - June 20 to August 20, 2002, and May 1 to August 14, 2003; and South Carolina - April 15 to July 16, 2003.

Traps baited with ethanol were attractive to 10 species of ambrosia beetles (Ambrosiodmus tachygraphus, Anisandrus sayi, Dryoxylon onoharaensum, Monarthrum mali, Xyleborinus saxesenii, Xyleborus affinis, X. ferrugineus, Xylosandrus compactus, X. crassiusculus, and X. germanus) and 2 species of bark beetles (Cryptocarenus heveae and Hypothenemus spp). Traps baited with (–)–α-pinene were attractive to 5 bark beetle species (Dendroctonus terebrans, Hylastes porculus, H. salebrosus, H. tenuis, and Ips grandicollis) and 1 platypodid ambrosia beetle species (Myoplatypus flavicornis). Ethanol-enhanced responses of 5 species (Xyleborus pubescens, Hylastes porculus, H. salebrosus, H. tenuis, and Pityophthorus cariniceps) to traps baited with (–)–α-pinene. The latter bait, (–)–α-pinene, interrupted responses of some ambrosia beetle species to traps baited with ethanol. Of 23 species of ambrosia beetles captured in our field trials, 9 were exotic and accounted for 70 to 97 percent of total catches of ambrosia beetles. Our results provide support for the continued use of separate traps baited with ethanol alone and ethanol with (–)–α-pinene to detect and monitor common bark and ambrosia beetles from the southeastern U.S.
ATTRACTANTS FOR LONGHORN BEETLES IN THE SOUTHEASTERN U.S. - PINE VOLATILES AND ENGRAVER PHEROMONES

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ABSTRACT

Our objective was to determine the effect of adding the binary combination of pine engraver pheromones, ipsenol and ipsdienol, to the binary combination of pine volatiles, ethanol and (-)α-pinene, on catches of some common pine longhorn beetles (Cerambycidae) in the southeastern U.S. Six trapping experiments were conducted in stands of mature pine in four states (Florida, Georgia, Louisiana, and Virginia). Four experiments were conducted in the summer of 2006 and two in the fall of 2006. In each experiment, thirty 8-unit multiple-funnel traps were grouped into 10 replicates of three traps per replicate with traps spaced 10 to 15 m within a replicate and replicates spaced 15 to 30 m apart. Sites contained various combinations of the following species of southern pines: Pinus taeda, P. echinata, P. palustris, and P. elliottii. Sites were selected based on recent history of thinning or prescribed burns in the past 12 months. In Virginia, replicates were divided equally between two locations (>500 m apart). The following treatments were randomly assigned to one of the three traps within each replicate: ethanol + (-)-α-pinene (EA); and ethanol, (-)-α-pinene, ipsenol and ipsdienol (EA + SD). The release rates for ethanol and (-)-α-pinene were about 1 to 5 g/day at 23 to 25 °C whereas ipsenol and ipsdienol were each released at 0.1 to 0.2 mg/d at 23 to 25 °C. Collection cups contained RV and Marine antifreeze. Summer collection periods in 2006 were April 4 to June 6 in Florida, April 11 to June 13 in Georgia, April 4 to May 31 in Louisiana, and May 25 to July 27 in Virginia. Fall collection periods were August 23 to October 4, 2006 in Georgia and September 6, 2006 to January 5, 2007 in Louisiana. Traps baited with the quaternary lure combination of ethanol, (-)-α-pinene, ipsenol and ipsdienol (EA + SD) were attractive to Acanthocinus obsoletus, Astylopsis arcuata, A. sexguttata, Monochamus titillator, M. scutellatus, and Rhagium inquisitor in the southeastern U.S. Attraction of Xylotrechus sagittatus to traps baited with ethanol and (-)-α-pinene (EA) was unaffected by the addition of ipsenol and ipsdienol (SD) to traps.
NOTES ON THE BIOLOGY OF SCYMNUS (PULLUS) CONIFERARUM: AN ADELGID PREDATOR

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ABSTRACT

The conifer lady beetle, Scymnus (Pullus) coniferarum Crotch 1874, was previously collected from five states in the western U.S. and from British Columbia. Whitehead, in his 1967 thesis, noted that all collection records of S. coniferarum were from pine and that he collected large numbers from lodgepole pine and Monterey pine infested with adelgids. In 2008-2009, we collected 303 S. coniferarum adults from several locations in the Seattle, WA, metropolitan area from western hemlock, Tsuga heterophylla, infested with the hemlock woolly adelgid, Adelges tsugae Annand. Sampling was done during the fall and winter in conjunction with sampling for the beetle Laricobius nigrinus Fender. We also sampled fir and western white pine infested with adelgid, but did not recover the lady beetle from these species.

We have reared S. coniferarum through two complete generations in the laboratory on A. tsugae. Adults fed on all stages of A. tsugae, eating a mean of 8.6 eggs, 2.8 nymphs, and 1.0 adult in 48 hours. Survival and development time of the immature stages are similar to those of Scymnus (Neopullus) beetles collected in China and reared on A. tsugae in the laboratory. However, S. coniferarum oviposits and develops best at a temperature range that is 5°C higher than the Chinese lady beetles.

Although S. coniferarum can be successfully reared on A. tsugae, this adelgid may not be its major host. Feeding preference tests were conducted in which adults were provided a choice between the hemlock woolly adelgid and the pine bark adelgid, Pineus strobi (Hartig). An adult ate an average of 2.0 and 0.8 eggs, and 0.7 and 0.1 adults, of P. strobi and A. tsugae, respectively, during the 20 hours it had access to the prey in a petri dish.

The appearance, biology, and feeding preference of S. coniferarum closely resemble another lady beetle in the subgenus, S. suturalis Thunberg. The latter is endemic in Europe, but was introduced (apparently both purposely and accidentally) in the eastern U.S. and is now widely established there. Although S. suturalis does occur on eastern hemlock as both larva and adults and has been reared on A. tsugae in the laboratory, it also favors Pineus spp. adelgids as prey and has been frequently recovered from pines infested with adelgids, in both Europe and the United States. While both of these lady beetles prey and reproduce on A. tsugae in nature and can be reared on this adelgid in the laboratory, neither would be considered ideal agents for biological control of A. tsugae, based on the information now available.
MONITORING ASIAN LONGHORNED BEETLES IN MASSACHUSETTS

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ABSTRACT

An operationally effective trap to monitor the Asian longhorned beetle (Anoplophora glabripennis or ALB) has been a goal of the ALB eradication program since the first beetle was found in New York in 1996. Ground surveying is only ~20 percent effective at identifying infested trees and, although tree climbing is more effective, it is also highly time-consuming and expensive. Our laboratory and greenhouse bioassays showed that the two male-produced compounds, (4-(n-heptyloxy) butan-1-ol) and (4-(n-heptyloxy) butanal), were attractive to both sexes, and several plant volatiles were also attractive to both sexes of ALB adults. Our results from field experiments conducted in 2008 in China showed that ALB virgin females were highly attracted to a mixture of five plant volatiles and male-produced pheromone in Intercept® panel traps. The goal of this current project was to determine the feasibility of a trapping system for detecting ALB in the United States.

In Worcester, MA, 84 Intercept® panel traps were set up on June 16-20, 2009, at the following sites: 42 on or adjacent to the Worcester Country Club, 7 randomly distributed in Dodge Park (major cut area where all infested trees were removed), 20 traps along Nelson Street, and 12 traps in Indian Hill Park. We then added 20 traps on September 10, 2009, at 2 sites: 7 traps on Tacoma Street and Constitution Avenue, and 13 traps between Interstate 190 and Ararat Street. Traps were placed on or near maple trees, within the lower part of the tree canopy, and checked at least twice a month, starting June 15-20, 2009, and ending November 15-20, 2009. Six different lure treatments and an unbaited control were replicated 15 times for a total of 105 traps. Climbers installed the traps on a simple pulley system so that a person on a ladder could release them and lower them to the ground and then return them to the canopy after they were checked. Lures were changed and treatments were rotated between the traps within each site every month. Trap cups were filled with environmentally safe antifreeze.

A total of 9 adult ALB, all females, were caught in the traps, relatively high number compared to a total of only 29 beetles observed in the Worcester area in 2009 by residents or surveyors. The highest number of beetles was caught in the same combination of male pheromone and plant volatiles that was found most effective in China in 2008. Two particularly important locations where beetles were caught were Dodge Park,
where 2 beetles were caught in an area thought to have been cleared of all infested trees, and on Doyle Street where host trees were scarce and scattered. Guided by these trap catches, two new infested trees were found in Dodge Park. To assess efficacy of the traps (as measured by what proportion of nearby beetles were trapped), a survey of the number of new exit holes in the five nearest neighbor host trees to each of the traps is ongoing, with the help of USDA APHIS and a team from Penn State University.
FORESTRY-RELATED PATHWAYS FOR THE MOVEMENT OF EXOTIC PLANT PESTS INTO AND WITHIN THE GREATER CARIBBEAN REGION

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ABSTRACT

Forests of the Greater Caribbean Region (GCR) are important ecologically and economically. These unique ecosystems are under increasing pressure from exotic pests, which may cause extensive environmental damage and cost billions of dollars in control programs, lost production, and forest restoration. Forests may serve as both the source of exotic species when wood or non-wood forest products are exported, and as the area at risk from the introduction of pests. Our objectives were to outline important forestry-related pathways of pest movement and to offer suggestions for improved safeguarding. We reviewed scientific literature, interception data, international forestry sites, and other sources of information to determine potential pathways. Forestry-related pathways include raw wood products (logs, chips, railway ties, firewood), non-wood forest products, and trees for planting. Pests may infest these commodities prior to harvest or transport, or may attach themselves as hitchhikers at any time during the extraction, transport, or trading process.

