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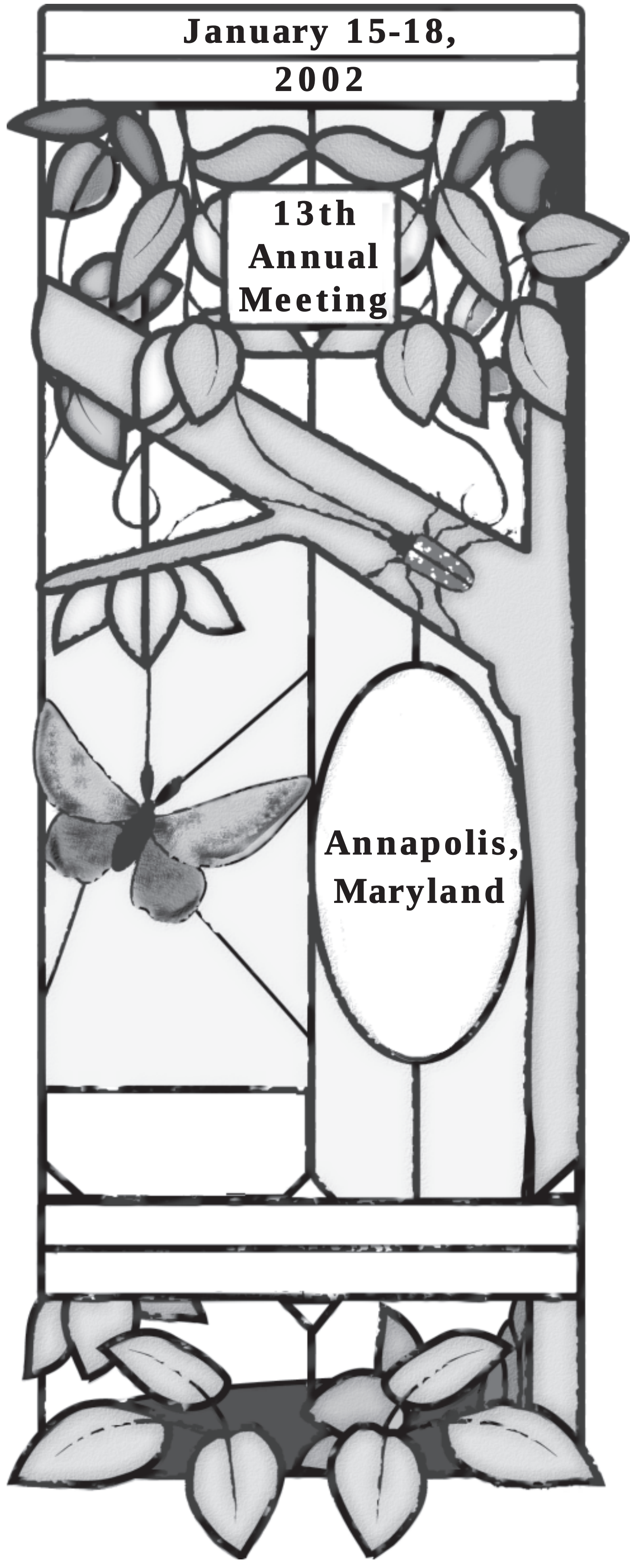

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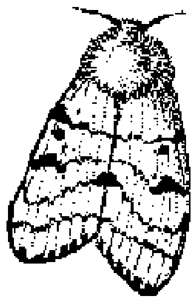
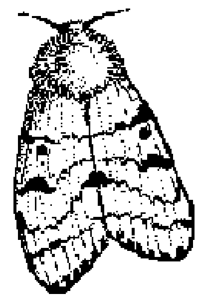


January 15-18, 2002
Loews Annapolis Hotel
Annapolis, Maryland



Edited by
Sandra L. C. Fosbroke and Kurt W. Gottschalk

Sponsored by:

Forest Service Research

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USDA

Foreword

This meeting was the thirteenth in a series of annual USDA Interagency Gypsy Moth Research Forums that are sponsored by the USDA Gypsy Moth Research and Development Coordinating Group. The title of this year's forum reflects the inclusion of other invasive species in addition to gypsy moth. The Committee's original goal of fostering communication and an overview of ongoing research has been continued and accomplished in this meeting.

The proceedings document the efforts of many individuals: those who made the meeting possible, those who made presentations, and those who compiled and edited the proceedings. But more than that, the proceedings illustrate the depth and breadth of studies being supported by the agencies and it is satisfying, indeed, that all of this can be accomplished in a cooperative spirit.

USDA Gypsy Moth Research and Development Coordinating Group:

Kevin Hackett, Agricultural Research Service (ARS)

Vic Mastro, Animal and Plant Health Inspection Service (APHIS)

Bob Nowierski, Cooperative State Research, Education and Extension Service (CSREES)

Robert Bridges, Forest Service-Research (FS-R), Chairperson

The program committee would like to thank the Heron Group, LLC, USDA-APHIS National Biological Control Institute (NBCI), and Valent BioSciences Corporation for their support of this meeting.

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Asian Longhorned Beetle in Austria: critical comments on phytosanitary measures and regulations
Hannes Krehan, Federal Forest Research Center, Vienna, Austria

Response of the brown spruce longhorn beetle, *Tetropium fuscum* (Fabr.) to host volatiles
Jon Sweeney, Natural Resources Canada, CFS

Hitchhikers with the invasive *Tetropium fuscum* (Fabr.) in Atlantic Canada
Karin Jacobs, Agriculture and Agrifood Canada, Ottawa; Ken Harrison, Natural Resources Canada, CFS

Wednesday Afternoon, January 16

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GENERAL SESSION .... Moderator: Kathleen Shields, USDA-FS
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Research Reports

Presenters: Robert Fusco, Valent Bioscience Corporation, Mifflintown, PA; Ghislain Rousseau, Society for Control of Forest Insects and Diseases, Quebec, Canada; David Lance, USDA-APHIS; Matteo Maspero, Scuola di Minoprio, Italy; Lawrence Hanks, University of Illinois

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GENERAL SESSION .... Moderator: Kathleen Shields, USDA-FS
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Presenters: Kier Klepzig, USDA-FS; Anne-Sophie Roy, European and Mediterranean Plant Protection Organization, Paris, France; Robert Fusco, Valent Biosciences Corporation, Mifflintown, PA; Hugh Evans, Forestry Research, Farnham, Surrey, UK

POSTER SESSION III

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Asian Longhorned Beetle

Presenters: Mike Stefan and Chris Markham, USDA-APHIS; Michael Smith, USDA-ARS; Ann Hajek, Cornell University; Baode Wang, USDA-APHIS; Melody Keena, USDA-FS; Kelli Hoover, Pennsylvania State University; Stephen Teale, State University of New York; Alan Sawyer, USDA-APHIS; David Nowak, USDA-FS

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Sudden Oak Death in California

David M. Rizzo

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Abstract

A new canker disease of *Lithocarpus densiflorus*, *Quercus agrifolia*, *Q. kellogii*, and *Q. parvula* var. *shrevei* in California has been shown to be caused by *Phytophthora ramorum*. The pathogen is a recently described species that was previously known only from Germany and The Netherlands on *Rhododendron* and *Viburnum*. This disease has reached epidemic proportions in mixed evergreen and redwood forests over an area approximately 300 km long along the central coast of California. The most consistent and diagnostic symptoms on larger trees are the cankers that develop before foliage symptoms become evident. Cankers have brown or black discolored bark, seep dark red sap and occur on the trunk at the root crown up to 20 m above the ground. Cankers do not enlarge below the soil line into the roots. Cankers can be over 2 m in length and are delimited by thin black zone lines in the inner bark. Foliage on affected trees often turns from a healthy green color to brown over a period of several weeks. In *L. densiflorus* saplings, *P. ramorum* was isolated from branches as small as 5 mm diameter. Lithocarpus densiflorus and *Q. agrifolia* inoculated with *P. ramorum* in the field and greenhouse developed symptoms similar to those of natural infections. The pathogen was re-isolated from inoculated plants, thereby confirming pathogenicity. The host range in California has been greatly expanded and now includes *Rhododendron* spp., madrone (*Arbutus menziesii*), huckleberry (*Vaccinium ovatum*), manzanita (*Arctostaphylos* spp.), California bay laurel (*Umbellularia californica*), buckeye (*Aesculus californica*), bigleaf maple (*Acer macrophyllum*), toyon (*Heteromeles arbutifolia*), coffeeberry (*Rhamnus californica*) and honeysuckle (*Lonicera hispidula*). On these hosts, *P. ramorum* causes a variety of foliar and branch symptoms. Significant dieback and plant death has been recorded on the ericaceous hosts (e.g., rhododendron, madrone). On hosts such as bay laurel and buckeye, only leaf symptoms have been noted. However, disease progression is poorly understood on most non-oak species.

In some mixed-evergreen forests nearly all woody plants can serve as host for *P. ramorum*. For example, at China Camp State Park in Marin County, the overstory consists of coast live oak, black oak, bay laurel, and madrone. Toyon is the primary understory shrub species. Buckeye and manzanita are found at the margins of closed canopy. Valley oak (in riparian areas and at the edge of the closed canopy) appears to be the only woody plant species unaffected by *P. ramorum* at this location. There are many other plant species that co-occur with oaks and tanoak that have not been tested for susceptibility to *P. ramorum*. This includes additional species in the Ericaceae (e.g., salal). As the geographic range of *P. ramorum* changes, it will potentially encounter other hosts that are not found in forests in the current range.

The most encouraging result from our inoculation studies was the finding that *P. ramorum* did not cause lesions on the white oaks, valley oak (*Q. lobata*) and blue oak (*Q. douglasii*) that differed significantly from the control wounds. However, the pathogen did survive in the discolored tissue for at least 6 weeks following inoculation. It is unknown if the pathogen can continue to survive for long periods in white oak tissue and eventually cause mortality of plants. To date, no species in the white oak group has been diagnosed with the disease in the field.

We have conducted a preliminary study comparing the susceptibility of California oaks and eastern North American oak species to *P. ramorum*. Challenging seedlings of northern red oak (*Quercus rubra*) and pin oak (*Quercus palustris*) with *P. ramorum* resulted in longer lesions in the bark than those that developed in the coast live oak and California black oak. Lesions in northern red oak were nearly twice as long as those observed in coast live oak and about the same length as those observed in tanoak. Tanoak is considered to be the most susceptible California tree species to *P. ramorum*. Extrapolation of results from seedling experiments to the potential effects on mature trees must be done cautiously. However, because lesion sizes in red oak and pin oak seedlings were much larger than in coast live oak seedlings (a species in which the adults are very susceptible), we suggest that it is likely that mature trees of northern red oak and pin oak will be susceptible to infection by *P. ramorum*. Therefore, efforts to prevent spread of *P. ramorum* to eastern North American forests are critical.

An important step in controlling *P. ramorum* involves understanding how it is spread. The presence of diseased oaks at all elevations on hillsides and the above-ground nature of the disease suggest wind-blown rain or rain splash as a common mechanism for movement of spores. Although viable spores have yet to be found on infected oak tissue, other hosts may serve as sources of rain-dispersed inoculum. In the laboratory, abundant sporangia form on moistened leaves of infected bay laurel and rhododendron within 72 hours. These sporangia break off and easily disperse in water. Chlamydospores are also observed on the surface of moistened bay leaves. Consistent with these results, *P. ramorum* has been recovered from rain, soil, litter and stream water from woodlands with infected oak and bay trees. Spores of *P. ramorum* do not survive drying, but in moist conditions can survive for at least one month.

The broad host range of *P. ramorum* that we have discovered in California forests suggest that this pathogen has the potential to cause similar, long-term landscape level changes in these forests. Loss of oaks and other overstory trees and shrubs will have cascading effects on these ecosystems including increased fire hazards, soil erosion, and loss of habitat for wildlife. Developing restoration plans for these ecosystems will represent a significant future challenge for forest managers. An understanding of the host range and spatial distribution of the pathogen is pivotal in formulating hypotheses for further epidemiological research, developing monitoring strategies and management guidelines that may either prevent further spread of the disease or ameliorate disease conditions where the disease may be only recently present.

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Evolution of Invading Forest Pathogens via Interspecific Hybridization

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Abstract

Traditional morphologically-based fungal species concepts have tended to go hand in-hand with a perception that fungal species are genetically 'fire-walled' units between which almost no gene flow occurs. Also, prior to 1990, known examples of interspecific hybridization in fungi were very rare. However, observations on the internationally invading Dutch elm disease pathogens suggested that intense ecological disturbance events, including introductions or invasions, could result in hybridization. Since this could also lead to changes in a pathogen's aggressiveness, host range or other fitness attributes, it has considerable implications for the health of forests and natural ecosystems.

A conceptual model for hybridization between introduced and resident plant pathogens was developed (Brasier 1995). This was based upon an assumption that barriers to gene flow might be weaker, or even non existent, between fungal species that had evolved in geographic isolation; and that human influences have increase the chances of such species coming into contact. The potential for hybridization would also depend on many other factors, such as the frequency of niche contact; the precise nature of any genetic barriers to hybridization; the degree to which the two genomes can successfully recombine; and the ability of any resulting hybrids to compete with the 'parent' species. Possible outcomes range from the acquisition of a single gene by one parent to a full species hybrid incorporating the genomes of both parents (reviewed in Brasier 1995) Subsequently, at least eight examples of 'ongoing' hybridization have come to light (reviewed in Brasier 2000, 2001). Seven of these involve economically important forest pathogens.

Among these are the Dutch elm disease pathogens. *Ophiostoma novo-ulmi* — responsible for the current DED pandemic — has migrated across the northern hemisphere in the past 30-40 years. It has rapidly replaced *O. ulmi*, responsible for the first pandemic (see Brasier 2001). During this process the two species have come into close physical contact in the bark around scolytid vector breeding galleries of diseased elms. *O. ulmi* and *O. novo-ulmi* probably originated in different geographic locations in Asia (Brasier and Mehrotra 1995) and are strongly, but not totally, reproductively isolated. It is now known that rare and transient (unfit) hybrids occur between the two species when they come into contact (Brasier *et al.* 1998). This allows *O. novo-ulmi* — which initially spreads at epidemic fronts as a genetic clone — to acquire new vegetative compatibility (vc) and mating type genes from *O. ulmi. O. novo-ulmi* probably discards any 'unwanted' *O. ulmi* genes it receives (Abdelali, Brasier and Bernier 1999). As a result, the *O. novo-ulmi* clones at the epidemic fronts rapidly become highly diverse in terms of vc types and mating type. This in turn probably allows the pathogen to control the spread resist deleterious fungal viruses that might otherwise have brought about its demise.

Another example is the new *Phytophthora* killing riparian and shelterbelt alders across Europe. This comprises a swarm of heteroploid hybrids between *P. cambivora*, and a *Phytophthora* close to *P. fragariae* (Brasier, Cooke and Duncan 1999). Neither of these is an aggressive pathogen of alder whereas the hybrids, especially the commoner hybrid types, are highly aggressive and specific to alder (Brasier and Kirk 2001) Since *Phytophthora* has not previously been recorded as a pathogen of alder the hybrids may therefore have acquired the ability to exploit a new host — a possibility demonstrated earlier with laboratory generated *Phytophthora* hybrids. ITS sequences and AFLPs of genomic DNA indicate the hybrids are recently evolved and still evolving. How their evolution will proceed is uncertain. Whether they pose a comparable threat to North American alders is unknown.

An equally important example is that of the hybrid *Melampsora* tree rusts (resident *M. medusae x* 'introduced' *M. occidentalis*) that have appeared on poplar varieties clones in the Pacific Northwest that were bred for resistance to *M. medusae* (Newcombe *et al.*, 2000). The *Melampsora* hybrids can attack *Populus deltoides*, the commercial 'resistant' clones and even the local *P. trichocarpa*. Furthermore, as a result of the hybridization *M. medusae* pathogenicity genes could be transferred into the local *M. occidentalis* population.

Such examples probably represent the tip of an iceberg. Interspecific hybridization between forest pathogens is a poorly monitored process. It may be accelerating as a

result of human mediated ecological disturbances. In particular, the increasing international movement of plants and their associated pathogens that will promote hybridization; and phenomena such as host stress, ecosystem stress and access to novel hosts which will enhance the survival of hybrids. Certain fungal groups such as *Phytophthora* species, tree rusts and insect-associated ascomycete pathogens might be more likely to give rise to hybrids.

Plant health legislation is often hailed as our first line of defence. However, many hybrids or introgressants are unlikely to be detected by conventional, mainly morphologically based diagnostic methods of international quarantine. Molecularly based tools will be needed, while some of the old 'lists of scheduled organisms' will either need to be modernised or discarded. Furthermore, the issue of how to formally taxonomically define a hybrid or swarm of hybrids in a way that has legal utility for quarantine legislation or is of practical value in diagnosis has yet to be adequately addressed by mycologists. Present day fungal nomenclature offers little of value in this respect. It too may need to be modernised to allow formal definition of particular genotypes and formal description of fluid, still evolving populations that may or may not approximate to species.

Even a small genetic modification to a pathogen as a result of a hybridization event, such as the acquisition of a single gene conferring a different host specificity or a different climatic tolerance, could have a profound effect on its behaviour. This is well illustrated by the recent demonstration that, when the *O.novo-ulmi* cerato-ulmin gene is artificially transferred into the common bark saprotroph *O. quercus,* the latter becomes a potential vascular wilt pathogen (Del Sorbo *et al* 2000).

Through opportunities for hybridization, introduced pathogens have a potential impact far beyond the initial disease outbreaks that they cause. Each introduced pathogen is a dangerous, uncontrolled and open-ended experiment in evolution; and a gamble with the long-term stability of our forests and other natural ecosystems.

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Asian Longhorned Beetle in Austria: Critical Comments on Phytosanitary Measures and Regulations

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Abstract

The ALB is indigenous to East Asia (China, Korea, Taiwan, but not actually present in Japan) It has been introduced twice into North America: New York City, N.Y (1996) and Chicago, Illinois (1998). The first record for Europe was in August 2001 in Braunau, Austria where it was likely introduced through wood packing material (i.g. pallets, crates, dunnage etc.) from Asia.

The first symptoms occurred on maples along a small street in Braunau in November 2000, but the agent of the symptoms was not determined; no adult beetles were found. The first beetles were identified in July, 2001 by experts at the Institute of Forest Protection.

Present Extent of Infestation

- 50 trees infested or with symptoms of maturation feeding andor oviposition sites (mainly *Acer saccharum* but also *A. platanoides* and *A. pseudoplatanus)*.
- 90 beetles caught so far in close proximity to the infested area.

Emergency Measures

All infested trees were felled, chipped and burned by the end of August 2001. Also more than 900 young maple trees without symptoms, occurring in a small forest-like strip in proximity to trees with ALB-feeding symptoms that had been cut, were felled, inspected and chipped between August 2001 and January 2002.

Federal Regulations: (in force since September 1, 2001):

- prohibition of transport of *Anoplophora glabripennis.*
- treatment (chipping and burning) of infested trees and wood.
- stronger import regulations for wood packing material including dunnage of deciduous trees (host trees of ALB) from areas of occurrence: wood must be bark-free <u>and</u> without grubholes > 3 mm <u>and</u> kiln dried below 20% moisture content.
- transport of non infested, susceptible wood including dunnage and plants older than 2 years out of the safety zone (political district of Braunau) possible only with plant passport and inspection at the district border.
- transport of non infested, susceptible wood within the district of Braunau only under official supervision to wood manufacturers.

Further Federal Regulations: (Decree of Federal Ministry for the Monitoring of ALB):

<u>Infested zone</u> = radius of 1000m around infested areas: visual examination of every susceptible and endangered tree using either a hoisting crane or tree climber, was done in August, September and January; 40 pheromone traps were deployed but no beetles were caught between mid-September and the end of October.

<u>Safety zone</u> = political district of Braunau: regular random sample examination of susceptible trees and use of pheromone traps

<u>Federal territory</u> = Austria: intensive monitoring of susceptible trees especially around known importers of wood packed products from Asia.

Main problems on Import Inspections of Wooden Dunnage

- The very high number of consignments makes a complete inspection at points of entry (ports,...) impossible.
- In many cases, no declaration of wood-packing material is added to the imported goods, therefore it is not easy to find wood when consignments are packed in plastic foil or inside containers.
- Many organisms cannot be detected and identified at first sight: highly qualified specialists and labs are necessary to conduct identification. One of the most important recommendations for efficient phytosanitary regulations is the establishment of international standards for wood packing materials including:
- marking of boxes, crates and pallets.
- definition of appropiate treatment.
- declaration of origin (country and producer).
- scientific name of tree species utilized.
- date of productions.

Phytosanitary inspections should be allowed everywhere and always. Most efforts should be focused to known importers of "high risk goods" (e.g. stones, cast iron, electronics from Asia). Wood packing material should be destroyed (burned or chipped) under official control soon after usage and not stored or distributed among private users. Regulatory inspections (of trees) in the areas surrounding warehouses and places where crates, pallets or other wood-packing material is stored, are necessary in order to detect infested wood packing material which has not been inspected previously at entry points.

The results of phytosanitary inspections of wooden dunnage in 2001 indicate that there are many living organisms within the investigated wood originating not only from PR. China but also from the U.S. and Canada. Wood Wasps, *Monochamus clamator* from U.S., *Monochamus alternatus* from China, different species of wood feeding weevils from China, *Dryocoetini*- and *Orthotomicus*-bark beetles from both China and the U.S., and *Sinoxylon*-powder post beetles from India are the species most frequently intercepted. No Pine Wood Nematodes (*Bursaphelenchus xylophilus*) have been detected so far but several other *Bursaphelenchus* species such as *B. Mucronatu* have been recovered.

Table 1: Wooden Dunnage Inspection 2001 Austria

	Number of inspected samples	**Number of samples with symptoms**	**Living stages of insects**	**Living stages of nematodes** others than *Bursaphelenchus*	***Bursaphelenchus* nematodes** not *B. xylophilus*
PR China	364	120 (33%)			
non coniferous wood		29	2 (6.9%)	2 (6.9%)	0
coniferous wood		96	22 (22.9%)	35 (36.5%)	20 (20.8%)
USA / Canada	219	57 (26%)			
non coniferous wood		19	0	1 (5,3%)	0
coniferous wood		43	5 (11.6%)	8 (18.6%)	3 (6.9%)
Asia without PR China n.c. & coniferous wood	100	29 (29%)	3 (10%)	2 (7%)	0
Brasil n.c. & coniferous wood	8	4 (50%)	0	0	0

Response of the Brown Spruce Longhorn Beetle, *Tetropium fuscum* (Fabr.) to Host Volatiles

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Abstract

Studies were undertaken to develop an attractant and trap for survey and detection of the brown spruce longhorn beetle, *Tetropium fuscum* (Fabr.) (Coleoptera: Cerambycidae), a European beetle recently found established in Halifax, Nova Scotia. Cortical volatiles of *T. fuscum*-infested red spruce, *Picea rubens* Sarg., were sampled *in situ* in May 2001 and analyzed by gas chromatography-mass spectrometry. A synthetic "spruce blend" lure was then made up approximating the relative concentrations of the major monoterpenes found in the analysis, i.e., (+) and (-) -pinene, (-) -pinene, (+) 3-carene, (+) limonene, and -terpinolene. Field trapping bioassays were conducted to determine the attraction of the spruce blend and to compare the efficacy of the Lindgren 12-funnel trap, IPM Intercept trap, and a cross-vane pan trap, for capture of *T. fuscum.* The spruce blend was significantly attractive to *T. fuscum.* More than 60 brown spruce longhorn beetles were captured in a total of 30 spruce blend-baited traps whereas 0 beetles were captured in 30 unbaited traps; 2-3 times more males were captured than females. Mean catch was greatest in the cross-vane pan traps, followed by the Intercept traps and funnel traps but differences among trap types were not significant. A second field bioassay designed to test the effect of combining ethanol with the spruce blend was inconclusive due to low overall catch. Experiments will be repeated in 2002 to confirm or reject the trends observed in 2001, and to test ethanol as a possible synergist to spruce blend attraction.

Hitchhikers with Invasive *Tetropium fuscum* (Fabr.) (Coleoptera:Cerambycidae) in Atlantic Canada

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Abstract

Tetropium fuscum (Fabr.) (Coleoptera:Cerambycidae) is native to Europe and apart from the Halifax, Nova Scotia area, is unknown elsewhere in North America (Smith & Hurley, 2000). *Tetropium fuscum* poses no primary threat to *Picea abies* Karst. in Europe and is regarded there as a secondary insect that usually attacks severely weakened or recently dead trees. An infestation of *Tetropium fuscum* was discovered in 1998 in red spruce trees (*Picea rubens* Sarg.) in the Halifax Regional Municipality in Nova Scotia, Atlantic Canada (Smith and Hurley, 2000). The trees had very little resistance and repeated attacks by the beetles ultimately led to the death of the trees. Several species of ophiostomatoid fungi were found in association with the *T. fuscum* infestation.

Very little is known about the fungal associates of *Tetropium* spp (Mathiesen 1951; Mathiesen-Käärik 1953; Kotýnková-Sychrová, 1966; Solheim, 1996). Mathiesen (1951) described *O. tetropii* associated with *Tetropium* sp. on spruce in Europe and later (Mathiesen-Käärik, 1953) listed a few more species isolated from old larval galleries of the beetle. *Ophiostoma tetropii* has been reported from *Picea abies* in Finland (Virri, 1997), Norway (Solheim, 1986) and Czechoslovakia (Kotýnková-Sychrová, 1966).

The aim of this study was to characterize the ophiostomatoid fungi associated with *T. fuscum* in Atlantic Canada. We also compared the associates of *T. fuscum* with ophiostomatoid fungi associated with *Tetropium* species in Europe. *Ophiostoma* species associated with the indigenous, transcontinental species (*T. cinnamopterum* Kirby), which occupies the same niche in North America, were also compared to those associated with *T. fuscum* occurring in Canada.

Ophiostoma species were isolated from the stained sapwood adjacent to the beetle galleries of *Tetropium fuscum* from wood bolts collected in Halifax, Nova Scotia. Additionally, ascospores from perithecia and conidia from sporulating anamorphic structures in the pupal chambers of *T. fuscum* were aseptically transferred to 2% MEA and 2% CSMEA. Isolations were also made by allowing living *T. fuscum* adults to move around on 2% CSMEA. The resulting colonies were purified by transferring to fresh 2% MEA and incubated at 20°C and 25°C. Similar isolations were also made from ascospores and conidia obtained from sexual and asexual stages occurring in the galleries of the native *T. cinnamopterum* on red spruce and white spruce in the Point Pleasant Park area. Isolated strains were grown on 2% MEA and Oatmeal agar (OA). All measurements and microscopic observations were performed on fungal structures produced on 2% MEA and OA.

DNA extractions from pure cultures grown on commercial potato dextrose agar (PDA), were made using the UltraClean™ Microbial DNA kit (MO BIO, USA). Amplifications of the ITS1-5.8S-ITS2 (internal transcribed spacer regions) were done using standard protocols for PCR reactions using Ready-to-go (RTG) PCR-beads (Amersham Pharmacia Biotech) (Primers: NS7 and ITS4) (White *et al.,* 1990). PCR products were purified using the UltraClean™ PCR-cleanup kit (BIO/CAN Scientific) and sequenced using the Big-dye terminator cycle sequencing pre-mix kit (Applied Biosystems) on an ABI PRISM 310 automatic sequencer (Perkin Elmer Applied Biosystems) (Primers: NS7, ITS2, ITS3 and ITS4) (White *et al.,* 1990). Sequence data were edited using Sequencher (Gene Codes Corporation, Michigan, USA) and a computer-generated alignment (Wisconsin Package Version 10.1, Genetics Computer Group (GCG), Madison, Wisconson; Canadian Bioinformatics Resource, http://www.cbr.nrc.ca/) was manually adjusted in PAUP* v.4.0b8 (Phylogenetic Analysis Using Parsimony) (Swofford 1999). Phylogenetic relationships were inferred using a heuristic search in PAUP* v.4.0b8. Confidence levels were estimated using Bootstrap analysis (1000 replicates).

Species of ophiostomatoid fungi sporulated in the tunnels of *T. fuscum* and *T. cinnamopterum.* Three species of ophiostomatoid fungi were associated with *P. rubens* infested by *T. fuscum* in Atlantic Canada: *Ophiostoma tetropii, Ophiostoma piceae* and *Pesotum fragrans.* One strain of *O. floccosum* was isolated from a girdled *P. rubens* tree in Point Pleasant Park. No evidence of *T. fuscum* infestation was observed in this tree. Three strains of unidentified *Pesotum* spp. were also isolated from *P. rubens* attached by *T. fuscum.*

Ophiostoma tetropium was abundant in *P. rubens* attacked by *T. fuscum.* This species is homothallic and produces perithecia prolifically in culture, even after several subcultures. In culture *Ophiostoma tetropii* produces a *Hyalorhinocladiella* anamorph as well as a *Leptographium*-like anamorph with a short stipe. The strains isolated from in the Halifax area corresponds morphologically and phylogenetically to a strain from Europe, recognized as *O. tetropii* (CBS 428.94). This strain also has a *Hyalorhinocladiella* as well as *Leptographium* –like anamorph. Mathiesen (1951) described *O. tetropii* from Norway spruce (*Picea abies*) infested by *Tetropium* species in Europe. Unfortunately, no type material was designated and a single living strain of this species was deposited at CBS (CBS140.51=MUCL9530). The strain in CBS, however, no longer corresponds to the original description of *O. tetropii* (Mathiesen, 1951). The *"Scopularia"*-like (=*Leptographium*) state reported by Mathiesen (1951) was not observed and a distinct *Sporothrix* state characterized by obvious denticles on the apices of the conidiogenous cells was observed in culture. In addition, the ITS sequence of this strain does not correspond to the strain from Europe that has been identified as *O. tetropii.* Analysis of the data, group CBS140.51 together with other species of *Ophiostoma,* such as *O. stenoceras., O. narcissi* and *O. rostrocoronatum,* all characterized by white colonies and *Sporothrix* anamorphs with distinct denticles (Upadhyay, 1981).

Based on the evidence, we no longer consider the ex-type strain of *O. tetropii* (CBS140.51=MUCL9530) as authentic for this species and propose that the European strain of *O. tetropii* (CBS428.94) be considered representative for *O. tetropii.* In the absence of a designated type specimen, we propose that BPI 595681, deposited by Hunt as *O. tetropii,* be designated as the epitype for *O. tetropii,* while the illustration accompanying the original description serves as the lectotype. Griffin (1968) reported *O. tetropii* from *Picea mariana* in Ontario. This report could not be confirmed as the herbarium material (MFB7394) was deteriorated and overgrown with *Aspergillus* sp.

Ophiostoma tetropii *does not appear to be pathogenic in North America and is not known to be major economic importance in Europe. In the near future, we intend to initiate inoculation studies on red spruce with fungal associates of* T. fuscum *in Canada as part of a comprehensive risk assessment.*

Ophiostoma piceae was frequently isolated from both *Picea rubens* and *P. glauca*, including from trees attacked by either *T. fuscum* and the native *T. cinnamopterum.* The presence of this species comes as no surprise as it is commonly found throughout North America (Uzunovic *et al.,* 1999; Harrington *et al.,* 2001) and is considered a generalist not associated with a specific niche. The strains from Atlantic Canada were morphologically similar to descriptions in literature of this species (Upadhyay, 1981; Harrington *et al.,* 2001). Strains identified as *O. piceae,* based on their morphology, grouped together in analyses of ITS sequences with other strains of *O. piceae,* including the ex-type strain of *O. piceae* (CBS 108.21).

