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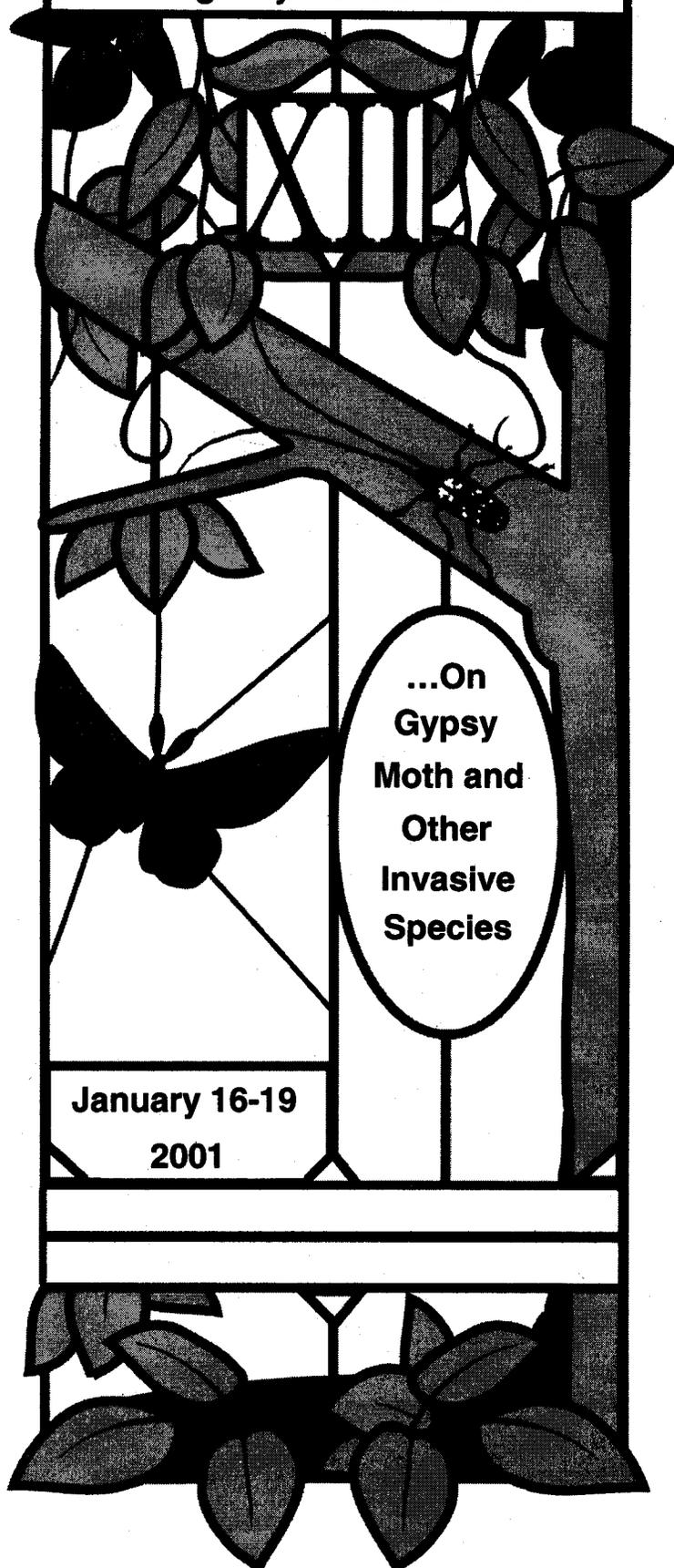
General Technical  
Report NE-285



## PROCEEDINGS

U.S. Department of Agriculture

Interagency Research Forum



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USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species  
January 16-19, 2001  
Loews Annapolis Hotel, Annapolis, Maryland

**AGENDA**

Tuesday Afternoon, January 16

REGISTRATION  
POSTER DISPLAY SESSION I

Wednesday Morning, January 17

PLENARY SESSION ..... Moderator: J. Robert Bridges, USDA-FS  
Welcome  
Michael McManus, USDA-FS

The Siege of Invasive Species in Midwestern Ecosystems  
Robert N. Wiedenmann, Illinois Natural History Survey

The Brown Spruce Longhorn Beetle in Halifax: Pest Status and Preliminary Results of Research  
Jon Sweeney, Natural Resources Canada

PLENARY SESSION ..... Moderator: Robert Mangold, USDA-FS  
The National Council on Invasive Species  
Lori Williams, Department of the Interior

A Multi-year Project to Detect, Monitor, and Predict Forest Defoliator Outbreaks in  
Central Siberia  
Max McFadden, The Heron Group, LLC

Wednesday Afternoon, January 17

GENERAL SESSION ..... Moderator: Cynthia D. Huebner, USDA-FS  
Invasive Plants: Organismal Traits, Population Dynamics, and Ecosystem Impacts  
Presenters: E. Nilsen, Virginia Polytechnic Institute & State University; D. Gorchov, Miami  
University of Ohio; F. Wei, State University of New York at Stonybrook; K. Britton, USDA-FS;  
C. D'Antonio, University of California at Berkeley

GENERAL SESSION ..... Moderator: Kathleen Shields, USDA-FS  
Research Reports  
Presenters: J. Colbert, USDA-FS; J. Elkinton, University of Massachusetts; J. Cavey, USDA-APHIS

POSTER DISPLAY SESSION II

Thursday Morning, January 18

GENERAL SESSION ..... Moderator: Victor Mastro, USDA-APHIS  
Asian Longhorned Beetle  
Presenters: M. Stefan, USDA-APHIS; D. Nowak, USDA-FS; S. Teale, SUNY College of Environmental Science and Forestry; B. Wang, USDA-APHIS; R. Mack, USDA-APHIS

GENERAL SESSION ..... Moderator: Kevin Thorpe, USDA-ARS  
Research Reports  
Presenters: S. Frankel, USDA-FS; B. Geils, USDA-FS; D. Gray, Natural Resources Canada

Thursday Afternoon, January 18

GENERAL SESSION ..... Moderator: Vincent D'Amico, USDA-FS  
Gypsy Moth in the Midwest  
Presenters: D. McCullough, Michigan State University; A. Liebhold, USDA-FS; W. Kauffman, USDA-APHIS; A. Diss, Wisconsin Department of Natural Resources; L. Solter, Illinois Natural History Survey; K. Raffa, University of Wisconsin

GENERAL SESSION ..... Moderator: Vincent D'Amico, USDA-FS  
Research Reports  
Presenters: B. Hrašovec, University of Zagreb, Croatia; E. Burgess, Hort-Research, Auckland, New Zealand; C. Maier, Connecticut Agricultural Experiment Station

Friday Morning, January 19

GENERAL SESSION ..... Moderator: Sheila Andrus, USDA-FS  
Asian Longhorned Beetle: Detection and Monitoring Panel Discussion  
Panel Participants: J. Aldrich and A. Zhang, USDA-ARS; R. Haack, USDA-FS; D. Lance and B. Wang, USDA-APHIS; D. Williams, USDA-FS; S. Teale, SUNY College of Environmental Science and Forestry; M.T. Smith, USDA-ARS; K. Hoover, The Pennsylvania State University

GENERAL SESSION ..... Moderator: David Lance, USDA-APHIS  
Asian Longhorned Beetle: Control Options Panel Discussion  
Panel Participants: V. D'Amico, USDA-FS; T. Poland and R. Haack, USDA-FS; A. Hajek, Cornell University; L. Hanks, University of Illinois at Champaign-Urbana; M. Keena, USDA-FS; B. Wang and W. McLane, USDA-APHIS; Z. Yang, Chinese Academy of Forestry; M.T. Smith, USDA-ARS

Closing Remarks

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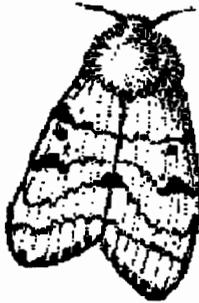
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# THE SIEGE OF INVASIVE SPECIES IN MIDWESTERN ECOSYSTEMS

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## ABSTRACT

Although coastal regions of North America are heavily invaded by exotic species, invasive species are not limited to the Atlantic or Pacific coasts. Here, I show that the midwestern U.S., as exemplified by ecosystems in Illinois, is equally predisposed to invasions, often by the same means. I cite traits of invasive species and the habitats they invade, offer some reasons why the Midwest is easily invaded and details about a few of the worst invasive species in the Midwest, then summarize our efforts to fight back by using biological control against purple loosestrife.

The recent publication by Pimentel et al. (2000) estimated that invasives cost the U.S. over \$137 billion per year. Invasive weeds and insects alone were estimated to cost over \$26 billion and \$16 billion, respectively. Calculating similar estimates of the ecological costs of invasive species is more difficult, but would be equally alarming. In the United States, approximately 40% of the species listed as threatened or endangered are thought to have been imperiled by the effects of invasive species (Wilcove et al. 1998). Other regions of the world fare even worse – as many as 80% of species endangered in some regions are thought due to invasive species. As many as 42% of vertebrates whose causes of extinction are known are attributed to invasive species.

In addition to directly affecting native species, invasive species can alter basic ecological processes in invaded habitats. Exotic plant species can displace native plants, affect nest sites for birds, alter nutrient cycles, or increase frequency of fires. Exotic animal invaders prey on beneficial organisms, disperse seeds that change landscapes, or have different grazing patterns that increase soil erosion. The aquatic invader zebra mussel (*Dreissena polymorpha*) actually increases water quality through filtration, predisposing the habitat to new invaders, such as the Eurasian fish, round goby (*Neogobios melanostomus*). In reverse, increased turbidity by common carp (*Cyprinus carpio*) alters water habitats needed by native plants and fish. Finally, the duration and extent of the effects of invaders may be felt long after the initial wave of invasions occurs, the so-called “ghost of invaders past.” Species that can persist in invaded habitats, the interactions among those species, and subsequent new invasions leave communities far different than they were originally. Consider the invasion of the Great Basin of the American West by European cheatgrass (*Bromus tectorum*). Large stands of cheatgrass enhance frequent fires, thus changing the shrub-steppe habitat to a near monoculture of the exotic grass (Kurdila 1995, Vitousek et al. 1996). Crossing that habitat now, one may not encounter many of the species that evolved in the shrub habitat. Instead, those plant and animal species that can persist in the new frequent-fire regimen will continue to structure this altered community.

What is special about the midwestern U.S.? Though geographically far from ocean coasts, we are easily reached by invasives. Economic trade has brought many invaders to the Midwest. Once the Great Lakes were isolated from oceans, but now are connected through the St. Lawrence Seaway. The opening of the Seaway brought goods via ocean-going vessels to ports on the Great Lakes, but also brought aquatic invaders in ballast water in the ships' holds. Sea lamprey (*Petromyzon marinus*) decimated Great Lakes fishing in the 1960s. Later invaders include zebra mussel and round goby, both considered native to the Black and Caspian Seas. Two species of tiny waterfleas (*Daphnia* spp.) are among the most recent invaders found in the Great Lakes and are impervious to predatory fish, giving them a leg up (figuratively, at least) on native species and reducing food for predaceous fish. Nor are these invaders of the Great Lakes confined to the lakes. Once, the Great Lakes and the Great River systems of the Midwest (Illinois, Mississippi, Ohio, Missouri, and Platte Rivers) were isolated. Not now. The Sanitary Ship Channel near Chicago connects Lake Michigan with the headwaters of the Illinois River. Some 250 miles downstream, the Illinois River joins the Mississippi. Once zebra mussel moved through the Great Lakes and into the Illinois River, the rest of the Great Rivers were open for invasion. Likewise, exotic fish that have found new homes in the river systems are poised to move the opposite direction, into the Great Lakes.

Land habitats are equally prone to invasion. Air traffic through major airports (e.g., Chicago's O'Hare Airport) connects the Midwest with the rest of the world in a few hours. Rail and truck traffic pass through the Midwest in all directions. A direct rail link from Nova Scotia in far-eastern Canada to Chicago may permit movement of brown spruce longhorned beetle (*Tetropium fuscum*) into the Midwest. Asian longhorned beetle (*Anoplophora glabripennis*) is one of the more notorious trade-enhanced invaders to threaten urban and rural Midwestern forests. The Chinese soybean aphid (*Aphis glycines*), first discovered in 2000, likely found its way into the U.S. through trade of bonsai plants from Asia. Once escaped (or moved through re-shipment of infested plants), the aphid found soybean habitats to invade, from Minnesota to Kentucky.

Invasion of Midwestern communities – whether aquatic or terrestrial – is enhanced by several factors. Many of our invasive pests are longitudinal migrants; that is, they arrived from a similar area of the world in a similar latitude and climate, traveling east to west, or vice versa. Invasible communities also often have some sorts of disturbance that predispose the habitats to invasion. Development, altered hydrology, siltation, fire, and grazing are all disturbances that contribute to invasion conditions. Or, communities may be early successional with a low diversity of native species, especially ecologically similar species. Illinois' remnant prairies can be considered early successional, and the Great Lakes were glaciated as recently as 10,000 years ago, thus having a fairly simple natural fauna. Thus, the Illinois landscapes and lakes were prime for invasion.

The other aspect of invasions relies on characteristics of the invaders themselves. Lists of traits associated with invasive species include high reproductive and dispersal rates, resistance to native natural mortality, and being a habitat or resource generalist. The perennial plant purple loosestrife, for example, produces >2.5 million seeds per year. Likewise, gypsy moth produces up to 1,000 eggs in a mass. Some Midwestern invasives

have high dispersal abilities. Round goby was discovered in the St. Clair River in 1990. Within five years, the fish could be found in all of the Great Lakes. Although gypsy moth females are flightless, the early instars can balloon on winds, and egg masses are especially prone to accidental movement by humans. Invasive plants, such as purple loosestrife or garlic mustard, are largely untouched by native herbivores.

The list of invasive species in the Midwest knows no taxonomic bounds. In Illinois, the diverse list of exotic invaders includes plants, insects, terrestrial vertebrates, aquatic organisms (both vertebrate and invertebrate), and even plant pathogens. Illinois has plant invaders such as garlic mustard (*Alliaria petiolata*), purple loosestrife (*Lythrum salicaria*), kudzu (*Pueraria lobata*), and teasel (*Dipsacus laciniatus* and *D. sylvestris*). Garlic mustard can reach densities of 2,000 plants/m<sup>2</sup> in many Illinois forests, displacing native spring ephemerals. Kudzu, whose infamy is known from the southeastern U.S., has over 90 known populations in Illinois. Teasel species apparently are increasing along roadsides and rights of way, also making incursions into pasture and prairie. I say apparently increasing, because any perceived increase in density and spread is anecdotal – due to a lack of economic cost, this species has not been monitored closely in the past (but the monitoring is beginning now). In addition, both common reed (*Phragmites australis*) and reed canary grass (*Phalaris arundinacea*), which are distributed throughout both Old and New Worlds, are represented in the state by apparently exotic genotypes with invasive properties. Several plants planted extensively along roadsides, either to stabilize hillsides (e.g., crown vetch, *Coronilla varia*) or as part of a wildflower mix (e.g., Dame's rocket, *Hesperis matronalis*), are now spreading and invading other areas. Finally, several species are either just arriving, such as Chinese yam (*Dioscorea oppositifolia*) moving northward, or anticipated to arrive soon, such as mile-a-minute weed (*Polygonum perfoliatum*) moving westward.

Insect invaders are equally diverse. Asian longhorned beetle in Chicago has become the poster-child for invasions due to trade. More than 1,500 trees in the Chicago area have been cut down to slow the spread; whether this has kept the beetle out of the area forest preserves is not yet certain. Gypsy moth was first detected in Illinois in 1973, but is acknowledged to have become far worse in the past decade. Another consequence of open trade, the Asian tiger mosquito (*Aedes albopictus*) invaded the state in used tires shipped from Asia. These cavity-nesting mosquitoes found the water that collected in tires and tree cavities to be perfect for their populations to increase. This mosquito can vector several kinds of encephalitis – themselves exotic species. Whether Chinese soybean aphid becomes one of the bad offenders is yet to be seen. Lacking many natural enemies, dispersing readily, and overwintering on another exotic species, buckthorn (*Rhamnus cathartica*), this aphid has many of the right (or wrong) traits. It also can vector soybean viruses, which may put this species toward the top of the list.

Aquatic invaders in the Midwest are more than adequately represented. Zebra mussel was first detected in the Great Lakes in 1988, but now reaches densities of >700,000/m<sup>2</sup> (Griffiths et al. 1991), often completely covering native mussels and clams, and clogging water intake pipes, causing an estimated \$100 million in damage and control costs. Round goby has been found at densities >50/m<sup>2</sup> along the littoral area of southern Lake Michigan – and this for a fish that can grow to 25 cm in length. Gobies also prey on many small fish and – in a quirk

that is one of the few rays of hope – will feed on zebra mussels (as well as native mussels). Other fish that threaten native mussels include the yet-to-arrive black carp, a species grown for aquaculture in nearby states. This species can grow to 1 m in length and as much as 36 kg – traits good for a food fish, but terrifying for an invasive predatory fish. Interestingly, black carp is only one of an array of six exotic carp species threatening invasion that occur at three trophic levels – herbivores, plankton feeders, and predators.

Invasive feral hogs (*Sus scrofa*), well established in southern Illinois, are poised to transmit livestock and human diseases. Even the seemingly benign Eurasian collared dove (*Streptopelia decaocto*) has been implicated in reductions in other native bird species. Finally, exotic, invasive plant pathogens have transformed the Illinois landscape. Cities once lined with American elms now are home to few, if any, after arrival of Dutch elm disease. And the Prairie State, though located on the edge of the eastern deciduous forest, still has felt the effects of chestnut blight fungus, though not so severely as the rest of eastern North America.

As bad as the picture seems, we are fighting back. Cutting trees and injecting insecticides seems to be slowing the spread of Asian longhorned beetle. Nearly all kudzu populations have been treated repeatedly with herbicides; many appear to be controlled. A barrier is planned for the Sanitary Ship Channel near Chicago to slow movement of round goby into the Illinois River (except that the fish has been found downstream of the barrier). Garlic mustard biological control agents are being studied in Switzerland and we hope to have some of them by 2002. And finally, we seem to be winning the fight against purple loosestrife in our wetlands. Since 1994, Illinois has been rearing *Galerucella* spp. beetles for biological control of the weed. Nearly 2 million beetles have been distributed to approximately 130 wetland sites in the state. At several sites, signs look promising. We have seen severe defoliation of loosestrife, followed by an increase in the native plant species, at one monoculture in northwest Illinois. At a more-diverse sedge meadow nature preserve in northeast Illinois, flowering and height of loosestrife plants have decreased, coupled with an increase in the native plants that defined the nature preserve. In 2000, there was no loosestrife flowering within a mile of the beetle releases. We also have engaged over 200 educators in understanding the value of Illinois wetlands and the problems of exotic species, and helped them grow loosestrife and rear *Galerucella* beetles in their classrooms for release. Educating the next generations of citizens about invasive species is part of our long-term plan for combating invasives in the state.

Though invasive species will continue to arrive in the Midwest – through trade bringing invaders via water, land, or air – we will continue to fight back. Statewide management plans are being developed to try to stem the threats imposed by current or future invaders. Whether we win the war (or at least a few battles) remains to be seen.

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THE BROWN SPRUCE LONGHORN BEETLE IN HALIFAX:  
PEST STATUS AND PRELIMINARY RESULTS OF RESEARCH

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ABSTRACT

In March 2000, it was determined that the brown spruce longhorn beetle (*Tetropium fuscum* Fabr.) (Coleoptera: Cerambycidae), a native of Central Europe and parts of Asia, had become established in Point Pleasant Park, Halifax, Nova Scotia, where it was killing red spruce (*Picea rubens* Sarg.) trees. The risk the beetle poses to the health of North America's forests resulted in the formation of the Brown Spruce Longhorn Beetle Task Force, comprised of members of several federal, provincial, and municipal agencies. In this presentation, we briefly describe the beetle's biology, the chronology of events leading to its discovery in Halifax, current efforts to eradicate it and contain its spread, and preliminary results of some of our research.

LIFE CYCLE AND BIOLOGY

In its native range, *T. fuscum* attacks mainly Norway spruce (*Picea abies* (L.) Karst.) but has also been reported infesting *Picea sitchensis* (Bong.) Carr. (Franke-Grosman 1954), *P. pungens* Engelm., *Abies alba* Mill., *Pinus sylvestris* L., and *Larix* spp. Mill. (Juutinen 1955 and references therein). While it is considered a secondary pest in Europe, usually infesting windthrown or living trees under stress from other factors such as root rots, drought, or bark beetles, *T. fuscum* occasionally appears in trees "weakened so negligibly that they are virtually the same as healthy trees" (Juutinen 1955). Throughout most of its range, *T. fuscum* is univoltine and overwinters as a larva. However, it can have two generations per year in the southern part of its range and can require 2 years per generation at its northern range limit. Adults emerge sexually mature from May to August, mate soon after emergence, and mate more than once. Females lay an average of 80 eggs, singly or in clusters, well hidden beneath bark scales (Schimitschek 1929). Larvae feed in the phloem and cambium and produce an extensive network of wide, irregular tunnels. Infestation eventually leads to girdling of the stem and increases the tree's susceptibility to other pests; once infested by *T. fuscum*, a tree usually dies within 1 to 5 years (Juutinen 1955). Mature larvae typically form a hook-like pupal cell 1-2 cm deep and 2-4 cm long in the sapwood, but may also pupate

between the wood and bark or entirely in the phloem. The adult beetle makes an oval to round exit hole about 4 mm in diameter (Wettstein 1951).

## CHRONOLOGY

Events leading to the discovery of *T. fuscum* in Halifax began in July of 1998 when representatives of Natural Resources Canada (NRCAN) and the Canadian Food Inspection Agency (CFIA) observed a large number of dying trees in Point Pleasant Park, located adjacent to a container port in the city. Surveys of the park in the fall of 1998 found many dead and living red spruce with copious resin flow down the trunk. During the winter of 1998-1999 and winter-spring of 1999-2000, more than 70 bolt sections were collected from the stems of 17 live, green spruce trees with copious resin flow and no evidence of attack by *Dendroctonus* spp., and incubated in containment facilities for insect rearing and fungal culturing (Pegler and Hurley 2001). A total of 125 adult specimens of *Tetropium fuscum* emerged from 14/17 trees and were confirmed by regional (NRCAN), national (AAFC and CFIA), and European experts. Other insects that emerged were native to North America, not known to cause tree mortality, and were found in only a few samples. In February 2000, examination of *Tetropium* specimens from the Nova Scotia Museum of Natural History revealed that 17 specimens of *T. fuscum* had been collected in Point Pleasant Park, Halifax, in 1990 and misidentified as a native species, *Tetropium cinnamopterum* Kirby. Morphological characters useful for distinguishing *T. fuscum* from *T. cinnamopterum* are available (Smith and Humble 2000). By March 2000, *T. fuscum* was considered established in Point Pleasant Park (Smith and Hurley 2000).

In parallel to the insect rearing studies, there were investigations into the incidence of root disease in the park's red spruce. Fungal culturing in February 1999 and field surveys in September 1999 found little evidence of root rots. However, pure cultures of an *Ophiostoma* sp. wood stain fungus were isolated from beetle-infested trees in April 1999 and confirmed by AAFC in March 2000. This *Ophiostoma* was isolated from infested trees again in April 2000. The species and pathogenicity of the *Ophiostoma* is unknown. Studies of *Ophiostoma* systematics and molecular methods for species identification are currently underway (NRCAN and AAFC).

On 30 May 2000, Point Pleasant Park was placed under federal plant quarantine by the CFIA and movement of wood from the park was restricted. On 5 June 2000, the Brown Spruce Longhorn Beetle (BSLB) Task Force was established with the main objective of eradication. The Task Force is led by the CFIA with representatives from Natural Resources Canada, Halifax Regional Municipality (HRM), Nova Scotia Department of Natural Resources (NSDNR), New Brunswick Department of Natural Resources & Energy (NBDNR&E), Maritime Lumber Bureau (MLB), and Dalhousie University. An intensive survey, beginning in the park and expanding outwards, was launched by the CFIA and NSDNR in which more than 52,000 conifers were examined on over 47,000 residential properties covering more than 170 km<sup>2</sup>. Surveys were also conducted within an 18- to 20-km radius of the park. Based on surveys, the CFIA expanded the BSLB quarantine zone on 13 October 2000. Trees suspected of BSLB infestation were felled and burned and bark was peeled from the stumps to the soil line. Cutting was halted by a court injunction in August 2000 but was overturned

on appeal in December 2000. To date, about 3,100 infested trees have been removed: 2,200 within Point Pleasant Park and about 900 outside the park. No BSLB infested trees have yet been found outside of a 15-km radius from Point Pleasant Park.

## RESEARCH

We present preliminary results from research on: (1) susceptibility of selected North American conifers to *T. fuscum*; (2) development of a trap for detection surveys; and (3) trap logs for containment of infestations. We also list other studies, proposed or underway, that are part of an overall research plan to increase our knowledge of the beetle's biology, behavior, and ecology, and to develop effective methods of detection and control.

Host susceptibility was investigated using: (1) small cage laboratory bioassays to compare the numbers of eggs laid on native spruce species in forced and choice tests; (2) a field experiment comparing infestation of 1-m vertical bolts of *P. rubens*, *P. glauca* (Moench) Voss, *P. mariana* (Mill.) B.S.P., *Abies balsamea* (L.) Mill., and *Larix laricina* (Du Roi) K. Koch; and (3) the rearing of insects from bolts collected from trees found displaying signs of *T. fuscum* infestation during field surveys. Given no choice, female *T. fuscum* laid eggs on *P. rubens*, *P. glauca*, and *P. mariana*; in choice bioassays, *P. rubens* was preferred to *P. mariana*. Lifetime fecundity averaged 85 eggs (0-151) and increased with female size. Adult *T. fuscum* successfully emerged from bolts of *P. rubens*, *P. glauca*, and *P. mariana* collected in field surveys; thus, all northeastern spruce species are susceptible. The results of the field experiment will be known when rearing is completed in late spring of 2001.

In an effort to develop a survey tool, we tested an unbaited control plus five combinations of  $\alpha$ -pinene, ethanol, and the 3-component aggregation pheromone for *Ips typographus* (as a potential kairomone) in Lindgren funnel traps for attraction of *T. fuscum*. The traps were placed about 1 m above the ground in Point Pleasant Park on 28 June 2000 and checked weekly until 24 August. Eight *T. fuscum* adults were captured and no lure was more attractive than the unbaited control. Twenty other species of cerambycid beetles were captured in the traps, including one specimen of *T. cinnamopterum*.

Because *Tetropium* spp. will attack freshly cut logs (Post and Werner 1988), trap logs were deployed operationally as a means of attracting ovipositing female *T. fuscum* in an attempt to reduce infestation in living trees. Over 100 log decks were spaced about 50 m apart along roads and pathways in Point Pleasant Park on 20-22 June 2000. Each deck consisted of six, 2.4-m long by 15- to 35-cm diameter red spruce logs stacked, parallel, three on the bottom, two in the middle, and one on top. All logs were cut from uninfested trees that were felled in late May to early June. We conducted two experiments. In the first, we baited 30 log decks with the same five combinations of host volatiles (plus unbaited control) that were tested in the funnel traps (five replicates per treatment). In the second, an unbaited, 6-log deck was compared with a: (1) spoke of 6 logs, each laying directly on the ground; (2) line of 6 logs, oriented vertically and braced between two trees; and (3) three freshly girdled, live red spruce trees. The remaining operational log decks were baited with high release rate lures of ethanol and  $\alpha$ -pinene (Phero Tech Inc.). Six of these log decks (=36 logs) were processed in November 2000 by milling the logs into 7- to 8-mm thick slabs using a portable bandsaw and

counting the numbers of pupal cells. We found a mean of about nine *Tetropium*-like (i.e., L-shaped) pupal cells per log. Logs on the bottom of the deck were infested more heavily than those exposed on top. From a subsample of 47 undamaged larvae, 46 were cerambycids and 38 (81%) were *Tetropium* spp. Infestation levels and the ratio of *T. fuscum* to native *Tetropium* will be determined in January 2001 by milling trap logs to count pupal cells and by rearing adults from bolt subsamples.

## FUTURE AND ONGOING STUDIES

In addition to the studies described above, research is underway in the following areas:

- Survey and diagnostic tools
  - morphological and molecular methods of identifying juvenile stages of native and non-native *Tetropium* spp. (NRCAN, CFIA, and AAFC)
  - comparative diagnostics of damage by *T. fuscum* and native *Tetropium* spp. (NRCAN, CFIA, and AAFC)
  - trap bolts/slabs as surveillance tools (NRCAN)
  - determining an effective trap and lure for detection of adult *T. fuscum* (NRCAN and NSDNR)
- Phytosanitary treatments
  - efficacy of chipping (NRCAN)
  - heat treatment protocols (UNB Wood Science & Technology Centre)
- Biological controls
  - microbial control through autodissemination of *Metarhizium anisopliae* (NRCAN and Anhui Agricultural University, China)
  - identity, origin (native vs. exotic), and impact of parasitoids/predators exploiting *T. fuscum* domestically; feasibility of classical biological control (NRCAN and CABI)
- Stem injection of systemic insecticides (NRCAN)
- Plant-insect interactions
  - adaptations of *T. fuscum* to new host species (NRCAN)
  - role of tree stress and host quality in host choice (NRCAN)
  - role of visual cues in short/long-range foraging and host selection (NRCAN)
- Chemical ecology
  - determining host-volatile composition from healthy and stressed red spruce (NRCAN)
  - screening of host volatiles in behavioral bioassays and field trials (NRCAN)
- Phenology of adult emergence, oviposition, and larval development (NRCAN)
- Identity and insect-fungal association of *Ophiostoma* sp. (NRCAN and AAFC)
- Current and potential socio-economic impacts of *T. fuscum* (UNB and NRCAN)
- Testing of feasible laboratory rearing protocols for *T. fuscum* (NRCAN)

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A MULTI-YEAR PROJECT TO DETECT, MONITOR, AND PREDICT  
FOREST DEFOLIATOR OUTBREAKS IN CENTRAL SIBERIA

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In 2000, the U.S. Agency for International Development (USAID) initiated a new Russia Forestry Resources and Technologies (FOREST) project which is planned for 5 years and has a budget of \$20 million. From the USA side, this project is implemented by Winrock International, Chemonics International, and The Heron Group, LLC, a group of international consulting companies who won the competition of proposals. Other cooperators include the new Ministry of Natural Resources of the Russian Federation which includes the former Russian Federal Forest Service, the World Bank, the Institute for Sustainable Communities, Sukachev Institute of Forest in Krasnoyarsk, Russian Far East Forest Research Institute in Khabarovsk, Regional Forest Service Centers in areas of project implementation, regional government offices, and other organizations.

The principal objectives of the project are to: (1) strengthen regional forest policy and legislation, (2) preserve and expand Russia's carbon sink (fire awareness and pest management), (3) more effective use of timber and non-timber forest products, (4) raise public awareness to reduce forest fires, and (5) promote the use of renewable biomass energy.

There are four technical components of the project (#1-#4) and three cross-cutting components (#5-#7):

1. Forest Fire Prevention
2. Pest Management
3. Secondary Wood Processing and Non-Timber Forest Products
4. Renewable Energy Alternatives
5. Forest Policy and Legal Reform
6. Applied Forestry Research (Carbon Cycle)
7. Forestry Grant/Loan Program

In this paper, we present only the Pest Management component, which is implemented by The Heron Group in cooperation with the Sukachev Institute of Forest and the Russian Forest Service. The goal of this component is to optimize the system of monitoring of major forest pest species based on their habitat preferences, intensive use of geographic information systems technology, and combined use of contemporary sampling methods. Pest monitoring will be funded by the FOREST project in the first 5 years, and later it will be continued by the Russian Forest Service.

The Pest Management component is focused on monitoring of the following major target pest species. The Siberian Moth (*Dendrolimus superans sibiricus*) is the most devastating defoliator in Siberia. Normally, each generation takes 2 years to develop, but during an outbreak, the population switches to a 1-year life cycle, which increases the population growth rate. This insect has a distinct outbreak cycle with a period of about 11 to 14 years. Between outbreaks, the population density is extremely low; it then gradually increases and finally leads to defoliation of millions of hectares of forests. The Siberian moth feeds on many coniferous host species but most economic damage occurs in fir and Siberian pine stands. After the last outbreak in 1993-1996, tree mortality occurred on 136,000 hectares despite an intensive treatment campaign.

Forest stands killed by the Siberian moth lose all their economic value; also, they are extremely flammable and often become sources of devastating forest fires. It appears that dead stands not only stop accumulating carbon dioxide from the atmosphere, but they also emit a large amount of carbon dioxide in the first 2 to 5 years after defoliation (Baranchikov and Kondakov 2000).

A second species is the gypsy moth (*Lymantria dispar* L.) which damages deciduous trees and larch. Its outbreak cycle is less pronounced than the Siberian moth.

The fir sawyer beetle (*Monochamus urussovi*) is mostly a secondary pest of firs. It often reaches a high population density in areas initially damaged by defoliators (e.g., Siberian moth) or fire. The beetle is a vector of several phytopathogenic fungi from genera *Leptographium* and *Ophiostoma*. It infects healthy firs with fungus during its maturation feeding on crown shoots. At high population densities, firs quickly become weakened by fungi and then become susceptible to beetle oviposition. Thus, the area of moth-killed fir stands is often doubled due to *Monochamus* activity.

One of the biggest problems in forest pest management in the Asian part of Russia is the ineffective pest monitoring system in most areas. In Krasnoyarskiy Kray, a set of permanent sampling plots was established in 1997 for monitoring of the Siberian moth and other pests. In these plots, the abundance of Siberian moth larvae is determined by cutting sample trees or shaking trees with a log and collecting fallen larvae. This is a very labor-intensive procedure because many trees in each site must be sampled to get a reliable estimate of larval density.

To reduce sampling efforts and to monitor larger areas of Siberian forests, we propose to use pheromone traps. Pheromone traps are routinely used in the U.S. for monitoring gypsy moth populations (Schwalbe 1981, Leonard and Sharov 1995). The technology of pheromone traps is well developed and is ready to be transferred to Russian Cooperators. Pheromone components are identified for the gypsy moth and recently for the Siberian moth (Klun et al. 2000) and can be synthesized in Russia.

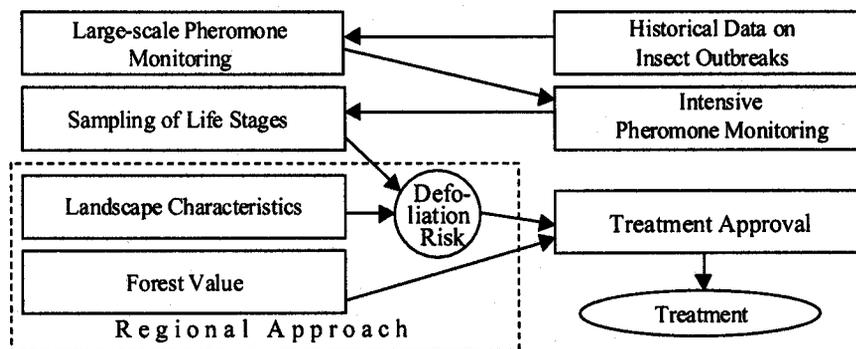
Monitoring forest defoliators with pheromone traps has the following advantages. (1) Trapping is less labor intensive than sampling of other life stages; thus, more sites can be sampled. (2) Moth counts in traps represent an average population abundance in the area. Thus, traps work well even if they are not placed in the most favorable habitats, which are

often difficult to access. (3) Traps capture a large number of individuals, thus, population trends can be determined with greater precision than with larval sampling.

But pheromone traps can not solve all problems with pest monitoring because they have several limitations. (1) Traps often get saturated with moths. This problem can be addressed, however, by increasing trap size, reducing the number of entrance holes, reducing attractiveness of the bait, or by electronic detection of moth counts. (2) Moth counts in traps do not represent local population abundance at a small scale. Thus, they can not be used for delineating areas for treatment. Additional sampling of other life stages may be necessary at high population densities. (3) Moth counts in traps do not represent additional population parameters (e.g., sex ratio, parasitism), but these characteristics can be determined using additional sampling methods.

We propose to develop a regional approach to forest pest monitoring in the area of Yenisey Siberia. This includes delineation of large-scale regions where monitoring is recommended for each target pest species, and specifications for monitoring activity in each region depending on climatic zone, vegetation patterns, and organizational structures of forest pest management. At a smaller scale, we will delineate areas with various risk of defoliation by the Siberian moth in individual forestry units. Maps will be generated with GIS technology to be used for optimizing placement of sampling areas that have to be located in the most favorable habitats. In post-outbreak periods, monitoring will be based mostly on pheromone traps. Larval sampling will be initiated only after a consistent increase in pest abundance has been determined with pheromone traps.

The pest monitoring program is a part of a larger decision-support system. Our draft version of a decision-support system for Siberian forests follows (Fig. 1). Based on historical records of pest outbreaks, areas are selected for large-scale pheromone monitoring. In areas where population numbers increase consistently, a denser grid of traps can be used. At high population density, it is important to sample life stages to evaluate the risk of defoliation. Treatments are approved based on information on pest abundance, landscape characteristics (tree species composition and terrain), and forest value.



**Figure 1. Proposed decision-support system for forest pest management in Russia.**

Another important component of the project is the use of information technology that includes database software, GPS recorders, GIS software, and presenting project results on a web site which will be available to all project participants and to the general public.

The Pest Management component will be implemented first in Krasnoyarskiy Kray, and then we plan to expand it to the Irkutsk and Khabarovsk regions. In 2001, we have the following tasks (some of which will continue through the life of the project).