Countries within the GCR import and export thousands of metric tons of raw wood products each year. Forest pests associated with raw wood products include bark and ambrosia beetles (Coleoptera: Curculioinidae: Scolytinae), wood boring beetles (Buprestidae), longhorned beetles (Cerambycidae), and horntail wasps (Hymenoptera: Siricidae), and pathogenic fungi. Minimally processed wood items, such as wooden handicrafts, musical instruments, tools, and toys may harbor pests. Non-wood forest products include food products, medicinals, bark for dyes and tannins, rattan, palms, and bamboo. We identified more than 300 species of forest pests with limited or no distribution in the GCR that are known to move on raw wood or NWFPs. While this review is not exhaustive, it demonstrates the variety of pests that move on raw wood and NWFP pathways.

Numerous exotic trees have become invasive throughout the GCR. Introduced for plantations or agroforestry, these species are chosen for traits such as rapid growth, high fecundity, and the ability to fix nitrogen – the same characteristics that may enhance their invasive potential. These species can form dense thickets or monocultures, replace native vegetation, disrupt activities of native fauna, and lower the water table. Some invade undisturbed forests, changing species composition and decreasing biodiversity.

The work presented here was carried out in the framework of the CISWG Caribbean Pathway Analysis. The full report is available at: http://carribean-doc.ncsu.edu/index.htm.
THOUSAND CANKERS PATHWAY ASSESSMENT: MOVEMENT OF 
GEOSMITHIA SP. AND PITYOPHTHORUS JUGLANDIS 
BLACKMAN FROM THE WESTERN INTO THE EASTERN UNITED STATES

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ABSTRACT

A newly recognized fungal canker disease of walnut, 
identified by state cooperators, may threaten the 
native range of eastern black walnut, Juglans nigra. 
The causal agent is a Geosmithia fungus (proposed 
name Geosmithia morbida) and the only known 
vector is the walnut twig beetle, Pityophthorus 
juglandis (WTB). The common name for the disease 
is “thousand cankers” due to the coalescing cankers 
surrounding multiple beetle entry points. The WTB 
and thousand cankers disease (TCD) has caused walnut 
mortality in Washington, Oregon, California, Idaho, 
Utah, Colorado, and New Mexico. The eastern edge 
of the disease is the Front Range of Colorado. Our 
objectives were to identify potential pathways for the 
movement of the Geosmithia sp. and its vector from 
the western states to the east; to identify potential 
alternate vectors; and to characterize the risk to the 
east. We identified pathways through consultation with 
experts, interception data, and review of the literature. 
We evaluated the potential movement of TCD east via 
these pathways using a geographic (GIS) approach. 

Potential pathways include timber, firewood, wood 
packaging material (WPM), nursery stock, scion 
wood for grafting, nuts, and natural spread. The most 
critical pathway is raw wood, which moves in a variety 
of forms, including raw logs, burls, stumps, and 
firewood. There is very little commercial movement 
of walnut from the western states into the east, but it 
does occur. Walnut logs, burls, are stumps often used 
by individuals for woodworking, but quantities and 
frequencies of movement are unknown. Low grade 
walnut may be used for WPM and is frequently used 
for firewood. There have been no reports of infested 
nursery stock, but if nurseries become infested with 
TCD, this could become another critical pathway. Nuts 
are not likely to move the disease or its vector. Infected 
scion wood could move the disease, particularly by 
individuals. Natural spread may be facilitated by 
walnuts growing along riparian areas from Colorado’s 
Front Range through the Great Plains States.

The complete pathway assessment may be accessed 
HOST RESISTANCE SCREENING FOR BALSAM WOOLLY ADELGID: EARLY RESULTS FROM 12 FIR SPECIES

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ABSTRACT

Nearly all Fraser fir (Abies fraseri) Christmas trees produced in North Carolina need to be treated one or more times during their 5- to 10-year rotation to prevent or lessen damage caused by the exotic balsam woolly adelgid (BWA) (Adelges piceae Ratz.). These pesticide applications result in an annual cost to the industry estimated at $US 1.53 million not including direct losses due to BWA damage or increased miticide control costs associated with BWA treatments. A BWA resistance screening trial was established in a greenhouse at the Upper Mountain Research Station in Ashe County, NC. The study included 13 fir species (4-year-old seedlings), some representing the range of known susceptibility and some of unknown susceptibility. We included three different seed sources of Fraser fir. Our long-term objective is to develop BWA-resistant Fraser fir trees for native stand restoration and the Christmas tree industry. Our short-term objective is to screen for resistance across multiple fir species (of equal age, grown under the same conditions, with insects from the same source) and to observe the reactions of both host and insect on the various species. The experimental design includes 4 blocks, 13 species (3 seed sources within Fraser fir), 5 treatment trees, in two repetitions (over time), for a total of 600 treatment trees, plus 120 control trees. After one season (summer/fall), one half of the study was dismantled and seedlings brought back to the lab for assessment of first instar settlement. The following spring, the remainder of the study was dismantled and the seedlings brought back for assessment of BWA development and egg production.

Early results indicate that BWA crawlers appear to settle preferentially on the buds of young fir trees. Development and egg production occur at the bases of buds, under old bud scales, and at the base of needles. BWA develops and produces eggs on each species, but the numbers of adults and eggs produced vary widely. Abies fraseri (Roan Mountain) is the most susceptible, in terms of the volume of eggs produced per cm branch, but A. lasiocarpa var. arizonica allows for the highest egg production per female (mean of 30 eggs per female). Abies veitchii is known as one of the most resistant fir species and exhibits very little development overall; the females that do develop lay only a few eggs. The three species of “unknown resistance” (Abies bornmuellariana, A. equi-trojani, and A. pindrow) appear to be susceptible to infestation by BWA (with a mean of 25, 16, and 7 eggs per female, respectively.)
INITIAL SURVEY OF PREDACIOUS DIPTERA ON HEMLOCKS IN JAPAN

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ABSTRACT

Some species of Coleoptera and Diptera are specialist predators of adelgids. Previously, we reported our survey of predacious Coleoptera on hemlocks in Japan (Shiyake et al. 2008). Two of these beetles, Sasajiscymnus tsugae and Laricobius sp. nov., have been exported to the U.S. for biological control. Here, we provide the first report of Diptera collected in Japan from its native hemlocks, Tsuga sieboldii and T. diversifolia.

Monthly surveys were made from 2005 to 2009 of hemlock in the Kansai Region and yearly at other areas on the map (Fig. 1). Nets on long poles that could reach up to 5 m were used to sweep the canopy. Hemlock woolly adelgid (HWA)-infested hemlock twigs also were collected for examination with a microscope in the lab. About 250 specimens of Diptera were collected and identified to family and morpho-species. Our primary source for nomenclature and background information on the feeding behavior of the family was the Manual of Nearctic Diptera (McAlpine et al. 1981). Twenty-six families of Diptera, representing >100 species, were collected from Japanese hemlocks. Ten of the families include some species with terrestrial predacious larvae, and four of these have species that feed on Aphidoidea (aphids, adelgids and phylloxera) and are of special interest to us: Cecidomyiidae is a family of midges, most of which feed on plants, but the genera Aphidoletes and Lestodiplosis are predators of Homoptera. Aphidoletes aphidimyza Rondani is used for biological control of aphids. These genera were collected in North Carolina from hemlock (Wallace and Hain 2000), but not in Oregon (Kohler 2007). This family was scarce in our aerial collections.

Syrphidae, or hover flies, have many well-known predators of Homoptera, but have had little success as biological controls. Heringia familiaris Matsumura was collected as a larva from heavily infested hemlock on Shikoku Island and reared to adult. Considering the abundance of hover flies in nature, we collected surprisingly few from hemlock.
**Chamaemyiidae**, or silver flies, is a small family that feed on scale insects and Aphidoidea. The genera *Neoleucopis*, *Leucopis*, *Lipoleucopis*, and *Cremiferania* have species that are adelgid specialists. *Leucopis taipiae* Blanchard has successfully been used for biological control of pine adelgid in several countries. Many species were released in North America for control of *Adelges piceae* and several reportedly established, but none have provided effective control. *Leucopis argenticollis* Zetterstedt, a cosmopolitan species, was collected from HWA in Oregon (Kohler 2007). This family seems to be rare on hemlock in Japan; we collected no adults and only one puparium.

