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# Proceedings

## XV U.S. Department of Agriculture Interagency Research Forum on Gypsy Moth and Other Invasive Species 2004

*The* *th*

USDA INTERAGENCY  
RESEARCH FORUM  
ON GYPSY MOTH & OTHER INVASIVE SPECIES

JANUARY 13-16, 2004  
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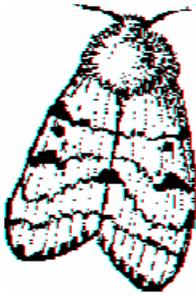
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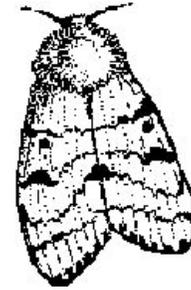
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## FOREWORD

This meeting was the 15th 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

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The program committee would like to thank The Heron Group, LLC, Valent BioSciences Corporation, Arborjet, Inc., Hercon Environmental, and the Management and Staff of the Loews Annapolis Hotel and the O'Callaghan Hotel for their support of this meeting.

**Program Committee:** Mike McManus, Kevin Thorpe, Vic Mastro, Joseph Elkinton, and Barbara Johnson

**Local Arrangements:** Kathleen Shields, Katherine McManus

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# Contents

<b>USDA APHIS Offshore Pest Information System.....</b>	<b>1</b>
<i>Robert J. Balaam</i>	
<b>Alder Decline in Austria Caused by a Hybrid <i>Phytophthora</i> .....</b>	<b>3</b>
<i>Yilmaz Balci and Thomas L. Cech</i>	
<b><i>Phytophthora</i> Diseases of Oak in Europe.....</b>	<b>4</b>
<i>Yilmaz Balci</i>	
<b>Invasive Plants of the Eastern United States: Identification and Control</b>	
<b>FHTET-2003-08. CD-rom.....</b>	<b>6</b>
<i>C.T. Barger, D. J. Moorhead, G.K. Douce, R.C. Reardon and A.E. Miller</i>	
<b>Studies on Microbial Insecticides for Suppression of Emerald Ash Borer Populations ..</b>	<b>7</b>
<i>Leah S. Bauer, Houing Liu and Deborah L. Miller</i>	
<b>Natural Enemies of the Emerald Ash Borer in Michigan and China .....</b>	<b>8</b>
<i>Leah S. Bauer, Houping Liu, Deborah L. Miller, Toby R. Petrice, Robert A. Haack, Ruitong Gao and Zhao Tonghai</i>	
<b>Life Cycle of the Emerald Ash Borer in Michigan .....</b>	<b>9</b>
<i>Leah S. Bauer, Deborah L. Miller, Houping Liu, Toby R. Petrice and Robert A. Haack</i>	
<b>Eradicating the Asian Longhorned Beetle <i>Anoplophora Glabripennis</i> from</b>	
<b>Woodbridge, Ontario—an Update on CFIA’s Efforts.....</b>	<b>10</b>
<i>Jon Bell</i>	
<b><i>Halyomorpha Halys</i>, (Heteroptera: Pentatomidae), the Brown Marmorated</b>	
<b>Stink Bug; are Trees the Primary Host for this New Invasive Pest? .....</b>	<b>12</b>
<i>Gary Bernon, Karen M. Bernhard, E. Richard Hoebeke, Maureen E. Carter and Leann Beanland</i>	
<b>Hemlock Woolly Adelgid (<i>Adelges tsugae</i> Annand) Phenology and Predators in</b>	
<b>the Pacific Northwest .....</b>	<b>13</b>
<i>Maggie K. Byrkit and Darrell W. Ross</i>	
<b>Reproductive Strategies of <i>Scymnus</i> Ladybeetle Predators of HWA.....</b>	<b>14</b>
<i>Carole A. S-j. Cheah, Kathleen S. Shields, Paul M. Moore, Jenny E. Ogradnick and Gregg L. Bradford</i>	
<b>Chemical Defenses in Garlic Mustard (<i>Alliaria petiolata</i>) and their Potential Role</b>	
<b>in Species Interactions in Forest Understories.....</b>	<b>15</b>
<i>Don Cipollini</i>	
<b>Development of Insect-killing Fungi for Management of Hemlock Woolly Adelgid ....</b>	<b>18</b>
<i>Scott D. Costa, Margaret Skinner, Svetlana Gouli, Michael Brownbridge, Vladimir Gouli, William Reid and Bruce L. Parker</i>	
<b>HWA Cold-hardiness: Towards Defining Limits of Range Expansion .....</b>	<b>19</b>
<i>Scott D. Costa, Margaret Skinner and Bruce L. Parker</i>	
<b>Comparing Systemic Imidacloprid Application Methods for Controlling</b>	
<b>Hemlock Woolly Adelgid .....</b>	<b>23</b>
<i>Richard S. Cowles, Carole S.- J. Cheah and Michael E. Montgomery</i>	
<b>Arborjet Methodology: Assaying New Systemic Formulations for Trunk Injection</b>	
<b>and Micro-infusion Technologies.....</b>	<b>24</b>
<i>Joseph J. Docola, Peter M. Wild, Christine Taylor and Eric Bristol</i>	

<b>Invasive.org: a Web-based Image Archive and Database System Focused on North American Exotic and Invasive Species .....</b>	<b>25</b>
<i>G.K. Douce, D.J. Moorhead, C.T. Bargeron and R.C. Reardon</i>	
<b>An Anisotropic Model of Hemlock Woolly Adelgid Spread .....</b>	<b>26</b>
<i>Alexander Evans</i>	
<b>Competitive Interactions Among Three Exotic Predators of the Hemlock Woolly Adeglid .....</b>	<b>29</b>
<i>Robbie W. Flowers, Scott M. Salom and L.T. Kok</i>	
<b>Development of Survey Tools for the Emerald Ash Borer .....</b>	<b>31</b>
<i>Joseph A. Francese, Victor C. Mastro, David R. Lance, Stephen G. Lavalley, Jason B. Oliver and Nadeer Youseff</i>	
<b>Effects of Parental Age at Mating on Sex Ratios of the Gypsy Moth Parasitoid <i>Glyptapanteles Flavicoxis</i> (Hymenoptera: Braconidae) .....</b>	<b>32</b>
<i>R.W. Fuester, K.S. Swan, P.B. Taylor, S.C. Wingard and G. Ramaseshiah</i>	
<b>A New Species of <i>Laricobius</i> from China (Coleoptera: Derodontidae), a Predator of Hemlock Woolly Adelgid.....</b>	<b>33</b>
<i>Holly A. Gattton, Gabriella Zilahi-balogh, Scott M. Salom and L.T. Kok</i>	
<b>Fruit Fate, Seed Germination and Growth of an Invasive Vine: an Experimental Test of “Sit and Wait” Strategy.....</b>	<b>34</b>
<i>Cathryn H. Greenberg, Evelyn Konopik, Lindsay M. Smith and Douglas J. Levey</i>	
<b>Emerald Ash Borer: Studies on Firewood, Host Range, Dispersal, and Occurrence in China.....</b>	<b>37</b>
<i>Robert A. Haack, Toby R. Petrice, Leah S. Bauer, Deborah L. Miller and Houping Liu</i>	
<b>Assessing the Safety of Microbial Biological Control Agents .....</b>	<b>38</b>
<i>Ann E. Hajek</i>	
<b>Field Study to Evaluate the Egg Parasitoid <i>Aprostocetus Anoplophorae</i> Sp. N. (Hymenoptera: Eulophidae) on Two <i>Anoplophora</i> Hosts.....</b>	<b>40</b>
<i>Franck Hérard, Christian Cocquempot, Jaime Lopez, Johanna Covi, Matteo Maspero and Mario Colombo</i>	
<b>Evaluation of Resistance of Asian and North American Ashes to Emerald Ash Borer... </b>	<b>43</b>
<i>Daniel Herms, David Smitley, Pierluigi Bonello and Deborah Mccullough</i>	
<b>Systemic Resistance in Gypsy Moth to LdNPV.....</b>	<b>44</b>
<i>Kelli Hoover, Diana Cox-Foster, Mike Grove and Jim Mcneil</i>	
<b>CFS-Atlantic Exotic Beetles and Associated Fungi Project 2002-2005 .....</b>	<b>45</b>
<i>J.E. Hurley, G.A. Smith, K.J. Harrison, A.W. Mackay, A.S. Doane, D.S. O'Brien and T.J. Walsh</i>	
<b>Influence of Light Condition on the Spatial Distribution an Ambrosia Beetle <i>Platypus Quercivorus</i> (Murayama) (Coleoptera: Platypodidae) Flying in a Natural Secondary Broad-leafed Forest.....</b>	<b>46</b>
<i>Yutaka Igeta, Kenryu Kato, Naoto Kamata and Kojiro Esaki</i>	
<b>Are Island Forests Vulnerable to Invasive Defoliators? .....</b>	<b>47</b>
<i>M. (Nod) Kay</i>	
<b>Alternate Iron Sources for Use in Gypsy Moth Artificial Diet .....</b>	<b>48</b>
<i>Melody A. Keena</i>	

<b>Mating Parameters Associated with Fertility in <i>Anoplophora Glabripennis</i> (Coleoptera: Cerambycidae)</b> .....	49
<i>Melody A. Keena and Vicente Sánchez</i>	
<b>Potential Distribution of the Emerald Ash Borer (<i>Agrilus Planipennis</i>)</b> .....	50
<i>Daniel A. Kluza and Eduard Jendek</i>	
<b>Use of an Artificial Inoculation Technique to Identify American Beech Trees with Resistance to the Beech Scale Insect</b> .....	51
<i>Jennifer L. Koch and David W. Carey</i>	
<b>Analysis of Japanese Oak Wilt Spread Using Aerial Photography and GIS</b> .....	52
<i>Ryotaro Komura, Andrew Liebhold, Koujiro Esaki, Ken-ichiro Muramoto and Naoto Kamata</i>	
<b>The Effect of Placement Height, Color and Release Rate on Trap Catches of the Asian Longhorn Beetle, <i>Anoplophora Glabripennis</i></b> .....	53
<i>Jennifer Lund, Joseph A. Francese and Stephen A. Teale</i>	
<b>Biology and Phenology of the Emerald Ash Borer, <i>Agrilus Planipennis</i></b> .....	54
<i>Barry Lyons, Gene C. Jones and Kerrie Wainio-Keizer</i>	
<b>Asian Longhorned Beetle Cooperative Eradication Program. Program Status Report Fy 2003</b> .....	55
<i>Christine K. Markham</i>	
<b>Comparison of the Palatability of Gypsy Moth Across Several Woody Species Native to Northern Japan</b> .....	59
<i>Sawako Matsuki, Hirohumi Hara and Takayoshi Koike</i>	
<b>Emerald Ash Borer Canadian Program Update</b> .....	60
<i>Mélanie Mecteau</i>	
<b>Ethanol and <math>\alpha</math>-pinene—Nationwide Survey of Responses of Bark and Wood Boring Beetles</b> .....	62
<i>Daniel R. Miller and cooperators</i>	
<b><i>Scymnus Ningshanensis</i> Yu Et Yao (Coleoptera: Coccinellidae) for Biological Control of <i>Adelges Tsugae</i> (Homoptera: Adelgidae)</b> .....	63
<i>Michael Montgomery, Hongbin Wang, Elizabeth Butin, Defu Yao, Wenhau Lu and Nathan Havill</i>	
<b>Evidence for Host Tree Resistance Against the Asian Longhorned Beetle</b> .....	64
<i>W.D. Morewood, K. Hoover, P.R. Neiner, J.R. McNeil and J.C. Sellmer</i>	
<b>Evaluation of Potential Host Trees for <i>Anoplophora Glabripennis</i> (Coleoptera: Cerambycidae)</b> .....	65
<i>W.D. Morewood, P.R. Neiner, J.R. Mcneil, J.C. Sellmer and K. Hoover</i>	
<b>Developing Attractants and Trapping Techniques for the Emerald Ash Borer</b> .....	66
<i>Therese M. Poland, Peter De Groot, Gary Grant, Linda MacDonald and Deborah G. McCullough</i>	
<b>Exotic Scolytids in North America: Who's Here and What are the Impacts</b> .....	67
<i>Robert J. Rabaglia</i>	
<b>Impacts of Imidacloprid on Target and Non-target Species on Hemlock</b> .....	68
<i>Michael J. Raupp, Ralph E. Webb, Adrianna Szczepaniec, Donald Booth and Robert Ahern</i>	

<b>Analysis of the Asian Longhorned Beetle Infestation in Jersey City, NJ</b> .....	70
<i>A.J. Sawyer, V. Mastro, B.C. Emens and T. Denholm</i>	
<b>The Asian Gypsy Moth Situation in Mongolia</b> .....	71
<i>Paul W. Schaefer, Baigal-Amar Tuulaikhuu, Clyde E. Goulden and Tatu Kira</i>	
<b>2002-2003 Winter Mortality of Hemlock Woolly Adelgid in the Northeastern United States</b> .....	72
<i>Kathleen S. Shields and Carole A.S-J. Cheah</i>	
<b>Control of the Browntail Moth in Maine with a Baculovirus</b> .....	73
<i>James Slavicek, Joseph Elkinton and John Podgwaite</i>	
<b>Research on Microsporidia as Potential Classical and Augmentative Biological Control Agents of the Gypsy Moth</b> .....	74
<i>L. Solter, V. D'amico, D. Goertz, G. Hoch, M. Hylis, A. Linde, M. Mcmanus, J. Novotny, D. Pilarska, P. Solter, J. Vavra, J. Weiser and M. Zubrik</i>	
<b>Recent Improvements in the Efficacy of Trapping the Brown Spruce Longhorn Beetle, <i>Tetropium Fuscum</i> (F.), Using Host Volatile-baited Traps</b> .....	76
<i>Jon Sweeney, Peter de Groot, Linda MacDonald and Jerzy M. Gutowski</i>	
<b>Efficacy of Gypsy Moth Mating Disruption Treatments in the Slow-the-spread (STS) Project</b> .....	77
<i>Kevin W. Thorpe</i>	
<b>The Spread of Gypsy Moth in Wisconsin: a New Paradigm for the Midwest Invasion?</b> .....	78
<i>Patrick C. Tobin</i>	
<b>Dispersal and Spatiotemporal Dynamics of Asian Longhorned Beetle in China</b> .....	80
<i>Patrick C. Tobin, Michael T. Smith, Guohong Li and Ruitong Gao</i>	
<b>Update on the Efficacy of Selected Systemic Insecticides for the Control of the Asian Longhorned Beetle</b> .....	81
<i>Baode Wang, Victor C. Mastro, Ruitong Gao and Richard C. Reardon</i>	
<b>Applied Baculovirus and <i>Entomophaga maimaiga</i> Provide Outstanding Control of Gypsy Moth</b> .....	83
<i>R.E. Webb and G.B. White</i>	
<b>Application of Hyperspectral Imaging to Survey for Emerald Ash Borer</b> .....	84
<i>David W. Williams, David W. Bartels, Alan J. Sawyer and Vic Mastro</i>	
<b>Poster Display 2004</b> .....	85
<b>Missing Presentation Abstracts from the 2004 USDA Research Forum</b> .....	87
<b>Missing Poster Abstracts from the 2004 USDA Research Forum</b> .....	88
<b>Attendees</b> .....	89
<b>Genetic Aspects of Invasive Leaf Miners—examples of Three Species Introduced Recently to Europe</b> .....	Addenda A
<i>Ferenc Lakatos, Zoltán Kovács, Christian Stauffer, Charles Covell and Donald R. Davis</i>	
<b>Comparison of Residue Levels in Green Ash and Norway Maple Between Six Trunk Injection Systems</b> .....	Addenda B
<i>Phillip A. Lewis, David M. Cowan, and John J. Molongowsk</i>	

# USDA APHIS OFFSHORE PEST INFORMATION SYSTEM

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## Abstract

International trade is rapidly expanding and the relevance of international borders as barriers to pest movement is diminishing. As trade expands, so does the risk of accidentally or intentionally spreading exotic plant pests. The Offshore Pest Information System (OPIS) is a process designed to collect, analyze, communicate and use relevant international information concerning pests that are not known to occur in the U.S. Using OPIS will enable USDA, Animal and Plant Health Inspection Service (APHIS) to meet its mission. That mission is to “safeguard American resources from exotic invasive pests and diseases.” By using OPIS, the Agency can quickly and effectively respond to imminent pest threats.

A stakeholder review of the USDA’s plant safeguarding system in 1999 found that a “broad range of highly reliable information on international pests is needed to enable APHIS PPQ to effectively safeguard America’s plant resources.” Likewise, the Animal Health Safeguarding Review found in 2001 “that the U.S. cannot achieve exclusion, detection, assessment of risk or eradication, and control of foreign animal diseases without adequate, scientifically sound, rapidly accessible and completely communicated international animal health information.” Both reviews recognized the importance of identifying pest threats.

The components of an effective safeguarding system include offshore risk management, port-of-entry exclusionary measures, quarantines, pest detection, and emergency response. One of the most important of these is pest detection. The Offshore Pest Information System provides for the detection of pests on a global scale for specific targeted plant and animal pests. Communication of this information through the designed system complements the other components of the effective safeguarding system.