1. Facilitate the production of pheromones and traps in Russia.
2. Use a grid of pheromone traps to monitor the abundance of Siberian moth and gypsy moth on the left bank of the Yenisey River to the south from Krasnoyarsk.
3. Develop a database on the abundance of forest pest insects accessible via the Internet. This objective will require training of Russian personnel. Initially the database will be set at the Sukachev Institute of Forest and later transferred to the Russian Forest Service.
4. Develop an algorithm for delineating regions with various habitat quality for the Siberian moth.
5. Compare captures of the Siberian moth in pheromone traps in various habitats. Traps will be placed in favorable, moderately favorable, and unfavorable habitats to test the effect of habitat on moth counts in traps.
6. Compare counts of the Siberian moth in pheromone traps with larval density.
7. Compare effectiveness of an attractant of the Siberian moth produced in Russia with one produced in the U.S..
8. Organize a workshop on methods for forest pest monitoring.

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# MODELING DISPERSAL OF THE ASIAN LONGHORNED BEETLE

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## INTRODUCTION

Numerous exotic invasive species pose a grave threat to the biodiversity and economic interests of the USA (Bright 1998). Typically, invasive species are introduced, establish breeding populations, and then spread rapidly (Parker et al. 1999). Inevitably, the introduction and establishment of some invasive species will occur. When an established species represents a major threat, it may still be eradicated. The Asian longhorned beetle (ALB), *Anoplophora glabripennis* Motsch., is a recent invader with extremely high risk to native deciduous forests (Pasek 1999). ALB serves as a test case for eradication because it produces relatively few progeny and is not prone to disperse from suitable host trees. Feeding by ALB larvae can girdle the cambium and kill a large host tree in about 4 years. Although immature beetles cause most of the damage, factors affecting adult dispersal, rather than development or reproduction, are the most important processes influencing the invasion of exotic species (Higgins et al. 1996; Fig. 1).

Once breeding populations of ALB were found in the U.S., state and federal agents undertook a major effort to eradicate the beetle. ALB readily attacks 29 species including healthy, full-grown maple, poplar, elm, and willow trees. If allowed to spread, losses for maples alone could potentially include destruction of the maple syrup industry, fall foliage tourism, and urban street trees (30% maples). Forest Inventory and Analysis (FIA) data show that the importance value of ALB host trees is extensive within the eastern U.S. (Iverson et al. 1998). Importance value is relative to 80 species in the eastern forest. The index ranked red maple 1<sup>st</sup>, American elm 3<sup>rd</sup>, and sugar maple the 4<sup>th</sup> most important species (Fig. 2).

Intensive survey for infested trees, followed by felling, removal, and chipping, is currently the only available method of population suppression. Effective surveys require establishment of boundaries around infestations (referred to as quarantined and/or

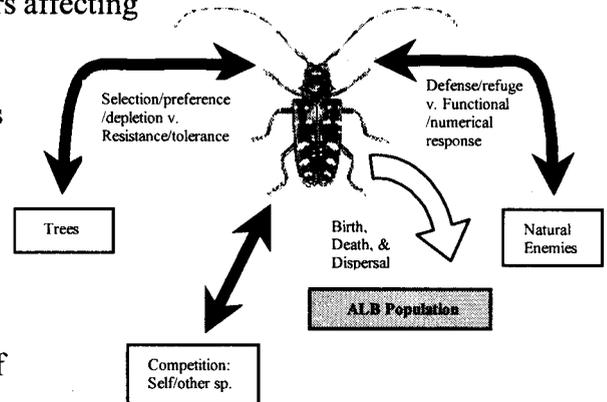


Figure 1. Interaction with host trees and conspecifics is the focus of current research. Specifically, we are investigating factors associated with dispersal in the U.S..

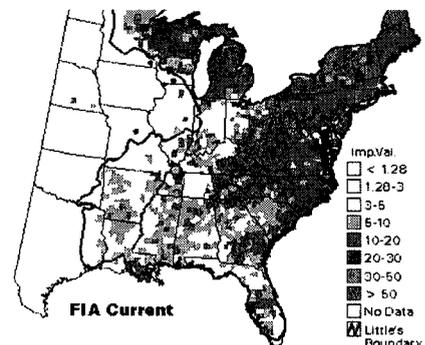


Figure 2. Sugar maple, a preferred host tree of ALB, is common in much of the eastern forests of the U.S..

eradication survey boundaries), inside of which surveys are conducted. However, delineation of boundaries is based upon the dispersal potential of ALB. Current guidelines for APHIS eradication surveys are 1/2 mile from the closest known infested tree (Dr. Alan Sawyer, per. comm.), which are based upon rate of detection of infested trees.

If ALB populations become large and spread beyond the urban areas, eradication of ALB is unlikely. In such an event, Cavey et al. (1998) suggests that \$137 billion could be needed for management of ALB. Immediate action is important to protect the vast hardwood forests of the upper Midwest and Northeast. Understanding dispersal by ALB is critical to the eradication effort. Accordingly, this study provides critical new information on the dispersal of ALB. This information forms a basis for the delineation of the quarantine boundaries and concentrating survey and detection efforts, and thereby lowers the detection threshold for nascent infestations. We describe our ongoing efforts to predict ALB distribution and enhance quarantine efforts.

### INDIVIDUAL-BASED SIMULATION

Environmental cues that are used by ALB have been programmed into an individual-based simulation. We are using the simulation of dispersal to create hypotheses about the importance of candidate dispersal mechanisms and predict ALB dispersal. In turn, the hypotheses are tested with discriminating experiments and lead to more accurate prediction and better control strategy. The simulation was designed for flexibility, which is facilitated by implementation in the object-oriented language C++. A simplified flowchart shows how the simulation runs (Fig. 3). Because the focus is on predicting dispersal for one generation, estimation of reproduction was not needed. However, high mortality reduces dispersal distance. Mortality rate over time was calibrated with data from an age-specific fecundity experiment<sup>1</sup>.

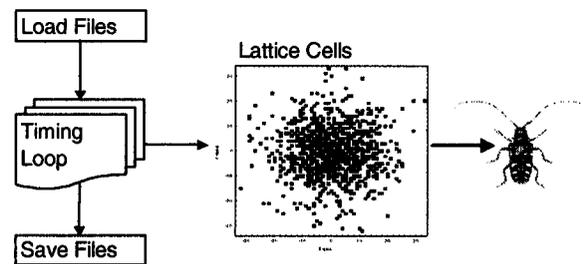


Figure 3. At each 1-hour time-step, all cells and beetles within a cell updated their age and used a random number to determine if their position changed.

$$P(\text{death}) = N(t, b, c) \text{ where:}$$

$N$  = Normal pdf

$t$  = time - start at age 0

$b$  = 92 (mean of normal distribution)

$c$  = 25 (variance of normal distribution)

Figure 4 shows that the fit of three common probability density functions offers adequate approximations of the mortality data. Field data on mortality is being acquired, which will improve the characterization of the mortality agents.

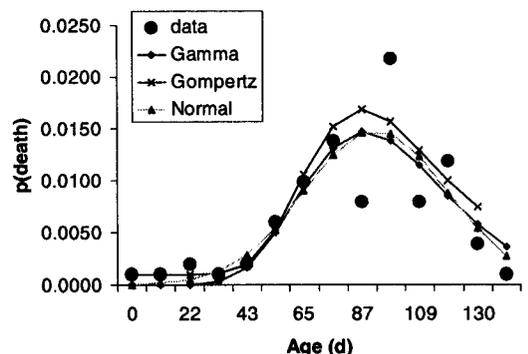


Figure 4. The probability density function for mortality.

<sup>1</sup> Smith, M.T.; Bancroft, J.S.; Tropp, J. 2001. Age specific fecundity of *Anoplophora glabripennis* on three hosts. In preparation.

The dispersal model was tested with data from dispersal experiments. Because the release of ALB is prohibited in North America, a mark-recapture experiment was conducted in Gansu province, China, in order to estimate ALB dispersal rate. A total of 188 marked ALB were recaptured in weekly samples (Smith et al., in press). Trapping data was fitted with a time-integrated, two-dimensional diffusion model (Turchin and Thoeny 1993), but more realistic dispersal may include individual variation in reaction to local abundance of either host trees or beetles of the same sex. Because data was gathered at discrete spatial and temporal intervals, an individual based model was a more intuitive approach.

A “random walk” model technique was used to simulate beetles moving on a grid of cells. The lattice of 30-m cells accommodates remotely sensed data, which will be discussed later in this paper. Conditions used by the model mimicked those from the dispersal experiment. The habitat or lattice size was set at 66 x 66. Each cell has a single “quality” that was set to a uniform value (128). The simulation runs were set to 56 d with samples taken every 7 d. The dispersal probability for a given cell was calculated as follows.

$p(\text{leave}) = i(b + r(D/Q))$  where:

$i$  = individual variation - uniform distribution

$b$  = base emigration rate

$r$  = scaling of ratio dependence

$D$  = density of same sex in cell

$Q$  = quality of cell

An interesting challenge to our understanding is evident in U.S. survey reports and our field data. Infestations in the U.S. have been largely located by the identification of infested trees, rather than by the collection of adult beetles. Similarly, in our field studies in China, while average annual emergence of adult beetles has been about 10 ALB / tree, numbers of resident adult beetles inhabiting trees has averaged only about 0.55 ALB / tree / week. Therefore, large numbers of ALB are either dying or dispersing without being captured. Furthermore, our studies have shown that ALB are capable of flights over a kilometer. Many explanations explain why live or dead adults are not observed. We restrict speculation to testable hypotheses in our experimental area in China. One possible explanation for this phenomenon is that many ALB are dispersing into the surrounding areas due to the lack of suitable hosts within our study area. In the U.S., host trees are plentiful, and vigilant surveys are needed to ensure that infested trees do not go undetected.

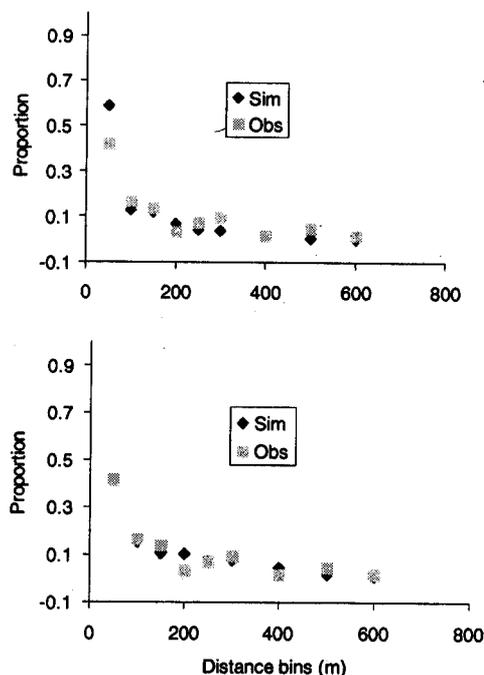
## RESULTS AND DISCUSSION

The test data set consisted of numbers of ALB captured at each of nine distances. These values were normalized by dividing by their total (188). Similarly, simulation data was grouped into distance bins and normalized to one. The proportion of population dispersing a given distance was then compared between the simulation and observed data. The response measure of simulation's fit consisted of the weighted sum of squared error at each of nine distances. Powell's algorithm is a well tested technique for fitting multivariate functions such as our individual-based simulation (Press et al. 1986). Dispersal parameters in the simulation were manipulated to find values that provided the best match of the simulation with observed data.

Numerous versions of the dispersal function were tested. A detailed explanation of the possible mechanisms of dispersal and the rationale behind the mathematical representation is beyond the scope of this paper (see Tilman and Kareiva 1997 for an excellent overview). In each case, parameters were fitted so as to minimize the mean square error of the ALB at the nine distances. We chose two versions of the simulation that show the process of evaluation and selection of best models. The first version is a simple random walk model (Turchin 1998). Using only one parameter, this model was able to explain 88% of the variance in the data (Fig. 5a). The second version includes three parameters that represent processes that likely influence dispersal (Fig. 5b). While the second version is slightly more complicated, it better explains the data ( $R^2=0.95$ ). The density parameter in the better model indicates that the abundance of host material per beetle will be important for dispersal and a fruitful avenue of continued research. Similarly, the other parameter, individual variation, suggests that studies of the propensity of individuals to disperse should help explain important population-level effects.

Our estimates for dispersal distance were much larger than previous estimates (Yan 1985, Wen et al. 1998) because of variation in size and arrangement of tree species. Huang (1991) and Huang and Zhou (1992) found ALB dispersal distance was generally within 200 m. However, their studies were conducted in a homogeneous, young poplar plantation (3- by 5-m tree spacing). ALB dispersal distance may be greater in the U.S. where preferred host trees are more widely spaced. Our future studies will strengthen the understanding of host-tree interaction and dispersal in response to landscape elements.

Simulation development has occurred concurrently with six experimental projects: mass-mark recapture, which provides large-scale seasonal movement; individual tracking, which provides daily movement and activity; flight propensity, which provides environmental impetus for movement behavior; host utilization in China, which provides adult emergence and death rates; host suitability, which screens possible U.S. host trees; and age-specific fecundity, which compares oviposition and death rates on host-tree logs in the laboratory. Each experiment fills a gap in our understanding. For example, dispersal differences among individual ALB were not evident in the data used to calibrate the current model, but the individual tracking study provides an independent measure of the importance of individual variation in dispersal. Generally, our studies provide a backbone of basic biology for predictions of ALB populations and development of control techniques.



**Figure 5. a) Point release 1000 ALB. No ratio dependence or individual variation. ( $R^2 = 0.88$ )**  
**b) Point release 16000 ALB - mimics experiment. Ratio dependence and individual variation. Mean dispersal =  $262 \pm 144$ m. ( $R^2 = 0.95$ )**

The results of simulations have determined gaps in our ecological knowledge that need further research. There are two major processes that we plan to experimentally quantify and incorporate into the dispersal model. When ALB of the same sex meet, repulsion due to fighting causes a local uniform distribution (contest competition within trees). We are addressing interaction in individual mark-recapture studies in 2001. At a larger spatial scale, attraction to preferred host trees results in congregation. Separate experiments on flight propensity, flight behavior, and host utilization are being used to understand interaction with host trees.

Data acquired on reproduction and mortality enables multi-year prediction of spread. We are pursuing host-tree preference and suitability as well as detailed data on trees in our field site (including species, size, position, and health). We are pursuing techniques to use biological control agents with pilot experiments on attack and culturing of natural enemies. The simulation will incorporate this information and make quantitative predictions of ALB abundance and distribution for successive years.

The use of satellites and remotely sensed data has become a promising tool for ecological habitat classification. These rapidly improving tools will help predict areas with high risk of infestation. This involves combining images from different spectral sensors and classifying the landscape to identify host trees. The development of high-resolution digital imagery involves geographic specialists at universities, the U.S. Geological Service, and private companies. These images, along with host-preference experiments, increase the applicability of ecological understanding to the predictions of ALB spread in the U.S.. This proactive approach will form the basis for development of adaptive management strategies for this and other invasive species.

Our studies use rigorous experimental designs to understand basic biology about the population dynamics of ALB in nature. This fieldwork provides a critical bridge between numerous laboratory efforts and the ongoing eradication efforts in U.S. landscapes. Collectively, these approaches enable reliable predictions of ALB infestations in the U.S..

In summary, the leptokurtic, or "fat tailed," redistribution allows for an accelerating spread that is seen in many exotic invasive species (Shigesada and Kawasaki 1997). Removal of infested trees in urban areas may provide containment. However, if ALB abundance in undetected populations is allowed to build, containment and eradication will be much more difficult. Our use of multiple experimental approaches and simulations has provided rapid progress in dispersal prediction, which is critical for eradication.

#### ACKNOWLEDGMENT

We thank R. Gao and G. Li for help in acquiring data in China. Without this data the model would not be testable.

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SIBERIAN MOTH SEX ATTRACTANT:  
TESTS IN DIFFERENT GEOGRAPHIC POPULATIONS

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ABSTRACT

In July 2000, a binary mixture of *Z,E*-5,7-dodecadienol and *Z,E*-5,7-dodecadienal was tested as a sex attractant of the Siberian moth (*Dendrolimus superans sibiricus* Tchtvrk.) (Lepidoptera: Lasiocampidae). The field trapping experiments were conducted with populations of the pest, representing the center and both the eastern and western peripheries of its range. Two populations were tested in each region: in Central Siberia (larch and fir forests), Urals (larch and pine), and in the Russian Far East (larch and pine forests). We used rubber septa with three concentrations of attractant: 20, 200, and 2000 µg per septum. At each site, we put a line of modified gypsy moth milk-carton traps in ABCABC sequence separated by 150 to 200 m; each concentration was replicated 8 to 10 times. The results showed the mixture was attractive to *Dendrolimus* males in all tested localities. The attractancy increased together with increasing concentrations of compounds in the septa. The attractant can be used for monitoring Siberian moth populations throughout their range in Northern Asia. It will be useful for early detection of this destructive pest at ports in the USA.

# GYPSY MOTH INSECT PREDATORS AND PARASITES IN CENTRAL SIBERIA

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## ABSTRACT

Gypsy moth (*Lymantria dispar* L.) in the United States is an invasive species of European origin and classical biological control efforts have been mounted against it. Explorations for natural enemies have been made in Europe, North Africa, and the Far East, but none in Central Asia. There is a vivid deficit of studies on the fauna and ecology of gypsy moth insect predators and parasites in Siberia. The work of N.G. Kolomeits (1987) summarized old publications but the new studies were performed recently.

We present the results of faunistic studies of entomophagous insects of the gypsy moth in six regions of Siberia: South-Western Altay Mountains, Kuznetskiy Alatau and Western Sayan Mountains, Tuvinian Republic, Minusinsk Valley, and Western Transbaikalia (Republic of Buryatiya). Collections and rearings of parasites were made during few years of field work in these regions. Identifications were performed by well-known Russian taxonomists G.A. Viktorov, A.N. Kirichenko, N.G. Kolomiets, M.N. Nikol'skaya, V.I. Tobias, A.A. Shtakel'berg, D.R. Kasparyan, B.A. Rikhter, K.B. Zinov'eva, and V.A. Tryapitzyn.

From South Siberia and Transbaikalia, N.G. Kolomiets (1987) mentioned only 24 species. We found this fauna is twice as large. There are 41 species from 2 orders and 8 insect families. The structure of the parasite community is rather complex: 26 species belong to primary parasites and 15 to hyperparasites. Among them only 2 species are ectoparasites and the other 39 are endoparasites. Thirty two species are solitary and 9 are gregarious parasites. Of the primary parasites, 4 attack pupae, 9 attack larvae and kill pupae, and 12 infest and kill only larvae. One species, *Anastatus japonicus* Ashm., is an egg parasite; it was found in unique specimens in Altay and Buryatiya.

Larval parasites belong to Ichneumonids (5) and Braconids (7 species). The most abundant were *Glyptapanteles liparidis* Bouche and *Cotesia melanoscelus* Ratz.; the last parasitised up to 40% of young larvae, but was heavily infested by hyperparasites. *Lymantrichneumon disparis* Poda dominated among pupal parasites. In Tachinids who infest larvae but kill pupae, *Blepharipa schinery* and *Exorista fasciata* dominated. In sparse gypsy moth populations, Braconids (*Apanteles*, *Meteorus*) dominated. At the peak of the outbreak and just after it, Tachinids are the most abundant parasites. The similarity of gypsy moth parasite faunas of Yenisey Siberia, Transbaikalia, and Altay is rather high; Chekanovsky coefficients of similarity are between 0.5 and 0.6.

# INVASIVE TERRESTRIAL SLUGS IN FOREST ECOSYSTEMS IN NORTH AMERICA

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## ABSTRACT

A complex of slug species evolved in European forest ecosystems and later adapted to disturbed habitats. Over the last 200 years, a number of these species have become established in North America; several are serious agricultural pests. However, the situation found in Europe was reversed in North America, as these species first invaded disturbed areas and later established in forests. The forests now serve as refugia for some species. The invasive and indigenous species complexes in the Pacific Northwest differ from the Northeast. The indigenous species in North America have not adapted to disturbed habitats, remaining primarily in the forests; there is an ecological concern about competition between these species and the invasive species.

The status of the invasive terrestrial slug fauna needs revision for several reasons. The United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA,APHIS), intercepts slugs on various commodities at international ports. A mission of APHIS is to prevent the importation of exotic agricultural pests. Unfortunately, a fair number of slug specimens intercepted at ports-of-entry are immature and are difficult to identify using morphological or anatomical methods. One goal is to develop molecular methods for taxonomic purposes to distinguish naturalized from exotic slug species.

However, current knowledge of slug species thought to be established in North America is assumed to be incomplete as the last thorough survey was limited to the Northeast 30 years ago. Therefore, it is difficult to evaluate risk species versus species of no concern (*i.e.* those already established). With recent increases in commerce and reduced trade restrictions, these same concerns are impacting other countries, especially those of Europe.

Collections have already identified several new records on the East coast. Laboratory colonies have been established as a result of these collections. These populations will allow us to better address molecular and ecological issues, and are available for ecological or applied studies. A preliminary synoptic collection of both naturalized and exotic risk species was completed in Europe. One key species that is rapidly spreading in Europe, *Arion lusitanicus*, is a serious risk to North America. A quarantine laboratory colony has been established to study this species.

If you are interested in helping with collections, we are requesting your help and can reciprocate with identifications. Please contact the author at: [gary.bernon@aphis.usda.gov](mailto:gary.bernon@aphis.usda.gov).

## POTENTIAL BIOCONTROL AGENTS FOR KUDZU FROM CHINA\*

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### ABSTRACT

A program to search systematically for biological control agents for kudzu in China has been established. Four survey sites in southeastern China were evaluated for stem and leaf damage every 10 days from June to October in 1999 and 2000. Over 50 different insect species were observed feeding on kudzu, and representative samples were collected for identification. A number of potential biocontrol agents have been identified, including stem and root boring beetles as well as leaf feeding insects. A destructive fungal disease called "imitation rust" was active at all four sites. Preliminary host testing on soybeans, string beans, and peanut was initiated for a few species in 2000.

### INTRODUCTION

Kudzu is a perennial, leguminous vine in the subtribe Glycininae. It was introduced into the United States as an ornamental plant in 1876 at the Philadelphia Centennial Exposition. At first it was popular to plant kudzu near the porch of southern homesteads to enjoy its luxuriant shade and sweet, grape-scented blossoms. Later it was planted as a forage crop, and many studies examined the optimum timing of mowing to maximize nutritional value and minimize damage to the kudzu. In the 1930s and 1940s, it was promoted for erosion control, and in a government landowner assistance program, over 73 million seedlings were produced and planted across the southern U.S., where erosion and top soil loss were major problems (Tabor and Susott 1941).

\* Thanks to the USDA Forest Service, Washington Office International Programs, and Forest Health Technology Enterprise Team, Morgantown, WV, for providing major funding for these efforts.

One recent estimate places kudzu acreage at 7 million acres, which stretch from Illinois to Connecticut and Oklahoma to Florida. At the edges of this range, kudzu is not yet much of a problem, but in areas where it has long been established, landowners deeply resent its intrusion into yards, fields, public lands, and right-of-ways.

In 1998, we surveyed landowners in the south who have kudzu problems and found that dissatisfaction with currently available control methods was universal. Utility companies spend enormous amounts to prevent kudzu from creeping up power poles and shorting out transformers. Forest landowners expect an annualized yield of \$100/acre; because it costs about \$350/acre to apply herbicides to kill kudzu, they cannot afford the treatment. The most effective herbicides are not selective and cannot be used near waterways. More selective herbicides are available, but cost more. In addition, unless a landowner owns the entire kudzu "patch," all efforts to control it are in vain. The 60 or so landowners and park managers who attended our public meeting, and another group that responded to our written questionnaire, unanimously supported a research program to look for biological control agents.

## METHODS

In 1999, three survey sites were established in Anhui Province and one in Guangdong. A site was added in 2000 in Shaanxi Province because different insects had been observed feeding on kudzu there. Defoliation was estimated on five vines at each location, using a pictorial key in 3 25-cm<sup>2</sup> quadrats per vine. The main vine and three attached branches were also evaluated for vine damage. Seed were collected and examined for feeding damage and internal insects.

Insects feeding on kudzu were carefully observed. Representative insects and herbarium specimens of damaged kudzu were collected and labeled to associate with behavioral observations. Larvae were collected and, when possible, reared to maturity. Insect specimens are being identified in China.

## RESULTS

Over 4,000 specimens have been collected from four areas in China. These represent about 500 species, all identified to family, some to genus, and some to species. A "short list" of about 25 potential biocontrol agents has been developed, and our collaborators are beginning to study their biology and feeding behavior.

***Stem and Root Borers.*** Many beetles, mostly Cerambycids, lay eggs in the vine, and the larvae tunnel down the stem. When the eggs are laid near the root crown, the larvae bore into the kudzu root, often killing it. Others pupate within the aboveground stem. At each survey site, two or three out of five main vines developed galls caused by stem-boring beetles. These galls were caged to capture emerging adults. Over 150 of these galls have been examined from other vines and other sites. Extensive feeding damage partially or completely disrupted stem tissues. The galls are being kept in cages, and as adult beetles emerge, they are sent to experts to confirm preliminary identifications.

Larvae attacked roughly 60% of kudzu roots in China in 1999. In Anhui Province, five different Cerambycid beetles, two moth species, one Buprestid beetle, and a very large Chrysomelid beetle laid eggs in above-ground stems. *Aristoba hispida*, *Paraleprodera diopthalma*, and a *Pterolophia* sp. are presently in "choice" host testing at Anhui Agricultural University. This year we are testing insects on soybean, peanuts, and string bean. In Shaanxi Province, six different Cerambycids and one unknown Buprestid were found in 1999, as well as two root beetles common to Anhui and Guangdong. A Shaanxi survey site was added in 2000 to study these new insects near Northwest China Forest University.

In 2000, an average of 39% of roots sampled in five locations were damaged by larvae. Larvae are most common in roots approximately 3 cm in diameter. Many of the roots damaged by borers appeared to contain cellulose-degrading fungi, and the possible synergy of borers and fungal pathogens as biocontrol agents deserves further exploration.

**Defoliators.** Leaf-feeding insects maintained an average of 10 to 20% defoliation in 1999. Many defoliators were polyphagous and have been excluded from consideration as biocontrol agents. Two Chrysomelid beetles and a sawfly remain to be studied.

Defoliation varies widely by site in China, but for most sites the level remains relatively constant over the season. This is similar to defoliation reported by Thornton (2001) for kudzu in the United States. They found that in the U.S., kudzu defoliators are generalists. The host specificity of kudzu defoliators in China has not yet been ascertained.

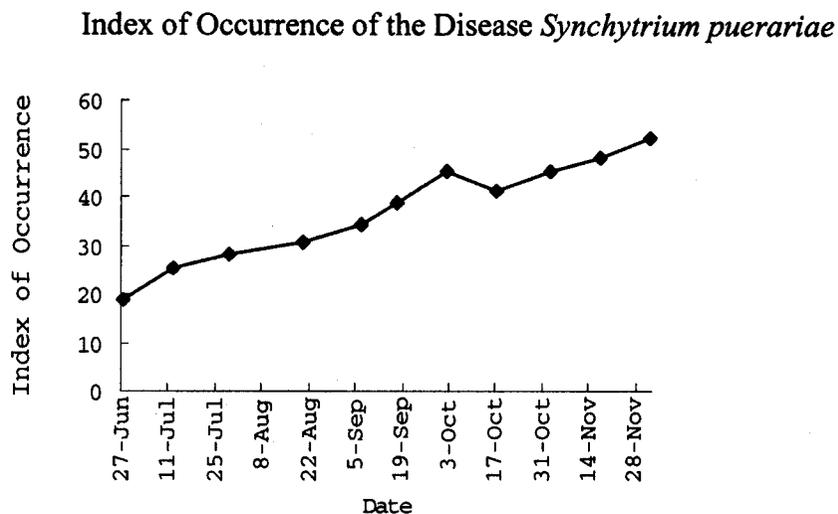
**Shoot Clippers.** Weevils (tentatively identified as *Deporaus* sp.) cut tips off kudzu vines and lay eggs in the cut portion. This later falls to the soil, where the larvae pupate over winter. These weevils were so common that 50 to 100% of the vine tips were clipped in some locations. Unfortunately, they attack soybeans as well as kudzu.

**Stem Gall Weevils.** *Alcidodes trifidus* is a black and white weevil that feeds on stems as an adult in a spiral pattern. The insect lays eggs in long slits it creates in green vines, which develop galls. Dr. Cai was able to establish a colony of *Alcidodes* on kudzu in his garden. Unfortunately, these weevils also attack soybeans.

**Seed Predators.** Bruchid weevils, a Pyralid moth, and stinkbugs' combined attack reduces seed germination by about 90%. This is similar to the situation in the United States (Thornton 2001). It is not known when the Bruchid arrived in this country, but most likely it was imported before World War II, when a great deal of kudzu seed was imported from Japan for propagation in nurseries.

**Diseases.** In 1999, Dr. Jiang Zide identified seven diseases on kudzu in Guangdong. The most promising of these is "imitation rust" caused by an unicellular fungus (*Synchytrium puerariae*) that forms motile, flagellate spores in pustules called sori embedded in the stem and leaves. Infection of leaves is most common along the leaf veins, and severely infected leaves are small and distorted. At one location in 1999, vine dieback was observed, apparently caused by disrupted translocation as the sori developed in the stem. Disease pressure was lighter in 2000 than in 1999, due perhaps to drier weather. Figure 1 shows the

increase in disease severity over the 2000 field season. We plan to assess the impact of imitation rust on kudzu growth and survival in the field and greenhouse and attempt to demonstrate whether or not it is host specific.



**Figure 1. Severity of imitation rust (*Synchytrium puerariae*) in Guangdong Province.**

**DNA Testing.** Three species or varieties of kudzu grow in Guangdong Province. The taxonomy is quite difficult because a single specimen can display floral and leaf characteristics of more than one kind. Originally we had hoped to conduct a broad survey of kudzu in China to determine through DNA analysis the center of kudzu biodiversity. The cost proved prohibitively high, and our plan was reduced to examining the variation in these three varieties or species and comparing their DNA and morphological characteristics with that of kudzu in the United States.

#### PLANS FOR 2001

We feel we have enough data on defoliating insects to choose candidates for host testing now. We must, however, continue to survey stems and roots because many more larvae will be needed to complete host testing. Many larvae are still feeding in cut roots maintained in collaborators' laboratories and cannot be identified until they emerge. Biology studies should be completed for some potential biocontrol agents that passed preliminary "no choice" tests this year. It is hoped that after one more field season in China, we can begin host testing in quarantine facilities in the United States.

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# PEST RESISTANCE GENES FOR THE CONTROL OF GYPSY MOTH AND OTHER FORESTRY PESTS

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## ABSTRACT

In order to identify resistance genes which could be engineered into *Pinus radiata* to protect trees from insect attack, purified insect resistance proteins were fed in artificial diet to gypsy moth and European pine shoot tip moth larvae. Experiments with these two insects were conducted in the USA and Chile, respectively. One protease inhibitor, two biotin-binding proteins, and three Bts were highly effective against gypsy moth and/or pine shoot tip moth.

## INTRODUCTION

The Gene-Based Insect Science Team of HortResearch, a New Zealand-based research and development company, is conducting a Forest Pest Project which aims to find resistance genes to protect New Zealand's *Pinus radiata* forests from attack by "biosecurity risk" insect pests. Such pests are defined as those not yet present in New Zealand, but which could threaten the economic future of the country's forestry industry if they were accidentally introduced. We aim to develop trees with built-in pest resistance by genetically engineering *P. radiata* with genes encoding proteins toxic to pest insects.

Resistance proteins from candidate genes were tested against two Lepidoptera identified as biosecurity risk pests for *P. radiata*: gypsy moth (*Lymantria dispar* (L.)) and European pine shoot tip moth (*Rhyacionia buoliana* (Den. and Schiff.)). Trials against gypsy moth were carried out in the USDA Forest Service Quarantine Facility at Ansonia, Connecticut, from September to October 1999, and against the pine shoot tip moth at Remehue Research Station near Osorno, Chile, from January to February 1999.

## CANDIDATE GENES FOR PINE PROTECTION

*Protease Inhibitors (PIs)*: These are proteins that regulate the activities of enzymes in all living things, so they can be sourced from plants, animals, and microbes. They are often quite specific in the enzymes to which they bind. Effective PIs inhibit protein digestion by the insects that feed on them, preventing growth and leading to insect death. By identifying PIs that bind tightly with pest insect digestive proteases in *in vitro* assays, we can deduce which are likely to be effective in subsequent bioassays.

*Biotin-Binding Proteins (BBPs)*: These bind to biotin and can cause a deficiency of this vitamin. We have found BBPs to be toxic to a wide range of insects. We have also patented a system for expressing these proteins in transgenic plants (Christeller et al. 1999). Since plants also have a requirement for biotin, the usual plant transformation methods will not work with these genes, as the plant itself would suffer biotin deficiency from exposure to expressed BBPs.

*Bacillus thuringiensis Proteins (Bts)*: Most of these bacterial proteins target Lepidoptera, though some are toxic to Coleoptera or other orders. Bts bind to midgut receptors, causing cell lysis and death.

## GYPSY MOTH BIOASSAYS

One of the early steps in making transgenic plants that express insect resistance proteins is to identify effective genes by testing the purified proteins, encoded by the genes, against the pest. The proteins are incorporated into artificial diets, which are fed to the insects, and survival and growth are then measured.

We fed one PI, aprotinin, to five strains of gypsy moth (Honshu, Russia Mineralni, Connecticut, Russia Black Lakes, and Lithuania), and another, potato protease inhibitor 2 (Pot-2), to one strain, Russia Black Lakes, which had been identified as the least susceptible to Bt (Melody Keena, pers. comm.). Gypsy moth has been reported to use trypsin and elastase as its major digestive proteases (Valaitas 1995). Aprotinin was selected because it binds with many forms of insect trypsin, and we have observed its effectiveness against other species, both in artificial diets and when expressed in transgenic plants. Pot-2 was selected because it binds with both elastase and trypsin, suggesting effectiveness against gypsy moth.

We fed one biotin-binding protein, avidin, to the same five strains of gypsy moth, and a second, streptavidin, to the Russia Black Lakes strain.

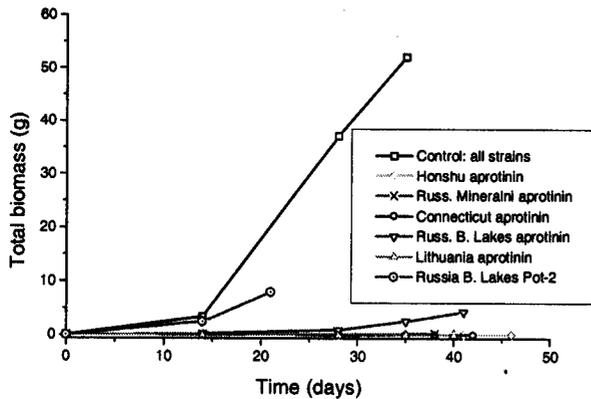
***Protease Inhibitor Results.*** Growth and survival data were combined to give biomass (Fig. 1), which measures the total weight of all surviving insects at any given time. Biomass gives a good indication of the pest's potential to cause plant damage.

Aprotinin-fed larvae had very poor growth, and survival in all strains was significantly reduced compared to controls, although less so in the Russia Black Lakes strain than in the other strains. After three weeks, there were few larvae of most strains left alive. Figure 1 shows that aprotinin is extremely effective against gypsy moth, with very little biomass accumulated in any of the strains over the course of the experiment.

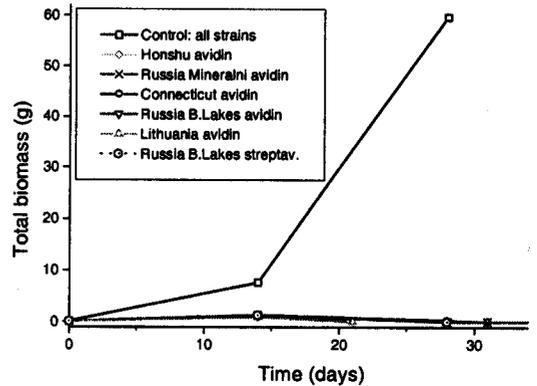
While survival of Russia Black Lakes larvae fed Pot-2 was no different from that of control larvae, growth was significantly reduced. Biomass (Fig. 1) was intermediate between controls and aprotinin-fed larvae. The Pot-2 trial was terminated after three weeks because larvae had consumed all the available Pot-2 diet by this time.

**Biotin-binding Protein Results.** All strains were highly susceptible to avidin (Fig. 2). Survival dropped sharply after one week. After three weeks almost all larvae were dead and none survived until the end of the experiment. There was very little growth of avidin-fed larvae, so that virtually no biomass accumulated in any strain throughout the trial.

Russia Black Lakes strain larvae fed streptavidin had very poor growth and survival, and accumulated minimal biomass, as noted for those fed with avidin.



**Figure 1. Effect of PI proteins on biomass of 5 strains of *Lymantria dispar*.**



**Figure 2. Effect of BBP proteins on biomass of 5 strains of *Lymantria dispar*.**

## EUROPEAN PINE SHOOT TIP MOTH BIOASSAYS

*Rhyacionia buoliana* is a tortricid that feeds exclusively on pine. It arrived in Chile in 1985, spread quickly throughout the country's extensive *P. radiata* forests and is now considered a serious pest. Young larvae drill into the base of shoots, creating tunnels as they feed, and make their way towards the tip as they grow. This causes death of the shoots and even whole seedlings, multi-leadering, and results in reduced tree growth and wood volume. A parasitoid was introduced with some success, but further control is needed.

As with the gypsy moth, resistance proteins were incorporated into artificial diet. We fed five Bts (coded 1, 2, 3, 7, and 8) to tip moth larvae, each at six different concentrations. Survival was measured at each concentration and  $LC_{50}$ s (the concentration which kills 50% of insects in a given period of time) were determined after 14 days.