**Chloropidae** are mostly phytophagous and known mostly as pests of cereal grasses. Some are predacious and several species in the genus *Thaumatomyia* feed on Homoptera. Chloropidae were very abundant in our sweep net collections and may have been attracted to the honeydew of HWA and *Cinera* sp. aphids feeding on the hemlock. We will use a recently acquired key (Kanmiya 1983) to see if any of the taxons we collected belong to predacious genera or species. Future work on Diptera will make more a thorough collection, including larvae on HWA infested foliage. Larvae will be observed for feeding on HWA and reared to adults for identification to species. We especially want to determine the family of the very small fly larvae we find on HWA infested foliage in Japan.

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**Literature Cited**


ANALYSIS OF AREA-WIDE MANAGEMENT OF INSECT PESTS BASED ON SAMPLING

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ABSTRACT

The control of invasive species greatly depends on area-wide pest management (AWPM) in heterogeneous landscapes. Decisions about when and where to treat a population with pesticide are based on sampling pest abundance. One of the challenges of AWPM is sampling large areas with limited funds to cover the cost of sampling. Additionally, AWPM programs are often confronted with cooperators/farmers that resist participating in those programs. In this study, we evaluated AWPM programs based on sampling to determine under what conditions they reduce pest abundance and decrease pesticide use. We focused on a general insect pest, but results should be relevant to other pests that can be sampled before control.

We created an abstract model of the population dynamics of a pest inhabiting 1,600 sites in a 40x40 unit region. The pest has multiple generations per year and can move from natal site to any of eight neighboring sites in each generation. Population abundance and population parameters vary from site to site. Accordingly, simulations were completed under assumptions about pest reproduction and biology that resulted in either low or high variation in pest abundance among fields. Fields were sampled to assess pest abundance on fixed intervals. Two AWPM programs were compared: synchronous and asynchronous. Synchronous AWPM treats all sites simultaneously once a spray threshold based on the mean pest density for the region is exceeded. Asynchronous AWPM permits cooperators to treat each site based on a sample from that site (the spray threshold is the same for all sites). In some simulations, site managers did not cooperate and those sites were never treated.

Synchronous AWPM can succeed even when a high proportion of fields are not sampled, but this type of AWPM allows pests in some fields to exceed acceptable levels as variation increases in the landscape. Because of high emigration from untreated fields in low variation scenarios, the number of treatments increases significantly as participation declines.
SCANNING ELECTRON MICROSCOPY
AS A TOOL IN UNDERSTANDING
HEMLOCK WOOLLY ADELGID
BIOLOGY, FEEDING BEHAVIOR,
AND HOST PLANT RESISTANCE

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ABSTRACT

Using scanning electron microscopy, details surrounding feeding mechanisms of the hemlock woolly adelgid (Adelges tsugae Annand) are studied in detail. Stylet bundles are inserted on the adaxial side of the hemlock needle, between the abscission layer and the stem and needle cushion junction. Sheath material, a gel-like material made by the salivary glands, is produced to surround the styles. It is observed on the surface of the host plant and is known to be used internally. Sheath material is similar in size and form to fungal hyphae (also found on the host), but can be distinguished by the bead-like appearance. Future studies will include analysis of resistant and susceptible hemlock species and more investigation into sheath material presence, location, and function.
NORTH AMERICAN HOST TREE RESPONSE TO
AMYLOSTEREUM AREOLATUM, THE FUNGAL
SYMBIONT OF THE WOODWASP SIREX NOCTILIO

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ABSTRACT

*Sirex noctilio* Fabricus (Hymenoptera: Siricidae), a wood boring wasp native to Europe, western Asia, and northern Africa, is now considered established in New York, Pennsylvania, Michigan, New Hampshire, and southern Quebec. A single *S. noctilio* adult was found in Vermont in 2007. *S. noctilio*, together with its obligate symbiotic fungi, *Amylostereum areolatum*, will likely negatively affect populations of North American pines, because these species are the preferred hosts of the wasp. Female *S. noctilio* deposit asexual spores of *A. areolatum* in a host tree during oviposition. The fungus then grows, serving as food for the wasp larvae and eventually killing the tree. It is unknown how vigorous and stressed pines respond to and defend against the fungus. The purpose of this study is to simulate *S. noctilio* attack and evaluate the interaction between conifer hosts and *A. areolatum*. Overall physiological responses in white pine (*Pinus strobus*) and red pine (*P. resinosa*), particularly tree defense mechanisms deployed against *A. areolatum*, are quantified. Information collected during this study will further understanding of the impacts that *S. noctilio* will have on North American forests, identify signs and symptoms of attack, and support the development of silvicultural practices that minimize damage caused by *S. noctilio*. Preliminary results are presented.
RELATIVE POTENCIES OF GYPSY MOTH NUCLEOPOLYHEDROVIRUS GENOTYPES ISOLATED FROM GYPCHEK

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ABSTRACT

Gypchek is a gypsy moth (Lymantria dispar L.) - specific biopesticide whose primary use is for treating areas where environmental concerns outweigh the use of broad-spectrum pesticides for gypsy moth management. Gypchek is a lyophilized powder produced from larvae that have been infected with the gypsy moth nucleopolyhedrovirus (LdMNPV). The product contains a mixture of closely related LdMNPV genotypes that, in combination, act as the active ingredient. The genotypes vary in quantity and quality (virulence) and account for the variability in viral occlusion body (OBs) yield from one in vivo Gypchek lot to the next. Which genotypes account for most of the in vivo replication is indeterminable. We continue to search for and test virulent strains of LdMNPV amenable both for large-scale in vitro (cell culture) production and for the development of a “single” genotype or a “cocktail” of genotypes, from which OB yield can be maximized and viral activity “standardized” from one in vivo production lot to the next. Plaque-purified viral genotypes (122b, 122hp, 203, B1B, and MPV) were isolated from Gypchek powder and propagated in fourth stage gypsy moth larvae. In bioassays, second stage gypsy moth larvae from a standard New Jersey colonized strain were challenged with a range of doses of Gypchek, 122b, 122hp, 203, B1B, and MPV OBs, incorporated into artificial diet. Probit analysis (PoloPlus, 2.0, LeOra Software) was used to determine and compare Lethal Concentration (LC) values from the larval mortality data.

Based upon calculated 95-percent confidence limits, both 122hp (LC50=1568 OBs) and 122b (LC50=2128 OBs) were equally potent and more active against the laboratory strain of gypsy moth than either Gypchek (LC50=6896 OBs) or any of the other genotypes tested: 203 (LC50=7414 OBs), MPV (LC50=13113 OBs), B1B (LC50=18079 OBs). Differences in potencies were supported by tests for parallelism and equality of slopes of the regressions. From that data, it appeared that 122b and 122hp were about three times as potent as Gypchek. Further laboratory studies are indicated to confirm the differences in potency over several in vivo and in vitro passages. If and when these elevated potencies are confirmed, field experiments to demonstrate efficacy of these genotypes as products formulated from either in vivo or in vitro productions will be designed and executed.
DETECTION OF EMERALD ASH BORER, 
AGRILUS PLANIPENNIS, AT LOW 
POPULATION DENSITY

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ABSTRACT

The exotic emerald ash borer (EAB), Agrilus planipennis (Coleoptera: Buprestidae), was first discovered in North America in Detroit, MI, in 2002. This beetle has killed millions of ash trees in several states in the United States and in Canada, and populations of this insect continue to be detected. EAB is difficult to detect when it invades new areas or occurs at low density. For the foreseeable future, trap tree and ground surveys will be important tools for detecting emerald ash borer populations. In 2008, a large-scale survey using baited purple prism traps was carried out in the United States. The effectiveness of baited purple traps compared with trap trees in areas with low EAB populations is unclear.

In 2008, a field experiment was established at Straits State Park, St. Ignace, MI, to characterize the effectiveness of different trap types in an area of known low-density EAB infestation. The questions studied included: (1) Are girdled trap trees, non-girdled trap trees, or purple prism traps hung in ash trees more effective at trapping adult beetles? (2) Are girdled trap trees, non-girdled trap trees, or purple prism traps hung in ash trees more effective at detecting EAB larvae? and (3) Do canopy assessments of trees indicate whether a tree is infested with EAB? A trapping survey and canopy assessment was conducted during the flight season of EAB. All trap trees, including those that had purple traps hung in them, were then cut and fully peeled in the fall to look for larvae.

Adult beetles were not detected using the baited purple prism trap during the trapping survey. By cutting and peeling the trap trees, it was shown that girdled trap trees acted as sinks for larvae at this low density site; however, the canopy assessment showed that the canopy condition of these trees was not predictive of whether they were infested or not. Of the girdled trees, infested trees were larger in diameter than the non-infested trees. Based on these findings, the use of large girdled trap trees may be the most effective tool for detecting EAB populations at low density.
DISJUNCT POPULATION OF REDBAY AMBROSIA BEETLE AND LAUREL WILT DISEASE DISCOVERED IN MISSISSIPPI

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ABSTRACT

Laurel wilt is an aggressive, non-native vascular wilt disease of redbay trees (Persea borbonia), sassafras (Sassafras albidum), and other plants within the Lauraceae family. The laurel wilt pathogen, (Raffaelea lauricola), is vectored by the redbay ambrosia beetle (Xyleborus glabratus), which was first detected in Georgia in 2002. Since then, laurel wilt disease has caused severe mortality to redbay trees in Georgia, Florida, and South Carolina. In June of 2009, a landowner in Gautier, MS, contacted the Jackson County, Mississippi Extension Service and reported dead redbay trees on his property. Signs and symptoms identical to those reported for laurel wilt along the Atlantic coast were observed at the home and several other sites in the surrounding area. In July 2009, chips of discolored sapwood were collected from three symptomatic redbay trees in Jackson County, MS. Molecular confirmation of the identity of the fungus was completed using 18S sequences of rDNA and exhibited 100 percent homology to previously submitted isolates of R. lauricola. Redbay ambrosia beetle was also positively identified at the site.