Pesotum fragrans was isolated a few times during the study. Mathiesen-Käärik (1953) described this species from galleries of *Ips sexdentatus* in *Pinus sylvestris* logs. This fungus is widespread and has been reported from USA, New Zealand and Australia (Harrington *et al.,* 2001). *Pesotum fragrans* does not appear to be exclusively associated with *Tetropium fuscum* or red spruce. It was also isolated from Balsam fir, ambrosia beetles and the native *Tetropium cinnamopterum.* On OA, a bright yellow pigment is produced, that appears to characteristic of this species. ITS sequences of the strains from Halifax are identical to the strain (CBS 279.54) that is considered to be authentic for this species (Harrington *et al.,* 2001). Although *P. fragrans* has not been reported from Canada before, it appears to have been present in Canada for a considerable period of time. Two of the strains (NLC 348, NLC 349) used were isolated from the sapwood of dead balsam fir trees, attacked by *Trypodendron lineatum* (Oliv.) in Newfoundland, Canada in 1982. We also examined a dried specimen from Quebec, Canada, collected in 1986. This suggests that *P. fragrans* is not a new introduction to Canada associated with *T. fuscum.*

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Chipping as a Phytosanitary Treatment For *Tetropium Fuscum*

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Abstract

A series of experiments were conducted to determine the efficacy of wood chipping on survival of the brown spruce longhorned beetle, *Tetropium fuscum*. Three experiments involved inserting known numbers of surrogate larvae (PVC-plastic, live buprestid larvae, and live dipteran larvae) into logs, chipping the logs, and then assessing damage and/or insect survival. The fourth experiment involved chipping logs known to be infested with *T. fuscum*, followed by rearing to assess adult survival. In a fifth experiment, known numbers of adult Scolytidae were inserted into logs, logs were chipped, and chips were held in emergence cages to assess adult survival. PVC larvae proved to be more durable than live larvae. Damage to plastic surrogates of 5, 10, 15 and 20 mm length was 31%, 66%, 90% and 98% respectively. No survival of the 200 buprestid larvae, 800 dipteran larvae, or *T. fuscum* larvae tested was observed. 1/2300 scolytid adults survived chipping. These results strongly suggest that chipping is an effective method of killing larvae and adult woodborers in the size range of *T. fuscum*.

Microsporidia from the Asian Longhorned Beetle in China

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Abstract

During the past two years, we isolated microsporidia from Asian longhorned beetle (ALB) larvae collected in Gansu, Hubei, Henan, Jiangxi Provinces of China. We recently completed studies on the morphology, life cycle, tissue specificity, and genetics of the microsporidian isolate from Gansu Province. This isolate infects only the epithelial cells of the ALB midgut; environmental spores are ovoid; ca. 1.8 x 3.8 µm in size; uninucleate; with 8-9 coils of the polar filament when observed in cross section. During sporogony, variable numbers of spores develop in a non-persistent sporophorous vesicle containing large crystalline inclusions; spores are excreted with the frass in host galleries. Phylogeny, based on small subunit ribosomal RNA using maximal parsimony analysis (PAUP), suggests this microsporidium is most related to the *Endoreticulatis-Eterocytozoan* taxonomic group. Spores of this microsporidium are also infective to a native cerambycid, the cottonwood borer, *Plectrodera scalator* (Fab.). Impact of these microsporidia on ALB are unknown, however, microsporidia typically increase host mortality, prolong developmental period, and reduce adult longevity and fecundity.

An Exotic Pest Threat to Eastern Hemlock: an Initiative for Management of Hemlock Woolly Adelgid

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Abstract

Hemlock woolly adelgid (HWA) is the greatest threat to the health and sustainability of hemlock in eastern North America. The potential ecological impacts of this exotic insect pest can be compared to those of gypsy moth, Dutch elm disease, and chestnut blight. The USDA Forest Service, with the support and cooperation of the National Association of State Foresters and the National Plant Board, is proposing an expanded program to develop and implement management strategies to reduce the impact and slow the spread of HWA. This 5-year initiative calls for expanded research and technology development combined with accelerated management efforts implementing existing and newly developed control techniques. This document briefly describes this initiative's goals and funding requirements.

Background

In the early 1950s, a small, aphid-like insect was first observed feeding on hemlock in Virginia. This insect was the hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), an exotic pest native to Japan and China. HWA has since spread to 11 eastern states where it attacks the eastern hemlock and Carolina hemlock. HWA is responsible for extensive decline and mortality of hemlock trees in the eastern United States. The insect has spread steadily north and west from its point of introduction and is a serious threat to hemlock throughout eastern North America.

Key Issues

Continued Spread

- Half the range of hemlock in the East is now infested.
- The entire range of eastern hemlock is at risk.
- In the past year, HWA was found in isolated locations in Maine, New Hampshire, and Michigan.

Resource Impacts

- Extensive tree decline and mortality are found throughout the infested region.
- Severe impacts to date are in Virginia, New Jersey, and Connecticut.
- In New Jersey, hemlock mortality in heavily infested stands is estimated at 48 to 92 percent.
- Scientists predict significant tree mortality throughout the range.
- Harvesting of hemlock is proceeding at a rapid pace in an attempt to salvage value from threatened and dying trees.
- Impacts to hemlock forests and ecosystems are expected to intensify.

Need for Accelerated Development and Implementation of Technology

- HWA is an exotic species with no native enemies to keep it in balance.
- Detection of low-level populations is difficult.
- Current survey and monitoring activities are inadequate.
- Control with insecticides is expensive and limited to accessible areas.
- Biocontrol presently offers the only means to manage HWA in forest environments.
- Additional investments are needed to develop, refine, and implement management tools and strategies.

Table 1.—Estimated funding needs for accelerated HWA initiative ($ in thousands)

Program Component	FY2003	FY2004	FY2005	FY2006	FY2007	Total
Research & Development	$3,000	$3,000	$2,500	$2,000	$1,500	$12,000
Management	$1,940	$1,900	$2,350	$2,800	$3,250	$12,240
Information Transfer	$ 60	$ 100	$ 150	$ 200	$ 250	$ 760
Totals	$5,000	$5,000	$5,000	$5,000	$5,000	$25,000

Program Components

Research & Development

Research will focus on the following:

- Identifying and providing environmentally safe control options
- Developing ways to recognize the susceptibility of individual trees and vulnerability of stands
- Improving methods to survey populations and damage and to predict spatial and temporal movement and impacts
- Developing a knowledge base for restoration of damaged stands
- Understanding HWA biology and interactions with other pests.

Management

The goals are to:

- Implement control tactics by expanding technical assistance and use of cost-share programs
- Establish a program that will slow the spread of HWA
- Identify ecological and economic impacts so that management activities can be directed properly
- Provide guidelines and demonstrations of best management practices for the harvest and reforestation of damaged stands; and
- Jointly with research, accelerate development of new management tactics and tools to assist forest managers, forest health specialists, and homeowners.

Funding Needs

Initial analysis and review indicates that an estimated $25 million in federal funds is needed over the next 5 years to develop necessary management tools to mitigate HWA impacts and slow the spread of this pest. Table 1 illustrates these estimates.

Comparative Impact of *Scymnus ningshanensis* and *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae) on the Hemlock Woolly Adelgid

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Abstract

The hemlock woolly adelgid (*Adelges tsugae* Annand) is an introduced pest thought to be native to Asia. Damage to eastern hemlock and Carolina hemlock can be serious (Salom et al. 1996), but western and Asian hemlocks are seldom damaged. Potential biological control agents have been observed in Japan and China (Sasaji and McClure 1997, Yu et al. 2000). We compared two of these which have been previously imported, *Scymnus ningshanensis* Yu et Yao, a coccinellid from China (Yu et al. 2000) and *Pseudoscymnus tsugae* Sasaji & McClure a coccinellid from Japan (Sasaji and McClure 1997).

For each lady beetle, we examined the host range and the numerical response to prey density (adelgid egg masses) in the laboratory, and in field studies, their ability to reduce hemlock woolly adelgid population growth. When given a choice between two prey species in the laboratory, *S. ningshanensis* preferred *A. tsugae* to *Adelges laricis* and *Prociphilus tesselatus* ($p<0.05$, 2 sample t-test) but preferences between *A. tsugae* and *Adelges cooleyi* or *Pineus strobi* were not different ($p>0.05$, 2 sample t-test). The host range results for *P. tsugae* were inconclusive because the beetles fed very little on all prey species provided, including the hemlock woolly adelgid. *S. ningshanensis* showed a positive numerical response ($p<0.05$, linear regression), and *P. tsugae* showed a density independent response ($p>0.05$, linear regression) to an increasing density of hemlock woolly adelgid egg masses. In the field, caged branches with a pair of *S. ningshanensis* resulted in a negative population growth of *A. tsugae*, while cages with a pair of *P. tsugae* adults and the control without lady beetles resulted in an increase in the population growth of *A. tsugae* ($p<0.05$, ANOVA).

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Effects of Herbicide (Round-up®) on *Alliaria Petiolata* (Bieb.) Cavara & Grande (Garlic Mustard), an Invasive Biennial, and Subsequent Effects on the Native Plant Community in Hueston Woods State Nature Preserve, Ohio

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Abstract

Alliaria petiolata (Garlic Mustard) is invasive throughout the northeast U.S. and has become established in the Nature Preserve at Hueston Woods State Park in southwest Ohio. The Park is attempting eradication by spot-spraying Round-up® herbicide each fall.

We investigated the effects of Round-up® on *Alliaria petiolata* and the effects of *A. petiolata* decline on forest floor plants in two stands: an old-growth *Acer-Fagus* stand and a second-growth *Liriodendron-Quercus* stand. We established 50 1x1m plots in each stand in May 2000 and randomly assigned half to be sprayed (Nov. 1, 2000). All plots were censused for density of *A. petiolata* in May, June, Aug., Oct. 2000 and 2001. Densities of herbs in leaf at the time of spraying were also determined in all plots in Oct. 2000 and 2001. Three times each growing season, we determined the percent cover of each species using a point frame. Peak cover values were used to calculate species richness and Shannon-Weiner diversity index values for each plot. Detrended correspondence analysis (DCA) ordination was done using peak cover data to show patterns in forest floor community composition among plots and treatments.

Density of the 2000 *Alliaria petiolata* cohort was significantly lower in sprayed plots in May 2001 in both the old- and second-growth stands (ANOVA: p=0.002, p=0.046 respectively). However, there was no treatment effect on density of the spring 2001 *A. petiolata* cohort, nor on densities of the most common herb in leaf at the time of spraying, *Stellaria pubera*. Spraying did not affect species richness or diversity in either stand. However, ordination revealed a significant compositional difference between experimental and control plots in 2001 in the old-growth stand (ANOVA on DCA Axis 1 scores: p=0.021), but not in the second-growth stand.

In conclusion, the first Round-up® treatment reduced the density of the sprayed cohort of *Alliaria petiolata* without affecting the density of *A. petiolata* seedlings or other herbs. Decline in *A. petiolata* density has already affected the forest floor community composition in the more mature of the two stands.

Rapid Detection of Exotic Lymantriids and Scolytids Pilot Study

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Abstract

Exotic invasive species, inadvertently introduced into North America through importation and travel, are threatening the integrity of North American forest ecosystems. The National Invasive Species Council in their 2001 Strategic Plan identified a collaborative program for early detection, diagnosis and response to high-risk, exotic, invasive insects, pathogens and plants as essential for minimizing pest damage to these ecosystems. In 2001, the Animal and Plant Health Inspection Service (APHIS) and the Forest Service (FS) established a multi-organizational Exotic Pest Rapid Detection Team. Team members included the National Plant Board, National Association of State Foresters, Oregon Department of Agriculture, Maryland Department of Agriculture, Cornell University, APHIS, and FS. The team developed national strategies for detecting exotic bark beetles (Coleoptera: Scolytidae) and nun moth (Lepidoptera: Lymantriidae *Lymantria monacha* Linneaus), implemented pilot tests to evaluate these strategies, and identified gaps and research needs. Components of the strategies include: developing and modifying detection technologies; enhancing detection and diagnostic capabilities; implementing a monitoring program; and developing and implementing rapid response plans for eradication and managements.

During 2001, pilot tests for 10 targeted exotic Scolytids were conducted near the ports of Erie PA, Oswego NY, Toledo OH, Long Beach CA, Seattle WA, Portland OR, Baton Rouge LA, Alexandria LA, and Houston TX. These tests were a success and demonstrated the feasibility of a national survey regionally coordinated for early detection of exotic pests. *Hylurgops palliatus*, a targeted exotic scolytid species and *Arhopalus pinetorum* a non-targeted Cerambycidae were detected for the first time in the United States. In addition, numerous new state and country records were established for many non-targeted exotic scolytids. Nun moth pilot tests, conducted in Portland, OR and at the Department of Defense Naval Weapons Station-Earle, NJ, were also successful because no nun moths were detected. Associated oversea nun moth lure trials, demonstrated that the lures attracted male nun moths and highlighted the need for additional oversea trials to delineate differences in European and far eastern moth behavior. Results of and lesson learned during the 2001 pilot test were used to modify and improve protocols for the 2002 exotic scolytid pilot tests and the oversea nun moth lure trials.

First Field Trials with Fungi Against *Anoplophora glabripennis*

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Abstract

We have been evaluating the use of entomopathogenic fungi against *A. glabripennis*; so far, 20 strains have been isolated belonging to three species, 14 strains have been tested in the laboratory, five strains have been evaluated in caged field trials and two strains have been tested in the open field.

In July 2001, new caged field trials were carried out near Bengbu, Anhui Province, China, with the four best strains from all laboratory bio-assays carried out so far: a North American *Metarhizium anisopliae* strain, a North American *Beauveria bassiana* strain, an indigenous Chinese *B. brongniartii* strain and a commercialized *B. brongniartii* strain from Nitto Denko (Osaka, Japan) being used routinely against *A. chinensis* in Japan. Based on previous caged field trials, we chose the application method developed by Nitto Denko, which markets its strain as non-woven fiber bands impregnated with fungal cultures, hung around the stems of trees, allowing reproduction of the fungus in situ. Forty willow (*Salix* sp.) trees were used for each treatment, with an additional 40 acting as a control group. Five adult beetles were released in a window screening cage around the stem of each tree. We provided the beetles with food and water daily and monitored their mortality. After 10 days, they were removed from the cages and further monitored in the laboratory. Fungus-exposed beetles died faster than controls. The time needed to kill 50% of the population (LT_{50}) was similar for *M. anisopliae*, *B. bassiana* and the Nitto Denko strain (9.4, 10.6 and 11.5 days, respectively) but was higher for the Chinese strain (14.8 days) because this strain produced fewer spores on the bands. The Nitto Denko strain performed better in July 2000 (LT_{50} = 7.8 days) when the maximum daily temperature was never > 33 °C and it rained at least 6 mm on all but one day during the experiment. During 2001, temperatures were routinely > 35 °C during drought conditions. After 15 days, five new beetles were released in the same cages for only 6 days and with only 10 cages per treatment. The LT_{50}s for each of the strains were the same, showing that the bands retain their full virulence in the field for at least 15 days.

Open field trials were carried out in Huayuan, Anhui Province, China, with the indigenous Chinese *B. brongniartii* strain and the commercialized *B. brongniartii* strain. Each of two field sites was divided into three treatments of 100 willow (*Salix* sp.) trees: a control area and two areas treated with a fungal band hung around the stem of each tree. The trees were subsequently monitored for one month. The normalized cumulative reproduction per beetle, measured on the individual tree level, acted as an indirect measurement of the population density. Additionally, beetles were collected in each of the field sites to assess time to death and fungal infection rate. In one of the field sites, reproduction in the fungus-treated trees slowed down, being more pronounced for the Nitto Denko strain than the indigenous Chinese strain, and beetles collected from the fungus-treated trees died faster compared to the control trees. In the other field site, the reproduction did not differ among the treatments and differences in time to death were less pronounced, probably due to other natural enemies already present. When data for the two field sites were combined, infection rates were much higher in the fungus-treated beetles than in the control ones.

Based on these results, we consider that both *B. brongniartii* strains, but especially the commercialized Nitto Denko product, are potentially effective in the field for reducing Asian longhorned beetle population densities and thus would provide a viable and environmentally sound control option to combat this pest in the urban and forest landscape.

Soil Properties and Exotic Plant Invasions: A Two-way Street

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Abstract

Invasions of exotic plant species have become not only widespread but also a major threat to the health of native ecosystems. In order to manage these invasions, it is important to understand the changes that exotic plants may cause in the environment, and the signs that such changes are taking place. Typically, studies of exotic invasions focus on two issues – the characteristics that render species invasive and communities invasible. Invasiveness is most commonly explained in terms of characteristics of the reproductive and physiological ecology of the plants – seed production and dispersal, tolerance of environmental conditions, etc. Invasibility is commonly related to the species richness, nutrient availability, and the frequencies of anthropogenic and natural disturbance. However, none of these patterns have proven generally true. In addition, only a small fraction (usually about 10%) of established exotic species become pests. The traits which may explain why some new species may become established may not be the same as the traits which allow some of them to become widespread pests. I suggest that one explanation for this transition may be the development of a positive feedback between the plants and the soil that promotes the growth of the exotics at the expense of the natives. Extensive studies of native plant species have demonstrated a variety of mechanisms by which plants alter the soil environment in which they live, making the plant-soil interaction a two-way street.

I have examined the evidence for such feedback processes for two invasive exotic species which have become abundant in eastern deciduous forests, the shrub Japanese barberry (*Berberis thunbergii*) and the annual grass Japanese stiltgrass (*Microstegium vimineum*). Barberry was introduced by the Arnold Arboretum in 1870 for horticultural use. It forms dense clusters of shrubs which in some places coalesce into dense thickets. Stiltgrass appeared in Tennessee in 1917, probably inadvertently introduced. It forms both patches and dense, continuous lawns in closed-canopy forest. Both species are currently found in over 30 states, and are continuing to spread.

I and my colleagues initially documented vegetation and soil properties in three forested areas (Morristown National Historical Park, Allamuchy State Park and Worthingon State Forest) in which heavily infested areas occurred adjacent to uninvaded forest. We found that soils in the invaded sites had strikingly lower amounts of litter on the forest floor, much thinner organic soil horizons, and higher pH values in the mineral soil than did the nearby uninvaded sites. These patterns were also documented across a landscape of adjacent invaded and uninvaded patches. This landscape-level study also suggested that in invaded areas, available nitrate (NO_3^-) was positively correlated with the abundance of the exotics.

We further examined the relationships of plant occurrence and nitrogen dynamics by sampling soils directly beneath barberry clusters, within stiltgrass lawns, and amongst the native Vaccinium stems of the uninvaded areas. These studies corroborated the patterns suggested by the previous results: both extractable nitrate and the net nitrification rate was higher under the exotics than under the native plants.

The patterns of changing soil characteristics can be related to differences between the exotic and native species in a variety of traits. A two-year experiment showed that the decomposition rate of *Berberis* litter is much faster than those of native canopy species (*Quercus alba* and *Betula lenta*); however, *Microstegium* litter decomposes at a rate about equal to *Betula* (*Berberis:* 90% mass loss in 1 year, versus about 30% mass loss for *Quercus* and 40-50% mass loss for *Betula* and *Microstegium*). The *Berberis* also has a much higher standing crop of nitrogen in the biomass than does the understory shrub species that it replaces; this reflects higher N concentrations in leaves, stems and roots. *Microstegium* has relatively low N concentrations in the leaves and culms, but its roots are also rich in N. The two exotic differ strikingly, however, in the amounts of root tissue present: Berberis has large amounts of N-rich fine roots, whereas the Microstegium has a remarkably small root biomass. The two exotic species also have high levels of activity of the enzyme nitrate reductase in

their leaves, unlike the native canopy and understory species; this enzyme is correlated with the ability to utilize nitrate as a source of nitrogen. These results suggest that the high levels of nitrate observed in the soil match an ability of the exotics to utilize this form of the nutrient, unlike the native species. Additional experiments done with plants grown in the greenhouse in previously uninvaded soils show that the microbial community, as indexed by a range of extracellular enzyme activities and also by the profiles of phospholipid fatty acids present in the microbial cell walls, are different under the three species (*Berberis, Microstegium* and the native *Vaccinium pallidum*). Together, these data show that Berberis changes ecosystem-level processes through differences in the nutrient content of its tissues, whereas Microstegium may change these processes by having so little root biomass that the soil volume is essentially unoccupied by active root biomass.

The widespread distribution of these species – virtually all protected natural areas, parks and forests in the New York metropolitan area are invaded, for example – combined with the results of these studies suggest that changes to soil properties and processes may be equally widespread. Thus, feedback processes involving soil-plant interactions may be an important component of the invasiveness of some exotic plant species, and may also contribute to the invasibility of some sites. These changes may, furthermore, cause changes in successional dynamics as native species adapted to low-nitrogen, acidic forest soils are outcompeted by weedy species able to take advantage of the higher amounts of nitrate and the lower acidity.

Acknowledgments

I thank Drs. Peter Kourtev and Weize Huang for their contributions to all aspects of this research. Many students, including Megi Kourtev, Lara Masker, Karin Tekel, Jeff Lambe, and Michelle Hughes, assisted with both field and laboratory studies. The research was supported by the USDA NRICGP.

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Kourtev, P.S.; Ehrenfeld, J.G.; Huang, W.Z. **Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey.** Soil Biol. Biochem.

Kourtev, P.S.; Ehrenfeld, J.G.; Haggblom, M. **Exotic plant species alter microbial structure and function in the soil.** Ecology.

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Alternatives to Methyl Bromide for Control of Quarantine Pests: Can Composting of Bark Provide Consistent Lethal Heat Accumulation?

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Abstract

Under the Montreal protocol (1991), methyl bromide has been recognized as a significant ozone depleting compound and a program to phase out its use has been agreed. This will involve a 25% reduction by 1999, a further 25% reduction by 2001, a 20% reduction by 2003 and a complete ban by 2005. There are exemptions for developing countries so that use will remain at average levels used during the period 1995 to 1998, followed by 20% reduction by 2005 and complete phasing out by 2015. Although there are further exemptions for quarantine usage, including pre-shipment treatments, there is now a world-wide effort to address the methyl bromide issue in an attempt to find viable alternatives for both pest management and for quarantine pre- or remedial treatments. This paper describes work under a joint program, part funded by the European Union, entitled *New Quarantine Treatments for Horticultural and Timber Products as Alternatives to Methyl Bromide Fumigation* (Project Code: FAIR CT98 4259) involving partners in the UK (Central Science Laboratory (Coordinator) and Forest Research) and the Netherlands (Research Station for Floriculture and Glasshouse Vegetables (PBG)). Further information can be found at http://www.csl.gov.uk/mebr/index.htm.

Purpose of the Research

Although exemptions for critical use of methyl bromide are to be allowed under the Montreal protocol, these are only allowed under specific criteria, including:

a. there are no available technically and economically feasible alternatives or substitutes that are acceptable from the standpoint of environment and health
b. work is underway to investigate, evaluate, field test, commercialise and, where necessary, facilitate regulatory approval for alternatives and substitutes, with a view to phasing out methyl bromide as soon as possible
c. methyl bromide has been regularly used as an integral part of fumigation operations in the crop and region concerned during the previous five years.

Within the current research program, the scientific consortium is studying a number of potential alternative measures that might be suitable as quarantine treatments. These include heat treatments, composting, extreme controlled atmospheres and alternative fumigants. The current paper describes work carried out by Forest Research on composting of bark as an alternative to methyl bromide for shipment of bark between quarantine zones.

There has been a long history of international trade in wood chips, mainly to serve the pulp and paper industries. In recent years there has been increasing interest in the use of bark and wood chips for mulches in the horticulture and amenity areas. Research into composting schedules has tended to concentrate on the rate of decay of the bark or wood and, particularly, on the suitability of the final product for plant growth, ensuring that quantities of toxins are below required levels. Research on composting as a plant health regime requires that temperatures throughout the compost heap should exceed the thermal death time for any quarantine organisms that might be present. Current heat treatment schedules specify 56°C for 30 minutes for wood and wood products and this has been used as the minimum criterion in carrying out research into compost heat treatments.

The baseline protocol used to assess compost heat treatment is the European and Mediterranean Plant Protection Organization (EPPO) Phytosanitary procedure PM 3/53 *Fermenting (composting) of bark of conifers*. This includes requirements such as milling the bark to below 50 mm mesh size, addition of up to 2% N and 4% P and the turning of the stack 3-4 times during a 4-6 week period (based on research by Solbraa (1979). The target is to achieve heat accumulation of >60°C throughout the compost heap. Although this process is likely to provide a high level of security in relation to various quarantine organisms associated with bark (mainly bark beetles), the physical limitations on bark particle size and the extended time period in which the bark actually is rendered into a compost tends to rule out many markets for unmodified bark with larger

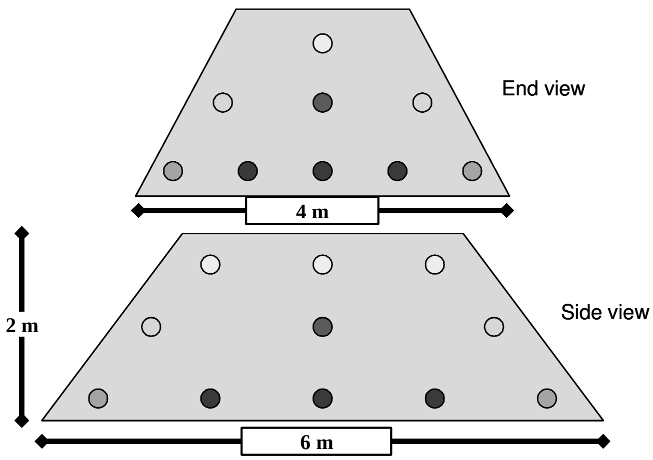

Figure 1.—Diagram of side and end views of experimental bark heaps showing positions of the 37 temperatures sensors. Additional sensors measured air and ground temperatures.

dimensions. Our research has, therefore, concentrated on the basic requirement that any bark, irrespective of particle size, should be capable of being *heat treated* using natural metabolic activity such that a temperature exceeding 56°C for 30 minutes is achieved throughout the bark heap. It is known that, under suitable conditions, metabolic activity can be very rapid, leading to temperatures exceeding 60°C in 2-3 days. Experiments on both small and larger scales were designed to test the ability of unmodified bark (i.e. bark generated by commercial debarking machines without further milling to smaller sizes) to support aerobic metabolic activity and to reach suitable temperatures for quarantine purposes.

Basic Experimental Approach

Bark heaps were constructed as indicated in Figure 1, where the positions of temperature probes at three layers are indicated. The probes were connected to a data logger taking regular readings throughout the experiment. Particular attention was paid to the spatial distribution of temperature accumulation, with the express aim of assessing whether temperatures exceeded 56°C throughout the heap. Both C and N concentrations were measured on some samples and moisture content was assessed in all cases. In some experiments, additional aeration was achieved by use of perforated plastic piping in the base of the heap.

Results

Initial experiments indicated that successful heating was achieved but that temperatures varied considerable throughout the heap. A typical result is shown in Figure 2.

Although temperatures in the top section of the heap reached required temperatures, the heating rates in the bottom layer, especially in the center were too low, thus indicating that at least 60% of the bark would not have reached the required lethal temperature. This is shown more clearly in Figure 3, which is a three dimensional representation of the temperature profiles.

Further experiments confirmed this initial finding and attempts were made to assess the reasons for the low metabolic activity in the bottom layer. The possibility that activity was purely anaerobic was investigated by use of additional aeration through perforated pipes in

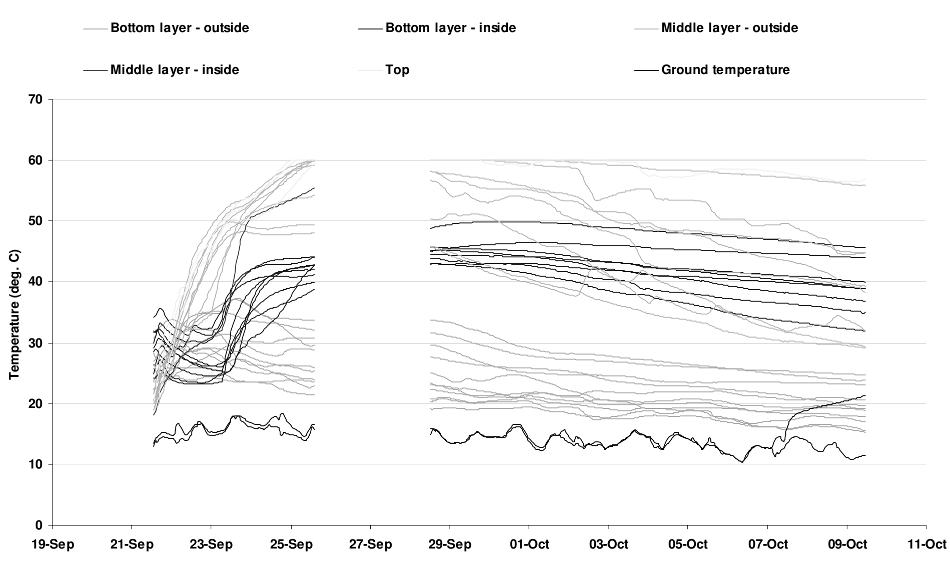

Figure 2.—Temperature profiles for representative bark heap showing differential heating by layer. Upper temperatures are limited to 60°C and there are missing data on 27 Sept.

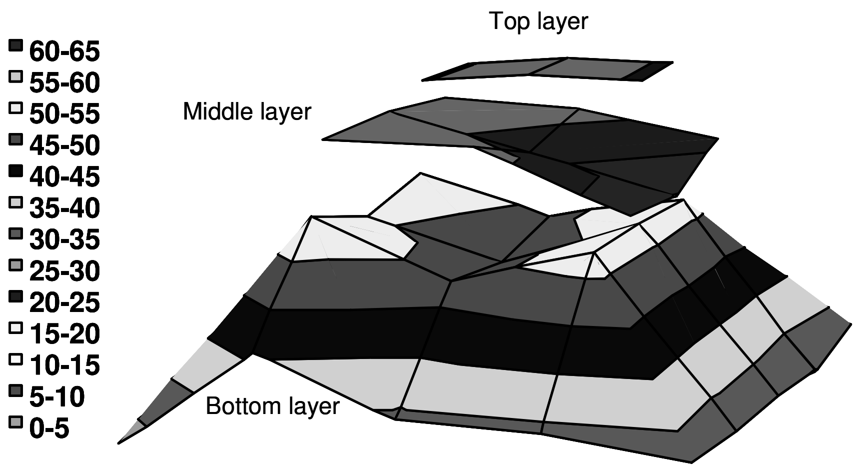

Figure 3.—Three dimensional representation of temperature profiles by layer within the bark heap.

the bottom layer. This exacerbated the problem and caused temperature depression below the ambient level, probably as a result of latent heat of vaporization as water was evaporated from the bark. It was also noted that bark differed in the rate of heat accumulation depending on the time of year, but this was not linked to ambient temperature. Experiments indicated that the moisture content of bark was the key variable in this respect and this was confirmed by addition of water to the heaps, which gave an increase in temperature in the upper layers but not in the lower layers. This was probably linked to the fact that bark pieces were generally impervious and also acted to divert moisture away from lower layers, preventing adequate re-moisturization.

Conclusions

The use of unmodified bark as a product will require adequate heat treatment before it can be transported in international trade. Current protocols for bark composting as a quarantine treatment rely on bark modification and use of a full composting procedure that might not be suitable for all commercial purposes. The experiments in this study have indicated that it is possible to achieve lethal temperature accumulation in the upper sections of bark heaps but that further measures are needed to ensure that the bulk volume of bark lower in the heap is heated adequately. This can be achieved by turning the heap, but this must recognize the fact that there are cool zones in both the outer layers on the surface of the heap and in the bottom middle layers. Addition of N and P sources to accelerate metabolic processes, particularly through lowering the C/N ratio of bark, can be contemplated, but these may be impractical and too expensive for the relatively low value market in unmodified bark. Further work is being carried out to address these issues.

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Flight Propensty of *Anoplophora glabripennis*, an Asian Longhorned Beetle (Coleoptera: Cerambycidae)

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Abstract

Anoplophora glabripennis (Coleoptera: Cerambycidae) (Motschulsky), is a recently introduced pest of hardwoods. Research to study its flight behavior was conducted in the field in Ningxia Autonomous Region, Peoples' Republic of China. To study the flight propensity of *A. glabripennis*, adult beetles were observed in population density and host preference experiments.

For the host preference studies, beetles were placed on one of 3 host types: *Populus nigra*, a preferred native host; *P. alba*, a non-preferred native host; or a model tree constructed of plastic leaves and a bamboo trunk. The trees were checked at 1, 2, 4, 7, and 23 hours following placement on the tree. Location and activity were recorded each period. Significantly higher numbers of both sexes remained on *P. nigra* than models during the first seven hours, while beetles on *P. alba* were intermediate. The proportion of females leaving models was significantly higher during the 0-1 hour, 1-2 hour and 2-4 hour periods than the proportions leaving *P. nigra*. The proportion of males leaving models was only significantly higher during the 0-1 hour period.

During the population density study, beetles were placed on *P. nigra* trees in groups of 1, 2, 3, 4 and 10. The trees were checked at 1, 2, 4, 7 and 23 hours following placement on the tree. Location and activity were recorded each period. There was no significant difference between the different density groups.

While host preference does appear to play a role in flight propensity, population density does not. We plan to perform more studies during summer, 2002.