Three protease inhibitors were selected based on our previous characterization of enzymes present in larval gut material, which showed trypsin to be the major digestive protease. *In vitro* trials showed that aprotinin bound well with this trypsin, inhibiting over 90% of activity, while potato protease inhibitor 1 (Pot-1) was also effective, though less so, inhibiting over 60%. Both these PIs were tested, along with a third, eglin-C, which, while relatively ineffective in inhibiting the trypsin, showed activity against another tip moth digestive protease, chymotrypsin. Aprotinin and Pot-1 were fed at two concentrations; eglin-C was fed at the lower concentration only.

The two biotin-binding proteins, avidin and streptavidin, were also tested, both at three concentrations. Survival and growth were measured for the PI and BBP treatments, and biomass determined.

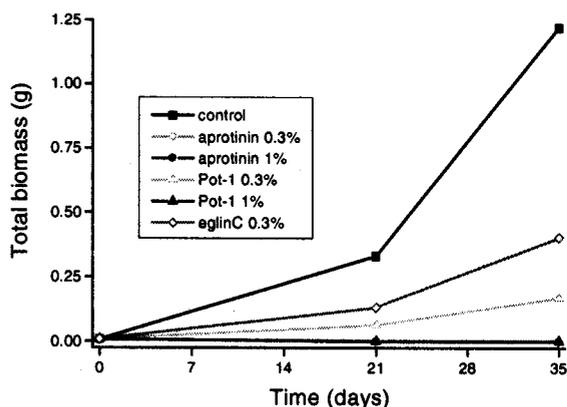
**Bt Results.** LC<sub>50</sub>s for the five Bts screened ranged from 10 to 0.2 µg/ml of diet (Table 1). The Bts with the three lowest LC<sub>50</sub>s are potentially good control agents.

**Table 1. Bt results**

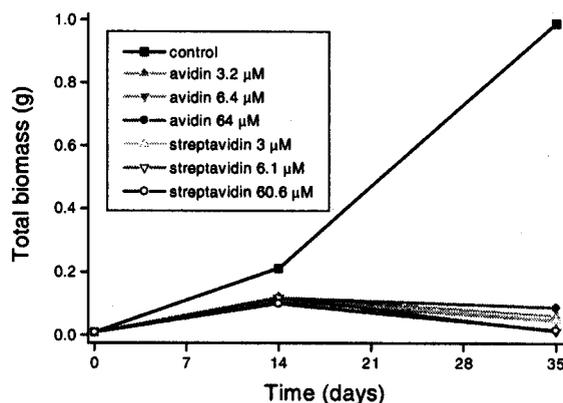
Bt Code	LC <sub>50</sub> (µg/ml)
1	1
2	10
3	5
7	0.2
8	0.6

**Protease Inhibitor Results.** Both concentrations of aprotinin and the high Pot-1 treatment were very effective, with essentially no accumulation of biomass (Fig. 3). The low concentration of Pot-1 and eglin-C were less effective, but still significantly different from controls. These results indicate that aprotinin has excellent potential as a resistance protein for this insect.

**Biotin-binding Protein Results.** As with the gypsy moth, control larvae survived and grew well, while those feeding on either avidin or streptavidin at each concentration failed to grow and died, resulting in minimal accumulation of biomass (Fig. 4).



**Figure 3. Effect of PI proteins on biomass of *Rhyacionia buoliana*.**



**Figure 4. Effect of BBP proteins on biomass of *Rhyacionia buoliana*.**

## DISCUSSION

We have identified a number of pest resistance genes suitable for engineering into *P. radiata* for pine pest control. We have found the protease inhibitor, aprotinin, and the biotin-binding proteins, avidin and streptavidin, to be effective against gypsy moth and European pine shoot tip moth, and three Bts to be effective against the tip moth. Furthermore, we have shown that aprotinin and avidin have similar effects on five different strains of gypsy moth.

Identifying more than one effective pest resistance gene allows the option of “gene pyramiding.” This strategy of engineering more than one protective gene into a plant reduces the selection pressure for a single characteristic that could allow the development of non-susceptible insect biotypes. The pyramiding of diverse resistance genes that encode proteins acting on different physiological targets in the pest insect reduces the likelihood of the development of non-susceptible pest strains. Given their different modes of action, PI, BBP, and Bt genes could be pyramided to increase the durability of pest resistance in the field. This is likely to be particularly advantageous in a pine tree crop that will stand in the ground for nearly thirty years.

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## HEMLOCK WOOLLY ADELGID IMPACT ASSESSMENT:

### HOW BIG OF A THREAT IS THIS CRITTER?

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### INTRODUCTION

The hemlock woolly adelgid (HWA) (*Adelges tsugae*) is an introduced invasive species that was first recorded in the western United States in 1924 and on the east coast in the 1950s near Richmond, VA. The insect spends the majority of its life in a fixed location except during the crawler stage when it moves to new growth. It is a sucking insect that feeds from the tissue of young hemlock (*Tsuga canadensis*) twigs; more specifically, it feeds on stored nutrients in the xylem ray parenchyma cells. HWA is bivoltine (two generations per year) and is parthenogenic, producing from 50 to 300 eggs/adult. Consequently, HWA populations can explode to extreme levels quickly where conditions are favorable.

As HWA populations increase, the ability of hemlocks to continue to produce new growth is significantly reduced and the current foliage becomes less functional in photosynthesis. Foliage grays and prematurely drops. After just a few years, the infested branches often lose the majority of their needles. Impacts to stem and branch growth typically progress upward from the lower crown and if the adelgid populations remain high, trees will die.

This study could not have been carried out without the strong support of our cooperators: Richard Evans, National Park Service, Delaware Water Gap National Recreation Area; John Quimby, Bradley Register, and James Unger, Pennsylvania Bureau of Forestry; Robert Rabaglia, Maryland; and Sherri Hutchinson, West Virginia. They directed the field crews that have collected data from permanent plots each year.

Brad Onken, together with Rich Evans, John Quimby, and Sherri Hutchinson, initiated this damage assessment study in 1993 (Work Plan on file: September 1994; USDA Forest Service, Northeastern Area Forest Health Management, Morgantown, WV). They were interested in assessing tree and stand susceptibility, rate of spread, and potential impacts of HWA on eastern hemlock forest stands. In 1993, very little information was available regarding how quickly hemlock would succumb to HWA once infested. Early reports by McClure (1991) and McClure et al. (1996) were that hemlocks succumb within 4 years, and yet some hemlocks were known to have survived a much greater period of infestation (Quimby, pers. comm.). The primary purpose of the study was to determine rates of tree mortality and determine if hemlocks are able to survive or recover following a HWA infestation.

## STUDY DESIGN AND METHODS

**Sampling Plan: 1993-1997.** The study was initiated by establishing a system of permanent plots within hemlock forests. Sites were selected to represent local hemlock forest stands that were large enough to contain a minimum of three plots. To capture the onset of HWA infestation, sites that were not currently infested but were likely to become infested within a few years were selected where possible. Plots consisted of 10 permanently tagged hemlocks that were predominantly dominant or codominant trees. Each year these trees would be revisited to make an annual assessment of their health. The study used the Forest Service Forest Health Monitoring Assessment Variables for rating crown health (EMAP FHM, Section 2: Crown Condition, Rev. No. 1, April 1998). This study established protocols to assess new growth on hemlock branches as well as a means to assess adelgid populations within these plots and stands. The plots were to be followed to monitor changes in HWA infestation levels and to identify other stress agents that may be contributing to the deterioration of the tree's health. A database was developed by the Forest Service to maintain all data and for use in analysis of the collected observations.

Stands were initially chosen such that hemlock would be a major component of stocking and with sufficient dominant and codominant hemlock trees to provide a sufficient sample. Site and stand information that can be used to evaluate potential differences in stand susceptibility and vulnerability were also collected. Stand species composition, stand density, elevation, soil type, slope and aspect, tree diameter at breast height, vigor rating, and crown position were collected as plots were established. These data were to be re-evaluated every five years.

**Forest Health Assessment.** Each year, each of the following five variables was recorded to assess crown health for each sample tree:

- Crown Diameter      measured along its widest axis and again at 90 degrees to that axis (cm)
  - Crown Ratio          the ratio of crown height to total tree height \*
  - Crown Density        part of expected total crown silhouette that is present \*
  - Crown Dieback        branch tip loss of foliage or fine twigs \*
  - Crown Transparency loss of expected foliage density in existing branches \*
- \* Percentage measured to the nearest 5 percent

Each tree was examined annually and other insect or disease problems were noted. Defoliation by other insects was assessed and rated to the nearest 5%.

**Branch Tip Assessments.** Where possible, 30-cm branch tips were selected from plot trees. If plot-tree crowns were not accessible from the ground, trees close by were used for branch-tip sampling. At least 10 branch tips per plot were to be sampled each year. All shoots were counted on each tip. A shoot consisted of the outermost branch stem section that derived from a single bud and resulted in a single year's growth, and that, in the absence of HWA, would be expected to contain needles or fruiting structures. The number of shoots, the number of shoots that produced new growth, and the number of shoots that have adelgid present were recorded. The fact that new growth production was recorded required that these

data be collected in late spring or early summer when bud flush could be determined. It should be noted that we were not counting the number of new buds or new shoots but rather the number of current shoots that produced new growth. Similarly, the adelgid index is the number of infested shoots. Thus, the number of shoots will always be equal to or greater than either of these counts, as it serves as the base for these counts. For example, out of 47 shoots on a branch tip, 32 produced new growth and 38 were infested (with one or more adelgid).

**Revised Plan: 1998.** Prior to the 1998 field season, we had seen very few newly attacked hemlock trees in our plot system. In 1998 we added plots where adelgid populations could be located in an attempt to better understand the relationship between the branch-tip data and the crown rating system. Plots contained a minimum of five dominant or codominant trees. If no canopy branches could be reached on plot trees, then neighboring trees that had accessible branches were added as plot trees. Branch tips were selected at cardinal directions and tagged to allow for annual remeasurement. A minimum of 12, 30-mm branch tips were selected on each plot, four per tree where possible.

### DATA AND ANALYSIS PROCEDURES

Plots were initially established over the 1993-1995 field seasons.

CROWN ASSESSMENT DATA*				BRANCH TIP DATA**			
Locations	Sites	Plots	Years	Locations	Sites	Plots	Years
DWG	6	81	1993-1999	DWG	6	81	1996-2000
PA-E	13	43	1995-1999	PA-E	12	38	1993-2000
PA-C	9	27	1993-1999	PA-C	9	27	1993,1995-1999
MD	14	45	1995-1999	MD	14	45	1995-2000
WV	3	9	1993-1999	WV	3	9	1993,1994,1996-1999

\*All crown ratings were done to the nearest 5%; to minimize between-crew variance, all field crews were provided annual field review of the assessment rating system.

\*\*New standards were initiated for the collection of data from permanently tagged branch tips starting in 1998.

All analyses were done using the SAS general linear models procedure with Type III errors due to the unbalanced nature of the data among the trees, plots, sites, and states. Because the HWA and branch data were not collected from the same trees that were assessed for crown attributes, all tests related to crown attributes were done at the plot level. The relationship between HWA index and new growth was carried out for branch tips.

### RESULTS

One result of this study has been the review of the pattern of change in crown variables over time. While the study was initiated using the full array of crown health variables, we have found no consistent pattern of change in crown diameter, ratio, or density. In 1999 we conducted a side study to examine the consistency of measurement procedures among and between crews and found that crew effects were not significantly biasing these data. From our analysis, we do not expect crown diameter, ratio, or density to contribute to HWA impact

assessment. Dieback and transparency have exhibited trends associated with HWA abundance. Statistically significant relationships have been found between mean adelgid index in plots and dieback in plot trees, as can be seen in Table 1.

Note that the effect of HWA across all locations increases from 1994 until 1996 when new sites were added that were not infested, decreasing the aggregate relationship. As adelgids increased in our plots, we began to see their effect in 1995 but by 1998 the HWA population had begun to decline. We suspect that this is because as infested trees began to produce new growth, HWA populations were affected by the lack of quality feeding sites. In 1998 and 1999, the effects of adelgid on the trees' ability to sustain new growth production, as exhibited in reduced numbers of shoots producing new growth and waning of adelgid population, reversed the trend. In the coming year, we will be examining ways to best explore the relationship between historical HWA population trends exhibited in these data and crown dieback. As adelgids increased in our plots, we began to see their effect on crown transparency in 1996 and 1997 but by 1998 the population had begun to decline (Table 2).

**Table 1. Adelgid on crown dieback**

Year	Slope	P value*	Prob. > F	df
1994	0.003	NS	0.90	38
1995	0.33	0.02	0.02	77
1996	0.11	0.003	0.003	138
1997	0.31	< 0.0001	< 0.0001	170
1998	0.04	NS	0.27	51
1999	-0.11	NS	0.06	55

\*NS = non-significant trend (5-percent level)

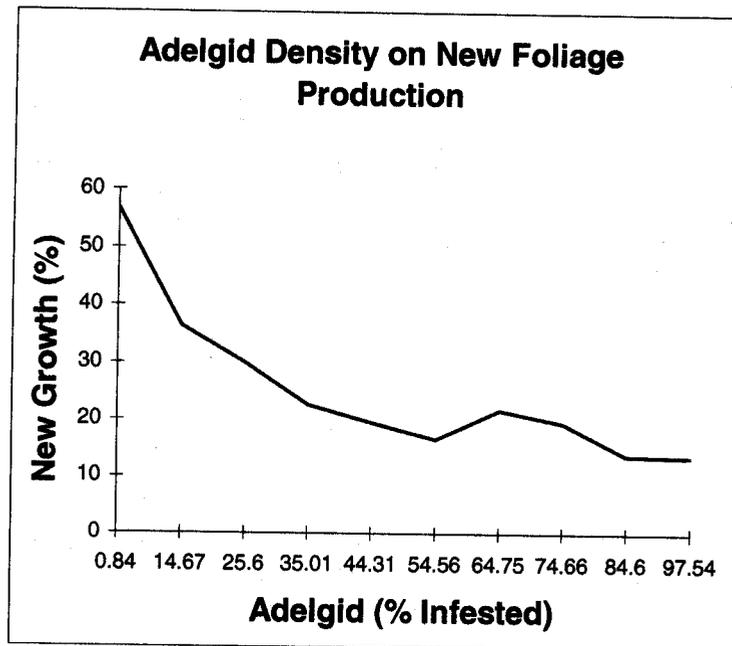
**Table 2. Adelgid on crown transparency**

Year	Slope	P value*	Prob. > F	df
1994	0.09	NS	0.41	38
1995	-0.26	NS	0.61	77
1996	0.44	0.01	0.0108	138
1997	0.30	< 0.0001	< 0.0001	170
1998	-0.04	NS	0.51	51
1999	-0.11	MS	0.06	55

\*MS = marginally significant trend (5-percent level)

The relationship between tip growth and crown transparency is significant each year but the interpretation is not as clear as for other factors. New growth did not show a consistent pattern with transparency among plots between years when aggregated across all locations. We will examine this relationship within areas where we have consistent tree and plot data for sites to determine what appears to be confounding from the dropping and adding of trees and plots to the dataset.

**Adelgid Effects on New Growth.** Looking at the two indices, shoots producing new growth and those infested with HWA, we are able to examine these data at the tip level, i.e., we can consider the branch tip as the sample unit. The relationship between adelgid population index and new growth index is complex. The initial infestation of a plot can happen quickly and the tree may be able to continue to flush previously set buds and produce new buds for a short period. Even with this in mind, the aggregate relationship shows that the productive capacity of eastern hemlock is highly variable. By looking at the local averages of new growth and HWA indices within each 10-percent interval of HWA index, a clearer picture of the relationship appears. Figure 1 shows the average decline in new foliage production on branch tips as HWA populations increase.



**Figure 1. Average index values for HWA population and new growth by 10% intervals of HWA index.**

Note that the values on the horizontal axis are the average values for the 10-percent intervals and in the extremes (0-10% and 90-100%) the averages are near the end points. This shows that the majority of tips in the 0-10% class were uninfested and slightly over half of all these shoots produced new growth. At the other extreme, when a branch tip had at least 80% of its shoots infested, it tended to have all shoots infested (97.54 was the average for that range). In all other classes the average was closer to the midpoint of the interval, showing a more regular distribution. This would indicate that populations are more randomly located on shoots until the density is extreme, at which time the population tends to be more uniformly distributed.

**Tree Mortality.** As mentioned earlier, we expected HWA to move more rapidly into our study areas than has happened. While some tree mortality has occurred, this has not been as pronounced as has been seen in other infested areas, particularly as heavy or as extensive as has been seen in New Jersey, Connecticut, or Virginia. Table 3 shows the tree mortality by location and year. Only Delaware Water Gap has seen substantial tree mortality to date among our plots. We do expect to see increases as we continue to follow these plots and locate new plots in areas where HWA is currently present. The total loss of trees to date is 95 dead among 2,050 canopy trees followed in this study.

**Table 3. Total tree mortality by year and location**

Year Location	1994	1995	1996	1997	1998	1999
DWG	10	0	13	25	5	19
MD	0	0	0	1	3	0
PA-E	2	3	4	5	0	0
PA-C	0	0	0	0	0	1
WV	0	0	0	0	2	2

Five additional trees were removed by chainsaw and are not accounted for in Table 3. Three trees were taken from Delaware Water Gap and two from central PA plots. Death of these trees cannot be attributed to HWA but these trees were in sufficient state of decline to have been considered dead by the woodcutters. If we look at the plots with some positive HWA presence at the time of death (at least one infested shoot), the number of dead trees falls dramatically to a total of 15 trees. This lack of impact in the originally established plot system was the main factor driving our change of protocols and work to expand the study to new areas.

## CONCLUSIONS AND DISCUSSION

Through the use of additional samples of hemlock and HWA from surveys using aerial or satellite imagery, we expect to be able to calibrate HWA mortality models in the coming year. Adelgid has been seen to cause significant damage in these plots but the most valuable data are those we are now collecting to more directly relate population to individual tree effects. Working at these two levels will provide information needed to complete these prediction models. We will be completing the entry and analysis of 2000 data and then assess the need for additional data under our new protocols. We will examine various other data to augment the information we have here.

Although the number of infested plots has increased since 1998, the impact of HWA on tree mortality has been minimal at the time of this analysis, including the few plots that have been infested longer than six years. Plots that have been infested the longest appear to be in significant decline and mortality rates are expected to increase significantly over the next several years. We plan to evaluate the change in crown conditions of these infested trees over time in the near future.

In decline and the absence of new growth, hemlocks become less suitable hosts and we suspect adelgid populations will only be present in low numbers, if at all, in the actual year tree mortality occurs. Rather, weakened trees are more vulnerable to other biotic stressors such as the hemlock borer (*Melanophila fulvoguttata*, Harris) or abiotic conditions such as drought. We hope to substantiate this hypothesis in future analysis.

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# INVADING FOREST INSECTS IN CENTRAL EUROPE

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## ABSTRACT

The spread of exotic organisms is usually followed with great interest, particularly if they have the ability to cause significant economic and ecological effects. In the 20<sup>th</sup> century, quite a few species of forest insects have expanded their range in Central Europe. While many exotic species were accidentally introduced either from North America or Asia to different locations in Europe, other species were already native to Europe and simply expanded their distribution northward. Many of these insects have become successfully established. These newly established insects include lepidopterans, homopterans, dipterans, hymenopterans, and hemipterans. While some of these species do not appear to cause significant economic or ecological damage, others have become significant pests. The invasions can be classified into three major categories.

- A. Northward range expansion of insects from Southern Europe following their earlier introduced host plant (*Phyllonorycter platani* – *Platanus* sp., *Phyllonorycter leucographella* – *Pyrracantha coccinea*)
- B. Accidental introduction (sometimes multiple) followed by range expansion with establishment on host plants deliberately introduced from the same origin (*Parectopa robiniella* and *Phyllonorycter robiniella* – *Robinia pseudoacacia*, *Argyresthia thuiella* – *Thuja* and *Chamaecyparis* spp.)
- C. Accidental introduction followed by range expansion on host(s) native to Europe (*Coleotechnites piceaella* – *Picea* spp., *Corytucha ciliata* – *Platanus* sp., *Hypanthria cunea* and *Quadraspidiotus perniciosus* – many different hosts native to Central Europe)

The most extraordinary invasion is probably that of *Cameraria ohridella*. The species feeds on horse chestnut (*Aesculus hippocastaneum*). Its origin is still unknown, but was first discovered near Lake Ohrid, Macedonia, in 1985, and was described as a new species. Its European invasion followed its deliberate introduction from Macedonia to Linz, Austria, which later served as the center point of invasion to different directions in Europe.

Based on experiences with Central European invasions, it appears that accidentally introduced specialist leafminers have an outstanding potential of becoming significant pests if their host is abundant enough in their new range.

# NONLINEAR TRANSMISSION OF THE GYPSY MOTH NPV

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## ABSTRACT

Previously we reported that transmission rates of the gypsy moth nuclear polyhedrosis virus (LdMNPV) are nonlinear and violate the mass-action assumption that is a key element of many models of disease transmission. The mass-action assumption is a feature of the "Anderson-May" models that have been used to describe many host-pathogen systems; we believe that an understanding of the idiosyncrasies of this system could improve use of LdMNPV as a biopesticide.

We discussed several mechanisms that might be responsible for the nonlinearity observed in our experiments: the effects of induced foliage responses such as tannins on LdMNPV, the effects of heterogeneity in host susceptibility to infection, and the effects of spatial distribution of pathogen (considered in detail at this forum). We tested the hypothesis that spatial clumping is a major cause of the nonlinearity of transmission rates that we have previously demonstrated. Spatial clumping is a pronounced feature of the transmission of nucleopolyhedrosis viruses in insects such as gypsy moth, because larvae become infected by feeding on foliage contaminated with polyhedral occlusion bodies (POBs) of the virus that are deposited when other larvae die from the virus. These POBs spread across the foliage to some extent, particularly under the influence of rain, but generally remain highly concentrated within the cadavers of virus-killed larvae that decompose on the foliage. We found that clumping significantly reduced mortality of gypsy moth from LdMNPV and presented preliminary findings suggesting that clumping may be the cause of nonlinear transmission.

# ECOSYSTEM IMPACTS OF INVASIVE EXOTIC PLANTS IN WILDLANDS – CHARACTERIZING THE CULPRITS

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## ABSTRACT

Until the past two decades, the focus of invasive plant research was largely on agricultural and rangeland weeds because of the direct economic costs associated with crop and grazing land losses. Over the past two decades, however, the continued decline of parks and wild lands in the face of invasion by non-native species has contributed to the rising awareness and concern over species that threaten native biodiversity and ecosystem functioning including many introduced plants. While non-native plants can cause the decline of native species by directly competing with them for limiting resources, they may also alter the fundamental processes that support native species assemblages. Species that alter ecosystem processes change the rules of the game for native species and potentially shift ecosystems down new trajectories from which it may be hard to return. They therefore represent serious management and restoration challenges.

Introduced plants affect ecosystem processes if they change rates of nutrient accumulation, retention, and turnover; alter patterns of energy flow and water movement; alter geomorphology and physical structure of the site; and/or alter the disturbance regime. Many plant invaders do more than one of these things at once (e.g., grasses that promote fire cause changes in N cycling and loss). Currently, we lack a framework for reliably predicting which species will have ecosystem-scale impacts. The purpose of this talk is to present an overview of the factors that will determine which species might have ecosystem impacts and highlight the interplay of invading species with the recipient community in determining the outcome of species invasions.

The impact of a plant species might be thought of as being a function of (1) its demography (birth and death rate, dispersal characteristics, etc.); (2) its morphology and physiology (this can be thought of as the effect at the individual level: how fast does it take up nutrients, what does it do with them, how does it affect heat flux, physical habitat structure, etc.); and (3) the recipient environment. Regarding the latter, a species is unlikely to have the same effect in each environment it enters, both because the environment affects demography and physiology of a species and because of varying degrees of resistance to change by the resident biota or abiotic features of the environment.

Efforts to characterize plant invaders in terms of their demographic traits date back to the 1960s when ecologist Herbert Baker wrote a now classic article, "On the modes and origins of weeds." But Baker did not distinguish high impact from low impact invaders and for the most part he focused on agricultural weeds, many of which do not have impacts in wildlands.

Since Baker's time, efforts to find general traits to explain "invasiveness" have not been fruitful. Perhaps this is because the term "invasiveness" itself is ambiguous and is generally defined as the ability to increase when rare; almost any species can do this under at least some circumstances. More fruitful efforts to characterize traits of invaders have focused on prediction within particular groups, such as *trees* (Reichard and Hamilton 1997) or specific genera such as *pinus* (*Pinus*) (Rejmanek and Richardson 1996). These authors distinguish species either as ones that tend to spread or do not tend to spread from planted sites. A consistent finding is that successful invaders have (1) fairly fast growth through the juvenile period, (2) fewer years between large seed crops, and (3) generally smaller seeds than non-invaders within their category.

While these studies give us some idea of what it takes to be an invader for trees, neither of the studies distinguish high- from low-impact invaders. It may be that there are no unique demographic traits associated with high-impact species, but this has not been specifically addressed. In California, there are an estimated 1,050 introduced plant species with self-replacing populations, yet only a small fraction of them actually cause measurable change in some feature of wildland ecosystems. The California Exotic Pest Plant Council (CalEPPC) concluded that 78 of these species merit listing as invasive and damaging in wildlands and an additional 50+ merit further study. We can consider these to be "high-impact" invaders. An interesting feature of this group that relates to their demography is that 78% of them were purposefully (as opposed to accidentally) introduced to California or the western USA. This is also true in Hawaii where 91% of the 107 introduced plant species considered a threat to natural areas were also purposefully introduced species. This stands in contrast to damaging introduced insects which largely come in as accidental introductions (an OTA report estimates 81% are accidentals). It also contrasts with the proportion of purposefully versus accidentally introduced plant species in the general flora (for example, in Hawaii only 58% of all of the established plant invaders were purposefully introduced.). Hence it seems that there is a disproportionate representation of purposefully introduced species in the "high-impact" group. This suggests that these high-impact species are not necessarily species with good long-distance dispersal (which is a good thing in terms of control) and because most of them are perennial, they are not likely to be ruderal species. In other words, local *persistence* is part of what these species are all about. In addition to being perennial, most of these species maintain large seed banks, have a wide tolerance for environmental conditions once established, and show rapid recovery after disturbance. There are several high-impact invaders on the CalEPPC list that are annual species and were accidental imports. Notable examples include yellow star thistle (*Centaurea solstitialis*) and cheatgrass (*Bromus tectorum*). Their persistence in part comes from large seed banks and wide environmental tolerance but it may also come in part from positive feedbacks that they generate (e.g., cheatgrass/fire cycles). Mechanisms promoting their persistence merit further study.

My field research has focused on two groups of high-impact invaders that I believe have some of the most long-term ecosystem effects: (1) those that alter N accumulation and cycling, and (2) those that affect disturbance regime. Among ecosystem ecologists, there is a great deal of work looking at how species traits (physiology/morphology) such as leaf litter chemistry, rates of N uptake, timing of uptake, and allocation affect rates of N turnover and retention. A question of great interest is how different does an invader have to be from

resident species to have an impact on rates of cycling, retention, or accumulation? With my former PhD student Michelle Mack, we investigated the effect of introduced perennial bunchgrasses on ecosystem N cycling in seasonally dry Hawaii woodlands which previously lacked a perennial grass component. These grasses are different from natives in their nutrient use efficiency and their leaf tissue chemistry. Some of these characteristics, such as lignin content and C:N ratio, are known to have a strong control over rates of litter decomposition. Hence we predicted that these grasses should cause a change in ecosystem N cycling. Yet we found that despite being in these sites for 25+ years, they are not having a measurable effect on soil nutrient cycling. We believe this is because their major interaction with native species is a competitive one—they compete for the same pool of limiting resources and are not different enough in traits that influence N cycling to create an effect despite their strong superficial differences. It may also be that it will take another decade for effects to develop because the soils in this area contain a large amount of organic carbon and it may take a long time for an invader to dilute this pool if the natives are still in the system. We suggest that species that are not discretely different from natives in their traits and interact with them largely through competition may take a long time to show ecosystem process changes.

By contrast, species such as the N fixing shrub, *Myrica faya*, which is invading these same sites, are very different from most of the species present in terms of litter chemistry and can much more rapidly alter soil N accumulation and cycling in these N-limited communities. Peter Vitousek found that *Myrica faya*, first introduced to Hawaii as a forestry tree and now widely invasive in mesic and seasonally dry forests and shrublands, can add up to 4 times as much N per year as all native sources combined. It also substantially elevates rates of N mineralization. *Myrica* does something qualitatively different from the other species present and this characteristic—fixing atmospheric N—makes its litter contributions substantially different in their chemistry from native species. In other words, *Myrica* has no functional analog relative to N cycling in the invaded system and most likely because of that it has a rapid and dramatic impact on ecosystem processes. In California, five out of seven shrubs on the CalEPPC “A” list are nitrogen fixers. Karen Haubensak, a graduate student in my lab, has documented that two of these species have a strong effect on soil N, but this effect seems dependent on the abundance of the invader and perhaps the duration of presence on the site. These shrubs are invading sites with native N fixers but these tend to be species with small stature. So these N fixing shrub invaders again appear to have no functional analog in the invaded sites. Elevated soil N has a long-term effect on community development. N fixing invaders are frequently the target of removal efforts or insect attack. A variety of investigators, including people in my lab, have demonstrated that N left over from these N fixers can have a long-term effect on community development by favoring fast-growing exotic species.

A second difficult-to-reverse impact appears to occur with species that alter disturbance regime. In the western U.S. and Hawaii, there are numerous introduced plant species that alter the frequency and intensity of fire (list presented). In general, alterations to fire regime are due to changes in the continuity of the fuel bed due to the way in which grasses fill in the space between woody species. In ecosystems where native species are not adapted to intense or frequent fire, the effects of this alteration on native species composition and ecosystem structure have been very dramatic such as in Hawaii where I have been working. However, the impacts of grass-fueled fires are dependent upon the recipient environment. We found that in

the coastal lowlands of Hawaii Volcanoes National Park, alien grass-fueled fire does not have a negative impact on cover of native species. We concluded that this was because the native species in these sites are fire tolerant whereas in higher elevation (submontane) sites, different native species are present and they are not fire tolerant. Grass/fire cycles can be very difficult to reverse due to the inevitability of fire in human-dominated landscapes, feedbacks from grasses to microclimate, and the ability of these grasses to regenerate quickly and suppress the recruitment of woody species either directly or because of high fire frequency.

Often traits that enhance the spread of fire may not be initially obvious. For example, *Tamarix*, or saltcedar, is a fast-growing, salt-tolerant tree that has invaded naturally saline river courses throughout the American West. Its salty litter and foliage would not appear to be fire promoting. Yet in a study of fire in riparian habitats in parts of the lower Colorado River and nearby drainages, D. Busch found a high, and increasing, frequency of fire in areas dominated by *Tamarix*. He accounts part of the apparent increasing fire frequency to the accumulation of dead litter of native species because *Tamarix*, a species highly consumptive of groundwater, gradually reduces available water in the system and slowly outcompetes or adds stress to life for native species. In this case, the interplay of *Tamarix* with the native community adds more fuel to the impending fire. These examples highlight that in order to really predict the impact a species will have, you need to know about the potential recipient environments and how native species in these sites will interact with the invader and respond to the altered ecosystem processes that will occur once the invader is established.

So do we simply throw up our hands and say it is close to impossible to predict which species will have ecosystem impacts because we need to know too much about both the species and the potential environments? I believe that we need to rely on empirical evidence to guide decision making. We know, for example, that N fixers have tremendous potential to impact sites. We should carefully review introduced N fixers in the horticultural trade and look for those species with traits that will promote spread and persistence using known high-impact invaders as a point of reference. Since we already know who many of these invaders are, we should reduce their abundance in the horticultural trade in states where they are a risk and look carefully at species that share many traits with them but might not be perceived as a threat yet. Likewise, we already know that many introduced grasses can cause difficult-to-reverse, ecosystem-level change. We need careful controls over further introductions of non-native grasses and better coordination among agencies so that agencies that promote outplanting of grasses are not in direct conflict with conservation groups.

I conclude by saying that our search for a general framework for predicting species impacts is still in an early stage; it may be that no such general framework will emerge that works for all plant species. Nonetheless, data on wildland weeds and *the conditions under which they create impact* is essential to helping managers identify areas most at risk from invader impacts and species that should be prioritized for control or removal. While it is difficult to remain optimistic in the face of dramatic changes in the composition and function of our forests and wildlands, it is important for research biologists to interact with managers, policy makers, and stake holders to help identify areas at risk, identify species that pose the greatest threats, and work in teams at a regional scale to find creative solutions to control, removal, and/or restoration.

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ASSISTED AND NATURAL SPREAD OF PARASITIDS AND DISEASES IN  
NEWLY ESTABLISHED POPULATIONS OF GYPSY MOTH:  
THE WISCONSIN EXPERIENCE

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ABSTRACT

Gypsy moth was isolated from the nearest long-established population in Michigan for years following establishment in Wisconsin. Given this isolation, we were concerned that natural spread of specialist enemies of this foreign pest might be slowed. We decided to conduct an initial survey of natural enemies of gypsy moth and implement a biological control introduction program.

In the summers of 1996 and 1997, workers from the Department of Natural Resources placed burlap bands around preferred host trees at sites with relatively higher catches of male moths. Sites were checked twice weekly starting the first week of June through the third week of July. This corresponded to the period of third instar larvae through pupation. Larvae found under the bands were sent to a rearing facility where the cause of death of any that died prematurely was determined. When gypsy moth egg masses were found during population monitoring, we checked them for the presence of parasitoids.

We found *Entomophaga maimaiga* at one site in the Door Peninsula. Nucleopolyhedrosis virus (NPV) and *Cotesia melanoscela* were present at a site near the southern end of Green Bay. Dipteran parasitoids were present throughout the range of gypsy moth and some of these were identified as *Compsilura concinnata*. *Ooencyrtus kuvanae* was present at five sites in the base of the Door Peninsula.

Following the survey of parasitoids and diseases already present, we started an introduction program to supplement natural spread. We used three criteria in selecting candidate species: a high degree of specificity to the gypsy moth, the ability to establish in low-density gypsy moth populations and to survive in our climate, and availability. Using these criteria, we selected *E. maimaiga*, *O. kuvanae*, and *C. melanoscela* for release. Introductions were made throughout the area where gypsy moth was established in Wisconsin for four years.

We introduced *E. maimaiga* using ground cadavers. Releases were done in September, October, and November. By using cadavers, we expected to provide a more consistent distribution of spores and a higher concentration. We also avoided the possibility of introducing soil-borne pathogens. We introduced *O. kuvanae* as adults collected off of infested gypsy moth egg masses supplied from wild populations in Michigan. Release of the

early fall generation of this species was emphasized to maximize the proportion of females. Captive bred *C. melanoscela* were released in late fall as overwintering cocoons as previous studies had shown this reduced mortality from parasitism. Despite this, emergence was an average of only 62%.

Post-release surveys were done using the same procedure as before. Sites were surveyed each year following an introduction through the summer of 2000. *E. maimaiga* was recovered at approximately 50% of its sites. Recovery appeared to be influenced by rainfall; natural spread also occurred into central Wisconsin in 2000. *O. kuvanae* was recovered at about 35% of the sites where we released it. Unassisted spread of this species has been rapid, however. We have not yet recovered *C. melanoscela* from sites where it was released or at other sites.

The spread of *E. maimaiga* appears to have been assisted by our introductions. Natural spread, however, will play a larger role during wet years now that gypsy moth is more generally established in the eastern areas of the state. This species is a good choice for introductions into newly established and isolated populations of gypsy moth. The risk of effects on non-targets is small, it can persist for many years, and while it can be difficult to make collections, it is relatively cheap. We would recommend to pest managers that the establishment rate might be improved by distributing spores soon after collection in mid summer to avoid desiccation. Variation in the likelihood of recovery emphasizes the need to conduct post-release surveys for several years.

The spread of *Ooencyrtus* was not greatly aided by our introductions. Most populations found during surveys were not associated with introductions. In terms of public relations though, introductions of this species were a success. *Ooencyrtus* is an easy species for the public to find when gypsy moth is at moderate levels, so it makes a good ambassador species for the concept of biological control. It is also easy to obtain, cheap, and fairly specific to gypsy moth when collected from wild populations.

We can't say how successful our introductions of *Cotesia* were due to limited efficacy of the burlap band survey technique at recovering this parasitoid. Simple searches of young larvae at release sites also failed to find evidence of this species, however. Pest managers might consider the ability to recover a species when selecting them because a lack of results could be a problem in maintaining support for a biological control program.

In order to improve biological control introduction programs, pest managers need more information on which natural enemies benefit from deliberate introductions, particularly when a species is expensive or difficult to obtain. More information on how to optimize conditions for establishment of each species would also be helpful.

# CONTROL OF *ANOPLOPHORA GLABRIPENNIS* WITH ENTOMOPATHOGENIC FUNGI

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## ABSTRACT

Two species of entomopathogenic fungi applied in two different ways for control of *Anoplophora glabripennis* adults were compared during field trials near Wuhe, Anhui Province, China, in July 2000. A strain of *Beauveria bassiana* originally isolated from beetles and presently marketed by Mycotech (Butte, MT) and a strain of *Beauveria brongniartii* isolated from a cerambycid in Japan and marketed by Nitto Denko (Osaka, Japan) were used. For application, use of non-woven fiber bands impregnated with fungal cultures and attached around tree trunks was compared with spraying comparable doses of fungal spores onto tree trunks. Thirty poplar trees were used for each treatment with an additional 30 untreated trees as controls. For each study tree, five field-collected *A. glabripennis* adults were placed within a cage made of window screening, 70 cm in length and wider than the diameter of the tree so that beetles could move freely, placed 1.5 to 2 m up the tree trunk (= 750 total adult ALB were used for this study). Beetles were counted and provided with food and water daily for 10 days, after which adults were removed and monitored further in individual cups. Fungal treatments always differed from controls. Oviposition rate per female and the number of oviposition sites per tree were significantly lower for cages where *B. brongniartii* was applied compared with *B. bassiana*, although results for *B. brongniartii* did not differ by application method. Time to 50% mortality (LT<sub>50</sub>) was significantly lower for cages where *B. brongniartii* was applied compared with *B. bassiana*. Once again, the method that *B. brongniartii* was applied did not yield statistically significant differences although we saw a trend of lower LT<sub>50</sub>s for bands (7.8 days) than for sprays (9.2 days).