This discovery of a disjunct infestation of laurel wilt disease in Mississippi is at least a decade ahead of previous predictions, and no records of laurel wilt have been reported from western Georgia, all of Alabama, or the Panhandle of Florida. At this time, the only confirmed infested locations in Mississippi are in Jackson County, along the Interstate 10 corridor and the Pascagoula River drainage. The mode of introduction is still unknown.

Twenty-six Lindgren funnel traps baited with phoebe and manuka oils were installed in the six southeasternmost counties of Mississippi and monitored bi-weekly from July to November 2009. Peak beetle capture occurred in early October, slightly later than reported in other parts of the U.S. The number of beetles captured per trap per day in Mississippi during 2009 ranged from 0.02 to 1.22. Previous reports of infestations in Georgia and South Carolina reported that “older infestations” usually yielded 0.04 to 0.12 beetles/trap/day, while “newer infestations” yielded 4 to 7 beetles/trap/day. This, along with the lack of fallen, decomposed redbays in Mississippi, seems to indicate that the Mississippi infestation began 2 to 3 years ago.
COMPARISON OF FECUNDITY AND SURVIVAL
OF HEMLOCK WOOLLY ADELGID (HEMIPTERA:
ADELGIDAE) IN NORTHERN
AND SOUTHERN POPULATIONS

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an introduced species first reported in the eastern United States in 1951. The infestation has since spread in all directions from its initial sighting in Virginia, to its current range from northern Georgia, to southern Maine, and westward into Tennessee, causing substantial mortality to eastern (*T. canadensis*) and Carolina (*T. caroliniensis*) hemlocks. Tree death often occurs in as little as 4 years, especially in southern populations; however, in New England, it has been shown that *A. tsugae* populations may often persist at relatively low density for many years. Various studies have shown that cold winter temperatures cause high mortality in northern populations and in some years are sufficient to cause major declines in *A. tsugae* population density, but whether overwintering mortality can account for the apparent low density stability of *A. tsugae* at these sites remains to be demonstrated. We initiated a study to quantify *A. tsugae* density and fecundity in southern sites (2003-2008). Furthermore, overwintering mortality increased significantly with *A. tsugae* density in the north (PROC Logistic SAS 9.13; logit p = 0.24+4.07*log10(x+1), df=1, Wald Chi sq = 267.7, P<0.001), while no density-dependent trend was evident in the south (PROC Logistic SAS 9.13; logit p=-0.6.878+0.1474*log10(x+1), df=1, Wald Chi sq= 0.7375, P=0.3905). Overwintering mortality may thus contribute to the evident stability of northern *A. tsugae* populations. We note, however, that this mortality may not increase with density in all years as reported by previous investigators.

We found no significant relationship between density and fecundity in either the north (F=0.0122, df=30, p=0.9127) or the south (F=2.1164, df=66, p=0.1505). This result was surprising in that McClure reported pronounced reductions in fecundity as HWA populations declined from high density. We chose populations of HWA at southern sites that had only recently been infested, so that we could document the entire course of the expected rise and fall of HWA populations. Thus, we may have quantified fecundity during the increasing phase of the HWA populations at these sites before the expected decline in density and associated reduction in fecundity. We plan to collect more data along these lines in the coming year to see if this expected pattern occurs.
THE GOLDSPTED OAK BORER: AN OVERVIEW OF A RESEARCH PROGRAM FOR “CALIFORNIA’S EMERALD ASH BORER”

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ABSTRACT

A new threat to oaks in California was identified in June 2008 following years of misdiagnosis. The goldspotted oak borer (GSOB), Agrilus coxalis auroguttatus Schaeffer (Coleoptera: Buprestidae), is aggressively attacking and killing three species of oaks in San Diego County (Coleman and Seybold 2008a, b; Hespenheide and Bellamy 2009). About 20,000 coast live oaks, Quercus agrifolia, California black oaks, Q. kelloggii, and canyon live oaks, Q. chrysolepis, have died in a 4,903-km² area centered near the Descanso Ranger District of the Cleveland National Forest and Cuyamaca Rancho State Park. Oak mortality has been continuous for the past 8 years and occurs on all land ownerships. Because the two subspecies of GSOB are native to southeastern Arizona, Guatemala, and southern Mexico, and the indigenous populations have never been reported to be associated with extensive tree injury or mortality, we hypothesize that elevated oak mortality in southern California has occurred because of an absence of evolved host resistance in native oaks and/or an absence of natural enemies found in GSOB’s native range. The native distributions of the California hosts of GSOB extend north through most of the state along the coastal foothills and the Sierra Nevada. Thus, this new pest to oaks has the potential to cause a more widespread decline of oaks in more northerly regions in California and perhaps beyond. The recognition of the national significance of this new mortality agent was highlighted in the most recent annual National Forest Pest Risk Assessment (U.S. Forest Service 2009).

To address this critical problem, we have drafted a research plan to stimulate and organize collaborative work among scientists from the U.S. Forest Service, Forest Health Protection-R5 and R-3, and the Northern, Pacific Southwest, and Southern Research Stations; USDA APHIS-CPHST, and the University of California, Davis and Riverside. The principal participants from the agencies are Tom Coleman, Joel McMillin, and Sheri Smith (FHP); Nancy Grulke and Steve Seybold (PSW); Rob Venette (NRS); Brian Strom (SRS); Damon Crook (USDA APHIS-CPHST); Mary Louise Flint (UCD), and Mark Hoddle, Tom Scott, and Richard Stouthamer (all UCR).

PROGRAM CONTENT

SURVEY AND DETECTION

- Establishing the distribution of GSOB (2009-2010)
  ○ We are trapping at more than 40 sites with unbaited purple prism traps to infer the current distribution and population densities throughout southern California, the Central Valley, and the central and southern Sierra Nevada.
  ○ Current Progress: In 2009, the distribution of GSOB continued to be confined to San Diego County, although new finds were made to the north, south, and west of the initially delineated distribution and the size of the infested area increased from 1,200 to 4,903 km². A satellite location was discovered near La Jolla through ground survey. Sites were selected and traps
installed before May 15 each year, and traps were sampled following peak activity of GSOB (late July).

- Products/Measures: This work will delimit the current extent of the infestation in California and provide the justification and scope for a proposed quarantine.
- Collaborators: Coleman and Seybold
- Supported initially in part by a Detection Monitoring grant from the National Forest Health Monitoring Program and Forest Health Protection (FHP), Region 5.

**Improving GSOB survey techniques (2009-2011)**

- We are assessing various trapping treatments that vary trap color, trap height, and potential lures in the field. We are conducting electroantennal detection and retinalgram work on GSOB adults following volatile and bark/foliage reflectance assessments.
- Current Progress: Trap color and height were assessed between January and September 2009; trap bait type was assessed between June and September 2009. In July 2009 and March 2010, oak bark volatiles were sampled from coast live, canyon live, and Engelmann oaks to guide us in future bait development.
- Products/Measures: Initial trapping results suggest that trap color (purple vs. green) and height do not have a strong effect on trap catch. None of the commercial emerald ash borer lures were very attractive.
- Collaborators: Crook, Coleman, and Seybold
- Supported by APHIS-CPHST (2010-2011) through a cooperative agreement with UC-Davis.

**BIOLOGY**

- **Establishing the life cycle and flight period for GSOB**
  - Adult trapping is being conducted at two elevational levels (1,000 and 1,800 m) in GSOB-infested areas in California.
  - Current Progress: Flight activity in California began during the week of May 22, 2009, and continued through October 2009. A method was developed for separating the sexes of adult GSOB based on external morphological characters (Coleman and Seybold 2010b).
  - Products/Measures: The results of this work will allow us to more accurately time future surveys and management activities.
  - Collaborators: Coleman and Seybold
  - Supported in part by a Detection Monitoring grant from the National Forest Health Monitoring Program

**IMPACT AND MANAGEMENT**

- **Surveying the native distribution of GSOB in Arizona (2009-2010)**
  - Current Progress: With the assistance of Coronado National Forest staff, we monitored purple EAB traps at seven sites in the Santa Rita and Huachuca Mountains from February to August 2009. No specimens of GSOB were recovered from these traps. Forest stand data were also collected to compare to California forest conditions (see “impact” below). Ground surveys were conducted in Arizona to determine the native hosts of GSOB and its impact in the native forest stands. Infested trees were examined and infested bark was collected.
  - Products/Measures: We have determined native oak hosts in Arizona and found several natural enemies (Coleman and Seybold in prep.). Collections of GSOB from this area will also allow us to determine the source of the California population (see below).
  - Collaborators: Coleman, Seybold, McMillin, and Coronado National Forest staff.
  - This work is being supported by the U.S. Forest Service FHP Forest Health Technology Enterprise Team and the Pacific Southwest Research Station.