There are four basic elements to OPIS:

1) Collecting the information

- 2) Synthesizing the information
- 3) Communicating the information, and
- 4) Supporting the system through research and data collection

The Offshore Pest Information System is designed to identify for American plant and animal regulatory officials based domestically and abroad, and our trading partners, a maximum number of targeted plant pests and animal diseases which the Agency has determined represent a current and significant threat to the U.S. agricultural and natural resources.

With the assistance of several professional scientific societies and industry groups, the USDA APHIS has developed a target pest list. This list is comprised of more than 600 insects, mites, pathogens, nematodes, mollusks, and weeds that the cooperating groups identified as the most threatening to U.S. plant and animal health. Approximately 100 pests have been identified as “priority” targets. Disclosure of these pests of concern by the U.S. helps U.S. trading partners become aware of American concerns and its desire to work with those countries to keep such pests out of their countries as well; this helps both the U.S. and its trading partners.

Information on the density and distribution of priority target pests is collected regularly in the foreign countries where they are known to occur. New detections of these organisms are also monitored and reported. As current status information is collected and reported on these priority target pests and diseases, safeguarding personnel in the U.S. and elsewhere adjust pest and disease risk management options. These options may include modifying the following:

- Initiating offshore pest management or risk mitigation
- Organizing early-detection surveys for these pests and diseases in the U.S.
- Improving port-of-entry inspection procedures

- Re-evaluating existing phytosanitary policies

The OPIS consists of the following components:

- Global pest and disease database—A reference tool on pests
- Target pest list—A list that is reviewed and updated periodically
- Pest/Pathway status in foreign countries—Monthly reports on target pests
- Pest interception data from U.S. ports—Information reported into the system
- Communication component—Getting the word out through monthly or emergency pest alerts and news releases
- Initiation of protective and mitigation measures—Actions by U.S. and foreign countries

To ensure timely, accurate, and secure management of this international pest and disease information, a web-based reporting and reference system has been developed. The system identifies and communicates to those who have a need to know, the target pests and diseases that have been identified by APHIS as a current potential threat to the U.S. Offshore safeguarding officers and others can report their findings immediately through the secure electronic system. Reported information is evaluated by key scientists and policy making officials within APHIS to determine credibility, spatial and geographic impact on American plant and animal resources, and to determine risk mitigation procedures or policies to be undertaken by safeguarding officials here in the U.S. Following this analysis, the collected information and appropriate mitigation or advisory measures are electronically reported through a secure system as ranked pest alerts or pest news items to federal and state safeguarding officials for subsequent commodity inspection, pest detection or quarantine activities.

The electronic system provides a link to basic pest information contained in a USDA APHIS Global Pest and Disease Database. The information contained in this database includes geographic distribution, host commodities, animals, or other material, taxonomy and identification information. The offshore pest and disease

status information reported by safeguarding officials is also stored electronically for use by risk assessors and policy makers when needed for future decision making.

Beginning in 2004, APHIS will conduct a pilot program of the Offshore Pest Information System in three countries or regions: Caribbean Region, Brazil, and South Africa. These countries/regions have been determined to be a significant threat of exotic pest introduction because of their proximity to the U.S., developing pest populations, and existence of potential pathways identified by present or planned trading activities. Safeguarding officers will be hired by APHIS and placed in these countries to cooperate with foreign officials to monitor the populations of the target pests and diseases in that region or in regions with which they conduct trade.

Several mutual benefits are anticipated from the pilot program in these countries. Included among those benefits is increased communication about specific pests with potential for disrupting trade between the U.S. and these pilot program countries. An example outcome of a pest monitoring system in the South American and Caribbean region would be the implementation of Caribbean regional exclusion measures designed to impede the northward spread of soybean rust from South America. Another mutual benefit from such a system would be the facilitation of pest mitigation or pest management programs for pests of concern. This benefit has already been demonstrated in at least one Caribbean country through the development and establishment of a biological control program for pink hibiscus mealybug.

## References

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- The Animal Health Safeguarding Review, Results and Recommendations**, October 2001, National Association of State Departments of Agriculture Research Foundation.

# ALDER DECLINE IN AUSTRIA CAUSED BY A HYBRID *PHYTOPHTHORA*

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## Abstract

Species of alder trees are important components of riparian ecosystems and have great conservation value especially in wetlands and along rivers where they stabilize riverbanks. For about 15 years they have suffered from a lethal *Phytophthora* disease. The species of *Phytophthora* that is infecting alder is considered to be a new one that represents a hybrid involving *P. cambivora* as one parent.

The disease is widespread in Austria but is particularly severe in the provinces of Burgenland and Styria, where most alder plantations are located. Extensive decline and symptomatic trees were observed in many plantations, where the disease has increased quickly with devastating consequences. However, in plantation settings the number of diseased or dead trees varied greatly from site to site. Affected alder trees also were found in high elevation native alder habitats along whitewater streams.

The alder *Phytophthora* (and *P. cambivora* (A<sup>2</sup>)) was recovered from soil samples taken from nursery beds providing evidence that nurseries maybe the inoculum source for infected plantations and native alder habitats adjacent to plantations. However, isolation success from

soil at infested sites was very low, whereas isolation frequencies from fresh expanding necrotic stem and bark tissue were high. The number of successful isolations from older necrotic tissues varied but primarily depended on whether or not the necrotic tissue was removed from fresher portions of the lesions.

Three previously described variants were detected among the 32 isolates collected from alder. Most isolates were placed in group I (43.7%) resembling the 'Swedish' variant. The second most common group of isolates (34.4%) resembled the 'standard' variant (group II) and a third group of isolates (9.4%), resembled a less common 'Dutch' variant. In this study, two isolates were recovered which differed from all others by producing very few and often aborted oogonia and hyphae that resembled *P. cambivora*. This is in contrast to a previous Austrian survey where almost all isolates resembled the 'standard' variant (group II). The degree of isolate variation noted at some sites (i.e. three variants out of four isolates at one site) suggests the unstable status of the *Phytophthora* population. This may indicate an ongoing genetic hybridization process.

# PHYTOPHTHORA DISEASES OF OAK IN EUROPE

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## Abstract

Decline and mortality of European oak ecosystems has been reported since the early 20th century. Among the various contributors to decline are species of *Phytophthora*, particularly *P. cinnamomi*, which is known to be involved in Iberian oak decline. However, recent investigations have shown there are a variety of other *Phytophthora* species associated with oak trees in Europe and Turkey.

Surveys of *Phytophthora* in oak forests in Europe and adjacent countries yielded a diverse assemblage of species including; *P. cinnamomi*, *P. cambivora*, *P. citricola*, *P. gonapodyides*, *P. megasperma*, *P. cactorum* and *P. cryptogea*. In addition, five new species were discovered; *P. quercina*, *P. europaea*, *P. uliginosa*, *P. psychrophila* and *P. pseudosyringae*, some other taxa await description. Some species occurred over a broad geographic area while others were isolated from specific locations. The two most common species were *P. quercina* followed by *P. citricola*.

*Phytophthora* species associated with oak trees probably require different site conditions to thrive in forest soils. The exotic pathogen *P. cinnamomi* was only recovered once in Turkey and never in Austria. In related studies, in Germany *P. cinnamomi* was not found but frequently was encountered in Italy and parts of France. Its recovery may indicate an existing climatic limit for the pathogen. Other *Phytophthora* species such as *P. gonapodyides* and *P. uliginosa* were encountered on sites where aquatic habitats and hydromorphic soils exist and *P. psychrophila* was associated to non-hydromorphic soils. *P. quercina* and *P. citricola* were isolated from sites with the greatest variation in site characteristics (e.g. soil type, soil moisture and pH). This may explain their wide distribution and frequent recovery over a wide geographic area. Noteworthy, *P. quercina* was recovered on very dry sites where *Phytophthoras* usually do not thrive.

*Phytophthora* species were more frequently recovered from rhizosphere of declining than healthy oak trees. Similarly, in Germany and Turkey a significant association existed between the presence of *Phytophthora* species and the decline status of oaks. In Italy *P. quercina* was the only species significantly associated with declining oak trees, whereas there was a mild evidence for connection between deteriorating crown status and the presence of *Phytophthora* spp. in Austria. Further, in Germany it was shown that the level of fine root damage was significantly higher in oaks infested with *Phytophthora* spp and particularly *P. quercina*. However, evidence obtained from forest conditions cannot be interpreted directly as a cause and effect relationship.

In various stem and root inoculation tests, *Phytophthoras* differed in their aggressiveness to oaks and in their host and tissue specification. The most frequently recovered species, *P. quercina* appear to be host specific and capable of causing fine root mortality of oak, but did not attacked the main bark of any host species tested. Most of the other recovered species were non-host specific while causing damage at different extent to their hosts. The order of aggressiveness among isolates as well as between the encountered species showed considerable variation on root inoculation and on stem inoculation tests. In soil infestation tests *Phytophthoras* were able to cause more damage on plants under stress condition (e.g. drought or flooding experiments).

Oak species differed in their susceptibility toward *Phytophthora* infection. Though, holm oak (*Q. ilex*) was much more susceptible to *P. cinnamomi* infection than any other oak species tested, where inoculated saplings displayed highest root loss and mortality. By contrast, pedunculate and red oak displayed low root susceptibility to *P. cinnamomi*.

The type of symptoms in *Phytophthora*-infected oak forests usually appears without mortality suggesting, at least for some species, a natural association. In addition to the surveys in throughout Europe, studies in Turkey revealed some evidence that the most common species

*P. quercina* is probably co-evolved with oak species occurring on a wide range of oak ecosystems including endemic forests. However, associated *Phytophthoras* could play a greater role under conducive soils and may cause more damage to their hosts under stress conditions.

# INVASIVE PLANTS OF THE EASTERN UNITED STATES: IDENTIFICATION AND CONTROL

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## Abstract

A biological invasion of non-native plants is spreading into our nations' fields, pastures, forests, wetlands and waterways, natural areas, and rights-of-way. Various referred to as exotic, non-native, alien, noxious, or non-indigenous weeds, invasive plants impact native plant and animal communities by displacing native vegetation and disrupting habitats as they become established and spread over time.

Drawing on recent publications by the USDA Forest Service, National Park Service, U.S. Fish and Wildlife Service, USDA-APHIS-PPQ and the Southeast Exotic Pest Plant Council, this web site covers identification characteristics, distribution, and control options for 97 tree, shrub, vine, grass, fern, forb, and aquatic plant species that are invading the eastern United States. For each species, a menu of control options is presented, including mechanical treatments, specific herbicide prescriptions, and, for selected species, recent advances in biological control. Also included on the CD are 486 digital images taken by 81 photographers that are available in digital form in multiple file formats and can be off-loaded and used for educational purposes at no cost to the user.

While this is not an official list of "invasive" plants throughout the eastern United States, it includes Federal Noxious Weeds and those listed by State regulatory agencies, pest plant councils and other organizations. Some of the plants on this list are often found in ornamental plantings and landscapes. In fact, many non-native plants introduced for horticultural and

agricultural use now pose a serious ecological threat in the absence of their natural predators and control agents. This publication will aid landowners, foresters, resource managers, and the general public in becoming familiar with invasive plants in their area to help protect our environment from the economic and ecological impacts of these biological pollutants.

Copies of this free CD-ROM can be ordered from [www.invasive.org](http://www.invasive.org).

## Citations

- Miller, J.H. 2003. **Nonnative Invasive Plants of Southern Forests: A Field Guide for Identification and Control**. USDA Forest Service, Southern Research Station.
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- Presented by G.K. Douce ([kdouce@uga.edu](mailto:kdouce@uga.edu)) and R. Reardon ([rreardon@fs.fed.us](mailto:rreardon@fs.fed.us)).

# STUDIES ON MICROBIAL INSECTICIDES FOR SUPPRESSION OF EMERALD ASH BORER POPULATIONS

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## Abstract

Programs designed by regulatory agencies to eradicate localized infestations of emerald ash borer (EAB), *Agrilus planipennis*, involve detection and removal of infested ash trees (*Fraxinus* spp.) and creation of an ash-free zone surrounding the epicenter. Conventional insecticides are being tested to aid in the eradication effort and to protect landscape ash trees, however, the use of such products is not an option in Michigan due to the extensive area infested by EAB, legal issues, and lack of public support. Due to the difficulty in identifying infested trees and the high rate of EAB movement, this invasive buprestid is likely to continue spreading in forests and parks throughout Michigan, resulting in rapid decline and death of the millions of ash trees in this region and beyond. Clearly, methods are needed to manage EAB in North America. To this end, we are studying the efficacy of biopesticides formulated with isolates of *Bacillus thuringiensis* (Bt) and a product formulated with *Beauveria bassiana* var. GHA (Bb). Public acceptance remains high for the use of biopesticides due to good safety records and compatibility with other management strategies, including biological control.

In 2003, we screened EAB adults with four registered Bt biopesticides, each with different Bt strains and host ranges. Mortality was observed for each product, but only at concentrations exceeding the maximum labeled rate. We are now cooperating with other Bt researchers to screen isolates and fractions of Bt for activity against EAB with the long term goal of developing a new Bt-based insecticide targeting EAB.

*Beauveria bassiana* var. GHA is the active ingredient of the biopesticide, BotaniGard<sup>®</sup>, registered for control of insect pests of forest and shade trees in 1999. We

are evaluating the potential use of BotaniGard for suppression of EAB populations by homeowners and land managers. The two formulations of this product, BotaniGard ES (petroleum based) and BotaniGard O (vegetable oil) were equally virulent against EAB adults with LC<sub>50</sub>s of 4.9 and 4.7 spores/cm<sup>2</sup>, respectively; LT<sub>50</sub>s ranged from 4 to 10 days, depending on spore concentration. Laboratory and greenhouse studies showed that pre-emergent trunk sprays of BotaniGard were a promising method for control of EAB. In spring 2003, we applied BotaniGard to EAB-infested tree trunks prior to beetle emergence at the rate of 2 and 20 qts BotaniGard/acre in a plantation of 20-yr-old ash trees in Ann Arbor. The trunks of treated and control trees were then caged, enclosing some epicormic shoots, and EAB were allowed to complete their life cycle within the cage. After death, EAB were cultured for fungal infection; at 0, 2, and 20 qts/acre, prevalence of *B. bassiana* infection among adults was 0%, 58.5%, and 83.0%, respectively. The maximum-labeled rate for BotaniGard is 17 qts/acre. In Oct 2003, we sprayed EAB-infested ash trees with 14 qts BotaniGard/acre to evaluate the impact on larvae inside infested ash tree trunks. These trees had visible cracks in the bark over EAB galleries, and we presumed BotaniGard may penetrate into the larval galleries via these bark cracks. In the winter, after tree dissection, we determined 10-20% of the larvae were infected with *B. bassiana*; no EAB larvae were infected in the controls. It is possible that higher levels of infection might be achieved if BotaniGard is applied to cracked tree trunks earlier in the fall, prior to EAB entering the sapwood to overwinter. In 2004, we plan to evaluate the application of BotaniGard over larger areas in the field and with foliar treatments.

# NATURAL ENEMIES OF THE EMERALD ASH BORER IN MICHIGAN AND CHINA

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## Abstract

In 2002, regulatory agencies in the United States and Canada adopted a strategy of eradication for the emerald ash borer (EAB), *Agrilus planipennis*, in an effort to protect New World ash (*Fraxinus* spp.). Should eradication fail, however, conventional biological control will be needed to suppress the populations of this invasive buprestid. To this end, we are studying the natural enemies of EAB in Michigan and in China, where EAB is a periodic pest of their native ash, as well as two locally planted New World ash species: *F. velutina* and *F. pennsylvanica*. Virtually nothing was known of EAB natural enemies until the recent report of *Spathius* sp. (Braconidae), a gregarious ectoparasitoid of EAB larvae in China (Xu Gongtian 2003).

In a woodlot in Livonia, Michigan, we surveyed the insect natural enemies and entomopathogenic fungi attacking EAB from August 2002 through July 2003. The most prevalent natural enemies were five species of fungi, although less than 2% of immature EAB were infected. Fungal species included: *Beauveria bassiana* (24 isolates), *Paecilomyces farinosus* (30 isolates), *Paecilomyces fumosoroseus* (7 isolates), *Verticillium lecanii* (36 isolates), and *Metarhizium anisopliae* (2 isolates). One egg parasitoid, *Pediobius* sp. (Eulophidae), was reared from 0.3% of EAB eggs collected in early July 2003. Seven potential larval parasitoids included *Heterospilus* sp. (Braconidae); *Phasgonophora sulcata* (Chalcidae); *Balcha* sp. and *Eupelmus* sp. (both in Eupelmidae). Two other parasitoids, reared from EAB-infested logs cut in different woodlots, included *Atanycolus* sp. and *Spathius simillimus* (both in Braconidae). The most prevalent parasitoid was *Balcha* sp., a solitary ectoparasitoid that we successfully reared to adult in the laboratory. This parasitoid is native to Asia and was recently discovered in Maryland and Virginia (Michael Gates, USDA SEL, personal communication). Coleopteran predators

included *Enoclerus* sp. (Cleridae), *Catogenus rufus* (Passandridae), and *Tenebroides* sp. (Trogossitidae); these predaceous beetles consume EAB during both larval and adult stages. Other EAB mortality factors included woodpecker predation, starvation, desiccation, and cannibalism, especially in heavily infested logs. Our results revealed that mortality of EAB in Michigan due to parasitoids is low compared to that reported for some of our native *Agrilus* spp.