Laboratory bioassays to compare additional fungal strains were conducted both with adult ALB from the colony in the USDA quarantine in Ithaca, NY, and with field-collected adult ALB in China. Ithaca bioassays comparing two isolates of *B. bassiana* and two isolates of *B. brongniartii* demonstrated faster LT<sub>50</sub>s for *B. brongniartii*; these results are consistent with field bioassay results. Bioassays in China compared five strains of *B. bassiana*, one strain of *B. brongniartii*, and one strain of *Metarhizium anisopliae*. LT<sub>50</sub>s for adults exposed to one *B. bassiana* strain, the *B. brongniartii* (Nitto Denko), and the *M. anisopliae* strain were all < 6 days.

# ASSESSING THE IMPACT OF NORWAY MAPLE ON NATURAL FOREST COMMUNITIES

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## INTRODUCTION

Unprecedented high rates of biological invasion are increasingly homogenizing the earth's biota, with serious consequences both for humans and for natural communities and ecosystems (Lodge 1993, Vitousek et.al. 1997). Invasions vary in the extent to which they alter the structure, function, and dynamics of natural ecosystems (Williamson and Fitter 1996). There are very few accepted generalizations about which kinds of invasive species cause the greatest ecological effects. One of them is the "superior competitor hypothesis." It predicts that the invader that has a superior competitive ability can cause a significant effect by drawing down resources to very low levels (e.g., Huenneke and Thomson 1995, Meekins and McCarthy 1999) and may lead to competitive displacement and numerical reductions in native species. This hypothesis is applicable to many invasive species because they tend to excel in a new area where their congeners have existed (Williamson and Fitter 1996). The superior competitor hypothesis can also be readily extended to any species (native or exotic) to address a more fundamental question: how does a single species interact with its neighbors at the individual level and produce consequences at the community and ecosystem levels? Current mass invasion and the rapid community/ecosystem changes as consequences of invasion have offered a great opportunity to answer this question.

Trees are the dominant life form and comprise the majority of the biomass and productivity in forests. Canopy trees drive succession and ecosystem functions in forests. Subsequently, invasive tree species may easily have a large effect on a forest at several organizational levels. However, it is often difficult to distinguish between the effects of tree invasion and effects of prior land use. Among the few studies on invasive tree species, only Vitousek and Walker (1989) pin down the mechanism of the invasion's effect by linking physiological processes and population dynamics to ecosystem properties. None of these studies has directly tested the superior competitor hypothesis in a field experimental setting.

In this study, I test the superior competitor hypothesis by assessing the impact of *Acer platanoides* (Norway maple, NM), an invasive tree species, on natural forest communities. I hypothesize that the superior competitive ability of *A. platanoides* at the individual level causes their negative effects at the levels of community and ecosystem. I further hypothesize such superior competitive ability of *A. platanoides* adults is achieved by three mechanisms: (1) aboveground competition for light, (2) belowground competition for water and nutrients, and (3) litter effect, including possible nutrient release and/or physical effects.

*A. platanoides* was introduced as an ornamental shade tree from Eurasia in 1762 and is now the most planted street tree in the U.S. (Webb 1997). It has been reported that its dense canopy reduces wildflower diversity (Wyckoff and Webb 1996, Webb et al. 2000) and that it exhibits higher water, nitrogen, and phosphorus use efficiencies than its congeneric species *Acer saccharum* (Kloppel and Abrams 1995), but no quantitative measurements were taken to assess these hypothesized effects. *A. platanoides* appears to be aggressively invading forests in the northeast U.S. and has even captured the attention of the popular media. *Acer rubrum* (red maple, RM), a congener of *A. platanoides* and a dominant canopy species in the mesic urban forest on Long Island, NY, will be compared as the representative native species in terms of effect.

The study is conducted in the nature preserves. The neighboring natural community is a typical mesic temperate deciduous forest. The dominant tree species are *Quercus rubra*, *Q. velutina*, *A. rubrum*, *Betula lenta*, *Cornus florida*, *Sassafras albidum*, and *Carya* sp. The shrub layer is dominated by *Viburnum acerifolium*, *Viburnum dentatum*, *Vaccinium vacillans*, and *Gaylussacia baccata*. The herb layer is carpeted by *Maianthemum canadense* and *Parthenocissus quinquefolia*, and scattered *Polygonatum biflorum*, *Smilacina racemosa*, *Smilax rotundifolia*, and *Aster divaricatus*. Under an *A. platanoides* dominated forest, however, these species are largely absent and the understory is dominated by *A. platanoides* juveniles, *Lonicera japonica*, and *Alliaria petiolata*.

## METHODS AND RESULTS

**Vegetation Survey and Mapping.** To detect whether *A. platanoides* has significant spatial associations with particular species, either natives or exotics, I mapped all individual adult trees (DBH > 2.0cm) in a 100x50 m<sup>2</sup> area (100m W-E) in Muttontown, NY. The mapping area covers the highly invaded area (west) and its neighboring native forest (east). I recorded species, DBH, and the X,Y coordinates of each tree. I then divided this area into 200 5X5 m<sup>2</sup> plots. I recorded the density of tree seedlings (height < 0.3m) of each species in each plot and recorded the coverage of each shrub, herb, and vine species in each plot as well.

An initial inspection indicated that (1) *A. platanoides* was negatively associated with native species diversity. (2) Very few species grew under *A. platanoides* monoculture except *A. platanoides* and two other invasive exotic species. They are much less abundant towards the interior of the native forest (east of the mapping area). (3) *A. platanoides* is invading the interior of the native forest (*A. platanoides* adult density drops to nearly 0 beyond 65 m eastward, while seedlings established beyond the full 100m).

**Belowground Competition Experiments.** To explore the competitive effects of *A. platanoides* roots on seedling performance of *A. platanoides* (NM) and *A. rubrum* (RM), I conducted two experiments with a manipulation of trenching the treated plot to 25cm deep to remove shallow root effects. Log-transformed growth data were analyzed with ANOVA, and the survival data were analyzed with Chi-square test.

**Experiment 1.** I transplanted a total of 240 seedlings under three NM and three RM adult trees in the summer of 1999. Two of four 1x1 m<sup>2</sup> plots under each tree were trenched. Five seedlings of NM and RM were transplanted in each plot. The results showed that (1) RM

seedlings grew better under RM trees, while NM seedlings grew better under NM trees; (2) all seedlings survived better under NM than under RM ( $P = 0.036$ ,  $n=6$ ), while NM seedlings survived better than RM seedlings ( $P < 0.001$ ,  $n=6$ ); and (3) seedlings grew better in trenched plots than control plots, more so under RM than NM, but not significant due to the high mortality of seedlings.

**Experiment 2.** I repeated the experiment with a total of 640 seedlings in the fall of 1999 with two additional components in the design: (1) I used two sites to see if the pattern from Muttontown was applicable in other mesic forest on LI; (2) in addition to four NM in monoculture and two RM in mixed area, I also used four NM adults in the same mixed area for each site. The results showed that (1) seedling growth did not differ significantly between the two sites ( $P=0.478$ , initial  $n=320$ ); it would not be the priority of my future experiment to use more than one site; (2) seedling survival under two NM canopy types was very similar, but significantly lower than under RM, which strongly suggested that NM adults, not the prior land use of NM monoculture, caused the lower seedling survival; (3) NM seedlings survived better than RM ( $P < 0.001$ ), but RM seedlings grew faster if they survived ( $P=0.008$ ); this implied certain life history trade-offs in the seedling performance; and (4) trenching (removing shallow root effects) significantly improved the growth of RM seedlings under NM, but not of NM seedlings. This suggests that the root of NM adults suppressed the native RM seedlings' growth, while did no harm to their own seedlings.

**Above + Belowground + Litter Experiment.** Based on what I had learned up to the spring of 2000, I set up a formal experiment to simultaneously test all three proposed mechanisms with which *A. platanoides* adults compete with understory seedlings, compared to its native congener *A. rubrum*. The performances of transplanted responding seedlings (e.g. phytometers) will measure the competitive hierarchy of the canopy species. The phytometer species are chosen according to the mapping study: four native species dominated in the mixed forest (*A. rubrum* (tree), *B. lenta* (tree), *P. quinquefolia* (vine), and *M. canadense* (herb)); three exotic species persisted under the *A. platanoides* monoculture (*A. platanoides* (tree), *L. japonica* (vine), and *Alliaria petiolata* (herb)); and one more exotic vine (*Celastrus orbiculata*, which is most invasive and destructive at many forests on Long Island and likely to be the next invader to Muttontown Preserve). All the phytometer seeds were collected from LI mesic forests (mostly from Muttontown) in 1999 and seedlings were germinated in the Stony Brook greenhouse in the spring of 2000.

The experiment tests the presence and the relative importance of the competitive effects of *A. platanoides* adults on seedling performance due to its canopy, root, and litter, in comparison to those of native *A. rubrum*. It also tests the competitive response hierarchies of eight phytometer species in various experimental conditions, which may predict the direction of community transition under the effect of *A. platanoides* invasion. Eight *A. platanoides* adults were randomly chosen in an *A. platanoides* monoculture, and another eight *A. platanoides* and eight *A. rubrum* were chosen in an adjacent mixed forest. Half of the trees had the south half of the canopy removed in May 2000. Under each tree, three 1.5mx1.5m plots were randomly located at 2m north of the trunk, one trenched, one with litter removed, and one intact. In each plot, five seedlings of each of the eight phytometer species were transplanted in May, 2000, with a total of 2,880. Their performances were recorded in late July and September of 2000.

The preliminary analysis of the seedling growth showed that (1) light is the most limiting factor under NM canopy; (2) root competition for resources is also limiting the understory; (3) litter, in general, protects understory seedlings from herbivory; and (4) different seedling species respond to experimental treatment differentially, suggesting that NM may potentially alter species composition.

## CONCLUSIONS

This proposed study is one of the first field experimental studies on the mechanism of invasive tree species' effects at both community and ecosystem levels. The challenge of distinguishing the effects of invasive tree species and land use history has prevented all except one existing case to demonstrate the mechanism of the effects by tree invaders. This study will use a combination of comparative and experimental approaches to address this issue, which will greatly enhance the power of hypothesis testing and interpretation of confounded empirical patterns. Results of the study will not only offer the much needed knowledge on the effects of invasive tree species and have broad implications on predicting the effects of invasive species in general, but also will shed new light on how species interaction at the individual level has consequences at higher organizational levels, e.g., community and ecosystem.

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# THE PHYTOSANITARY ALERT SYSTEM OF THE NORTH AMERICAN PLANT

PROTECTION ORGANIZATION: WWW.PESTALERT.ORG

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## ABSTRACT

The introduction of exotic plant pests into North America has resulted in both ecological and economic costs of immense proportion. These pests include arthropods, mollusks, pathogens, and weeds. Quarantine efforts are under increasingly intense pressure from factors including globalization and the accompanying movement of people, increases in international tourism and trade, and smuggling. As a result, there is a need for more effective exclusion and detection measures to defend against increased pressure from pest invasions. A new tool for those on the front line of safeguarding North American plant resources is an information network that provides alerts about emerging pest threats. The North American Plant Protection Organization (NAPPO), which is comprised of members from Canada, Mexico, and the United States, has established the Phytosanitary Alert System. This web-based resource consolidates international pest information of significance to North America. It provides concise pest alerts enabling focused inspections on high-risk cargo, passenger baggage, and other pathways of entry.

NAPPO panel members have constructed the Phytosanitary Alert System located at [www.pestalert.org](http://www.pestalert.org). Sources of information for the System may include: the world-wide web, port of entry interception records from the NAPPO countries, journals, newsletters, databases, conferences, and personal networks, as well as international organizations and plant protection services. The website also solicits input from the scientific community and others who have information to contribute about emerging pest threats. Quality control filters will ensure that only accurate, reliable information is posted. The System will strive to focus on that which is truly important to Mexico, Canada, and the United States. In addition to posting concise pest alerts, the site displays or offers links to relevant information sources, data sheets, and photographs. Eventually, the System may offer an email capability similar to that used by ProMed to actively notify personnel. Access by hand-held device is currently available. Finally, not only will the System serve as a practical tool, it can become a valuable archive of the historical record of pest threats to North America.

TECHNOLOGIES FOR PREVENTING INTRODUCTIONS OF  
EXOTIC WOOD-BORING INSECTS INTO THE UNITED STATES

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ABSTRACT

The use of microwaves to destroy Asian longhorned beetles in wood used for making pallets and crates was investigated in China. Our preliminary research found that green wood is an excellent absorber of microwave energy regardless of wood type studied, whereas dry wood is a much poorer absorber, but a better transmitter, of microwave energy. Consequently, because larvae and pupae contain a high volume of water, insects are killed much more quickly in dry wood than wet wood using microwaves. Initial experiments conducted in China on 4"x4"x1" and 4"x4"x4" blocks of poplar showed that irradiation at 100% power using a 900 W microwave oven kills ALB larvae and pupae in 5 to 30 seconds in dry poplar and 3 minutes or less in wet poplar. Our preliminary data suggest that microwaves are a feasible, practical alternative for eradication of exotic wood-boring insects in wood used to construct solid wood packing materials. We are currently working on optimizing the parameters of this technology and the inclusion of chemical indicators that can be applied to wood to verify that effective treatment of the wood has occurred.

RELATIONSHIPS BETWEEN ENDOGENOUS JUVABIONE IN FRASER FIR AND  
BALSAM WOOLLY ADELGIDS

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ABSTRACT

Balsam woolly adelgid (*Adelges piceae* (Ratzeburg)) is an introduced pest of little concern in its native range, but devastating to Fraser fir. Balsam woolly adelgids (BWA) typically attack the trunk of Fraser fir. Loss of apical dominance usually occurs early in a BWA infestation. Old growth Fraser fir stands are virtually eliminated, but individual trees still survive. In many cases vigorous Fraser fir reproduction has replaced the old growth as seen at the top of Mt. Mitchell, NC. In central Europe, fir trees tolerate a BWA infestation by producing thick outer bark, which is an unsuitable substrate for further development. There is some indication that Fraser fir may have a similar mechanism. Juvabione, which is found in the wood and bark of Fraser fir, may also play a role in resistance to BWA. Juvabione concentrations increase through the growing season. Juvabione concentrations increase with tree age. Juvabione concentrations may be induced to increase with BWA infestation levels. Juvabione has been correlated with reduced BWA egg production. When topically applied, juvabione can eliminate BWA egg production. Additional questions to be explored are: Does juvabione within the tree influence BWA reproduction and metabolism? Can we select for trees with high juvabione levels that will be resistant to BWA?

DEVELOPMENT OF ODOR-BASED MONITORING SYSTEMS  
FOR EXOTIC PESTS

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ABSTRACT

The ability to rapidly detect and delimit incipient populations is critical to efforts to protect managed and natural ecosystems from invasive species. We are developing semiochemical-based monitoring technology for exotic pests that are recently established in, or pose a significant threat to, U.S. ecosystems. Current projects, with one exception, involve forest pests. Our general approach is: (1) collect and identify potential attractants (pheromones and kairomones), (2) assay them to determine their biological activity, and (3) develop lure/trap combinations that can be used in action programs.

*Callidiellum rufipenne* (Coleoptera: Cerambycidae) (Motschulsky) has been found in North America to infest Eastern red cedar (*Juniperus virginianensis*) and Northern white cedar (*Thuja occidentalis*). Several host-produced compounds elicit antennal responses, and this spring we plan to perform field assays to test the behavioral activity of these compounds.

*Dendrolimus superans sibiricus* (Lepidoptera: Lasiocampidae) Butler is an important pest of fir, spruce, and larch in its native range of northern Asia. Although it is not established in the U.S., it poses a significant threat to our coniferous forests. An attractant has been found for this pest<sup>1</sup>, but current work involves determining the exact structure of the sex pheromone.

*Copitarsia* spp. (Lepidoptera: Noctuidae) (Walker) are polyphagous pests of ~40 crop plants in their native range of Mexico, Central America, and South America. We have obtained permits to import these pests and are making arrangements to collect them.

*Anoplophora glabripennis* (Coleoptera: Cerambycidae) (Motschulsky) is a serious pest of poplar, maple, and willow in its native range of China. In the past few years, infestations have been discovered in the New York and Chicago areas. Current research involves, among other things, the development of lures and traps (in collaboration with groups from SUNY-ESF, Syracuse, NY; USDA ARS, Beltsville, MD; and Beijing Forestry University, China) to be used in the survey and detection effort.

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# SUDDEN OAK DEATH IN COASTAL CALIFORNIA

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## ABSTRACT

Thousands of tanoak (*Lithocarpus densiflorus* Hook. & Arn. Rehd.), coast live oak (*Quercus agrifolia* Nee), and California black oak (*Quercus kelloggii* Newb.) have died in coastal areas of California. This unprecedented oak die-off is believed to be caused by a previously unknown *Phytophthora* species discovered by David Rizzo, UC-Davis, in June 2000. Western oak bark beetle, Ambrosia beetles, and *Hypoxylon thourasianum* are commonly found on infected trees. Considered secondary, these organisms accelerate deterioration of the weakened trees.

The disease was named by Pavel Svhira, UC-Cooperative Extension Horticultural Advisor in Marin County in 1995 when he first reported dying tanoak in Mill Valley, CA. Affected trees show a rapid foliar color change with the entire crown changing from green to yellow to brown in a few weeks.

Sudden oak death has been found in the following six coastal counties: Marin, Sonoma, Napa, San Mateo, Santa Cruz, and Monterey. Infested areas are close to the Pacific coast with the farthest inland extent being 35 miles in Napa County. We do not know if this distribution pattern is due to temperature and humidity constraints or because it is a recent introduction.

In January 2001, it was confirmed that the unknown *Phytophthora* was genetically identical to an unknown *Phytophthora* discovered in 1993 that is causing a dieback in rhododendron in Germany and the Netherlands. The *Phytophthora* causing Sudden oak death was also isolated from rhododendron in a commercial rhododendron nursery in Santa Cruz County.

In August 2000, the California Oak Mortality Task Force was created to address Sudden oak death for the state of California. The task force goals are to provide a safe environment for the citizens in infested areas and to limit the spread of Sudden oak death. Further information on Sudden oak death may be found at the task force web site [www.suddenoakdeath.org](http://www.suddenoakdeath.org).

MALE-BIASED SEX RATIOS IN *GLYPTAPANTELES FLAVICOXI*  
(MARSH) (HYMENOPTERA: BRACONIDAE) PARASITIZING  
GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE)

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ABSTRACT

Efforts to obtain biological control of this pest have involved the importation, study, and release of parasitoids, predators, and pathogens. One of the parasitoids imported for trial against the gypsy moth was *Glyptapanteles flavicoxis* (Marsh), a gregarious larval endoparasite of the Indian gypsy moth (*Lymantria obfuscata* (Walker)). Laboratory studies in quarantine showed that *G. flavicoxis* readily attacked the gypsy moth, and releases of this species were made in the Middle Atlantic States, but did not result in its establishment. Because of its gregarious development, large numbers of this parasitoid can be reared with relatively few hosts, and the species was considered to have potential for inundative releases directed at specific gypsy moth populations.

Unfortunately, sex ratios in laboratory rearings of *G. flavicoxis* are usually male-biased. Male-biased sex ratios in populations of parasitic wasps used in biological control are undesirable, because they can prevent the establishment of introduced species or hinder commercial production of species used for augmentative control. Studies were conducted on potential factors contributing to male-biased sex ratios in laboratory rearings of the braconid endoparasitoid *Glyptapanteles flavicoxis* (Marsh) with the gypsy moth (*Lymantria dispar* (L.)) as a host. Sex determination in this wasp is arrhenotokous, a system in which fertilized (diploid) eggs give rise to female progeny, and unfertilized (haploid) eggs, male progeny. In the first experiment, we found that sex ratios did not differ among progenies of parents stored at 13 or 16°C and allowed to mate at 20 or 25°C, but that many females produced all male progeny, indicating that they had not mated. In the second experiment, females were exposed to hosts soon (0-60 min) after mating or 23-25 h later. Sex ratios were higher in progenies of females provided with a rest period than in those which were not. In a third experiment, females were allowed to mate from one to four times with a given male. Although differences between these groupings were not statistically significant, the data suggested that more than two matings might depress sex ratios of progeny. An alternative analysis with only two groupings (1-2 matings and 3-4 matings) suggested that more than two matings might be detrimental. Therefore, it is concluded that matings of this species be controlled (limited to 1 or 2) and that females should be provided with a period of repose after mating before they are offered hosts for parasitization.

REVIEW AND PROSPECT OF RESEARCH ON *ANOPLOPHORA GLABRIPENNIS*  
(COLEOPTERA: CERAMBYCIDAE) IN CHINA

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ABSTRACT

**Geographic Distribution of *Anoplophora glabripennis* in China**

*Anoplophora glabripennis* (Motsch.) is one of the most important wood borers damaging a great number of broad-leaved tree species in China. It has broadly expanded in 24 Provinces of China, including Hebei, Shaanxi, Gansu, Ningxia, and Inner Mongolia, etc. The main tree species damaged by *A. glabripennis* are poplar (*Populus*), willow (*Salix*), elm (*Ulmus*), and maple (*Acer*).

**Research History of *A. glabripennis* in China**

The species was reported briefly by Liu Hechang in 1937 and named as *Melanauster glabripennis* Mots. In the 1950s, Qin Xixiang started to study and describe the life cycle in detail, biology, and control methods of *A. glabripennis* (Motsch.). In the 1960s and 1970s, only less than 10 papers were published in study of *A. glabripennis*. After the 1980s, along with the increase of poplar plantation area in China, the damage caused by *A. glabripennis* (Motsch.) became more and more serious. Therefore, the Chinese government began to pay more attention to the problem and more entomologists started to participate in studies in finding effective methods for controlling the beetle. More than 200 papers have been published during this period.

**Research Status**

The research on *A. glabripennis* by Ruitong Gao and Ping He showed that the adult's life span and fecundity depend on different tree species fed for replenishing nutrition. And a laboratory study on mating behavior showed that sex pheromone and vision acted together on the male beetle to find its mate. Results of research on reproductive behavior of *A. nobilis* in field conducted by Kebin Zhang showed that adult activity in one day could be divided into five periods. Moisture, sugar, and protein contents as well as sclereid contents in tree bark are important factors that influence host selection as reported by different researchers.

According to the widely distributed newspaper in China, the Guangming Daily, about 40% of the 2,330,000 ha poplar plantation in China had been damaged by the beetle. About 50,000,000 trees had been cut down from 1991 to 1993 in Ningxia province alone, and the direct economic lose was 300 millions Yuan RMB. It also caused severe damage to the ecosystems of Northwest China was destroyed severely.

Natural enemies of the beetle include *Dastarcus longulus*, *Iphiaulax imposter*, *Tetramorium caespitum*, *Lasioseius ometes*, *L. sp.*, *Proctolaelaps cossi*, *Dendrolaelaps sp.*, *Paecilomyces farinosus*, *Pseudomonas alcaligenes*, *P. putida*, *Enterbacter agglomerans*, *Beauveria*

*bassiana*, *B. brongniartii*, and *Metarhizium anisopliae*. The woodpecker *Dendrocopos major* feeds on many larvae of insects in the Cerambycidae, Scolytidae, Buprestidae, Sesiidae, Limacodidae, and Notodontidae families.

Peaks of adult emergence and larval hatch were monitored by using development stage method, phenology method, and bait tree method, etc. Gao Ruitong (1997) determined appearance period of *Anoplophora glabripennis* adults by using *Acer negundo* as the bait tree in Henan Province. The results showed that adult elementary period in the middle 10 days of June, peak time in the last 10 days of July, and telophase period in the last 10 days of September.

The most important beetles in the *Anoplophora* genus are *A. glabripennis* and *A. nobilis*. *A. chinensis* is widespread in China and infests many tree species. *A. chinensis* mainly infests *Casuarina* spp. seriously in China. But we have never seen other reports on economic losses of damages caused by *A. chinensis*. Just a few reports about other species can be found in China.

### **Control Methods**

Controls of both *A. glabripennis* and *A. nobilis* are based on cultural practices, with emphasis on IPM to build a healthy forest ecosystem. Other methods, such as plant inspection and quarantine, biological control, chemical control, and physical control may also be integrated into the management strategy, which aims to regulate the interaction among host plants, the beetle, and its natural enemies to minimize damage by the beetle.

### **Remaining Problems**

*A. glabripennis* bionomics and its hosts records accord with those of *Apriona germari* (Hope) and *Anoplophora chinensis*, other species of longhorned beetles. Taxonomy problem of *A. glabripennis* and *A. nobilis* is as a focus along with their research work. As the description of taxonomy, adults whose scutellum and elytra have white patches of hairs are *A. glabripennis*; adults with yellowish or yellow patches are *A. nobilis*. But according to our long-term observation and crossbreeding experiment from 1998 to 2000, we have made the conclusion that the two species are at most two different subspecies within one species.

### **Research Prospect**

Emphasizing studies on adjusts and control action of natural factors in forest ecosystem, using the interacting relations of tree-pest-natural enemy in forest ecosystem, adjusting defensive function, repair and reduced resistance of tree to high-point by resistant selection, cultivation and water as well as fertilizer management.

Selecting resistant tree species to plant, make it unsuitable for longhorned beetle to eat and develop. We think it is most important to select the tree species inhibiting eggs and young larvae to develop. Emphasizing studies on high and new technology, taking full advantage of genetic engineering technique and leading insect-resistant genes in the tree makes it resistant to the pest; protecting and utilizing natural enemy. That is to say, we need to create the advantage of subsistence and propagation of natural enemy, and should be selective to use insecticide or bio-insecticide. Researching the behavior and biology of longhorned beetle adults in more detail, the relations between longhorned beetle adult and host, and special information relations between male and female adults. This research will be the base for developing artificially synthesized bio-insecticide.

# IMPACTS OF WHITE PINE BLISTER RUST

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## ABSTRACT

The introduction of *Cronartium ribicola* Fisher to North America early in the 1900s began the first major forest disease outbreak American forestry seriously confronted. White pine blister rust has caused significant impacts in eastern North America, the Lake States, Pacific, and Rocky Mountain forests. Blister rust is still a damaging pathogen and important ecological factor in forests of those regions. It is now spreading in the Great Basin, Southern Rocky Mountains, and Southwestern forests, and may soon reach northern Mexico (if it has not already). Because white pines are so highly valued, their loss to blister rust and our efforts to mitigate those losses have generated incalculable impacts—socio-economic, political-administrative, ecological, scientific, and aesthetic. The history of blister rust in North America has already gone through two management cycles (eradication and integration), and I propose it is entering a third (adaptation).

Before blister rust was discovered in 1906 in Geneva, New York, forest pathologists knew of its destructive potential to American white pines (Spaulding 1922, 1929). American white pines had been planted widely across cool, moist Europe where nearly every rural family cultivated black currant. When *Cronartium ribicola* was introduced from Asia, it found both a suitable environment and the two hosts it required to complete its life cycle. The disease eventually caused European foresters to abandon the highly susceptible white pines. In America, however, forestry was white pine forestry. When the white pine of a region was exhausted, logging moved west. The timber industry had just set up in the Pacific and Inland Empire forests of western white pine and sugar pine. In the East and Lake States, replanting eastern white pine was underway using cheap and plentiful nursery stock from Europe. By the time the rust was discovered, it was already widely established. There had not been much which could be done to prevent the other catastrophic outbreak of the times, chestnut blight. But, white pines were worth saving (Pack 1933) and this pathogen had a weakness (Spaulding 1922). The first attack on blister rust consisted of quarantines (especially to keep the rust out of western North America) and eradication (first, cultivated black currant and then wild *Ribes*). Some of the first impacts of blister rust were political-administrative—passage of our first quarantine and weed laws and regulations and the first organization of agencies and cooperative federal-state programs dealing with forest pests (Maloy 1997). The laborers employed in *Ribes* eradication no doubt saw the opportunity as an economic boon (especially during the Depression when the eradication program reached its peak in employment and expenditure).

In the Eastern states, conditions were especially suitable for *Ribes* eradication to succeed and it continues to be effective for protecting young plantations (see Ostrofsky et al. 1988, Lombard and Bofinger 1999). *Ribes* was plentiful in the Lake States and could be removed with reasonable effort. Regional and landscape features, however, made the environment for the rust more variable (Van Arsdel 1961, Charlton 1963). Where conditions were not suitable for infection, *Ribes* eradication was not economically efficient (Anderson 1973). Although the Office of Blister Rust Control (BRC) was dedicated to *Ribes* eradication, the strategy was severely tested in the West (Benedict 1981). In eastern Washington, northern Idaho, and western Montana, wild *Ribes* were not only important sources of rust inoculum, they were large and abundant (Mielke 1943). Considering transportation and labor, eradication became a Herculean task, met by a determined BRC with chemicals, heavy machinery, and explosives.

Although some of the first research in biological control was undertaken, managers were mostly concerned with improving the efficiency of eradication. Accomplishments were reported as acres treated. In some areas only a few white pines survived exposure to the rust; many were salvaged. Control probably did reduce the velocity and severity of the outbreak. Research was begun on hazard rating (Van Arsdel 1961), genetic breeding (Bingham 1983), and silvicultural control. With improvements in fire suppression, reserve crews of rust busters were no longer needed. Eradication moved to areas of marginal benefit (Toko et al. 1967). The crisis for the BRC was probably the deployment of an antibiotic strategy without an adequate understanding of canker development and without controlled, replicated testing (Leaphart and Wicker 1968). Not only was the blister rust program reorganized under the Forest Service, but the entire strategy of forest disease control shifted to integrated management as a part of forest operations.

The rust outbreak and salvage created new conditions. Other forest tree species came into economic and ecological importance. Successional changes and new disturbance regimes required foresters to deal with new pests—defoliators, bark beetles, and root diseases. The rust spread into the Sierra Nevada and high-elevation pines of the Northern Rockies. Supported by new research (McDonald et al. 1991), new approaches were developed for integrating pest management into forest practice (Hagle et al. 1989). Tactics included: silviculture to reduce regeneration of *Ribes* (Moss and Wellner 1953), genetically improved planting stock, thinning and pruning (Hunt 1998), and decision-support tools (McDonald et al. 1981). The paradigm of this era was “if we can quantify the economic costs and ecological impacts of forest pests, we can implement appropriate, intensive-management policies.” Unfortunately, this approach did not adequately consider “surprises” (Holling and Meffe 1996) and change in ecosystems and institutions (Gunderson et al. 1995). Although the rust in North America began with a genetic bottleneck (Hamelin et al. 2000), it seemed to be evolving (McDonald 1996). Society’s demands from public forests also raised the importance of biological diversity and ecological sustainability. Forest insects and pathogens were no longer viewed as “pests,” they were “natural disturbance agents.”

*Cronartium ribicola* is an exotic, invasive species which has proven capable of instigating catastrophic ecological change. Our challenge is to design research for and adapt management for a dynamic, unpredictable environment. The issues are likely to include

impacts in sensitive ecosystems and on dependent species (Hoff and Hagle 1990), bridging genetic barriers of the rust (e.g., by introduction of new Asian races), accommodating the cultivation of *Ribes* (Hummer and Snieszko 2000), and expansion of the rust into new regions (such as Mexico). Our most important tool will no longer be the hodag (developed for eradication) but communication and cooperation.

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*ENTOMOPHAGA MAIMAIGA* AT THE SOUTHERN EDGE OF THE  
GYPSY MOTH RANGE

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ABSTRACT

The fungus *Entomophaga maimaiga* Humber, Shimazu & Soper was originally introduced in 1910 for gypsy moth control. However, it was first recovered from North American populations in 1989. The fungus is a very effective pathogen and very host specific. Therefore, when gypsy moth is driven to low levels by *E. maimaiga*, there may be too few hosts to maintain high levels of *E. maimaiga* in the soil. Low levels of the fungus can create a delay in the negative feedback loop. A time lag may exist between gypsy moth recovery and *E. maimaiga* recovery. Our objective was to introduce sterile gypsy moth egg masses to sites where *E. maimaiga* is present and observe what effect host augmentation has on *E. maimaiga* levels in the soil. Three sites were selected in Camden County and two in Currituck County, NC. On 13 March, 2000, soil was collected from around bases of three trees at each site and processed to extract pre-season resting spore density. On 20 April, 2000, sterile gypsy moth egg masses were distributed at three different levels. On 5 July 2000 soil samples were collected again and processed to determine post-season resting spore density. We concluded that (1) host augmentation can be used successfully to increase resting spore densities in the soil; (2) high levels of egg masses are more effective than low levels; (3) host augmentation is ecologically safer and less labor intensive than other resting spore enhancement techniques; and (4) host augmentation could be incorporated into long-term management plans for selected sites.

EFFECTS OF THE INVASIVE SHRUB, *LONICERA MAACKII*, AMUR HONEYSUCKLE,  
ON NATIVE PLANTS IN EASTERN DECIDUOUS FORESTS

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ABSTRACT

Invasive plants are often associated with reduced cover of native plants, but rarely has competition between invasives and natives been assessed experimentally. The shrub *Lonicera maackii* (Rupr.) Herder (Caprifoliaceae) is native to northeastern Asia and has invaded forests and old fields in numerous parts of eastern North America (Luken and Thieret 1995). In southwestern Ohio forests, it is associated with reduced tree seedling density and herb cover and diversity (Hutchinson and Vankat 1997) and reduced species richness and basal area of native shrubs (Medley 1997). *Lonicera maackii* expands leaves earlier and retains them later than any of the native woody species in this area (Trisel and Gorchov 1994).

We hypothesized that the early spring leaf expansion of *L. maackii* causes it to have greater negative effects on those native species that depend most on early spring photosynthesis. This hypothesis has been supported by studies by our lab on forest annuals (Gould and Gorchov 2000), but effects on tree seedlings were uncorrelated with leaf phenology (Trisel 1997).

We used field experiments to test the effects of *L. maackii* on the survival and growth of three species of perennial forest herbs of contrasting leaf phenology: a spring ephemeral, *Allium burdickii* (Hanes) A.G. Jones (Wild Leek), and two species that retain leaves through the summer, *Anemonella thalictroides* L. (Rue-Anemone) and *Viola pubescens* Aiton (Yellow Violet). For each species, 240 individuals were transplanted into each of two stands near Oxford, OH: Western Woods and Gregg's Woodlot. Western Woods is a mature stand (24.9 m<sup>2</sup>/ha basal area (BA)) dominated by *Quercus rubra*, *Fraxinus* spp., *Acer saccharum*, and *Fagus grandifolia*. Gregg's Woodlot (21.4 m<sup>2</sup>/ha BA) was selectively cut about 100 years ago, was regularly burned and grazed by cattle until about 1960, and is dominated by *Carya ovata*, *C. laciniosa*, *Fraxinus* spp., and *Quercus rubra* (Gould and Gorchov 2000). Herbs were transplanted into three treatments in Western Woods: *L. maackii* present, *L. maackii* removed, and *L. maackii* absent in a blocked design (each of 20 blocks had one plot of each treatment). In Gregg's Woodlot, we used 30 blocks, but only the first two treatments because high density of *L. maackii* precluded the "absent" treatment. Rhizomes or bulbs of the herbs were weighed and transplanted in 1995 (*Allium*), 1996 (*Anemonella*), and 1997 (*Viola*), and above-ground structures were monitored throughout each of the subsequent growing seasons.

Most transplants emerged the next spring, and subsequent survival was compared among treatments using survival analysis (PROC Lifetest, SAS version 8). Treatment effects on size (number of leaves or leaflets) and reproduction (number of seeds or fruits per flowering individual) each year were assessed using 2-way mixed model ANOVA, with block considered a random effect. In most cases, these variables were log-transformed to improve the normality of the distribution. Treatment effects on the proportion of individuals flowering each year were assessed with the G-test of independence.

Survival of *Allium* over the past 5 years was high regardless of *L. maackii* treatment, but survivors grew larger where *L. maackii* was removed. This effect was significant beginning in the second year after transplanting (1997) at Gregg's Woodlot, but not until the fifth year at the less-disturbed Western Woods. The proportion of survivors flowering was higher in the removal treatment in 3 of 5 years at Gregg's Woodlot, and in 1 of 5 years at Western Woods. Seed number per flowering plant was significantly greater in removal plots at Gregg's Woodlot (every year beginning in the second year) and Western Woods (only in the fifth year).

Survival over the past 4 years of *Anemonella* at Gregg's Woodlot was greater where *L. maackii* was present, whereas there was no difference at Western Woods. However, survivors at Gregg's Woodlot grew larger in the removal plots (significant beginning the third year after transplanting). While the proportion of survivors that flowered did not differ between treatments, flowering individuals averaged more seeds in the removal treatment at Gregg's Woodlot (significant beginning the second year) and Western Woods (only in 1999).

Removal of *L. maackii* did not affect *Viola* survival, but did increase size (number of leaves) of survivors in the second year after transplanting (1999) at both sites. Similarly, the proportion of plants flowering was higher where *L. maackii* had been removed (both sites, both second and third year). At Gregg's Woodlot, the number of fruits per flowering individual was also higher in the removal plots, in both the second and third years.

To estimate the overall effects of *L. maackii* on fitness of each herb species, we integrated these data on survival and reproduction to obtain the cumulative number of fruits (or seeds for *Allium*) produced per individual since transplanting. For each species at each site, fitness was greater in the *L. maackii* removal treatment. Furthermore, for each species the fitness difference was greater in Gregg's Woodlot, the more disturbed site where *L. maackii* occurred at greater density.

To more comprehensively assess the effects of this invasive shrub on the population biology of herb species, we recently initiated experiments to test the effects on seedling emergence and survival of these three species.