Male-biased Sex Ratios in Laboratory Rearings of Gypsy Moth Parasitoids

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Abstract

Male-biased sex ratios in laboratory colonies of parasitic wasps used in biological control are harmful because they can prevent the establishment of introduced species or hinder commercial production of species used for augmentative control. Over 20 species of parasitic wasps, most in the families Ichneumonidae and Braconidae, were imported and released against the gypsy moth, *Lymantria dispar* (L.), during 1971-1999. We checked quarantine records to see how often this problem developed in laboratory rearings of gypsy moth parasitoids. In the Ichneumonidae, incoming sex ratios from field collected material were usually 50:50 or female-biased, and marked shifts towards male-biased sex ratios were not observed in outgoing shipments following laboratory rearings (Fig. 1). In the Braconidae, incoming shipments were also about 50:50 or female-biased, but four species, *Aleiodes indiscretus*, *Apanteles* sp., *Cotesia melanoscelus,* and *Glyptapanteles flavicoxis*, showeded marked shifts towards male-biased sex ratios (Fig. 2).

The rest of my presentation will focus on sex ratio studies in *G. flavicoxis*, a gregarious larval parasitoid. This species, collected from Indian gypsy moth, *Lymantria obfuscata* (Walker), was selected for study because (1) it can be reared in large numbers using relatively few hosts, (2) it has high dispersal power and host finding ability at low gypsy moth densities, and (3) it might have potential for inundative releases directed at specific gypsy moth populations (Krause et al. 1991). In fact, *G. flavicoxis* was integrated into a successful urban forest IPM program for gypsy moth in Virginia, but Bt and releases of *C. melanoscelus* were used also, so it is hard to say how much it lent to the suppression effort (Ticehurst and Finley 1988). We report here the results of monitoring sex ratios in a new colony from India, an experiment testing the effects of holding and mating temperatures for adults on sex ratios in their progeny, a second experiment testing the effects of offering hosts to mated females with or without a post-mating rest period, and a third experiment testing the effects of the number of times a female has copulated.

Methods and Materials

Monitoring New Colony. Pedigrees were created for all but the F_1 generation, because many P_1 founders had emerged *en route* and could have mated earlier. In this and ensuing studies, cocoons of *G. flavicoxis* were isolated in gelcaps, and emergees were sexed and held in separate cages with honey and water, so that virgin females were available for controlled matings. In this study, adults were stored at 16°C until mating. Each pair of adults was placed in a 7-cm shell vial containing a droplet of honey and a cotton plug. After mating, females were given a 15-24 h rest period, then placed in

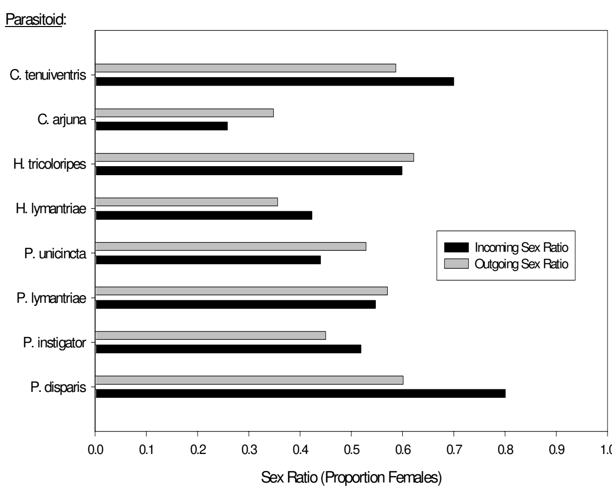

Figure 1.—Incoming and outgoing sex ratios in ichneumonid parasitoids of the gypsy moth.

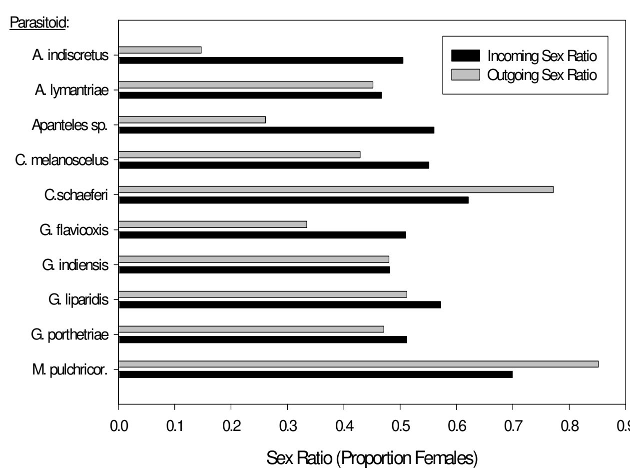

Figure 2.—Incoming and outgoing sex ratios in braconid parasitoids of the gypsy moth.

a sting unit with five unparasitized larvae (instars IV-V) of *L. dispar* for 48 h. In this and ensuing studies, parasitized hosts were fed high-wheat germ artificial diet and held at 25° C; 14:10 (L:D) photoperiod, and 50-60% RH. We monitored the colony for five generations, observing the overall frequencies of sex ratios, and compared sex ratios between generations and between sib-mated and outcrossed females. We also tested for correlation between sex ratios among a pair's progeny and those of the parental generation.

Effects of Storage and Mating Temperatures. After emergence from their cocoons, virgin adults of *G. flavicoxis* were stored at 13°C or 16°C for 16-24 h. Virgin males and females of *G. flavicoxis* that had been held for 16-24 h at 13° C or 16° C were placed in holding cages at 20°C or 25°C under fluorescent lights for three hours. Fifteen males and five females were placed in the mating cage associated with each combination of holding × mating temperature; then, each female was placed in a sting unit containing 10 fourth-instars of *L. dispar* for 48 h. The sex ratio (expressed as % females) of each female's progeny was determined. Data were subjected to two-way ANOVA with holding and mating temperatures as grouping variables.

Effects of Post-Mating Rest Period. Emerging male and female parasitoids were stored separately in holding cages (described above) containing droplets of honey and atomized with distilled water at 16°C until ready for mating trials. Each pair of adults were placed in a 7-cm shell vial containing a droplet of honey and a cotton plug. Vials were watched carefully, and the pair separated as soon as the first copulation was completed. After the observation period, usually 1-60 min, one half of the females were placed individually in sting units containing 10 unparasitized 4th-instars of *L. dispar* for 24 h, and transferred to fresh sting units daily until the female died. The remaining females mated on that date were placed in similar sting units on the following day (23-25 h later) and treated the same thereafter. The experiment was run twice with colony founders received from India in 1999 and 2000. Sex ratios compiled over a female's lifetime were subjected to two-way analysis of variance with treatment and year as grouping variables.

Effects of Multiple Matings. Matings were conducted by placing three males and one virgin female in a 7-cm shell vial containing a droplet of honey and a cotton plug. Extra males were removed once a copulation was observed to negate any genetic variation that might be caused by some females mating with more than one male. Mated pairs were moved to different trays as successive matings were observed. After the observation period, usually 90-120 min, males were removed, and vials containing mated females marked with the number of times the female therein had copulated. No females were allowed to copulate over four times. Each female had a 15-17 h rest period prior to host exposure, then was placed in a sting unit with 5 unparasitized 5th-instars of *L. dispar* for 48 h. Sex ratios of progeny for each female were recorded.

Results and Discussion

Monitoring of New Colony. Figure 3 shows the frequency distribution of sex ratios over five generations. Sex ratios (stated as % females) ranged from 0 to 68%, averaging 9.3% (SEM = 1.1%), and were not normally distributed but highly skewed at the lower end. Nearly 60% of the mated pairs had no female progeny, and only 28% had sex ratios Æ5%. The usual host of *G. flavicoxis* is *L .obfuscata*, so *L. dispar,* though a close relative, is a novel host. The host switch might have induced *G. flavicoxis* females to allocate fewer female eggs to *L. dispar*. Sex ratios differed among generations, averaging 13-17% in the F_1, F_2, and F_4 generations, but <4% the F_3 and F_5. Proportions of parental females yielding progeny of both sexes followed a similar trend. The reason for these differences is unknown. When grouped by cross type (sib-mating or outcross), sex ratios did not differ significantly. We looked at the relationship between sex ratios in filial (3-5) and parental (2-4) generations by regressing the sex ratio of each pair's progeny against those of its parents. A model with two independent variables sex ratios of the maternal and paternal families) and one dependent variable (sex ratio of pair's progeny) was not statistically significant.

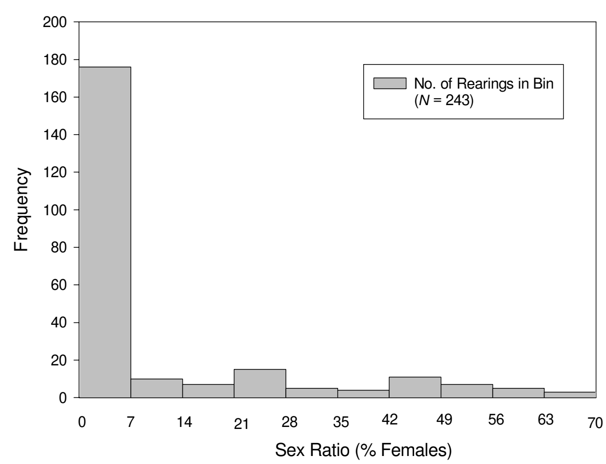

Figure 3.—Histogram for overall distribution of sex ratios in *G. flavicoxis* across five generations.

Effects of Storage and Mating Temperatures. Temperatures in holding or mating cages for parental adults did not affect sex ratios in their progeny (Fig. 4). Sex ratios were highly variable, because about half of the females had no female progeny, presumably because they failed to mate. In most species of parasitic wasps, unfertilized (haploid) eggs result in male progeny, while fertilized (diploid) eggs result in female progeny, causing unmated females to produce only male rather than mixed progeny.

Effects of Rest Period and Year. Sex ratios for total progeny produced over a female's lifetime differed between treatments (F = 4.35; df = 1,31; P < 0.05), being over twice as high in females having a rest period than in those which did not (Fig.5). Year and treatment-year interaction effects on sex ratio were not significant. Six of the 16 females without a rest period failed to produce any female progeny, whereas only one of 19 given a rest period failed to do so. These proportions differed significantly (Fisher's exact test, P = 0.024). Because all of the females in this experiment, had copulated before the tests began, these results indicate that copulation does not always ensure that a female is impregnated and that *G. flavicoxis* might need a period of inactivity following copulation.

Effects of Number of Copulations. ANOVA showed that the number of times (1-4) a female had copulated did not appear to influence the sex ratios in her progeny. Figure 6 suggests that sex ratios might tail off when the number of matings exceeds two, so we grouped them into only two categories (1-2 and 3-4) and attempted to retest with a one-way ANOVA. With only two groupings, the Kolmogorov-Smirnoff normality test failed (P = 0.002), so we subjected the data to Kruskal-Wallis one-way ANOVA on ranks. The difference in rankings was statistically significant (H =4.302; df = 2; P = 0.038). The median sex ratio for those females mating 1-2 times (n = 20) was 36.3%, whereas that for females mating 3-4 times (n = 19) was only 1.3%. These results suggest the sex ratio of *G. flavicoxis* might be adversely affected when the number of copulations exceeds two.

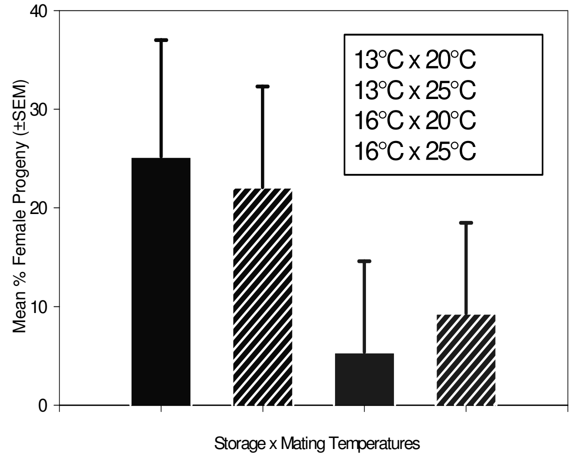

Figure 4.—Effect of storage and mating temperature on sex ratio of *G. Flavicoxis*.

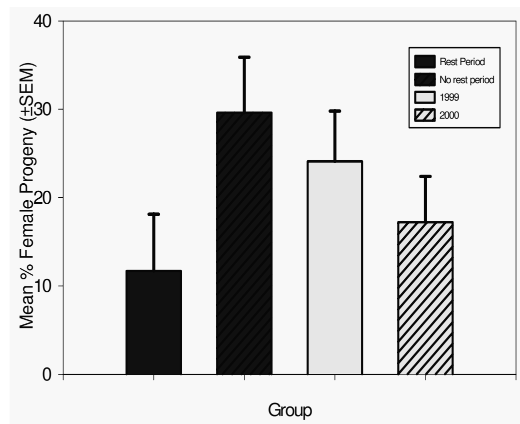

Figure 5.—Effect of rest period and year on sex ratio of *G. flavicoxis*.

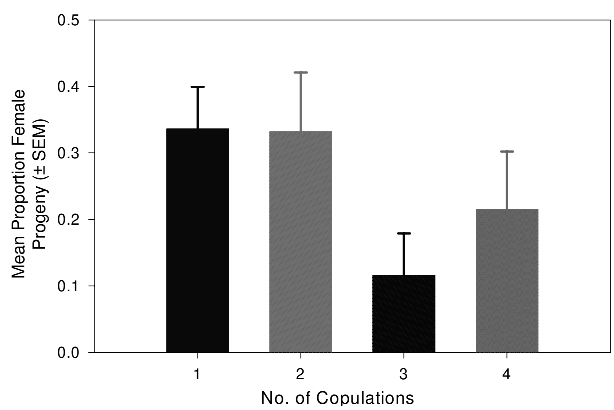

Figure 6.—Effect of number of copulations on sex ratio in *G. flavicoxis*.

Summary and Conclusions

Sex ratios were very low in the new colony, suggesting the host switch (*L. obfuscata* to *L. dispar*) was responsible, but other factors cannot be ruled out. Sex ratios differed among generations, but not in a consistent pattern. Holding and mating temperatures for parasitoid adults did not affect the sex ratio of their progeny. Females given a post-mating rest period had higher sex ratios in progeny than those which did not, but over two copulations appeared to depress sex ratios in their progeny. While the best treatments enhanced the sex ratio, the sex ratios were lower than those in incoming shipments. More work is needed.

Acknowledgment

We thank Dr. Gujjanadu Ramaseshiah for providing field collected *Glyptapanteles flavicoxis.*

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Update on Security Plans to Prevent Bioterrorism Attacks During Agricultural Bacilli Programs

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Abstract

In the aftermath of September 11, security in aerial application suppression programs utilizing *Bacillus thuringiensis* has become a critical concern for State pest control managers and aerial applicators.

An update of security plans of those States involved in the 2002 Gypsy Moth State-USDA-FS Suppression Programs was reviewed. The following issues were discussed:

1. Insecticide: Including chain-of-custody documentation from manufacture to aerial applicator, factory evident seals, testing of insecticides for contaminants, insecticide-sampling protocols.

2. Equipment: Security of aircraft and spray equipment, flushing of equipment, 24-7 security at airport or LZ's.

3. Operations: Restricted access to loading and storage areas, FAA approval for flights in restricted areas, background checks on pilots and ground crew, identification documents for access to operations areas, aircraft coded transponders.

4. Public Awareness and Project Communications: Conduct public meetings, meet with local authorities and emergency management officials, media notification prior to treatment.

Low Volume Undiluted Btk Application Against Heavy Gypsy Moth Population Densities in Southern Corsica

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Abstract

Low volume undiluted applications of *Bacillus thuringiensis* are common and efficacious against coniferous forest pests such as pine processionary moth and spruce budworm, but have not been common practice against deciduous forest pests due to coverage issues. With the introduction of higher potency Btk formulations and the advancements in aerial application technology, Gypsy Moth Forest Pest Managers have recently been able to successfully lower volumes to around 3 liters/ha with Foray 76B® (20.1 BIU/liter) and still achieve suppression program objectives. The objective of this study was to determine if foliage protection could be achieved with a single application of a higher potency Btk formulation applied at 2 liters/ha against a heavy gypsy moth population.

In 2001, a 5 hectare stand of cork and holly oak was treated with Foray 96B® (25.4 BIU/L) applied undiluted at 2.0 L/50 BIU/ha against a heavy gypsy moth population in Southern Corsica. The application was made with a Piper Pawnee equipped with 4 Micronair AU-5000 rotary atomizers rotating at maximum RPM's to produce droplets with volume medium diameter (VMD) of 100 µm. Evaluations included reduction of gypsy moth larval density measured by frass traps and defoliation estimates. The results showed that 2 L/ha of Foray 96B® was able to reduce larval density and protect Mediterranean *Quercus spp* in Corsica. Further trials are planned at 2 L/ha in more dense forest stands containing larger oak species of trees.

Molecular Genetics of Asian Longhorned Beetles: Introduction, Invasion, and Spread in North America

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Abstract

We have used molecular techniques to study the genetic structure of Asian longhorned beetle (ALB) populations in North America, allowing us to assess the dispersal behavior of the adult beetles, the extent to which populations have spread in urban areas, and the potential for future spread. We have extracted and sequenced DNA from individuals of six populations; four from China, one from New York and one from Chicago. Three regions of the mitochondrial genome have been assessed for variation among populations. Sequenced gene regions include cytochrome oxidase I and II, and a portion of the large ribosomal subunit of the 16S rRNA. Unfortunately, screens of over 500 base pairs of sequence from each of these gene regions either showed little variation (telling us little about population structure) or were unreadable when sequenced.

We have begun examining population structure using Random Amplified Polymorphic DNA (RAPD) analysis. We screened 20 RAPD primers for variation among the six populations, scored eleven loci from 2 primer sets, and subjected these data to discriminant function analysis. We found that the Gansu population from China shares a significant portion of its band variation with Illinois and New York, and Illinois and New York populations share some similarities among individuals. Results from sequencing the mitochondrial sodium dehydrogenase 4 (ND4) gene corroborated the RAPD data. Sequence data from 242 base pairs of ND4 for beetles from the six sites identified 12 informative sites. Preliminary phylogenetic analyses using neighbor-joining methods indicate that the Illinois and New York populations are related to the Gansu population.

Our findings confirm that there is considerable genetic variability among ALB populations, and this variation can be used for continuing our search for populations in China that were the origin of North American infestations. This information will be critical for evaluating international quarantine practices and preventing introduction of other invasive pest species.

Comparative Studies on the Influence of Bulgarian *Nosema* Isolates on the Gypsy Moth, *Lymantria dispar* L.

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Abstract

During an outbreak in 1995 to 1997, two microsporidian isolates were recovered from gypsy moth (*Lymantria dispar* L.; GM) populations in the vicinities of Veslec and Levishte in northern Bulgaria. Preliminary studies have confirmed that both isolates are morphologically very similiar and belong to the genus *Nosema*. In this investigation, we studied the influence of both isolates on the development and performance of the host in laboratory bioassays.

The two isolates were fed to GM third instar larvae at 5 different dosages ranging from $2x10^2$ to $5x10^4$ spores/µl. Larvae were reared individually on standard artificial diet and the following parameters were recorded every second day: larval mortality, larval stage and weight, day of pupation, pupal weight, and day of adult eclosion.

Both isolates were highly infective to GM larvae. Control mortality was below 3%. The isolate from Veslec caused higher mortality than the isolate from Levishte (89-98% vs. 79-93% respect.) Dosage did not have a significant influence on the mortality rates of either isolate. The stage-specific mortality caused by the two isolates was very different: the Veslec isolate acted faster and killed infected individuals as larval instars, while the Levishte isolate caused mortality mainly during the prepupal and pupal stages.

The isolate from Veslec did not have an effect on the development time, however, the time from infection to pupation of male larvae infected with the isolate from Levishte was significantly prolonged by 2 to 3 days. Neither isolate had an effect on the weights of female pupae, however, infected male pupae were significantly heavier than uninfected male pupae.

Our results indicate that both isolates have a different impact on individual larvae and therefore, might have a different influence on the population dynamics of gypsy moth in the field.

Potential Susceptibility of Eastern Forests to Sudden Oak Death, *Phytophthora ramorum*

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Abstract

Sudden oak death is caused by the fungus-like organism, *Phytophthora ramorum*, and was first discovered in central coastal California in 1995. The non-native disease has killed large numbers of oaks (coast live oak (*Quercus agrifolia*), California black oak (*Q. kelloggii*), and Shreve oak (*Q. parvula* var. *shrevei*)), tanoaks (*Lithocarpus densiflorus*), and Pacific madrone (*Arbutus menziesii*) and has recently been found in coastal Oregon. The organism has not affected California species in the white oak group. Greenhouse tests of eastern oak species pin oak (*Q. palustris*) and northern red oak (*Q. rubra*) have shown these species to be just as susceptible to sudden oak death as their west coast relatives. We developed a preliminary map of the potential risk to eastern forests should this organism become established in the East. The map was developed using FIA plot data for all species in the red oak and live oak groups. Kriging was used to develop a map of potential susceptibility of forests containing those two oak groups for the eastern United States.

Evaluation of the Viability of the Butternut Resource

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Abstract

Butternut (*Juglans cinerea* L.), a widespread but rare tree, is being affected by a lethal canker disease caused by the *Sirococcus clavigignenti-juglandacearum* fungus. The fungus was probably introduced from outside North America and is possibly spread by insects. The first butternut deaths were reported in 1967 and butternuts of all ages are dying throughout the range of butternut in North America. We evaluated the distribution of butternut in the eastern United States using U.S. Forest Service Forest Inventory and Analysis (FIA) plot data. Butternut occurrence was then classified by ecoregion province and section levels. Significant differences in butternut occurrence existed at both levels. Kriging was used to initially derive a probability map of butternut occurrence across the eastern United States. This map was then overlaid by forest density data, resulting in an adjusted probability map of butternut occurrence in eastern forests. Candidate areas for butternut reintroduction have been identified by this analysis. In addition, field plots evaluating the progression of butternut canker in natural and planted seedlings in young stands were established.

Intercepted Bark Beetles (Scolytidae) at U.S. Ports of Entry: 1985 – 2000

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Abstract

Since 1985, USDA APHIS (U.S. Department of Agriculture, Animal and Plant Health Inspection Service) has maintained an electronic database for all plant pests intercepted at U.S. ports of entry. The database is known as the Port Information Network or PIN. When I accessed the PIN database in August 2001, there were 577,829 insect records of which 73,649 were for Coleoptera and 6992 were for Scolytidae. When I restricted the database to the years 1985-2000, there were 6827 scolytid records. Of the 6827 scolytid interceptions, 5077 (74%) were identified to the genus or species level, while 1750 (26%) were identified to the family level only (i.e., Scolytidae).

Overall, 49 genera of scolytids were identified. The 10 most commonly intercepted scolytid genera were *Hypothenemus* (821 interceptions), *Pityogenes* (662), *Ips* (544), *Coccotrypes* (520), *Orthotomicus* (461), *Hylurgops* (327), *Hylurgus* (266), *Tomicus* (194), *Dryocoetes* (166), and *Hylastes* (142). For the 2740 scolytid interceptions that were identified to the species level (40% of 6827), the 10 most commonly intercepted species were *Pityogenes chalcographus* (565 interceptions), *Orthotomicus erosus* (385), *Hylurgops palliatus* (295), *Ips typographus* (286), *Hylurgus ligniperda* (217), *Ips sexdentatus* (157), *Tomicus piniperda* (155), *Hylastes ater* (75), *Hypothenemus hampei* (62), and *Polygraphus poligraphus* (48).

The intercepted scolytids originated from at least 118 different countries. The top 12 countries were Italy (1090 interceptions), Germany (756), Spain (4570), Mexico (425), Jamaica (398), Belgium (352), France (261), China (255), Russia (247), India (224), UK (151), and Portugal (150).

The scolytids were intercepted at 97 U.S. ports of entry in 35 U.S. states. The top 12 U.S. states were TX (1203 interceptions), FL (1102), GA (612), LA (467), NY (451), MD (421), OH (327), SC (278), CA (240), KY (232), NC (202), and NJ (192). The top 10 port cities were Houston, TX (822 interceptions), Miami, FL (685), Savannah, GA (466), New Orleans, LA (463), Baltimore, MD (421), Brooklyn, NY (353), Toledo, OH (287), Ft. Lauderdale, FL (250), Charleston, SC (244), and Erlanger, KY (227).

Overall, 72% of the scolytids were associated with solid wood packing material, 20% with food or plants, and 8% other or unspecified. Some of the imported products most commonly associated with infested wood packing were tiles, marble, machinery, steel, parts, ironware, granite, aluminum, slate, and iron. Some of the foods and plants that were commonly infested with scolytids were nutmeg, palms, coffee beans, cola nuts, and macadamia nuts. When considering all insect interceptions associated with wood products, beetles (Coleoptera) comprise the bulk of the interceptions, with the scolytids being the most commonly intercepted insect family (Haack and Cavey 1997, 2000).

Currently, 44 species of exotic scolytids are known to be established in North America (Haack 2001), including 2 species of *Ambrosiodmus*, 7 *Coccotrypes*, 1 *Crypturgus*, 1 Dryoxylon, 1 *Euwallacea*, 1 *Hylastes*, 1 *Hylastinus*, 1 *Hylurgops*, 1 *Hylurgus*, 1 *Hypocryphalus*, 11 *Hypothenemus*, 1 *Pityogenes*, 1 *Premnobius*, 3 *Scolytus*, 1 *Tomicus*, 1 *Trypodendron*, 1 *Xyleborinus*, 5 *Xyleborus*, and 3 *Xylosandrus*.

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Hylurgus Ligniperda (Scolytidae): A New Exotic Bark Beetle in New York State

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Abstract

An established population of the red-haired pine bark beetle, *Hylurgus ligniperda* (F.) (Coleoptera: Scolytidae), was found in the United States in November 2000 near Rochester, NY (Hoebeke 2001). During surveys in 2001, *H. ligniperda* was detected in three counties in New York.

Our objectives in 2001were to determine: (1) which trap design is most efficient at capturing *H. ligniperda* adults, (2) which lure is the most attractive to *H. ligniperda* adults, and (3) what is the pattern of seasonal flight and seasonal development in New York?

Traps. We tested three trap designs [12-unit funnel traps (PheroTech), panel traps (IPM Technology), and Theysohn traps (El-Tech)], using standard ethanol and alpha-pinene lures. Overall, during the first 6 weeks of adult flight, funnel traps captured about twice as many adults than did the other two trap types.

Lures. Using standard 12-unit funnel traps, we tested five lures that consisted of various combinations of alpha-pinene, beta-pinene, ethanol, and the "exotic ips lure" (PheroTech). Overall, during the first 6 weeks of adult flight, traps baited with high-release ethanol (1000 mg/d) and high-release alpha-pinene (750 mg/d) captured the most adults. Traps baited with other combinations of alpha-pinene, beta-pinene, and ethanol captured intermediate numbers. The exotic ips lure captured very few adults.

Seasonal flight and seasonal development. Using funnel traps baited with ethanol and alpha-pinene, we trapped for *H. ligniperda* during the period 5 April to 16 November 2001. During that period, adults were collected from 18 April through 16 November 2001. Trap catches were highest during late summer and fall. We also dissected pine stumps at 2-week intervals from April through September 2001. New adults were first found in July. *Hylurgus ligniperda* completed at least two generations during 2001 under New York conditions.

Literature Cited

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Activity of *Entomophaga maimaiga* in the Field

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Abstract

In long term plots in central New York gypsy moth populations have remained at extremely low densities since 1991. From 1992 to 2001, we collected larvae throughout the field seasons to evaluate prevalence of disease. Each year, we detected infection by the fungal pathogen *Entomophaga maimaiga*, with >70% infection during 5 of the 10 years. However, in MI, *E. maimaiga* has not seemed as effective at controlling gypsy moth. Also, along the northeastern U.S. coast and in central Pennsylvania, gypsy moth populations rebounded during the 2001 field season, causing defoliation.

We established 33 sites throughout Michigan where *E. maimaiga* had been released or had been recorded. To evaluate the effect of site, stand and weather on *E. maimaiga*, we caged larvae over soil to compare infection in the field with infection under optimal conditions in the lab. Levels of infection in the field were much lower than under optimal conditions, but in both instances, infection levels increased from 1999-2001. Counts of resting spores in soil have been compared with results from lab bioassays to develop a bioassay-based method for estimating resting spore density in soil. During this study, *E. maimaiga* was released in some of the plots either as field-collected or lab-produced (in vivo) resting spores. We see a trend toward greater infection at fungal release sites but results are not significantly different due to variability among plots.

To continue our studies of non-target effects of this fungus, counts of resting spores in soil to evaluate persistence of *E. maimaiga* have shown that titers have declined over 4 years, unless epizootics added resting spores to the soil. From 1997-2001, we sampled larval lymantriids in the forests of VA and WV during the gypsy moth field season to evaluate infection in this susceptible group of insects. Seven species of endemic lymantriids (EL) were collected but densities were always very low. Gypsy moth populations were also scarce from 1997-1999 and no infection was found in any larvae during this period. However, during 2000 and 2001, of the six species of EL collected, infection by *E. maimaiga* occurred in three of them, with maximal infection at 33% for *Dasychira obliquata* (5 of the 15 larvae collected over the 2 years) and *D. vagans* (one of three larvae). In summary, *E. maimaiga* infects EL in the field but infections were not found all years; of the total 152 EL collected over the 5 years, only seven individuals were infected.

Natural Enemies of *Saperda* spp. (Col.: Cerambycidae, Lamiinae) in Europe, Envisioned as Potential Agents in Biological Control of *Anoplophora* spp. in Europe and the U.S.A.

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Abstract

Project Objectives/Approach. Our rationale is founded on the evaluation of possible new associations between the Asian longhorned beetle (ALB) and natural enemies of European cerambycids that have similarities with ALB in terms of taxonomy, host-plants, and behavior. Our first objective is to search, among the biocoenoses associated with selected European cerambycids, natural enemies (specifically early stage parasitoids) accepting ALB as hosts. The European pests that we selected for these studies are *Saperda populnea* (L.) and *Saperda carcharias* (L.).

Project Update. *Natural enemies of S. populnea*: They were surveyed in Southern and Eastern France, Denmark, Southern Sweden and Southern Finland. The egg parasitoid *Euderus caudatus* Thomson (Hymenoptera, Eulophidae), cited in the literature, was not found in the field, yet. So far, two parasitoids of early larvae were found: a yet to be identified tachinid, which was obtained from hosts collected in Southern and Eastern France, and in Finland, and *Euderus albitarsis* Zetterstedt (Hymenoptera, Eulophidae), which attacked first instar larvae of *S. populnea*. *Euderus albitarsis*, was obtained from first instar hosts, mainly, and sometimes from second instar hosts. It fully develops on the attacked instar. It was obtained from material collected in late July in Southern Finland. Two parasitoids whose adults emerged from full grown larvae of *S. populnea* were found: *Billaea irrorata* (Meigen) (Diptera, Tachinidae), and *Dolichomitus populneus* (Ratzeburg) (Hymenoptera, Ichneumonidae). An entomopathogenic fungus *Beauveria* sp. was isolated from an adult *S. populnea*. Rate of parasitism by each species in the various sites could not be fully processed yet as the duration of total development of the host is 2 years, and the duration of development is particularly lengthy for some of the parasitoid species. In the literature, 37 other parasitoids were cited on both hosts. The biocomplex of enemies of these two cerambycids constitutes a great reservoir of species that can be tested against *Anoplophora* spp.

The following predatory Diptera larvae were found by dissection of branches in *S. populnea* galleries: *Odinia xanthocera* (Collin) (Diptera, Odiniidae), and *Lasiambia baliola* Collin (Diptera, Chloropidae), both new for France, and *Thaumatomyia elongatula* (Becker) (Diptera, Chloropidae), new for continental France.

Development of laboratory rearing techniques: In the laboratory, techniques for rearing *S. populnea* on rooted cuttings of poplars were developed. Fecundity and longevity of adults of *S. populnea*, initially collected in the field as pupae in host plant material then emerged in the laboratory, were studied using fresh cuttings of poplar as oviposition sites, at 22°C. Fresh foliage was supplied to the adults for feeding and maturation. Forty percent of females had a low longevity (21-32 days) and laid 50-90 eggs. Sixty percent of females had a high (42-60 days) longevity. Among them, thirty percent of females laid 100-170 eggs, and thirty percent laid 200-230 eggs.

Several plantation conditions were tested in order to get healthy rooted cuttings. Laboratory experiments were designed to compare two types of soils, two watering systems, two types of fertilization, and several combinations of these factors. The best treatment was as follows: soil was a mixture of compost, sand, and vermiculite in the proportions 0.50, 0.25, 0.25 of the volume, using 6-litre containers; watering was supplied by saturating each clump with water once a week from the top of it, and the culture was conducted in a quarantine greenhouse at constant temperature (23 ± 1°C), under natural lighting. In growing *Populus tremula*, *Populus deltoides*, *Populus alba*, and *Salix capraea*, not adding any fertilizer was an important factor for the successful cultivation conditions.