- **Evaluating impact of GSOB in forest stands (2008-2010)**
  - Long-term plots have been established in southern California and Arizona to record tree mortality, percentage of infested trees,
species composition, diameter class, tree health, regeneration, etc.

○ Current Progress: Plots have been established on the Cleveland and the Coronado National Forests. Additional forest stand data are being collected this year.

○ Products/Measures: Current GSOB infestation rates are reaching ~90 percent in areas with tree mortality around Pine Valley, CA. The GSOB infestation is expanding to the north, south, and west from the Descanso Ranger District (Coleman and Seybold in prep.). In Arizona, infestation and mortality rates are very low, but native hosts have been identified.

○ Collaborators: Coleman, Flint, McMillin, and Seybold

○ Supported in part by a cooperative agreement between the U.S. Forest Service, Pacific Southwest Research Station, and UC-Davis.

● Biological Control: Evaluating the population genetics of GSOB and its potential natural enemies

○ Current Progress: Molecular analyses are being conducted on California, Arizona, and Mexican populations of GSOB by Stouthamer’s lab in the Department of Entomology at UC-Riverside to establish the source of the California population. Contacts have been made with Mexican partners to evaluate the impact of GSOB in Mexico. Surveys were conducted in Arizona for natural enemies, and wasps were reared from parasitized GSOB larvae collected from an Emory oak in Arizona. Specialists have identified two species: Calosota sp. (Eupelmidae) (more abundant) and Atanycolus simplex Cresson (Braconidae) (less abundant) (Coleman and Seybold in prep.). The former species appears to be new to science and is being described. Survey trips to discover additional natural enemies have been made to Arizona and Mexico by Hoddle and Coleman.

○ Products/Measures: This study represents a potentially long-term management solution by re-uniting the parasite complex from verified source populations of GSOB in Arizona with the introduced population of GSOB in California.

○ Cooperators: Hoddle, Stouthamer, Seybold, and Coleman

○ Supported by U.S. Forest Service FHTET, USDA FHP, U.S. Forest Service FHP International Activities and Travel, and the U.S. Forest Service Western Wildlands Environmental Threat Assessment Center (WWETAC). Funding is being used to support the Ph.D. program of a UC-Riverside graduate student, supplies, and travel to Arizona and Mexico.

● Evaluating the drought stress status of oaks within and outside of the zone of infestation (2009-2011)
○ We are assessing drought stress in uninfested and infested coast live oaks at four sites along a north-south transect that passes through the zone of infestation.

○ Current Progress: Plots were established in June 2009 and one season of data were collected. Data collection will continue in 2010 and 2011.

○ Products/Measures: Initial results suggest that oaks in the survey plots are not significantly water stressed, which implies that GSOB is injuring and killing healthy trees.

○ Cooperators: Grulke, Coleman, and Seybold.

○ The U.S. Forest Service FHP National Forest Health Monitoring Program began supporting this work in 2010.

• Developing technology to more accurately determine the potential distribution and impact of GSOB (2010-2013)

○ We are determining the intrinsic flight dispersal of GSOB by mark-recapture of beetles marked with fluorescent powder; establishing the physiological host range by using lab rearing studies; and conducting a pest risk assessment for California and Oregon.

○ Products/Measures: Project will begin in June 2010.

○ Cooperators: Venette, Coleman, Flint, and Seybold.

○ Project is being supported by the U.S. Forest Service Special Technology Development Program (2010-2013). Funding will support the research of a postdoctoral associate (A.D. Graves) from the UC-Davis Department of Entomology.

• Testing the efficacy of systemic and topically applied insecticide treatments

○ Three systemic insecticides are being assessed to prevent tree mortality from GSOB.

○ Current Progress: Trees were treated in 2009 and 2010, and foliage was sampled three times in 2009 for efficacy. In 2009, systemic insecticide treatments included soil and stem injections, whereas in 2010, two types of stem injections are being assessed. Topically applied insecticides are also being tested in 2010.

○ Products/Measures: This study will determine the effectiveness of systemic insecticides in preventing tree mortality and provide insight on treatment timing and duration.

○ Cooperators: Strom, Smith, and Coleman.

○ Supported by U.S. Forest Service FHP, R5.

• Evaluating tree health and time of mortality following GSOB infestation

○ Current Progress: Tree health and time of mortality are being assessed to determine management strategies. A health class rating system has been developed to determine tree decline in coast live oak. Trees were tagged across a range of health classes and will be followed in the future.

○ Products/Measures: Information from this project will allow land managers to determine the probability of whether a tree can be treated and saved.

○ Cooperators: Coleman, Grulke, and Seybold.

○ Supported by the U.S. Forest Service FHP National Forest Health Monitoring Program and the Pacific Southwest Research Station.

PROGRAM PRODUCTS

Forty-one presentations (scientific/technical or outreach) on GSOB have been delivered since late 2008 by Coleman, Seybold, or Flint. Three trainings have been held to “train the trainer” on the Cleveland National Forest. A Pest Alert, several outreach and educational materials, six symposium proceedings, and three peer-reviewed publications were produced between 2008 and 2010.


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Coleman, T.W.; Seybold, S.J. In prep. *Collection history and comparison of the interactions of the goldspotted oak borer, Agrilus coxalis auroguttatus* Schaeffer (Coleoptera: Buprestidae), with host oaks in southern California and southeastern Arizona. The Coleopterists Bulletin.


USING A MULTICRITERIA RISK MODEL TO GUIDE GROUND SURVEYS FOR EMERALD ASH BORER

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ABSTRACT

The emerald ash borer (EAB) (Agrilus planipennis) is an exotic wood boring beetle from Asia that has become a major pest of ash trees (Fraxinus spp.) in the Midwest. The insect has spread from its initial invasion site in southeastern Michigan aided by human movement of firewood, nursery stock, and other wood materials. Well-established populations of EAB have been found in the Upper Peninsula (UP) of Michigan, an area relatively isolated from the initial invasion site. This has prompted questions about the likelihood of other existing populations in the UP and the possible use of landscape characteristics to predict the locations of EAB satellite invasions.

Known locations of EAB were used to determine common characteristics of invaded sites and develop a multicriteria risk model. Known locations of EAB were divided into two groups, one to build the model and one to validate the predictions. The model was assessed by calculating accuracy as the percent of known locations correctly predicted to be at moderate to high risk. Important parameters in the model were distance to roads, land cover type, and proximity to campgrounds. The accuracy of the points used to build the model was 77 percent, with 19 percent of the land area of the UP and northern Lower Peninsula (LP) of Michigan at moderate to high risk. Validation of the model was made using the known EAB points set aside during development with 83 percent of the validation points accurately predicted to be at moderate to high risk.

Using the predictions, we developed a ground survey to visit random points across the UP focusing on high risk areas. At each point, basal area by species was determined and ash trees within a 1/10-acre plot were counted and assessed, recording species, diameter, percent dieback, and vigor as well as any signs and symptoms of EAB including sprouts, splits in the bark, D-shaped exit holes, serpentine galleries, or woodpecker damage. During the ground survey, no new populations of EAB were discovered. The lack of detection could in part be due to the difficulty in locating ash, because only 22 percent of points had ash present. Further information on the spatial distribution of ash resources should improve the model accuracy and success of the ground survey. It is likely that the model overpredicts the area of high risk; it is more realistic to assume that only 4 percent of the UP is at high risk as a result of a limited amount of ash. Future work will involve refining the model and incorporating it into monitoring efforts. The predictions should also prove useful for locating infested trees when detection is made on artificial traps.
FACTORS AFFECTING THE DISTRIBUTION AND
ABUNDANCE OF EXOTIC EARTHWORMS
IN THE HURON MOUNTAIN CLUB,
UPPER PENINSULA, MICHIGAN

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ABSTRACT

Exotic earthworms are becoming established in previously earthworm-free areas of the Great Lakes region with the potential to alter forest ecosystems. Understanding the factors controlling their distribution and abundance across the landscape will aid in efforts to determine their consequences and potential forest management solutions. Presence and abundance of earthworms were measured at the Huron Mountain Club (HMC) in the Upper Peninsula (UP) of Michigan to characterize factors influencing their distribution and abundance.

A preliminary GIS-based risk model for earthworm invasion was developed and used to select randomly located sample points stratified across risk levels and focusing on high risk areas. Earthworm presence was confirmed by earthworm signs and middens created by the deep burrowing *Lumbricus terrestris* (common night crawler). At each sampling point, site characteristics and aspects of earthworm invasion were recorded including midden count, forest floor condition (ranked from 1-highly altered to 5-minimally altered), earthworms signs (castings, salt and pepper, missing layers), and presence of earthworms.