This is one of the first experiments to confirm negative effects of an invasive plant on native plant populations. While perennial herbs with different leaf senescence times were affected similarly by *L. maackii*, all of these expanded leaves in early spring. We predict that perennial herbs that expand leaves later in the season will not be as strongly affected by this invasive shrub.

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ASSESSING GYPSY MOTH SEASONALITY AND RISK OF ESTABLISHMENT  
USING A GEOGRAPHICALLY ROBUST MODEL OF PHENOLOGY

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ABSTRACT

Among the requisites of each insect is the availability of food during the feeding stage(s) and suitable temperatures to complete its life cycle. These requisites can be summarized as a requisite seasonality. Seasonality is the predictable occurrence of a life stage event at roughly the same time each year (Lieth 1974). Implicit in this definition is that first instar gypsy moth (GM) (*Lymantria dispar* L.) larvae will hatch coincidentally with the emergence of new foliage of host plants, and that winter will coincide with the cold hardy diapause stage (Leonard 1968) of development, and that these events will coincide sufficiently each year for the continual survival of the population. These same requisites exist for the successful establishment of an introduced population to a novel environment.

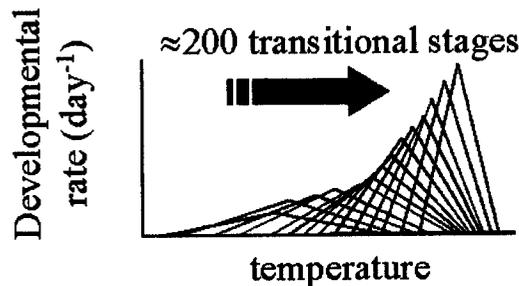
The ontogeny of poikilothermic insects is controlled by temperature, and a phenology model simulates the developmental (ontogenetic) response to temperature. Thus a phenology model can directly assess one criterion of the risk of establishment of an introduced population to a novel environment. It can make this assessment by quantifying the likelihood that temperature regimes in the environment will consistently produce seasonal development. This method of assessing the risk of establishment has been utilized at least twice in the case of GM. Allen et al. (1993) used a partially developed three-phase model of GM egg phenology (Gray et al. 1991) and concluded that winter temperatures would limit the southern extent of GM by not satisfying diapause requirements. Phero Tech Inc. (1994) used the same partially developed phenology model and concluded that temperatures in at least a portion of British Columbia would probably satisfy the requirements of GM, and, therefore, a moderate risk existed for establishment.

The appropriate level of confidence that should be placed on estimated risks of establishment that are derived from phenology model output is directly related to the expected accuracy of model predictions for each location. This expected accuracy, measured on a large landscape scale, is the geographic robustness of the model. Gypsy moth egg hatch is a critical life-stage event in establishing seasonality. Unfortunately, the majority of GM egg phenology models have limited geographic robustness. This limited geographic robustness is largely due to the methods employed in modelling the multi-phase nature of GM egg development.

Gypsy moth egg development has been described as being comprised of three distinct phases. Embryos begin development in a prediapause phase that is characterized by high respiration rates (Gray et al. 1991), abundant morphological development (Leonard 1968), and developmental rates that are favoured by high temperatures (Gray et al. 1991). After

spending 25 to 14 days in prediapause (assuming a constant temperature regime of 20 to 30°C), gypsy moth enters the diapause phase as fully differentiated pharate larvae (Bell 1996). Diapause is characterized by low respiration rates (Gray et al. 2001), a virtual absence of morphological development, and developmental rates that are favoured by low temperature (Gray et al. 2001). During the postdiapause phase respiration rates are again high, and developmental rate is favoured by high temperatures (Gray et al. 1995). Eggs hatch upon completion of the postdiapause phase. Due largely to difficulty in observing, either directly or indirectly, the transition between successive phases, model developers have modelled only the postdiapause phase after assuming that diapause is completed by a particular day. Such an assumption weakens the geographic robustness of the model.

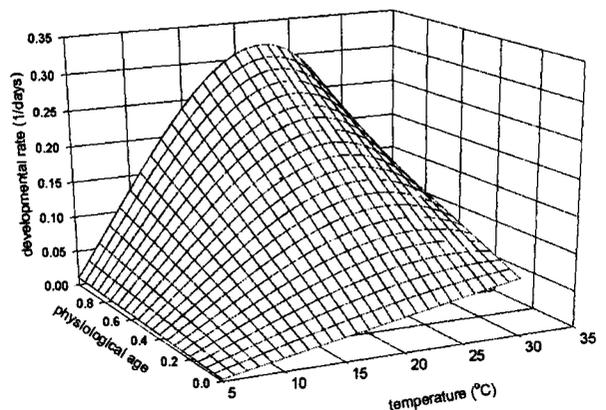
A notable exception is the model of Sawyer et al. (1993) that models diapause and postdiapause as a composite phase, without a clear distinction. After assuming that prediapause is completed by a particular day, development is modelled as a gradual transition between a low temperature developmental response and a high temperature developmental response by using 200 transitional sub-phases (Fig. 1). However, such a model can not produce the age-specific developmental responses estimated by Gray et al. (2001).



**Figure 1. Illustration of the gradually increasing optimum temperature and maximum developmental rate of the Sawyer et al. model.**

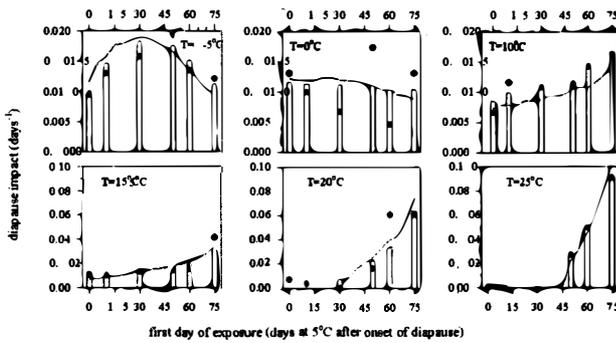
Another exception is the three-phase, sequential model of Gray et al. (1991, 1995, 2001) that includes no assumptions regarding completion of any phase. Utilizing precise measurements of respiration rates of individual eggs, they were able to distinguish clear transitions between successive phases. Age-specific developmental responses were estimated in the diapause (Gray et al. 2001) and the postdiapause (Gray et al. 1995) phases. An age-independent developmental response relationship with temperature was estimated for the prediapause phase (Gray et al. 1991).

In the three-phase, sequential model, the developmental response at the onset of postdiapause increases only slightly with increasing temperature (Fig. 2). A physiological trait such as this reduces the likelihood that eggs will hatch prematurely with exposure to the occasional warm day that commonly occurs during early spring. Developmental responses during diapause displayed extremely complicated patterns that were

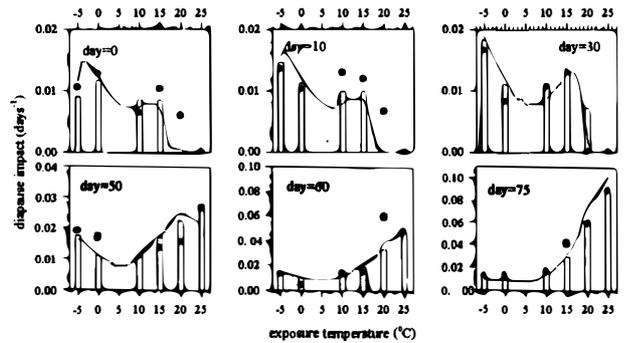


**Figure 2. Age and temperature dependent developmental rates in postdiapause.**

simulated by a dual process model (Gray et al. 2001). Developmental response at  $-5^{\circ}\text{C}$  doubled during the first 30 days of diapause and then declined to original level after another 45 days. Developmental response at  $25^{\circ}\text{C}$  was absent for the first 50 days of diapause and then rose sharply. Developmental response at  $20^{\circ}\text{C}$  was also absent at the onset of diapause and then rose sharply (Fig. 3). However, it is interesting to note that the increase in response occurred *earlier* at  $25^{\circ}\text{C}$  than at  $20^{\circ}\text{C}$ . The model of Sawyer et al. (1993), which uses a gradually increasing optimum temperature (Fig. 3), will not produce these observations. When presented in a different fashion (Fig. 4), these same data display another interesting feature. At day 50 (for example) of the experiment, developmental response was minimal at approximately  $5^{\circ}\text{C}$ , and was higher at either higher or lower temperatures. A dual process model of inhibitor-mediated development and removal of the inhibitor explained 94% of the variability in these observations (Gray et al. 2001).

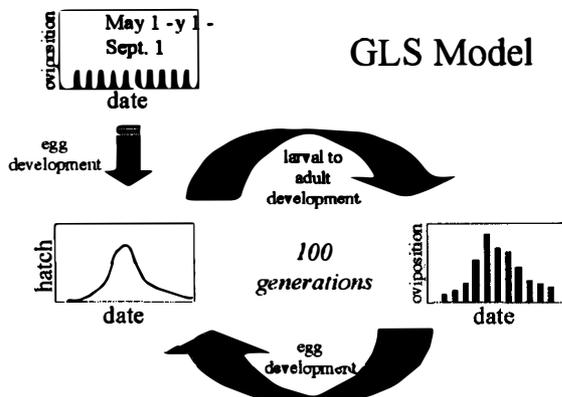


**Figure 3.** The effect of an exposure to the experimental temperatures during diapause. See Gray et al. (2001) for a full explanation.



**Figure 4.** The effect of an exposure to the experimental temperatures during diapause. See Gray et al. (2001) for a full explanation.

A complete GM life cycle model is now possible with an egg development model that includes no arbitrary dates for completion of any phase. The Gypsy Moth Life Stage (GLS) model is a composite model of the stage specific models developed for the egg to adult stages. The acronym also designates the authors of the stage-specific models: Gray et al. (1991, 1995, 2001) for the egg stage; Logan et al. (1991) for the early larval stages; and Sheehan (1992) for the late larval to adult stages.

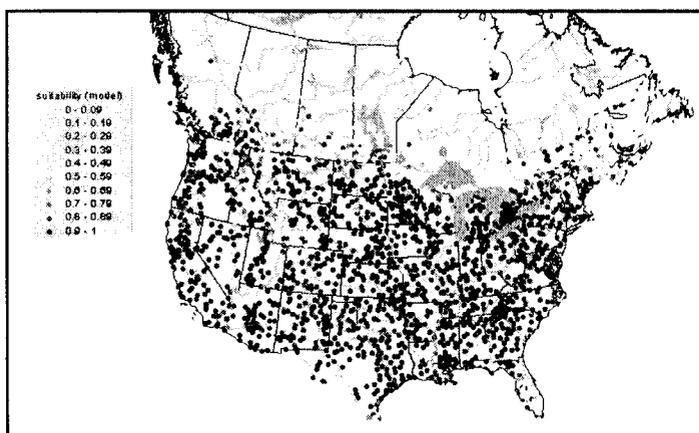


**Figure 5.** A schematic representation of the Gypsy Moth Life Stage Model.

The GLS model operates in the following manner (Fig. 5). The possibility of successful egg hatch in a location is maximized by simulating a protracted oviposition period, May to September, with 15 cohorts. The successful egg hatch constitutes the originating population and temporal distribution of hatch. Daily egg hatch creates larval cohorts. Each female adult lays 2 eggs 2 days after female adult emergence. The complete distribution of egg oviposition is described by 15 egg cohorts of variable sizes.

The GLS model can be run for any number of locations, for any number of generations. Specific years can be selected for which daily minimum and maximum temperatures exist. Alternately, GLS can be run in random mode where the user specifies a *number* of years, and GLS selects a random sequence of years from the available data. In this manner, GLS can be run for an infinite number of years in order to estimate mean dates for specific life-stage events and variability in those dates. The random mode is also useful to estimate a “climatic suitability index” (CSI) for the location, which is defined as the average proportion of the population that completes its life cycle in the climatic regime.

Climatic suitability is  $\geq 0.9$  for much of the United States (Fig. 6). Climatic suitability declines sharply in Florida, in Canada, and in a band along the Rocky Mountains, though not for the same reason in all locations. For example, cool and short summers in Combermere, Ontario (45:22N, 77:37E), cause slow larval and pupal development. Mean date of maximum egg oviposition is Sept. 6. Development in prediapause is not rapid at the low temperatures which are common at this time of year. Consequently, in many years only 50% of the population is able to complete prediapause and subsequently develop further to egg hatch. Conversely, hot summers in Ocala, Florida (22:12N, 82:5E), cause rapid larval and pupal development. Mean date of maximum egg oviposition is July 10, and eggs quickly complete prediapause in the high temperatures that still prevail. However, winter temperatures are not commonly low enough to satisfy diapause requirements, and in many years only 20% of the population is able to complete diapause and subsequently develop further to egg hatch.



**Figure 6. Climatic suitability of approximately 2000 North American locations for gypsy moth establishment.**



**Figure 7. Climatic suitability of Florida for gypsy moth establishment.**

Estimated mean dates for specific life-stage events, or a CSI, can be graphically displayed as point data (Fig. 6), or an interpolative method can be employed to create a more continuous coverage. The risk of establishment of gypsy moth in Florida, based on CSI, is illustrated in Figure 7. To create Figure 7, a logistic function was fit to the CSI data using latitude and longitude as independent variables, a spherical semivariogram model was fit to the residuals, and the residuals were kriged using elevation as an external drift variable.

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ACOUSTIC DETECTION OF *ANOPLOPHORA GLABRIPENNIS* AND  
NATIVE WOODBORERS (COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT

Wood-infesting insect larvae generate acoustic signals as they feed and tunnel in trees. Studies on acoustic detection of *Anoplophora glabripennis* (Motschulsky) (Cerambycidae) larvae were conducted in 1999 and 2000. In the U.S., we have recorded acoustic data from *A. glabripennis* and several native cerambycid larvae, including cottonwood borer (*Plectrodera scalator* (Fabricius)), linden borer (*Saperda vestita* Say), locust borer (*Megacyllene robiniae* (Forster)), whitespotted sawyer (*Monochamus scutellatus* (Say)), red oak borer (*Enaphalodes rufulus* (Haldeman)), and sugar maple borer (*Glycobius speciosus* (Say)). In China, we have recorded acoustic data from *A. glabripennis*-infested elm, poplar, and willow trees. The specific objectives of this study are to determine how vibration data varies by insect species, tree species, larval instar, wood moisture content, and distance between the larva and the sensor. The goal of this work is to develop a field-portable acoustic detector that can identify trees infested with *A. glabripennis*.

Scientists at the Oak Ridge National Laboratory conducted extensive sound analyses on the vibration data and developed a mathematical algorithm that would recognize the acoustic signature produced by *A. glabripennis* larvae feeding in wood. The algorithm has been successfully tested on a variety of infested materials, including infested log samples and standing trees in both the U.S. and China. The algorithm is a real time filter that is optimized to respond to vibrations that match closely with pre-selected data sets of actual larval feeding vibrations. Incoming data first passes through the algorithm (filter). If the output is a close match to the pre-selected data set, then a high amplitude response is generated and a "bite" is recorded. On the other hand, if there is no match between the input data and the pre-selected data set, then no "bite" is recorded.

The detection algorithm has been incorporated into a data collection and analysis system that has been installed on a laptop computer. The system is fully portable. Data analysis is completed in real time. An indication of infestation (number of beetle "bites" detected) is displayed on the computer screen in real time. All components utilized in this system can be miniaturized and integrated into a smaller package.

Initial results confirm that larval feeding in wood produces detectable vibrations. The vibrations from *A. glabripennis* and other cerambycids are similar, although there are some unique acoustic features of *A. glabripennis* feeding. Vibrations are larger in amplitude for larval feeding in wood compared with larval feeding in inner bark.

Preliminary studies were conducted in 2000 to compare feeding vibrations among different sized larvae, different wood species, and different insect species. More detailed studies will be conducted in each of these areas in 2001. In addition, more detailed studies will be conducted in 2001 to (1) compare acoustic signals of larvae in live trees compared with infested crating, (2) determine how feeding vibrations vary with air temperature, and (3) determine over what distance feeding vibrations can be detected in trees. We are currently using trunks of cottonwood trees, 7 to 8 m long, into which we have inserted *P. scabator* larvae. In this way, we can vary the distance between the sensor and the larvae. We also hope to field test a prototype acoustic detector in 2001 on actual infested trees in the U.S..

ASIAN LONGHORNED BEETLE (*ANOPLOPHORA GLABRIPENNIS*)  
IN THE UNITED STATES:  
HISTORIC TIMELINE OF KEY DETECTION/INTERCEPTION EVENTS

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ABSTRACT

The Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)) was first detected in the United States in August 1996. At the time of its discovery, it was heavily infesting various hardwood trees, mostly maples, along streets and in parks in Brooklyn, New York. It was found again in Chicago in July 1998. All historical and key detection/interception events surrounding the presence of this highly destructive exotic forest pest in the United States are highlighted.

# THE GYPSY MOTH IN CROATIA

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## ABSTRACT

Gypsy moth (*Lymantria dispar* L.) has a long history of being the most important species among several defoliators in Croatian forests. It causes serious detrimental impact in the highly valued and ecologically complex lowland stands of pedunculate oak (*Quercus robur*). These forests grow along main inland river flows, spreading over surrounding plains and merging into a higher zone of sessile oak forests (*Q. petraea*). Periodical flooding during the high water levels, from late fall until late spring, is considered a major ecological factor defining the tree species composition and viability and competitive status of *Q. robur*. Climatically, the area is characterized by mean annual precipitation between 700 and 1,000 mm and mean annual temperatures between 10.5 and 11.5 °C. These naturally growing forests once occupied more than 70% of the region (by late 19<sup>th</sup> century more than 350,000 ha were covered by this continuous "green sea"). Today's forests of pedunculate oak cover little more than 200,000 ha, this area being about 1/10 of the total forest cover in Croatia (2,100,000 ha or 37% of the total country area being under closed forest canopy). Most of the gypsy moth's (GM) outbreaks, in intensity as well as territorial spread, occurred and still occur in these forests. No wonder that the vast majority of GM research and closely related suppression activities focus on the same part of the country.

The recorded history of GM outbreaks goes back into the late 80's in 19<sup>th</sup> century. The largest outbreak periods lasted more or less continuously from 1874 until 1892, and from 1903 until 1935, with the heaviest attack from 1922 to 1927 during which infested forests comprised a more than 400-km long non-disrupted outbreak area. There was again one big outbreak from 1948 to 1950 with 187,000 ha of *Q. robur* forests and about 2,5 mil orchard trees defoliated. In 1953 another outbreak followed, covering nearly 200,000 ha, and during which the first sign of outbreak loci eastward shift (Vojvodina, northern Serbia) was noticed. One of the major characteristics of these continental outbreaks is a typical four-year duration period followed by 5 to 8 years of latency. This fluctuating gradation type persisted until these days. However, some changes on the long run are noticeable. The latest data on outbreaks in this lowland area most probably reflect those changes. The shrinkage of an area being once a continuous forest region, and several important ecological changes that have occurred during the past 30+ years have had some impact on GM populations and the latest outbreak history. Absence of periodical flooding, dropdown of groundwater level, a general shift toward dryer and warmer climate, loss of some constituent tree species (Dutch elm disease), occupancy of vacant niches by other tree species, rising outbreaks of other defoliators (formerly not recorded), and influences of suppression measures are among a few of the most documented and striking ones.

The average GM outbreak area in the last decades dropped from 156,732 ha in 1970s, to 97,185 ha in 1980s, and to 52,008 ha during the last decade of the 20<sup>th</sup> century. The last serious outbreak peaked in 1974 when over 100,000 ha were under defoliation threat. Aside from eastward shift of the outbreak loci, there was a new development on an European scale. By the beginning of the 1990s, outbreaks occurred in some central and western European countries, and recently in area east and southeast from Croatia (eastern and southern Serbia). Throughout Croatia, new outbreaks loci evolved. Some were known formerly and existed for years but new appeared and initiated dramatic outbreaks that swept the whole coastal area, again in the NW to SE direction. Susceptible xeric forests of the coastal margin are characterized by holm oak (*Q. ilex*) and downy oak (*Q. pubescens*) and are growing in maritime climate. They are quite different in a structural and ecological way if compared with inland *Q. robur* forests but have recently been defoliated by GM in much more drastic way than in the past (versus the decline of outbreaks in inland Croatia).

One important factor, being closely related with GM outbreaks and its ecology, are several other defoliators that have been recorded both inland and in coastal, maritime forests. From the early outbreak recordings, the green leaf roller (*Tortrix viridana*) and the brown tail moth (*Euproctis chryorrhoea*) were known to have periodic outbreaks. Loopers (*Erannis defoliaria* and *Operophtera brumata* as the most important two), the oak sawfly (*Apethymus abdominalis*), and the lackey moth (*Malacosoma neustria*) appeared later on, their outbreaks being new in a sense of outbreak magnitude known formerly for those species. Today, we experience an almost continuous outbreak condition where at least one of the above listed defoliators is in the higher population levels, causing local or more widely spread damages.

As one might expect, the natural enemy complex of GM in its natural habitat is quite rich and diverse. NPV, Bt, mycoses and microsporidia have been recorded during quite many outbreaks in the past, and among pathogens, NPV appears to have the biggest influence on outbreak cessation. Parasitoid fauna is well represented and some of the more often found species are: *Anastatus japonicus*, *Ooencyrtus Kuwanai*, *Phobocampe disparis*, *Apanteles glomeratus*, *Glyptapanteles liparidis*, *G. fulvipes*, *Exorista larvarum*, *Compsilura conncinata*, *Drino inconspicua*, *Carcelia lucorum*, *Blepharipa pratensis*, *Pimpla instigator*, *P. turionellae*, *Brachymeria intermedia*, and *B. femorata*. Predators are also well represented and some of the most common are: *Calosoma sycophanta*, *C. inquisitor*, *Carabus cancellatus*, *Silpha quadripunctata*, *Megatoma pici*, *M. pubescens*, *M. undata*, *Ctesias serra*, *Globicornis nigripes*, *Dermestes erichsoni*, *Julius floralis*, and *Malachus bipustulatus*. There are also records on avian predation but interestingly, no serious work or data on small mammal predation which is being well addressed and documented in the case of GM spread in the United States.

Suppression activities against GM are undertaken when classical monitoring procedures (yearly done egg masses surveys) predict an outbreak situation for the subsequent spring. Spraying are the most commonly used techniques and thermal fogging has been replaced with LV and ULV applications of chemicals, the greater part of them belonging to synthetic pyrethroids. In some more vulnerable areas, like watersheds and periurban areas, IGR's and Btk based formulations are used instead. Compared with the past, much smaller areas are treated today. Average yearly-sprayed area against GM and other defoliators varies between

2,000 and 20,000 ha. One important role of the spraying is the prevention of the oak decline and growing cases of tree mortality. The typical chain of events is: defoliation – heavy attack of an oak mildew (*Microsphaera alphitoides*) – attack of bark beetles, cerambycids and agrilids – girdling and final attack by root rot fungus (*Armillaria mellea*). Successful control of GM and avoidance of defoliation is the key factor to the continuance of the heavily struck and generally more susceptible oak forests of today.

# COMPARISONS OF NATURAL ENEMY POPULATIONS IN EASTERN AND MIDWESTERN GYPSY MOTH POPULATIONS

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## ABSTRACT

In 1996 and 1997, we sampled eggs, small larvae, and large larvae of gypsy moth (*Lymantria dispar* (L.)) at 12 forest sites in the upper Midwest and the East Coast of the United States. Our objective was to determine what pathogens and parasitoids, known to be established in North America, now occur in gypsy moth's older, resident populations (6 sites in New Jersey [NJ]), later established populations (4 sites in Michigan [MI]), and newly established populations (2 sites in North Carolina [NC]). Species richness averaged 5.5 for NJ, 4.5 for MI, and 2.0 for NC. Two common pathogens, nuclear polyhedrosis virus (NPV) and a fungus (*Entomophaga maimaiga*), accounted for the highest mortality (25 to 35%) of larvae annually in the three regions sampled. NPV was present at all sites, whereas *E. maimaiga* was present at all sites in NJ and MI but absent at NC sites. An egg parasitoid (*Ooencyrtus kuvanae*) was present at all sites with <3.5% parasitism of eggs. Another egg parasitoid (*Anastatus disparis*) was present at all NJ sites with 8.9% parasitism of eggs, present at only one MI site (<1% parasitism), and absent at all NC sites. At least one larval parasitoid was collected from each site in NJ and MI but none from NC sites. *Cotesia melanoscelus* was present at all NJ sites (0.8% parasitism of larvae), present at two MI sites (2.3% parasitism of larvae), and absent in NC. *Compsilura concinnata* was present at only one site in NJ (18.9% parasitism), present at all MI sites (9.4% parasitism), and absent in NC. *Phobocampe disparis* was present only at two NJ sites (0.1% parasitism). We believe that the greater species richness of pathogens and parasitoids in older, resident gypsy moth populations in NJ is responsible for more stable, long-term biological control than experienced in newer, established populations in the midwestern and southeastern U.S.. This disparity warrants efforts to establish a more complete guild of natural enemies in these newer gypsy moth populations.

*ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)

FLIGHT PROPENSITY IN THE LABORATORY

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ABSTRACT

*Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) is one of the more recently introduced nonnative invasive species to become a pest in the United States. Current surveys in urban areas are estimated to be only 30% successful because of uncertainty about the characteristics of *A. glabripennis* dispersal and capacity to spread. The studies described here document the proportion of the population that initiates flight under laboratory conditions, and the life history and host quality factors that lead to flight initiation (including age, feeding and mating status, and host moisture content). Flight-tested adults were from laboratory strains or infested wood obtained from the Chicago (IL) or Queens (NY) infestations. Thirty-two males and 30 females were flight tested on *Acer saccharum* logs dried for 3+ weeks, and 18 females and 14 males on fresh cut *A. saccharum* logs. Each individual was flight tested four times during the adult stage (always on the same log type): (1) at emergence, never fed, and unmated; (2) 1 week after emergence, unmated, and fed; (3) 1 day after mating and fed; and (4) 3 weeks after mating and fed. Each bioassay consisted of 1 to 4 adults on separate logs that were observed for 45 minutes.

Adults initiated flight more slowly at lower than at higher % RH. Males tended to fly more than females during the 45-minute observation period. A significantly higher percentage of females flew from dry logs than from fresh logs at all stages. Females chewed significantly more pits on fresh than on dry logs; of the females that chewed a pit, only 16% flew. Newly emerged and newly mated females were more likely to fly from fresh logs than 1-week-old virgins or females mated for 3 weeks. Newly emerged males were more likely to fly from fresh logs than males at the other stages. When disturbed, a higher percentage of both male and female beetles flew a longer distance than when they were allowed to initiate flight on their own. This result was most pronounced in the fresh log group. The results suggest that about half of the newly emerged adults of both sexes will fly from a log that has no twigs attached, probably in search of food. Nearly every beetle walked up and/or down on the log before initiating flight, so on living trees, fewer may have flown because they would encounter twigs to feed on. Well-fed females appeared to remain on a good-quality host and chew oviposition pits rather than fly. When the host quality is poor, >50% of females flew within the first 30 minutes and additional females would likely have flown after chewing pits and assessing host quality. Because > 85% of all males flew in this study, they would be expected to fly at some life stage regardless of host quality. Males were more likely to remain on a log than fly when they recently mated a female. Therefore, when populations are small and host trees are fresher, more males may fly in search of females while females likely would fly less than at higher population densities on deteriorating well-colonized hosts.

*ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)

DEVELOPMENT ON CUT LOGS OF FOUR SPECIES OF *ACER*

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ABSTRACT

*Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) was first discovered in 1996 in the New York City metropolitan area and has since been found in the Chicago (IL) metropolitan area. In China, it is considered a major pest of maple (*Acer*), poplar (*Populus*), and willow (*Salix*). There is a critical need for information on the basic biology of *A. glabripennis* to predict the timing of biological events fundamental to the improvement of exclusion and eradication methodologies. This study reports on the attack rate and relative developmental rate of *A. glabripennis* on four species of *Acer*: *saccharum* (sugar), *rubrum* (red), *saccharinum* (silver), and *negundo* (boxelder).

Freshly cut logs (10 to 15 cm diameter and 90 cm long) with both ends waxed were exposed for 1 week to a single mating pair of *A. glabripennis* from a strain established from the Queens (NY) infestation. At 14, 28, 56, 84, and 112 days, three logs of each of the tree species were stripped and split to assess the larvae and eggs present on the log.

The number of eggs laid per log in 1 week was significantly lower on red maple than on the other three maple species. Significantly more oviposition pits were chewed on the boxelder and sugar maple logs than on the red or silver maple logs. The female beetles did not oviposit in all pits that were chewed, and the number of sites without eggs differed between tree species. Differences in attack rate and oviposition may be due both to log moisture levels and sap-sugar content since these beetles have been shown to prefer trees with a higher sugar content. Survival of *A. glabripennis* was significantly higher and development faster on the two softer wood species, boxelder and red maple, than on the harder wood species, silver and sugar maple. Survival significantly declined with log age and larval weight within each instar was reduced in the 112-day logs. Three 5<sup>th</sup>-instar larvae on 56-day boxelder were the only ones to enter the heartwood of the logs. These larval development results are consistent with our observations for cut infested wood and suggest that the beetle larvae are more likely to survive and develop from egg to adult in cut logs of softer than harder wood maples. This is particularly important since softer maples are more likely to be used for firewood or packing materials such as crates.

SYSTEMATICS OF ASIAN LONGHORNED BEETLES  
OF THE GENUS *ANOPLOPHORA*

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ABSTRACT

The Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)), native to mainland China and the Korean peninsula, was discovered infesting various hardwood trees along streets and in parks in the New York City area in August 1996 and in Chicago in July 1998. This represents the first known infestation of standing trees in North America by any member of the lamiine genus *Anoplophora*. This exotic wood-boring beetle also has been intercepted on multiple occasions in wood crating and pallets at many U.S. ports of entry. In addition, infested solid wood packing material has been detected at approximately 30 importer warehouse sites in at least 14 states of the U.S. and Canada. To better enable accurate and timely identification of the adult and immature stages of this important exotic forest pest, as well as those of the approximately 45 other described species of the genus, we are conducting a comprehensive taxonomic revision of the species of the Asian genus *Anoplophora* and producing an illustrated handbook for their identification. This research is funded by USDA's Agricultural Research Service (ARS).

CHARACTERIZING THE RESPONSE OF ARTIFICIALLY INSERTED  
ASIAN LONGHORNED BEETLE LARVAE IN THREE HOST TREE SPECIES  
UNDER GREENHOUSE CONDITIONS

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ABSTRACT

The introduction of Asian longhorned beetle into the United States has resulted in the destruction of thousands of street trees in Chicago and New York. Presently, our understanding of the susceptibility of trees to feeding and oviposition by ALB remains limited to field observations in China, Chicago, and New York and laboratory experiments on excised branches and cut logs. In China, the highly preferred trees include poplar (*Populus* spp.), willow (*Salix* spp.), and maple (*Acer* spp.), whereas chinaberry (*Melia* spp.), mulberry (*Morus* spp.), black locust (*Robinia pseudoacacia*), elm (*Ulmus* spp.), and some fruit trees (i.e. plums and pears) are occasionally attacked. Observational reports from the New York and Chicago infested sites listed several *Acer* species (Norway, sugar, silver, sycamore, and boxelder) as primary hosts. In addition, several new larval hosts were identified in these infestations, including birch (*Betula* spp.), horse chestnut (*Aesculus hippocastanum*), and green ash (*Fraxinus pennsylvanica*). Research results from a survey under laboratory conditions using cut logs by USDA/APHIS/PPQ suggest that the potential host range may be larger, although maples remain the preferred target in their studies. It is our goal to evaluate the host range of the Asian longhorned beetle among commonly planted urban landscape trees including: paperbark birch, gray birch, American beech, sweetgum, yellow-poplar, blackgum, Norway spruce, white spruce, American basswood, river birch, Whitespire birch, European hornbeam, common hackberry, yellowwood, Turkish filbert, hawthorn, ginkgo, honeylocust, Kentucky coffee tree, Golden raintree, Callery pear, bur oak, pin oak, willow oak, English oak, Tree lilac, Littleleaf linden, Silverleaf linden, alder, and Zelkova.

A preliminary experiment was conducted to develop a method for artificially inserting larvae into trees to evaluate larval survival under greenhouse conditions. Larvae were artificially inserted into container grown sugar maple (*Acer saccharum*), green ash (*Fraxinus pennsylvanica*), and red oak (*Quercus rubra*) trees. The trees were grown under greenhouse conditions for six months prior to the experiment. Two trees of each species were placed into two separate screened cages (3 x 2.7 x 2.1 meter). The larvae were inserted into the trees through 5-mm deep incisions made through the bark near the bark-cambium-phloem interface. Four insertions were made in each tree at 1, 1.5, 1.75, and 2 meters above the soil

line. At implantation, the tree species varied in caliper width: sugar maple was  $25 \pm 2.6$  mm, green ash was  $34 \pm 3.5$  mm, and red oak was  $21 \pm 2.2$  mm.

Insertion sites were monitored daily until the larvae were no longer visible. Sixty days after implantation, the trees were destructively harvested to determine the progress, viability, and size of the larvae. The implanted section of trunk was removed from the tree and each implanted area was carefully split open with a chisel and hammer. Care was taken not to damage the larvae. Each larva was removed and weight recorded.

Fifteen days after insertion, a 19% mortality rate was observed for larvae inserted into both sugar maple and green ash. No larval mortality was recorded in the red oak. Larval mortality that occurred through day 15 was most likely due to injury during handling or the insertion process. The percent larval mortality from day 10 to 60 in each tree species was sugar maple 15.4%, green ash 69.2%, and red oak 37.5%. Larvae reared on green ash weighed significantly more (18 mg) than larvae reared on sugar maple (9 mg) or red oak (12 mg).

The larval insertion technique used for this study resulted in low mortality during the first two weeks of the study. We observed significant sap flow in green ash using this technique, and it is likely that this was responsible for the high larval mortality we observed in green ash. Red oak and sugar maple also exhibited sap flow, but not to the same extent as green ash. Larvae reared in green ash weighed more than the larvae reared in sugar maple or red oak. This indicates that the larvae were capable of surviving in ash if they were not “drowned” initially by high sap flow.

Over the next three years, we will screen common landscape trees for susceptibility to ALB oviposition and larval development. This research will provide vital information for field identification of susceptible trees and permit recommendation of non-susceptible trees. By characterizing oviposition activities, identifying susceptible trees, and confirming non-susceptible trees, we can reduce the direct financial and indirect economic impact this pest may have on forest, landscape, and nursery industries. Identifying non-susceptible cultivars will accelerate the re-greening of infested sites throughout the U.S., thereby assisting in controlling the spread of the beetle.

METHODS DEVELOPMENT FOR THE EXCLUSION OF  
ASIAN LONGHORNED BEETLE

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ABSTRACT

Methods developed to exclude Asian longhorned beetle (ALB) from future shipments containing solid wood packing materials were initiated in China during the spring of 2000 as part of a cooperative agreement between China Inspection and Quarantine (CIQ) and USDA-APHIS-PPQ. Heat treatment experiments were conducted on solid wood packing material at a commercial pallet manufacture plant in Xiaoshan, Zhejiang Province, China. Poplar wood in thicknesses of 2.5, 5, 7.5, 10, and 20 cm was artificially seeded with late instar larvae of ALB and heated to 60 °C in a kiln. Results are forthcoming and will serve to strengthen the existing wood products treatment schedule (T404-b-4) printed in the USDA-APHIS-PPQ Treatment Manual. Fumigation trials were conducted in Hohhot, Inner Mongolia, using fabricated Lexan chambers (15 cu. ft.) placed in refrigerated shipping containers. Poplar timbers (10 cm x 10 cm), both naturally infested and artificially seeded with larvae of ALB, were exposed to currently accepted concentrations of methyl bromide, sulfuryl fluoride, and phosphine at temperatures of 4.4, 10, 15.5, and 21.1 °C. Preliminary results indicate that the current treatment schedule for methyl bromide on wood products (T404-b-1-1) is adequate. Sulfuryl fluoride trials proved inconclusive due to poor fumigant penetration as a result of the high moisture content of freshly sawn wood. The evolution of CO<sub>2</sub> gas from green wood also had influence on the readings of both methyl bromide and sulfuryl fluoride. Phosphine trials resulted in reduced efficacy at lower temperatures and were hampered by slow evolution of the Chinese phosphine formulation. Further testing of fumigants on solid wood packing is planned for the 2001 season.

DISTRIBUTION, HOSTS, AND SEASONAL ACTIVITY OF THE EXOTIC  
JAPANESE CEDAR LONGHORNED BEETLE, *CALLIDIELLUM RUFIPENNE*  
(COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT

The small Japanese cedar longhorned beetle (*Callidiellum rufipenne* (Motschulsky)) is an eastern Asian cerambycid recently discovered in the wild in the eastern United States. The first record was represented by a single adult found at a coastal location in Dare Co., North Carolina, in 1997 (E.R. Hoebeke, personal communication). In the fall of 1998, infestations of larvae and adults were found in live landscape plants in garden centers in southern Connecticut (Maier and Lemmon 2000). These findings were the first records of larval boring in live, apparently healthy, plants. Shibata (1994) previously had reported that *C. rufipenne* bored into only dead and dying cedars. After its discovery in live landscape plants in the United States, federal officials reclassified *C. rufipenne* as an actionable insect (J. Cavey, personal communication), and regulatory officials in Connecticut quarantined the infested counties. Maier and Lemmon (2000) reported that most of the live plants infested by *C. rufipenne* were arborvitae (*Thuja occidentalis* L.) which had been balled and burlaped before infestation. It is possible that the infested live plants had been stressed by root breakage during removal from the ground; inadequate watering before, during, or after shipping; branch breakage during shipping; or inadequate care during or after planting.

Based on my observations in southern Connecticut, *C. rufipenne* has one generation per year. The adults emerge from wood in spring and mate on the host. Females lay eggs singly or in small batches in cracks and crevices in the bark of branches and trunks. In the laboratory, females lay an average of 65 eggs. After hatching, larvae bore into the cambium and phloem, completing their development by late summer or early autumn. They then bore into the sapwood and carve an ellipsoidal pupal chamber. Before pupating, larvae plug the entrance to their individual pupal cells with chewed wood. Adults eclose in autumn, but they remain in the pupal cells until the following spring.