Duration of incubation of the eggs of *S. populnea:* Egg incubation duration was studied in branches of living aspens was studied in the field, under natural Mediterranean climatic conditions. This informed us about the time available for egg parasitoids to attack their hosts. This information was also needed to

determine the appropriate time of exposure in the field of living branches, preliminarily laboratory infested with eggs of *S. populnea* in the laboratory, when we want to capture egg parasitoids from various sites. We showed that most eggs hatched after 5 days of incubation in the temperatures that occurred at Montpellier in the period 23 May through 2 June, 2001.

Incomplete work or areas needing further investigation:
– Continue explorations in some of the same areas and in other regions in Europe to complete inventory of the early stage parasitoids of *S. populnea* and *S. carcharias*.
– Finalize *S. populnea* and *S. carcharias* rearing techniques using rooted cuttings as host plants.
– Implement ALB rearing techniques in 5-10 cm diameter rooted cuttings.
– Test *Saperda* spp. parasitoids on ALB, in quarantine at Montpellier.
– Survey ALB and *Anoplophora chinensis* populations in sites where these 2 species were accidentally introduced in Europe, for possible occurrence of parasitism by the local species.

Products anticipated. Parasitoids of longhorned Beetles from the Western Palearctic region which are attracted to the early stages of ALB, accept ALB as hosts, utilize it for their development cycle, are promising agents to control ALB in the Nearctic region, and which have no negative impact on North American ecosystems.

Physiological effects of a microsporidian infection: Alterations in carbohydrate and fatty acid levels in *Lymantria dispar* larvae

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Abstract

Vairimorpha sp. is a virulent microsporidian pathogen isolated from *Lymantria dispar* L. in Central Europe. The fat body is the target tissue of the infection; there, mass proliferation of *Vairimorpha* occurs and, finally, environmental spores are formed. The host is usually killed in the larval stage. Microsporidia are obligatory intracellular pathogens and thus completely depending on resources provided by the host. Little is known, however, on the physiological effects of a microsporidian infection on the host or the nutritional requirements of these pathogens themselves. In our study, we used an infection of the gypsy moth, *L. dispar*, with the microsproridium *Vairimorpha* sp. as a model to study the effects on the level of carbohydrates and fatty acids in the host.

Vairimorpha infection caused a total depletion of trehalose from host hemolymph during the course of infection. A steep, significant decrease of trehalose levels in the hemolymph of infected larvae occurred 5 days post infection (dpi). At that time mass proliferation of *Vairimorpha* began in the fat body, and first environmental spores were formed. Concentrations did not increase again during the subsequent sampling periods, while in uninfected larvae, hemolymph trehalose levels increased steadily during the fourth instar. Also a pronounced decline in the content of the storage carbohydrate glycogen was observed in infected hosts in the late phase of the infection. Consequently, the glycogen content was significantly lower than in controls 13 and 15 dpi. Moreover, *Vairimorpha* infection led to a reduction of fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid) in the hemolymph; levels were significantly lower 5, 7, and 9 dpi.

The results show that the microsporidian infection severely affects host metabolism. There are several possible explanations for our findings. This can be a consequence of nutrient uptake by the pathogen from the host cell. Also the infected host cell itself may use carbohydrates and fatty acids to support its increased energy metabolism; resulting ATP could be harnessed by the microsporidia. Finally, lower carbohydrate and fatty acid levels could result from a severely disturbed synthesis in infected fat body cells.

Performance of Asian Longhorned Beetle Among Tree Species

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Abstract

Two procedures were evaluated for assessing susceptibility of a variety of tree species to *Anoplophora glabripennis*. In the first procedure, adult beetles were caged with a section of sugar maple, northern red oak, white oak, honeylocust, eastern cottonwood, sycamore or tulip poplar wood and allowed to oviposit. Results showed that females laid viable eggs on sugar maple, red oak, white oak and honeylocust. Oviposition did not occur on cottonwood, sycamore, or tulip poplar. Eighty-seven percent of the first instar larvae survived in white oak, followed by sugar maple (82%), honeylocust (50%), and red oak (39%). In the second procedure, first instar larvae were manually inserted into potted sugar maple, green ash, and red oak trees and allowed to feed for 60 or 90 days. Significantly more larvae survived for 90 days within the red oak (67%) compared to green ash (17%). Larvae recovered from red oak weighed significantly more than larvae from sugar maple or green ash. Larval survival was positively related to height of insertion. These results indicate: 1) controlled laboratory and greenhouse-based procedures can be used to assess tree susceptibility to *A. glabripennis* and 2) *A. glabripennis* will oviposit and larvae can develop in northern red oak for up to 90 days making it a potential host.

Detection Trapping for Scolytidae in Northeastern China

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Abstract

At least 550 species of Scolytidae are known to occur in China and the adjacent regions of eastern Russia, Japan and Korea. Our collaboration has initiated field testing of commercially available pheromones and kairomones of Scolytidae to determine the potential of such lures to detect bark and ambrosia beetle species found in NE China and non-indigenous to Canada. Half of the 46 species of Scolytidae currently acknowledged as introductions in North America, including two recently discovered species, *Hylurgus ligniperda* (Fabricius) and *Hylurgops palliatus* (Gyllenhal), as well as numerous species of quarantine concern occur in east Asia.

The objectives of our ongoing studies include determining which species can be detected with commercially available lures, obtaining voucher material to aid in identification of non-indigenous Scolytidae and development of collaborative research projects for the detection of bark beetles of quarantine significance which do not respond to existing lures. Results from trapping experiments conducted in 2000 and 2001 are presented.

Ophiostomatoid fungi associated with invasive *Tetropium* spp. (Fabr.) (Coleoptera:Cerambycidae) in Atlantic Canada.

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Abstract

Species of the Ascomycete genus *Ophiostoma* are well-known for causing stain in living trees and lumber, as well as devastating pathogens, for example *O. ulmi* and *O. novo-ulmi*, the causes of Dutch elm disease. *Ophiostoma* spp. occur in close association with insects, especially bark beetles in the family Scolytidae, which may function as vectors. In 1998, red spruce trees (*Picea rubens*) in a small area of the Halifax Regional Municipality in Nova Scotia, Atlantic Canada, were discovered infested by *Tetropium fuscum*, an Eurasian species of longhorn beetle in the family Cerambycidae. *Tetropium fuscum* was infesting and killing red spruce tree species absent from the insect's native range. Several ophiostomatoid fungi occurred with this beetle. The aims of this study were to identify the fungi associated with *T. fuscum* in Halifax and compare them with ophiostomatoid fungi associated with this insect in Europe. *Ophiostoma* species associated with *T. cinnamopterum* (indigenous to and transcontinental in Canada) were also compared to those with *T. fuscum*. Species were identified based on morphological and molecular characters. *Ophiostoma tetropii* appears to be the major species associated with *T. fuscum* in Europe and Canada and was probably introduced into Atlantic Canada by the insect. Other species isolated from trees attacked by *T. fuscum* include *Ophiostoma piceae,* a ubiquitous secondary colonizer of conifer sapwood, and the rarely reported *Pesotum fragrans.*

Sampling Seasons for Exotic Woodboring Insects: How Long is Long Enough?

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Abstract

Introduction. Several injurious exotic woodboring insects (EWBI) have recently been introduced into North America, such as the Asian longhorned beetle, *Anoplophora glabripennis* (Motschuslsky) (Coleoptera: Cerambycidae), the brown spruce longhorned beetle, *Tetropium fuscum* (F.) (Coleoptera: Cerambycidae), and the pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera: Scolytidae).

The influx of intercontinental and regional EWBI continues unabated. Of the 81 Scolytid species recorded in Oregon since 1997, 29 % are exotic. Recently detected exotic Scolytid species in OR (eight) outnumbered more "historical" ones (seven) detected prior to 1997. The agricultural, ecological, and economic consequences of many of these species are currently unknown. Development of EWBI survey protocols is receiving increased attention.

Since survey resources are limited, an important consideration is: What is the optimum survey season for EWBI? This question was explicitly addressed during the development of protocols for the USDA 2001 Rapid Detection of Exotic Scolytidae Pilot Project (RDESPP). Based upon available data and a short list of target species, a survey period of early March through the end of July was selected. The question we address here is: Could rapid detection of some newly introduced EWBI be impaired by ending sampling in July?

Methods. Three Oregon sites were selected for the RDESPP survey. Following RDESPP protocols, three 12-funnel Lindgren traps, each utilizing a different lure, were placed at each site. Samples were collected bi-weekly. In order to ascertain any benefits of an extended survey period, trapping was continued until the end of the Oregon Dept. of Agriculture's (ODA) standard EWBI survey season, mid-October. Analysis was based upon the proposed RDESPP survey period, the "Season" ending July 31, versus the period thereafter, the "Post Season" ending mid-October. Data from ODA's 1999 and 2000 EWBI surveys in western Oregon, which included five times as many traps and sites as the RDESPP Oregon survey, were also analyzed in this manner. Both native and established exotic species of Cerambycidae, Scolytidae, and wood wasps (Sircidae and Xiphydriidae) were considered.

Analysis. We compared the percentage of species of Cerambycidae, Scolytidae, and wood wasps detected within "Season" and the percentage of additional species detected "Post Season". Percentages of cerambycid species added after July 31 ranged from substantial (25%) to minor (7%). Additional scolytid species were relatively few (2 % to 8%), although it is worth noting that for the RDESPP the increase was almost 10%. In sharp contrast, the bulk of wood wasp species, from 40%-67%, were detected "Post Season".

Several species were only detected "Post Season". These included *Ortholeptura valida* (LeConte) and *Plectura spinicauda* Mannerheim (Cerambycidae), and *Scolytus oregoni* Blackman (Scolytidae). Fully half of the wood wasp species were only detected "Post Season": *Sirex cyaneus* F. and *Urocerus gigas flavicornis* (F.) (Siricidae), and the exotic *Xiphydria prolongata* (Geoffroy) (Xiphydriidae). *Sirex cyaneus* is very similar in appearance to the notorious *S. noctilio* F., which has caused extensive damage to pine plantations in Australia, New Zealand, and South America. Furthermore, individuals of some woodboring insect taxa were predominant only "Post Season". Two-thirds of *Scolytus* individuals and over 90% of wood wasp individuals were collected "Post Season".

Conclusions. Data based largely upon indigenous insects or those of a particular region, as in this case, must be used with care when extrapolating to the behaviors of exotic species. Nonetheless, some useful conclusions can be drawn:

- An EWBI rapid detection survey season of mid-March through July may detect most but not all species of Scolytidae.

- Some species of wood borers were only found after the end of July. Phenologies of some species of EWBI may thus make rapid detection unlikely if surveys are conducted only through July.

- The vast majority of individuals of *Scolytus* and wood wasps were collected after July. These, and

other, important groups of potential EWBI may be virtually overlooked by surveys terminating before August.

- If some species of WBI indigenous to Oregon are introduced into other countries or regions, they may not be readily detected by surveys terminating in the middle of local summers.

Detection of *Tetropium castaneum* L., an Exotic Longhorned Beetle in The Dalles, Oregon

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Abstract

Introduction. Concerns about the unintentional introduction of exotic woodboring insects prompted surveys of high-risk sites in Oregon beginning in 1997. Ports, port areas, mills and businesses known to have received imported wood or wood products and urban forests have been monitored statewide. These surveys have produced new state and regional records for exotic woodborers (Mudge et al. 2001).

In 2000, two *Tetropium castaneum* L. (Coleoptera: Cerambycidae) were collected from a Lindgren funnel trap placed at a railroad tie processing mill in The Dalles, Oregon (Wasco County) and identified by Steve Lingafelter (U.S. National Museum). These specimens are the first known records of this species being trapped in the U.S. *Tetropium castaneum* is a Palearctic species known from most of Europe, northeastern Asia, China, and Japan. In Europe, this species attacks stressed, dying, or recently dead spruce, fir, larch and pine. *Tetropium fuscum* (F.), which attacks stressed or dying spruce in Europe and Asia, was recently found to be attacking large numbers of live, healthy red, white and Norway spruce in Halifax, Nova Scotia.

Survey Methods. Lindgren funnel traps and bait logs have been used in surveys for exotic woodboring insects in Oregon since 1997. A set of Lindgren traps (12 funnel) was placed at either end of the mill yard. Trap locations were selected based on proximity to likely sources of introduction, availability of suitable hosts, and lack of interference with mill operations. Each set consisted of three traps, one baited with 3-component exotic bark beetle lure (cis-verbenol, methyl butenol and ipsdienol), one with alpha-pinene and ethanol (AP-EtOH), and a third with ethanol only (PheroTech®). Trap collecting cups contained 50:50 propylene glycol:water. Traps were placed and sampled biweekly from mid-February until mid-October. Each *T. castaneum* was in the same AP-EtOh-baited trap on June 5 and July 5, 2000.

In 2001, Lindgren funnel traps baited with AP-EtOh were placed at the mill site (in mid-March) and in the surrounding two square miles (in mid-April) to determine if a population of *T. castaneum* was present in this area. Due to lack of suitable hosts and good trap sites, only 15 additional traps outside the mill site were placed. Seventeen sets of sitka spruce bait logs (3 logs/set) were either paired with the additional traps or placed separately, also in mid-April. Traps were checked biweekly until October. Bait logs were removed in September and placed in emergence tubes until September 2002 for adult emergence.

Discussion. Detection of *T. castaneum* is limited by available survey tools. Visual surveys are unreliable, especially for low populations. Spruce bait logs have been used to detect *T. fuscum* in Halifax, Nova Scotia, but are labor intensive and require special rearing equipment and facilities. The lag time between oviposition and adult emergence further delays results. The effectiveness of bait logs and funnel traps as detection or delimitation tools for *T. castaneum* and other cerambycids is unknown.

Answers to several critical questions would help the ODA and USDA address the detection of *T. castaneum* and other invasive cerambycids more effectively:

1) *Where did the T. castaneum found in The Dalles come from?* Softwoods from Arkansas, Idaho, Missouri, Texas, Washington, British Columbia and Mexico have all been processed at the mill. In 1998, fumigated softwood railroad ties from Russia were received. Prior to fumigation, the ties apparently had live adult ambrosia beetles, as well as cucujid larvae under the bark of stickers. Apparently, no wood from Europe and Asia has been received within recent years.

2) *Are funnel traps effective survey tools?* Bark beetles studies indicate a very low recapture rate (0-10%) for funnel traps. Surveys with large numbers of funnel traps and sorting the resulting samples are expensive and time consuming. Trapping density guidelines and lures for specific species are lacking. Specific kairomones or pheromones will improve the effectiveness of funnel or similar traps. Despite these drawbacks, funnel (or similar) traps are one of the few detection tools available for woodborers.

3) *Were the two individuals trapped the only ones present?* Cerambycids can be captured in funnel traps. Seventy-seven species and 2,977 individuals have been collected in funnel traps in Oregon from 1997-2000. Given the low efficiency of funnel traps in capturing bark beetles, a substantial population may likely have been present for two individuals to be trapped. However, additional traps placed in 2001 caught no more *T. castaneum*. Does this mean *T. castaneum* is not now present? Bait log results will not be available until next summer.

4) *How can the efficacy of bait logs, traps and visual survey be improved?*

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Synthesis Report on Rearing Asian Longhorned Beetle

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Abstract

Since not all research on *Anoplophora glabripennis* (Motschulsky) (ALB) can be conducted in China or at North American sites where it is being eradicated, the ability to mass rear the Asian longhorned beetle is critical to rapid progress on research necessary for exclusion, detection, and eradication of this serious pest. Large numbers of beetles in all life stages are needed year round. Currently, research on rearing methods is being conducted at three primary rearing sites: Cornell University, Ithaca, NY; USDA Forest Service, Quarantine Laboratory, Ansonia, CT; and USDA Animal and Plant Health Inspection Service (APHIS) Otis Plant Protection Center, Otis Air National Guard Base (ANGB), MA. The advantages and disadvantages of the modified diets currently in use are summarized in Table 1. Current methods and diets used at the three sites are summarized in Table 2.

ALB can complete its development on all three diets in use; these are modifications of previously published diets. The formulations in use are the result of studies that compared available diets or modifications to them. Studies at Ansonia showed that use of the modified red oak borer diet reduces handling and subsequent contamination, shortens developmental time, and results in pupation that exceeds 60 percent. These studies also showed that higher levels of available iron in the diet slow larval development, increase larval mortality, and reduce percent pupation.
Larval growth and adult parameters were compared at Ithaca using artificial diets developed for three species of *Lamiini*, including one diet developed for ALB in China. The only difference in performance of larvae and adults reared on these three diet types was that nondiapausing larvae reared on *Monochamus carolinensis* (Oliver) diet (Necibi and Linit 1997) requireed less time to pupate than larvae reared on ALB diet. Comparisons of diets with cellulose and wood products, also studied at Ithaca, showed that males grew fastest on diets with sawdust or phloem-cambium and remained for the shortest period as pupae on ALB diet. Females grew faster on diets with cellulose than sawdust and lived the longest as adults on the ALB diet.

Mass rearing of ALB requires not only on a good artificial diet but one that can be dispensed into many containers relatively quickly. A twin-screw extruder is being installed at the APHIS quarantine laboratory that can dispense diets with a wider range of physical characteristics than can be accommodated with diets that must be "pourable" before fully solidifying. Diets dispensed by this machine will be tested as part of an effort to develop systems that provide appropriate larval nutrition and environment but are more resistant to degradation and can be dispensed easily.

A great concern in laboratory rearing is that the individuals reared on artificial diet may not be comparable to those reared on host material. Adults from larvae reared on the modified red oak borer diet at Ansonia have been used for both flight and oviposition studies, and performed as well or better than adults that emerged from infested wood.

We have also developed methods for holding and handling each life stage of ALB. Eggs can be allowed to hatch under the bark of oviposition bolts, though this is not always desirable because bolts can dry out quickly. This increases egg mortality, larval mortality during establishment, microbial contamination of the diet when the larvae are taken from wood, and asynchrony of larval development. As a result, methods for collecting and in vitro hatch of ALB eggs have been developed and are being used at both the Forest Service and APHIS quarantines. When larval rearing temperatures were compared at Ansonia, mortality over the first 8 weeks was higher at 20°C than at 25°C despite lower microbial contamination. Development at 25°C was more rapid than at 20°C, but there was the potential for heavier pupae at 20°C than at 25°C. Methods for retrieving and holding prepupae and pupae

Table 1.—Advantages and disadvantages of the three modified diets currently in use

Item	Modified ALB diet	Highly modified red oak borer diet	Modified tilehorned *Prionus* diet
Advantages	1) Resists microbial contamination well 2) Less frequent diet changes required 3) Fairly rapid development 4) No host material	1) No special mixing required; can be poured directly into containers 2) Fairly rapid development 3) No host material	1) No special mixing required; can be poured directly into containers 2) No host material
Disadvantages	1) Must be hand mixed and packed into containers 2) Contains ingredient that is irritating to humans	1) Diet must be changed frequently 2) Can develop microbial contamination	1) Diet must be changed frequently 2) Can develop microbial contamination 3) Fairly slow development

Table 2.—Current methods and diets used at three quarantine rearing sites

Item	Cornell University Ithaca, NY	USDA Forest Service Ansonia, CT	USDA APHIS Otis ANGB, MA
Diet in use	Modified ALB diet (Diet D, Zhao et al. 1999)	Highly modified red oak borer diet (Galford 1985)	Modified tilehorned *Prionus* diet (Diet 3; Payne et al. 1975) and modified ALB diet
Rearing temperature	22.5 ± 2.5°C	25 ± 2°C or 20 ± 2°C	23 ± 2°C
Rearing relative humidity	Larvae, pupae, and logs > 75% RH, Adults 60% RH	First 2 weeks ~100% RH; then 60 ± 5% RH	First 4 weeks ~100% RH; then 55 ± 15% RH
Larval rearing containers	59-ml plastic cups 237-ml glass jars	59-ml, 118-ml, and 237-ml plastic jars	50 ml wells in plastic trays and 237 ml glass jars
Frequency of diet changes	First change at 12 weeks, all subsequent changes at 4 weeks; diet change just prior to chill is the last	Every 2 weeks at 25°C and every 3 weeks at 20°C	Every 2 weeks (first 2 changes), then every 3 weeks
Larval chill	At 180 days chill at 5°C for 30 days	At ~100 days chill at 10°C for 112 days	None
Adults containers	926-ml (singles) or 3.9- liter (pairs) glass jars	950-ml (singles) or 3.8-liter (pairs) glass jars	2-liter clear, vinyl jars (singles) or screen cages (pairs)
Host used for adult feeding and oviposition	*Acer saccharum*	*Acer saccharum*	*Acer* spp., usually *Acer pensylvanicum*

developed at Ansonia greatly reduced eclosion abnormalities that occur when larvae pupated in the diet or were exposed to low humidity. Additionally, methods for holding adults in use at Ithaca and Ansonia have increased survival time and curtail aggressive behavior. Because some late instar larvae stopped developing and did not resume development until after a cold period, research continues at both Ithaca and Ansonia to determine ideal conditions and durations of chill.

Some research requires bioassaying even-aged and even-sized larvae, so methods to synchronize development are being sought. Methods for stockpiling eggs by chilling oviposition bolts and synchronizing egg hatch have been developed at Ansonia. These methods provide even-aged larvae and lengthen the time that small larvae are available. A chill period during the later larval instars also has been shown to shorten the time to pupation and improve the synchrony of adult eclosion.

It would be beneficial to have adults available for research year round and eliminate the need for using fresh host material for feeding and oviposition. Prototype artificial logs for oviposition have been developed at the APHIS quarantine. Logistics for making and using the logs, along with their acceptance by female beetles, is less than ideal, so additional work in this area is needed.

Because the supply of ALB for research is limited and there are few quarantine laboratories with permits for this species, researchers have sought a surrogate species that need not be quarantined. The cottonwood borer, *Plectrodera scalatoa*, a closely related native cerambycid, was chosen for this purpose. Leah Bauer and Debbie Miller (USDA Forest Service, East Lansing, MI) developed a method for rearing the cottonwood borer in the laboratory using the tilehorned *Prionus* diet.

Much progress has been made toward maintaining a readily available supply of healthy ALB for research purposes. However, to mass rear ALB, we need to: 1) Reduce the time and cost of rearing individuals from egg to adult; 2) Increase the resistance of some diets currently in use to microbial contamination; 3) Better understand the requirements for large larvae to initiate pupation; 4) Increase quarantine space and resources to rear larger numbers of beetles at some sites; 5) Develop an artificial diet and oviposition substrate for adults or methods to retain host material over the winter so that all stages are available year round; and 6) develop a method for rearing larvae on cut host material or for obtaining naturally reared individuals for studies that require them.

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Effects of Temperature on the Life History Parameters of *Anoplophora Glabripennis* (Coleoptera: Cerambycidae)

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Abstract

There is a critical need for information on the basic biology of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), to provide the biological basis for predicting developmental phenology in order to optimize the timing of exclusion and eradication treatments and to predict attack rates under different environmental conditions. In these studies, we used individuals from Bayside, NY and Ravenswood, IL to assess temperature effects on developmental rates and survival at 10°C (larvae only), 15°C, 20°C, 25°C, 30°C (egg and larvae only) and 35°C (larvae only).

There were no significant differences in female longevity between populations or temperatures. Males tended to lived longer than females at all temperatures and lived longer at 20°C than 15°C or 25°C. The time the females began laying eggs and the order of average number of eggs laid was significant: 25°C > 20°C > 15°C. Bayside females laid fewer eggs at both 20°C and 25°C than Chicago females. The order of mating success, as measured by the percentage of females that laid eggs that hatched, was: 25°C > 20°C > 15°C for both strains. The percentage of eggs that were viable did not vary between temperatures or strains, but the percentage that hatched at 15°C was significantly less than at the other temperatures. Some of the eggs laid at 15°C did not hatch until moved to 25°C. A nearly linear relationship existed between developmental rate of eggs and temperatures between 15°C and 30°C. Using this relationship, we would predict that eggs would not hatch at temperatures of 10°C or less. Based on the lower percentage hatch of viable eggs at 30°C compared to 25°C, the upper temperature at which egg development ceases and eggs die is at or above 35°C.

The minimum developmental threshold for instars 1 to 8 is projected to be close to 10°C. There is some development at 10°C, at least for early instars; about 20 percent of the larvae will molt to the second instar after about 5 months. The upper threshold for larval development, the temperature at which development stops and death occurs, is probably between 35 and 40°C. Larval survival was higher at 25°C than at lower temperatures and was zero for 35°C by the beginning of the fifth instar. Larvae held at 15°C, 30°C, and 35°C had narrower head capsules and weighed less than those held at 20°C or 25°C from the third instar. This might indicate that these temperatures are less than optimal for growth. The Chicago larvae gained weight faster than those from Bayside in the later instars.

The Mexican Pine Beetle (*Dendroctonus mexicanus*), Our "Newest" Invasive Species

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Abstract

The Mexican pine beetle, *Dendroctonus mexicanus* Hopkins (XPB), is recorded here for the first time as a new introduction for the U.S. Individuals of this species are occupying the same logs of *Pinus leiophilla* and several other pines in the Chiricahua mountains, AZ with the sibling species of XPB, the southern pine beetle, *D. frontalis* Zimmermann (SPB). Both species are also caught in Lindgren traps baited with southern- and western pine beetle attractants, both of which contain the pheromone frontalure. XPB outnumbered SPB 20-1 in the traps, perhaps because XPB is better adapted to the 2000m elevations. Both XPB and SPB are highly destructive to pines, and XPB could pose a real threat if accidently introduced to pines the higher elevations of the the eastern U.S.

Transparency for International Trade

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U.S. Department of Agriculture - Animal and Plant Health Inspection Service – Plant Protection and Quarantine (USDA-APHIS-PPQ) has developed a Regulated Plant Pest List (RPPL). This provides trading partners with an official list of plant pests of concern to the U.S., along with providing greater transparency of Agency actions. The rationale is that through greater transparency, better information is made available, and unjustified phytosanitary trade barriers will be revealed, challenged, and eliminated. Additionally, the RPPL provides focus to APHIS' safeguarding activities, including inspection of commodities for pests in its pre-clearance programs and at U.S. ports-of-entry, surveys for exotic pests, methods for pest risk mitigation, and pest eradication programs.

The list was derived from pests identified in Title 7, Code of Federal Regulations, Parts 300-399. In addition, due to changes in pest status or new information, certain pests detected through inspection or survey may no longer be regulated. The list was updated to reflect recent taxonomic nomenclature and pest status. APHIS continually detects threatening new pests through its inspection and survey activities. Therefore, the RPPL does not include all pests for which APHIS would necessarily take action.

The RPPL is a living document to which additional species may be added as they are identified and their pest status documented. Pathways for pest taxa being added to or deleted from the RPPL are:

Pathway 1) Regulated pests frequently intercepted from imported agricultural commodities

Pathway 2) Regulated pests identified by APHIS or stakeholders as potentially causing serious economic or environmental damage in the United States.

Candidates for the PPQ Pest List are limited to regulated pest taxa as defined by the International Plant Protection Convention of the Food and Agricultural Organization.

Activity Patterns of Adult *Anoplophora glabripennis* in China

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Abstract

Activity patterns of adult Asian longhorned beetles (ALB), *Anoplophora glabripennis* (Motschulsky) were monitored in a shelterbelt of *Populus* and *Ulmus* spp. in Ningxia A. R., P. R. of China. Trees were searched daily at each of four time periods (0800-1100, 1100-1300, 1400-1600, and 1700-1900 hours) on each of 18 d during July and August, 2001. At each check, the following data were taken on all adult beetles that were located: sex, type of activity, substrate (stem >2 cm diameter, twig, leaf), height off of the ground, height of tree, and whether the beetle was in sunlight or shade. Data were recorded on averages of 9.7 ± 2.8 (S.D.) females (total = 1398) and 16.2 ± 3.6 males (total =2333) per observation period.

Environmental conditions appeared to affect choice of microhabitat by the beetles. The beetles showed a strong tendency to remain in the shade; also percentages of beetles in direct sunlight decreased from 17-20% (overall) at temperatures of 25° C and below to <5% when temperatures were 29°C or higher ($P < 0.01$, Kruskal-Wallis test). Beetles also showed a lesser (though significant) tendency to be found lower on trees (measured as percentage of tree height) during the hotter 1100-1300 and 1400-1600 hour periods than at other times of day (ANOVA; *d.f.* = 3, 51; $P < 0.01$). There were also some significant differences in percentages of beetles on different substrates at different times of day, although most beetles (65-85%) were on larger stems, with higher proportions of the remaining insects on twigs than on leaves, at all time periods.

At all times of day, more beetles were observed resting (includes feeding) than in any other activity. Roughly half of the males were either walking or participating in mating or mate-guarding at all time periods. Almost half of females observed in this study were either mating or being mate guarded, and this activity peaked in the 1400-1600 period (53% of females). Females spent approximately 20% of their time chewing oviposition pits and 5% ovipositing.

Overall activity levels of beetles and their choice of microhabitat suggest that spot-applied insecticides on limbs or within-tree traps could potentially be valuable to ALB eradication programs. Such systems could be either “passive” or incorporate short-range attractants.

Anoplophora chinensis Introduction in Tukwila, Washington

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Abstract

The apparent introduction of several adult Citrus longhorned beetles, *Anoplophora chinensis* (= *malasiaca*) (Forster), was recently documented at a bonsai nursery at Tukwila, Washington. In August 2001, the owner of the nursery called plant health officials with reports of what he believed to be *Anoplophora glabripennis* (Motschulsky), the Asian longhorned beetle that has been found in the New York and Chicago areas. Washington State Department of Agriculture (WSDA) personnel went to the site and obtained the suspect beetle along with two additional beetles that they found on bonsai *Acer*. The *Acer* were being held outdoors in a fenced-in "post-entry quarantine" (designed for disease, not insects). A fourth beetle was heard (then seen) taking flight but unfortunately escaped the nursery and flew into a nearby wooded area. The 369 *Acer* were subsequently examined and then destroyed. In total, eight exit holes and substantial adult feeding damage were discovered, indicating that up to five beetles escaped into the surrounding area. Examination of the spermatheca of the one female that was recovered suggested that she had been mated.

A. chinensis, like *A. glabripennis*, is a polyphagous pest of forest and shade trees that attacks primarily broadleaves. Unlike many cerambycids, these beetles are quite capable of attacking (and subsequently killing) live, apparently healthy trees. The host range of *A. chinensis* is quite broad, including maples, alders, willows, citrus, *Casuarina*, elms, and trees from many additional taxa. Their geographic range tends to be more tropical than that of *A. glabripennis* but still extends well into areas where freezing temperatures are common in winter. *A. chinensis* tends to attack lower on trees than does *glabripennis*, and its oviposition scars are, if anything, more difficult to detect. Historically, *A. chinensis* has entered the United States primarily in imported bonsai and/or penjing materials rather than in solid wood packing materials.

A. chinensis can likely survive well in Seattle's climate and can potentially feed on locally dominant woodland trees, including *Alnus* and *Acer* spp. As a result, WSDA and APHIS-PPQ believe that there is a significant chance that this introduction will result in the local establishment of a population if measures are not taken to prevent it. Economic and ecological consequences of establishment would likely be dire (at least as serious as the establishment of *A. glabripennis* would be in that area). WSDA and PPQ convened a Science Advisory Panel to provide technical information on the pest, and WSDA established a quarantine zone around the site of the infestation. The agencies secured initial funding are currently planning a program that will include survey, preventative treatment, and public outreach components.

APHIS (PPQ) Exotic Pest Detection

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Abstract

The legally mandated responsibilities of APHIS Plant Protection and Quarantine (PPQ) include: (1) Protect American agriculture from foreign plant pest introduction and establishment, (2) facilitate export of American agricultural products, and (3) control or eradicate pests as authorized by legislation and regulation. To that end, PPQ strives to minimize risk associated with introductions of exotic pests into the United States by: (1) excluding those pests through quarantines and related activities, (2) detecting any populations of newly established exotic pests, and (3) mitigating the effects of newly established pests through eradication or other management options.

APHIS-PPQ has a number of programs and activities designed to detect incipient populations of foreign agricultural pests. These include:

1. Cooperative Agricultural Pest Survey (CAPS) programs, which includes most of the exotic pest trapping and other survey activities that are run cooperatively with state and local agencies.

2. Fruit fly detection programs.

3. Survey portions of emergency programs, conducted to determine the extent of newly detected infestations and track the effectiveness of mitigation efforts.

4. Public outreach and awareness, though such avenues as fliers, participation in meetings, working with growers or other industry groups, PSA's, etc.