In north and northeastern China, ash trees in woodlots, cities, roadsides, and nurseries were surveyed for EAB and its natural enemies in Heilongjiang, Jilin, Liaoning, Hebei, Tianjin, and Shandong Provinces from late October and early November 2003 (H. Liu and T. Petrice). The previously reported *Spathius* sp. was collected at two locations, with 1 to 50% parasitism of EAB larvae. We also discovered an unknown gregarious endoparasitoid of EAB larvae in Changchun City in Jilin Province (Oct. 25), and Benxi County in Liaoning Province (Oct. 30), with a parasitism rate of 2.7 to 50% (H. Liu). Mature larvae, pupae, and adults were collected and later identified as *Tetrastichus* sp. (Eulophidae) (M. Gates, USDA ARS SEL). The numbers of *Tetrastichus* from each parasitized EAB larva ranged from 4 to 29. We gave specimens of this *Tetrastichus* to Prof. Dawei Huang at the Institute of Zoology, Chinese Academy of Sciences, Beijing, China for identification or description. Based on the results of the 2003 survey, we established plots in Jilin and Liaoning Provinces, in cooperation with local foresters, to survey EAB natural enemies during the 2004 EAB season.

Xu Gongtian. 2003. *Agrilus marcopoli* Obenberger, pp. 321-322, In Xu G-T (ed.), Atlas of ornamental pests and diseases. China Agriculture Press, Beijing, China.

# LIFE CYCLE OF THE EMERALD ASH BORER IN MICHIGAN

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## Abstract

In 2002, following the discovery of emerald ash borer (EAB), *Agrilus planipennis*, in Michigan, we began research on its biology and rearing. From field and laboratory studies of EAB in Michigan, we learned their eggs are ca. 1 mm in diameter, gradually changing from white to amber in color after being laid between layers of bark and in bark crevices on ash trees (*Fraxinus* spp.). Larvae hatch in about a week and tunnel directly through the bark to the cambial region where they feed, etching a serpentine gallery in the phloem and outer sapwood. Based on measurements of sclerotized larval structures, we identified four larval stages. In late summer and fall, fourth-instar larvae enter the sapwood or outer bark and excavate a pupation chamber where they overwinter as prepupae; diapause is facultative. Pupation generally occurs in late spring, although larvae too young to prepupate in the fall spend the winter *in situ* and complete development the following summer.

EAB pupae are exarate (naked) and gradually develop to adults in the pupation chamber. In the laboratory at constant 24°C, maturation time of an EAB pupa to a functional adult is ca. 25 d. When mature, adult EAB chew out of the tree through tunnels initiated by the larva during excavation of the pupation chamber. Adults emerge through the characteristic D-shaped exit holes in the tree bark and are capable of immediate flight upon emergence. EAB adults feed on ash foliage throughout their lives, and are most conspicuous on hot sunny afternoons, hovering about ash tree trunks, and landing to mate or oviposit. In the laboratory, EAB adults mate soon after emergence, however, they maturation feed for ca. 3 weeks on ash foliage before oviposition starts. EAB adults can live for several weeks. In 2003, the peak oviposition period was late June to early July, and most eggs were eclosed by mid-July.

# ERADICATING THE ASIAN LONGHORNED BEETLE *ANOPIPHORA GLABRIPENNIS* FROM WOODBRIDGE, ONTARIO —AN UPDATE ON CFIA'S EFFORTS

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## Abstract

Since the first interceptions of the Asian Longhorned Beetle (ALB) *Anoplophora glabripennis* in cargo at Burnaby, BC in 1992, the Canadian Food Inspection Agency (CFIA) has been actively involved in screening cargo and surveying high risk sites. Fortunately, the level of preparedness within CFIA and other government agencies, to such an exotic, is high due to a number of emergency simulations held during the last 5 years.

In September 2003, a resident of Woodbridge, Toronto submitted to the CFIA, an insect, later confirmed as *Anoplophora glabripennis*. A quick survey of the site, indicated a localized introduction of ALB in an industrial park. An emergency response was initiated by CFIA which include commencement of delimitation surveying, mobilization of staff and communications with media and partners. CFIA is the lead agency in partnership with the Cities of Vaughan and Toronto, York Region, Toronto Region Conservation Authority other local municipalities, Canadian Forest Service, and the Ontario Ministry of Natural Resources.

The Eradication Team is divided into three sections: Operations, Communications, and Science. The Operation group is responsible for survey direction and data collection, host removal and disposal, quarantine zone enforcement, mapping and GIS and costing. The communications group is responsible for press releases and media interviews, information line for public assistance, website management, public open houses and community involvement. The research group has been tasked with finding answers to and making recommendations as to host tree susceptibility, eradication protocols, disposal options and chemical treatments.

The original site of introduction was in an industrial area between warehouses on private property. Estimates

on the introduction date is 6 years ago which predates the regulations enacted to prevent the movement of untreated wood from Hong Kong and China. The infestation has expanded throughout the industrial area to an area of 3 km east/west by 2 km north/south with three satellite sites just beyond the main area. The area originally surveyed was 160 km<sup>2</sup> of delimitation survey of all public or street trees, equating to 50% of all trees (private and public) in the area. An intensive (approx 100 km<sup>2</sup>) visual survey and tagging of public and private trees in the infested core is ongoing and includes the primary, secondary and tertiary zones as well as a containment area with a radius of 1600 m from the infested trees.

After extensive consultation and debate, the recommendations for eradication are to remove all host material within a radius of 400 m (secondary) from an infested tree (primary). An additional, a further area of treatment of 400 m (tertiary) is to be treated with Imidacloprid, ( Canadian registration pending) or host removal. The areas where trees are to be removed include the generally infested industrial park, a newer residential area, a cemetery, and an older well treed neighborhood. The time frame for eradication commenced with the first tree removal November 20 and a projected completion for host removal by May 15, 2004. Insecticide treatment by soil injection under host trees into the root ball will commence in the spring but is dependant upon the registration of the insecticide by the Canadian Pesticide Management Regulatory Agency. In areas where the insecticide cannot be used i.e. in riparian zones, host removal will be the option. A Ministerial Order will be issued to authorize the removal or treatment of all host trees in the primary, secondary and tertiary areas.

The projected number of trees to be removed within the 400 m host removal area is estimated to be 11,000+ trees. The host list that is being used includes *Acer*, *Aesculus*,

*Salix, Ulmus, Betula, Platanus, Celtis, Populus,* and *Sorbus* but leaves open the possibility of including some possible genera cited in literature as *Fraxinus, Malus* and *Prunus* should these hosts prove positive after extensive surveying. Host trees within the industrial park have been removed (600 trees as of early January) and 120 trees have been confirmed positive. Approximately 10% of the asymptomatic trees removed were found positive with ALB oviposition sites.

Continued surveys are expected for the next 3 to 5 years depending upon the results of this years eradication program. CFIA legislation enables the funding the survey and eradication costs but does not permit covering the costs of site restoration in the eradication areas. Other levels of government and agencies are assessing their levels of commitment in replacing ALB host trees with suitable nonhost species.

# ***HALYOMORPHA HALYS*, (HETEROPTERA: PENTATOMIDAE), THE BROWN MARMORATED STINK BUG; ARE TREES THE PRIMARY HOST FOR THIS NEW INVASIVE PEST?**

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## **Abstract**

*Halyomorpha halys*, (Heteroptera: Pentatomidae), is a pest in eastern Asia on soybeans and woody plants, including broadleaved trees and fruit trees. In 2001, a univoltine population was discovered in Allentown, PA, but had apparently been established since 1996. *H. halys* is now reported from five counties in PA and two abutting counties in NJ. In 2003, isolated populations were discovered in Harrisburg, PA, and Hagerstown, MD.

Mitochondrial DNA was analyzed and preliminary analysis suggested only one maternal haplotype in the U.S. Sequence identity with Taiwan and Japan populations was 96.2% and 99.3% respectively. However, specimens from other potential source populations in China and Korea need to be analyzed to show a conclusive pattern (Carter, unpublished data).

*Paulownia tomentosa* was found to be a host in PA and is a known host in Japan where *H. halys* is a vector of witches' broom, a phytoplasma disease of this tree. Preliminary analysis using universal phytoplasma primers did not detect the presence of phytoplasma in the PA population (Beanland, unpublished data).

Host plant surveys in Allentown indicated that *H. halys* is polyphagous with patchy and sometimes dense populations, but limited to landscaped urban areas. Damage to fruit trees and feeding on vegetables was observed in gardens. Two parasitoids, *Telenomus podisi* and an *Anastatus* sp. were identified from field collected eggs. Until populations reach commercial growers, population dynamics in agroecosystems will not be apparent. Woody plants, including ornamentals and trees are primary hosts in landscaped areas.

# HEMLOCK WOOLLY ADELGID (*ADELGES TSUGAE ANNAND*) PHENOLOGY AND PREDATORS IN THE PACIFIC NORTHWEST

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## Abstract

Thirty-one western hemlock (*Tsuga heterophylla*) infested with hemlock woolly adelgid (HWA) in western Oregon are visited monthly. Tree health, HWA development and adelgid density are monitored. Beat samples are used to detect the presence of predatory arthropods on infested branches. From April 2003 to present, HWA have developed through life stages concordant with those in eastern states, with similar timing. HWA populations in Oregon are small. No decline in tree health due to HWA has been observed. Small numbers of several predatory insect taxa have been collected from HWA infested branches, including *Laricobius nigrinus*, mirids, other hemipterans, earwigs, coccinellid beetles and lacewings.

# REPRODUCTIVE STRATEGIES OF SCYMNUS LADYBEETLE PREDATORS OF HWA

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## Abstract

Hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera:Adelgidae) is an introduced Asian pest of native Eastern and Carolina hemlocks, *Tsuga canadensis* Carriere and *Tsuga caroliniana* Engelman, in eastern North America. Current efforts to manage the spread of *A. tsugae* have focused on developing biological control programs based on the release of predators from Japan, China and Canada. While species from Japan and Canada have been released, a history of colony and production difficulties have delayed field release trials for *Scymnus* species from China. Colonies of two Chinese ladybeetle species, *Scymnus (Neopullus) sinuanodulus* Yu & Yao and *Scymnus (Neopullus) ningshanensis* Yu & Yao (Coccinellidae:Tribe Scymnini) have been maintained in Connecticut since 1996 and 1998, respectively, but have experienced mass mortality in recent years. In October 2002, improved colony maintenance and production procedures were revised significantly as a result of a new cooperative project between the USDA Forest Service and the Connecticut Agricultural Experiment Station. Some of the challenges facing the development of improved mass rearing technology for *S. sinuanodulus* and *S. ningshanensis* are presented here: biased sex ratios & adult parasitism, and a possible interpretation provided in terms of reproductive strategy.

Insect sex ratios are generally balanced, with exceptions in some orders. Female biased sex ratios in larger coccinellids are known to be distorted by maternally transmitted cytoplasmic male-killing bacteria of the genus *Wolbachia*. Biased sex ratios can also arise from differential larval survival and inbreeding. There are few

published studies on sex ratios of Scymnini species. Sex ratios of F1 progeny reared in early spring 2002 were extremely skewed towards females in *S. ningshanensis* (11:1) and in *S. sinuanodulus*, by 2.2:1. After colonies of *S. sinuanodulus* and *S. ningshanensis* were stabilized in 2003, overall sex ratios of the F1 2003 generation produced in the spring and summer were still biased towards females, but much less so than in 2002, when the colonies experienced highly stressed conditions (2.4:1 for *S. ningshanensis* and 1.9:1 for *S. sinuanodulus*).

Parasitized male adults were discovered in fall 2002 field collections of *S. sinuanodulus* adults from China. Each of two cadavers contained one parasite larva. This is the first record of adult parasitism in *S. sinuanodulus*. Examination of F1 2002 summer cadavers recorded 16.8% of males and 14% of females with the same displaced elytra and sunken abdomens, suggesting previous contamination by parasites. In winter 2003, under quarantine conditions, an unidentified adult parasite was found to have successfully emerged from a parasitized male *S. sinuanodulus*, which had died in July 2003. In China, native Chinese hemlocks are a minor component of highly diverse forests which support a large diversity of coccinellid species on low, scattered densities of *A. tsugae*. The native abundance of *S. sinuanodulus* may be the result of an adaptive reproductive strategy, reflected in a biased sex ratio, that favors the production of females when there is abundant intra- and interspecific competition for limited prey resources and natural population regulation by parasites.

# CHEMICAL DEFENSES IN GARLIC MUSTARD (*ALLIARIA PETIOLATA*) AND THEIR POTENTIAL ROLE IN SPECIES INTERACTIONS IN FOREST UNDERSTORIES

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Garlic mustard [*Alliaria petiolata*] (M. Bieb) Cavara & Grande; Brassicaceae] is a European native biennial herb, first recorded on Long Island, NY in the 1860s, and is expanding rapidly in northeastern and midwestern forests in the U.S. and in southern Canada. Garlic mustard flourishes in moist woodlands with moderate exposure to light, but it can grow in a diversity of other habitats. It is found in natural areas, woodlots, and along edges of agricultural fields and lawns throughout North America. Several life history traits likely contribute to the invasiveness of this species. It has a high inbreeding rate and can produce numerous seeds. It exhibits remarkable morphological plasticity to local environmental conditions. It can exude allelopathic chemicals (glucosinolates and their hydrolysis products) that can reduce seed germination and growth of some species, and that can affect mycorrhizal potential of soils. Garlic mustard has been shown to outcompete some ecologically and commercially important hardwoods in short-term experiments, and its presence in natural areas is associated with reduced native herb abundance and diversity. Garlic mustard can also negatively impact salamander populations that rely on litter dwelling animals for food, and it can endanger populations of the rare butterfly *Pieris virginiensis* by serving as an oviposition site by adults on which larvae can not survive. Because of its known or potential negative impacts in natural and agricultural ecosystems, garlic mustard is an important target for chemical and biological control efforts.

The Evolution of Increased Competitive Ability (EICA) Hypothesis predicts that invasive plants in novel habitats lacking substantial pressure by natural enemies will evolve reduced expression of costly, unneeded chemical defenses to the benefit of growth and reproduction. We tested predictions of this hypothesis in garlic mustard, a European native that lacks substantial specialist herbivory in North America, where it is also largely resistant to generalist herbivores (Figure 1). We grew plants from

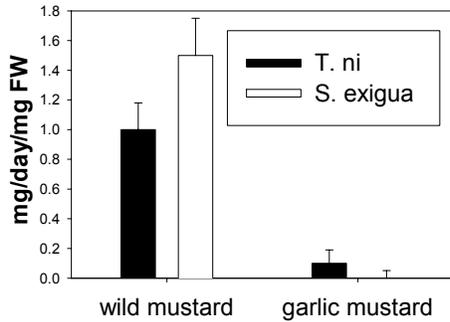
four North American populations from Ohio and Pennsylvania, and seven European populations from the United Kingdom and the Netherlands in the greenhouse from field-collected seed. Plants were grown for 35 days, at which time length and width of the third true leaf were taken and half of the plants were treated with a foliar spray of jasmonic acid (JA). Jasmonic acid is a wound-related hormone involved in the induction of several chemical defenses associated with insect and pathogen resistance. Exogenous treatment of this hormone can induce defenses in a manner similar to herbivore attack, without the confounding effects of leaf damage. Four days later, samples from the fourth true leaves were harvested for the analysis of several constitutive and JA-inducible defense proteins and secondary metabolites that range from general resistance factors to defenses that are unique to garlic mustard. Levels of some of these defenses have been shown to vary among populations in the field, which may explain variation in herbivore resistance among natural populations in the field (Figure 2). Data were analyzed with mixed model ANOVA with continent, population within continent, and JA treatment as main effects.

Glucosinolates, secondary compounds characteristic of the mustard family, are involved in numerous species interactions including specialist herbivore attraction, generalist herbivore resistance, and interactions with soil fungi. Total glucosinolates (of which sinigrin is a major component in garlic mustard) were assessed using the glucose release method. Total glucosinolate content differed significantly among the populations within continents (Figure 3a). In addition, the response of populations to JA treatment by continent was marginally significant, with North American populations tending to be more inducible by JA than European populations. Activity of the phenolic oxidizing enzyme, peroxidase, was assessed in soluble protein extracts using a spectrophotometric assay with guaiacol as a substrate. No variation among continents in peroxidase activity was

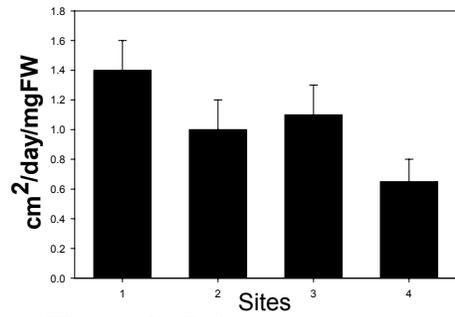
found, although variation was found among populations within continents (Figure 3b). Although not significant, an interesting pattern was present in the response of populations to JA treatment. Two North American populations displayed higher peroxidase activities after JA treatment, and two populations displayed lower peroxidase activities. Six of seven European populations displayed lower peroxidase levels after JA treatment. Trypsin inhibitors, capable of competitively inhibiting digestive serine proteases of animals, were assessed in soluble protein extracts using a radial diffusion assay through a trypsin-containing agar. Garlic mustard expressed substantial activity of trypsin inhibitor. There was significant variation in trypsin inhibitor levels among the populations within each continent, but no trends could be significantly attributed to their continental origin (Figure 3c.) JA treatment significantly increased trypsin inhibitor expression, but there was no significant variation among populations in their response to JA. Unique secondary compounds of garlic mustard that have been shown to impart resistance to specialist herbivory include the cyanoallyl glucoside, alliarinoside, and the flavone glycoside, isovitexin 6''-O-B-D-glucopyranoside. Levels of these compounds in water-soluble fractions of ethanol extracts were analyzed by HPLC. A representative HPLC chromatogram is shown in Figure 4. North American populations had more variable amounts of alliarinoside (Figure 5a) and isovitexin 6''-O-B-D-glucopyranoside (Figure 5b) than European populations, and generally expressed higher amounts of isovitexin 6''-O-B-D-glucopyranoside. JA did not consistently induce higher expression of either compound. Due to

low samples sizes, levels of these two compounds were not statistically analyzed. Length (Figure 6a) and width (Figure 6b) of the third true leaf, measured prior to JA treatment, significantly varied among populations within continents, but did not vary with continental origin. However, specific leaf weight of the fourth true leaf varied by continent, and among populations within each continent (Figure 6c). In particular, North American populations had higher specific leaf weight than European populations.