During an initial survey in 1999, I determined that *C. rufipenne* was established in three coastal counties in Connecticut; that it bored into live or dead wood of cupressaceous plants in garden centers, in residential yards, and in the wild; and that it was attracted to trap-logs of eastern red cedar (*Juniperus virginiana* L.) and northern white cedar (*T. occidentalis*) (Maier 2000).

To expand my initial research on distribution and host range, I reared adults of *C. rufipenne* from >140 samples of dead wood collected in northeastern states between autumn 1999 and spring 2000. One or more beetles emerged from wood sampled in Massachusetts, Rhode Island, Connecticut, New York, and New Jersey. Based on data from the rearing project and from other sources (Ken Ahlstrom, personal communication; T. Denholm, personal communication; NAPIS 2000), *C. rufipenne* now occurs in the coastal states of Massachusetts (1 county), Rhode Island (1 county), Connecticut (4 counties), New York (5 counties), New Jersey (5 counties), and North Carolina (1 county). The exotic beetle apparently is confined to counties that border the Atlantic Ocean or a major river.

In spring 2000, I reared over 3,800 cerambycid beetles of eight species from the dead wood of Atlantic white cedar (*Chamaecyparis thyoides* (L.) B.S.P.), common juniper (*J. communis* L.), *J. virginiana*, and *T. occidentalis*, collected in the northeastern states of Vermont, Massachusetts, Rhode Island, Connecticut, New York, and New Jersey. Nine of 18 (50%) of the host records were new. Adults of *C. rufipenne* emerged from the dead wood of *C. thyoides*, *J. virginiana*, and *T. occidentalis*. Based on these host records and others (Maier 2000, unpublished data; Maier and Lemmon 2000), *C. rufipenne* is now known to bore into the wood of seven species of Cupressaceae in the Northeast.

To determine the seasonal activity of *C. rufipenne* in groves of eastern red cedar in Connecticut, I captured adults with sticky bands placed on logs (0.7 m long), cut trees, and live trees, and with unbaited sticky yellow traps put on live trees. At Middletown (Middlesex Co.), most of the 45 adults were caught on cut trees (57.8% of total) and trap-logs (26.7%). No beetles were caught on sticky bands on live trees, suggesting that *C. rufipenne* may not be attracted to healthy trees.

Based on weekly captures on trap-logs or cut trees at Stamford (Fairfield Co.), Milford (New Haven Co.), and Middletown, the period of adult activity in 2000 lasted 7 weeks between early April and mid June. The period of activity was later as the latitude of the sites increased. Males outnumbered females in catches at every location, and the mean date of capture tended to be earlier for males than females.

To record adult emergence, logs of *T. occidentalis* were removed from Stamford in spring and monitored daily in screened cages at Hamden (New Haven Co.). Beetles emerged between 6 April and 15 May in 1999 and between 25 March and 10 May in 2000. In both years, the pattern of emergence was erratic, although most of the beetles emerged during dry, warm days in May. The start of emergence preceded the first catch on trap-logs at Stamford in 1999 and 2000 by 1 to 2 weeks. In both years, the emergence was significantly earlier in males than females, and the sex ratio was 1.0.

In the three years since its discovery in the eastern United States, the distribution, hosts, and seasonal activity of *C. rufipenne* have been investigated in Connecticut and, to a lesser extent, in a few other northern states. It would be desirable to elucidate the seasonal activity of *C. rufipenne* in the southernmost part of its range. With information from the northern and southern end of its range, it would be possible to predict more accurately when adult surveys at in-between locations should be conducted.

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STUDIES ON MULTIPARASITISM OF *GLYPTAPANTELES PORTHETRIAE* AND  
*GLYPTAPANTELES LIPARIDIS* (HYM., BRACONIDAE) ON THE HOST LARVA,  
*LYMANTRIA DISPAR* (LEP., LYMANTRIIDAE)

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ABSTRACT

The effect of interspecific competition between the solitary endoparasitoid species, *Glyptapanteles porthetriae*, and the gregarious species, *Glyptapanteles liparidis*, was investigated in their multiparasitized host larva, *Lymantria dispar* L. Gypsy moth larvae were parasitized either by both species simultaneously in premolt to the second or third host instar on the same day or with a delay in parasitization of 4 days by the second wasp species. Five days after oviposition of the first wasp species, development of host larvae and parasitoid larvae were monitored by dissecting individual larvae every second day.

Host selection experiments for oviposition revealed that both wasp species do not discriminate between host larvae that have been parasitized previously by the same or competing species or by unparasitized larvae; rather, the wasps usually parasitized the first caterpillar that they encountered. In delayed parasitism experiments, developing larvae of the primary species (first oviposited) never attacked eggs of the secondary species (those which were oviposited after four days). In these same experiments, larvae of the primary wasp species generally were successful in completing their development. However, larvae of the competing species were frequently attacked and killed or exhibited reduced growth. When *G. porthetriae* was the secondary parasitoid species, their larvae were never able to complete their development and to emerge successfully from host larvae. However, when *G. liparidis* was the secondary parasitoid species, their larvae completed development successfully from 12.5% of multiparasitized hosts.

When host larvae were parasitized more or less simultaneously by both wasp species, the rate of successful development of one or both species was dependent on the state of development of the host. In premolt to second instar parasitized hosts, 44% of *G. liparidis* and 28% of *G. porthetriae* emerged successfully, while both parasitoid species emerged successfully in 20% of the host larvae. Both species emerged successfully only when premolt to 2nd instar larvae were used. When host larvae were parasitized in the premolt to the third instar, *G. liparidis* emerged successfully from 90% of the multiparasitized hosts, as compared to only 8% of *G. porthetriae*. However, unlike the situation where larvae were parasitized in the premolt to second instar, we never observed successful emergence of both parasite species.

FACTORS AFFECTING THE SUCCESS OF THE GYPSY MOTH  
BIOLOGICAL CONTROL *ENTOMOPHAGA MAIMAIGA* IN MICHIGAN

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ABSTRACT

*Entomophaga maimaiga*, an entomopathogenic fungus, has become an important natural enemy of gypsy moth in much of the northeastern region of the U.S.. This pathogen has been established in Michigan since 1991 but its effects on gypsy moth dynamics have been less consistent than in the Northeast. We initiated major projects to (1) evaluate the suitability of climatic factors in Michigan and the north central region for *E. maimaiga* and (2) identify site or stand-related factors that may influence *E. maimaiga* persistence, germination, or infection rates.

Preliminary results of climatic modeling using 30-year mean temperature and precipitation data indicate that much of the north central region is similar to northeastern areas that have experienced repeated *E. maimaiga* epizootics. These areas should, therefore, be highly suitable for *E. maimaiga*. More intensive modeling using additional weather data from Michigan suggest that cold weather experienced in areas of northern lower Michigan and the western Upper Peninsula may limit the effectiveness of *E. maimaiga*. Precipitation is less likely to be a limiting factor. We intend to continue this work by comparing additional climatic data sets from Michigan and the north central region to conditions known to favor epizootics in the northeastern states.

To evaluate potential effects of site or stand factors on *E. maimaiga*, we selected 32 oak-dominated stands across northern Michigan; *E. maimaiga* was previously recovered from all stands. Stands were classified based on location and climatic regimes (e.g. lake effect, inland, or central frost pocket) and included low- to high-density gypsy moth populations. Results from field bioassays conducted in 1999 using freshly molted 4<sup>th</sup> instar larvae indicated that *E. maimaiga* infection rates were generally low, with means ranging from 5 to 7%. Laboratory bioassays were conducted under optimal conditions for the fungus, using

soil collected in each stand. Mean infection rates were roughly 25%, indicating that germination and/or larval infection was likely limited by weather or field conditions. Quantification of resting spores is in progress; results to date indicate that density was high enough in most stands to result in outbreaks if conditions were suitable.

Field and lab bioassays, resting spore analysis, and examination of gypsy moth cadavers collected from wild populations was repeated in 2000; dissection of cadavers and analysis of results are in progress. Bioassays will be repeated in 2001. Results from bioassays will be related to soil, vegetation, and other site or stand traits to identify factors associated with *E. maimaiga* success.

INTERCEPT™ PANEL TRAP, A NOVEL TRAP FOR  
MONITORING FOREST COLEOPTERA\*

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ABSTRACT

A novel trap, the Intercept™ Panel Trap, has been developed for monitoring forest Coleoptera. The trap is made from corrugated plastic. It is lightweight, water proof, and durable. The trap performance was tested in the field for several forest Coleoptera and compared to that of the Phero-Tech 12-unit Multi-Funnel Trap. The trap was tested against the spruce beetle (*Dendroctonus rufipennis*), Douglas-fir beetle (*D. pseudotsugae*), western pine beetle (*D. brevicomis*), western balsam bark beetle (*Dryocetes confusus*), pine bark beetles (*Ips* sp.), larger pine shoot beetle (*Tomicus piniperda*), pine sawyers (*Monochamus* spp.), Asian longhorned beetle (*Anoplophora glabripennis*), buprestid beetles, wood wasps, and several other exotic forest pests. The Intercept™ Panel Trap captured equal numbers or more of most tested bark beetle species except for the spruce beetle when compared to captures in the multi-funnel trap. The Intercept™ Panel Trap captured a substantial number of Cerambycid and Buprestid beetles and Siricid wood wasps. In tests for the capture of exotic forest pests in Oregon, the Intercept™ Panel Trap captured substantially more insect species than the multi-funnel trap.

\*The assistance of Dr. Steve Munson, USDA, FS, Ogden, UT is gratefully acknowledged. This is a report of research only. No endorsement by USDA of products or services mentioned is intended or implied.

ATTRACT AND KILL TECHNOLOGY FOR MANAGEMENT OF  
EUROPEAN PINE SHOOT MOTH, *RHYACIONIA BOULIANA*, AND  
WESTERN PINE SHOOT BORER, *EUCOSMA SONOMANA*\*

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ABSTRACT

Last Call™, an attract and kill bait matrix, was deployed for management of European pine shoot moth (*Rhyacionia buoliana*) and Western pine shoot borer (*Eucosma sonomana*) in pine plantations and tree nurseries. *R. buoliana* is an introduced pest of native and ornamental pines throughout the northeastern and northwestern United States and adjacent Canadian provinces, and a serious pest of pine plantations in South America and Europe. *E. sonomana* causes substantial economic losses in ponderosa, lodgepole, and Jeffrey pine in the western United States. Due to the cryptic larval habits of these shoot borers, conventionally sprayed insecticides are not very effective. Attract and kill (A&K) technology very selectively removes male moths of the target species from the ecosystem with negligible impact on non-target organisms. Baits combine the selectivity of pheromone (only 0.21 g/ha, compared to 3.5-20 g/ha for mating disruption) with rapid toxicity of insecticides (only 7.92 g/ha, compared to 500-800 g/ha for conventional sprays). Last Call™ retains the insecticide within a hydrophobic matrix that precludes run-off or drift, thus preventing ecosystem contamination and damage. The toxicant is partitioned so that non-target organisms, such as beneficial parasites and predators, are not harmed.

The pheromone trap capture data showed that, in comparison to the number of male *R. buoliana* captured within untreated plots, male moth populations in plots treated with a formulation containing 0.16% (w/w) of pheromone and 6% permethrin were reduced by 86.6% to 100%. The trap captures of male *E. sonomana* in A&K treated blocks were reduced 76% to 93% when compared to that of untreated blocks. Damage to Ponderosa pine by *E. sonomana* was significantly reduced in Last Call™ treated plots over two seasons, while damage in untreated plots remained unchanged.

\*This abstract reports the findings of research only. No endorsement by USDA of products or services mentioned is intended or implied.

NOVEL NEW ATTRACTANT AND FORMULATIONS FOR CONTROL AND  
MONITORING OF TEPHRITID FRUIT FLIES\*

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ABSTRACT

A novel plant-based attractant (EGO) has demonstrated activity as an attractant for three species of *Ceratitis* fruit flies: Mediterranean fruit fly (Medfly, *C. capitata*), Natal fruit fly (Natal fly, *C. rosa*), and Marula fruit fly (Mango fruit fly, *C. cosyra*). In field trials in Hawaii, South Africa, and Kenya, EGO captured more Medfly males than commercial formulations of trimedlure. It has attracted Marula fruit fly and Natal fruit fly to traps. EGO can be obtained very economically from natural sources. EGO was formulated with a toxicant in IPM Technologies' Last Call™ paste matrix and demonstrated attracticidal reduction of Marula fruit fly and medfly numbers and damage to citrus in South Africa.

The multispecies activity of EGO presents a very useful new tool for regulatory agencies and others involved in monitoring and control of *Ceratitis* species.

Also, the Last Call™ matrix has been used successfully to dispense cuelure and methyl eugenol to attract and capture Oriental and related fruit flies (*Bactrocera* species) in field traps. Tephritid fruit fly attractants can be easily and precisely dispensed in the field for either control or monitoring purposes using highly portable, stable, and environmentally safe Last Call™ formulations.

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# ATTRACTANTS FOR THE LARGER PINE SHOOT BEETLE,

## *TOMICUS PINIPERDA*\*

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### ABSTRACT

A new and more effective lure for the larger pine shoot beetle (*Tomicus piniperda*) has been developed. Five candidate beetle attractants ( $\alpha$ -pinene,  $\alpha$ -pinene oxide, nonanal, myrtenol, and *trans*-verbenol) were tested singly and in binary, tertiary, and quaternary combinations as the bait in 12-unit multi-funnel traps. Trap captures of *T. piniperda* were increased by from 175 to 433% in traps baited with binary or greater combinations when compared to  $\alpha$ -pinene alone. Trap captures with tertiary and quaternary blends were more effective and consistent from year to year than captures with binary combinations. This study demonstrated that, at the least, a tertiary combination of  $\alpha$ -pinene,  $\alpha$ -pinene oxide, nonanal, and *trans*-verbenol released at high rates is required to assure optimum trap captures.

\*This is a report of research only. No endorsement by USDA of products or services mentioned is intended or implied.

# FIELD EVALUATION OF A CHINESE LADY BEETLE FOR BIOLOGICAL CONTROL OF THE HEMLOCK WOOLLY ADELGID

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## ABSTRACT

The lady beetle (*Scymnus (Neopullus) sinuanodulus* Yu et Yao) was imported from China to the USDA Forest Service quarantine facility in Ansonia, CT, for evaluation as a potential biological control agent against *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA). The impact of the beetle on HWA was evaluated for 2 years by caging individual, gravid females on infested branches.

Beetles were caged on branches using white nylon mesh bags. The experiment consisted of three treatments: (1) unbagged branch, (2) bagged branch without lady beetles, and (3) bagged branch with a single, previously mated female beetle. The experiment was initiated early in the spring, and branches were collected and brought to the laboratory for examination twice during each field season to coincide with the least overlap between HWA generations. Branches were examined under a microscope and HWA nymphs and *S. sinuanodulus* progeny were counted.

In 1999, bags and beetles were placed on trees in mid-April when the adults of the overwintering HWA sisten generation were at peak oviposition. In 2000, treatments were placed in the field earlier when adelgids were beginning to lay eggs and larger bags were used to enclose more foliage because of lower adelgid densities due to high winter mortality.

This field study and current laboratory experiments show that beetle oviposition is a function of prey density, quality, and phenology. More beetle progeny were present on branches with higher initial HWA densities. The presence of a lady beetle resulted in decreased HWA population growth, but the impact was lower when measured across both HWA generations. Despite reduction in the growth of HWA populations of 66% (1999) and 76% (2000) compared to bagged controls, the impact was not sufficient to prevent an increase in HWA populations.

Tri-trophic feedback from the host trees may have influenced beetle performance. Deterioration in the health of the hemlock trees was visibly dramatic during the course of the experiment, and the quality of HWA as food may have been reduced, lowering lady beetle fecundity. The timing of beetle placement in the field experiments may not reflect the beetle's natural synchronization with HWA phenology, and thus may have reduced the impact of the beetle on HWA.

# ADAPTIVE PHYSIOLOGICAL TRAITS AND CONSTRAINTS ON THE DISTRIBUTION OF INVASIVE LEGUMES

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## ABSTRACT

While traversing the planet for many centuries, humans have introduced non-native species to many ecosystems. Some of these introduced species were hitchhikers while others were carried for agricultural or horticultural reasons. However, naturalization of non-native plant species has greatly accelerated in the past few decades (Lodge 1993), resulting in a homogenization of species unprecedented in geological time. Naturalized, non-native species become an ecological problem when the results of the naturalization ramify throughout the invaded community and ecosystem. Some of the common ecological consequences of non-native plant species invasions are a reduction of native plant diversity, a loss of animal habitat, a change in the frequency and severity of disturbances such as fire, a reduction in resource availability to native species, and an alteration of ecosystem services. In fact, the changes in diversity, species distributions, extinctions, and ecosystem services due to non-native species invasions dwarf the effects of global climate change on these processes. Non-native invasive species are widely recognized worldwide as posing a threat to diversity and ecosystem stability second only to habitat destruction. A majority of the research on non-native introduced plant species directly concerns mechanisms for control of their population growth and persistence in the community. However, some recent studies have attempted to develop a body of ecological theory about non-native plant introductions.

The two major questions addressed by this burgeoning theory are: (1) What characteristics of ecosystems make them prone to invasion by non-native species? (2) What characteristics of non-native species make them likely to naturalize and induce ecological change? Davis et al. (2000) have recently addressed the first question in a synthesis article. They concluded that ecosystems become vulnerable to invasion when they experience an increase in resource availability induced by either a loss of vegetation or an increase in resource input. Therefore, the vulnerability of ecosystems to invasion is not constant and successful invasions will occur only when there is a rise in resource availability that coincides with the availability of invading propagules.

The second question has resisted the development of theoretic consensus. The diversity of naturalized species, diversity of habitats invaded, lack of research on unsuccessful invasions, and the focus on remediation rather than prevention are all causes for our inability to characterize the traits that make plants invaders. However, there are some generalized traits that are common to most invading non-native species. Baker (1965) was the first to formally

compile a list of traits common to invading species. His list of 14 traits was focused on reproductive characters such as fast maturation to reproductive age, high output of seeds, extended seed longevity, discontinuous seed germination, the ability to produce seed over wide environmental conditions, both long and short distance seed dispersal mechanisms, and the ability to reproduce by vegetative means from rootstock. Another study by Rejmanek and Richardson (1996) on invasive and non-invasive members of the Pineaceae also identified reproductive traits as those that characterize invasive species (short juvenile period, short interval between seed crops, and small seed mass). There are many different combinations of plant traits that can promote a successful invasion, but our ability to decipher and quantify those suits of traits remains poor (Mack et al. 2000). More recently we have come to recognize the importance of mutualistic relationships among species (such as mycorrhizal or rhizobial mutualisms) for successful naturalization of species (Richardson et al. 2000). However, reproductive traits and mutualisms are only two aspects of the entire cycle required for successful naturalization.

The processes required for a successful invasion can be presented as a spiral of increasing population size with specific checkpoints along each cycle. The most significant checkpoints in this spiral are the presence and effectiveness of a distribution vector, abundance of safe sites for establishment, appropriate abiotic conditions for growth, the presence of a pollination vector, and the absence of strong propagule predation. Any one of these checkpoints can hinder the success of an invader. Moreover, changes in these checkpoints may be effective mechanisms of abating the population rise of an invader. An additional complication to this scenario in plant systems is their ability to utilize vegetative as well as sexual reproductive means. In species that rely on vegetative reproduction, abiotic conditions, safe sites, and propagule predation become the most important checkpoints.

Physiological characteristics of the invading plants are most significant for the establishment, growth, and reproductive phases of the invader's population cycle. In particular, the physiological ability to withstand the stresses imposed by the ecosystem and the ability to effectively acquire resources are of paramount importance to invasion success. Although there are many studies on the reproductive traits of invasive non-native species, generalities on physiological characters are poorly developed. The best generalities we can state now is that invasive non-native species have a high resource demand, often depend on mutualism to improve resource acquisition, and often have a wide tolerance of environmental stressors. One of the best ways to begin to improve our understanding of physiological traits in non-native invasive species is to examine a phylogenetically constrained group of invasive plants.

Legumes are one of the most important invasive, woody plants in non-agricultural systems. Of the 78 species considered the most invasive plants in California, 9 are legumes (Bossard et al. 2000). Are there common physiological traits among these species that make them particularly effective invaders? Do these traits also constrain their possible expansion into a diversity of habitat types? Answers to these two important questions can elucidate the potential extent of a particular species invasion, the likelihood that other legumes with a similar physiology will be invasive, and the potential that certain management protocols against plant species invasion will be successful. Here I will focus on the invasive and

indigenous legumes in California; nevertheless, the physiological traits and constraints on distribution apply to many legumes in other systems.

The nine invasive non-native legumes in California are predominantly spiny, drought tolerant shrubs with green stems inhabiting disturbed sites or old fields. Patterns of photosynthetic response to abiotic environmental conditions in these species could provide an understanding of physiological adaptation to climate and habitat preference. Moreover, a comparison between the invasive non-natives and indigenous species that have similar growth forms may lead to an understanding of important traits of invasive plants.

Temperature tolerance of the invasive legumes in California is relatively broad. Photosynthesis is maintained between 5 °C and 40 °C in many species. On the other end of the scale, several of these invasive legumes are very sensitive to cold-induced damage.

Light response of photosynthesis is one of the most dramatic characteristics of these species. Light saturation points are high (greater than 1200  $\mu\text{E}/\text{m}^2/\text{s}$ ) for both leaf and stem photosynthesis. In addition, light compensation points are relatively high in leaf and stem indicating a relatively high respiration rate and an intolerance of low light conditions. In fact, we could consider invasive legumes as obligate heliophytes.

One of the most prominent characteristics of invasive legumes in Mediterranean systems is stem photosynthesis. All nine of the non-native invasive legumes in California have prominent green stems, and many of these have documented net carbon gain by photosynthesis. Photosynthetic stems provide several advantages for legumes colonizing disturbed sites. They provide increased drought tolerance, increased tolerance of leaf herbivory, effective use of nitrogen, and increased carbon acquisition capacity. Several native legumes also contain this trait, but are not invasive. Therefore, this trait does not define an invasive plant, but it does enhance a plant's ability to withstand the stresses of a recently disturbed site.

The invasive, non-native legumes do show significant drought tolerance. The ability to avoid drought stress by defoliating and tolerate drought stress by osmotic adjustment in the stem tissues is a characteristic of both non-native and indigenous legumes.

Photosynthetic responses to light indicate a limited ability to tolerate lower radiation. However, these photosynthetic properties could change in shaded habitats affording a higher degree of growth than predicted by light response curves of plants in disturbed habitats. An examination of photosynthetic flexibility to different light availability of two non-native, invasive legumes indicated no capacity to change photosynthetic light response when grown under low light conditions. This lack of physiological flexibility caused severe effects on growth and resource allocation in lower light environments. Fifty percent of full sun or lower caused significant decreases in growth for both species. Allocation away from stem area to leaf area and weak stem development suggested greater sensitivity to drought and cold stress for plants growing in shaded conditions.

Nitrogen availability is a major limiting factor for plants in natural systems. Legumes have an advantage over many other plants because they form mutualisms with bacteria, and these associations (nodules) are able to convert di-nitrogen gas into amino acids. Therefore, legumes have the capacity to survive in low nitrogen sites. When soil has a relatively high amount of nitrogen, the association between bacteria and legumes is inhibited and the legumes survive on soil nitrogen. Increases in soil ammonia inhibit the growth of these invasive legumes (Nilsen 1992).

These physiological traits of invasive legumes will constrain their distribution in nature. The lack of flexibility to shade restricts these species from forested regions. Moreover, their growth form in shaded habitats limits their stress tolerance. Their sensitivity to ammonium limits their range to regions of low fertility and relatively high pH soil. As a consequence most invasive legumes are restricted to high light sites with low nutrient and are unlikely to invade into established communities from the edges of disturbances.

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# IMPORTANT GENERA OF LONGHORN BEETLES NATIVE TO SLOVAKIA:

## PEST STATUS AND NATURAL ENEMIES

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### ABSTRACT

More than 20,000 species of Cerambycidae are known worldwide and have been described in the literature. A relatively small number of species from this insect family (2,000 to 2,500 species) are native to the palearctic region (Europe and Asia). In Slovakia, there are 200 species of Cerambycidae represented in 86 genera. Of this number, only 20 species from several genera are considered to be forest pests.

Four genera are recognized as important forest pests in Slovakia: *Cerambyx*, *Monochamus*, *Saperda*, and *Tetropium* (syn. *Isarthron*). Species within these genera and their hosts are as follows: ***Cerambyx***: *C. cerdo* L., *C. scopoli* Füssly - pests of broad-leaved trees (oak, beech, etc.). ***Monochamus***: *M. sartor* F. (spruce stands), *M. sutor* L. (conifers – spruce, pine, fir). ***Saperda***: *S.* (syn. *Anarea*) *carcharias* L. (poplars), *S. populnea* L. (poplars). ***Tetropium***: *T. castaneum* L. and *T. fuscum* F. (spruce, fir rarely), *T. gabrieli* Weise (larch).

Natural enemies play an important role as bioregulation factors that influence the population density of Cerambycids in Slovak forests. The following natural enemies have been recognized and described from Central European longhorn beetle populations:

***Cerambyx* sp.:** Ichneumonidae - *Ephialtes mesocentrus* Grav., *Ephialtes tuberculatus* Foucroy, *Ephialtes manifestator* L., *Rhyssa amoena* Grav., *Rhyssa persuasoria* L., *Rhyssa superba* Schrnk. Braconidae - *Helcon dentator* F.

***Monochamus* sp.:** Ichneumonidae - *Mesostenus gladiator* Scop., *Ephialtes tuberculatus* Foucroy, *Perithous divinator* Rossi, *Pyracmon austriacus* Tschek. Braconidae - *Coelobracon initiator* Nees., *Iphiaulax flavator* F., *Doryctes striatellus* Nees.

***Saperda* sp.:** Ichneumonidae - *Proscus suspicax* Wesm., *Cryptus viduatorius* F., *Xylophrurus lancifer* Grav., *Goniocryptus analis* Grav., *Brachycentrus brachycentrus* Grav., *Ephialtes populneus* Rtzb., *Ephialtes messor* Grav., *Ephialtes manifestator* L., *Glypta rostrata* Homgr., *Glypta teres* Grav., *Ischnocerus filicornis* Kriechb., *Deuteroxorides albitarsus* Grav., *Xorides praecatorius* F., *Rhimphoctona fulvipes* Holmgr. Braconidae - *Coelobracon denigrator* L., *Bracon discoideus* Wesm., *Ascogaster rufipes* Latr. Chalcidoidea - *Habrocytus tenuicornis*

Forst.. Larvaevoridae - *Atropidomyia irrorata* Meig., *Masicera silvatica* Fall., *Dionea nitida* Meig.

***Tetropium* sp.:** Ichneumonidae - *Ephialtes aciculatus* Hellen, *Ephialtes dux* Tschek., *Ephialtes terebrans* Rtzb., *Rhyssa persuasoria* L., *Poemia notata* Holmgr., *Neoxorides nitens* Grav., *Neoxorides collaris* Grav., *Xorides praecatorius* F., *Xorides irrigator* F., *Xorides niger* Pf., *Rhadinopimpla brachylabris* Kriechb., *Rhadinopimpla atra* Grav., *Coleocentrus caligatus* Grav., *Pyracmon austriacus* Tschek., *Pyracmon xoridiformis* Strobl.. Braconidae - *Coelobracon denigrator* L., *Coelobracon initiator* Nees., *Coelobracon neesi* Marsch., *Atanycolus initiator* Nees., *Doryctes leucogaster* Nees., *Doryctes mutillator* Thunb., *Helcon aequator* Nees., *Helcon dentator* F., *Helcon tardator* Nees., *Baeacis dissimilis* Nees.

Information about entomopathogens that infect species of Cerambycidae is seldom mentioned in the literature. We found no reference of entomopathogens for the genera *Cerambyx* spp., *Monochamus* spp., and *Tetropium* spp. In the genus *Saperda*, we found references to the following pathogens: Bacteria - *Pseudomonas aeruginosa* Schroeter and *Enterobacter cloacae* Jordan; Nematoda - *Pristionchus lheritieri* Maupas.

Most of the natural enemies listed are not commonly found in field collections because the population density of most Cerambyriid species is low. The higher number of natural enemies recorded for the genera *Saperda* and *Tetropium* reflects the greater abundance of species in those genera that are encountered commonly in the field.

POTENTIAL EFFECT OF AN ASIAN LONGHORNED BEETLE  
(*ANOPLOPHORA GLABRIPENNIS*) ON URBAN TREES IN THE UNITED STATES

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ABSTRACT

An Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) was recently found in New York City and Chicago. In an attempt to eradicate this beetle, thousands of infested city trees have been removed. Field data from nine U.S. cities and national tree cover data were used to estimate the potential effects of the Asian longhorned beetle on the urban forest resource. For the cities analyzed, the potential tree resources at risk to Asian longhorned beetle attack (based on host preferences) range from 12 to 61% of the city tree population, with an estimated value of \$72 million to \$2.3 billion per city. The corresponding canopy cover loss that would occur if all preferred host trees were killed ranges from 13 to 68%. The estimated maximum potential national urban forest impact of the Asian longhorned beetle is a loss of 34.9% of total canopy cover, 30.3% tree mortality (1.2 billion trees), and a value loss of \$669 billion. More detailed information on this study is given in: Nowak et al. [In press]. Potential effect of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on urban trees in the United States. Journal of Economic Entomology.

EVALUATION OF SYSTEMIC INSECTICIDES TO CONTROL  
*ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT

The use of systemic insecticides may prove useful in controlling Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)) (Coleoptera: Cerambycidae) adults during maturation twig feeding and larvae when feeding in the cambium and sapwood. In 1999, we evaluated imidacloprid as a systemic insecticide against *A. glabripennis*. We used four injection methods: Mauget (Imicide, J.J. Mauget, Co.) and Wedgle (Pointer, Arbor Systems, LLC) trunk injectors, ACECAP trunk implant (Creative Sales Inc.), and Kioritz soil injector (Merit, Bayer Corp.). All injection methods were tested in four tree species which were known to be hosts in China and were infested in New York City and Chicago: American elm (*Ulmus americana* L.), boxelder (*Acer negundo* L.), quaking aspen (*Populus tremuloides* Michx.), and silver maple (*Acer saccharinum* L.). Tree samples were collected 4, 8, and 16 weeks after injection and presented to *A. glabripennis* adults and larvae. Testing of *A. glabripennis* was conducted in the USDA Forest Service quarantine facility in Ansonia, CT. Branch sections were presented to mating pairs of adults to test oviposition success, twigs were presented to individual adults to assess mortality during maturation feeding, and artificially reared first instar larvae were inserted into branch sections to assess larval mortality. Chemical residue analyses were conducted on foliage and twig samples at the USDA APHIS laboratory in Gulfport, MS. Overall, little adult or larval mortality occurred during the 2- to 6-week feeding tests. Adults fed on the outer bark of the twigs and thus not deep enough into the wood to receive a lethal dose. The highest levels of *A. glabripennis* larval mortality occurred in branch samples from boxelder trees that were collected 16 weeks post-injection. The chemical residue analyses suggested that the imidacloprid did not spread evenly throughout the trees. Of the four injection techniques, imidacloprid was most commonly detected in trees treated with the Mauget trunk injection method.

In 2000, in addition to imidacloprid, we also evaluated azadirachtin (the active ingredient in neem seed extract) and emamectin benzoate in both the U.S. and in China. In the U.S., we tested two doses of imidacloprid using Mauget trunk injection devices (Imicide), two doses of azadirachtin (Oranzin, Amvac Chem. Corp.) using both the Kioritz soil injector and systemic tree injection tubes (STIT), and one dose of emamectin benzoate using trunk injection (Shot One, Novartis). In the U.S., we tested imidacloprid in both boxelder and silver maple trees, whereas azadirachtin and emamectin benzoate were tested only in boxelder trees. Samples of trees injected with azadirachtin were collected 4 weeks after injection and larvae were inserted under the bark in the Ansonia quarantine lab. Larvae were

allowed to feed for 8 weeks prior to dissection of the branch samples. Samples from trees injected with imidacloprid and emamectin benzoate were collected 12 weeks after injection and larvae were allowed to feed for 4 weeks prior to dissection. Overall, we found very low mortality in all lab bioassays in 2000. Residue analyses are still being completed.

We are currently conducting laboratory bioassays with *A. glabripennis* and cottonwood borer (*Plectodera scalator* (Fabricius)) (Cerambycidae) feeding on artificial diet treated with various concentrations of imidacloprid and azadirachtin. The cottonwood borer is being reared in a non-quarantine laboratory in East Lansing, MI, as a surrogate for *A. glabripennis*. Preliminary results demonstrate a strong anti-feedant effect for both insecticides. As concentrations of imidacloprid and azadirachtin increase in the diet, larvae feed less and lose weight. For *P. scalator*, no mortality was seen until 8 weeks of feeding. After 12 weeks, several larvae feeding on diet treated with concentrations of imidacloprid or azadirachtin greater than 50 ppm had died, while larvae on doses as low as .005 ppm lost weight and displayed various signs of decline. For *A. glabripennis*, mortality was first seen after 4 weeks of feeding and after 8 weeks, a few larvae had died and similar reductions in feeding, weight loss, and signs of decline were observed. These results may help explain why we observed such low larval mortality in the insecticide-treated branch samples in 1999 and 2000, i.e., that the larvae may require more time to feed before branch dissection and assessment of mortality. In addition, the small larvae that were inserted into the branch samples may not have received a lethal dose because they feed very little and primarily in the phloem tissue.

In June 2000, we injected 48 elm trees, 48 poplars, and 48 willows in Gansu Province, China. For each tree species, 24 of the trees were currently heavily attacked by *A. glabripennis* and 24 were lightly attacked. Equal numbers of trees were injected with imidacloprid using Mauguet trunk injectors (Imicide, high dose), azadirachtin using systemic tree injection tubes (Ornazin, high dose), or emamectin benzoate using trunk injection (Shot One). In July, 4 weeks after injection, four mating pairs of *A. glabripennis* were caged on each of the lightly infested trees to ensure that some larvae would be present. In October, 4 months after injection, we cut down and dissected half of the trees. Mortality varied by *A. glabripennis* life stage, tree species, and insecticide. Overall, moderate levels of mortality were found for all three insecticides tested. The highest larval mortality rate (81% of the larvae that were in the sapwood) occurred on poplar trees that had been injected with imidacloprid. The remaining trees will be cut and dissected in May or June 2001. Additional doses and compounds will be tested in 2001. In addition, trees will be injected simultaneously with dye and each of the different insecticides using different doses and number of injection sites to determine translocation patterns and the optimal delivery protocols to ensure complete coverage.

THE USE OF MOLECULAR GENETICS TO IDENTIFY AND  
MONITOR INVASIVE PESTS

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ABSTRACT

Our group has been involved with pest identification issues that require the use of molecular techniques that complement morphometric and anatomical methodologies. The types of problems range from population differentiation (*Ceratitis capitata*) to strain differentiation (*Lymantria dispar*) to species differentiation (terrestrial slugs). Medfly can be invasive to California, Florida, and Texas. The Asian strain of the gypsy moth can be invasive to the forests of North America and exotic mollusks can be invasive to both disturbed and undisturbed habitats. These three identification problems require different methodologies using genetic markers specific to the task. We summarize here the results from these three projects and briefly present the differing methodologies.

# EXOTIC AMBROSIA BEETLES IN THE SOUTHEASTERN UNITED STATES

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## ABSTRACT

Native bark and ambrosia beetles (Scolytidae) are among the most important forest insects, causing the loss of millions of dollars in forest products each year. Exotic species are also having an impact. In 1977, it was estimated that there were 37 species of Old World scolytids established in North and Central America. Since then, 10 additional species have been reported new to the United States. Recent surveys have found an increasing number of exotic ambrosia beetles established in the United States. Ambrosia beetles are usually considered secondary pests in their native habitats; however, increasing evidence has suggested that some species of ambrosia beetles are aggressively attacking healthy saplings and twigs of healthy trees. An important first step in evaluating the impact of exotic ambrosia beetles on the health of forests is to determine what species are present. Surveys in the Northeast and mid Atlantic have found six species of exotic Xyleborini (the largest and most important group of ambrosia beetles) new to the area within the past 10 years. The purpose of this Forest Health Evaluation Monitoring project was to conduct a preliminary survey of the Southeast to determine the occurrence of exotic ambrosia beetles. Nine states in the southeastern United States (U.S. Forest Service Region 8) participated in this survey (Alabama, Florida, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and Virginia). In most states, ethanol-baited funnel traps were co-located with traps for the southwide southern pine beetle survey and were placed in pine stands at the time of dogwood bloom. Forest health workers in each state monitored traps and collected samples weekly for eight weeks. Fifty-five new state records for scolytids were recorded for 26 species during this survey. A total of 41 of these new state records were for ambrosia beetles and 28 for exotic species. Among the ambrosia beetles, this is the first record of *Xyleborus pelliculosus* Eichhoff in the South. *Dryoxylon onoharaensum* (Murayama), *Ambrosiodmus rubricollis* (Eichhoff), and *Xyleborinus saxeseni* (Ratzburg) are all exotic ambrosia beetles and were collected in every state during this survey. Two other exotic ambrosia beetles, *Xyleborus californicus* Wood (previously reported only from South Carolina in the South) and *Xylosandrus crassiusculus* (Motschulsky), were collected in eight of nine states. A total of 12 exotic species was collected, 10 of which were ambrosia beetles and of these, five have been reported for the first time in the Southeast in the past 10 years. Most of these "new" introductions are from Asia. Nine of the 12 exotics are from Asia and the two European exotics were introduced over 100 years ago. During this survey, 8,273 scolytids were collected. Ambrosia beetles accounted for 7,104 of these specimens (86%). The most numerous was *Xyleborinus saxeseni* with 4,375 specimens (53%). This exotic ambrosia beetle was found in every state and dominated most trap samples. Members of the tribe Xyleborini made up 77% (6,438) of the specimens collected. In the United States, there are currently 33 species of xyleborines; 14 of these are exotics.