The CAPS program includes surveys for pests not known to occur in the U.S. as well as the survey components of domestic programs such as gypsy moth, Japanese beetle, imported fire, pink bollworm, and noxious weeds. Data from these survey efforts are entered into the NAPIS database (National Agricultural Pest Information System). The CAPS program provides funding, coordination, and data support for exotic pest surveys, although funding support has been less than ideal.

In the early 1980s, CAPS was envisioned as a system for national coordination of all pest surveys. This vision proved to be overly ambitious, and in 1992, CAPS was redirected toward the following goals: (1) detect exotic pests before they can become well established, (2) facilitate the export of U.S. agricultural products, and (3) collect and manage survey data from PPQ cooperative programs. Along with the NAPIS database, the CAPS system includes a 3-tiered committee system: a national committee, two regional committees (corresponding to PPQ's Eastern and Western Regions), and state committees, along with their associated trapping teams and efforts. The National and Regional Committees are responsible for prioritizing and coordinating CAPS survey work, and the State Committees design and implement surveys within their states. The Committee system is currently being reorganized and "upgraded" (see below), so specific responsibilities of the different committees are still being decided and delegated.

Funding for the CAPS program comes from a variety of sources. Much of the exotic pest survey money comes from a Congressional line item ("Pest Detection") that goes to PPQ and is administered, at present, by the APHIS regions with input from the committee system. Additional CAPS funding comes from monies that are targeted for the management, containment, or exclusion of specific pests, such as gypsy moth, imported fire ant, and pink bollworm.

Programs to detect incursions of exotic tephritid fruit flies are, in a sense, part of the CAPS effort in that data from those programs are entered into the NAPIS data base. Fruit fly detection programs, though, are relatively autonomous, large, and well-organized, and they are funded through appropriations that are specific to protecting American agriculture from exotic tephritids. Four categories of trapping systems are included: (1) trimedlure-baited traps for *Ceratitis* spp. such as medfly and Natal fruit fly, (2) methyl-eugenol-baited traps for oriental fruit fly and other closely related *Bactrocera* spp., (3) traps for cuelure-responding *Bactrocera* spp. such as melon fly, and (4) food-lure-baited traps (either liquid protein lure or synthetic food odors) for species that don't respond to the other lures. Species in the last category include, among others, *Anastrepha* spp. such as the Mexican, Caribbean and South American fruit fly. Trapping for exotic tephritids is concentrated in Florida, California, and Texas, although programs are also carried out in high-risk portions of other southern states. The effort is concentrated on residential areas, which is where risk of introduction of exotic tephritids is greatest. Fruit fly trapping protocols are designed to detect incipient tephritid populations while they are still small enough to be eradicated. The intensity (cost) of fruit fly trapping is balanced against potential program, environmental, and political costs associated with eradication, with the understanding that higher survey effort will find populations when they are smaller and thus reduce eradication costs. Over the past 25 years, resistance to aerial applications of insecticide bait sprays has increased continually, leaving releases of sterile insects as the eradication strategy of choice for medfly and Mexican fruit fly. Because sterile releases work best against small, isolated infestations, this trend has placed a premium on having a highly sensitive detection program. As a result, medfly traps, for example, are now maintained at 10 traps per square mile throughout thousands of square miles of high-risk areas.

Recently, the National Plant Board coordinated a review to identify ways that APHIS-PPQ could improve its ability to protect American agriculture from exotic pests and ensure that produce from the U.S. would be accepted in overseas markets. The resulting report, "Safeguarding American Plant Resources," detailed over 300 recommendations for changes to PPQ's structure, priorities, and mode of operation. PPQ formed a series of committees to consider the recommendations and, where appropriate, develop action plans for their implementation.

The Safeguarding recommendations included several suggestions on how to enhance PPQ's capability to detect newly established populations of invasive pests. Action plans for most of these suggestions have been developed, and some are in the implementation phase. These recommendations included: develop a national surveillance system, establish a National Survey Coordinator within PPQ, establish a 3-tiered committee system to coordinate and prioritize survey activities, and develop a broad-based pest database that would contain information on pest biology and risk, interception data, survey methodology, survey results, emergency action plans, and other pertinent information. To a degree, these components of the safeguarding system already existed, but they need to be upgraded and, in some cases, better organized to meet the standards set in the Safeguarding Report. Other suggestions included ideas on incorporating pest risk information into the design and coordination of detection programs, and how to ensure that appropriate guidelines are available for conducting surveys. In the end, though, PPQ's ability to enhance its pest detection capability will be limited by funding. Temporary funds that will allow PPQ to start implementing improvements to its pest detection capabilities are available, but more permanent funding that will allow for sustained enhancements of the survey effort has yet to be secured.

Mapping Forest Risk Associated with Beech Bark Disease

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Abstract

Beech bark disease is an alien pest species complex consisting of the scale insect, *Cryptococcus fagisuga*, and at least two species of *Nectria* fungi, *N. coccinea* var. *faginata* and *N. galligena*. The scales typically achieve large numbers feeding on sap in the inner bark and allow the pathogenic *Nectria* fungi to invade the xylem, often resulting in dieback or tree mortality. The disease was apparently introduced to North America near Halifax around 1890 and has been slowly expanding its range. As this disease invades new areas, large proportions of American Beech, *Fagus grandifolia*, are often killed. In order to plan for the management of beech bark disease in the future, there is a need to delimit the distribution of susceptible stands in areas that are currently uninfested. American Beech has a very large range and beech bark disease has only invaded a fraction of that area. While the greatest concentrations of this tree species occur in northeastern North America, this species exists through much of the Southeast as well. We expect that the impacts of this disease are likely to increase in the future.

Mapping Forest Risk Associated with the Gypsy Moth

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Abstract

The gypsy moth was originally introduced near Boston in 1868 or 1869; it has been slowly expanding its range mostly to the south and west. Its slow spread through the Northeast can be attributed to the limited dispersal capabilities of this insect (females do not fly). It is inevitable that the gypsy moth will continue to spread to the south and east over the next century. In order to plan for the management of the gypsy moth over the next decade and beyond, there is a need to delimit the distribution of susceptible stands in areas that are currently uninfested.

The gypsy moth is a polyphagous insect; North American populations feed on over 300 different shrub and tree species. Despite this wide breadth of host preference, there is considerable variation among trees in their susceptibility to defoliation; the most preferred gypsy moth hosts are in the genus *Quercus*, *Populus*, and *Larix*. We used USDA Forest Service Forest Inventory and Analysis (FIA) data to calculate the proportion of basal area at each plot that is composed of susceptible species. The areas with the highest concentration of susceptible forests were in the central and southern Appalachians, the Cumberland Plateau, the Ozark Mountains, and the northwestern lake states. The finding that the gypsy moth has not yet invaded most of the susceptible forests in the US suggests that there still may be considerable value in limiting the future spread of the gypsy moth. It also indicates that both the impacts of defoliation and costs of gypsy moth management are likely to increase in the future.

Mapping Forest Risk Associated with the Hemlock Woolly Adelgid

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Abstract

The hemlock woolly adelgid, *Adelges tsugae*, is native to Asia and was first introduced to North America in British Columbia in the 1920s and was later discovered in the Shenandoah Mountains of Virginia in the 1950s. It has gradually been expanding its range, largely to the North. Adelgids feed by sucking sap from hemlock twigs and when they reach very high densities they can cause dieback and mortality of their hosts. In the eastern U.S., the adelgids' principal host is Eastern hemlock, *Tsuga canadensis*. The range of this species is largely limited to moist, cool sites; it is most abundant in the New England states. The adelgid has only recently invaded southern New England and is now poised to expand its range into northern New England. Thus, the hemlock wooly adelgid is likely to cause considerable damage in the future as it expands into areas with large quantities of hemlock.

False Indigo (*Amorpha fruticosa* L.) – An Invasive Plant Species in Croatia: Control Strategies

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Abstract

False indigo (*Amorpha fruticosa* L.), is a shrub that reaches up to 5 m in height. Originally from North America, it was introduced to Europe in 1724. It covers large areas of lowland oak forests and makes the regeneration of these forests more difficult and expensive. Control by cutting provides poor results. Systemic herbicides can be applied only in areas where there are no young oak plants since they also are susceptible to herbicides. However, polypropylene tree shelters, combined with a reduced use of herbicides, provides excellent results. This method is both economically and ecologically acceptable, and does not require much human labor. Furthermore, the shelters prevent oak seedlings from being attacked by oak mildew (*Microsphaera alphitoides* Griff et Maubl.) or by defoliating caterpillars thus eliminating the need to apply fungicides and insecticides.

Spruce Aphid in High Elevation Habitats in the Southwest U.S.

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Abstract

Spruce aphid, *Elatobium abietinum* (Walker) (Homoptera: Aphididae), is a new invasive pest in the interior Southwestern United States. This insect is causing extensive and severe damage on dormant Engelmann spruce, *Picea engelmannii* Parry, and Colorado blue spruce, *P. pungens* Engelm., in high elevation forests in late fall and winter. Engelmann spruce is more susceptible than is Colorado blue spruce. Average mortality on heavily defoliated plots is 28-42%, with 100% mortality on some plots. In other regions, where the insect develops high density populations in the spring, it's mostly parthenogenic population dynamics and damage are limited to areas where temperatures seldom fall below freezing. In the interior Southwestern U.S., populations increase in the fall, and a sexual life cycle and greater cold-hardiness are possible factors contributing to the insects success under more severe conditions. Outbreaks appear to be associated with dry winter and spring weather prior to the fall and winter in which feeding occurs.

Invasive History

In 1976, spruce aphid was found in urban Santa Fe, New Mexico, 1300 km from the Pacific coast. A wildland outbreak occurred over the 1987-1988 winter in the White Mountains of Arizona, the first outbreak documented in interior wildland forests. Since then the spruce aphid range has expanded to five mountain ranges in Arizona and New Mexico, and three of the last six winters have incurred outbreaks (Lynch *et al*. in press). The 1999-2000 outbreaks were alarming for their severity and extent. In that year, approximately 57,000 ha in the White Mountains were defoliated, with severe defoliation on 9,300 ha. At the same time, 120 km to the south, the first outbreak in the Pinaleño Mountains severely defoliated two-thirds of the host type in a single season.

The host species in the Southwest U.S. are Engelmann spruce and Colorado blue spruce. Both species occur naturally in mixed-conifer and spruce-fir forests above 2400 m. Both species, but especially Colorado blue spruce, are commonly planted in urban forests.

Spruce aphid, known in Europe as green spruce aphid, has a history of causing problems in maritime forests, principally in the United Kingdom, Fennoscandia, Denmark, Iceland, and the North American Pacific Northwest coastal areas (Day *et al.* 1998). In those areas the most important hosts are Sitka spruce, *P. sitchensis* (Bong.) Carr., white spruce, *P. glauca* (Moench) Voss, and Norway spruce *P. abies* (L.) Karst. In many areas, both the host species and the insect are exotic.

Spruce aphid has been found in North America since 1916 (Carter & Halldórsson 1998, Keen 1939, Koot & Ruth 1971), probably introduced from Europe (Bejer-Petersen 1962, Carter & Halldórsson 1998). On Sitka spruce in coastal forests of western North America, spruce aphid has been a chronic pest, but occasionally causes severe tree mortality in local areas (Koot 1992). Recently there has been some concern that the spruce aphid problem has become more extensive and severe in the North American Pacific Northwest area, contributing to decline in shore-line stands as well as defoliating stands further inland and at higher elevations than in the past.

Life Cycle And Weather

In maritime climates, spruce aphid population dynamics are limited by cold temperatures, with starvation occurring below 6°C, cold damage below 0°C, and freezing at -5°C. Outbreaks do not occur in years or places with any monthly mean temperature falling below freezing or with ambient temperature falling below -7 to -14°C (different temperatures are specified by different authors). Damage occurs from high density populations that develop parthenogenically in the late winter and spring (see various papers and references cited in Day *et al.* 1998). A critical factor in population dynamics is that aphid populations increase to damaging levels only on dormant trees.

In the Southwestern U.S. mountains, ambient and monthly mean temperatures fall well below the temperatures that limit aphid populations in maritime climates. Population increases occur in the fall and early winter, and populations may persist over the winter. Although population increases appear to be from parthenogenic reproduction, numerous sexual males are produced each year, especially in early winter (late November or December). I assume that cold-hardy eggs are produced by the sexual forms, and that this stage allows survival during the coldest winter temperatures. A holocyclic life history prevails in Scandinavia and in the native territory in Germany, where the insect is mostly innocuous (Bejer-Petersen 1962; Bevan 1966; Carter & Halldórsson 1998).

The production of cold-hardy eggs could explain population persistence in mountain areas with cold winters, but does not explain population increase and survival during the fall. Aphid populations in the Southwest U.S. are clearly surviving, increasing, and causing severe defoliation and related tree mortality under conditions that maritime populations cannot. Mountain populations appear to exhibit additional cold-hardiness than do maritime populations. At this time I do not know what contributes to the additional cold-hardiness, but suspect a) that individuals develop more cold-hardiness under fluctuating diurnal temperatures, b) that host foliage chemistry may provide additional protection (aphids lose some cold-hardiness after feeding on Sitka spruce, and Engelmann spruce is much more cold-hardy than Sitka spruce), or c) a genetic change has occurred. Understanding the mechanisms behind spruce aphid cold-hardiness is critical to determining how much of the Engelmann spruce range is at risk from this insect.

In the mountains, there is a tendency for spruce aphid outbreaks to follow dry winter and spring weather (Fig. 1). This tendency does not appear to be influenced by the amount of moisture received later during the summer monsoon. Spruce aphid populations increase when the host trees are dormant, both in maritime climate (in the spring) and in temperate climate (in the fall). I speculate that minimal snowpack accumulation, or early loss of snowpack, affects soil temperature such that tree phenology is affected: early soil warming cause trees to break dormancy early, subsequently causing them to enter dormancy early in the fall. This would provide dormant foliage as a food resource over a sufficiently long, and relatively warm, period for populations to increase before extremely low temperatures occur. Also, moisture stress early in the growing season might alter foliage chemistry so that it is a more suitable food resource. In all likelihood, susceptibility is related to a combination of factors including prior winter and spring weather, autumn temperature regimes, host response to previous feeding, and the size of the pre-existing spruce aphid population.

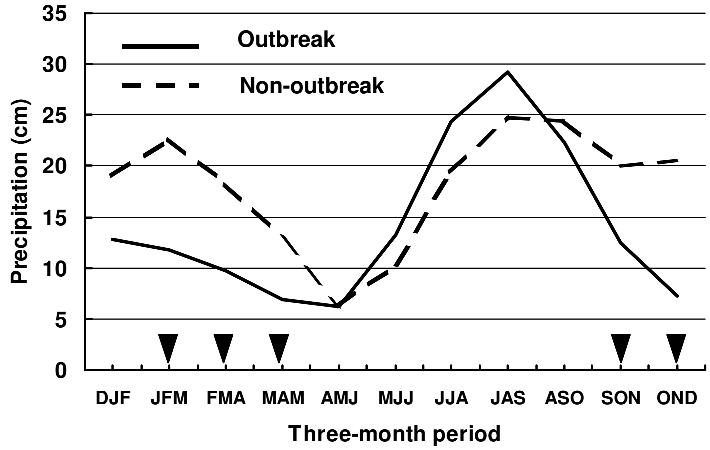

Figure 1.—Three-month running average of precipitation in the White Mountains of Arizona, for years with and without fall spruce aphid outbreaks (DJF through JJA would precede the outbreak) (1981-2001). Triangles indicate significantly different means ($P < 0.10$).

Tree and Stand Damage

Defoliation is variable from watershed to watershed, with less variability within each stand. Individual tree defoliation frequently approaches 100%. All size and age classes of trees are affected, and Engelmann spruce is more susceptible than Colorado blue spruce (Fig. 2). On trees 4 cm or larger in dbh, tree size does not influence the severity of defoliation (Fig. 2). Quantitative data on regeneration size classes is inadequate, but reconnaissance indicates that seedlings and saplings are more severely defoliated than larger trees. In the White Mountains, average plot mortality on heavily defoliated plots (mean defoliation index of 6 or greater, a rating that indicated that all crown thirds were 34% or more defoliated) was 28-42% three years after defoliation, with 100% mortality on some plots. On an individual tree basis, those most likely to die (67% mortality) were spruce of either species with severe defoliation plus heavy infections of spruce dwarf mistletoe, *Arceuthobium microcarpum* (Engelmann) Hawksworth & Wiens (Viscaceae).

Management concerns include resource values affected by tree mortality (particularly timber and recreation), increased risk of wildfire, increased risk of bark beetle outbreak, and impacts to wildlife habitats. In the long run, the possibility of repeated events leads to concerns about the loss of established regeneration and anticipated failure of defoliated trees to set viable seed, and subsequent effects on tree population dynamics.

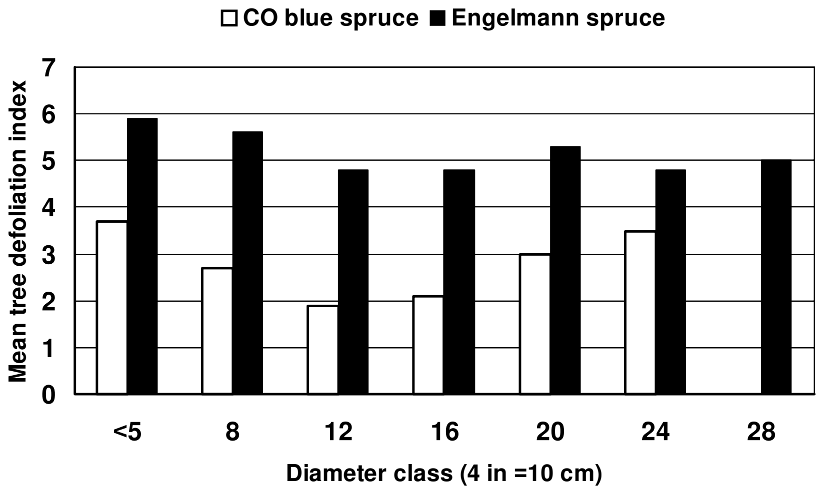

Figure 2.—Defoliation of spruce with dbh of 4 cm or greater in the White Mountains. Over the 1996-1997 outbreak. Defoliation Index is a sum of three ratings, where each crown third is rated as 0, 1, 2, or 3, by 33% defoliation (an index of 9 indicates that each crown third was 67-100% defoliated).

Future Prospects

If outbreak frequency is low, then many trees should be able to survive and reproduce. Spruce aphid feeds on foliage that was produced in the growing season prior to autumn feeding (years *n*-1, *n*-2, etc.). The current year's foliage (year *n*) is not a suitable food resource until at least late autumn. This feeding pattern allows trees to gather nutrients and set buds prior to defoliation. In some less severe episodes, not all of the year *n* foliage is lost. Therefore, in year *n*+1, buds will flush on surviving trees and branches, and some of year *n* foliage might be retained as well, providing a photosynthetic base until the next defoliation episode. The fate of spruce, particularly Engelmann spruce, is dependent upon the frequency of spruce aphid outbreaks. Repetitive outbreaks will result in extensive mortality, loss of established regeneration, and loss of reproductive potential. Outbreaks can occur two years in a row in the same general area, but I do not yet know if the same trees are heavily defoliated in both outbreaks.

Research is needed on this insect, focusing on insect biology, population dynamics, insect-host physiology, impact, disturbance ecology, and host resistance. Population dynamics on interior host species under mountain conditions are obviously different than population dynamics on coastal species under maritime climatic conditions. The association of epizootic population behavior with prior weather conditions makes it likely that outbreaks can be predicted 6 months in the future with good confidence, and even longer with less confidence. Such prediction would require a more detailed model than "aphid outbreaks tend to follow dry winters", but is realistically attainable and would allow managers take protective action. This information would also allow us to evaluate the frequency of outbreaks that is likely under contemporary climatic patterns. Population dynamics need to be modeled in both ecosystems, and the weather and cold hardiness mechanisms determined Insect-host interaction research should focus on insect cold-hardiness and reproductive capacity and on foliage phenology, chemistry, and seasonal suitability as a food resource. Sap sucking causes additional tree damage than does defoliation from a leaf chewing insect, as the tree replenishes and subsequently loses more sap before the needles die. Impact assessment studies need to quantify the relationships between feeding pressure, needle death, and tree mortality. A metric for relating a relatively easy field observation to the likelihood of tree mortality is needed. Local risk and hazard conditions as well as geographic areas of the host range that are at risk need to be identified. The impact of defoliation on seed production and viability needs to be known in order to project long-term effects of this new disturbance agent to mixed-conifer and spruce-fir disturbance ecology. Resistant trees should be identified (there appear to be some, but spruce beetle outbreaks threaten mature individuals), and used either for developing planting stock or for a tree improvement program. Also, coastal aphid populations should be evaluated to determine if the greater extent and severity of damage seen in those areas is due to altered population genetic character or to weather patterns.

This insect will likely impact the natural disturbance regimes in mixed-conifer and spruce-fir forests. It will alter fuels accumulation, bark beetle hazard, and future stand development. Long-term stand effects may be severe. Repeated defoliation events on all size classes of trees will not only remove regeneration, but will in all likelihood prevent viable seed from being produced. If this is the case, Engelmann spruce representation in the forest will be greatly diminished over time.

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Asian Longhorned Beetle Cooperative Eradication Program Program Accomplishments 2001

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Abstract

APHIS spent approximately $3 million to continue ALB eradication activities in New York and Illinois in FY 2001. In New York 6,615 trees were removed, over 4,500trees replanted, and approximately 121 square miles were under quarantine. In Illinois 1,529 trees were removed, over 2,200 trees replanted, and approximately 31 square miles were under quarantine. Surveys conducted through December 2001 resulted in only 616detection's of infested trees (547 in New York and 69 in Illinois). In NY, APHIS began trunk injection treatments with the systemic insecticide, Imidacloprid, this year. The outermost boundaries of the infested areas were targeted for the NY treatment. In IL, a second year of treatment was completed, with all infested areas receiving treatment. There were 23,740 trees treated in NY and 35,490 in IL.

A very aggressive treatment program is planned for 2002. The program is expanding the treatment area from 1/8 mile beyond an infested area to ¼ mile. In addition, soil injection treatment will be used for the first time operationally within limited areas of the IL eradication project. Over 40,000 trees are planned for treatment in IL and over 111,000 trees in the NY infested area.

Areas targeted for survey were increased in 2001 and will continue in 2002. The survey area now extends to a 1-½ mile radius outside the infested core. In addition, surveys were completed primarily with bucket trucks and tree climbers. APHIS implemented five survey contracts in New York and one in IL. However, due to a delay in funding and the contracting process, the contracts were implemented after mid year. Table 1 shows the survey results in positive trees detected each program year.

Table 1.—ALB-Infested Trees

State	FY 1996	FY 1997	FY 1998	FY 1999	FY 2000	FY 2001
NY	812	841	978	1,798	715	547
IL	0	0	521	728	209	69
Total	812	841	1,499	2,526	924	616

As a result of expanded surveys, the size of the infested area has increased. Approximately 21 square miles of new infested area (Table 2) was discovered in FY 2001.

Table 2.—Size Of ALB-infested Areas (NY & IL) (in square miles)

FY 1997	FY 1998	FY 1999	FY 2000	FY 2001
70	113	149	157	178

The regulated boundaries increased in November 2001. In NY, 19 square miles were added for a total of 121 square miles. The regulated area boundary expansion now covers the width of the lower end of Manhattan and includes the area between the Brooklyn/Queens regulated area and the Bayside/Flushing regulated area. In Illinois, the total regulated square miles is 31, an increase of 9 from FY2000. The regulated area in Illinois now covers primarily the Ravenswood, Loyola, and Kilbourn Park areas on the north side of Chicago and the suburbs of Addison, Summit, Park Ridge, and an area near O'Hare Airport.

ALB Research is a joint effort of ARS, APHIS, FS, and several State Universities, in cooperation with Chinese research institutions. A concerted effort was made this year to align all research activities toward the eradication goals. The following research efforts should be available for operational use within the next few years.

1) Acoustical Detection Devices – To hear beetles chewing through the wood. This technology, which is being researched by FS and ARS, could be used for delimiting around infested trees. Removing trees earlier will hasten eradication. Field-testing from

2001 yielded promising results for a prototype in the spring of 2002.

2) Entomopathogens – A fungal pathogen, *Beauvaria brongniartii*, is being used to control *Anoplophora malasiaca/chinensis* in Japan and has shown promise against ALB in laboratory studies. A Cornell researcher has placed commercially prepared bands impregnated with the pathogen on NYC trees in the summer of 2001 as part of a larger project to develop this technology for use against ALB. This could be operational next flight season if efficacy, operational, and environmental issues are satisfied.

3) New Chemicals – Several new chemicals and formulations are being tested to replace Imidacloprid if necessary, or for specialized uses such as forested areas or potted plants on rooftops.

4) New Application Methods – Imidacloprid spikes or drenches for potted plants should be available in the summer of 2002. The program is also developing a spot treatment, but operational use is several years away.

Present Situation of *Anoplophora malasiaca* Thomson Occurring in Italy

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Abstract

The enormous increase in the international trade of goods and plants has also increased the probability that pests and pathogens can spread from their native habitats to new countries throughout the world. This fact is demonstrated in the literature and is of increasing concern to regulatory officials. Because of the difficulty associated with conducting phytosanitary inspections at borders and at ports of entry, the Phytosanitary Service of Regione Lombardia provided financial support for research to develop a post-entry survey program that addresses "New pest identification and monitoring". This program was initiated in 2000 and has allowed us to identify seven new pests, some of which are new introductions to Italy and Europe. The most important pest discovered was *Anoplophora malasiaca* which is included on a quarantine list for Europe. Its presence represents a serious threat for both the ornamental nursery industry and for native tree species.

The first detection of this pest occurred in a nursery located in Parabiago, in the vicinity of Milan, which trades with China mainly for importation of bonsai. During inspections carried out over a quarantine period of three months by the Phytosanitary Service, devoted mainly to detect and identify parasitic nematodes, other pests and pathogens, we observed exit holes made by adults of *A. malasiaca* on *Malus cerasifera* bonsai. After the first detection, a specific survey program was initiated which consisted of setting wire mesh traps in the ornamental nursery fields and inspecting six other nurseries to look for the damage and presence of this pest. *A. malasiaca* was collected only in the Parabiago nursery and in the immediate vicinity on *Acer* spp., *Fagus sylvatica*, *Platanus acerifolia*, *Carpinus betulus*, *Quercus robur*, *Aesculus hyppocastanum*, *Lagestroemia indica* and *Zizyphus sativa*, most of which are species commonly used for plantings in gardens and parks.

This presentation provides information on the current situation and distribution of *A. malasiaca* in Lombardy and provides initial data about the life cycle and emergence period of adults. Logs, both with and without exit holes, were dissected to destroy infested material and to provide us with more information on the biology and damage associated with this pest. Preliminary results of the survey program indicate that dispersal of *A. malasiaca* is rather limited however more investigations are needed, mainly to determine its occurrence on native ornamental species. Phytosanitary measures will be implemented in cooperation with Regional and National Phytosanitary Services after additional studies are conducted on the biology and distribution of this pest and we determine the effectiveness of chemical control techniques. Extension service and outreach activities should be improved in order to enhance the education and involvement of growers and citizens about the significance of this pest; this will be facilitated by publication of technical sheets and bulletins and their placement on the Lombardy Agricultural Department web site.

The Ecology of Garlic Mustard in Eastern Hardwood Forests

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Abstract

Upwards of 25% of the plant species in many eastern floras have been introduced. Many of these species have become invasive. Many of the worst invasives are native to Eurasia and were brought to the U.S. either intentionally for forage, fiber production, erosion control, ornamentals, or unintentionally via crop seed contaminant, transportation, or nursery stock. Typical examples include multiflora rose (*Rosa multiflora* Thunb.; Rosaceae), kudzu (*Pueraria lobata* (Willd.) Ohwi; Fabaceae), autumn olive (*Eleagnus umbellata* Thunb.; Eleagnaceae), purple loosestrife (*Lythrum salicaria* L.; Lythraceae) and Japanese and Amur honeysuckles (*Lonicera japonica* Thunb. and *L. maackii* (Rupr.) Maxim.; Caprifoliaceae). New species are being constantly added to the list as botanists and ecologists recognize new and emerging threats.

Attempts to characterize the life-history of these species has been hindered by the broad array of species and different phylogenetic origins. Many, but not all, of the most problematic species have small, easily dispersed seed that has few germination requirements and is able to maintain a seedbank once established. These species often exhibit rapid growth, vegetative reproduction, and considerable phenotypic plasticity. In addition, the pollination syndrome is often generalized or abiotic, and many of the species are self-compatible. All of these traits aid in the establishment and growth of these non-native species in their new environments.

Likewise, attempts to characterize and make generalizations regarding invaded habitats is also complicated by the individualistic nature of the species and habitats involved. Some have suggested that old-growth forests may be less susceptible to invasion due to increased stability. Ecosystems with frequent breaks in their cover, due to natural or anthropogenic disturbances, may be more susceptible to invasion. The data to support these suggestions and observations is scant.

Garlic mustard (*Alliaria petiolata* [Bieb.] Cavara & Grande; Brassicaceae) is an invasive species that has now made it on to many of the eastern U.S. most problematic species lists. It shows many of the characteristics of weedy species, including: self-compatibility, high fecundity (5,000-20,000 seeds m^{-2}), seed viability >95%, dispersal by gravity & water, maintains a seedbank from 1-5 years, and requires only 100d of cold stratification for germination (most likely factor limiting southern migration). Throughout most of the U.S., the species has an obligate biennial life-cycle. The seeds are dispersed during mid-summer, they are cold-stratified over the following winter and germinate in the early spring; during their first summer they grow as a basal rosette and maintain that form over the second winter; they will bolt during the spring of the following year, flower, set seed, and repeat the cycle. Curiously, the species has a growth habit which is completely different in its natural and invaded ranges. In its natural range, the species usually grows in a solitary fashion and is quite unassuming. In the eastern U.S., the species often grows quite robustly in very dense patches and will spread to occupy large patches in a variety of shaded and open habitats.

My lab embarked upon a series of studies to evaluate various aspects of the ecology, genetics, and biogeography of garlic mustard. One of my first studies with this species (McCarthy 1997) was designed to evaluate the community level impact of this species in a heavily infested floodplain. Garlic mustard was removed from paired plots (vs. control) and the response in understory species diversity and abundance monitored for three years. Plots with garlic mustard removed showed an increase in native species diversity and overall compositional change. Responses were most pronounced among annuals and woody perennials (the latter was unexpected). This experiment demonstrated community level suppression of native species by garlic mustard. Subsequent experiments were designed to evaluate the mode of interference (competition vs. allelopathy).

We assessed the allelopathic potential of garlic mustard on seed germination and seedling establishment via two experiments utilizing a dilution series of shoot and root extracts (McCarthy and Hanson 1998). Regardless of extract concentration or seed type, seed germination was largely unaffected by garlic mustard extracts. Only one target species exhibited slight inhibition of seedling growth when watered with the most concentrated

dilution of garlic mustard extract. Overall, we concluded that allelopathy does not have a strong direct effect in its interference with other species. However, a subsequent competition experiment did demonstrate a strong competitive effect of this species on native species, even amongst newly germinating oak seedlings (Meekins and McCarthy 1999). Garlic mustard was shown to be very aggressive and able to capture considerable resources.

Subsequently, we embarked upon a molecular-based population genetic and biogeographic study to evaluate the likely origin of garlic mustard and to assess its genetic diversity (Meekins et al. 2001). Using inter-simple-sequence repeat (ISSR) analysis of populations from Europe and the U.S. we found that there was a strong match between our Scottish (putative source) population and many populations in the central Appalachians. But, there have likely been a number of introductions from different sources. We also found strong population structuring, with the greatest variance among populations (61%) compared to between continents (16%) and within populations (23%).

The two most recent studies on garlic mustard were designed to evaluate what resource levels garlic mustard requires to grow and reproduce and to assess how this relates to habitat invasibility and spread. In Meekins and McCarthy (2000) we employed a complete factorial design (manipulating density, nutrients, and light) and determined that site fertility may play a role in invasion and establishment. Moreover, light availability may be the most critical factor in producing large, highly fecund individuals that could spread to dominate an otherwise intact understory. We then brought these results in to a field experiment, to examine invasion success at forest edges (vs. interior) in upland and lowland habitats. Litter disturbance was also applied to evaluate localized biotic effects and light availability on seed germination. While litter disturbance showed no effect, moist lowland sites and edge habitats were quite susceptible to establishment and growth of garlic mustard (Meekins and McCarthy 2001).