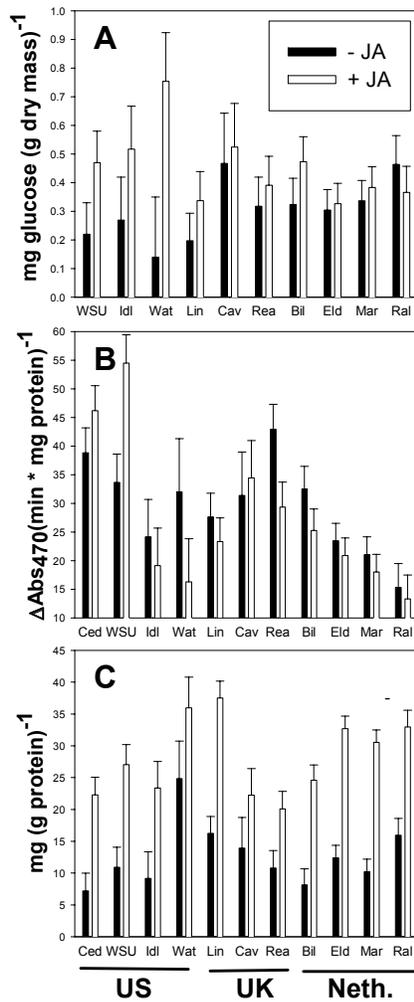
Our results provide mixed support for predictions of the Evolution of Increased Competitive Ability hypothesis in garlic mustard. Leaf growth traits, such as higher specific leaf weight, were suggestive of increased productivity in North American populations as predicted, but this must be verified with longer-term studies. In contrast to predictions, no evidence of reduced expression of chemical defenses was found in North American populations relative to European populations. In fact, greater inducibility of glucosinolates by JA and tendencies for peroxidase activity to be higher in North American populations suggest that the opposite may be true for some defenses. Invasive garlic mustard populations may both grow fast and defend well, despite the tradeoff typically posited between these traits. Future experiments will include a greater biogeographical representation of garlic mustard. In addition, chemical defenses will be assessed more thoroughly throughout the life cycle, and quantitatively related to herbivore resistance and seed production as determined in laboratory bioassays and field studies.



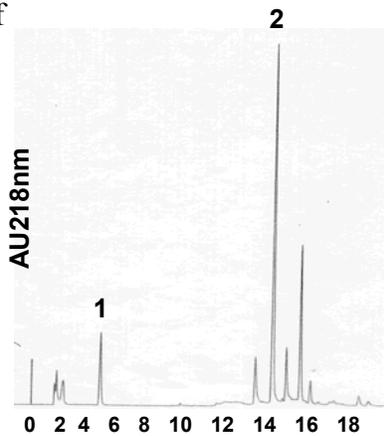
**Figure 1.** Relative growth rate of 7-day-old *Trichoplusia ni* and *Spodoptera exigua* on leaves of wild mustard and garlic mustard. N = 6-10.



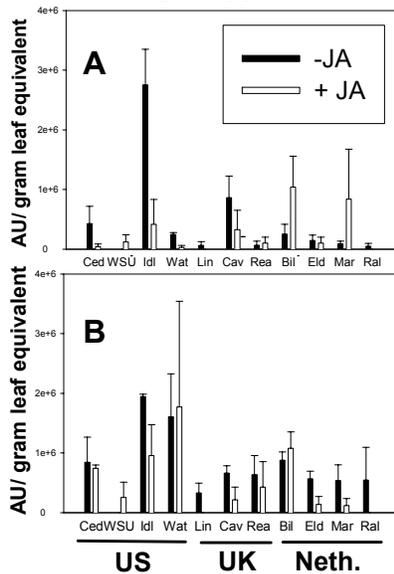
**Figure 2.** Relative consumption rate of *Trichoplusia ni* on garlic mustard leaves from different sites in the field. N = 9.



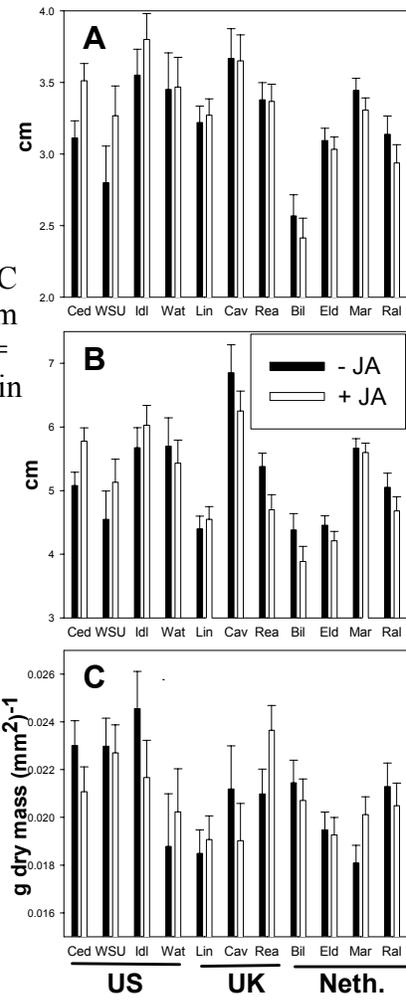
**Figure 3.** A. Glucosinolate, B. peroxidase and C. trypsin inhibitor levels in third true leaves of garlic mustard from the US, UK and Netherlands. N = 5-10.



**Figure 4.** Representative HPLC trace of flavonoids isolated from garlic mustard leaves. Peak 1 = alliarinoside, Peak 2 = isovitexin 6''-O-B-D-glucopyranoside



**Figure 5.** A. Alliarinoside and B. isovitexin-6''-O-B-D-glucopyranoside levels in third true leaves of garlic mustard from the US, UK and Netherlands. N = 5-10.



**Figure 6.** A. Leaf length, B. leaf width and C. specific leaf weight of third true leaves of garlic mustard from the US, UK and Netherlands. N = 5-10.

# DEVELOPMENT OF INSECT-KILLING FUNGI FOR MANAGEMENT OF HEMLOCK WOOLLY ADELGID

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## Abstract

The potential of insect-killing fungi for suppression of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is highlighted by the observed impact of *Entomophaga maimaiga* Humber, Shimazu and Soper on gypsy moth populations in North America. Fungal isolates showing activity against HWA in laboratory trials were evaluated in a variety of forest trails during spring and fall of 2001-2003. Our goal was to select the most virulent fungal isolates and determine the best timing and concentration for fungal applications. Our fungi were also assayed against *Pseudoscymnus tsugae* Sasaji and McClure, a predator being developed for HWA management, to evaluate the compatibility these biological strategies.

Forest trials were conducted at Mt. Tom Reservation, Holyoke, MA. Fungi were applied to HWA infested branches using hand-held and ULV sprayers (5-6 branches/treatment) at concentrations from  $5 \times 10^7$  to  $2 \times 10^8$  spores/ml as either single or multiple applications. Horticultural oil (0.1%) was used as a positive control, in addition to both non-treated and formulation blank controls. Survival and density of HWA were examined 3-4 weeks post application. The non-target effects of fungi to *P. tsugae* were evaluated in the lab using a petri-dish assay and in the forest on caged hemlock branches infested with HWA.

We found that fall applications were optimal for targeting HWA and resulted in measurable reductions in HWA survival. For instance, in both 2002 and 2003, a single fall application of ARSEF 6010 [*Verticillium lecanii* (Zimmerman) Viegas] at  $1 \times 10^8$  spores/ml significantly reduced HWA populations. A second isolate, CA 603 [*Beauveria bassiana* (Balsamo) Vuillemin], had activity only in 2002. In laboratory trials there was little or no effect of fungi on survival of *P. tsugae* and, when examined under forest conditions, fungi having activity against HWA did not reduce *P. tsugae* survival.

Targeting the sistens in late summer and fall offers a wide window during which HWA populations are relatively stable. In spring, HWA populations are rapidly expanding, which may allow HWA to overrun control effects, while also making evaluation of efficacy difficult. The reductions of HWA populations obtained with fungi and fungal compatibility with *P. tsugae* demonstrates the promise of fungi as a component in the integrated management of HWA. Future research is needed to develop novel formulations for delivery by ULV application, determine spore persistence and dispersal, and expand evaluation of nontarget effects.

# HWA COLD-HARDINESS: TOWARDS DEFINING LIMITS OF RANGE EXPANSION

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## Abstract

Cold temperature is probably the most important factor limiting the range of insects in temperate regions (Salt 1936 and others since then). The relationship between insects and low temperature is dynamic, making it difficult to assign specific temperatures at which an insect won't survive, or more importantly, become established in a particular region. Steinhaus considered cold to be one of several agents of noninfectious disease (Steinhaus 1962)—a useful paradigm for evaluating effects of biotic and abiotic factors on insect susceptibility to cold and changes in fitness. Table 1 lists some factors to consider when evaluating insect responses to cold stress.

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, responds uniquely to the change of season in the progression of its life cycle. The sisten generation aestivates or rests as a first instar during summer months and resumes development in fall, which is heralded by development of their woolly coat. As temperature permits, the adelgids develop through the winter into adults. The sistens surviving overwinter to adulthood begin egg production in early spring, producing the progredien and sexuparae generation—sexuparae are a winged form that presumably don't contribute to population growth or expansion in the northeastern U.S. because they lack a required alternate host. The progredien generation in turn produces a new sisten generation in late spring that will ultimately overwinter.

Evidence of the cold-tolerance of HWA overwintering sistens has been reported in a series of publications produced by researchers at the Entomology Research Laboratory, University of Vermont (Parker et al. 1998 & 1999; Skinner et al. 2003). Experiments were conducted by collecting winter-acclimated insects in the forest and exposing them to cold in a low temperature bath in the laboratory. Using this methodology, HWA survival was examined in relation to intensity and duration of cold exposure, and the geographic location and season in which collections were made. The use of HWA that had experienced a natural change of season before cold exposure enhances the usefulness of these findings for gauging responses of forest populations to winter climates.

Several critical temperatures for HWA survival have been observed. The lower lethal threshold where little or no survival occurs in mid-winter (January) is between -30 and -35° C. During the same season -25° C was identified as a transitional point where substantial mortality begins to occur. However, as spring approaches this transitional temperature increases to -15° C, which has no effect on survival during mid-winter. In more southerly locations the loss of cold-acclimation occurs earlier in the year, most likely related to earlier onset of spring. However, there is also evidence of a genetic component to variation in cold-tolerance (Butin 2003).

**Table 1.—Important factors relevant to insect responses to cold.**

Variables to Consider	Example
Insect stage of development	Sensitivity of overwinter vs. reproductive stages
Seasonal acclimation	Change of season increases cold tolerance
Intensity and duration of cold	Very low temperatures for short times
Genetic influences on tolerance	Genetic populations can vary in cold tolerance
Interactions with other stressors	Unhealthy trees increase sensitivity to cold
Individual vs. population responses	Few survivors expand population rapidly
Sub-lethal effects of cold exposure	Reduced reproduction and longevity of survivors

Extensive data is available on ambient temperatures and other environmental parameters for most areas threatened by HWA. Temperature is monitored in weather stations protected from sunlight and other elements and does not necessarily represent conditions in the HWA microhabitat— the tips of branches on hemlock trees. Understanding the relationship between ambient and microhabitat temperatures is necessary in order to infer geographic range limits from cold-hardiness data. We are in the second year of a project (2002-2004) to elucidate this relationship and have sites at Mt. Tom Reservation (yrs 1 & 2) Holyoke, MA and Kettle town State Forest (yr 2), Southbury, CT. We are monitoring temperature (15-min. intervals) on the north and south aspect of hemlock trees (yr 1 – 6 trees; yr 2 – 12 trees) from late December through March with probes placed on the underside of the current year's growth. In addition, ambient temperature is monitored in three weather stations per site along with observations of solarization, rainfall, leaf wetness, relative humidity and wind speed. Data taken on HWA survival will not be presented.

During the winter of 2002-2003 (yr 1) the ambient temperature at Mt. Tom Reservation never dropped below  $-25^{\circ}\text{C}$ , the transitional temperature where major negative effects on HWA survival can be expected to occur (Fig. 1). On three occasions the minimum daily temperature dropped below  $-15^{\circ}\text{C}$ , the point where previous data suggests reductions in survival would occur later in the season. Currently we have no way to predict when the change in acclimation that reduces cold tolerance takes place. However, there is substantial scientific background to develop meaningful research in this area.

We found only small differences between daily minimum ambient and microhabitat temperatures (Fig. 2) and 90% of differences were less than  $1^{\circ}\text{C}$ . These results suggest that minimum ambient temperature may provide insight for course delineation of HWA potential range relative to the critical threshold of  $-25^{\circ}\text{C}$  and lower. In contrast, daily maximum temperature generally tracked higher in the HWA microhabitat relative to ambient (Fig. 3). The temperature increase was most pronounced on southerly aspects of trees, and when days were sunny. These data

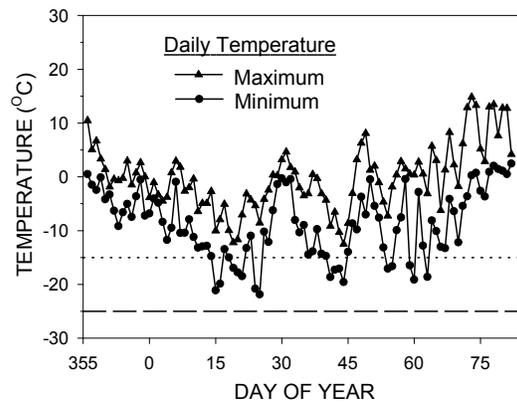


Figure 1.—The average minimum and maximum daily temperature at Mt Tom Reservation, Holyoke, MA.

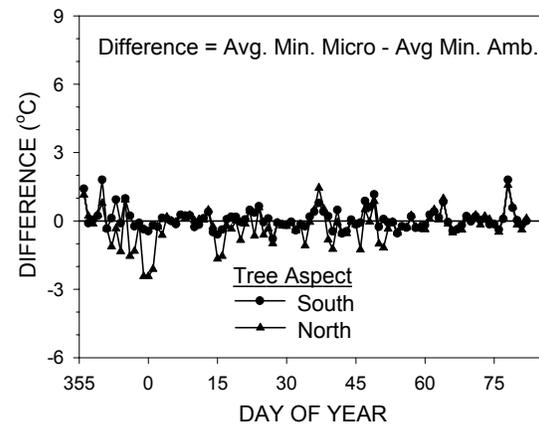


Figure 2.—Daily differences between minimum ambient (zero reference line) and minimum microhabitat temperatures on two of hemlock trees.

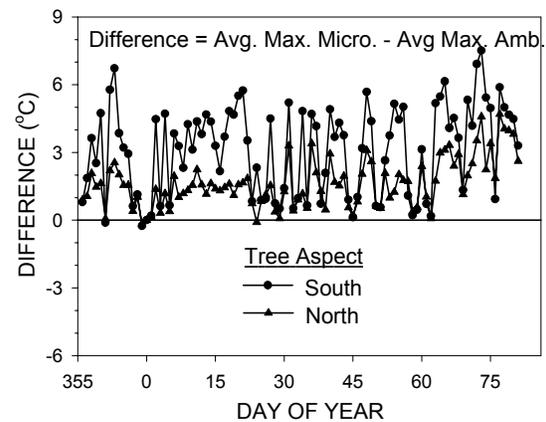


Figure 3.—Daily differences between maximum ambient (zero reference line) and maximum in HWA microhabitat temperatures on two aspects of hemlock trees.

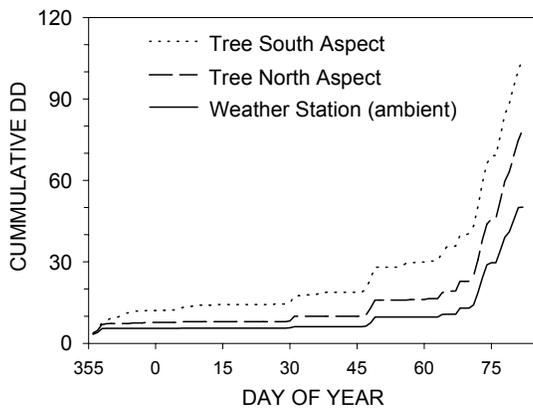


Figure 4.—Cumulative degree-days in HWA microhabitat on north and south aspects of hemlock trees and in weather stations.

indicate that HWA are experiencing more warming than ambient temperatures would indicate. The accumulation of warmth by insects is strongly associated with physiological changes and progression of development. Loss of cold-acclimation by HWA may be influenced in this way.