## RECENT STUDIES ON MIDGUT BACTERIA OF THE GYPSY MOTH

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### ABSTRACT

Internal microbial symbionts are widely found among insects and play important roles in their basic physiological, developmental, and behavioral processes. Insect functions affected by endosymbionts include the ability to exploit various host plants and their relative susceptibilities to pathogens. Because microflora are so intimately linked with insect survival and reproduction, the midgut community may offer a largely unexplored target for pest management. We currently know very little about the microflora of Lepidoptera, particularly in comparison to Heteroptera and Isoptera. Information from other systems suggests that most internal symbionts are bacteria, and that most bacteria are not culturable. Therefore, we used a complementary series of traditional (culture independent) and molecular methods, including culturing, PCR, and TRFLP. We present here a preliminary list of bacteria identified from the gypsy moth midgut. The composition of the community is altered by the host tree species on which gypsy moth larvae from a common laboratory colony feed. The community is similar among gypsy moths from various field and laboratory sources. The linear aminopolyol, zwittermicin A, alters the midgut microflora and provides a useful tool for subsequent studies. Addition of zwittermicin A synergizes the activity of Btk, but is not toxic by itself to gypsy moth. Efficacy appears to vary little among population sources of gypsy moth, but varies with host plant. Our future work on midgut microbial communities will emphasize (1) comparisons of Lepidoptera vs. Coleoptera, (2) comparisons of foliage feeders vs. woodborers, (3) mechanisms of zwittermicin A synergism, and (4) comparisons of synergism of various forms of Bt among susceptible and resistant Lepidoptera and Coleoptera species and biotypes.

THE NEW PEST ADVISORY GROUP: A COMPONENT OF SAFEGUARDING  
U.S. AGRICULTURAL AND NATURAL ECOSYSTEMS

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ABSTRACT

The New Pest Advisory Group (NPAG) is a process within the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Center for Plant Health Science and Technology (CPHST), designed for response to new plant pest detections in the United States.

The NPAG assesses new detections of exotic plant pests in the U.S. to recommend an appropriate course of action. These exotic plant pests include arthropods, mollusks, pathogens, and weeds. During the evaluation process, Federal, State, and University personnel with regulatory and scientific expertise for the particular pest assemble to form an ad hoc panel. In addition, representatives from appropriate scientific societies attend. The NPAG then makes consensus recommendations to PPQ management using information gathered through literature evaluations, panel discussions, and risk analyses. The immediate goal of the NPAG is to communicate, document, and ask strategic questions that need to be addressed by PPQ program staff. The NPAG ultimately provides necessary information for management decisions. In addition to exotic pests detected within the country, the NPAG also investigates exotic pests that are likely to be introduced into the United States. A database of NPAG pest information, created this year, will be a tool for retrieving archived information and for analyzing trends in pest introductions.

NPAG procedures for responding to plant pests and a preliminary analysis of the 1998-2000 introductions are presented. The NPAG assessed a total of 27, 16, and 68 cases in 1998, 1999, and 2000, respectively. For all three years, arthropods comprised the largest group of new and imminent pests. In 2000, recommendations for the pest situations were: no regulatory response, 40%; State action alone, 18%; and APHIS PPQ and State cooperation, 42%. Currently, the NPAG is assessing 35 exotic pests.

AUTOMATED RECORDING TRAPS TO ASSESS GYPSY MOTH  
FLIGHT PHENOLOGY ALONG THE ADVANCING FRONT

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ABSTRACT

The National Gypsy Moth Slow the Spread (STS) Project is a cooperative venture involving USDA Forest Service and state agencies. STS reduces the rate of spread of gypsy moth by intensive monitoring and timely control of growing, isolated populations along the advancing front of the generally infested area (currently from North Carolina through Michigan). Data on capture of male moths in pheromone traps are used to guide management activities the subsequent year. However, capture of migrant moths, which occasionally travel long distances on the wind, can reduce the value of trapping data for decision making. Gypsy moth development rate is strongly affected by local temperature, so suspect migrants can be identified by comparing the seasonal timing of capture to predictions of local flight activity generated by temperature-driven phenological models. Currently, this requires visiting traps two to three times weekly, which is prohibitively expensive.

We are developing traps that automatically record the date and time of each capture. The trap bodies are plastic and designed after the standard milk carton trap. They house HOBO<sup>®</sup> event-recording data loggers (Onset Computer Corp.) with one of two types of sensors to detect captured moths: IR (6 month battery life) or piezoelectric (1 year). Sixty prototype traps were built at a total cost of \$400 per unit, but production units would likely cost much less.

In 2000, the 60 traps were field tested across four states. None were broken or lost, and all sensors and event recorders were functioning at the end of the season. The IR and piezoelectric versions performed comparably and, when placed near one another, showed similar patterns of seasonal and diurnal captures. Both types tended to undercount where moths were abundant, probably because moths entered more frequently than the pre-programmed 3-minute lockout interval (intended to prevent single moths from triggering the device more than once). Shorter lockout periods will be tested. Traps in Massachusetts and Michigan typically showed close agreement between numbers of events recorded and moth capture, but those in Wisconsin and Indiana generally did not. The cause of these discrepancies is under investigation. Some traps in Massachusetts and Michigan showed evidence of influxes of males after the main flight period, whereas some traps in northern Michigan appeared to catch early season migrants. We will continue to evaluate the potential strategic value and cost efficiency of these traps.

DISTANT EFFECT OF PHEROMONE USED FOR  
MATING DISRUPTION OF GYPSY MOTH

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ABSTRACT

Mating disruption with synthetic pheromones is used increasingly to eradicate or suppress isolated low-density populations of the gypsy moth (*Lymantria dispar* L.) in the United States. It is used both in APHIS eradication projects and in the U.S. Forest Service Slow-the-Spread Project that is focused on reducing the rate of population spread to the west and south. Additional research is needed to determine possibilities for reducing the cost of pheromone treatment. One of the questions is how far from sprayed dispensers mating can be disrupted. Currently, dispensers are distributed uniformly over the entire area in the same way as pesticides. However, if mating can be disrupted at some distance away from sprayed dispensers, then it would be possible to use wide-swath spraying that can considerably cut the cost of applications.

Field experiments were done from June to August 2000 near Millboro Springs, VA. Six 25-ha blocks were sprayed with Hercon® pheromone flakes or with 3M microcapsules, both containing racemic disparlure at 75 or 37 g AI/ha. The pheromone suppressed male moth capture in traps baited with (+) disparlure up to 2,000 m away from the nearest plot treated along the valley and 350 to 450 m away towards mountain ridges. The seasonal peak of moth capture rate in traps located 300 m away from the boundary of treated plots (and hence, affected by the pheromone) was on July 20, which is 8 days later than the peak of moth capture rate in control groups of traps. This indicates that the effect of pheromone at 300 m away from treated plots was strongest immediately after treatment and then declined.

Mating success was measured by exposing tethered laboratory-reared virgin females on tree boles for 1 day from July 17 to 21. Fertilization was determined by female dissection and by the analysis of egg embryonation. Lines of tethered females, 10 females in each line, were set at various distances from plots treated. The proportion of fertilized females increased with increasing distance from plots. Reduced mating success was observed up to 1 km from the treated area. The proportion of fertilized females was closely related to the moth capture rate in pheromone traps in the same location. This relationship was modeled by the equation  $F = 1 - \exp(-s \cdot M)$ , where  $F$  is the proportion of fertilized females per day,  $M$  is the male moth capture rate per trap per day, and the parameter  $s$  is a relative attractiveness of females compared to traps. Parameter value was estimated as  $s = 0.23 \pm 0.03$  ( $\pm$ SE). This relationship can be used for predicting the mating success of females based on moth counts in traps.

# *ANOPLOPHORA GLABRIPENNIS* ANTENNAL SENSORY RECEPTORS:

## A RESEARCH UPDATE

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### ABSTRACT

Work is in progress to delineate sensory structures on the antennae of male and female Asian longhorned beetle (ALB) (*Anoplophora glabripennis*) that might be responsive to chemical attractants. Antennae have been examined using light microscopy and scanning and transmission electron microscopy, and a number of types of receptors have been identified.

Beetles emerged within the USDA Forest Service Quarantine Laboratory, Ansonia, CT, from infested logs harvested in Chicago, IL, and New York City. For light and transmission electron microscopy, antennal segments were fixed in glutaraldehyde, paraformaldehyde, and acrolein; post-fixed in osmium tetroxide; dehydrated; and embedded in Spurr's resin. Thick sections were stained with toluidine blue. For scanning electron microscopy, specimens were fixed in ethanol/dimethoxypropane and air-dried or in glutaraldehyde/paraformaldehyde and osmium tetroxide and critical point dried.

The antenna of each sex has 11 segments – a scape, pedicel, and nine similar annuli that make up the flexible flagellum. Because the scape and pedicel bear few surface structures, it is unlikely that they are involved in chemoreception. The flagellum is covered with dense hairs arranged in broad, alternating black and white bands. The hairs are socketed and their surface is uniformly sculptured with fine ridges running along the axis. The hairs measure approximately 8  $\mu\text{m}$  x 55  $\mu\text{m}$  and appear identical to hairs found on the elytra. Three other types of setae are found on the flagellum. Long hairs (10  $\mu\text{m}$  x 250  $\mu\text{m}$ ) are located at the junctions of annuli. There are 12-25 of these per segment; their position indicates that they likely function as mechanoreceptors. Somewhat shorter setae (8  $\mu\text{m}$  x 120  $\mu\text{m}$ ) are located just proximal to the junction of annuli (20-40 per segment), and several (2-40 per segment) long setae (8  $\mu\text{m}$  x 350  $\mu\text{m}$ ) are located in the midregion of each annulus. Their histology indicates that the setae function as mechanoreceptors. To date, there is no evidence of pores or pore canals within the setae that would be indicative of chemoreception.

Several structures have been identified on the flagellum of male and female ALB antennae that may function as chemoreceptors. The terminal annulus of both male and female antennae has at its apex 11-20 basiconic pegs, approximately 2.8  $\mu\text{m}$  in diameter and height. Pores generally are located near the pegs. However, histological examination of the pegs reveals they closely resemble contact chemoreceptors described in other insects. Observations of adult ALB behavior lend support to the hypothesis that these pegs function as contact chemosensilla.

Styloconic pegs are distributed along all of the annuli in both sexes. These pegs are approximately 3  $\mu\text{m}$  in diameter and 13.5  $\mu\text{m}$  long. They are most abundant on the six distal annuli (> 700 per annulus); fewer styloconic pegs are located on each of the three more proximal annuli, with < 10 on the most proximal annulus. Although relatively abundant, these pegs are partially covered by the larger and longer sculptured setae that are distributed along the flagellum. Pores have not been elucidated in the styloconic pegs, but preliminary histological work suggests the existence of pore canals. These structures are similar to chemosensilla that have been described in other insect species.

Our research indicates that it is likely that the tip of the most distal antennal segment of both male and female ALB is used for contact chemoreception. In addition, the most distal six segments of the antennae (and to a lesser extent, all of the flagellum) of both sexes may be used for chemoreception of volatile compounds. Work is in progress to further define the ultrastructure of the putative sensilla and determine whether their structure is indicative of chemoreception.

OLFACTORY RESPONSES OF ANTENNAL SENSE ORGANS OF THE FEMALE  
SPHINX MOTH (*MANDUCA SEXTA*) TO HOST-ASSOCIATED PLANT ODORANTS

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ABSTRACT

The flagellum of female *Manduca sexta* (L.) (Lepidoptera: Sphingidae) detects diverse odors (mixtures of volatile organic compounds or odorants) by means of olfactory receptor cells (ORCs) that reside in six types of cuticular sensilla. Females oviposit preferentially on various plants in the family Solanaceae (Yamamoto et al. 1969) and use olfactory cues present in odor emitted by those plants for selection of an oviposition site. The antennal flagellum of females is about 2 cm long, comprises  $\geq 80$  subsegments or annuli, and is sexually dimorphic (Sanes and Hildebrand 1976). Each annulus may bear 2,100-2,200 sensilla (Lee and Strausfeld 1990, Shields and Hildebrand 1999b). Each antenna has approximately  $3.0 \times 10^5$ - $3.4 \times 10^5$  ORCs (Oland and Tolbert 1988) and is associated with about  $10^5$  sensilla (Sanes and Hildebrand 1976; Keil 1989; Lee and Strausfeld 1990; Shields and Hildebrand 1999a, 1999b). In female *M. sexta*, the longest trichoid sensilla (type-A) average 34  $\mu\text{m}$  in length and are innervated by two unbranched ORCs (Shields and Hildebrand 1999a). They are most abundant in a narrow band along the distal and proximal margins of the leading, dorsal, and ventral surfaces of each annulus, and are also distributed at lower density over each of these three surfaces (Shields and Hildebrand 1999a, 1999b). Extracellular electrophysiological tip-recordings were made from individual type-A trichoid sensilla. A single annulus bears about 1,100 of these sensilla (Shields and Hildebrand 1999b). We tested the responses of these sensilla to a panel of 102 volatile compounds, as well as three plant-derived odor mixtures, chosen to represent a broad range of floral and vegetative plant volatiles and two key components of the sex pheromone of female *M. sexta*. We discerned three different functional types of type-A trichoid sensilla: one subset of receptor cells exhibited an apparently narrow molecular receptive range, responding strongly to only one or two terpenoid odorants; the second subset was activated exclusively to aromatics and responded strongly to two to seven odorants; the third subset had a broad molecular receptive range and responded strongly to odorants belonging to several chemical classes. We also found receptor cells that did not respond to any of the odorants tested but were spontaneously active. Anterograde labeling of the ORCs of type-A trichoid sensilla in female antennae with rhodamine-dextran permitted tracing of their axonal projections via the antennal nerve into the ipsilateral antennal lobe (AL). The main target of these sensory-afferent axons was the pair of large, distinct, and sexually dimorphic glomeruli (large female glomeruli, LFGs) (Rössler et al. 1998, 1999; Rospars and Hildebrand 2000). In addition, a relatively small fraction of ORC axons from type-A trichoid sensilla projected to some of the 60 "ordinary," sexually isomorphic glomeruli in the AL. Based on these preliminary anatomical findings and on our evidence that the ORCs of type-A trichoid sensilla are tuned mainly to terpenoids and aromatic esters, we hypothesize that information about odorants belonging to those chemical classes is processed in the LFGs. Supported by NIH grant DC-02751.

A MUTATION IN ORF 134 OF THE LDMNPV CAUSES PRODUCTION OF  
ABNORMALLY LARGE POLYHEDRA

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ABSTRACT

During investigations of the formation of *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus (LdMNPV) few polyhedra mutants, a virus that generated polyhedra with abnormal morphology was identified and designated as isolate PFM-2. LdMNPV isolate PFM-2 generates abnormally large polyhedra that range from approximately 2-8 micrometers in diameter, either lack or possess a fragmented polyhedron envelope, and contain virions. The shape of PFM-2 polyhedra was often irregular and sometimes exhibited protrusions or holes. Isolate PFM-2 produced approximately three-fold fewer polyhedra compared to wild-type virus. A greater percentage of Ld652Y cells infected with PFM-2 contained polyhedra in comparison to cells infected with wild-type virus. Marker rescue studies localized the region containing the mutated gene in isolate PFM-2 to an area containing ORF 134. Sequence analysis of the region revealed a single nucleotide change that caused a histidine residue to be replaced by an arginine residue in ORF 134. Rescued PFM-2 virus generated wild-type polyhedra. However, a greater percentage of Ld652Y cells infected with rescued PFM-2 virus contained polyhedra compared to cells infected with wild-type virus. This result suggests that the abnormal polyhedron phenotype and the trait of an increased percentage of infected cells containing polyhedra exhibited by isolate PFM-2 are the consequence of different mutations.

# DISPERSAL POTENTIAL OF *ANOPLOPHORA GLABRIPENNIS* MOTSCH.

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## INTRODUCTION

The Asian longhorned beetle (ALB) (*Anoplophora glabripennis* Motsch.) is native to China and Korea. As a recent invader, ALB is a candidate for eradication because infestations are currently thought to be limited in size and scope. The aim of eradication is the elimination of all reproductively viable ALB from North America. Intensive survey for infested trees, followed by felling, removal, and chipping, is currently the only available method of population suppression. Effective surveys require establishment of boundaries around infestations (referred to as eradication survey and delimitation survey boundaries), inside of which surveys are conducted. Current guidelines for the eradication surveys, as per USDA Animal Plant Health Inspection Service (APHIS), are 1/2 mile from the closest known infested tree. These guidelines are based upon rate of detection of infested trees. However, delineation of boundaries should be based upon the dispersal potential of ALB, likely the most important factor for invasion by exotic species (Higgins et al. 1996). Therefore, the objective of these studies was to determine the dispersal potential of adult ALB, thereby providing a basis for the delineation of the quarantine boundaries and the concentration of survey and detection efforts. In turn, this should lower the detection threshold for incipient populations, vastly improve the operational cost:benefit ratio of APHIS's eradication program, and greatly enhance the potential for successful eradication.

Because the release of ALB is justifiably prohibited in North America, mass mark recapture (MMR) field experiments were conducted in Gansu Province, China, in order to estimate ALB dispersal characteristics. In the event that ALB becomes uncontainable in the U.S., this research, when coupled with other current investigations (i.e., colonization behavior, host preference, natural enemies), will provide estimates of ALB dispersal parameters that are applicable in other landscapes at risk (i.e., urban and forests) in North America. In so doing, therefore, this proactive approach will form the basis for development of adaptive management strategies for this and other invasive species.

## MATERIALS AND METHODS

These studies were conducted 1 km west of the town of Liu Hua, bordering the Yellow River in Gansu Province, north central China. This field site was selected because it possessed landscape characteristics similar to those of the urban infestations in the U.S., particularly site-specific factors that are thought to most likely influence dispersal distance. The general landscape is composed of both host (72.3%) and non-host (27.7%) tree species of mixed age

classes. Known ALB hosts are dominated by *Populus nigra* L. var. *thevestina* (Dode) Bean, comprising ca. 87% of the ALB hosts, followed by *Salix* sp. and *Ulmus* sp., at 9% and 4%, respectively. The study site was composed of isolated trees and trees planted along paths amid dwellings, such as homes and greenhouses, as well as trees planted as wind-rows (generally 2 m spacing within rows and 50 m spacing among rows) bordering agricultural fields. Greenhouses and small dwellings were also commonly found within or adjoining agricultural fields.

ALB used in these studies were marked and released from the center of the study areas. Of these ALB, those that had emerged from logs were released daily, while those that were collected outside the study areas were released weekly. Transects radiated from the center release site in 8 directions: north, northeast, east, southeast, south, southwest, west and northwest. Recapture locations lay along each transect at 50-, 100-, 150-, 200-, 250-, 300-, 400-, 500-, and 600-m intervals in the 1999 study, and at 100-m intervals from 100-1,000m in the 2000 study. However, landscape heterogeneity (presence of obstacles) sometimes required that recapture positions be modified accordingly. Each recapture location was composed of a fixed group of poplar trees (average of 12 trees per location). Trees at each recapture location were sampled weekly for adult *A. glabripennis* by shaking. This passive recapture method was preferred since it did not influence the dispersal behavior of ALB (as is common where pheromone traps are used to recapture insects in MMR studies). In addition to recapturing beetles along the transects mentioned above, beetles were also sampled weekly at random positions beyond the 600-m and 1,000-m radius in the 1999 and 2000 studies, respectively. Each marked ALB recaptured was preserved, and the location, release date, and body length and width recorded. In addition, marked female ALB were dissected and the number of mature eggs recorded. Unmarked adult ALB collected were recorded and released.

## RESULTS AND DISCUSSION

Distances at which marked beetles were recaptured during 1999 and 2000 are shown in Figures 1 and 2, respectively. In 1999, the average distance that ALB dispersed was 266 m, while the maximum distance was 1,450 m. Analysis showed that the 98% recapture radius was 560 m (n=188 recaptured beetles). In 2000, the average distance that ALB dispersed was 498.02 m, while the maximum distance was 2,664 m (n=401 recaptured beetles). Among these recaptured ALB, 20 (10.6% of the recaptured ALB in 1999) and 76 (19.0% of the recaptured ALB in 2000) were recaptured beyond 600 m and 1,000 m in 1999 and 2000, respectively.

Distances at which marked female ALB dispersed with eggs during 1999 and 2000 are shown in Figures 3 and 4, respectively. Surprisingly, there was no significant correlation in eggs remaining in females as distance increased. One explanation is that ALB emerge from trees with their full complement of eggs, disperse, settle, and then begin to oviposit. However, female ALB held no more than 25 eggs at recapture, but are capable of producing as many as 80 eggs (Gao, per. comm.; Smith, unpublished data). Therefore, this may indicate that female ALB develop eggs continuously or in batches. Thus, mated females may disperse great distances (1,442 m and 2,664 m in 1999 and 2000, respectively) and then deposit eggs. We suspect that the distribution in Figure 4 shows evidence for serial oogenesis.

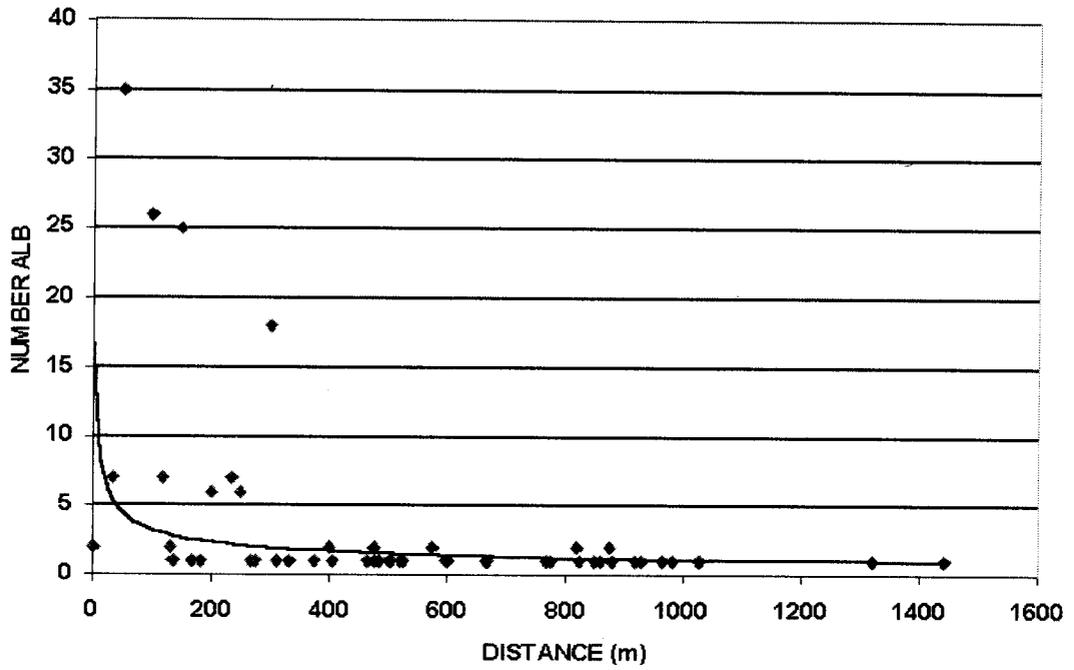


Figure 1. Dispersal distance of adult ALB (1999).

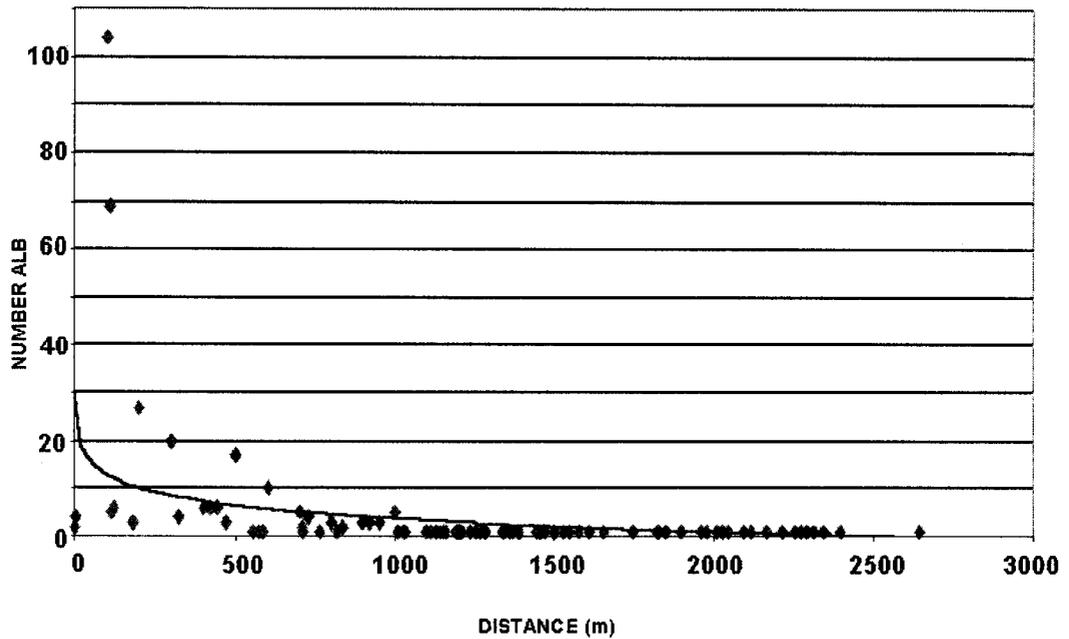


Figure 2. Dispersal distance of adult ALB (2000).

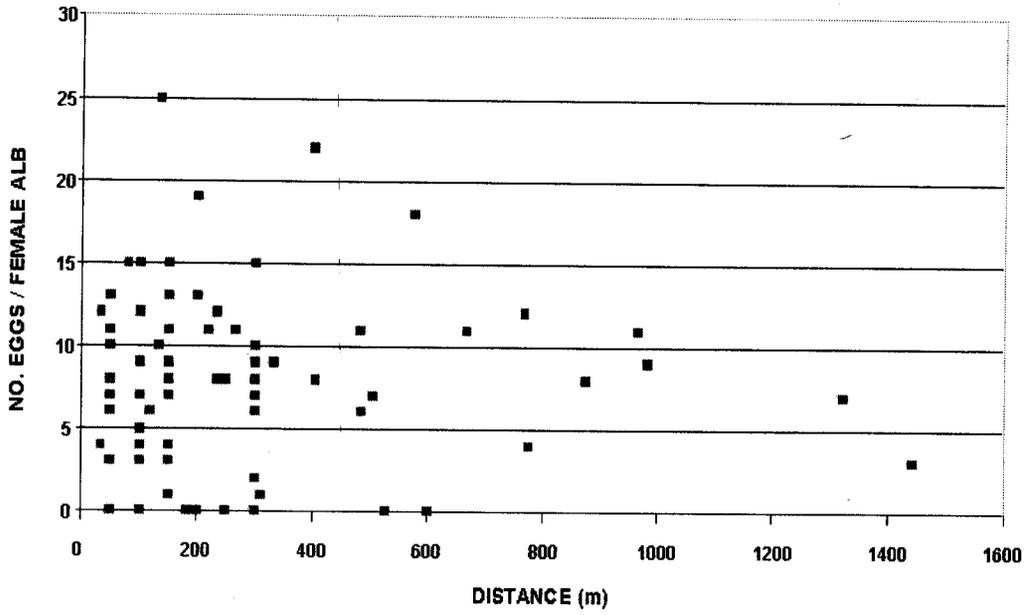


Figure 3. Distance ALB disperse eggs (1999).

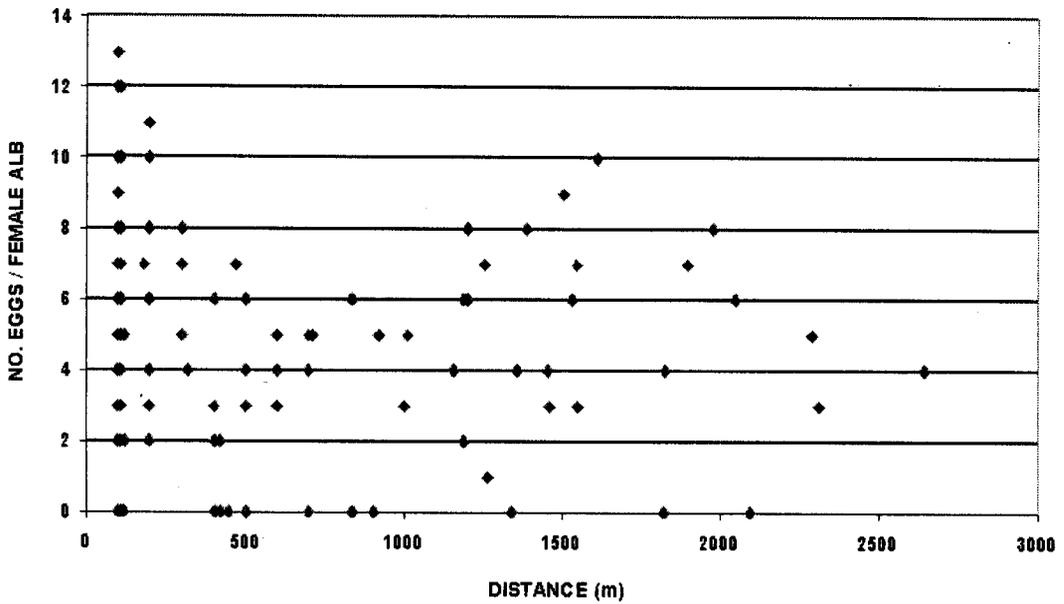


Figure 4. Distance ALB disperse eggs (2000).

Previous studies have generally reported lower ALB dispersal distances than were found in the study reported here. These differences may be based upon a variety of factors. First, recapture sampling at high frequency over an extensive area may trap out dispersing individuals (Turchin 1997). This may have contributed, in part, to the lower average dispersal distance of 106 m reported from the mark recapture study by Wen et al. (1998), in which they recaptured ALB daily or every other day. Weekly recapture sampling was used in the study reported here. Secondly, recapture sampling duration, both in terms of the entire life-span of an insect, as well as across an entire season, provides a more accurate measure of population dispersal. Wen et al. (1998) used unknown-aged ALB and extrapolated dispersal distance from the first 28 days of recapture. As many insects are known to decrease movement behavior with age, this may account, in part, for their shorter dispersal distance. Both life-time (use of newly emerged ALB) and season-long (recaptured for ca. 100 d) ALB dispersal potential were ascertained in the study reported here. Finally, landscape heterogeneity, especially variation in size and arrangement of tree species, is likely to have strong effects on ALB dispersal. This, too, may account (at least in part) for the lower ALB dispersal distance (generally within 200 m, but not more than 300 m) reported by Huang (1991), where they conducted their experiment in a homogeneous young poplar plantation (3- by 5-m tree spacing). ALB dispersal distance may tend to be relatively low in plantations where preferred host trees are proximal, but greater where preferred host trees are more widely spaced. Our field site (described above) contained heterogeneity in key features that are likely to be important to ALB dispersal. Our future studies will strengthen the understanding of host tree interaction and dispersal in response to landscape elements.

The most important implication for eradication of ALB is that the maximum dispersal distance recorded was 2,664 m (1.5 miles) by a female ALB carrying mature eggs. It must be assumed that ALB can disperse at least 2,664 m in the U.S.. Therefore, surveying or treatment of trees should extend to this distance so that incipient colonies do not prevent eradication. Current APHIS detection and survey guidelines are as follows: (1) each year, all host trees within 0.5 miles from an infested tree are inspected; (2) each year, 50% of all host trees that are between 0.5 miles to 1.5 miles from an infested host tree are inspected; and (3) over a 3-year period, 18 host trees/square mile (two host trees at each of nine inspection points, within each 1- by 1-mile grid) that are between 1.5 miles and 25 miles from each infested host tree are inspected.

The data reported here show that 89.4% and 81.0% of ALB dispersed less than 600 m (0.37 miles) and 1,000 m (0.62 miles) in 1999 and 2000, respectively, suggesting that most beetles occur close to previously infested trees. On the other hand, the data also show that 10.6% and 19.0% of ALB dispersed beyond 600 m (0.37 miles) and 1,000 m (0.62 miles), and that 0.53% and 0.25% of ALB dispersed 1,450 m (0.9 miles) and 2,664 m (1.6 miles) in 1999 and 2000, respectively. Collectively, these beetles represent long dispersers that may initiate new infestations. For eradication to be successful, ALB near previous infestations must obviously be killed. However, one must also detect and kill rare, newly founded infestations resulting from the long dispersers as well. Therefore, one of the greatest challenges facing eradication will be to effectively partition finite resources between efforts to kill all beetles in local infestations and efforts to detect and kill the more rare, distant infestations that represent foci for potential future breeding populations. Spatially explicit models being developed here at

BIIR, using data from a number of complementary studies, will provide a detailed understanding and prediction of ALB spread within landscapes at risk in the U.S..

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COMPARISON OF THE REPRODUCTIVE POTENTIAL OF  
*ANOPLOPHORA GLABRIPENNIS* (MOTSCH.) AMONG HOST TREE SPECIES

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INTRODUCTION

Potential spread of the Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) (Coleoptera: Cerambycidae: Lamiinae: Lamiini) (ALB) in the United States is dependent upon its rates of reproduction and dispersal, particularly among host tree species that it encounters within suitable climatic regions. Therefore, the goal of this study was to measure the reproductive potential of ALB on three host tree species. More specifically, investigations of the age-specific fecundity and survivorship and the intrinsic rate of increase of ALB were undertaken.

This study of the individual performance of adult female ALB, which was under optimal conditions of the abiotic and biotic environment, represents the first of the three basic steps in the research approach in nutritional ecology outlined by Price (1997). The species of host tree colonized obviously plays an important role in the reproductive success and population dynamics of ALB. Therefore, from among the tree species thus far reported attacked by ALB in the U.S., Norway maple (*Acer platanoides* L.), red maple (*Acer rubrum* L.), and black willow (*Salix nigra* Marsh.) were used. Norway maple is widely planted as an ornamental in urban landscapes, while red maple is prevalent among maple species in many northeastern U.S. forests. Willow is planted as an ornamental and is among the three most commonly attacked tree genera in China.

MATERIALS AND METHODS

**ALB-Infested Logs.** ALB-infested logs were obtained from Chicago, IL, in February of 1999 and transported to the USDA-ARS BIIR quarantine facility in Newark, DE. Both ends of the logs were sealed with melted paraffin wax and then placed into 189.2 l metal trash cans. Cans were vented and held under quarantine conditions at 22°-25°C, 50-60% RH, and a photoperiod of 16:8 (L:D) h. Newly emerged ALB were collected daily.

**Experimental Cages and Oviposition Logs.** Experimental cages were 24 cm wide, 45 cm deep, and 41 cm high with a removable plexiglass front door. Cage sides and top were screened with saran. Cages, open on the bottom, were placed atop metal trays (35 cm x 50 cm and 2 cm high) filled with fine, sterilized sand. Sand was kept moist daily and cages were held at 22°-25°C, 50-60% RH, and a photoperiod of 16:8 (L:D) h.

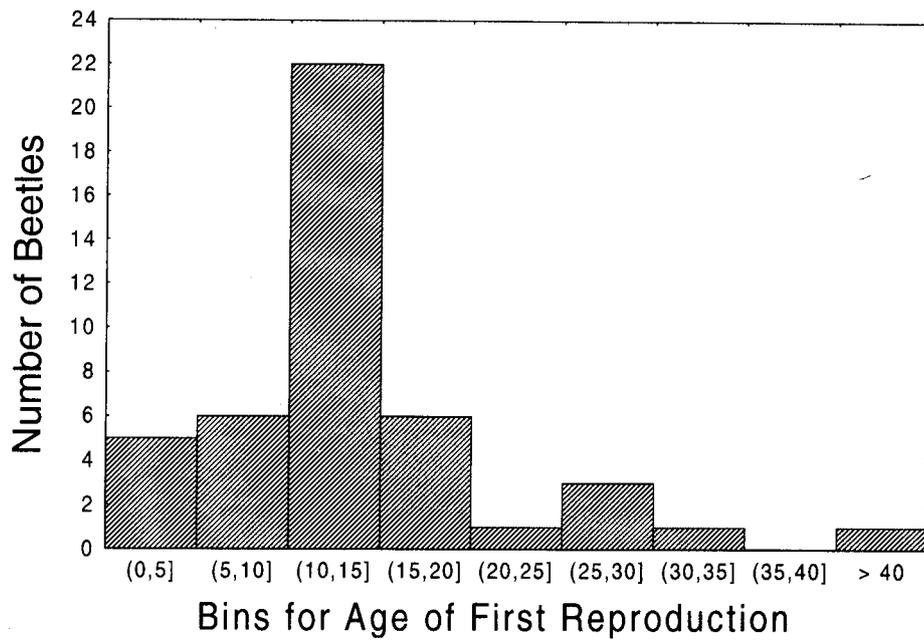
Logs of *A. platanoides*, *A. rubrum*, and *S. nigra* were cut from live, healthy trees and returned to BIIR. Tops of logs were sealed with paraffin wax and then assigned at random (unsealed end down into the moist, sterilized sand) to experimental cages. Freshly cut twigs and foliage bouquets in distilled water-filled flasks of each tree species were also placed into their respective cages in order to provide food for adult ALB and were changed daily or as needed. Newly emerged ALB (0-24 h old), obtained from the ALB-infested logs, were randomly assigned to cages (one pair per cage), and a total of 15 pairs was evaluated for each tree species. Because female ALB are normally longer lived than males, replacement males (1-3 d old) were provided so as to maintain mate availability.