In conclusion, garlic mustard appears to behave quite differently in its native and invaded populations, despite the qualitatively similar habitat and genetic structure. The species appears to be quite plastic, readily able to capture resources, and aggressively competitive. Allelopathy does appear to be a strong factor in structuring invaded plant communities. Despite its ecological amplitude, garlic mustard responds rapidly to high light conditions on fertile sites. Because the species does negatively affect community structure, control should be attempted, but probably in a triage fashion. Lower quality, closed canopy sites will be easier to control than highly fertile sites with open canopies (or edges) and higher light conditions.

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Intercept™ Panel Trap, Modified for Monitoring Forest *Cerambycidae* Coleoptera*

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Abstract

The Intercept™ Panel Trap, was modified and field tested for enhanced monitoring of forest Coleoptera, and especially for Cerambycidae. The trap is made from corrugated plastic. It is light-weight, water proof, and durable. Trap efficacy was measured in comparison to the 12-unit funnel trap and the original panel trap design. Captures of longhorn beetles were significantly higher in two modifications of the new prototype of the Intercept panel trap than in the old version of the trap or the Funnel trap. The best performance of the Intercept panel trap for monitoring longhorn beetles was achieved by using: 1) trap with 5 cm hole in the collecting funnel, 2) wet-cup option of collection cup, and 3) increasing the slipperiness of the trap surfaces.

Exotic Plant Invasions in Successional Systems: The Utility of a Long-term Approach

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Abstract

Introduction. The limited temporal duration of most studies constrains our understanding of the dynamics of invasion, and the subsequent function of exotic species in plant communities. While most plant invasions occur over time periods of decades or longer, most experimental studies of invasions are brief, lasting only 1-2 years. These short-term studies, while providing detailed, mechanistic information, are expensive and time consuming and can only be done for a handful of problematic exotics. Similarly, observational sampling studies frequently only capture a single snapshot in time of community associations, yielding little information on pattern development. Both of these approaches do not account for the temporal variation characteristic of plant communities. We propose long-term vegetation monitoring as a complementary approach that provides information on pattern development at a whole-community level and can yield crucial management information. To illustrate the utility of this approach, we draw examples from a long-term study of successional dynamics.

The Buell-Small Succession Study (BSS). Since exotic plants commonly dominate abandoned agricultural land, successional studies, with continuous species invasions and losses, provide a unique opportunity to study the invasion of exotic species. Since 1958, a series of abandoned agricultural fields within the Piedmont region of New Jersey have been continuously sampled (Small *et al*. 1971; Pickett 1982). This study was initiated by Murray Buell, Helen Foot-Buell and John Small of Rutgers University and is located at the Hutcheson Memorial Forest Center in Somerset, New Jersey. The initial objective of their research was to document vegetation patterns in secondary succession. This landmark study has continued uninterrupted for 43 years, making it the longest, continuous study of secondary succession in existence.

The study consists of 10 fields, each containing 48 permanently marked 1 m^2 plots. The fields were abandoned over an 8-year span and with a variety of pre-abandonment agricultural treatments. Since abandonment, data collection has occurred every year until 1979, when sampling was switched to alternate years. At each sampling, the percent cover of all species present in each permanently marked 0.5 x 2.0 m plot is recorded. While the study was originally designed to examine successional changes in community composition, it is an ideal framework from which to address the problem of exotic plant invasions.

Successional patterns. Analysis of the BSS data has shown that the proportional abundance and diversity of exotic species has declined over the first 40 years of succession (Fig. 1; Meiners et al. in press). These declines appear to be in response to increased shading from canopy closure. Successional patterns suggest that management for later successional systems or for closed canopy forests should help to reduce the importance of shade-intolerant exotic plants (Robertson et al. 1994). While this result is encouraging from a management perspective, we have also observed recent increases in several species of shade tolerant exotics such as *Alliaria petiolata*, *Lonicera maackii* and *Microstegium vimineum*. Norway maple (*Acer platanoides*), an exotic tree species problematic in the Northeastern United States, has invaded the old- and second-growth forests of the

Figure 1.—Change in the proportion of exotic plant cover during succession in the 10 fields of the BSS.

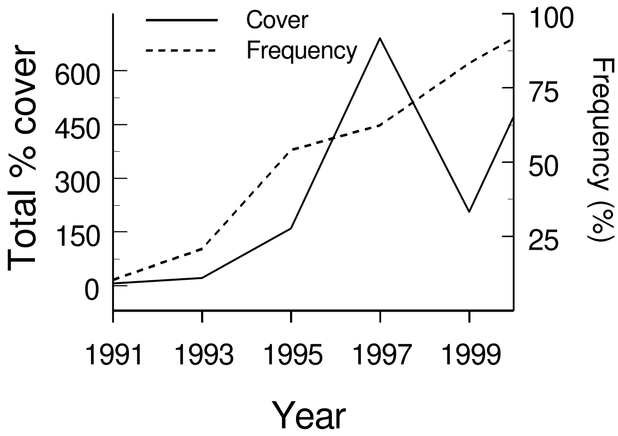

Figure 2.—Increase in cover and frequency of *Alliaria petiolata* in one BSS field.

Hutcheson Memorial Forest Center and has just begun to appear in the BSS fields. This new suite of invaders has the potential to persist within closed canopy forests and represents future threats to the biotic integrity of northeastern forests.

Invasion impacts on diversity. Community-wide vegetation sampling may also be used to set management priorities. Problematic exotic species are often found to cause declines in local diversity. To assess these impacts, we examined the influences of invasions on species richness for a large suite of native and exotic species. In this analysis, we focused on all species that 1) attained an average cover of at least 1% in a field and 2) became locally abundant (>66% cover) in at least five 1 m^2 plots. For each plot, the increase in cover of the invading species was related to the change in species richness during the invasion. Of the 25 native and exotic species studied only the native *Solidago canadensis* and the exotics *Agropyron repens*, *Lonicera japonica*, *Rosa multiflora*, and *Trifolium pratense* were associated with detectable decreases in species richness. These analyses revealed that relatively few species invasions have detectable influences on the diversity of plant communities. By determining which species are potentially damaging and thus management concerns, long-term vegetation monitoring can provide crucial information for land managers.

How should we measure invasions? Assessment of population growth is a crucial step in identifying new invasions and determination of the need for management intervention. Species invasions generally occur over fairly long time periods at regional scales but within-site spread may be quite fast. Knowledge about the rate of spread of a species within a site would be very helpful in determining the speed necessary for an effective management response However, it is not clear how these assessments should be made. There are two ways in which invasion rate may be assessed: as an increase in the frequency of an exotic species (plot occupancy) or as the increase in cover of a species (plot dominance). These two methods may be complementary or yield quite different results. In Figure 2 we show rates of increase for *Alliaria petiolata*, a shade-tolerant understory herb that has recently invaded the BSS fields. Within this field, the two indices of invasion differ in their temporal pattern. While cover of the plant was severely impacted by the 1999 drought, plot occupancy continued to increase. If we limit analysis to pre-drought increases, we find a linear pattern of increase for frequency and an exponential increase for cover. At least for this species in this field, the two indices are not analogous and describe different processes. Therefore, visual assessment of this invasion based on coverage would lead to problematic delays in management response that may result in the establishment of an uncontrollable population.

Rates of invasion. As previously mentioned, knowledge about rates of invasion can be very useful in timing control efforts. Invasions often show lag times, periods with little or no population growth, followed by rapid population expansion. The presence of a lag time may provide a useful window of opportunity during which management strategies can be devised and implemented. As an example, we provide an analysis of the rate of invasion for two woody vines common in the BSS fields (Fig. 3). Years 2-5 show a lag time for both species, with little increase in frequency. If analyses are restricted to the period following the lag phase, the rates of colonization are roughly linear. *Lonicera japonica* has a greater rate of spread than *Parthenocissus quinquefolia*.

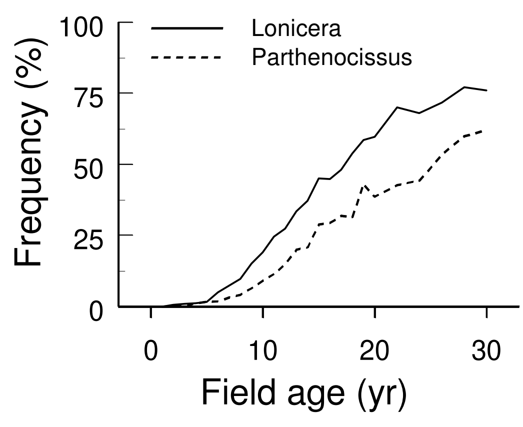

Figure 3.—Rates of invasions for the native *Parthenocissus quinquefolia* and the exotic *Lonicera japonica* in one field of the BSS.

However, the rate of spread for *Lonicera japonica* is much slower than that of *Alliaria petiolata* (Fig. 2), suggesting that management intervention to control *A. petiolata* would need to occur much more quickly.

Early detection of species invasions. Long-term vegetation data can have additional utility in detecting new invasions. Once vegetation monitoring protocols are in place, new species invasions can be detected before they become problematic at a local level. Continuous monitoring of permanent plots can also yield information about conditions associated with the species invasion and the subsequent consequences of the invasion. This information may allow land managers to address an invasion very early while management costs would still be quite low.

Conclusions

Long-term vegetation monitoring represents an approach to the study of exotic plant invasions that is complementary to detailed manipulative studies. While direct mechanistic results are not possible with monitoring data, these studies provide 1) data directly addressing temporal variation in exotic populations, 2) methods for assessing management priorities, 3) estimates of within-site rates of spread, and 4) a framework for the detection and evaluation of new species invasions. These data, coupled with manipulative case studies of exotic plants, can provide a well-rounded knowledge base from which to make effective management decisions.

Acknowledgements

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Effects of Trap Design on the Capture of Large Cerambycidae in Northern Florida

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Abstract

Four experiments were conducted in the spring and fall of 2000 and 2001 in stands of mature longleaf and slash pine in the Osceola National Forest in northern Florida. In each experiment, traps were baited with devices releasing ethanol and (-)-α-pinene at high rates of 1-3 g/day. Traps were grouped into 8-10 replicates of 4 treatments per replicate. Trap catches were collected at 2-3 week intervals, with propylene glycol or dichlorvos strip replaced on each occasion. Trap design had little influence on catches of *Arhopalus rusticus* (ARU), *Monochamus titillator* (MTI), *Xylotrechus sagittatus* (XSA) and *Acanthocinus nodosus* (ANO) (Coleoptera: Cerambycidae) (Figs. 1-3). Similarly, trap and funnel width had little influence on catches of ARU, XSA and ANO (Fig. 3). Traps with collection cups containing glycol (RV antifreeze) outperformed those with cups containing dichlorvos (VaporTape) (Fig. 4).

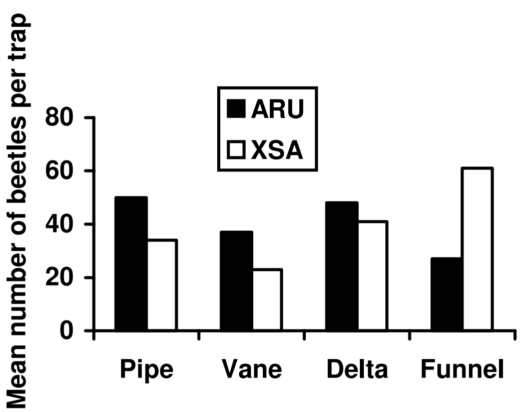

Figure 1.—Pipe vs.vane vs. delta vs. funnel design

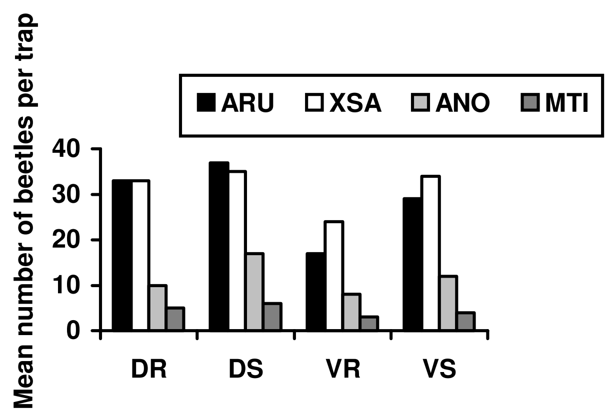

Figure 2.—Soft (S) vs. ridig plastic (R) with Delta (D) and vane (V) designs

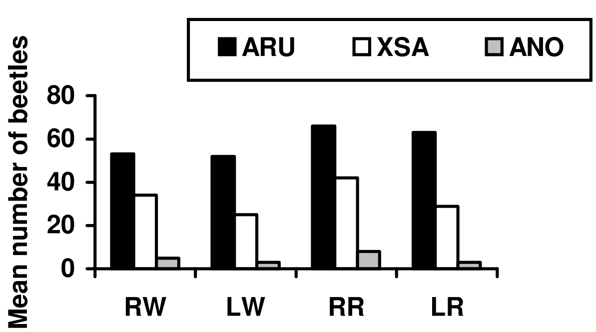

Figure 3.—Wide (W) vs. regular (R) with and large (L) vs. regular (R) funnel

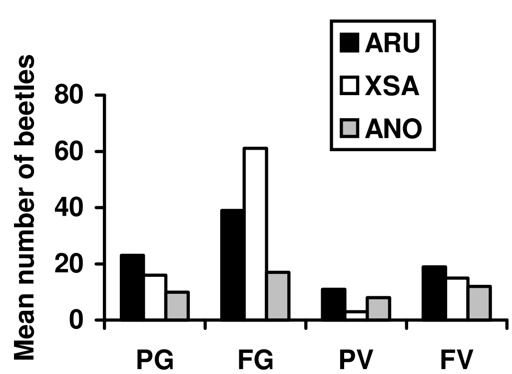

Figure 4.—Glycol (G) vs. dichlorvos (V) in pipe (P) and funnel (F) designs

Hemlock Woolly Adelgid Biological Control Research

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Abstract

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an introduced pest that causes mortality of hemlock in the eastern U. S. Three laboratories have imported and are evaluating predacious beetles for biological control of the adelgid.

The lady beetle, *Pseudoscymnus tsugae* Sasaji & McClure, was imported from Japan by the Connecticut Agricultural Experiment Station in 1994 and was first released in 1995. It has now been released in 11 states from North Carolina to New Hampshire and is considered to be established in seven states. This predator has two generations per year in the field in Connecticut and the adults overwinter on the hemlock foliage during mild winters. Active oviposition occurs from late April through July in Connecticut. Its predation impact is continuous from April to September on the three *A. tsugae* generations that occur in a calendar year.

The USDA Forest Service, Hamden, CT, imported several species of lady beetles from China between 1996 and 1998. Two species, *Scymnus sinuanodulus* Yu et Yao and *S. ningshanensis* Yu et Yao, have been studied in the quarantine laboratory and are undergoing field evaluation in cages. They lay eggs in the spring and are univoltine. The adults become inactive at low temperatures but do not enter diapause. Field cage studies on the numerical impact of the lady beetles on HWA found that a pair of lady beetles confined on a branch with 200-400 ovipositing adelgids significantly reduced subsequent HWA generations compared to controls without beetles. The impact of the beetles was influenced by negative density dependent effects of the adelgid on the host.

The derodontid beetle, *Laricobius nigrinus* Fender was imported from British Columbia between 1998 and 2001 by Virginia Tech for evaluation in quarantine. This species is univoltine. It becomes active in the fall after undergoing an aestival diapause in the adult stage. Adults feed on developing sistens nymphs throughout the fall and winter at temperatures above 0°C. Females begin to oviposit early in the spring. Oviposition by *L. nigrinus* corresponds with onset of oviposition by HWA sistens. Larvae feed on the eggs of the progrediens generation. Mature larvae drop to the soil and pupate. Emergent adults aestivate in the soil during the summer months. Field cage studies, initiated in March 2001, evaluated the impact of a single pair of adults caged on branches infested with ~500 HWA. These consumed all of the HWA in the cages by June, whereas the HWA populations in cages without beetles increased.

Future research plans include evaluation of the efficacy of these predatory beetles in combination on HWA, improving rearing procedures for the beetles, use of field insectaries of infested living hemlocks to produce predators, and developing an optimal release strategy for the predators.

Overview of Asian Longhorned Beetle Research by the USDA Forest Service, North Central Research Station, in East Lansing, Michigan

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Abstract

Our current research on the Asian longhorned beetle (ALB), *Anoplophora glabripennis*, focuses on acoustic detection, systemic insecticides, and natural enemies.

Acoustic detection. Over the past 2 years, we have conducted a number of studies in collaboration with researchers at the Oak Ridge National Laboratory to identify unique acoustic signal descriptors associated with ALB larval feeding in live trees and wood packing materials. We have recorded feeding sounds from ALB larvae as well as larvae of several native cerambycids: cottonwood borer, linden borer, locust borer, red oak borer, sugar maple borer, and whitespotted sawyer. Overall, feeding sounds of cerambycid larvae are quite similar but ALB feeding sounds do have unique signal descriptors. We have developed real-time filter algorithms that recognize sounds of feeding larvae in both trees and cut logs. In China, we have recorded ALB larvae feeding in infested elm, poplar, and willow trees. Recordings have been of larvae that were feeding at distances of up to 7 m away from the sensor. We have also developed a prototype field-portable ALB acoustic detector.

Systemic insecticides. Over the past two years, we have tested the efficacy of various systemic insecticides to kill ALB larvae and adults. In China, we have injected infested elm, poplar, and willow trees. We have tested imidacloprid (Imicide, J.J. Mauget Co.), azadirachtin (Ornazin, Cleary Chemical Corp.), emamectin benzoate (Shot One, Novartis), and thiacloprid (J.J. Mauget Co.). Overall, mortality rates of the within-tree ALB life stages were highest for imidacloprid. In addition, when dead adult beetles were counted around the base of each tree, the imidacloprid treated trees had the highest number of dead ALB adults. In the laboratory, we reared cottonwood borer larvae (CWB), a surrogate for ALB, on artificial diet treated with various concentrations of imidacloprid and azadirachtin. Both insecticides had strong antifeedant effects, which resulted in larval weight loss. Complete mortality occurred at the highest doses of imidacloprid (160 ppm) and azadirachtin (50 ppm) after 12 weeks of feeding. Some mortality occurred at lower doses (0.16 ppm imidacloprid and 0.5 ppm azadirachtin). After 18 weeks, surviving larvae were able to complete development when placed on untreated diet.

Natural enemies. Potential natural enemies for ALB were obtained from field-collected ALB in North America and China, from ALB reared in quarantine laboratories, and from native cerambycids. Necropsy of ALB revealed microsporidia, entomopathogenic fungi, nematodes, and a dipteran parasitoid. Microsporidia were found in larval midguts and in adults from several provinces of China. Fungal pathogens included *Beauveria* spp. and *Metarhizium* spp. in ALB adults from North America and China; resting spores of an Entomophthorales in ALB adults and eggs from North America; and *Verticillium* spp. in ALB larvae from China. Endoparasitic dipteran larvae were found in 4% of ALB larvae collected in Chicago in June 2001 (n = 76); some ALB contained multiple parasitoids. Unidentified nematodes were found in liquefied ALB found in Chicago and New York; ALB cadavers from China also contained nematodes. Identification of natural enemies and studies on their impact on ALB survival and performance are in progress.

Status of Beech Bark Disease Establishment and Research in Michigan

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Abstract

Beech bark disease was first discovered in Michigan in spring 2000 in Ludington State Park on the shore of Lake Michigan in the lower peninsula. Soon thereafter it was found in the upper peninsula of Michigan in the Bass Lake campground. Since then surveys have found it in six counties in Michigan. Beech bark disease involves two exotic organisms: the beech bark scale (*Cryptococcus fagisuga* Lind.; Eriococcidae) and fungal pathogens in the genus *Nectria*. Both the sap-feeding scale insect and the fungus are thought to have originated from Europe and were accidentally introduced into Nova Scotia on ornamental beech imported from Europe in 1890. Since then the scale and the disease have slowly spread through the New England states, Pennsylvania, West Virginia, Virginia, North Carolina, Tennessee, Ohio, Quebec and Ontario.

The beech scale is parthenogenic and has one generation a year. Eggs are laid in midsummer and hatch by early fall. The first stage crawlers are mobile and feed on sap in the tree's inner bark through the fall. They overwinter as second stage crawlers which are legless, immobile, and secrete wax. In the spring they resume feeding and become immobile adults in late spring. When trees are heavily infested they appear to be covered by white wool. The minute feeding wounds caused by the scale insects enable *Nectria* fungi (both native species and European species) to enter the tree. The European *Nectria* species kills areas of woody tissues, sometimes causing cankers on the tree stem and large branches. If enough tissue is killed, the tree becomes girdled and will die. Trees may become chlorotic with thin ragged leaves. Branches and trunks of some infected trees break off in heavy winds resulting in "beech snap."

Beech bark disease will likely continue to spread throughout Michigan killing up to 50% of the large beech trees in Michigan during the first wave of the disease. Another 25% may become infected but survive as weak, defective trees. Some beech trees escape infection and may be at least partially resistant to beech scale. Research is underway to determine the mechanisms and genetics of potentially resistant trees.

Several techniques were evaluated for controlling the beech scale on high value trees including scrubbing scales off of trees, spraying horticultural oil on the trunks of infested trees and treating infested trees with systemic insecticides. The systemic insecticides tested included trunk injection with imidachloprid (Imicide, J.J. Mauget Co.), a combination of imidachloprid and fungisol (Imisol, J.J. Mauget Col), azadirachtin (Ornazin, Cleary Chemical Corp.), and soil injection with imidachloprid (Merit, Bayer). Initial results one month after application of treatments indicate that trees that were scrubbed remained free of scales, and spraying with horticultural oil resulted in approximately 50% mortality of scales present on the trees. Efficacy of the injected insecticides was evaluated in early fall when first stage crawlers began actively feeding. Scale density remained very low on the scrubbed trees and was signifantly lower than on untreated trees. Scale densities were also greatly reduced on trees injected with imidacloprid (Imicide, Imisol and Merit) but were not significantly different from untreated trees. Further evaluations will be conducted to determine long term efficacy of treatments.

Both *Lymantria Dispar* Nucleopolyhedrovirus Enhancin Genes Contribute to Viral Potency

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Abstract

Enhancins are a group of proteins first identified in granuloviruses (GV) that have the ability to enhance nuclear polyhedrosis virus potency. We had previously identified an enhancin gene (E1) in the *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus (LdMNPV) (Bischoff and Slavicek, J. Virol. 71:8133-814, 1997). Inactivation of the E1 gene product within the viral genome lowered viral potency by an average of 2.9 fold. A second enhancin gene (E2) was identified when the entire genome of LdMNPV was sequenced (Kuzio et al., Virol. 253: 17-34, 1999). The E2 protein exhibits approximately a 30% amino acid identity to the LdMNPV E1 protein as well as the enhancins from *Trichoplusia ni* GV, *Pseudaletia unipuncta* GV, *Helicoverpa armigera* GV, and *Xestia c-nigrum* GV. Northern analysis of viral RNA indicated the E2 gene transcripts are expressed at late times post-infection from a consensus baculovirus late promoter. The effect of the enhancin proteins on viral potency was investigated through bioassay using two recombinant viruses, one with a deletion in the E2 gene (E2del) and a second with deletion mutations in both enhancin genes (E1delE2del). The enhancin gene viral constructs were verified by Southern analysis and were shown not to produce enhancin gene transcripts by northern analysis. The E2del virus exhibited an average decrease in viral potency of 1.8 fold compared to wild-type virus. In the same bioassays the recombinant virus E1cat, which does not produce an E1 gene transcript, exhibited an average decrease in viral potency of 2.3 fold compared to control virus. The E1delE2del virus exhibited an average decrease in viral potency of 12 fold compared to wild-type virus. Collectively, these results suggest that both LdMNPV enhancin genes contribute to viral potency, that each enhancin protein can partially compensate for the lack of the other protein, and that both enhancin genes are necessary for wild-type viral potency.

Evaluating the Treatment Efficacy of *Bacillus thuriengiensis* Var. Kurstaki: Reliability of Various Tools

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Abstract

The success of an operational or pilot insecticide spraying program against the spruce budworm (*Choristoneura fumiferana* Clemens) depends to a great extent on a variety of factors, and, in particular on: spraying efficacy and the meteorological conditions during and immediately following spraying. Among other things, spraying efficacy depends on: knowledge of the volume of insecticide necessary to kill this insect (droplet imbibing assays); adjustments to the spraying system in order to produce droplets containing a lethal dose (AT-802 calibration); and, finally, the capacity to achieve target objectives (optimization trials)—that is, the deposit of these droplets on foliage.

Droplet imbibing assays on the spruce budworm. Rousseau and Lemieux. 2001.

In Québec, the Société de protection des forêts contre les insectes et maladies (SOPFIM) is the organization mandated to efficaciously protect forests from certain forest pests. It performs quality control on products during all its spraying programs, including pilot programs. The quality control program devoted to formulations consists in: 1) determining insecticidal power; 2) searching for certain pathogenic microorganisms; and 3) identifying insecticidal species. All three aspects are the subject of laboratory analyses. Since 1997, SOPFIM has used a specific bioassay technique, developed by Kees van Frankenhuyzen and his team (van Frankenhuyzen et al. 1997.), in order to verify the insecticidal power of formulations. This technique, called an "imbibing assay," consists in measuring the mortality rate occurring after each insect has ingested a micro-droplet containing a known quantity of pesticide. In the present study, however, the results of imbibing assays are presented from the perspective of the efficacy of spraying and not that of the quality control program, whose purpose is to ensure that the insecticidal power of pesticide deliveries complies with the information printed on the label.

During 1999, the other members of the SOPFIM research team and I noted that the presence of an antibiotic in the diet used to raise insects appeared to have an impact on LD_{50}. The decision was thus made to verify in greater detail the effect of the antibiotic and its impact on calculations performed with a view to maximizing the efficacy of a treatment. In addition, droplet imbibing assays were also performed using Mimic™ (a tebufenozide-based insecticide) so as to compare its toxicity with that of Foray 76B™ (an insecticide having a base of *Bacillus thuringiensis,* var. kurstaki). The results of these assays were thus used to calculate the size of the droplets required in order to carry out an efficacious spraying program. Depending on the target efficacy level, most droplets should, at the least, contain a lethal dose for 50% of the individuals that ingested one of these droplets.

To begin with, spruce budworms were raised on a diet that either did or did not contain aureomycin, the antibiotic.Then, the assay method consisted in causing these budworms (instar V) to ingest a known quantity of the product. Generally speaking, groups of 40 insects were subjected to treatment involving each of the six serial dilutions (1:3) made using a diluted formulation. One group of insects that ingested only the dilution buffer served as a control during each assay. Individuals that either did not ingest the product or regurgitated it were eliminated; the others were put on a diet that either did or did not contain antibiotic. Then, larvae were placed in an environmental chamber: temperature was 25°C; relative humidity was approximately 45%; and photoperiod lasted 16 hours. Mortality was measured after 5 days. The ideal target mortality rate is 100% for the strongest dose and about nil for the weakest dose. Data were then processed using Polo™ software so as to establish the statistical validity of the lethal dose calculations. Assays were also performed using spruce budworms that had originated in a natural environment. This group of budworms were put on an aureomycin-free diet following ingestion of pesticide.

The antibotic has an effect on the results, the LD_{50} is 20 times lower when antibiotic is not used and the larvae continue to feed after ingesting a non mortal dose. In the case of a formulation of a Foray 76B™ type, a droplet as small as 29 µm in diameter at the time of release appears to have contained a lethal dose for 50% of individuals who absorbed it. These laboratory results fit with those obtained with spruce budworms taken from a natural forest environment during the pilot program conducted in the Outaouais region. With respect to Mimic™, the toxicity of this product following ingestion is low compared to that of Foray 76B™. Thus, in order to kill 50% of a population (Instar V), droplets having a diameter of 72 µm at release are required (Sundaram and al. 1998.). Thus, a Mimic™ droplet this size represents a volume 15 times greater than that of a Foray 76B™ droplet (LD_{50} : 0.25 IU for instar V).

The toxicity data of this study should be used as inputs when mathematical calculations are performed in connection with treatment efficacy. The results of such calculation should be used prior to performing certain calibration tasks and wind tunnel tests so as to determine the best settings on a spraying system. In addition, this droplet imbibing assay technique could be used in order to discriminate between various *B.t.k.*-based formulations with respect to their insecticidal power and shelf life, thus constituting a valuable tool for spray program managers.

AT-802 calibration. Rousseau and Lemieux. 2001.

In collaboration with Forest Protection Limited of New Brunswick, SOPFIM performed a characterization program centering on deposits of Foray 76B™ when this pesticide is sprayed from an AT-802 aircraft fitted out with 10 Micronair AU4000™ sprayers. This project was conducted during summer 2001 on the very location of the Longue-Pointe-de-Mingan (Québec) airport.

Before the plane arrived on the site of characterization work, FPL staff performed a series of adjustments on the spraying system. Wind tunnel test results were used to adjust flow rates and atomizer rotation speeds. As a result, settings on the spraying system were to produce droplets smaller than 100 µm in diameter for more than 90% of sprayed volumes (VMD=50 µm). These settings were established in relation to a swath of 70 meters. Testing was conducted into the wind and also under crosswind conditions for a swath of 70 meters. Testing was also performed into the wind for a swath of 100 meters. Prior to flying the plane, Kromekote™ papers and Petri dishes were placed on the ground every 2.5 meters for flights performed into the wind. The sampling system was set perpendicular to the wind, and aircraft generally flew over the centre of the sampling system, into the wind. During tests conducted under crosswind conditions, papers and dishes were set out every 5 meters. The sampling system was set parallel to the wind direction with the plane flying perpendicularly over the edge of the sampling system on the windward side. Ten minutes following flyover, Petri dishes and Kromekote™ papers were gathered. Papers were analyzed using the original Swath Kit™ equipment, using a modified version of the Swath Kit™, and by a hand count performed with a binocular microscope. These analyses were designed to determine the number of drops per area unit. Droplet diameter was measured with the microscope in order to identify the volume median diameter (VMD) and the number median diameter (NMD). Petri dishes were flushed and flushing liquid was analyzed using the Adam Kit™ so as to determine the active ingredients. Reference ranges were devised using a sample originating from the aircraft. The results of these analyses were expressed in BIU/ha.

The analysis of Kromekote™ papers using the Swath Kit™ proved to be rather unreliable. The system was thus modified, but once again, the results did not fit with the results produced by a “hand count” performed with a microscope fitted out with a filar micrometer. The difficulty, when using cameras, of differentiating dust from among the droplets made it necessary to read papers with a microscope. Depending on the equipment used, the results could vary by upwards of 100% and even 200%. The results of an analysis of the flushing liquid originating in the Petri dishes made it possible to accurately characterize the deposit in terms of the active ingredient. Using the Adam Kit™, it was possible to measure the presence of pesticide in droplets of less than 20 µm in diameter on Kromekote™ papers, the amount was sufficient to kill the larvae.

After having simulated several flyovers with planes equidistant to one another at 70 meters, the results obtained show an average deposit of 34 droplets/cm^2, for a coefficient of variation (COV) of 0.47. The VMD and DNM values were, respectively 95 and 33µm. In terms of active ingredient, 10.4 IU/hectare were detected, for a COV of 0.50.The results of trials done into the wind indicate a little overlap of the deposit occurs between spray lines, possibly denoting an overwide swath. With a narrower swath, the COV will decrease and the deposit will increase. For the crosswind trial, the results show that for wind speeds of approximately 8 km/h, practically no active ingredient or droplets were found on the papers or on the Petri dishes. It is difficult to target the product at the appropriate locations whenever very small droplets are released from a plane. Tests should be conducted over the forest in order to verify the deposit of pesticide on fir needles under normal spraying conditions, considering the low level of deposit recorded under crosswind conditions.

Optimization trials for insecticide spraying into small blocks. Mickle and Rousseau. 1998.

Deposit for dyed Foray 48B™ and Foray 76B™ to two 6-hectare (200m x 300m) collocated blocks was compared for two spray strategies. One block was sprayed using a normal application strategy of equally spaced swaths across the block. The other block was sprayed using an optimization technique developed from model (FSCBG, AgDRIFT™) predictions based on application parameters (Cessna 188, aircraft height, 4 Micronair AU4000™ rotary atomizers, drop size distribution) and local meteorology (winds, temperature and relative humidity). The two blocks were arranged as one 15-hectare (500m x 300m) block to allow for continuous spraying across both blocks on equivalent lines thereby reducing meteorological differences during the application. Five trials were realized.

Optimization strategies resulted in multiple passes (up to 6) on a single line displaced up to one half swath upwind of the block boundary. Swath displacement and multiple passes increased with increasing wind speed.