Signs of earthworm activity were observed at 57.6 percent of the points visited, while middens (exclusive to *L. terrestris*) were observed at 20.3 percent of points. As expected, forest floor condition was related to midden count: those sites with more middens had lower quality forest floors as altered by *L. terrestris*. Points with middens present were significantly closer to roads than those without (*p* < 0.001). The number of preferred trees present (e.g., basswood, maple, ash, and balsam fir) was significantly greater on sites with middens than without (*p* = 0.009), as was the percent of preferred trees of all those present (*p* = 0.012). Points with middens had significantly higher soil pH than those without (*p* < 0.001), although pH may not affect the abundance of middens, suggesting a possible threshold effect. No relationships were found between earthworm activity and distance to hydrologic features, drainage index, elevation, or slope. Analysis of the field and available GIS data is ongoing, and additional factors may be found to be significant. The results will be used to develop a spatial model using significant factors to predict the potential distribution of earthworms across the UP.
FINE STRUCTURE OF SELECTED MOUTHPART SENSORY ORGS OF GYPSY MOTH LARVAE

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ABSTRACT

Gypsy moth larvae, Lymantria dispar (L.), are major pest defoliators in most of the United States and destroy millions of acres of trees annually. They are highly polyphagous and display a wide host plant preference, feeding on the foliage of hundreds of plants, such as oak, maple, and sweet gum. Lepidopteran larvae, such as the gypsy moth, depend largely on their gustatory and olfactory sensory organs (sensilla) to find food sources. Feeding behavior is controlled by input from the mouthpart gustatory sensilla. The majority of these larvae possess four types of bilateral gustatory sensilla. One type, the lateral and medial styloconic sensilla, is thought to play a decisive role in host plant selection behavior. These sensilla are in continuous contact with plant sap during feeding and are capable of detecting different phytochemicals. These sensilla were examined using both scanning and transmission electron microscopy. Ultrastructural examination of the styloconic sensilla of fifth instar larvae revealed that each sensillum is comprised of a small cone inserted into a tall style. Each sensillum is ≈ 70 um in length and 30 um in width and consists of a single sensory peg inserted into the socket of a large style. Each peg bears a slightly subapical terminal pore averaging 317 nm in lateral and 179 nm in medial sensilla and houses five bipolar neurons. The proximal dendritic segment of each neuron gives rise to an unbranched distal dendritic segment. Four of these dendrites terminate near the tip of the sensillum below the pore and bear ultrastructural features consistent with contact chemosensilla. The fifth distal dendrite terminates near the base of the peg and bears ultrastructural features consistent with mechanosensilla. Thus, each sensillum bears a bimodal chemo-mechanosensory function. The distal dendrites lie within the dendritic channel and are enclosed by a dendritic sheath. The intermediate and outer sheath cells enclose a large sensillar sinus, whereas the smaller ciliary sinus is enclosed by the inner cell. The neurons are ensheathed successively by the inner, intermediate, and outer sheath cells. The results of this morphological research allow us to more easily characterize the neurophysiological responses of gustatory receptor neurons housed within these sensilla, which is research currently ongoing in the laboratory. It also allows us to further our understanding about the processing of gustatory information by these taste organs.

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ABSTRACT

The Pennsylvania Department of Agriculture (DOA) participates in a variety of insect surveys each year to detect new and potentially invasive species affecting plants in Pennsylvania. Surveys are carried out by seasonal survey crews, permanent DOA staff, and cooperating agencies. Regardless of the target organism of a particular survey, specimens of the following groups are submitted to and identified to species by entomologists at the Pennsylvania DOA: Curculionidae (Scolytinae), Cerambycidae, Buprestidae, Pentatomoidae, Membracidae, Siricidae, and Apiodea. Surveys for exotic wood destroying insect pests were implemented in 2008 and 2009 targeting *Agrilus planipennis* Fairmaire, *Agrilus biguttatus* (Fabricius), *Anoplophora glabripennis* (Motschulsky), invasive Scolytinae, and invasive Cerambycidae. In some instances, experimental lure combinations proposed by cooperating partners were used. Most surveys are conducted in cooperation with the USDA and U.S. Forest Service as part of national cooperative programs. Results for Cerambycidae, Buprestidae, and Scolytinae are compiled and presented. The combined surveys recorded 134 species of Cerambycidae totaling 4,373 specimens, 66 species of Buprestidae totaling 7,131 specimens, and 74 species of Scolytinae totaling 16,889 specimens.

Special acknowledgments are given to Dr. Larry Hanks and Dr. Jocelyn Millar for the use of “general Cerambycidae lures” and trapping protocols, to Dr. Kelli Hoover and Maya Nehme for *Anoplophora glabripennis* pheromone/plant volatile lures and trapping protocols, and to Robert Androw for sugar bait attractant and trapping protocols.
DEVELOPMENT OF RESTRICTION ENZYME ANALYSES TO DISTINGUISH WINTER MOTH FROM BRUCE SPANWORM AND HYBRIDS BETWEEN THEM

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ABSTRACT

Elkinton et al. recently completed a survey of northeastern North America for the newly invasive winter moth, *Operophtera brumata* L. The survey used traps baited with the winter moth pheromone, which consists of a single compound also used by Bruce spanworm, *O. bruceata* (Hulst), the North American congener of winter moth. Our traps filled with both species, and they are difficult to tell apart because wing characters are unreliable. Eidt et al. showed that the two species could be distinguished based on different shapes of the male genitalia. However, we found that many moths in the region where the two species co-occurred had intermediate genitalia, suggesting that they might be hybrids. To develop a more reliable identification, we extracted DNA and amplified and sequenced the barcoding gene, cytochrome oxidase subunit I (COI) and showed that the two species differed by 7.4 percent of their nucleotides. To distinguish hybrids, we amplified and sequenced the nuclear gene glucose-6-phosphate dehydrogenase (G6PD). We found the two species differed by six nucleotides in the G6PD fragment that we sequenced of which three were completely reliable. Hybrids (at least F1 hybrids) were heterozygotic at all six sites. Here we demonstrated a G6PD restriction enzyme assay based on the Taq I enzyme that cut the 233 bp G6PD fragment for Bruce spanworm into two fragments at ~179 and 54 bp. The winter moth G6PD sequence doesn’t have this enzyme site so the band size is at ~233 bp as an uncut PCR product. The hybrid samples, having the DNA from winter moth and Bruce spanworm parents, had all three fragments with the size of 233, 179, and 54 bp. Running the two assays together allowed one test to confirm the species ID with the other. In the case of hybrids, the G6PD test would identify the hybrid and the COI test would identify the female parent.

Based on the COI sequence previously obtained by Elkinton et al., we demonstrated expected fragments for the digest with Sac I enzyme for the winter moth samples (~241 and 453 bp). The Bruce spanworm samples don’t have a Sac I enzyme site; therefore, the bands size are at ~694 bp as an uncut PCR product. Because COI is a mitochondrial gene identifying the maternal line, it is not sufficient to distinguish the hybrids between these two species. To identify hybrids, Elkinton et al. sequenced the nuclear gene glucose 6 phosphate dehydrogenase (G6PD) and found six nucleotides out of the 233 bp G6PD fragment that reliably distinguished between the two species. Hybrids (at least F1 hybrids) were heterozygotic at all six sites. Here we demonstrated a G6PD restriction enzyme assay based on the Taq I enzyme that cut the 233 bp G6PD fragment for Bruce spanworm into two fragments at ~179 and 54 bp. The winter moth G6PD sequence doesn’t have this enzyme site so the band size is at ~233 bp as uncut PCR product. The hybrid samples, having the DNA from winter moth and Bruce spanworm parents, had all three fragments with the size of 233, 179, and 54 bp. Running the two assays together allowed one test to confirm the species ID with the other. In the case of hybrids, the G6PD test would identify the hybrid and the COI test would identify the female parent.
DEVELOPING REARING METHODS FOR *TETRASTICHUS PLANIPENNISI* (HYMENOPTERA: EULOPHIDAE), A LARVAL ENDOPARASITOID OF THE EMERALD ASH BORER (COLEOPTERA: BUPRESTIDAE)

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ABSTRACT

Classical biological control efforts against emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) in North America primarily have focused on introduction and releases of exotic parasitoid species collected from northern parts of China. Recently, field surveys in Michigan, Pennsylvania, Ohio, and Ontario also indicate that some existing parasitoids in North America (either indigenous or inadvertently introduced) have already become associated with EAB and may play a supplementary role in suppressing the populations of EAB. *Balcha indica* (Hymenoptera:Eupelmidae) is one such parasitoid, recently recovered from various stages of EAB larvae, prepupae, and pupae collected in Michigan, Pennsylvania, and Maryland, and found to reproduce thelytokously (i.e., virgin female reproducing daughters). In the present study, the longevity, oviposition rate, and fecundity of adult females, as well as development time of immature stages were determined in the laboratory. *Balcha indica* is a solitary ectoparasitoid attacking larvae, prepupae, and pupae of EAB. Lifetime fecundity of 27 females averaged 36 eggs with a maximum of 94 eggs, and adult longevity averaged about 59 days. Mean adult survivorship was 8.58 weeks with lower and upper 95-percent confidence intervals of 6.95 and 10.21 weeks, respectively. It is important to note that one female lived 98 days without laying a single egg. Throughout exposure bouts, parasitism averaged 30.9 percent (range from 0 to 100 percent). Under the normal rearing condition (25±°C and D: L 14:10 hour photoperiod), *B. indica* took approximately 89 days to complete its life cycle (from egg to adult emergence), with a short development time for egg (less than 2 days) and first instar larvae (less than 4 days), and a prolonged development time for the remaining larval stages (56 days) and pupal stage (27 days).
VISITATION RATES TO THE APOSTLE ISLANDS NATIONAL LAKESHORE AND THE INTRODUCTION OF THE NON-NATIVE SPECIES *LYMANTRIA DISPAR* (L.)