**Protocol.** Scars made by adult *A. glabripennis* on the surface of oviposition logs were differentially marked and recorded daily. Oviposition logs were replaced every 7 d with freshly cut logs until death of the adult female beetle. Once replaced, the removed oviposition logs were held (with their base in moist sand and under identical environmental conditions) for 21-28 d, after which each scar was dissected and categorized as (1) nicks, (2) aborted oviposition sites (interface of inner bark and phloem with a roughly circular area which is discolored or stained and slightly sunken or depressed), (3) nonviable eggs (unhatched), and (4) viable eggs (presence of larvae and/or frass). Upon death, female body width and length were measured, and body size was calculated as a cylinder ( $\pi r^2 L$ ). Length and circumference of each oviposition log was also measured in order to calculate log surface area.

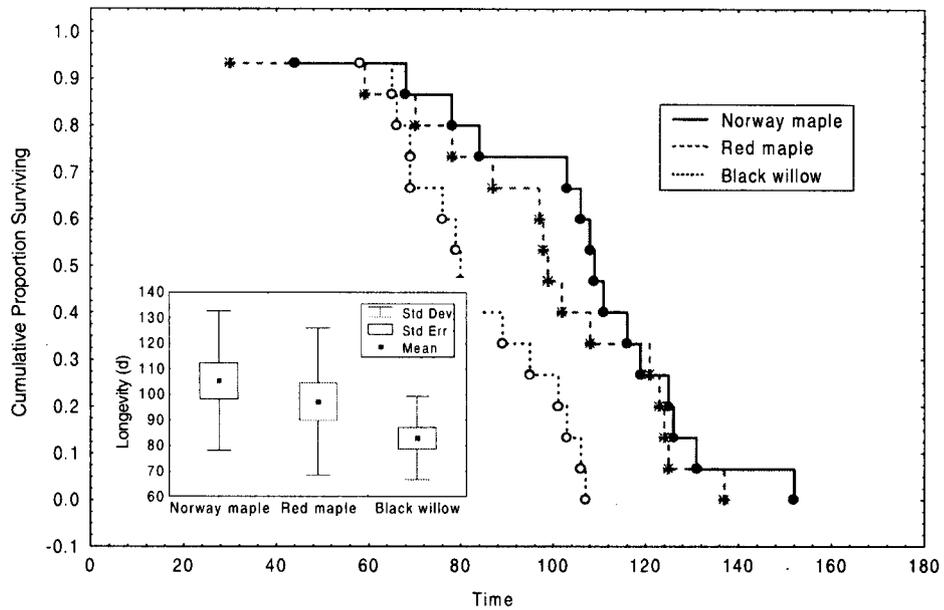
The data were used to test whether reproduction or mortality varied among ALB provided the three tree species. Analysis of variance (ANOVA) was used to test for an effect of tree species. Means of oviposition sites produced by ALB on each of the three tree species were then used to normalize the data and compared using Tukey's HSD test. A general linearized model was used to test for effects of log area, beetle size, and beetle age on female oviposition site production. A Kaplan-Meier analysis was performed to test for effects of tree species on survival. Finally, a life table was calculated with age-specific survival ( $l_x$ ) and age-specific egg viability ( $m_x$ ) of females. Because rearing techniques have not been fully developed for this univoltine species, the number of viable eggs were used as a proxy for reproductive success. The net reproductive rate ( $R_0$ ) and the intrinsic rate of increase ( $r$ ) were estimated for ALB on each of the three host tree species.

## RESULTS

Data analysis of the daily fecundity of *A. glabripennis* showed that *A. glabripennis* performed differently among three host tree species. Preovipositional period (Fig. 1) averaged 10.6 d, 16.7 d, and 15.8 d on Norway maple, red maple, and black willow, respectively. Collectively, however, preovipositional period was generally between 10 to 15 days of age. Longevity of adults averaged 103.9 d (44-131 d), 97.2 d (30-137 d), and 83.0 d (58-107 d) on Norway maple, red maple, and black willow, respectively (Fig 2). Daily and lifetime oviposition were significantly higher on Norway maple (1.80eggs/day; 193.3 eggs/lifetime) than on red maple (0.99eggs/day; 98.5 eggs/lifetime), which was in turn significantly higher than that on black willow (0.54eggs/day; 45.9 eggs/lifetime) (Figs. 3 and 4). Approximately 90.3% of all oviposition sites contained an egg. Oviposition rate was negatively correlated with age (Fig. 5).



**Figure 1. Preovipositional period.**



**Figure 2. Survival of ALB.**

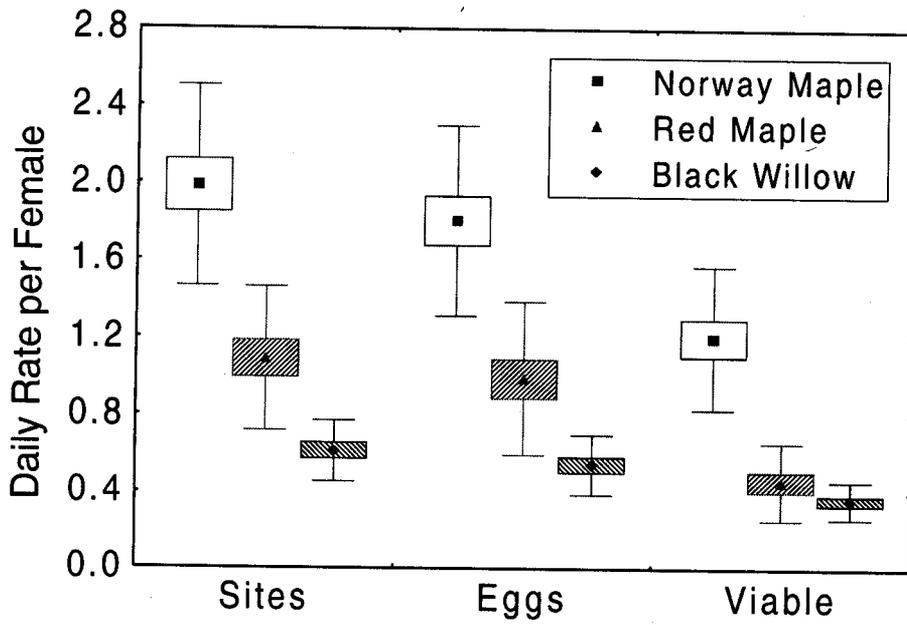


Figure 3. Daily reproductive rate.

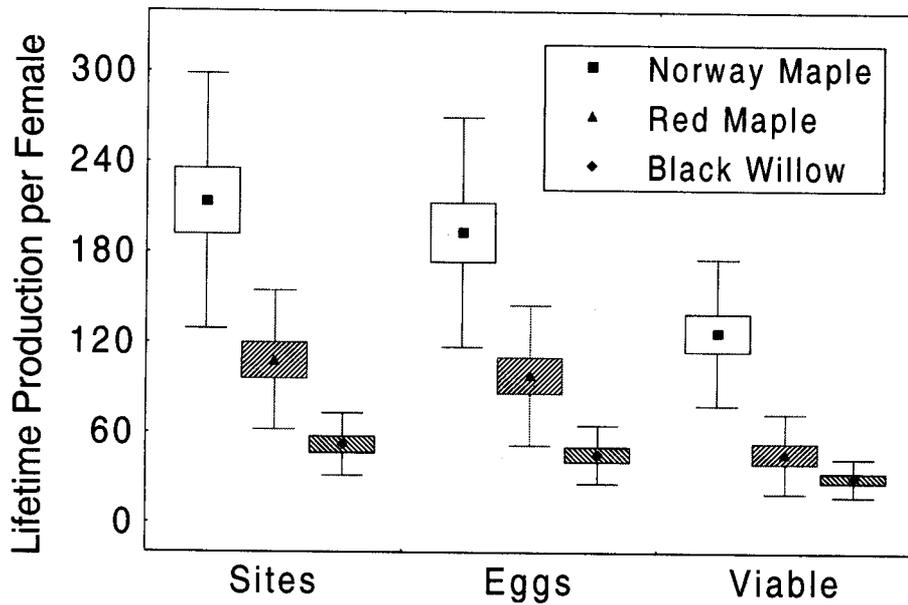
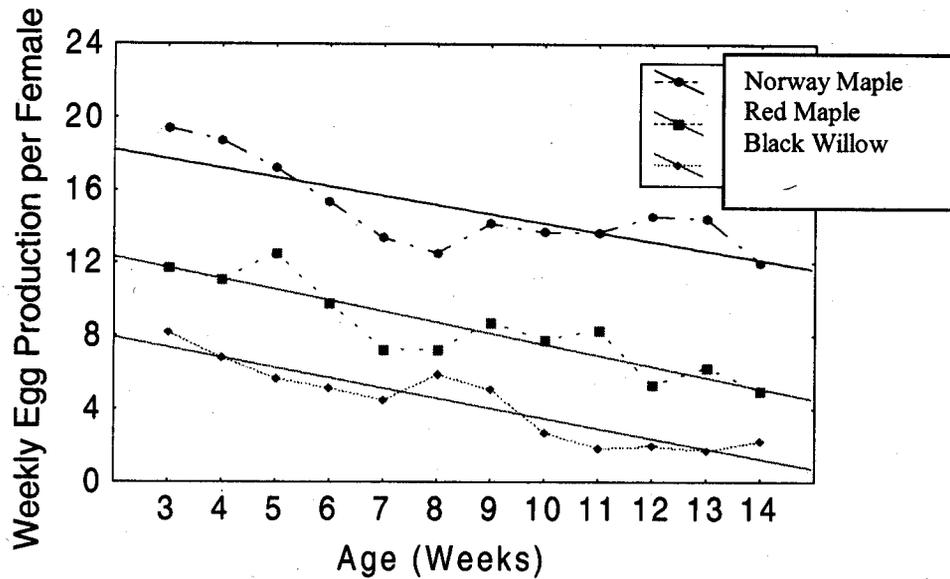


Figure 4. Lifetime reproductive rate.



**Figure 5. Age-specific fecundity.**

Percent egg viability was 60.4% on Norway maple, 60.5% on black willow, and 42.5% on red maple, which translates into an average lifetime production of 127.3, 46.8, and 30.7 viable eggs on Norway maple, red maple and black willow, respectively. The annual intrinsic rate of increase on Norway maple, red maple, and black willow was 4.1, 3.1, and 2.7, respectively. These likely over estimate intrinsic rate of increase since larval, pupal, and adult mortality are not included. However, these results show that, in terms of adult ALB survival and reproductive capacity, the maples were more suitable than willow, with Norway maple somewhat more suitable than red maple. We hypothesize that woody-tissue characteristics (i.e., nutritional substances, secondary substances, structural features) caused the observed differences in *A. glabripennis* survival and reproduction.

## DISCUSSION

The differences among the three host tree species reported here represents the initial assessment of the impact of ALB after its invasion and establishment, and is among the studies suggested by Hanks (1999). This new information provides insights into the reproductive strategies of ALB, and by discriminating the potential effects of available trees on reproduction, one aspect of ALB impact on various ecosystems in the U.S. is measured. We are incorporating these data into an individual-based simulation model of ALB spread. We suggest studies of dispersal with respect to mating and food preference will further this assessment of invasion. Future studies should also include the evaluation of host suitability of various tree species in terms of development from egg to adult, with particular attention to host stress. Collectively, these studies will contribute to the development of management guidelines (eradication and otherwise) that are sensitive to insect-host interactions under various landscapes at risk in the U.S..

## ACKNOWLEDGMENTS

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# PATHOGEN-HOST AND PATHOGEN-PATHOGEN INTERACTIONS:

## MICROSPORIDIA VS. THE GYPSY MOTH

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### ABSTRACT

Physiological effects of microsporidian infection of the gypsy moth (*Lymantria dispar* (L.)) were investigated by comparing nutrient utilization values of infected and uninfected larvae. The measured values included the relative consumption rate, approximate digestibility, relative growth rate, efficiency of conversion of ingested food, and efficiency of conversion of digested food. Gypsy moth larvae were infected with *Vairimorpha* sp., a relatively virulent microsporidium that naturally occurs in Central European gypsy moth populations, and the values were measured during 24-hour time periods for 12 days. In addition, larvae were dissected during the course of the experiments to assess the progression of the disease and to compare stages of the infection and pathogen proliferation with the nutrient utilization values.

Between 4 and 7 days post inoculation, the time period during which *Vairimorpha* sp. invaded the fat body tissues and began to rapidly proliferate in the cells, infected larvae consumed significantly more food and had significantly higher approximate digestibility as well as higher levels of proteases recorded from frass (6-7 days) than uninfected control larvae. The relative growth rate and efficiency of ingested and digested food, however, were significantly lower for infected larvae. The *Vairimorpha* sp. produced the strongest effects during this early period of pathogen development but continued to affect the growth and development of the larvae until death occurred. It is possible that the pathogen is either competing for host resources, disrupting the metabolically important fat body tissues, or both, resulting in lack of weight gain and normal development of the larvae.

The interactions between three microsporidian genera, all isolated from Bulgarian gypsy moth populations, were studied by measuring and comparing the weight gain of infected gypsy moth larvae. Larvae were fed one of the following spore solutions: *Nosema* sp., *Endoreticulatus* sp., *Vairimorpha* sp., *Nosema* + *Endoreticulatus*, *Nosema* + *Vairimorpha*, or

*Endoreticulatus* + *Vairimorpha* at minimum dosages that produced 100% infection. All microsporidia and combinations of microsporidia significantly reduced weight gain in the infected larvae compared to the controls ( $p < 0.0001$ ). The *Nosema* sp. isolate significantly moderated the effects of the *Vairimorpha* sp. and the *Endoreticulatus* sp., suggesting that some antagonism occurred. Observations of the silk glands, however, suggested that *Vairimorpha* sp. out-competed *Nosema* sp. in the silk glands, the primary target tissue of the *Nosema* sp. Further studies are being conducted to determine whether introduction of more than one microsporidian species into a gypsy moth population would result in competition between the pathogens for the host.

INFECTIVITY OF RHABDITOID NEMATODES TO THE  
ASIAN LONGHORNED BEETLE

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ABSTRACT

The Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)), recently introduced to the United States, is a pest of many species of trees in the urban and woodland environment as well as a threat to the sugar maple industry (USDA Forest Service Publ. NE-INF-140-00, 1999). The boring activity of larvae in the cambium and through heartwood causes extreme stress to the host trees which, when added to pre-existing stress conditions extant in the urban environment, frequently leads to their death. Efforts are underway to develop environmentally safe, biologically based methods to control *A. glabripennis* that do not entail destruction of the entire infested tree. Rhabditoid nematodes have been used successfully as microbial insecticides in control programs for other cryptic pests. The restricted movement of *A. glabripennis* larvae and moist protected environment within their galleries suggest that nematodes, particularly searching species, may have some potential as a control method. Several studies in China found that entomopathogenic nematodes reduced the number of new *A. glabripennis* emergence holes when they were introduced into trees through existing emergence holes.

Four species of rhabditoid nematodes produced by Integrated BioControl Systems, Inc., were tested for their ability to kill and reproduce in *A. glabripennis* larvae: *Steinernema carpocapsae* (Weiser) 1955, Sal strain; *Heterorhabditis bacteriophora* Poinar 1976, Lewiston strain; *H. indica* Poinar, Karunakar and David 1992, HOM-1 strain; and *H. marelatus* Liu & Berry 1996, IN strain. The *A. glabripennis* larvae were permissive to all four nematode species; however, host mortality, and survival and reproduction of nematodes were highest for *H. marelatus* and *S. carpocapsae*. Bioassays with *H. marelatus* estimated that the lethal dosage (LD<sub>50</sub>) was approximately 19 infective juvenile nematodes for second and third instars and 347 nematodes for fourth and fifth instars. *H. marelatus* infective juveniles on moistened sponges were stapled to oviposition sites on cut logs and were able to locate and invade host larvae within 30-cm galleries.

# INFLUENCE OF MALE AND FEMALE MATING AGES ON THE PERCENTAGE OF EMBRYONATED EGGS IN GYPSY MOTH EGG MASSES

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## ABSTRACT

Gypsy moth (*Lymantria dispar* (L.)) (Lepidoptera: Lymantriidae) egg masses containing few embryonated eggs are often found in plots where disparlure was used as a mating disruption technique. The presence of embryonated eggs indicates that the females mated; however, the cause for the low embryonation rate is unknown. Field release of disparlure increases the time it takes a male to find a mate, thus a greater proportion of the matings could involve older males and females. Older males may transmit fewer sperm or fewer viable sperm to females and if the females are unable to attract other males, egg masses with few embryonated eggs could be deposited. We conducted an adult aging study to determine if matings by older adults would result in egg clusters (masses and/or spewed eggs) with low numbers of embryonated eggs.

In this study, fewer pairings resulted in embryonated eggs when one of the mating pair was aged 96 or more hours. The primary cause was the death of one or both of the adults shortly after pairing; however, one mating pair (144 hr males x 144 hr females) did survive 8 days without the female producing embryonated eggs. In pairings resulting in embryonated eggs, the oldest treatment age (192 hr males x 192 hr females) produced the fewest eggs and the lowest percent embryonation, but overall there was not a consistent reduction in the number of eggs and the percent embryonation with an increase in treatment ages. Twenty-four of 615 pairings produced egg clusters with only a few (1 to 50) embryonated eggs. Some pairings produced egg clusters containing a high number of eggs with few embryonated eggs, while other pairings produced clusters containing few eggs with either a high or low proportion of embryonated eggs. When both of the adults were aged less than 48 hours at the time of pairing, none of the pairings produced egg clusters with few embryonated eggs. However, increasing the male age ( $\geq 48$  hrs) at the time of pairing resulted in progressively younger ( $< 48$  hrs at pairing) females depositing egg clusters with low numbers of embryonated eggs. Likewise, increasing the female age ( $\geq 48$  hrs) at the time of pairing resulted in low embryonated egg clusters being produced in pairings with progressively younger ( $< 48$  hrs) males. These data suggest that the egg masses found with low numbers of embryonated eggs in mating disruption plots could have been the result of matings between adults where one of the mating pair was aged 48<sup>+</sup> hrs before pairing.

# TESTING MATING SUCCESS OF GYPSY MOTH FEMALES IN WISCONSIN

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## ABSTRACT

Mating success of the gypsy moth (*Lymantria dispar* L., Lepidoptera: Lymantriidae) is an important factor that limits the establishment and growth of isolated colonies beyond the advancing population front. Although it was analyzed in Virginia by Sharov et al. (1995), no studies of mating success have been conducted in northern regions where climate and landscapes are different. In Virginia, mating success of females was closely related to the daily male moth capture in pheromone traps. This study was planned to check if this relationship holds in northern areas and can be used to predict mating success from moth counts in traps.

Experiments were conducted in the Kettle Moraine State Forest (southeastern WI) and nearby areas from July 25 to August 6, 2000. Seven study plots were established at various distances from the advancing gypsy moth front. Each plot had 20 to 26 tethered females arranged in two lines and separated by 15 to 20 m, and two pheromone traps 100 m away from females. Females were exposed for 1 day and then fertilization was determined by dissection and analysis of spermatheca. The relationship between the proportion of fertilized females,  $F$ , and the average moth counts in a trap per day,  $M$ , was modeled using equation

$$F = 1 - \exp(-s \times M),$$

where parameter  $s$  is the relative attractiveness of a female compared with a trap. Also, we recorded the proportion of females removed by predators per day.

In Wisconsin, parameter  $s = 0.23$  (c.i. from 0.16-0.30,  $P = 0.05$ ) was estimated using non-linear regression (least square method). In Virginia, the parameter value was slightly lower ( $s = 0.15$ ) (c.i. 0.09-0.23,  $P = 0.05$ ) (Sharov et al. 1995). Analysis of variance indicates that the difference is not significant ( $F = 2.41$ ; d.f. = 1, 40;  $P = 0.13$ ). Thus, the relationship between mating success of females and moth counts in pheromone traps did not depend on geographic location.

Predation on gypsy moth females (mostly by ants) was lower in Wisconsin ( $16 \pm 3\%$  per day) ( $\pm$ SE) than in Virginia ( $52 \pm 5\%$  per day), as determined by Sharov et al. (1995). Thus, females live longer and have higher chances to be mated in Wisconsin. The high rate of population spread in Wisconsin may have resulted from an increased mating success of females which is caused by increased long-distance dispersal of males and increased longevity of females.

# TOXICITY AND EFFICACY OF IMIDACLOPRID TO

## *ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)

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### ABSTRACT

The efficiency of the insecticide imidacloprid against *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) was evaluated in both laboratory and field conditions in China. In the laboratory, adult beetles were provided with twigs or leaves of host trees treated with different concentrations of imidacloprid to evaluate the efficacy of the insecticide through oral or contact or both entries. Beetles were checked once every 24 h and the areas of twigs and leaves consumed by tested beetles were recorded immediately after the beetle was found dead. The actual level of imidacloprid in twigs and leaves was analyzed, and this level was plotted against the applied concentration of imidacloprid to determine the relationship between the two. The  $LC_{50}$  values for applied levels of imidacloprid to adult beetles for 24 h, 48 h, and 72 h was 87.4 ppm, 43.1 ppm, and 27.3 ppm, respectively. These values correspond to 5.0, 2.9, and 1.9 ppm of the actual level of imidacloprid detected in twigs. Our results indicated that mortality of adult beetles resulted not only from the oral and contact poison, but also from their refusal of feeding. In field test, adult beetles were caged with live twigs of trees treated with imidacloprid through soil injection, trunk injection, and trunk implanting. The status of adult beetles caged with live twigs of treated trees was checked every day and the level of imidacloprid in leaf, twig, and bark and xylem area of the treated trees was analyzed different days following the insecticide application. The results indicate imidacloprid caused high mortality of adult beetles, but not larvae of the beetle, although mortality of adults and larvae may differ for different application methods and timings of applications. The levels of imidacloprid in leaf, twig, and bark and xylem of treated trees in the field were comparable to the  $LC_{50}$  values detected in treated twig and leaf tests in the laboratory.

# INTEGRATING A BIOLOGICAL CONTROL PROGRAM INTO EDUCATION AND OUTREACH

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## ABSTRACT

Purple loosestrife is an exotic weed that severely threatens the natural composition of wetlands in northern Illinois. The most promising method to restore the natural state of these wetlands is using biological control to reduce weed populations to levels low enough to allow native vegetation to flourish. The purple loosestrife biological control program at the Illinois Natural History Survey (INHS) has flourished as a result of a strong partnership among state, county, and city land managers; administrators; educators; and scientists from a variety of organizations. Although we rear the insects at INHS, we train and support others in rearing their own – thereby weaning them from us. The long-term sustainability of the Survey's project relies on engaging partners to take more responsibility for the project's implementation — and enjoy the project's successes. One major benefit of this project and its partnerships has been appreciation of the role of biological control by diverse partners, and strong recognition of and support for the Natural History Survey on other projects.

Biological control is, at its heart, a hands-on discipline. However, processes associated with biological control can seem arcane to the general public. We have developed and implemented curriculum materials for Illinois classrooms, using the purple loosestrife project to bring greater awareness and understanding of, and participation in, the Survey's biological control project. Using "Biodiversity in Illinois" as a starting point, we train educators about the importance of the state's native biodiversity, the value of wetlands and native wetland organisms, and how exotic, invasive species affect Illinois' wetlands. We teach them about the processes of biological control and ways to implement biological control of purple loosestrife into their classroom curricula. Educators are trained and students are engaged in the process of understanding how biological control fits into maintaining biodiversity. Students raise and release *Galerucella* beetles in partnership with the Survey's Biological Control Program, thus greatly multiplying our efforts. To date, over 200 educators have been trained and have used these materials in their classrooms, with the majority rearing their own beetles and releasing them into nearby wetlands. Even more heartening, approximately 75% of educators previously trained continue to use the materials two or three years after first participating.

# OBSERVATIONS ON *ANOPLOPHORA GLABRIPENNIS*

IN SOUTH KOREA IN SUMMER 2000

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## ABSTRACT

The Asian longhorned beetle (*Anoplophora glabripennis* (Motschulsky)), which was introduced into the United States from China about 10 years ago, continues to pose a serious potential threat to hardwood forests in North America. Because it is not known to be established, all research in the U.S. must be carried out in quarantine facilities. Thus, we chose to continue our investigations of the ecology of *A. glabripennis* in South Korea, an area where it is endemic. We carried out experiments and observations in a planting of about 40 silver maples (*Acer saccharinum* L.) in Mt. Sorak National Park in Kangwon Province during July and August of 2000. Our two primary research objectives were to assess the effectiveness of ultraviolet (UV) light in attracting adult beetles and to investigate the dispersal of adults. Those objectives address two critical needs for the eradication program in the U.S.: a method to monitor for *A. glabripennis* and an assessment of how far it may have spread since its introduction. We tested a UV light apparatus with a water trap to catch any beetles attracted to the lamps. We investigated adult dispersal using two approaches: harmonic radar and capture-mark-recapture of individual beetles. The first technique involved attaching radar-reflective antennas to individual beetles and tracking them with a portable radar detection device. The second technique involved marking individual beetles with numbers in sequence as they were encountered and noting their subsequent movements. Because silver maple is exotic to Korea, an additional objective this year was to try to identify native host tree species of *A. glabripennis*. Doing so would help to resolve the question of whether the beetle is native or introduced in South Korea and provide a basis for beginning to investigate its ecology in natural forest stands. Investigating the ecology of *A. glabripennis* in healthy, closed forest stands in South Korea may provide insights as to how big a problem the beetle may pose should it become established in similar habitats in the U.S..

Experiments carried out in quarantine at the USDA ARS Beneficial Insects Introduction Research Laboratory in Newark, DE, in 1999 suggested that adult beetles move toward sources of UV light. Following up on this result, we developed a UV light water trap for

testing in South Korea. The trap consisted of two 4-Watt UV lamps powered by rechargeable batteries and mounted over a pan containing water and a few drops of liquid detergent. Our hypothesis was that beetles would be attracted from the host tree toward the UV light source and drown in the pan of water. Each of our two experimental replicates included an unlighted pan as a control. Trials were carried out for about four hours per night (~2230-0230 hr) on nine nights without rain. Although the UV light water traps caught considerable numbers of Lepidoptera and other insects and arthropods, they failed to trap any *A. glabripennis* under trees in which beetles were known to be present. We concluded that either beetles were not attracted to the UV light sources or the traps failed to capture them if they were attracted.

Tracking *A. glabripennis* with harmonic radar involved attaching small radar-reflective antennas to individual beetles, releasing them, and then relocating them periodically using a portable radar device. Two new types of harmonic radar antennas were tested. Both were light but relatively strong and did not seem to hinder flight. An improved technique for attaching the antennas was developed that kept them on beetles for up to a week. Antennas were tied transversely across the dorsal side of the pronotum. However, the beetles were very rough on antennas, and those that remained attached tended to become twisted or broken and, thus, had a short detection range. Our biggest limitation in this work was the low beetle population, which constrained tests to just six individuals. Beetles were found up to a week after release, and none of the beetles moved beyond trees very near those on which they were released initially. The harmonic radar system needs definitive testing where beetles are plentiful, preferably in China.

At the start of the study on dispersal using a capture-mark-recapture approach, all silver maple trees in the study area were numbered. Beetles were painted sequentially with red numbers as they were encountered. The beetle population was censused twice daily over the course of three weeks and tree locations of numbered individuals were recorded. The advantage of this approach over other capture-mark-recapture methods is that it permits monitoring of the movement of individual beetles. Of the 29 beetles marked during the first week of the study, 12 were recaptured many times up to 14 to 17 days after their initial capture. All but two of them remained on the same tree or on neighboring trees (within about 10 meters) over the course of the observations. The exceptions, a female and a male, moved to trees about 100 m across an open space from the trees on which they were marked. Of the same group of 29 beetles, five were marked but never recaptured and five were recaptured only once during three weeks. It is not known whether they left the observation area or died unobserved. We conclude tentatively that *A. glabripennis* individuals did not move much within our study area. However, the 10 beetles that were recaptured only once or not at all may have been dispersers that left the area. Our inability to assess the fates of those individuals is a clear limitation of this capture-mark-recapture approach in assessing dispersal.

In an effort to identify native host trees of *A. glabripennis*, we searched forest stands around the study area intensively. Beetles were found attacking nearby *Acer mono* Maximowicz, a common tree species in the surrounding natural forest. Subsequently, we found beetles on *A. mono* at another location about 20 km north of our study area. The geographical range of *A.*

*mono* extends from South Korea to Manchuria in the northeastern corner of China. After learning that *A. mono* was a host, we traveled to Mt. Chiri National Park, which is near the southern coast of South Korea, to explore a new area for *A. glabripennis*. The common maple in that park was *Acer truncatum* Bunge, a species closely related to *A. mono*. We examined many trees that were tapped for sap production, but were unable to find any beetles or obvious evidence of their activity. Later, we found *A. glabripennis* attacking *A. truncatum* at Mt. Songni National Park in the center of the country. Based on our observations, we speculate that *A. glabripennis* is a specialist on native Asian *Acer* species in South Korea and is itself a native species. *Anoplophora glabripennis* appears to be a rather rare species in its natural habitats, leading us to speculate that its populations are under some form of natural control and that stands of healthy native host trees are relatively resistant to it. Apparent differences in susceptibility of the native host species, *A. mono*, and the exotic host species, *A. saccharinum*, which was heavily attacked by *A. glabripennis* at our study site, may result from intrinsic physiological or biochemical differences between them and from water stress on the latter trees, which were planted around a parking lot.

INVESTIGATIONS OF NATURAL ENEMIES FOR BIOCONTROL  
OF *ANOPLOPHORA GLABRIPENNIS* (MOTSCH.)

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ABSTRACT

The Asian longhorned beetle (ALB) (*Anoplophora glabripennis* Motsch.) is a recent invader to the U.S. from China, with known infestations in New York (New York City and Long Island) and Illinois (Chicago). Although ALB is currently limited in distribution within the U.S., its potential for spread into other North American landscapes at risk is alarming and demands greater attention. The only method used to control ALB in China and the U.S. at present is through the removal of infested trees, and the current emphasis of much research is directed towards eradication. However, in the event that eradication is not successful, either in the known infestations in New York and Chicago, or in as yet undetected infestations elsewhere, alternative pest management approaches must be developed. In addition, even with complete eradication, new introductions are likely to occur as a result of the challenges of interception of infested cargo. For example, current interception efforts are focused on cargo that enters the U.S. directly from China, while cargo from other countries, which in fact originated in China, is extremely difficult to track and intercept. Collectively, therefore, survey, evaluation, and mass rearing of natural enemies of ALB in China, as well as similar investigations of natural enemies of related cerambycids in the U.S., have been initiated. The objectives of this research include the identification of highly effective and host-specific, self-propagating natural enemies of ALB that possess a high potential for establishment (classical biocontrol, which tends to be the most cost effective approach for biological control), as well as those natural enemies that could be easily reared and utilized in inundative-release programs. In addition, since a long-term management goal may more realistically be to slow the ecological damage of this invader, native natural enemies (to the U.S.) that adapt to ALB and/or its host trees may be of particular interest.

Compared with other longhorned beetles, relatively few natural enemies of ALB have thus far been identified. Prior to the initiation of these studies, no egg parasitoids of ALB had been reported. On the other hand, larval parasitoids had been reported, including *Dastarcus longulus* Sharp (Coleoptera: Colydiidae), *Scleroderma guani* Xiao et Wu (Hymenoptera: Bethylinidae), *Bullaea* sp (Diptera: Tachinidae), and *Megarhyssa* sp. (Hymenoptera: Ichneumonidae). Likewise, pupal parasitoids had also been reported, including *D. longulus*, *S. guani*, and *Aprostocetus* sp. (Hymenoptera: Eulophidae). Among these, *D. longulus* and *S.*

*guani* appeared to be the most important among these natural enemies of ALB since they were reported to be larval-pupal parasitoids.

In many areas, *D. longulus* has been reported to have parasitization rates of 50-70%. Female *D. longulus* lay eggs in frass and sawdust in a host gallery or on the host gallery wall. First instar larvae possess thoracic legs and crawl about in search of a host. Upon finding an acceptable host, the larvae lose their thoracic legs and attach to the body of its host for feeding. It is an ectoparasite, feeding singly or gregariously on its host (1-27 individuals per host), but in all cases the host is killed. *D. longulus* is considered to have the highest potential for use in biological control of ALB.

*S. guani* usually parasitizes longhorned beetle species whose larvae are small, ca. 15 mm in length. It is an idiobiont ectoparasitoid. Female wasps first paralyze their host by stinging, which immobilizes the host, and then lay eggs on the host body. Larvae are gregarious while developing on their host. After hosts are consumed, mature wasp larvae spin cocoons and pupate. Parental wasps remain with their young until they have completed their development and emerged as adult wasps. Should their eggs or larvae become separated from the host, parental wasps have been observed to return them to the host. Most female wasps are apterous. *S. guani* can be mass reared for biocontrol. Therefore, *S. guani* has great potential for use in the biological control of ALB larvae, specifically 1<sup>st</sup> to 3<sup>rd</sup> instars.

#### 1999

Surveys for natural enemies were conducted in Shaanxi, Shanxi, Hebei, Xinjiang, NeiMongol (Inner Mongolia), Heilongjiang, and Shandong Provinces. As such, while over 560 ALB eggs were collected, no ALB egg parasitoids were recovered. However, four ALB larval parasitoids were found, including: *D. longulus*, *S. guani*, *Zombrus sjostedti* (Fahringer)(Hymenoptera: Braconidae), and *Megarhyssa* sp..

Initial studies of *D. longulus* resulted in parasitization rates of 25-95% in Shaanxi Province. Furthermore, these studies showed that 1-18 individual *D. longulus* completed development on a single host larva and resulted in 100% ALB larval mortality. Preliminary studies of *S. guani* showed that it could parasitize both ALB larva (3<sup>rd</sup> and 4<sup>th</sup> instar) and pupa. Preliminary studies of the biology and behavior of *Aprostocetus prolixus* LaSalle et Huang (Hymenoptera: Chalcidoidea: Eulophidae, Tetrastichinae), an egg parasitoid of *Apriona germari* (Hope)(Coleoptera: Cerambycidae), indicated that it may have potential as an ALB egg parasitoid, and thus studies were planned for 2000.

#### 2000

A total of 1,256 ALB eggs was collected in Shaanxi, Hebei, and Ningxia Provinces, but no egg parasitoids were again identified. However, an egg parasitoid of *Batocera horsfieldi* (Hope), another important longhorned beetle pest of popular in China, was collected and appears to be a new species. Description of this species is in progress.

Studies of *D. longulus* were continued and showed that it overwinters as an adult in the crevice of old bark as well as in the soil near ALB-infected trees. A total of 650 overwintering adults were collected during this survey. Results indicated that its life span

may exceed 5 months and that it can be reared with artificial diet in 30 days. Finally, indications are that *D. longulus* population levels are lower in monocultural stands than in species rich stands. This corresponds with higher ALB population levels in monocultural stands than in species rich stands. Although studies are still in progress, results indicate that *D. longulus* may be selected as an effective biological control agent of mature ALB larvae.

Studies of the larval parasitoid, *S. guani*, resulted in the identification of an excellent substitute host for mass rearing *S. guani*. The substitute host is inexpensive and easily obtained. In addition, lab and fields experiments were conducted, and results confirmed that *S. guani* can control young ALB larvae. A parasitization rate of approximately 65% was obtained in lab studies, and field studies are still in progress.

Studies of *A. prolixus*, an egg parasitoid of *A. germari*, indicated that this parasitoid does not diapause. While control temperature experiments showed that *A. prolixus* emergence could be adjusted to coincide with ALB oviposition and that the wasp could parasitize 20-50% of *A. germari* eggs, it did not parasitize ALB eggs. Additional studies are planned.

#### SUMMARY

BIIR is currently the only U.S. lab examining insect parasitoids, and several promising ALB-specific biological control agents have already been identified. The research on rearing natural enemies is an important weapon for pest management and current results are encouraging. Collectively, these studies should contribute greatly to the development of an Integrated Pest Management Program for ALB in the U.S..

## PROGRESS ON ALB SEMIOCHEMISTRY

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### ABSTRACT

We isolated, identified, and synthesized two male-specific compounds from the Asian longhorned beetle (ALB). July 1999 field tests in China failed to demonstrate attraction of flying beetles to these compounds, with or without a mixture of six host volatiles. However, Y-tube olfactometer tests conducted during the 2000 season showed that the synthetic alkyl ethers are significantly attractive to walking ALB females and males. A patent has been granted (<http://ott.ars.usda.gov/inv/A347907.htm>) for the use of these heretofore unknown compounds (4-(*n*-heptyloxy)butanal and 4-(*n*-heptyloxy)-1-butanol) to assist in trapping the beetles. Negotiations are ongoing with potential licensees/CRADA partners to develop the technology. Traps are being designed to catch beetles walking on host trees; these new traps, baited with the male-specific dialkyl ethers, will be tested in China by CAIBL scientists during the 2001 season. In addition, past laboratory and field observations indicated that ALB males are territorial and that males recognize females upon antennal contact. Therefore, we plan to chemically identify the ALB female contact recognition pheromone since, for example, these chemicals may also be useful in inducing beetles to enter traps.

**USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species  
January 16-19, 2001  
Annapolis, Maryland**

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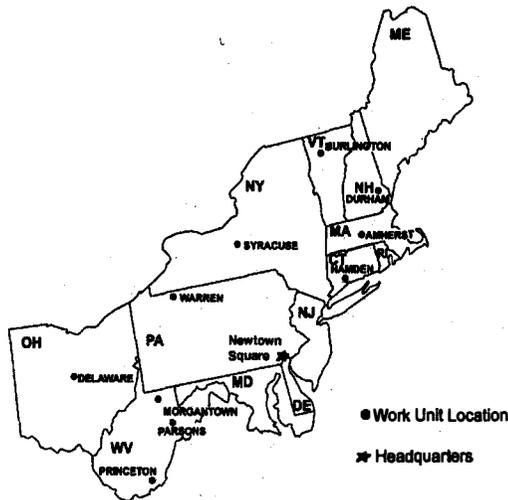
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