Measured deposit within the optimized block showed increases of 13% to 185% over deposit in the regular block (equal swath spacing). With increased deposit, average COV decreased from 0.64 to 0.43 reflecting the more uniform deposit across the block sprayed using the optimization strategy. Similar results were found for drop density with optimized to regular block ratios ranging from 1.0 to 2.87. Average COV declined from 0.75 in the regular block to 0.41 in the optimized block.

Acknowledgments

Special thanks go to Forest Protection Limited, Valent Corporation, and Sylvie Lemieux of SOPFIM, an important collaborator, for their support.

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Introduced Pests: the European Experience

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Abstract

Throughout the last two centuries, Europeans have experienced the introduction of many exotic pests into their crops. Past introductions of pests like *Phytophthora infestans*, *Viteus vitifoliae*, *Leptinotarsa decemlineata*, *Globodera* spp. or *Erwinia amylovora* have had major economic and social consequences. With the ever continuing increase in trade exchanges and travel movements, the risk of introducing new pests into new areas has clearly increased. Several examples of recently introduced pests will be given to illustrate this fact. Past and present pest introductions showed that introduced exotic pests can have catastrophic consequences, that preventing introduction is the most effective protection and that international cooperation is essential. In Europe, a regional plant protection organization (EPPO) was created in 1951 to promote international cooperation and to prevent entry and spread of dangerous pests. Within this forum, European countries have defined common plant quarantine strategies against alien pests. The identification of potential risks presented by pests and the establishment of phytosanitary measures based on a sound scientific basis are key points in these strategies. When, despite all efforts, pests are introduced into new areas, European countries have to develop eradication and/or containment measures. The example of *Diabrotica virgifera virgifera* will be presented to illustrate the practical difficulties of combating an invasive species that is spreading rapidly through several countries.

Last Call™ Attract and Kill Formulations in South African Citrus and Subtropical Fruit Pest Management

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Abstract

A novel plant based attractant bait is effective in management of 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 South African citrus. *Ceratitis* fruit flies were well controlled in replicated field trials in citrus and mango. This multispecies activity also presents a very useful new tool for regulatory agencies involved in monitoring and control activities. In parallel trials, false codling moth (*Cryptophlebia leucotreta*) was controlled with a novel sex pheromone + insecticide bait formulation.

2-Methyl-(*Z*)-7-Octadecene — The Sex Pheromone of Allopatric *Lymantria serva* and *Lymantria lucescens*: Two Potential Invasive Species in the Orient

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Abstract

Our objective was to identify the sex pheromones of two allopatric *Lymantria* species (Lepidoptera: Lymantriidae): (1) *L. serva* (Fabricius) in Taiwan whose larvae attack and occasionally defoliate *Ficus* spp. and (2) *L. lucescens* (Fabricius) in Honshu, Japan, whose larvae feed on *Quercus spp.* Coupled gas chromatographic-electroantennographic (GC-EAD) analyses of pheromone gland extracts revealed one antennally-active compound produced by female *L. serva*, and the same compound by female *L. lucescens.* This compound was identified as 2-methyl-(*Z*)-7-octadecene (2me-*Z*7-18Hy) by retention index calculations on DB-5, DB-23 and DB-2 10 columns, and by comparative GC-mass spectrometric (MS) and GC-EAD analyses of the insect-produced candidate pheromone and synthetic 2me-*Z*7-18Hy. Field trapping experiments in the Taipei Botanical Garden, Taiwan, and in mixed oak forests in Mifune, Toyota City, Aichi Prefecture, Japan, confirmed attraction of respectively male *L. serva* and male *L. lucescens* to 2me-*Z*7-18Hy. Allopatric distribution of *L. serva* and *L. lucescens* seems to allow both species to use the same sex pheromone without compromising specificity of either species. Synthetic 2me-*Z*7-18Hy can now be utilized for pheromone-based detection surveys of these exotic moths.

Ultrastructure and Histology of Antennae of *Anoplophora glabripennis*

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Abstract

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), known in the U.S. as Asian longhorned beetle (ALB), is a recently introduced pest of considerable economic importance and eradication efforts are under way in New York and Illinois. Identification of specific chemical attractants for ALB would facilitate survey and detection efforts in the U.S., but little is known about this insect's response to olfactory cues. In this study, antennae of male and female ALB were examined using light microscopy and scanning and transmission electron microscopy to provide information on the location, number, and description of cuticular sensilla that might function in olfaction.

Beetles emerged within the U.S. Department of Agriculture Forest Service Quarantine Laboratory, Ansonia, CT, from infested logs harvested in Chicago, IL, and Bayside, Queens, NY. For light and transmission electron microscopy, antennal segments were fixed, dehydrated, embedded in Spurr's resin, and sectioned. For scanning electron microscopy, specimens were fixed and either air-dried or critical point dried.

This study focused on the antennal flagellum (segments 3-11). The flagellum is densely covered with socketed hairs arranged in alternating black and white bands. The surface of these hairs is uniformly sculptured with fine ridges running along the axis of the hairs. Two other broad categories of socketed hairs are found on ALB antennae. Chetiform-1 sensilla are long hairs located at the junctions of segments and on the ventral surface of segment 11, at a special flex point on this segment. The proximity of these hairs to areas of antennal articulation indicates that they are likely to be mechanoreceptive. Even longer sensilla with articulatory sockets are found midway along the segments. These chetiform-1a type sensilla also are likely to serve in mechanoreception. Smaller socketed chetiform-2 type sensilla are dispersed among the larger sculptured hairs on all of the segments of the flagellum. These sensilla are articulated at a broadly acute angle to the axis of the segment on which they occur. As articulated hairs, they likely respond to physical stimulation; it is not known if they could be olfactory as well.

The sensilla that are most likely to have an olfactory function fall into two morphological classes – basiconic pegs and trichoid sensilla. Basiconic pegs occur primarily on segment 11, and nearly all are located at the distal end of that segment. Their number and location suggest that they could function in contact chemoreception. Trichoid sensilla occur in abundance on the distal segments and in gradually decreasing numbers on the more proximal segments of the flagellum. Many pores perforate the thick cuticular walls of the trichoid sensilla and pore tubules extend to the shaft lumen where there are numerous distal dendritic branches. The abundance and distribution of trichoid sensilla on the antennal segments and their histology and ultrastructure suggest an olfactory function.

There appear to be few differences in the number, type, or location of cuticular sensilla on male and female ALB antennae. Although the basiconic sensilla could be contact chemoreceptors and the trichoid sensilla are likely to be olfactory receptors, neither of these receptor types necessarily function in communications between the sexes. They could serve in host plant selection or other ecological roles.

Determining Feeding Preference Hierarchy in Gypsy Moth Larvae (*Lymantria dispar*) Using Choice Test Bioassays

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Abstract

The larva of the gypsy moth *Lymantria dispar* L. is considered to be one of the most serious forest pests of North America. Today, the gypsy moth can be found in many parts of the eastern and mid-west United States, as well as in Canada. Despite efforts to control this insect, it has persisted and continues to extend its range. It has significant economic impact since the larvae defoliate millions of acres of forest annually. To better understand the feeding behavior that this polyphagous insect displays during tree defoliation, we examined feeding preferences of fifth instar larvae using a two-choice leaf disc assay. Seven overstory tree species were selected from the Towson University campus. These included sweetgum (*Liquidambar styraciflu*a), sugar maple (*Acer saccharum*), tulip poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), American basswood (*Tilia americana*), red oak (*Quercus rubra*), and black walnut, (*Juglans nigra*). Intercomparisons made between each species revealed that sweetgum and red oak were highly preferred over the other species, whereas tulip poplar was least preferred. Our results reveal a clear hierarchical order of feeding preference for these larvae.

Genetic Analysis of Asian Longhorned Beetle Populations from Chicago, New York, and China Using the RAPD Technique

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Abstract

Anoplophora glabripennis samples were collected in the Ravenswood area of Chicago, near the Mt. Zion cemetery in Queens, New York (provided by Leah Bauer) and the Gansu Province in northwest China (provided by Leah Bauer). DNA isolation procedures have been developed for use on *A. glabripennis* larval samples fixed in ethanol. Random amplified polymorphic DNA (RAPD) fragments were generated and screened for use as markers. Most primers yielded DNA fragments from Asian longhorned beetle samples (ALB) with low to moderate size heterogeneity. A few primers analyzed to date yielded DNA fragments exhibiting extensive size heterogeneities. Primers yielding DNA fragments with moderate heterogeneities separated the Chicago and New York ALB samples from ALB samples from the Gansu province of China. Primers yielding highly heterogeneous sized fragments separated the Chicago samples from the New York samples, and the Chicago and New York samples from the samples from the Gansu province of China. These results suggest that the Chicago and New York populations represent genetic bottlenecks, which arose from introduction of a relatively small number of individual beetles. Phylogenetic analysis grouped the samples from the Gansu province of China with the samples from New York, indicating that the New York samples exhibit a greater genetic similarity to the Gansu province samples than to the Chicago samples. This result may suggest that the New York infestation arose from ALBs originating from or near the Gansu province of China, and that the Chicago infestation arose from ALBs originating from a different region of China. Additional analysis with more primers and ALB samples from different regions of China are needed to determine if this preliminary conclusion is valid. DNA primers yielding highly heterogeneously sized fragments also appear useful for analysis of ALB samples from individual host trees. Phylogenetic analysis suggests that ALB females may exhibit host preferences or "imprinting' behavior. In addition, several fragments have been identified that may be useful for distinguishing *A. glabripennis* from other cerambycids and other wood boring insect species. Samples of *Plectrodera scalator* (cottonwood borer), *A. chinensis* (citrus longhorned beetle), *Tetropium castaneum* (brown spruce longhorned beetle), and other species are being sought for these investigations.

First North American Record of the Palearctic Species *Tetropium fuscum* (Fabr.) (Coleoptera:Cerambycidae)

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Abstract

The first confirmed presence in North America of the palearctic cerambycid, *Tetropium fuscum* (Fabr.) (the brown spruce longhorn beetle) is reported. It was discovered attacking red spruce, *Picea rubens* Sarg., at Point Pleasant Park in the port city of Halifax, Nova Scotia. Biological notes pertinent to its infestation of red spruce at the Park are presented. In response to the observed tree mortality, bolts from living trees with green crowns and excessive resin flow have been reared repeatedly since 1999 with consistent results: emergence of *Tetropium fuscum*. Research on the North American establishment of this species has yielded novel information.

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Biological Control of *Anoplohora glabripennis* Motsch.: A Synthesis of Current Research Programs

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Introduction

Anoplophora glabripennis Motschulsky (Asian longhorned beetle) (ALB) (Coleoptera: Cerambycidae: Lamiinae: Lamiini), is among a group of high-risk exotic woodborers native to Asia, specifically China and Korea (Nowak et al. 2001). In China, *A. glabripennis* is considered one of the most important forest pests, having been reported from 25 provinces and extending from 21°- 43° N Latitude and 100°-127° E Longitude (Yan 1985). This region extends across climatic zones that correspond to the climatic zones in North America from southern Mexico to the Great Lakes, and includes virtually all of eastern U.S. Feeding by larvae in the cambium and xylem causes widespread mortality among many deciduous broadleaf tree species in China (Yang et al., 1995), particularly *Populus* spp., *Salix* spp. and *Ulmus* spp. (Xiao 1992).

Within the U.S., *A. glabripennis* has been intercepted in 14 states, but established infestations are currently only known to exist in New York City and on Long Island (first discovered in 1996), and in Chicago, Illinois (first discovered in 1998). Utilizing the most effective method currently proven to limit its spread, approximately 5,286 and 1,509 infested trees have been located, cut and removed in the New York and Chicago infestations, respectively, as of May 2001 (U.S. Forest Service 2001). Furthermore, *A. glabripennis* has thus far been reported to attack 18 deciduous tree species in 12 genera within these two U.S. infestations (Cavey et al. 1998; USFS 2001). Most notably among these are maples (*Acer* spp.). In addition to the ability to attack and kill apparently healthy trees, *A. glabripennis* also structurally weakens trees, which poses a danger to pedestrians and vehicles from falling limbs or trees.

Although quarantines and eradication programs exist in New York and Chicago, *A. glabripennis* possesses the potential for introduction into the urban, suburban and forest landscapes, particularly in eastern U.S. Based upon field data from nine U.S. cities, national tree cover data and proposed host preference of *A. glabripennis*, the estimated potential tree resources at risk to *A. glabripennis* attack ranges from 12 to 61% of the city tree population, with an estimated value of $72 million-$23 billion per city. The corresponding loss in canopy cover that would occur if all preferred hosts were killed ranges from 13-68%, with an estimated maximum potential impact of 34.9% of total canopy cover, 30.3% tree mortality (1.2 billion trees), and value loss of $669 billion (Nowak et al. 2001).

Therefore, efforts to develop control strategies that represent alternatives to the felling and chipping of infested trees were initiated within the past three years. Included among these are biological control strategies, which are the focus of this paper. These strategies have two broad objectives. The first objective is focused on the development of mass rearing and mass production technologies, coupled with inundative release and application technologies, respectively, for various biological control agents found to be effective against *A. glabripennis*. The resulting technologies are intended to complement existing or other currently developing technologies (i.e. insecticidal controls) for use in the eradication program. The second objective is to develop technologies that could be utilized in managing *A. glabripennis* populations should eradication fail to succeed. As such, this paper will provide an update on the biological control research, with the exception of fungal pathogens, which is the subject of a companion paper in these proceedings.

Research Summaries

***Nematodes* (Solter and Keena).** Entomopathogenic nematodes may offer an alternative and/or complementary method for the control of ALB, specifically targeting the larval and/or pupal stages. Therefore, four entomopathogenic rhabditoid nematode species, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, *H. indica*, and *H. marelatus*, were tested for their ability to kill and reproduce in larvae of the ALB. The larvae were permissive to all four species but mortality was higher and production of infective juveniles (IJ) was greater for *S. carpocapsae* and *H. marelatus*. The lethal dosage of *H. marelatus* was determined to be 19 IJ's for second and third instar larvae, and 347 IJ's for fourth and fifth instar larvae. *H. marelatus* infective juveniles, applied via sponges to oviposition sites on cut logs, located and killed host larvae within 30 cm galleries, and reproduced successfully in several of the larvae. *H. marelatus* killed fifth instar host larvae in 2-6d [@ 500 (n=3) and 2000 IJ/larva (n=5)]. *H. marelatus* reproduction within fifth instar host larval cadavers ranged from 158-321 x 10^3 nematodes per cadaver. While the *S. carpocapsae* isolate is currently being evaluated in ID-50 and LD-50 studies and data have not as yet been quantitatively analyzed, it appears that *S. carpocapsae* kills its hosts somewhat faster than for *H. marelatus*. However, it also appears that *S. carpocapsae* reproduction within the dead hosts may be lower than for *H. marelatus*. Results of the initial studies have been published (Solter et al. 2001).

***Bacillus thuringiensis* (D'Amico).** *Bacillus thuringiensis* (Bt) was evaluated as a microbial insecticide against larval and adult ALB. Studies in which existing commercial Bt products ("whole" Bt requiring activation) were incorporated into diet and fed to larvae and adult ALB, showed that they were not effective against either ALB life stage. Voltage clamp assays resulted in the identification of several Cry toxins that were effective against ALB larval midgut in vitro, especially Cry 1B. Assay of available Cry 1B showed that it is not effective against ALB larvae in vivo. It was noted that midgut environment in vivo may not be suitable for Bt MOA, which may cause a discrepancy between in vitro work and bioassays. Brush Border Cell Assays (conducted by D. Dean, OSU) provided positive results that led to preparation of Cry toxin for use against adult ALB. This preparation, as well as new commercial products will be evaluated against adult ALB in 2002.

***Microsporidia* (Bauer).** Working with Deborah Miller and Houping Liu, a new microsporidium isolate from ALB larvae was collected in Henan Province, China (December 2001), but it is as yet unidentified. Infection prevalence of larval samples was ca 2% (n=97).

***Predators and Parasitoids* (Smith et.al.; Herard et.al.; Bauer et.al.).** A number of natural enemies of the Cerambycidae have been reported worldwide, including predators belonging to the Cucujidae, Ostomidae, Cleridae, Colydiidae, and Elateridae beetles; Asilidae, Xylophagidae, and Rhagionidae flies; Phymatidae and Reduviidae bugs; and predaceous thrips and carpenter ants, as well as parasitoids belonging to the Braconidae, Ichneumonidae, Bethylidae, Encyrtidae, Eulophidae, Gasteruptiidae, Pteromalidae, Eupelmidae, and Eurytomidae, wasps; and Tachinidae and Sarcophagidae flies (Linsley 1961). As such, identification of effective parasitoids for use in eradication and/or management of ALB has been underway, both within the country of origin (China in particular), as well as outside the country of origin (including Europe and North America). Two approaches are being utilized in these efforts: (1) evaluation of natural enemies known to attack ALB in China; and (2) evaluation of possible new associations between ALB and natural enemies of other cerambycids in China, Europe and North America. However, in order to focus efforts on those natural enemy species with a greater probability of providing biological control of ALB, selection and prioritization of these natural enemies are based largely upon the relatedness of their respective longhorned beetle hosts to ALB at three levels: (1) phylogenetic relatedness; (2) ecological relatedness (e.g. climate; habitat; host tree species and condition); and (3) behavioral relatedness (e.g. larval feeding behavior) (Smith 1999). Certain aspects of each of these likely play a major role in determining the potential efficacy of a given natural enemy to control ALB, as well as other *Anoplophora* species.

China. In China, parasitoids have been identified that are known to attack longhorned beetles that share a common host tree with ALB, as well as those known to attack ALB and/or other *Anoplophora* species. Among the first group are several egg parasitoids, including: the encyrtids *Oophagus batocerae* and *Zaommoencyrtus brachytarsus*, parasitoids of *Batocera horsfieldi*, and *Austroencyrtus ceresii*, a parasitoid of *Ceresium sinicum*; and the eulophid *Aprostocetus prolixus*, a parasitoid of *Apriona germarii*. Also among the first group are larval parasitoids, including: the tachinid *Bullaea* sp., the bethylid *Scleroderma guani*, a parasitoid of *Saperda populnea*, *Semenotus bifasciatus* and *Semenotus sinoauster*; the braconids, *Ontsira palliates*, a parasitoid of *Semenotus bifasciatus* and *Semenotus sinoauster*, and *Zombrus bic*olor and *Zombrus sjoestedti*, larval parasitoids of cerambycid spp.; and the ichneumonids *Xylophrurus coreensis*, *Schreineria* sp and *Megarhyssa* sp., larval parasitoids of cerambycid spp. Among the second group is the egg parasitoid *Aprostocetus fukutai* (Eulophidae), which parasitizes both *Anoplophora chinensis* and *A. germarii* (Liao et al, 1987; Wang and Zhao, 1988). However, no egg parasitoids have as yet been reported from ALB or *A. nobilis* (Yan and Qin, 1992; Zhou, 1992). Also among this second group are several larval parasitoids, including the braconids *Ontsira* sp. parasitizing *A. chinensis* larvae, and *Ontsira anoplophorae* sp. nov., parasitizing Anoplophora malasiaca on citrus; as well as the Colydiidae beetle Dastarcus longulus, a larval-pupal parasitoid of ALB, *A. nobilis*, *B. horsfieldi, A. germarii, Monochamus alternatus*, and *Trirachys orientalis* (Qin and Gao, 1988).

To date, investigations by Smith et al. have found no egg parasitoids of ALB. Therefore, their efforts have focused in large part on two of the species mentioned above, *S. guani* and *D. longulus*. Primary objectives have been to evaluate their relative efficacy to parasitize ALB, and to develop mass rearing technology. Results from studies of *S. guani*, to date, have shown that *S. guani* is an idiobiont ectoparasitoid, and females 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. An average of 45 adult *S. guani* emerged from a single mature host larva of *Saperda populnea*. In nature, *S. guani* was found parasitizing 41.9 - 92.3% of *S. populnea* larvae in poplar stands in many areas. 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, and *S. guani* usually parasitizes longhorned beetle species whose larvae are small, ca. 15 mm in length. Therefore, *S. guani* would be used to specifically target ALB 1st to 3rd instar larvae. Results from studies of *D. longulus*, to date, have shown that it is an ectoparasitoid, with females laying eggs in frass and sawdust in host gallery or on the host gallery wall. First instar larvae possess thoracic legs and crawl 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 feeds singly or gregariously on its host, and as many as 30 individuals of this parasitoid are capable of successfully completing their development on a single ALB larva or pupa, which usually kills the ALB within 10 days. In many areas, parasitization rates of ALB by *D. longulus* has found to reach between 50-70%, and in locations where *D. longulus* is established in relatively high numbers, ALB is said to be under natural control. Investigations focused on development of mass rearing technologies for both of these species are in progress and are promising. Given their respective optimal preferences for different sized larvae, as well as the wingless nature of *S. guani*, inundative releases of these two species, in tandem, appears to offer a possible complementary approach to the existing strategies in the ALB eradication program. However, prior to releases, non-target studies are planned and will be conducted at BIIR. Results of the initial studies have been published (Smith et.al. 1999; Yang and Smith 2000, 2001)

Europe. Herard et. al. recently initiated investigations of potential natural enemies of ALB in Europe, with an initial emphasis on studies of *Saperda populnea* (L.) and *Saperda carcharias* (L.). These two species were selected because they share common traits with ALB: (1) both are Lamiinae; (2) both attack trembling aspens, poplars, and willows, trees that are among the preferred hosts of ALB in China; and (3) both attack healthy trees. While no egg parasitoids have been found in France to date, the eulophid *Euderus caudatus* has been reported as an egg parasitoid of *S. populnea* and *S. carcharias*. Two early larval parasitoids have been found thus far: a tachinid (not yet been identified) from France (southern and eastern) and Finland, and the eulophid *Euderus albitarsis* from southern Finland, where it was found parasitizing 1st instar *S. populnea* larvae. Two parasitoids whose adults emerged from full-grown larvae of *S. populnea* were found in 2001: the tachinid *Billaea irrorata* and the ichneumonid *Dolichomitus populneus*, previously mentioned from *S. populnea* and *S. carcharias*. Although *B. irrorata* emerges fairly late during its host development, its ability to attack very early larval instars will be elucidated. Rate of parasitism by each species in the various sites has not as yet been determined.

In addition, the following predatory Diptera larvae were found by dissection of branches in *S. populnea* galleries: *Odinia xanthocera* (Diptera, Odiniidae), *Lasiambia baliola* (Diptera, Chloropidae), and *Thaumatomyia elongatula* (Diptera, Chloropidae). While no braconids have been found to date, four species are known parasitoids of *S. populnea* and one species is known to parasitize *S. carcharias*. Among tachinids, two other species are known (one from *S. populnea* and one from *S. carcharias*). Among Ichneumonids, 22 other species are known from *S. populnea*, and 11 other species are known from *S. carcharias*. Consequently, it appears that the biocomplex of enemies of these two cerambycids in Europe constitutes a great reservoir of species that can be tested against *Anoplophora* spp.

In concert with identifying and selecting candidate natural enemies for evaluation against ALB, development of laboratory rearing techniques for the cerambycid species, specifically on live plant material, was initiated. Studies that are planned for 2002 include: (1) continue exploration and develop an inventory of early stage parasitoids of *S. populnea* and *S. carcharias* across Europe; (2) finalize *S. populnea* and *S. carcharias* rearing techniques using rooted cuttings; (3) implement ALB rearing techniques in 5-10 cm diameter rooted cuttings; (4) test *Saperda* spp. parasitoids on ALB, in quarantine at Montpellier; and (5) survey ALB and *Anoplophora chinensis* populations in sites where these 2 species were accidentally introduced in Europe, for possible occurrence of parasitism by local species. The anticipated product(s) from these studies are parasitoids of the Western Palearctic region cerambycids that show promise as efficacious biological control agents against early stages of ALB, and which can be used in the Nearctic region without significant non-target effects on North American ecosystems.

North America: Smith and Fuester, and Bauer et al. recently initiated investigations of potential natural enemies of ALB in North America. Both groups, to date, have found a dipteran parasitoid associated with ALB-infested trees (Bauer et al. from Chicago, 2001) (Smith et al. in Norway maple trees from New York, 1998), which has not yet been identified. In addition, however, to searching for natural enemies associated with ALB-infested trees in New York and Chicago, efforts to evaluated parasitoids of selected North American cerambycids are underway. To reiterate, in an effort to identify North American natural enemies that are most likely to adapt to ALB, Smith (1999) proposed that priority should be given to those natural enemy species whose cerambycid hosts are most similar/related to ALB (phylogenetically, ecologically and behaviorally).

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Forest Macrolepidopteran Parasitoid Host Range and Richness in the Central Appalachian Range

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Abstract

In a long-term nontarget study initiated in 1995 on the effects of *Lymantria dispar* (L.) and its control with applications of *Bacillus thuringiensis* var. *kurstaki* Berliner (Btk), parasitoids were sampled by Malaise traps and through rearing of macrolepidopterous larvae. Sampling was performed on 18 200-ha study plots located in the George Washington National Forest (GWNF) in Virginia and Monongahela National Forest (MNF) in West Virginia in the central Appalachians.

During 1995 and 1996, a total of 13,310 larvae were collected on foliage and under canvas bands with 5,213 of these reared for parasites and disease symptoms. Gypsy moth accounted for 6,856 larvae collected and 2,202 larvae reared. The caterpillars reared represented 114 species in 12 families, with each family having species parasitized. Tachinid flies, Hymenoptera or nematodes parasitized a total of 69 species of caterpillars. The tachinids had the widest host range, attacking 52 caterpillar species in eight families. The other two commonly encountered parasitic families, Braconidae and Ichneumonidae, parasitized 28 and 33 host species, respectively, with a combined total of 47 species parasitized in seven families. Of the 24 species of reared tachinids, 60 new hosts associations were determined, including first-known hosts for seven tachinids. This is a significant increase to our knowledge of tachinid associations and supports the possibility that Tachinidae and Ichneumonidea may be equally important in regulating macrolepidopteran larval populations in forest environments.

Reared species of tachinids were represented in Malaise samples as well, where 186 species have been identified in the first four years of sampling. Species richness is similar between the two forests with 158 species collected in the GWNF and 138 species in the MNF, with 103 (55%) shared between the two forests. The majority of all reported tachinid host relationships are with lepidopteran larvae, and this was true in our tachinid collections in these forest environments as well with 133 species represented: 112 on the GWNF, 91 on the MNF, and 70 shared between the forests. Temporal patterns of species richness and abundance of tachinids and their caterpillar hosts were similar.

The most diverse tachinid subfamily, Goniinae, made up 66% of the species richness and 67% of the abundance in the Malaise samples. Most Goniinae, Tachininae, and Dexiinae are parasitoids of lepidopterous larvae, and combined make up 96% of the species richness and 95% of the sampled abundance. Nearly all Phasiinae host associations known are with Hemiptera. The same 10 tachinid species were most abundant on both the GWNF and MNF, making up approximately 50% of the total sampled abundance on each forest. The most abundant tachinid on the MNF, *Compsilura concinnata* (Meigen), quickly increased with the movement of gypsy moth into the study plots in 1995. It has since declined in abundance with the decline of gypsy moth following the impact of the gypsy moth fungus, *Entomophaga maimaiga* (Humber, Shimazu & Soper).

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Weeds Gone Wild: A Web-based Public Information Project

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Abstract

The Plant Conservation Alliance's Alien Plant Working Group (APWG) was formed in August 1995 in order to address the problem of invasive exotic plants on a national level, to produce educational materials, and to forge cooperation with local and regional groups. The focus of the group is the threat and impacts of invasive alien (exotic, non-native) plants to the native flora, fauna, and natural ecosystems of the United States. Formal guidance for the APWG's future direction and activities was developed during the Plant Conservation Alliance's "Taking Initiative" workshop sponsored by the Lady Bird Johnson Wildflower Center in Austin, Texas, in January 1999. The resulting product, "An Action Agenda for Invasive Plants" is available on the web page (http://www.nps.gov/plants/alien/apwgaction.htm) and identifies the following eight focal issues: 1) Communication, Networking and Partnerships; 2) Funding; 3) Information Management; 4) On-The-Ground Management; 5) Outreach and Education; 6) Policy and Guidance; 7) Research; and 8) Resources and Training.

The group's first project was development of a web page, "Weeds Gone Wild: Alien Plant Invaders of Natural Areas" (http://www.nps.gov/plants/alien), online since August 1997, that is useful to the general public, land managers, researchers, and others. A background section explains the invasive species problem, defines terms, and provides suggestions for things people can do to help. The site also provides the most comprehensive list of invasive plant species affecting natural ecosystems through the U.S., to date running well over 1,000 species. Illustrated fact sheets provide plant descriptions, native origins, U.S. distribution and types of habitats invaded, biology and methods of spread, management options, alternative plants, and links to experts. An information links section helps connect users to invasive species contacts and other valuable resources worldwide. Recent additions include an electronic list-serve and electronic events calendar (http://www.eventcal.net/weeds_gone_wild). And last but not least, a theme song, "To Every Weed, Turn, Turn, Turn", is provided to encourage weed busters in their pulling, cutting, spraying and other weed-killing activities.

Participation is open to anyone interested in being involved and partners include federal, state, and local government agencies, non-governmental organizations, universities, private firms and individuals. Fact sheets and other materials are produced largely through efforts of volunteers. The National Park Service, one of 10 federal agencies that signed the Memorandum of Agreement establishing the Plant Conservation Alliance in May 1994 (http://www.nps.gov/plants/coop.htm), provides the internet service for the project.

Host Preference of the Brown Spruce Longhorned Beetle, *Tetropium fuscum* (Fabr.) on Selected North American Conifers

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Abstract

The brown spruce longhorn beetle, *Tetropium fuscum* (Fabr.) (Coleoptera: Cerambycidae), native to Europe, was recently discovered infesting and killing red spruce, *Picea rubens* Sarg. in Point Pleasant Park, Halifax, Nova Scotia. Our objective was to determine the relative susceptibility of selected North American conifers to oviposition and infestation by *T. fuscum.* In laboratory 2-choice bioassays, *T. fuscum* females laid significantly more eggs on *P. rubens* than on white spruce, *P. glauca* (Moench) Voss, black spruce, *P. mariana* (Mill.) B.S.P., balsam fir, *Abies balsamea* (L.) Mill., or tamarack, *Larix laricina* (Du Roi) K. Koch. When 1-m long bolts of the same five conifer species were exposed to *T. fuscum* in Point Pleasant Park from June to September 2000, successful development and adult emergence was observed from *P. rubens* and *P. glauca* bolts only. Bolts were also collected from a number of living trees exhibiting resinosis typically associated with *T. fuscum* infestation (5 *P. glauca,* 1 *P. mariana* and 1 white pine, *Pinus strobus* L.) and incubated at 20°C for 12 weeks in containment facilities; adult *T. fuscum* emerged from 4 of 5 *P. glauca*, and from *P. mariana* but not from *Pinus strobus*.

Picea rubens was clearly preferred to other conifers by ovipositing *T. fuscum* females, suggesting it is the best species to use in a bait log strategy. Infestation and successful development of *T. fuscum* in *P. glauca* and *P. mariana* greatly extends its potential spread and impact in North America, because these conifer species extend from Atlantic Canada to Alaska.

The Effect of Various Doses of Pheromone on Mating Disruption in Gypsy Moth Population

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Abstract

An experiment was conducted in June-August 2001 in the Cumberland and Appomattox-Buckingham State Forests, Virginia to evaluate the effects of various doses of synthetic pheromone (racemic disparlure) on mating disruption of the gypsy moth, *Lymantria dispar* (L.). The pheromone was applied aerially in two formulations: a microcapsule formulation (3M Canada Co., London, Ontario) was applied at 0.15, 0.75, 3, 15, 37.5, 75 g a.i./ha, and pheromone in a plastic laminated flake formulation (Disrupt II, Hercon Environmental Corp., Emigsville, PA) was applied at a rate of 15 g a.i./ha. All doses were replicated twice, except for the 15 and 37.5 g a.i./ha doses of the microcapsule formulation, which were unreplicated. Mating disruption was evaluated using laboratory-reared tethered virgin females and the release and recapture of males with USDA milk-carton traps baited with (+) disparlure. Each study plot had two male moth release points with one at the center of the plot and the other at 175 m to the north. Fifteen tethered females were placed in a circle around the central release point. One trap each was placed 150 m to the south, east and west of the central release point and four traps were placed around the northern release point at a distance of 25 m from the point. Adult females were placed on tree boles for 1 day and protected from ant predation by a band of the tanglefoot glue. Fertilization was determined by the analysis of egg embryonation. Male moth recapture in pheromone traps was studied over three periods: 15-45, 50-62 and 64 days after pheromone treatment. The proportion of fertilized females was significantly lower in plots treated with the plastic flake formulation at 15 g a.i./ha and the microcapsule formulation at 15, 37.5 and 75 g a.i./ha ($P < 0.01$) than in plots treated with lower doses of pheromone and in control plots. Male moth catches in pheromone-baited traps were significantly lower in plots treated with the plastic flake formulation at 15 g a.i./ha and the microcapsule formulation at 37.5 and 75g a.i./ha ($P < 0.01$) compared to male moth catches in all other plots. However, male moth recapture rates at 64 days after pheromone application were significantly lower on the plots treated with plastic flakes than on the plots treated with any of the doses of pheromones in the microcapsule formulation. As a result of this study, the dose-response relationship among pheromone dose, female mating success, and male moth catch in pheromone traps was obtained for a range of pheromone doses. The results also suggest that the plastic flake formulation may have a longer lasting effect on mating disruption of gypsy moth than the microcapsule formulation.