A degree-day model may be a useful approach for predicting changes in HWA cold-acclimation. These models are based on the accumulation of warmth above a minimum developmental threshold and a thermal constant necessary for a particular physiological event to occur. No developmental threshold is available for HWA sistens, but Salmon et al. (2002) determined the value to be 3.8° C for 2nd instars of progridien HWA. An exercise using this value to calculate degree-day accumulation finds ambient temperature a poor predictor for degree-days in the HWA microhabitat (Fig. 4). No thermal constant is available for loss of cold-acclimation by HWA, and factors such as photoperiod may also be involved.

Customarily, USDA plant hardiness zone maps (USDA 1990) are consulted for linking experimental data on HWA mortality to climatic conditions within a region. We know HWA are already established in areas corresponding to zones 5a and 5b, and experimental results suggest survival may be possible in zone 4b and potentially zone 4a. Survival in zone 3 seems unlikely. However, this course approach doesn't take advantage of

the abundance of climatic data that is available, nor can it incorporate developing understanding about the effects of duration and timing of cold exposure on survival, or changes in HWA susceptibility to cold relative to season and factors mentioned in table 1. For minimum temperatures, ambient data may have utility without adjustment for microclimatic effects for predicting geographical range limits of HWA. For maximum temperatures, our data will help define the ambient and microhabitat relationship, but understanding the role warmth has for modulating HWA susceptibility to cold is essential for narrowing predictions of HWA range limits.

There are numerous practical incentives for defining potential limits of HWA range, including better allocation of resources for surveillance and management. Several avenues of investigation are needed to enhance this effort. The response of HWA to cold needs to be defined in increments smaller than 5° C, which is the precision currently available. Threshold temperatures for sisten development and thermal constants for loss of cold-acclimation are essential for predicting lethal effects of the less severe temperatures in late winter. Data are needed from early winter on microhabitat temperature and HWA survival. Research on sublethal effects of cold, genetics of HWA populations, and range expansion in colder regions would better relate individual responses to consequences for populations. Finally, a synthesis of environmental, geographical and forest cover data is essential for characterizing the risk of invasion of northern hemlock forests by hemlock woolly adelgid.

## Acknowledgments

We would like to thank the following people for their continuing support of our HWA research program: Brent Teillon, Charlie Burnham, Mike Garyk, Kathleen Shields, David Mikus, Bradley Onken, David Orwig and staff at Mt. Tom Reservation Holyoke, MA. This work is funded through Cooperative Agreement with the U.S. Forest Service (USDA 02CA11242343102).

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# COMPARING SYSTEMIC IMIDACLOPRID APPLICATION METHODS FOR CONTROLLING HEMLOCK WOOLLY ADELGID

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## Abstract

We compared imidacloprid application methods to measure their efficacy against hemlock woolly adelgid (HWA, *Adelges tsugae* [Annand]) in Connecticut forests. The methods compared were Kioritz soil injection with (1) placement near the trunk or (2) placement near the trunk and out to the drip line, (3) drench near the base of the trunk with Bayer Tree and Shrub Insect Control (all three soil application approaches applying 1 g active ingredient per 2.5 cm DBH), and trunk injection with the (4) Arborjet, (5) Wedgle, and (6) Mauget systems, giving 0.1, 0.09, and 0.15 g a.i. per inch DBH, respectively. Along with the untreated check, these treatments were part of a  $7 \times 2$  factorial design, which included a comparison of fall vs. spring application timing. Six replicates were located at five sites for a total of 84 trees. Insecticides were applied between October 1-29, 2002 and between May 28 - June 6, 2003.

Cold temperatures resulted in 85 - 95% overwintering mortality at study sites, so mortality evaluation related

to insecticide treatments was delayed until July 7-15 when the following generation (progreddens) had developed. Mortality was also assessed in late November 2003. Site variability and natural mortality affected adelgid survival and obscured insecticide treatment effects in the July assessment. Adelgid mortality ranged from an average of 64% for the Wedgle-treated trees, 69% mortality in the untreated checks, and 80% for the Kioritz, near trunk imidacloprid placement. The November evaluations determined that soil applications of imidacloprid were more effective than trunk injections for long-term reductions in adelgid populations. The Kioritz near-trunk placement of Merit in the fall of 2002 resulted in undetectable adelgid populations measured more than a year later. Fall and spring application timing were not significantly different. The soil applications resulted in long-term moderate concentrations of imidacloprid in the sap and a reliable, highly effective suppression of HWA populations.

# ARBORJET METHODOLOGY: ASSAYING NEW SYSTEMIC FORMULATIONS FOR TRUNK INJECTION AND MICRO-INFUSION TECHNOLOGIES.

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## Poster Abstract

Arborjet, Inc. screens new systemic formulations for compatibility with delivery devices and tree transport tissues. Formulation analyses conducted include pH., viscosity (i.e., flow characteristics), flammability, behavior at temperature extremes, compatibility with other formulations and shelf life. Internal components of the Arborjet VIPER device(s) are exposed to new formulations at concentration for a prolonged period and evaluated for corrosion, abrasion and swelling. Laboratory bioassays are conducted using a number of tree species, including *Acer platanoides*, *Pinus strobus* and *Tsuga canadensis*, to evaluate trunk treatments. Five cm caliper, 3 m tall trees are cut and placed in 190 liter aerated hydroponics system, and placed in a controlled environment under 400 watt metal-halide grow lights. Evaluations include efficacy of delivery, uptake and distribution of active. Distribution of injected or infused formulations is modeled using fluorescent dyes. Analysis of distribution is performed using UV light. Differential distribution within the system is evaluated based upon branch:stem ratios. Further evaluations include foliar phytotoxicity and formulation residue evaluated using

HPLC, ELISA techniques or by bioassay. Following initial laboratory screening, promising formulations are incorporated into field trials to further evaluate performance and efficacy.

Methods illustrated include:

### A. Formulations

1. pH
2. viscosity
3. temperature range
4. compatibility
5. shelf-life
6. components
7. ignition
8. abrasion and/or corrosion

### B. Bioassay

1. ease of application into tree transport tissues
2. uptake and distribution through stem and canopy
3. phytotoxicity of foliar tissues
4. efficacy: plant health response or insect mortality

# INVASIVE.ORG: A WEB-BASED IMAGE ARCHIVE AND DATABASE SYSTEM FOCUSED ON NORTH AMERICAN EXOTIC AND INVASIVE SPECIES

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## Abstract

Invasive.org ([www.invasive.org](http://www.invasive.org)) is a web-accessible archive of high quality images driven by a fully-searchable taxonomic database based upon the ForestryImages ([www.forestryimages.org](http://www.forestryimages.org)) framework. Images in the system can be downloaded in the desired resolution, format and file size needed by the user and can be used with no royalties and no fees for all educational applications. The system was developed as a portal to high resolution images with links to species-based information. Invasive.org is one of 15 websites maintained by the Bugwood Network ([www.bugwood.org](http://www.bugwood.org)) on a broad range of topics, including invasive species, forestry and forest health, and entomology dealing with both natural systems and more directly managed systems.

Invasive.org and the associated ForestryImages system (as of December 2003) make over 17,000 images taken by over 460 photographers on more than 3,200 subjects available to users. During the period of January 2002 through September 2003, over 9.65 million pages of information were served to more than 961,000 users through these systems. The content of the systems and the user base continues to expand. We invite you to visit, utilize and contribute to these sites. Invasive.org is a joint project between the University of Georgia, USDA Forest Service and USDA-APHIS-PPQ.

Presented by G.K. Douce ([kdouce@uga.edu](mailto:kdouce@uga.edu)) and R. Reardon ([rreardon@fs.fed.us](mailto:rreardon@fs.fed.us)).

# AN ANISOTROPIC MODEL OF HEMLOCK WOOLLY ADELGID SPREAD

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## Abstract

The hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) continues to spread through the range of eastern hemlock (*Tsuga canadensis* (L.) Carrière) leaving dead hemlocks in its wake. Current estimates are either isotropic, 20 - 30 km per year (McClure et al. 2001), or vary by cardinal direction, 3.6 km per year west and 5.8 km per year north and south (Morin et al. 2003). Decades of infestation records and regional geographic data offer the opportunity to model HWA spread as a dispersion that varies with site conditions. This paper is a preliminary attempt to create an anisotropic model of HWA spread within a geographic information system (GIS). The GIS includes both county and township level infestation records along with digital maps of geographic variables including plant hardiness zone, elevation, forest cover type, urbanization, precipitation, temperature, and hemlock range. I use three statistical approaches to explore the connections between the site variable and spread rate: cluster analysis, principal components analysis, and classification and regression tree (CART) modeling. The resulting estimates of past and predicted spread are preliminary and their failings may be as informative as the final results.

The first step in modeling future HWA infestations is to measure historic spread. The infestation records available to measure historic HWA spread are the U.S. Forest Service county records and township based records for Pennsylvania and northward (Anhold 1990, Bofinger 2003, Burnham 2003, Denham 2003, Ouellette 2003, Sior 2003, Teillon 2003, U.S. Forest Service 2003). Neither the county nor the township dataset is exact, because records of HWA infestation are not exact. HWA's passive dispersal and tree crown habitat make it hard to detect and record the date of first infestation. There are also inconsistencies between the two datasets, perhaps because the methods of recording and reporting infestation vary by state and surveyor. Unfortunately, these data sets are all that is currently available for estimating spread.

There are a number of ways of delineating the front of infestation and the rate of spread (e.g. Andow et al. 1990, Liebhold et al. 1995, Sharov et al. 1996). I will use a straightforward approach where the median Euclidean distance from the previous infestation front to the far edge of the newly infested township is the spread distance. Using this metric, the average rate of HWA spread since 1990 in North America based on county data is 16 km per year. Excluding two infestations in western New York where infested nursery stock cause HWA movement, the rate is 15 km per year. Employing the same spread metric for the township-based data the estimate of spread is 8.4 km per year. For comparison, measuring spread with the county-based data for the same geographic area (Pennsylvania and northward) the spread rate is 12 km per year. The township-based rates of spread for Pennsylvania and New York are 20 km and 7.7 km per year respectively. The variation in these rates of spread shows that the data scale and the geographic area used to calculate the rate of HWA spread have a large impact on the estimated rate. Although 15 km per year may be an improved estimate of regional HWA spread, on average only 8% of the counties within 15 km of a previous infestation have HWA the next year.

The 15 km per year rate of spread does not vary with geography and so is an isotropic model of HWA infestation. Distance from previous infestation is the only variable considered in predicting infestation. However, observed HWA dispersal appears to vary geographically. For example, HWA has spread more slowly in the northern edge of its range in New Hampshire than it has in Georgia. There are sociological and biological explanations for anisotropic spread. Two examples are humans assisted dispersal of HWA and reduced HWA survival in extreme cold (Parker et al. 1998, Costa 2004). This study attempts to translate these types of influences on HWA dispersal into an anisotropic model via regional geographic variables. The variables included in the GIS and in the modeling of spread are:

- List of states and counties with known HWA infestations (U.S. Forest Service 2003)
- USDA Plant Hardiness Zone Map (U.S. National Arboretum 1990)
- Global Land 1 km Base Elevation Digital Elevation Model (National Imagery and Mapping Agency 1996)
- Forest cover type (U.S. Geological Survey and U.S. Forest Service 2000)
- The National Atlas urbanization map (U.S. Geological Survey 2000)
- The National Atlas road map (U.S. Geological Survey 2000)
- United States annual maximum temperature, 1961-90 (Day & Taylor 2000)
- Population in 1990 per square mile (U.S. Census 1990)
- Distance from previous infestation
- Direction of spread from previous infestation

The geographic variables and the infestation records are not independent observations, which limits the number of analytical techniques appropriate to the data. The first statistical method I used to examine the data was cluster analysis. Cluster analysis makes a good first step in data analysis because it does not define relationships between variables, rather it groups data points based on their similarity. There are a number of algorithms available to build the groups of data points (SAS Institute Inc. 1999, McGarigal et al. 2000), but unfortunately none of them defined groups of counties or townships that match well with infestation history.

The next analysis I tried was principal components. The basic idea of principal components is to combine many variables into a few important axes of variation and then organize samples within these axes (SAS Institute Inc. 1999, McGarigal et al. 2000). The goal in using principal components analysis is to compare the axes of maximum variation with HWA spread gradients. However, the principal components analysis failed provide insight into the geographic variation in rates of HWA spread.

The most fruitful analytical technique for examining HWA spread was Classification and Regression Trees

(CART) (Brieman et al. 1984). CART is a modeling technique that uses a training data set to assign new data into groups. The training data defines a classification tree that then splits the new data into classes based on a series of attributes. Some of the advantages of CART modeling are that it does not make any assumptions about the distribution of the data, it is robust to outliers and misclassifications in the training data, and it combines both categorical and continuous data in its classifications (Brieman et al. 1984).

Those counties or townships that had HWA before 2001 and those counties well beyond the reach of HWA were the training data to build a classification tree. The geographic variables determined each split in the classification tree. The counties infested in 2002 provided a test of the classification tree. The CART model correctly predicted 92% of the uninfested counties and 88% of the infested counties in 2002. Prediction based on the pre-2003 data for 2003 infestations was much less good, showing the need for refinements in the model. The biggest modeling limitation is the historic HWA infestation data. Infestation records could be improved by gathering more township records of HWA from the south and by consistent regional surveys of current HWA locations. Adding other geographic variables and incorporating annual weather variation might also improve the model.

## Acknowledgments

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# COMPETITIVE INTERACTIONS AMONG THREE EXOTIC PREDATORS OF THE HEMLOCK WOOLLY ADEGLID

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## Abstract

The objective of this study was to evaluate competitive interactions among three exotic predators of the hemlock woolly adelgid (HWA) under simulated natural conditions in the laboratory. *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) from western Canada and *Pseudoscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) from Japan are two specialist predators being released into infested eastern hemlock stands in the United States for classical biological control of HWA (Salom et al. 2001). In addition, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), an imported generalist predator of aphids, is found in increasing numbers in infested hemlock stands (Wallace and Hain 2000). Because these species may occur in the same ecosystem and competitive interactions can decrease the efficacy of biological control (Rosenheim et al. 1993), we examined conspecific and intraguild predation so that release strategies for *L. nigrinus* and *P. tsugae* may be optimized.

Laboratory studies were conducted using petri dish bioassays containing clipped, infested eastern hemlock twigs held in environmental chambers under two simulated environments corresponding to early and late spring in southwestern Virginia. The susceptibility of the eggs of each predator species to predation by conspecific and heterospecific larvae and adults was tested using no-choice and choice tests at a range of HWA densities. For the assays, the predator eggs were incorporated onto HWA infested foliage in their natural context and a single predator was then introduced for 48 h. The results showed that *L. nigrinus* eggs were highly susceptible to conspecific predation and intraguild predation by *H. axyridis*. Likewise, *P. tsugae* eggs were susceptible to intraguild predation by *L. nigrinus* and *H. axyridis*. However, predation of these two species was inversely HWA density dependent, primarily occurring when HWA density was either very low or absent. In contrast, *H. axyridis* eggs were only susceptible to conspecific

predation, and this occurred at high levels independent of HWA density.

Effects of competition among predators were analyzed by a series of tests comparing single predators alone to groups of three conspecifics or heterospecifics. Survival, feeding and reproduction were measured over a 6-day period with moderate HWA densities available throughout. Results showed that adults and larvae of each species survived as well alone as with conspecifics or heterospecifics. Larval feeding was additive regardless of the species combination. Adult feeding during the early spring simulation showed that *L. nigrinus* and *H. axyridis* had higher feeding rates than that of *P. tsugae*. In the late spring simulation, *H. axyridis* had the highest feeding rates followed by *L. nigrinus* and *P. tsugae*, which were similar. In terms of reproduction, *L. nigrinus* had the highest oviposition rates for both the early and late spring simulations. However, although *P. tsugae* and *H. axyridis* had very low oviposition rates during early spring, these increased dramatically in late spring. Oviposition was non-additive for *L. nigrinus* during both environmental simulations, but only during the late spring simulation for *H. axyridis*. Both *L. nigrinus* and *H. axyridis* demonstrated conspecific competitive interference. In contrast, *P. tsugae* oviposition was additive during both environment simulations.

It appears that eggs of *L. nigrinus* and *P. tsugae* are not chemically defended, but are preyed upon in the absence of sufficient HWA. Eggs of *H. axyridis* appear to be chemically defended, but suffer substantial conspecific predation. In terms of predator interactions, the results suggest that these three predator species may be more complementary than competitive. *L. nigrinus* appears to be an efficient predator in the colder conditions present in the early spring, while *P. tsugae* feeds more in warmer conditions as in late spring. *H. axyridis* has the potential to be a significant intraguild competitor, but in these

short-term studies with moderate HWA available, it did not cause significant mortality to *L. nigrinus* or *P. tsugae*, nor disrupt feeding or reproductive activities of these species. *L. nigrinus* and *H. axyridis* may regulate their population density through a combination of conspecific egg predation and decreasing oviposition in the presence of conspecifics.

Additional studies under way include using fabric branch enclosures to examine predators under more natural conditions in southwestern Virginia hemlock stands over 6 weeks during the overwintering and spring generations of HWA. Detailed video analysis of daily activity patterns are planned to better elucidate possible competitive interactions.