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ABSTRACT

The introduction of non-native species has accelerated due to increasing levels of global trade and travel, threatening the composition and function of ecosystems. Upon arrival and successful establishment, biological invaders begin to spread and often do so with considerable assistance from humans. Recreational areas can be especially prone to the problem of accidental non-native species transport given the number of visitors that arrive from geographically diverse areas. In this poster, we examined camping permit data to the Apostle Islands National Lakeshore in northwestern Wisconsin from 1999 to 2007 relative to gypsy moth distribution, phenology, and outbreak data. During this time, gypsy moth populations became established in this area ahead of the moving population front of the gypsy moth, suggesting possible anthropogenic introduction. The permit data revealed that most visitors arrived from outside of the gypsy moth established area. However, there was a consistent yearly trend of visitors who arrived from areas of high gypsy moth populations and who arrived during the gypsy moth life stage (egg masses) most likely to be successfully introduced. Using available data on the gypsy moth and its relationship to camping permit data, we describe how recreational managers could optimize park strategies to mitigate unwanted introductions of the gypsy moth as well as develop analogous strategies for managing other biological invaders in recreational areas.
WHAT DOES “LOCAL” FIREWOOD BUY YOU?
MANAGING THE RISK OF INVASIVE
SPECIES INTRODUCTION

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ABSTRACT

Firewood can serve as a primary vector in the transport of non-native species, particularly of wood boring insects that can be transported surreptitiously in firewood. State and Federal governments have enacted limitations on the movement of firewood as a means to limit accidental introduction of invasive species. However, it can be challenging for governments to determine an allowable distance for moving firewood, such as for recreational camping use, and regulations vary from state to state. We were motivated by this challenge and explored the risk associated with moving firewood for recreational campground use. We quantified the number of campgrounds at varying distances from a hypothetical range of a non-native species. We did this by developing a simulation model using ESRI ArcObjects to randomly select 2, 4, 8, 16, or 32 spatial locations, or “infested sites,” in Wisconsin, Illinois, Iowa, and Minnesota. The number of campgrounds within a 10-, 25-, 50-, 100-, or 200-mile radius of each infested site was determined, and we used a bootstrap method (500 replications) to estimate the mean and 95-percent confidence intervals of the number of campgrounds by the number of infested sites and radius. The mean number of all campgrounds, publicly owned campgrounds, and privately owned campgrounds increased nonlinearly within each radius relative to the number of infested sites for the four-state region. When considering a state-by-state simulation approach, an allowable radius of 100 miles for moving firewood encompassed almost as many campgrounds as a 200-mile radius when the number of infested sites $> 8$. 
LONG-TERM WEATHER VARIABILITY AND SHIFTING DISTRIBUTION LIMITS OF THE INVASIVE HEMLOCK WOOLLY ADELGID (ADELGES TSUGAE ANNAND)

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ABSTRACT

The hemlock woolly adelgid (HWA) is a small, aphid-like piercing-sucking insect native to Asia and northwestern North America (Havill et al. 2006, 2007). First documented in 1951 in the eastern United States near Richmond, VA, the HWA has spread to infest at least 17 states along the Appalachian Mountains from Georgia to southern Maine, where infestations have been limited to coastal regions with more moderate temperatures. Currently, the northern regions of Vermont, New Hampshire, and Maine remain uninfested.

Although hemlock species native to Asia and northwestern North America are either resistant to, or tolerant of, infestation by the adelgid, the two species native to eastern North America (Tsuga canadensis and T. caroliniana) are highly susceptible and can be killed within a few years following infestation. To date, native predators have failed to control populations of the adelgid, and no parasites are known in either the native or introduced ranges. Without these biotic regulators, the hemlock woolly adelgid in eastern North America may be free to infest the range delimited by host availability and the abiotic tolerances of the adelgids. Past work has shown that the adelgid is vulnerable to low temperatures, and winter temperatures can reduce adelgid population densities at the landscape scale (Parker et al. 1998, 1999; Trotter III and Shields 2009). However, little is known about how the geographic distribution of temperature-dependent population boundaries vary through time (although see Paradis et al. [2008] for estimates of boundaries based on global circulation model based projections of climate change). To fill this knowledge gap, weather records were extracted from the NOAA (National Oceanic and Atmospheric Administration) NCDC (National Climate Data Center) CDO (Climate Data Online) database for ~1,700 weather stations in the eastern United States. Minimum monthly temperatures for these stations were loaded into a GIS (ArcGIS) and interpolated (kriged) to produce temperature surfaces estimating the annual minimum temperature from 1960 through 2008. These annual temperature maps were then converted to estimates of adelgid survival using equation 4-E in Trotter and Shields (2009) and were in turn used to produce contour maps of adelgid survival. The 2-percent adelgid survival isocline was selected as the functional population boundary, and the boundaries from each of the 49 included years were combined into a single map. This composite map of annual, temperature-driven adelgid population boundaries provides a spatial probability distribution of the conditions suitable for adelgid population survival. From this output, several patterns emerge. First, the map indicates that approximately 2.2 percent of the eastern hemlock population range in the continental United States occurs in regions in which none of the included 49 years were well suited for adelgid survival; these regions are found in northern Maine, New Hampshire, and Wisconsin. Second, the data suggest large inter-annual variation in the geographic location of the boundary, which is found as far south as the Georgia/North Carolina border, and as far north as upper New England and Michigan. Third, when evaluated by decade, the weather data suggest a northward-trending shift in the conditions suitable for adelgid survival, in agreement with the findings of Huntington et al. (2009). These shifts could pose a threat to the climatic refugia for hemlock found in Maine, New Hampshire, and Wisconsin. Finally, these
data suggest the spatial distribution of adelgid survival probabilities, which, in combination with an improved understanding of population dynamics and processes (such as Allee effects) that drive them, can facilitate a probabilistic approach to evaluating the spatial risk of this invasive species.

**Literature Cited**


SYNERGISTIC EFFECT OF DUAL IMIDACLOPRID–METARHIZIUM ANISOPLIAE APPLICATIONS AGAINST ASIAN LONGHORNED BEETLES (ANOPLOPHORA GLABRIPENNIS)

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ABSTRACT

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), a longhorned beetle species native to Asia, has been introduced into several North American and European cities. Currently, eradication and preventive measures are limited to identifying and destroying infested trees and protecting uninfested trees with trunk or soil-injections of the systemic insecticides imidacloprid. Because entomopathogenic fungi like Metarhizium anisopliae (Metsch.) Sorokin have been identified as virulent against these beetles, we conducted several tests to determine the compatibility of the two agents in combination. In a 2x3 factorial experiment investigating potential interactions between exposure to imidacloprid and M. anisopliae, we observed no effect of imidacloprid alone on beetle survival at a single dose of 10 or 100 ppm compared to control insects, a significant effect of exposure to M. anisopliae, and a significant interaction between imidacloprid and M. anisopliae representing a synergistic (not additive) effect of dual treatment. Beetles exposed to the fungus alone lived significantly longer than insects treated with a single dose of 100-ppm imidacloprid (9.5 vs. 6.5 days). Consumption of twigs by beetles exposed to imidacloprid and M. anisopliae in a factorial experiment revealed a significant reduction in consumption (48 percent and 16 percent) over the 6-day test period as a function of exposure to M. anisopliae and imidacloprid, respectively. Beetles fed 100-ppm imidacloprid consumed 32 percent less over the first 3 days compared to beetles not exposed to imidacloprid and thereafter consumed as much as beetles not fed 100-ppm imidacloprid, whereas M. anisopliae-exposed beetles consumed significantly less food throughout the test period.
HISTOCHEMICAL STUDY OF LECTIN BINDING SITES IN FOURTH AND FIFTH INSTAR GYPSY MOTH LARVAL MIDGUT EPITHELIUM

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ABSTRACT

There is evidence that the gypsy moth, *Lymantria dispar*, midgut epithelial brush border membrane has membrane-bound glycoconjugates, such as BTR-270 and aminopeptidase N (APN), which function as high affinity binding sites (receoptors) for the insecticidal proteins produced by *Bacillus thuringiensis* (*Bt*). As gypsy moth larvae become older, they become resistant to the entomocidal activity of *Bt*, suggesting there may be a change in the expression of these toxin-binding glycoconjugates in older larvae.

To examine the possibility that the expression of cell surface glycoconjugates may be altered in older larvae, paraffin-embedded midgut tissues of fourth and fifth instar gypsy moth larvae were probed with a panel of lectins. Lectin binding to the tissue sections was assessed using fluorescence microscopy. Lectins are sugar-binding proteins that bind to specific carbohydrate structures in tissues. Specific and very small changes in the carbohydrate moiety of glycoconjugates attached to the midgut epithelial cells can be identified using lectins.