Measurement of Pheromone Concentration Using a Portable Electroantennogram

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Abstract

Mating disruption is an increasingly important tactic against the gypsy moth in the United States. Since the full implementation of the federal Slow-the-Spread of the Gypsy Moth program in 2000, mating disruption has become the predominant method used. Support for this tactic is strong because it is species specific, environmentally benign, and has been shown to be highly effective. However, because funding for the program is limited, efforts are underway to reduce the cost of mating disruption applications by using lower doses, developing less costly and/or more efficient formulations, modifying application methods, etc. During 2001, a dose response test was conducted with an experimental microcapsule formulation developed by 3M Canada, London, Ontario to obtain data to help determine to what extent operational doses could be reduced. Application rates ranging from 0.15 to 75 g a.i./ha were tested in 25-ha plots in central Virginia. There were two replicate plots for each rate. Treatment effectiveness was assessed by moth capture in pheromone traps and mating success of laboratory-reared virgin females tethered to tree trunks. To ensure uniform moth populations across plots, laboratory-reared male moths were released in the plots. Application rates lower than 15 g a.i./ha did not reduce trap catch or mating success. Trap catch and mating were disrupted at the 15 g rate, but not as much as they were at 37.5 and 75 g/ha.

For the past 3 years and in conjunction with other STS research efforts, work has been under way to determine the feasibility of using a portable electroantennogram device (EAG) to measure pheromone levels in the air in areas treated with mating disruptants as a way of assessing the quality of the treatment. An EAG uses a moth antenna connected to electrodes to measure pheromone levels in the air. The strength of electrical impulses produced by the antenna indicate the amount of pheromone in the air. The development of the portable EAG as an analytical tool to directly measure pheromone concentration within blocks treated with gypsy moth mating disruptants could simplify and streamline the process of evaluating new mating disruption formulations and use patterns. The availability of this tool could facilitate the development and testing of new formulations capable of releasing pheromone more efficiently so that the applied dose can be reduced. In 2001, two portable EAG devices manufactured by Syntech, Hilversum, The Netherlands were purchased and tested. EAG readings were taken with both units in all dose-response test plots on a weekly basis from June 23 to August 6. EAG readings were highest in plots treated at the two highest application rates (37.5 and 75 g/ha). These higher EAG readings corresponded to higher levels of biological efficacy as determined by reduced moth capture in pheromone traps and decreased mating among monitor females. These findings support the conclusion that it is possible to detect and measure disparlure levels in plots treated at 75 or 37.5 g/ha (currently the most commonly used rate), but further refinement may be needed to detect pheromone at lower levels. Based on these results, and the results of previous EAG work, it appears that the Syntech EAG units may not be sensitive enough to detect pheromone at low application rates (<37.5 g/ha), even though these rates may be sufficient to disrupt mating. However, it appears that the units are capable of detecting pheromone at the application rates currently in operational use. Therefore, the EAG continues to show promise as a potential method to assess pheromone concentrations in treated plots, and work will continue to further refine procedures for its use.

Evaluation of Insecticides for Controlling the Asian Longhorned Beetle, *Anoplophora glabripennis* - a Synthesis Presentation

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Abstract

Since 1998, we have evaluated different insecticides as well as various application methods for the control of the Asian longhorned beetle (ALB), *Anoplophora glabripennis*. Evaluations have been conducted by two separate cooperative projects in China, one between USDA Animal Plant Health Inspection Service (APHIS) Otis Laboratory, USDA Forest Service (FS) Forest Health Technology Enterprise Team, Morgantown and the Chinese Academy of Forestry Institute of Forest Ecology Environment and Protection (CAF IFEEP), the other between USDA FS North Central Research Station and CAF IFEEP. In addition to the studies in China both the Otis laboratory and the North Central Research Station have conducted research in the U.S. This presentation summarizes all major activities and results of the above mentioned work.

1. Cover Insecticides Tested in China by APHIS Otis Laboratory and IFEEP

In 1999, eight insecticides (bifenthrin, deltamethrin, permethrin, acephate, chlorpyrifos, lindane, bendiocarb, and fipronil) were evaluated. Four concentrations were tested for each insecticide. Two insecticides, permethrin and fipronil, were tested for their residual effect. These insecticides were applied by cover spray onto twigs of trees, which were then cut and presented to adult beetles. The 2-day total adult ALB mortality was 100% for all insecticides tested except for bifenthrin (biflex 2EC, 0.02 ml/l), which was 63.3%. However, Mortality of adult ALB dropped to 30% when they were exposed to treated twigs collected more than 2 weeks post-treatment. In 2000, four insecticides (permethrin, chlorpyrifos, sevin, and acephate) were tested for short term and residual effects on adult ALB, the results again showed high mortality, but poor residual after 2 weeks, although there were some differences among the insecticides.

2. Systemic Insecticides tested in China by APHIS Otis Laboratory and IFEEP

a. Systemic insecticides and application methods

A total of nine insecticides with different application methods have been tested for their systemic action since 1998. The insecticides included: (1) imidacloprid, (2) methamidophos, (3) disyston, (4) metasystox–r, (5) acephate, (6) acetamiprid, (7) thiacloprid, (8) bidrin, and (9) thiamethoxam. The delivery methods evaluated were soil injection (Kioritz & pressurized soil injector; with or without prior watering of the soil) for disyston, imidacloprid, metasystox–r, methamidophos, and thiamethoxam; trunk injection (Mauget) for acetamiprid, bidrin, imidacloprid, metasystox-r, and thiacloprid; and trunk implant (Acecaps & Medicaps) for imidacloprid and acephate. Insecticides were applied in March, April, May, June, July, September, and October. Generally, imidacloprid applied through trunk and soil injection showed the most promise for ALB adult control. Acetamiprid through trunk injection and thiamethoxam through soil injection resulted in similar efficacy to ALB adults with imidacloprid. However, larval mortality (especially, later instars) was found to be low for all insecticides tested.

b. Dose-response of ALB adults and larvae to imidacloprid

The LC_{50} values for the applied level of imidacloprid to adult beetle were 87.4 ppm, 43.1 ppm, and 27.3 ppm at 24h, 48h, and 72h, respectively. These values correspond to 5.0, 2.9 and 1.9 ppm for the actual level of imidacloprid detected in the twigs. Our results indicated that mortality of adult beetles resulted not only from oral and contact poison, but also from their refusal to feed. Although the dose-response test of ALB larva to imidacloprid is still in progress, preliminary results indicated that it would take 6-7 weeks to achieve 50% larval mortality even with high doses (>50 ppm applied, >0.50 ppm detected). Antifeedant activity of imidacloprid to ALB larvae was also observed.

c. Translocation of imidacloprid in trees
Samples of leaves, twigs and bark/xylem of tree trunks were collected from as early as 15 days to more than 2 years post imidacloprid treatments. Imidacloprid levels detected trees differed with different application dates and delivery methods. In some cases, the imidacloprid level detected in all tree parts exceeded the LC_{50} within one month post application and remained above the LC_{50} level several months to one year after application. However, levels of imidacloprid in trees were from non-detectable to around 0.5 ppm 2 years after application.

3. Systemic Insecticides tested in China by FS North Central Station and IFEEP

a. Systemic insecticides and delivery methods
Imidacloprid (10% and 15% a.i., and thiacloprid (10%, and 15% a.i.) through trunk injection using Mauget devices, emamectin benzoate through trunk injection using shot one bottles and azadirachtin (4% a.i.) through systemic tree injection tubes were applied to elm, poplar and willow trees in 2000. Half of the trees were felled 4 months post-injection and the remaining trees were felled 1-year post injection. Mortality of all stages of ALB was assessed. Although there were differences among tree species in terms of ALB mortality, average mortality of all stages combined for all tree species were: imidacloprid = 22.1%; emamectin benzonate =16.7%, azadirachtin = 11.3% and control = 7.9%. The mean number of dead adults found under elm and poplar trees were 67.5, 31.5, 8.8, 35.9 for trees treated with imicide, 10% thiacloprid, 15% thiacloprid and azadirachtin, respectively, and 13.6% for the control.

b. Translocation of imidacloprid and other chemicals
Samples of leaves and twigs of elm, poplar and willow were collected 4 weeks post injection of imidacloprid and azadirachtin and analyzed to determine the level of each compound. Residues level were found to range between 1.10 (willow twig)-1.54 ppm (elm twig) for imidacloprid and from 1.21 (willow twig) to 30.65 ppm (elm leaf) for azadirachtin.

In an attempt to understand the translocation of insecticide, especially, imidacloprid in trees, poplar and willow trees were injected with acid fuchsin stain. Injected trees were felled and dissected 24 h later and the presence of dye was examined. It was found that the presence of dye was variable along main trunk and there was no clear trend with height above injection site, indicating dye moved up and down. Dye penetrated up to four rings deep, but did not reach the cambium until 2 m above the injection site.

4. Systemic insecticides tested in the US by APHIS Otis Laboratory

a. Mortality of adult ALB feed on twigs of Norway maple nursery stock treated with imidacloprid through either soil injection (Merit 75WP), or trunk implant (Medicaps) or treated with trunk implant of acephate (Acecaps) were compared in 1997. Soil injection of merit 75 WP killed more adults than trunk implants.

b. In 1999, insecticides were applied to red and sugar maple through different delivery methods (imidacloprid through Mauget, wedgle, Acecaps, soil injection, abamectin through wedgle, bidrin through Mauget). Samples of twigs, leaves, and wood were collected 2-4 months post-application and imidacloprid levels were determined. The highest level of imidacloprid (2.1 ppm) was detected in wood of trees treated with imidacloprid through Mauget injection devices. Abamectin through wedgle, and bidrin through trunk injection did not yield detectable levels of each insecticide.

c. In 2000, imidacloprid was injected into trunks of red maple using CO2 tree tunk injection system at 300 PSI. Samples of tree parts were collected 2 weeks, 4 weeks, and 8 weeks post application, and imidacloprid levels were determined. This injection system is time consuming and resulted in non-detectable level (<0.03 ppm) of imidacloprid in trees. Also in 2000, different Mauget imicide treatment methods (i.e., deep vs shallow, 1 hr vs 2 hrs vs 3 hrs vs 4 hrs delivery time) were compared. No significant differences were found among treatment methods in terms of imidacloprid levels in trees 1-month post application.

d. In 2001, the efficacies of prophylactic treatments with imidacloprid (Mauget imicide and soil injection) were evaluated in IL and NY. In addition, imidacloprid treatment methods (drench, granular and soil plug) for containerized stock in two species of trees (birch, Japanese red maple) were evaluated. Comparison of Mauget imicide and Merit 75 WP soil injection (with and without wetting agent) in spring and fall was initiated in NY. Chemical analysis to determine imidacloprid levels in these trees is still in progress.

e. In 2001, levels of imidacloprid in leaf, twig and wood of different sizes (small =2-4" DBH, medium = 8-11" DBH, large = 12-32" DBH) of trees (American and slippery elm, Norway and silver maple, white ash) were compared 2 months post application for 3 different application rates (doses). The results indicated that higher application rates often yielded higher levels of imidacloprid in trees. Levels of imidacloprid were much higher in small trees than large ones. Generally, levels of imidacloprid detected in elm were higher than those in maple, which were higher than those in ash.

5. Systemic Insecticides tested in the US by FS North Central Station

a. Laboratory bioassay of imidacloprid and thiamethoxam.
In 1999, American elm, poplar, silver maple, and boxelder were treated with imidacloprid (through Mauget, Acecaps, Wedgle, and soil injection using the Kioritiz injector), and thiamethoxam (soil injection using the Kioritiz injector). Mortality of larvae transferred into branches of trees of different treatment were compared. The highest mortality was found for larvae transferred into boxelder treated with Maugets (= 70%).

Another bioassay was conducted in 2000. Insecticides (imidacloprid through Mauget and Kioritz soil injector, emamectin benzoate through Shot One bottles, and azadirachtin through systemic tree injection tubes) were injected into silver maple and boxelder. Overall mortality for ALB larvae transferred into branch sections of treated trees was very low. Samples of leaves, twigs and wood were collected. Residue analysis to determine levels of imidacloprid, emamectin benzoate, and azadirachtin are still underway.

b. ALB and cottonwood borer larvae reared on diet treated with imidacloprid and neem extracts.
Larvae of ALB and cottonwood borer were fed with artificial diet mixed with several concentrations of imidacloprid and azadirachtin extracts. The results indicated that both insecticides have strong antifeedant effects. It took 12 weeks to achieve 100% mortality on the highest doses and some mortality also occurred on the lowest doses.

Orientation of an Asian Longhorned Beetle, *Anoplophora glabripennis*, Towards Objects of Different Shapes and Colors

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Abstract

Silhouettes of different colors, shapes and sizes made of bamboo frames covered with cloth, paired in different color sets, were placed equidistantly around the perimeter of a circle with a 7.5 m radius, in an open area. About 50 -100 caged beetles were released in the center of the circle at the rate of about 1 beetle per 2 minutes for each release period. The flight paths of individual beetles were visually tracked and some were videotaped. The number of adult beetles that landed on each type of silhouettes as well as those left the test arena was recorded together with other types of behavior. Weather conditions, such as temperature, humidity, solar radiation, wind speed and direction during all observation periods, were recorded.

Although only about one third of the released adult ALB landed on the silhouettes, this proportion is significant considering that the space these silhouettes occupied is less than 10% of the arena. Among the beetles that landed on silhouettes of same size cylinders, more landed on silhouettes in black than landed on those of other colors. This, together with the behavior of adults flying close to the silhouettes, indicates that the beetle can perceive at least some color within short distances and prefer objects in black. When black silhouettes of different shapes and sizes were placed in the arena, more beetles landed on taller silhouettes, and those with the larger surface area at the top half. More beetles landed on cylinders whose top half or third was green than those that were black. The speculation is that the silhouettes whose top half or third were green probably look more like a tree to ALB adults than the ones whose top half or third were black. More studies will be conducted in 2002.

Measuring Stress and Recovery in Limed Sugar Maple on the Allegheny Plateau of North-Central Pennsylvania

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Abstract

A long-term study to evaluate the effects of lime fertilization on growth, crown vitality, and flower and seed production in declining sugar maple was established in stands in the Susquehannock State Forest on the Allegheny Plateau in north-central Pennsylvania in 1985. A single application of commercial pulverized dolomitic limestone (Ca=21%, Mg=12%, CaO equivalent=58.8) was applied at 22.4 Mg/ha^{-1} to plots in four stands on the forest. Recent evaluations of these study plots for growth and crown vitality in 1993 indicated that liming had a significant and positive effect on these health variables. This positive response of sugar maple to liming offered a unique opportunity to evaluate other indicators of tree stress/vitality and determine if they also indicated a positive response to lime additions. Foliar polyamines (low molecular weight organic polycations), starch and soluble sugar concentrations in root tissues, and cambial electrical resistance (CER) measured with a Shigometer were evaluated. Polyamines were measured in July 1997 and 1998. Root tissue for starch and sugar analyses was collected in November 1997 when starch concentration was anticipated to be highest and sugar levels lowest; starch in root wood was measured both visually and chemically. CER was measured in July 1998. We hypothesized that all measures would indicate an increase in vitality for trees in the limed plots; values of all stress indicators confirmed this hypothesis. Polyamine levels in the foliage, soluble sugar concentration, especially glucose and fructose, and CER decreased and starch content in both root bark and wood tissue increased. These changes were correlated with increases in tree vitality as measured by increased growth (dbh) and improved crown condition. Indicator levels were also significantly correlated with changes in soil and foliar cations in response to liming. Polyamines, soluble sugars, and CER decreased and starch content increased as concentrations of Ca and Mg and molar ratios of Ca/Al and Mg/Mn increased and as concentrations of Al and Mn decreased in both soil and foliage. Favorable changes in these stress/vitality indicators were also linked to an increase in soil pH. Our study confirms the beneficial effects of lime additions on sugar maple vitality, and the utility of physiological and biochemical stress indicators to assess tree vitality.

Evaluation of Production Method and Formulation for Optimizing In-vitro Produced Gypchek

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Abstract

Gypchek* (USDA Forest Service, Washington, DC), a product with the *Lymantria dispar* multi-enveloped nucleopolyhedrovirus (LdMNPV) as the active ingredient, is a general use biopesticide for use against the gypsy moth. Successful field trials with Gypchek incorporated with the commercially-produced Carrier 038 (Valent BioSciences Corp., Libertyville, IL) and environmental concerns over the effects of non-specific insecticides applied to forest ecosystems have stimulated interest in the use of Gypchek. Gypchek, as currently produced in vivo, has some limitations. It is expensive to produce, contains extraneous material, and lacks potency at affordable dosages. A strain of LdMNPV (strain 203) has been developed at the USDA Forest Service's Forest Science Laboratory at Delaware, OH, that is as potent as the strain LDP 226 currently used in Gypchek, kills a bit faster, and is stable in cell culture. Its use would result in an inherently clean product with the potential for industrial-scale production at reduced cost. Reduced cost would permit a higher dosage application that should result in improved efficacy. Gypchek produced in-vivo has a half-life of about 12 hours in direct sunlight. An in-vitro produced commodity should be more susceptible to degradation by sunlight since it would lack the insect melanins, etc., present in the partially purified in-vivo product. Valent BioSciences Corp. has addressed the sunlight degradation problem along with improving mixing characteristics in their new product, Carrier 038A. Production techniques may affect potency possibly explaining some variable results seen in earlier studies with strain 203 produced in-vitro.

We thus designed a test with the following goals: (1) We sought to evaluate in vivo produced LDP226 against in-vitro produced strain 203. (2) Strain 203 was evaluated as produced by three different methods to determine if method of production affects performance. (3) We sought to evaluate the effectiveness of Carrier 038A for protecting the virus products from sunlight.

We designed a study that compared in-vivo produced strain LDP226 against strain 203 produced by three different methods. All strains were tested at two doses: 1 x 1012 polyhedral inclusion bodies (PIBs) per ha and 1 x 1011 PIBs per ha. All of the above combinations were applied with and without Carrier 038A, and all the above were evaluated as 1-hour, 1-day, and 2-day residues. There was a Carrier 038A control and a distilled water control. We report the results of 18 treatments arranged in six randomized blocks.

We used a "bugs-in-bags" approach (developed at the University of Massachusetts by Joe Elkinton and students) at a research site at Cedar Swamp, DE, where we have access to large swathes of low, accessible oak

foliage. Two branch tips were pre-selected at each point for each treatment combination and residue date. A cloth bag was placed over each branch tip with leaves bearing treatment residues, and the larvae were added. Larvae were allowed to feed on the leaves for 7 days, were removed from the bags, placed individually in 30-ml diet cups half filled with gypsy moth diet, and held until death or pupation. All cadavers were necropsied.

Analysis of variance revealed that time (residue period) effects, dose effects, and carrier (with or without) effects, but not method of production effects were significant at P = 0.05. We concluded that: (1) In-vitro produced 203 was equivalent in potency to in-vivo produced LDP226. (2) Any affect of production method on potency of strain 203 was minor. (3) Product potency (either strain produced by any method) was greatly enhanced when applied with Carrier 038A. (4) Carrier 038A provided excellent protection from sunlight for at least 2 days.

Distribution and Abundance of *Anoplophora glabripennis* in Stands of its Native Host in South Korea and China

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Abstract

Following up on our discovery in 2000 that *Acer mono* is a native host of *Anoplophora glabripennis*, we spent eight weeks searching for the beetle in natural forest stands in South Korea and China. We wanted to assess the distribution and abundance of beetles in closed forest stands of native hosts. We searched for beetles on *A. mono* for seven weeks in South Korea. The first author then spent four days in Jilin Province, China, searching for beetles in natural stands of *A. mono*. *Acer mono* grows naturally in forests throughout South Korea and is most frequent in mountainous riparian habitats. It grows in steep, rocky ravines where water often is present and in more level terrain along or near streambanks.

We carried out extensive searches for beetles in five national parks, two recreational forests, and other forested areas of South Korea. We examined thousands of *A. mono* trees for beetles and their oviposition pits, larval sawdust holes, and adult emergence holes. We used binoculars because beetles live high in trees. Although we observed what appeared to be beetle damage in several places, we found adult beetles in just three. Because of the prevalence of beetles, we chose two locations in Mt. Sorak National Park for intensive study: the Young-Dae recreational forest and the Oknyo-Tang rest stop. We developed detailed maps of all *A. mono* trees in the two areas.

Beetles were very difficult to find in natural habitats in South Korea. We found none in China, although our search was short, by no means extensive, and probably too late in the season to find adults. More thorough searches are needed next season. In South Korea, we generally did not find beetles in closed stands in rocky ravines containing a high density of *A. mono* trees. We typically found them in more open habitats near roads and streams. Thus, it appears that *A. glabripennis* probably is a denizen of relatively open habitats, most likely attacking trees along streambanks under natural conditions. This may facilitate its movement and location of new hosts.

Tomicus piniperda (Scolytidae): a Serious Pest of Yunnan Pine in Southwestern China

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Abstract

The pine shoot beetle, *Tomicus piniperda* (L.), is native to the pine growing regions of Europe, Asia, and northern Africa (Bakke 1968; Långström 1983; Ye 1991). Established populations of *T. piniperda* were first found in North America in 1992 (Haack and Kucera 1993), and as of January 2002, it now occurs in 318 counties in the United States and 51 counties in Canada (Haack and Poland 2002). *Tomicus piniperda* occurs throughout China, but has been most destructive in Yunnan Province in southwestern China (Fig. 1; Haack et al. 1998), where it attacks primarily Yunnan pine, *Pinus yunnanensis* (Ye 1995, 1998, 1999). In Yunnan, the first reports of *T. piniperda* killing Yunnan pine began in the early 1980s (Ye 1991). Since then, more than 200,000 ha. of Yunnan pine forests have been severely damaged and killed (Ye 1999). In recent years, *T. piniperda* populations have been increasing in Yunnan, with more than 100,000 ha of Yunnan pine forests seriously infested as of 2001.

Tomicus piniperda completes one generation per year throughout its entire range. In Yunnan, the new generation adults begin to shoot feed in May and continue through March of the next year (Ye and Li 1994, Ye 1996). When 60-90% of the shoots on individual trees have been attacked and killed by *T. piniperda*, trees become highly susceptible to trunk attack by *T. piniperda* (Hui and Lieutier 1997). Because daily wintertime temperatures in Yunnan are usually >10°C, *T. piniperda* adults overwinter in the shoots rather than at the tree base as they do in the colder parts of their geographic range (Bakke 1968; Långström 1983; Ye 1994, 1996). Reproduction in Yunnan typically begins in November, peaks in February and March, and ends in May. Adult flight is common when air temperatures exceed 15°C. In some cases, *T. piniperda* adults first aggregate in the crowns of selected trees prior to trunk attack (Hui and Lieutier 1997). Trunk attack on these trees usually starts along the upper trunk near the base of the crown, and later spreads downward to the lower trunk (Ye 1995, 1999). Overall, most trunk attacks on Yunnan pine occur in the upper- and mid-bolts, which is opposite to the attack pattern found on Scots pine, *Pinus sylvestris* (Ye 1995). Overall, *T. piniperda* can seriously injure Yunnan pine because of their extended period of shoot feeding and their ability to directly attack and breed in the trunks of living trees.

Tomicus piniperda occurs throughout much of Yunnan, with the most severe damage occurring in a zone that extends from northwestern to southeastern Yunnan (Ye 1998, 1999). *Tomicus piniperda* is found at elevations between 600-3000 meters in Yunnan, with the highest populations occurring at about 2000 meters. Yunnan pine is the preferred host of *T. piniperda* in Yunnan. The *T. piniperda* outbreak area in Yunnan mirrors the geographic range of Yunnan pine. The average annual precipitation throughout most of *T. piniperda*'s Yunnan range is 700 to 1100 mm. About 20% of the annual rainfall occurs from November to early May when *T. piniperda* adults attack and reproduce in the trunks of live pine trees. Drought stress may be one of the key factors that increase tree susceptibility to *T. piniperda* attack (Ye 1999). The average annual temperature in central Yunnan is 15°C, and varies from an average of 9°C in winter to 23°C in summer. Such warm temperatures allow *T. piniperda* to be active year round in Yunnan.

In Yunnan, *T. piniperda* populations first increased to damaging levels in the early 1980s (Ye 1991). Populations peaked in 1987 and then declined through 1996. In 1997, populations increased again, reaching their highest levels in 2001 (Fig. 1). Moreover, in 2001, *T. piniperda* infested forested areas of Yunnan where it had never been previously reported. The high populations in 2001 may in part be related to several consecutive years of warm and dry winters that preceded 2001. Another factor that may be related to the *T. piniperda* buildup is the widespread cutting of infested trees with no immediate reforestation. As the stands became more open, they apparently became drier, which further increased their susceptibility to *T. piniperda* attack. In addition, during the late 1990s, tree removal often occurred after the brood adults had

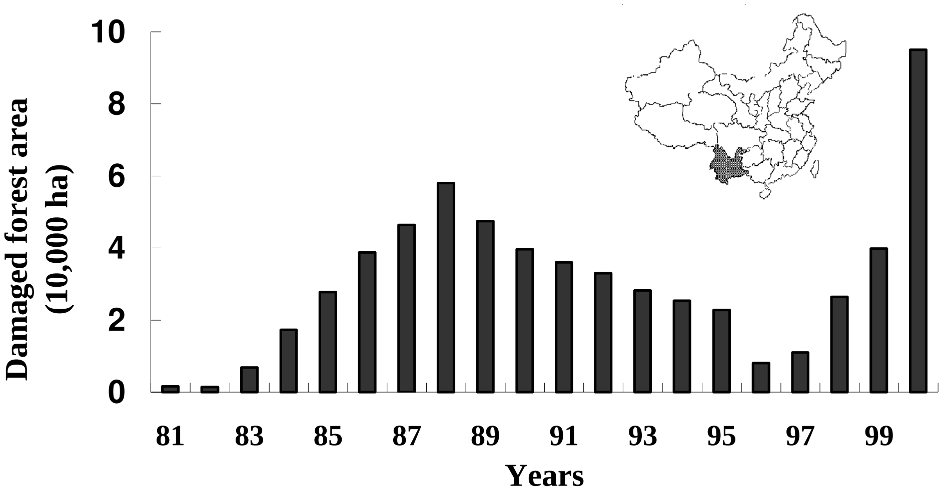

Figure 1.—Total area of Yunnan pine forest severely affected by *Tomicus piniperda* in Yunnan during the period 1981-2000. In addition, an outline map of China is shown, indicating the location of Yunnan Province (shaded area).

already exited the trees, which therefore did not help in lowering local *T. piniperda* populations.

The pathogenic fungus, *Leptographium yunnanense*, was first reported from galleries of *T. piniperda* in Yunnan in 2000 (Ye and Zhou 2000; Zhou et al. 2000). This bluestain fungus can kill Yunnan pines when artificially inoculated in the trunk, suggesting that this fungus can aid *T. piniperda* in overcoming tree resistance. The bluestain fungus *Ophiostoma minus* can also be isolated from *T. piniperda* adults and their galleries in Yunnan (Ye and Zhou 2000). At high densities, *Ophiostoma minus* can kill Yunnan pines. However, the frequency of isolating *Ophiostoma minus* from *T. piniperda* is much lower in Yunnan than in Europe.

Tomicus minor has the same geographic distribution as does *T. piniperda* in Yunnan. *Tomicus minor* tends to attack and breed in trees already attacked by *T. piniperda* (Ye 1997). Because *T. piniperda* typically initiates attack in the upper trunk, *T. minor* tends to colonize the lower and mid trunk (Ye 1997). The within-tree distribution pattern of these two *Tomicus* species in Yunnan is opposite to that reported on Scots pine in Europe. It is suggested that *T. minor* assists *T. piniperda* in overcoming tree resistance and accelerating tree death (Ye 1997; Ye and Ding 1999).

Thanasimus formicarius (Coleoptera: Cleridae) usually completes one generation per year in Yunnan. Adult *T. formicarius* oviposit in trees that have been attacked by *T. piniperda* (Ye et al. 1999). Oviposition begins in spring and continues through mid-summer. Clerid larvae prey on the immature stages of *Tomicus* along with other organisms living under the bark. Until now, *T. formicarius* populations have rarely reached high enough levels to influence *Tomicus* populations in Yunnan (Ye and Zhao 1995). One possible reason for this lack of impact is that most *T. formicarius* remains in the larval stage long after *T. piniperda* have matured and exited the trees. As the results, little food in the tree allows *T. formicarius* larvae to complete their development. Woodpeckers, such as *Dendrocopos major* and *Picus canus*, have also been found feeding on the immature stages of *T. piniperda*. However, the impact of woodpeckers on *T. piniperda* populations may be limited due to low bird populations in most areas of Yunnan.

Removal of the trunk-infested trees is the primary approach used to control *T. piniperda* in Yunnan. Most trunk-infested trees are cut in April before the new generation of *T. piniperda* have completed development and exited the trees. However, 3-4 cuttings per year are often needed due to *T. piniperda*'s extended period of trunk attack and production of sister broods. As a long-term control strategy, establishment of mixed forests has also been initiated in recent years to increase habitat diversity and abundance of natural enemies, which is hopeful to reduce *T. piniperda* outbreak. Fir (*Cryptomeria fortunei*, *Cunninghamia lanceolata*), cypress (*Platycladus orientalis*, *Calocedrus macrolepis*), and other pine species such as *Pinus armandii* are planted after removal of Yunnan pine. In addition, plans are now underway to augment natural enemy populations in Yunnan pine forests, using in particular the clerid *Thanasimus formicarius*.

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Exotic Species Patterns and Function in Urban Landscapes

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Abstract

Mack et al. (2000) state “Biotic invaders are species that establish a new range in which they proliferate, spread, and persist to the detriment of the environment.” This statement is true for many natural landscapes. In urban landscapes, however, exotic species are critical components of the landscape and enhance its livability. Exotic species provide aesthetic beauty, recreational opportunities, and ecosystem services and benefits.

Urbanization directly and indirectly affects natural ecosystems and species composition (McDonnell and others 1997). The most obvious direct effects are deforestation and fragmentation. Deforestation reduces forest cover, creates new forest edge, simplifies edges, decreases of forest interior habitat, and increases patch isolation (Zipperer 1993). Examples of indirect effects include urban heat island effect, soil hydrophobicity (White and McDonnell 1988), air pollution (Lovett and others 2000), and altered disturbance regime (Pickett 1998). Because of direct and indirect effects, native species may not be able to grow or survive on sites previously occupied. Although exotics species do displace native species, exotic species may be occupying niches left empty by native species and new niches created by urbanization.

In forested systems, studies of exotic species distribution reveal an increase in density and number as a landscape becomes more urbanized. This pattern, however, may be dependent on the strata of the forest. For example, for riparian forest habitats, the canopy stratum (woody stems ≥ 2.5 cm dbh) had greater stem density and higher number of exotic species in the urban landscape than in rural landscapes (Brush and Zipperer in review). For the shrub stratum (woody stems <2.5 cm dbh and ≥ 1 m in height), a different pattern was observed. The rural landscape had a greater stem density but a fewer number of exotic species than the urban landscape. These differences were attributed to site legacy and species performances.

In urban landscapes, exotic species contribute to the ecosystem benefits. An analysis of net carbon sequestration for the entire city of Syracuse, New York revealed that exotic species account for 45 percent of the net carbon sequestered (Nowak and Crane 2000, Nowak and others 2001). This percent varied by land use. In commercial land use, exotic species sequestered only 14 percent of the total net carbon sequestered by vegetation in that land use, whereas in residential land use, the percentage was 56 percent. Similar observations have been observed for air pollution removal. The removal of exotic species from the urban landscape potentially could create an ecological disaster for urban residents and significantly impair air and water quality.

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