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# DEVELOPMENT OF SURVEY TOOLS FOR THE EMERALD ASH BORER

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## Abstract

Currently, detection of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) Fairmaire (EAB), consists of visual inspection and identification of characteristic damage or possibly beetles. The objective of this study is to develop a trap and a semiochemical lure system for EAB. An effective trapping system would improve the sensitivity and efficiency of EAB survey and aid the overall program in achieving its goals.

A four panel trap design was employed to test four colors simultaneously. Corrugated plastic panels (0.6 cm thick) were 37.5 cm x 60.0 cm, and were coated with Pestick insect trapping glue. Two sets of four colors [Black - Yellow - White - Purple and Red - Green - Navy - Silver] were tested at two heights [1.5 m and 7.0 m]. Over 500 beetles were caught in a 3-week period. More beetles were caught on purple traps than on any other color. Red and yellow traps caught significantly fewer beetles than any of the other colors. More beetles were caught on low traps than on high traps for all colors except black and yellow. More females were caught on purple and black traps than males. Later in the field season, we also deployed a third set of four colors [Brown - Orange - Teal - Light

Blue]. These colors, however, did not catch many beetles as the deployment occurred after the peak flight. Single color, three panel traps were also tested but placement was too late in the field season, and catch was low. We are currently developing single color traps to be tested in 2004.

Commercially available semiochemical lures were tested in conjunction with two trap designs [a single purple panel (37.5 cm x 60.0 cm) and the IPM Tech Intercept Panel Trap]. The single panel trap caught 34 beetles compared to zero for the IPM Tech trap. There was no significant difference in catch between the eight lures and the blanks, although traps baited with lures that contained ethanol as an ingredient accounted for 20 of the total beetles caught.

We began collecting volatiles from beetles and host material in 2003. We plan to continue this work in 2004. Using gas chromatography coupled with an electroantennographic detector (GC-EAD) we hope to identify antennally-active compounds which will then be tested in lab and field bioassays.

# EFFECTS OF PARENTAL AGE AT MATING ON SEX RATIOS OF THE GYPSY MOTH PARASITOID *GLYPTAPANTELES FLAVICOXIS* (HYMENOPTERA: BRACONIDAE)

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## Abstract

*Glyptapanteles flavicoxis* (Marsh) is a gregarious larval parasitoid of the Indian gypsy moth *Lymantria obfuscata* (Walker) that readily attacks the gypsy moth. Though attempts to establish *G. flavicoxis* in the U.S. were unsuccessful, it is believed to have potential for inundative releases against gypsy moth populations, because it can be reared in large numbers with relatively few hosts. Unfortunately, sex ratios in laboratory reared *G. flavicoxis* are usually male-biased. Male-biased sex ratios hinder efforts to mass release parasitic Hymenoptera for biological control by making the production of females costly. Because parental age at time of mating is known to affect the sex ratio in some Braconidae, we crossed haploid males and virgin females 0-, 1-, 4-, 9-, and 16-days-old with at least 10 trials for each of the 25 combinations. Numbers and sex ratios of progeny produced by females each day were recorded and subjected to two-way analysis of variance. We used Tukey's test to detect differences in sex ratios among

progeny of differently aged parents and G-tests to test for treatment differences in proportions of females producing mixed and all male progeny. Both progeny and sex ratios (% females) among progeny produced by ovipositing females of *G. flavicoxis* decreased markedly over time, so only the first days production need be used in mass rearing. Thus, we focused our analyses on sex ratios in progeny produced on the first day hosts were provided to females. Females in all age classes mated to newly emerged males (day 0) were more likely to produce all male progeny (30%) than those mated to older males (10-15%). When crosses with only male progeny were excluded from the analysis, females mated to males 1 day old had higher sex ratios than those mated to males in other age classes. In addition, females mated the day that they emerged tended to have the highest sex ratios. Therefore, one should not use newly emerged males in rearing this species, but newly emerged females appear to be good candidates for a rearing program.

# A NEW SPECIES OF *LARICOBIVS* FROM CHINA (COLEOPTERA: DERODONTIDAE), A PREDATOR OF HEMLOCK WOOLLY ADELGID

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## Abstract

In April 2002 an expedition was made to Sichuan Province, China to search for potential predators of the hemlock woolly adelgid (HWA). A second collecting trip is planned for 2004. New species of *Laricobius* are of particular interest because *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), native to western North America, has been released in the eastern U.S. for control of HWA. Twenty-three larvae of a new species, *Laricobius* sp. n., discovered at the Luoxiba Deng Forestry Station, Kangding County in China, were imported to the Quarantine facility at Virginia Tech and reared on HWA-infested eastern hemlock. From these larvae, eight females and two males provided the founding generation for our lab colony.

Basic biological studies were initiated in fall 2002 and temperature-dependent development of the first entirely lab-reared generation was tested at 12 and 15° C, 12:12 h (L:D), in spring 2003.

Mean ( $\pm$  SD) fecundity, activity period and ovipositional period was  $196.4 \pm 53.4$  eggs,  $219.5 \pm 49.9$  days, and  $184.5 \pm 49.9$  days, respectively. Total development time ( $\pm$  SD) at 12° C was  $97.2 \pm 5.1$  days and  $64.5 \pm 2.3$  days at 15° C. These development times are comparable to *L. nigrinus*, although the new Chinese species develops significantly slower at 12° C but faster at 15° C. This suggests that *Laricobius* sp. n. may be better suited for warmer temperatures than *L. nigrinus*, and could work as part of a predator complex in the different climatic regions infested with HWA.

Future studies of *Laricobius* sp. n. will focus on host range testing and monitor temperature-dependent development at 6, 9, and 18° C to create a degree-day model for comparison with that of *L. nigrinus*. Balsam woolly adelgid, pine bark adelgid, eastern spruce gall adelgid, pine needle scale, elongate hemlock scale, and woolly alder aphid will be tested in choice and no-choice bioassays.

# FRUIT FATE, SEED GERMINATION AND GROWTH OF AN INVASIVE VINE: AN EXPERIMENTAL TEST OF 'SIT AND WAIT' STRATEGY

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## Abstract

Oriental bittersweet (*Celastrus orbiculatus*) is a vertebrate-dispersed exotic, invasive woody vine in the USA. Using a "sit and wait" strategy, it establishes in closed-canopy forest, and responds to canopy disturbance with rapid growth, often overtopping trees. We studied bittersweet's distribution, abundance, and effect on native plant diversity along the Blue Ridge Parkway (BRP) in relation to topographic characteristics, elevation, distance from Asheville, NC and distance from roadside. We also experimentally tested its mechanisms for dispersal and establishment. The occurrence of bittersweet along the BRP decreased with elevation, which also corresponded with distance from Asheville; we observed it as high as 1,994 m elevation. Bittersweet cover and number of stems were unrelated to distance from the roadside, but growth height was greater along roads than in the forest. It occurred more often on moist, north-facing than on drier, south-facing slopes, and percent cover was negatively related to native plant diversity. Seventy-six percent of marked fruits were removed; 24% fell to the ground. Fruit crop density did not affect removal rates. Seeds that were scarified by bird ingestion exhibited delayed germination, but seeds germinated in similar proportion to manually defleshed seeds. Germination of seeds within intact fruits was also delayed, and fewer germinated compared to other treatments. The amount of photosynthetically active radiation (PAR) received (20, 37, 53, 70, or 100%) did not affect the proportion of seeds germinating or germination rates. However, seedlings in  $\geq 70\%$  PAR had more leaves, heavier shoots, and longer, heavier roots than seedlings at lower PAR levels. Results show that bittersweet appears to be spreading outward from Asheville, and it reduces native plant diversity. Most (>75%) seeds are dispersed, seedlings can establish in dense shade, and plants grow rapidly when exposed to high light conditions. Control strategies should focus on minimizing seed dispersal.

Invasive, exotic plant species can reduce native plant species diversity and alter ecosystem processes such as disturbance types and regimes (Vitousek 1990). Many invasive species are associated with disturbed sites, where competition is reduced and greater resources are often available. Some can invade undisturbed habitats, presumably posing a more serious threat than invasive species that are restricted to disturbed areas.

Oriental bittersweet (*Celastrus orbiculatus*) was introduced to North America from southeast Asia in 1860 (Dreyer 1988). By 1974 it had spread to 33 states (Patterson 1974); in the southern Appalachians, it is particularly abundant near Asheville, North Carolina. It is highly shade tolerant, and can spread by root suckering. It grows rapidly in response to high light conditions, such as canopy openings from disturbance. It annually produces prolific crops of orange fruits that are dispersed by birds and mammals. When released, bittersweet overtops and kills native vegetation at all strata by blocking light.

We report here on a series of studies that address (1) the distribution of bittersweet in relation to its local primary source (Asheville), distance from the roadside, and topographic variables (Konopik 2002); (2) the effect of bittersweet on native plant diversity (Konopik 2002); (3) mechanisms for seed dispersal (Greenberg et al. 2001); and (4) conditions required for seed germination, establishment, and seedling growth (Greenberg et al. 2001). The studies were conducted at the Bent Creek Experimental Forest and nearby Blue Ridge Parkway (BRP), located in the Asheville basin near Asheville, North Carolina. Statistical significance indicates  $P < 0.05$ .

**Distribution of bittersweet in relation to Asheville and topographic variables, and impact on native plant diversity:** We established three, 1 X 5-m plots parallel to

and at three distances from the roadside on both sides of the BRP, where possible, at each milepost from mile 388-407. Our plots ranged from about 671 to 1,474 m in elevation. Distances from the roadside were: 1-3 m (edge); 9-12 m (transition), and > 30 m (interior). We counted the number of bittersweet stems and growth height in all plots. Aspect, elevation, slope, canopy cover, and percent cover of all vascular plant species were also recorded.

Logistic regression indicated that bittersweet was negatively associated with elevation. However, increasing elevation corresponded with increasing distance from Asheville. We noted its occurrence at the Mt. Pisgah parking area, elevation 1,994 m, indicating that distance from Asheville, rather than elevation, is most important in governing the distribution of bittersweet along that stretch of the BRP. Its occurrence in the parking lot of this highly visited hiking trail also suggests that humans may facilitate bittersweet dispersal. The occurrence, percent cover, and number of bittersweet stems were not related to distance from the roadside. Growth height was greater along the roadside edge (where canopy cover was high - averaging 80%), yet significantly lower than in the transition or interior zones. Bittersweet was more likely to occur on north-facing (90% probability) than on south-facing (30% probability) slopes. We also found a significant, negative relationship between bittersweet cover (>20%) and native plant species diversity ( $H'$ ).

**Mechanisms for seed dispersal, and conditions required for seed germination, establishment, and seedling growth:** In Experiment I we compared removal, damage, and fall rates of non-indigenous bittersweet and native American holly (*Ilex opaca*) fruits by marking fruits at replicate sites ( $n = 13$ ) along roadsides where both species occurred  $\geq 30$  m apart. Both species produce bright orange fruits that ripen in late fall. We conducted univariate repeated measures ANOVA to compare disappearance rates. American holly retained fruit longer (median 15.5 weeks) than bittersweet fruits (median 10.5 weeks). The amount of fruit remaining on plants also differed among sites and sample dates, and the sample date X species interaction was significant. More bittersweet fruits than holly fruits fell, and more were damaged; damaged fruits were apparently consumed and

dispersed by vertebrates. The temporal pattern of fruit fall and removal differed between the species due to more bittersweet fruits falling beginning in mid-December, and because American robins (*Turdus migratorius*) removed much holly fruit from some sites in March. Fruits of both species were consumed most during winter. In Experiment II we compared marked fruit removal rates in high- vs. low-density bittersweet patches ( $n=10$ ) located  $\geq 0.2$  km apart along roadsides. ANOVA detected similar fruit retention between high- and low-density bittersweet fruit patches.

In Experiment III we tested in a greenhouse whether seed treatment affects the proportion of bittersweet seeds germinating, number of days until germination, and growth of seedlings. The four seed treatments were: (1) scarified: fruits were quartered and fed to 31 captive Yellow-rumped warblers (*Dendroica coronata*); defecated or regurgitated seeds were sown singly ( $n = 43$ ), (2) defleshed-1 seed (DF1): pulp and skin were removed manually; seeds sown singly ( $n = 40$ ), (3) defleshed-all seeds (DFA): pulp and skin were removed manually; all seeds from the fruit were sown together (ca. 4.3 seeds per fruit), and (4) intact: one intact fruit was sown per pot. The mean number of days until germination differed among the four seed treatments (Chi-square test). Seeds within intact fruits took longer to germinate, followed by scarified seeds. Defleshed seeds took the fewest number of days to germinate regardless of whether they were sown individually (DF1) or with all seeds from a fruit (DFA). The proportion of seeds germinating within 61 days also differed among treatments (ANOVA). We found no differences in the proportion of seeds germinating among scarified, DF1, or DFA treatments (total 82.0%), but significantly fewer (51%) seeds of intact fruits germinated than in other treatments. Seedlings from the DF1 and scarified treatments showed no differences in root length, shoot length, or total height (Student's t-test). The high germination rate (total 82%) of defleshed (including scarified) bittersweet seeds indicates that seeds without fruit pulp or skin have a high probability of germinating, and that the presence of fruit pulp or skin inhibits germination; birds probably disperse bittersweet seeds, but do not aid in germination by gastrointestinal scarification.

In Experiment IV we tested the effect of 5 natural light intensities on germination and growth of bittersweet in the greenhouse by manipulating shade cloth. Treatments were full sun (100%) and photosynthetically active radiation (PAR) of 70%, 53%, 37%, and 20%. We recorded the date of germination, number of leaves and length and dry biomass of roots and shoots (n = 16 to 22 per treatment, destructively sampled) 100 days after germination. Varying light treatments did not produce significant differences in the proportion of seeds germinating (Chi-square test), the number of days until germination, the root:shoot weight ratio, or the root:shoot length ratio (ANOVA). Seedlings in  $\geq 70\%$  PAR generally had more leaves, heavier shoots, and longer, heavier roots. More defleshed (including scarified) seeds that were sown in late February for Experiment III (stratified 60 days) germinated, compared to those sown in late May for this experiment (stratified 150 days). Light intensity did not affect the proportion of seeds germinating, the time until germination, or seedling survival. This high level of germination over a wide range of conditions likely facilitates the establishment of seedling banks under closed canopy conditions.

Our experimental results clarify the mechanisms by which bittersweet employs this “sit and wait” strategy for invading undisturbed temperate forest. Most fruits (>75%) are apparently removed by vertebrates, and presumably dispersed. Most (82%) defleshed seeds, including scarified seeds germinate, although viability decreased with time. Many (51%) seeds within intact fruits also germinate. Thereby, fallen fruits also contribute to the viable seed pool. Seed germination and seedling survival is similar across a wide range of light intensities allowing establishment of a seedling bank even under closed canopy. However, seedling “vigor” is greater under high light conditions, suggesting that canopy disturbance aids the vegetative and clonal spread of established seedlings.

Our results show that viable bittersweet seeds are dispersed in large numbers, are capable of successful establishment under closed canopy conditions, and grow rapidly when exposed to high light conditions. This “sit and wait” invasion strategy allows bittersweet to invade intact forest and await a canopy disturbance for the opportunity to proliferate. In this manner bittersweet is becoming an increasingly serious threat to plant communities across the eastern United States. Control strategies for this highly invasive species should likely focus on minimizing seed dispersal by vertebrates.

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# EMERALD ASH BORER: STUDIES ON FIREWOOD, HOST RANGE, DISPERSAL, AND OCCURRENCE IN CHINA

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## Abstract

Highlights of our 2002-2003 research projects on the emerald ash borer (EAB; *Agrilus planipennis*) were presented. With respect to firewood, we found that EAB were able to develop and emerge from firewood cut at various times from July to October. Nevertheless, EAB survival was significantly lower in firewood cut during July and August vs. September and October. Similarly, EAB survival was lower in firewood stored in direct sunlight vs. shade. Overall, cutting infested trees early during larval development and placing the logs in full sunlight dramatically lowered EAB survival, but did not kill all larvae. A larger study was initiated in 2003, which incorporated the following treatments: different months of felling, sun vs. shade, split vs. whole bolts, and tarped vs. not tarped.

Although ash (*Fraxinus*) is the only known host of EAB in North America, the Japanese and Korean literature also reports species of elm (*Ulmus*), walnut (*Juglans*) and wingnut (*Pterocarya*) as hosts. In 2003, we evaluated foliage of several trees and shrubs as food for EAB adults in Michigan, in a series of no-choice and choice tests that were conducted in the laboratory. We tested members of the olive family (Oleaceae: *Chionanthus*, *Forestiera*, *Forsythia*, *Fraxinus*, *Ligustrum*, *Syringa*), elm family (Ulmaceae: *Celtis*, *Ulmus*), and walnut family (Juglandaceae: *Carya*, *Juglans*). Overall, EAB adults fed readily on ash, although blue ash (*F. quadrangulata*) was the least preferred of the ash species tested. EAB adults

did a small amount of feeding on shrubs in the ash family, including forsythia, fringe tree, lilac, privet, and swamp privet. There was almost no feeding on elm, hackberry, hickory, or walnut.

Using computer-monitored flight mills and tethered EAB adults, we measured flight distance over 24 hours. For the first 28 adults tested (flying without food or water during the test period), half flew less than 50 m while half flew more than 50 m. Of those flying over 50 m, their flight distances were 53, 71, 281, 649, 674, 716, 804, 814, 1110, 1216, 1418, 1653, 2426, and 4258 m.