In this study, striking differences were found between fourth and fifth instar gypsy moth larvae with several lectins, including peanut, *Arachis hypogaea* (PNA), ricin, *Ricinus communis* (RCA120), and wheat germ, *Triticum vulgaris* (WGA). In contrast to fifth instar larvae, cell surface molecules on the microvilli of fourth instar gypsy moth larvae did not interact with peanut (PNA) and ricin (RCA120) lectins, which have high affinity and specificity for terminal β-galactose residues on glycoproteins and glycolipids. In addition, the fourth instar microvilli and the basal lamina were labeled with WGA, but WGA lectin binding is restricted to the basal lamina in the fifth instar larval gut tissue. These results provide evidence that there are ontogenetic changes in the expression of specific carbohydrates on the surface of brush border microvilli during larval development that may have functional significance to the decrease in susceptibility to *Bt* insecticidal proteins in older insect larvae.
COMPARATIVE ANALYSIS OF *BACILLUS THURINGIENSIS* TOXIN BINDING TO GYPSY MOTH, BROWNTAIL MOTH, AND DOUGLAS-FIR TUSSOCK MOTH MIDGUT TISSUE SECTIONS USING FLUORESCENCE MICROSCOPY

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ABSTRACT

Many strains of *Bacillus thuringiensis* (Bt) produce insecticidal proteins, also referred to as Cry toxins, in crystal inclusions during sporulation. When ingested by insects, the Cry toxins bind to receptors on the brush border midgut epithelial cells and create pores in the epithelial gut membranes resulting in the death of susceptible insects. Different Bt strains produce a wide variety of Cry toxins. The type of toxins present in the crystals determines the insecticidal potency and specificity of a particular strain.

Several derivatives of a Bt strain, HD1 subsp. kurstaki (Btk), have become the major biopesticide used to control forest pests such as the gypsy moth (*Lymantria dispar*) and the spruce budworm (*Choristoneura fumiferana*) in North America. Bt products can be as effective as conventional broad-spectrum insecticides with little or no effect on non-target organisms. However, control of some forest pests such as the Douglas-fir tussock moth, *Orgyia pseudotsugata*, and the browntail moth, *Euproctis chrysorrhoea*, has not always been effective. These inconsistencies may in part be related to differences in the profile of the toxins present in different Bt formulations.

Because a critical step in the mode of action of Bt is the binding of Cry toxins to specific cell receptors, we examined, using fluorescence microscopy, Cry1Aa and Cry1Ac toxin binding and determined the presence of two Bt toxin receptors, BTR-270 and APN, in tissue sections of early fourth instar gypsy moth, Douglas-fir tussock moth, and browntail moth larvae. This experimental approach can be valuable for preliminary screening of Bt Cry proteins to discriminate between potentially lethal and nontoxic Bt Cry proteins. Although specific toxin binding of Cry proteins to midgut brush border membrane in target insect pests is indicative of toxicity, results of these experiments require confirmative testing.
MANAGING *SIREX NOCTILIO* POPULATIONS IN PATAGONIA (ARGENTINA): SILVICULTURE AND BIOLOGICAL CONTROL

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**ABSTRACT**

*Sirex noctilio* is a primitive wood boring solitary wasp with a univoltine life cycle. Characteristic of this species is the occurrence of severely damaging, pulse-like eruptive population outbreaks. During outbreaks, the damage to pine plantations can be severe; tree mortality may reach levels close to 80 percent. Outbreak behavior is thus important when designing management strategies to minimize the impact of woodwasps on the forest resources. Essentially, the aim of any management plan consists of either reducing the intensity of the outbreaks (also minimizing spread) and/or pushing outbreak probability away from the plantation cycle (Corley and Villacide 2005). In Patagonia (Argentina), as in other *Sirex*-affected regions, remarkable outbreaks have been observed. Based on empirical and simulation modeling studies, silvicultural treatment of plantations including usual thinning practices (removing stressed and/or suppressed trees) and sanitary thinning have resulted in a strong reduction in the intensity of damage and/or its occurrence (Villacide and Corley 2006). In turn, biological control by the nematode *Beddingia siricidicola* has reported highly variable parasitism rates, reaching 80 percent in some sites (Villacide and Corley 2006). However, these values do not appear to be directly linked to the effective *Sirex* decrease in tree mortality. A significant *Sirex* impact has been recorded in Patagonian plantations, even in the presence of high levels of parasitism by the nematode (between 60 and 80 percent). Parasitism rates by parasitoids have been reported only for *Ibalia leucospodes*. In field conditions, *Ibalia* may parasitize up to 40 percent of its hosts. Because of this, *I. leucospoides* is generally reported as a successful control agent of *S. noctilio*; however, some studies suggest that under some environmental conditions its regulatory role is secondary to that of other parasitoids. Because the parasitoids *Rhyssa persuasoria* and *Megarhyssa nortoni* have been introduced only recently, their contribution to *Sirex* control has not yet been reported. Despite this, *M. nortoni* was found in other sites far from the original release points and can prove a significant contribution to woodwasp mortality in Patagonia (Corley and Bruzzone 2009). We note that, among an array of tools used for the management of woodwasp populations, an integrated pest management protocol with strong involvement of silviculture practices is likely to obtain the best results.

**Literature Cited**


EFFECTS OF HOST WOOD MOISTURE ON THE LIFE CYCLE DEVELOPMENT OF THE ASIAN LONGHORNED BEETLE

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ABSTRACT

The Asian longhorned beetle, *Anoplophora glabripennis*, is an alien invasive species recently introduced to the United States in larval form within solid wood packing material from China. Larvae develop within the xylem of apparently healthy trees. In the U.S., the species has been found infesting 21 species of hardwoods, across 12 genera, with maple species being the most preferred hosts. While there is seasonal variability in the moisture content of the xylem of living maples, when the trees are cut for firewood, moisture levels can decrease rapidly and reach levels significantly lower than in living trees. Such environmental perturbation could have life history consequences for this xylem feeding insect. It could also mean that increased concern for further anthropogenic dispersal is warranted.

In the study described here, we sought to evaluate the effects of declining host wood moisture on larval development and adult fitness. We hypothesized that decreasing moisture in the larval environment could cue larvae to accelerate development in order to escape declining host wood quality. Adults from larvae that pupated earlier would have lower weights, and there would be correlated reductions in fitness, as measured by total egg production and viability of offspring.

We reared larvae from two populations (Worcester, MA, and Chicago, IL) on artificial diets with experimentally manipulated moisture contents. Beetles developing within both low moisture and incrementally decreasing moisture environments pupated sooner than beetles reared in the standard, high moisture diet. Earlier pupation resulted in smaller adults, but contrary to expectations, there was no significant treatment effect on total egg production. This may be due to the lack of significant difference between the treatment groups in longevity, given the strong correlation between longevity and fecundity shown in a previous study.

These results highlight the need for further work to determine if there are significant tradeoffs between size and some other aspect of fitness (e.g., egg size or mate selection). From an applied perspective, firewood and pallets represent potential host wood with low and declining moisture contents. *A. glabripennis* developing within these low moisture environments may develop earlier than existing models predict, thus emerging earlier in the spring and potentially having a longer time to reproduce and disperse. This may also increase the risk of establishment associated with movement of firewood as a pathway for human-mediated dispersal.
SIMULATING THE INTERACTIONS OF FOREST STRUCTURE, FIRE REGIME, AND PLANT INVASION IN THE SOUTHERN APPALACHIANS USING LANDIS

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ABSTRACT

Southern Appalachian forests face multiple environmental threats, including periodic fires, insect outbreaks, and more recently, exotic invasive plants. Past studies suggest these multiple disturbances interact to shape species-rich forest landscape, and they hypothesize that changes in fire regimes and increasing landscape fragmentation may influence invasive processes. However, long-term impacts of these multiple factors, landscape-scale processes that drive invasion, and forest management practices required to reduce damage from invasive plants are still unclear. We have developed a modeling approach to investigate the synergistic effects of wildfires and landscape fragmentation on the spread of two major exotic invasive plants, princess-tree (Paulownia tomentosa) and tree-of-heaven (Ailanthus altissima). LANDIS-II, a spatially explicit forest succession model, was used to simulate forest dynamics and plant invasion in a xeric pine-oak landscape, the predominant vegetation in the southern Appalachian Mountains. We parameterized a pool of the 36 dominant tree species including three major invasive tree species, and using a variety of forest fragmentation and fire scenarios, simulated changes in the abundance of the two invasive plants over a 200-year period. Species establishment coefficients were derived through rescaled spatial constancy data from the Carolina Vegetation Survey. We found that intermediate levels of fire frequency promoted spread of the invasive species, but that low and high extremes of fire frequency limited their invasions. Increased fire frequency promoted the growth of yellow pine species (pitch pine, Table Mountain pine, and shortleaf pine), while reducing less fire tolerant white pine. We also found that under the same level of fragmentation, a higher proportion of initial forested patches resulted in an increase in abundance of the invasive species in the landscapes because of greater seed availability. These results suggest that the synergistic effects of wildfires and landscape fragmentation are complex, and increasing fire frequency (or re-introducing fires) could maintain xeric pines, but it also may promote plant invasion. Under increasing fragmentation, a tradeoff between lower fire frequencies to minimize plant invasion and higher frequencies to promote pines in the southern Appalachian landscape may be required for integrated forest vegetation management. More generally, the modeling framework we have developed will allow us to investigate the factors that promote the spread of other invasive species, and in return, help foresters manage the invasive plant problems.
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