In autumn 2003, two of the authors (H. Liu and T.R. Petrice) visited 29 field sites in Heilongjiang, Jilin, Liaoning, Hebei, Tianjin, and Shandong Provinces in northeastern China. The field sites included natural forests, nurseries, plantations, city parks, and streets. The ash species included *Fraxinus chinensis* subsp. *chinensis*, and *F. chinensis* subsp. *rhynchophylla*, and *F. mandshurica*. In addition, the North American species *F. pennsylvanica* (green ash) and *F. velutina* (velvet ash) have been planted in eastern China and are both readily infested by EAB. Active EAB infestations were found in five of the six provinces (all but Shandong) and in 20 of the 29 field sites. EAB commonly attacked ash trees in open areas and along forest edges. Evidence of larval parasitism was common.

# ASSESSING THE SAFETY OF MICROBIAL BIOLOGICAL CONTROL AGENTS

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Over the last two decades, the environmental safety of biological control has been questioned. Surveys in the literature have shown that although there are some examples of negative impacts, negative generalizations across biological control as a whole are largely unjustified. The next step taken by researchers in Europe has been to develop procedures to ensure that continued use of biological control is safe for the environment. Between 1998-2003, the Environmental Risks of Biological Control Introductions in Europe (ERBIC) approach for safe use of biological control was developed. While concerns in the U.S. have principally focused on classical biological control, the ERBIC approach was developed for inundative biological control; in Europe, classical biological control is not commonly used and there is very active use of inundative biological control, especially in glasshouses. Organisms released inundatively for biological control in one European country could spread to another country, so regulations are coordinated. The European Union (EU) funded development of this risk assessment plan with involvement from nations participating in the EU. In the following description of the ERBIC system, I will focus on insect pathogens, which are usually applied inundatively.

For a full risk assessment of a biological control agent to be used inundatively, there are three steps: (1) identification of the risk; (2) a risk management plan dealing with risk reduction or mitigation; and (3) a risk/benefit analysis comparing use of the natural enemy with current and alternative pest management. Risk is composed of the likelihood of adverse effects versus the magnitude of potential effects.

Risk identification is the step that involves knowledge of the biology and ecology of the natural enemy. The attributes needed to identify risk are (1) establishment; (2) dispersal; (3) host range; (4) direct effects (e.g., effects on non-target herbivores and intraguild predation); and (5) indirect effects (e.g., competition with native natural enemies). To test insect pathogens, where host finding behavior is virtually never an issue (as compared with

arthropod natural enemies), host range would be tested in petri dishes in the laboratory. If infection is rare or does not occur, then the hazard rating is low. Otherwise, testing would proceed to more realistic microhabitats and, if substantial levels of infection occur, the next stage would be field testing. Of course field testing is only possible for new exotic species that cannot establish or field studies must be conducted in the area of origin, to derive an idea of host range. To choose species to test, first species in the same genus would be tested and then, the same subfamily, etc. In addition, non-related non-targets of interest in the release area or areas to which the natural enemy might spread as well as any threatened and endangered species should be tested.

Detailed tables in van Lenteren et al. (2003) present numerical risk index values for likelihood of establishment, dispersal, host range and direct and indirect effects that range from 1 to 5. For example, if the distance moved per release is < 10 m the rating is 1 while a species moving > 10,000 m per release would be ranked 5. Likewise, the magnitude of these different factors is scored, e.g., host range restricted to one species ranks 1 while host range restricted to the order ranks 4. Then, for each of the five attributes (e.g., establishment, host range, dispersal, etc.), the values for likelihood and magnitude are multiplied and then all five attributes are summed. The lowest possible sum for the risk index is 5 and the highest is 125. van Lenteren et al. (2003) suggest that for organisms with less than 35, no objection regarding release would be raised while for those ranking 35-70, you might want to study them further so the risk is better understood. To see how this rating system works, 31 natural enemies are ranked by van Lenteren et al. (2003), and a table is provided showing the decisions behind the final sums. These natural enemies are ranked based on where they will be used, e.g., glasshouse, open field, etc. The lowest ranked (safest) natural enemies were very small and host specific parasitoids while the least safe were generalist predators. Among the natural enemies, three entomopathogenic fungi and one entomopathogenic nematode were rated. These received

ratings near 50, largely due to broad host range. I applied the rating system to *Entomophaga maimaiga* with a resulting rating of 34. The gypsy moth NPV ranked still lower at 17. Use of non-woven fiber bands impregnated with cultures of *Metarhizium anisopliae*, a novel application method we are investigating for control of Asian longhorned beetle (Dubois et al. 2004), ranked 24, in part due to the limited area of application. At present, these are my ratings and I welcome others to repeat my rating.

After risk identification, risk management could take the form of labeling to restrict type of crop for application or use of specific application techniques, e.g., spraying from the ground, application to soil, application as bands on trees. Finally risk must always be weighed against benefit, comparing the different alternatives for control along with not controlling the pest.

In summary, rigid regulation of biological control releases would only allow use of highly specific natural enemies. However, the industry producing biological control agents often must produce less host specific natural enemies that are regularly available, to have a large enough market to stay in business. There must be a balance somewhere between these two extremes for biological control to be a viable option. The goals in developing this ERBIC rating system were to develop a

system so that it would be possible to choose the safest control agent, when more than one is available, decide if more information is needed to understand whether a natural enemy is safe and conclude whether some natural enemies are not suitable for use.

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# FIELD STUDY TO EVALUATE THE EGG PARASITOID *APROSTOCETUS ANOPLOPHORAE* SP. N. (HYMENOPTERA: EULOPHIDAE) ON TWO *ANOPLOPHORA* HOSTS

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## Abstract

Our current main objective is to evaluate possible new associations between *Anoplophora glabripennis* (ALB) or *Anoplophora chinensis* (CLB) and natural enemies of European Cerambycids that have similarities with the Asian pests in terms of taxonomy, host plants, and behavior. In Europe, two CLB-infested sites were found, at Parabiago, Italy, in 2000, and at Soyons, France, in 2003, and 2 ALB-infested sites were found, at Braunau, Austria, in 2001, and at Gien, France, in 2003. So far, the infested trees are located in urban areas, in streets, parks and private yards. During the eradication operations, which started in most of the sites, we dissected some infested plant material to eventually discover possible associations between ALB/CLB and local or inadvertently introduced natural enemies. On 20 February, 2002, at Parabiago, CLB eggs were found to be parasitized by a gregarious endoparasitoid, which was identified by G. Delvare (CIRAD, Montpellier, France) as a new species of Eulophidae (Hymenoptera), and described under the name *Aprostocetus anoplophorae* sp. n. (in press). The probable origin of the parasitoid is Japan or China, and its introduction pathway likely is the importation of bonsais infested with eggs of *A. chinensis*.

At Parabiago, CLB adults emerged and escaped from the greenhouses where bonsais had been imported (supposedly around 1992 – 1995) and attacked various local tree species in the surrounding green spaces. During the first surveys made in 2001 through 2003 in the Parabiago area we observed that the plant species attacked by CLB were *Acer saccharinum*, *Acer palmatum*, *Acer pseudoplatanus*, *Aesculus hippocastanum*, *Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, and *Prunus laurocerasus*. CLB eggs parasitized by *A. anoplophorae* were found on

*A. saccharinum*, *A. hippocastanum*, *B. pendula*, and *P. laurocerasus*. From the 30 trees dissected during winter 2002-2003, 133 CLB young larvae and 40 parasitized eggs were collected, which represented 23.1% egg parasitism among the eggs laid in summer 2002. From the overall emergence of *A. anoplophorae* adults, a skewed sex ratio, with 1 male for 7.5 females, was observed.

The presence of well established populations of CLB and its egg parasitoid in the field at Parabiago gave us an opportunity to evaluate ALB eggs as potential target hosts for *A. anoplophorae*. For this purpose, we first obtained from the French and Italian Plant Protection Services the necessary permits to move potted plants infested with eggs of ALB/CLB to Italy to expose them in a secured woodlot. The sentinel plants were rooted potted boles (7 through 10 cm diameter, and 55 cm height boles) of *Populus nigra*, or *Acer negundo*, which had been planted in pots during the previous winter. Prior to exposure in the field, the plants were caged during 5 days with a pair of adults CLB, or ALB, for egg deposition, in EBCL quarantine greenhouse, at Montpellier, France. Every 14 days, mid-July through mid-September, 2003, a dozen of infested potted plants were transported to Italy and exposed for 2 weeks in the study plot, inside a woodlot where the presence of CLB and its egg parasitoid had been observed during summer 2002. The plants were positioned in plastic buckets embedded in ground, and watered as needed. The plot was covered with a plastic mesh screen to prevent bird predation.

Data reported in Table 1 show that *A. chinensis* eggs were parasitized by the females of *A. anoplophorae* naturally occurring in the habitat. This result validated the

**Table 1.—Parasitization of eggs of *Anoplophora* spp. exposed in sentinel plants at Parabiago (MI), Italy, during summer 2003**

	Dates of exposure in 2003	No. of plants exposed	Plants exposed	No. of eggs exposed	No. of eggs parasitized by <i>Aprostocetus</i> on each plant species	No. of eggs parasitized by <i>Aprostocetus</i>	Percent parasitism
<i>A. chinensis</i>	10-24 Jul.	5	5 <i>P. nigra</i>	29	2 / <i>P. nigra</i>	2	6.9
	24 Jul.-7 Aug.	6	4 <i>P. nigra</i> + 2 <i>A. negundo</i>	41	6 / <i>P. nigra</i> + 4 / <i>A. negundo</i>	10	24.4
	7-20 Aug.	5	4 <i>P. nigra</i> + 1 <i>A. negundo</i>	23	1 / <i>P. nigra</i>	1	4.3
	20 Aug.-3 Sep.	5	2 <i>P. nigra</i> + 3 <i>A. negundo</i>	21	0	0	0
	3-17 Sep.	-	-	-	-	-	-
<i>A. glabripennis</i>	10-24 Jul.	6	6 <i>P. nigra</i>	26	0	0	0
	24 Jul.-7 Aug.	7	6 <i>P. nigra</i> + 1 <i>A. negundo</i>	30	0	0	0
	7-20 Aug.	8	6 <i>P. nigra</i> + 2 <i>A. negundo</i>	35	0	0	0
	20 Aug.-3 Sep.	8	8 <i>P. nigra</i>	22	0	0	0
	3-17 Sep.	3	3 <i>P. nigra</i>	5	0	0	0

technique using infested sentinel host-plants to capture parasitoids. The parasitoid showed a very high level of specificity for its original host: all the attacked hosts were *A. chinensis* eggs. As no egg of *A. glabripennis* was attacked by *A. anoplophorae*, the eulophid species has no potential as a biological control agent against ALB.

Some information on host-plant effect on parasitization of CLB eggs was obtained from the plants exposed 24 Jul.-7 Aug., 2003. The parasitized CLB eggs were distributed 40 % on *A. negundo* and 60% on *P. nigra*. *A. anoplophorae* showed a similar ability to find and attack its host in both plant species.

Some information on host age effect on parasitization of CLB eggs was obtained from laboratory and field tests. At 22° C, duration of incubation of the CLB eggs was 15-20 days. During laboratory tests, some 2- through 5-day-old CLB eggs were parasitized by *A. anoplophorae*, successfully. Older hosts were not tested in the laboratory. In field tests, all the eggs exposed were older than 5 days but some were parasitized. Moreover, observations made during dissection of host eggs showed us that a fully developed embryo of CLB also can be parasitized

successfully. Therefore, it appears that parasitization may occur during most of the host egg incubation period.

Some information on the number of generations per year in *A. anoplophorae* was collected. From the overwintering parasitized eggs collected in April 2003, adults of *A. anoplophorae* emerged July through August 2003. This cohort represented a first generation with a diapause lasting from summer 2002 through June, July, or August 2003. In early August 2003, parasitized eggs collected in the field under freshly made oviposition scars produced adult parasitoids 28 August through 6 September 2003. Moreover, CLB eggs laid in the laboratory 17 through 23 July 2003, then exposed and parasitized in the field 24 July through 7 August, produced adult parasitoids on 5 September 2003. Consequently, at least one summer generation, with a 4-week larval development, occurs in *A. anoplophorae*.

Some information on the level of host specificity in *A. anoplophorae* was inferred from field observations made at Parabiago where both hosts *Saperda carcharias* and *A. chinensis* live sympatrically. *S. carcharias* was parasitized by *Euderus caudatus* (Hymenoptera: Eulophidae), and *A.*

*chinensis* parasitized by *A. anoplophorae*; however, their respective egg parasitoids were never found in a non original host.

The gregarious egg parasitoid, *A. anoplophorae*, was fairly abundant in all the sites infested with *A. chinensis* around

Parabiago. It showed a high level of host specificity. It attacked hosts during a long period in summer. It showed at least two generations per year. Consequently, it has the major traits of a promising biological control agent against *A. chinensis*. On the other hand, it appears to have no potential against *A. glabripennis*.

# EVALUATION OF RESISTANCE OF ASIAN AND NORTH AMERICAN ASHES TO EMERALD ASH BORER

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## Abstract

Emerald ash borer (*Agrilus planipennis*) is an aggressive killer of ash (*Fraxinus* spp.) in North America, killing even healthy trees. However, limited reports suggest that emerald ash borer does not devastate ash in Asia, but rather that isolated outbreaks occur in response to stresses such as drought. This implies that Asian ashes may be generally resistant, with weakened trees preferentially colonized. Thus, emerald ash borer seems to behave in Asia much as its close native relatives do in North America, including bronze birch borer (*Agrilus anxius*) and two-lined chestnut borer (*A. bilineatus*), which preferentially colonize stressed trees.

Native trees may be more resistant to native pests because of natural defenses that have developed over their long coevolutionary history. Hence, Asian ashes may be a source of resistance genes to emerald ash borer. This hypothesis is supported by a 20-year study of birch resistance to bronze birch borer conducted by David G. Nielsen at the Ohio State University's Ohio Agricultural Research and Development Center in Wooster. Birches native to North America (*Betula papyrifera*, *B. populifolia*, and *B. nigra*) were found to be highly resistant to bronze birch borer (more than 75% of 200 replicate trees of each species survived at least 20 years), while European (*B. pendula* and *B. pubescens*) and Asian species (*B. platyphylla japonica* and *B. maximowicziana*) were extremely susceptible, with every individual (200 replicates of each species) killed in less than 10 years.

To test the hypothesis that Manchurian ash is a source of resistance genes, a replicated ash planting was established in Novi, Michigan in May of 2003 with the following

objectives: (1) compare resistance of native and Asian ashes to emerald ash borer; (2) identify mechanisms of resistance / susceptibility of ashes to emerald ash borer through comparative profiling of constitutive phloem chemistry, as well as induced biochemical responses of phloem to emerald ash borer attack; (3) identify any associated biochemical markers that can be used easily to screen for resistance; and (4) determine the effects of drought and other stress on borer susceptibility. The planting includes white (*Fraxinus americana*) and green ash (*F. pennsylvanica*), Manchurian ash (*F. mandshurica*), with which emerald ash borer shares an evolutionary history in Asia, and Northern Treasure ash (*F. x 'Northern Treasure'*), which is a hybrid between native black ash (*F. nigra*) and Manchurian ash. The inclusion of this hybrid may provide insight into patterns of inheritance of resistance genes, and facilitate their identification. Our major *a priori* hypotheses are (1) Manchurian ash is a source of resistance genes to emerald ash borer; and (2) stress will decrease resistance of Manchurian ash to emerald ash borer, but will have no effect on resistance of North American ashes, which will be highly susceptible regardless of their physiological state.

Identification of resistance mechanisms and their relationship to whole tree physiology will facilitate screening, selection, and/or breeding of resistant trees, as well as identification of silvicultural practices that enhance tree resistance to emerald ash borer. Ash genotypes with resistance to emerald ash borer will be critical to long-term reforestation efforts and preservation of the ash resource in forests, woodlots, and cities.

# SYSTEMIC RESISTANCE IN GYPSY MOTH TO LdNPV

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## Abstract

Fourth-instar gypsy moth (*Lymantria dispar*) larvae become markedly more resistant to its host-specific baculovirus, *L. dispar* nucleopolyhedrovirus (LdNPV) as the insect ages within a stadium. This resistance is systemic because it cannot be overcome when bypassing the midgut and delivering the virus directly into the hemocoel. We report here that larvae were able to clear virus, and this was markedly more pronounced in insects inoculated at the most resistant stage (48 hours post-molt). Larvae were inoculated intrahemocoelically with budded virus of a recombinant of LdNPV that expresses lacZ under control of the hsp70 from *Drosophila* (LdNPV-hsp70/lacZ) either immediately after molting (4<sup>0</sup>s) or at 48 hours post-molt (4<sup>48</sup>). Insects were bled at 24-hour intervals until larvae began to die (Day 11). Hemolymph from each insect was measured for (1) expression of lacZ in hemocytes; and (2) progeny BV titer in cell-free plasma by plaque assay. In both 4<sup>0</sup>s and 4<sup>48</sup>s, evidence of viral infection (hemocytes signaling lacZ and infectious budded virus) was detected at 3 days post-infection (dpi), but in both stages the proportion of insects having infected hemocytes decreased late in

infection. Also, virus titers dropped to undetectable levels during the course of infection in 4<sup>48</sup>-stage insects; whereas, titers increased in 4<sup>0</sup>-stage larvae. Our data suggest that both stages of insects can overcome viral infection by some form of immune response and/or apoptosis, but that resistant-stage insects clear virus far more effectively. We hypothesize that clearing of virus occurs by induction of immune responses against the virus itself or against virally-infected tissues. A potential immune response is indicated by 1) hemocytes encapsulating the tracheal system servicing the midgut; 2) chemical immuno-suppression by diethyldithio-carbamic acid decreasing developmental resistance in a dose-dependent manner; and 3) an increased activation of enzymes associated with immune responses. Intrastadial developmental resistance may also involve host tissues becoming refractory to infection and/or failure of hemocytes (and/or other tissues such as fat body) to amplify the virus. Preliminary studies in our lab, and published reports in other systems, suggest that these anti-viral responses are hormonally mediated.

# CFS-ATLANTIC EXOTIC BEETLES AND ASSOCIATED FUNGI PROJECT 2002-2005

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## Abstract

The arrival of “biological pollution” in the form of invasive insects like the brown spruce longhorn beetle in Halifax, Asian longhorn beetle in New York, Chicago and recently in Toronto, and emerald ash borer in Detroit and neighbouring Windsor, Ontario are the most recent examples of threats to the forested ecosystems of Canada. With Atlantic Canada’s 500-year-history of European settlement, and with the continuous movement of people, their goods and trade cargo into and through the region since that time, we have experienced many damaging forest pest introductions (e.g. Dutch elm disease, beech bark disease, etc).

Across North America exotic beetles and their ability to introduce damaging fungal associates (e.g. bluestains), have recently been of great interest. Surveillance for beetle species of quarantine significance has been led by the Canadian Food Inspection Agency principally in and around major Canadian ports. This CFS-Atlantic team is directing its research activities in a range of

forested locations, ensuring there is no duplication of effort.

The project goals include:

- determine the non-native versus native species composition and numerical relativity of Scolytidae and Cerambycidae;
- determine the presence of selected Scolytidae, Cerambycidae and Buprestidae of current North American regulatory concern; and
- establish validated culture and distribution records for ophiostomatoid fungal species (Ophiostoma, Ceratocystis and related genera) and their associated insects.

CFS-Atlantic is sensitive to the current high-risk environment for as yet undetected new introductions. This poster presents an overview of our overall project objective, goals and a general description of the methodology employed.

# INFLUENCE OF LIGHT CONDITION ON THE SPATIAL DISTRIBUTION AN AMBROSIA BEETLE *PLATYPUS QUERCIVORUS* (MURAYAMA) (COLEOPTERA: PLATYPODIDAE) FLYING IN A NATURAL SECONDARY BROAD-LEAFED FOREST

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## Abstract

Field surveys and laboratory experiments were carried out to investigate the influence of light condition on the spatial distribution of flying *Platypus quercivorus* adults around gaps. In the laboratory experiments, both male and female adults showed a positive phototaxis. In the field, many adults were captured under forest canopies near forest edges. The positive phototaxis of newly emerged beetles appeared to be one of the causes of this phenomenon. However small numbers were captured inside gaps, although newly emerged adults of *P. quercivorus* are positively phototactic. Peaks of trap captures appeared in places where relative light intensity was ca. 0.2. The distribution of *P. quercivorus* at the stand level was influenced by light conditions. The behavioral response of adults to light may therefore explain their tendency to invade trees around road and gaps. We conclude that adult *P. quercivorus*, in general tends to be associated with forest edges which is a trait shared by several other bark and ambrosia beetles.

# ARE ISLAND FORESTS VULNERABLE TO INVASIVE DEFOLIATORS?

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## Abstract

It has become axiomatic to represent oceanic islands as simplistic, fragile ecosystems, which, because of their evolution in isolation from continental biota, are particularly susceptible to loss of endemic biodiversity. This largely untested premise is based on the equilibrium theory of island biogeography, which fuels the paranoia of New Zealand's border controls. It is patently obvious that simple stable ecosystems do exist (e.g. bogs, boreal forests and oceanic islands) and remain stable if left undisturbed by man. The high rate of endemism and co-evolution of island biota is proof of their long-term stability.

Recent risk assessments of the threat posed by invasive Lymantriids to the New Zealand flora, indicate that despite the accepted paradigms, New Zealand's flora is largely resistant to exotic invertebrate defoliators. As an explanation of this apparent anomaly, the Island Resource Allocation (IRA) hypothesis was developed which states that 'the palatability of plants to invertebrate herbivores is proportional to the geographic range of the plant'.

The basis for the IRA hypothesis is a redefinition of the fundamental ecological principle of the species: area relationship. Species diversity does increase with area, but the relationship is more pronounced at higher trophic levels. Higher trophic levels (natural enemies) are lost prematurely as ecosystem area shrinks. The redefined species: area relationship means that islands, or similarly geographically constrained ecosystems, which support lower biodiversity, have fewer trophic levels, and

consequently have a lesser regulation of herbivores by natural enemies. The IRA hypothesis is an acceptable alternative to the argument that biodiversity maintains ecosystem stability, in that it argues that island ecosystem stability is achieved through bottom-up processes, such as plant defences, rather than top-down predation.

Ecosystem stability is the most rapidly growing theme in ecology as we grapple to understand the consequences of the loss biodiversity on the functioning of ecosystems. The most influential empirical research on biodiversity/ecosystem function relies upon the manipulation of grassland or microbial communities, and the measurement of productivity or nutrient fluxes. These studies have been the subject of persistent criticism because of the anthropogenic interference and the lack of trophic complexity involved. Like so many issues in ecology, the current debate on the causative relationships between the loss of diversity and possible effects on ecosystem stability is becoming polarised. This is at a time when locally, nationally and globally, species and genetic diversity is being lost at an alarming rate and there is an urgent need for criteria to help recognise the consequences of such loss. As well as resolving the problems associated with the manipulation of complex terrestrial ecosystems, a validation of the IRA hypothesis will provide an empirical anchor for biodiversity studies and allow predictors for forest ecosystem management and conservation programmes.

# ALTERNATE IRON SOURCES FOR USE IN GYPSY MOTH ARTIFICIAL DIET

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## Abstract

The consistent and predictable development of laboratory strains of gypsy moth is critical for production of insects for research and applied programs. In the past there have been periods of poor hatch, reduced survival, and slow asynchronous development resulting from lack of available iron in the artificial diet. Production of gypsy moth has been stabilized by using Wesson salt mix without  $\text{FePO}_4$  and adding the required amount of amorphous  $\text{FePO}_4$ , however, since this form of iron is no longer available from any known vendor, this study was designed to find alternatives before the usable stock was exhausted.

All insects used in this study were the New Jersey Standard Strain (NJSS) of gypsy moth. Nine different gypsy moth high wheat germ diets were prepared by incorporating the following iron compounds: no iron,  $\text{FePO}_4$ ,  $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2$ ,  $\text{FeC}_6\text{H}_5\text{O}_7$ ,  $\text{C}_4\text{H}_2\text{FeO}_4$ ,  $\text{Fe}_4(\text{P}_2\text{O}_7)_3$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$ , and  $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_8\text{FeNa}$ . Gypsy moth development and survival were assessed at 10 and 34 days for two successive generations reared at 25° C, 60% RH, and a 16:8 (L:D) h photoperiod. Egg masses produced in the first generation were chilled at 7-8° C for 168-175 days; hatch and embryonation were determined and larvae were fed the same diet as the first generation.

Gypsy moths reared on the no iron (insufficient iron) diet had significantly reduced pupation, lower fecundity, lower percent embryonation of eggs and reduced hatch of embryonated eggs compared to those reared on the other diets. In the first generation, the only differences between gypsy moths reared on alternate iron compounds and the amorphous  $\text{FePO}_4$  were significantly lower fecundity for individuals reared on the  $\text{Fe}_2(\text{SO}_4)_3$  diet and slightly more variation in percent embryonation of eggs produced by individuals reared on diets containing ferrous compounds ( $\text{C}_4\text{H}_2\text{FeO}_4$  and  $\text{Fe}_2(\text{SO}_4)_3$ ). In the progeny generation, gypsy moths reared on the no iron diet developed significantly slower and less synchronously, and had higher larval mortality than those on other diets. In the progeny generation, there were no significant differences in larval survival or development between the amorphous  $\text{FePO}_4$  and alternative iron compounds but larvae on the  $\text{Fe}_2(\text{SO}_4)_3$  diet did develop less synchronously. Based on these results, diet that contains the proper amount of available iron (85-115 mg/liter) is suitable for NJSS development and survival, regardless of the source of the iron. The results for the ferrous compounds were slightly more variable and the  $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_8\text{FeNa}$  diet darkened rapidly, suggesting that  $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2$ ,  $\text{FeC}_6\text{H}_5\text{O}_7$ ,  $\text{Fe}_4(\text{P}_2\text{O}_7)_3$ , and  $\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3$  are the best choices among the compounds tested as replacements for amorphous  $\text{FePO}_4$ .

# MATING PARAMETERS ASSOCIATED WITH FERTILITY IN *ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)

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## Abstract

There is a critical need for information on the mating behavior of *Anoplophora glabripennis* to provide the biological basis for predicting population dynamics, especially as beetle population size declines due to eradication efforts. To estimate the number of females a male can successfully mate in its lifetime and determine how male age affects mating success, one male from each of three laboratory strains (Illinois, New York, and China) was caged with 1-5 unmated females every weekday for its first 6 weeks of life, then with two or three females per week for the remainder of its life. To determine the frequency and duration of matings associated with sustained female fertility, 10 pairs from each strain were mated in each of four mating schemes: a single mating encounter, one mating encounter per week for 5 weeks, two mating encounters per week for 5 weeks, and unlimited mating encounters (paired for life). All mating encounters were terminated after one or two copulation events of 3 minutes or longer. A mating was considered successful if the female produced eggs that hatched.

Male beetles between 11 and 104 days of age were able to mate successfully. Males generally had one or more unsuccessful matings prior to the first success. Male fertility and mating success peaked for all three males

between 3 and 5 weeks of age, then slowly declined until stopping at about week 15. When the males reached 12 weeks of age, they were less agile and less able to grasp and hold a female, which in nature would probably result in a female's escape without mating. The shortest interval between two successful matings was 5 minutes (when a second female was presented immediately after another mating). The shortest intervals between matings occurred when males were 18 - 26 days old. The most females successfully mated by one male was 27, resulting in a total of 1,366 progeny.

On average, females mated 10 times or more had a significantly higher percentage of viable eggs than did females mated one or five times, but fecundity was unaffected. The number of mating encounters did not have a significant effect on female longevity. These results suggest that greater than 1 hour of total time *in copula* (excluding mate guarding time) is needed for maximum sustained fertility, as measured by percentage hatch. In nature, this copulation time requirement could be satisfied by one or more mating events of longer duration (multiple copulations), rather than through a series of short encounters. Shorter total time *in copula* decreases the likelihood that sufficient sperm will be transferred for maximum female fertility over her entire life.

# POTENTIAL DISTRIBUTION OF THE EMERALD ASH BORER (*AGRILUS PLANIPENNIS*)

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## Abstract

The emerald ash borer (EAB; *Agrilus planipennis*), native to temperate forests of eastern Asia, has become established in the states of Michigan and Ohio, and the Canadian province of Ontario. In North America, this beetle attacks and kills ash trees (*Fraxinus* spp.); three species (white [*F. Americana*], black [*F. nigra*], and green [*F. pennsylvanica*]) are known to be vulnerable to EAB. Lacking an evolutionary history with this pest, many North American ash species may lack defenses against this herbivore and be particularly susceptible to attack. To determine the extent of EAB potential geographic distribution, we used the Genetic Algorithm for Rule-set Prediction (GARP) to model the ecological niche of this species in its native range, and then projected this model onto North America. Our preliminary analyses indicate EAB potential distribution overlaps > 50% of the geographic range of nine North American ash species. Spread of EAB in North America poses serious socioeconomic and biodiversity consequences such as the loss of urban shade trees and potential elimination of *Fraxinus* spp. across much of their geographic range.

# USE OF AN ARTIFICIAL INOCULATION TECHNIQUE TO IDENTIFY AMERICAN BEECH TREES WITH RESISTANCE TO THE BEECH SCALE INSECT

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## Abstract

Beech bark disease begins when bark tissues attacked by the scale insect (*Cryptococcus fagisuga* Lind.) are rendered susceptible to infection by fungi of the genus *Nectria*, leading to the weakening and eventual death of the tree. A small percentage of American beech (*Fagus grandifolia*) trees remain disease free in stands long-affected by beech bark disease and challenge trials have shown that they are resistant to the scale insect (Houston 1982, USDA Forest Serv. Res. Pap. NE-507, 8 p.). Increasing the number of resistant beech trees while reducing the proportion of susceptible trees is currently thought to be the best management approach to minimize the overall impact of beech bark disease [Mielke ME; Houston DR; Bullard AT (1986) In: Proceedings, Integrated Pest Management Symposium for Northern Forests: 272-280]. Previous work by David Houston (1982), reported an artificial infestation technique that was successfully used to infest 1-year-old seedlings and to confirm the resistance of older, scale-free trees. We have initiated experiments to determine if this technique will be an effective tool in distinguishing resistant from susceptible individuals, particularly at the seedling or sapling stage. To directly compare resistant and susceptible individuals we are using two different tree sources: root sprouts and seedlings, both from open-pollinated seeds (half-sibs) and from controlled cross-pollinations (full-sibs).

Field trials have been initiated at both the Allegheny National Forest (ANF) in Pennsylvania and Ludington State Park (LSP) in Michigan. Insect eggs were collected from traps placed in 2002 and used to challenge a cluster of 12 putatively resistant trees in the ANF and a cluster of 20 in LSP. At both sites, clusters of susceptible trees were included as controls. Although the individuals within the clusters appear to be from root sprout origin, DNA analysis will be used to confirm the clonal identity of all individuals included in these studies. An artificial challenge experiment using a total of 438 six-month old seedlings, both full- and half-sibs was also initiated. Open-pollinated seedlings from both resistant and susceptible trees were included as well as 35 seedlings resulting from the cross-pollination of a resistant and susceptible tree and 40 seedlings that were the result of a controlled cross between two resistant trees.

Results from these studies will allow guidelines to be established for the use of the artificial infestation technique as a management tool and as a method to “screen” progeny for resistance, a prerequisite for genetic analysis and the development of a breeding program.

# ANALYSIS OF JAPANESE OAK WILT SPREAD USING AERIAL PHOTOGRAPHY AND GIS

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## Abstract

Japanese oak wilt (JOW) is an apparently exotic disease complex that has caused increasing levels of mass tree mortality in Japanese forests. The disease complex consists of the ambrosia beetle, *Platypus quercivorus* (Murayama) and the Ascomycete fungus *Raffaelea quercivorus* that coexist in a symbiotic relationship. The ambrosia beetle carries the fungus to uninfected host oak trees where it colonizes sapwood, causing necrosis, which restricts water conductance and ultimately leads to the death of host oak trees. We analyzed historical spread of JOW both at the regional scale and at the stand level. There were considerable differences in the patterns of spread observed at the regional vs. stand level. At the regional level, spread was more uniform and clearly separated four distinct foci. In contrast, spread at the stand level was much less uniform. It is also interesting that the rate of spread observed at the local scale (151 - 238 m / year) was substantially less than rates of spread observed at the regional scale (1805 - 5599 m / year). This difference is most likely a result of the fact that at the local scale, spread rates reflect both the gradual expansion around foci and to a limited extent (limited by the spatial extent of the study area) the formation of new colonies.

# THE EFFECT OF PLACEMENT HEIGHT, COLOR AND RELEASE RATE ON TRAP CATCHES OF THE ASIAN LONGHORN BEETLE, *ANOPLOPHORA GLABRIPENNIS*

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## Abstract

The effects of trap height, color and semiochemical release rates on trap catches of the Asian longhorn beetle, *Anoplophora glabripennis* in China were evaluated. In 2001, black Intercept Panel Traps™ were baited with a synthetic chemical combination (1-butanol, 1-pentanol and 2-pentanol) and hung at varying heights (bottom cup at ground level, 1 m, 2 m and at tree top) in a stand of *Populus opera*. Results of this experiment indicated no significant differences in trap catches among placement heights. In 2002, traps of various colors (black, green, gray, white, yellow and brown) were hung at breast height and baited with a combination of 1-butanol, 1-pentanol, 2-pentanol and cis-3-hexen-1-ol. Catches were significantly higher for black traps than any other color trap, with green ranking second. Also in 2002, experiments to verify attractiveness of host volatiles at different release rates were undertaken. A chemical combination of 1-butanol, 1-pentanol, cis-3-hexen-1-ol and 2-pentanol was tested at varying release rates (3 mg/day, 30 mg/day, 300 mg/day), with height and color held constant. There were no differences in the number of beetles caught among the different release rates. Further research is necessary in the development of a semiochemical based sampling technique to help with the eradication of this important pest insect.

# BIOLOGY AND PHENOLOGY OF THE EMERALD ASH BORER, *AGRILUS PLANIPENNIS*

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The seasonality of events in the life history of the emerald ash borer was investigated under field and controlled-laboratory conditions. The emergence period of adults was determined, under field conditions, by rearing adults from log bolts in cages in an outdoor insectary. Adults were collected weekly from the cages and their sex was determined. Temperature conditions were monitored onsite using data-logging equipment. The first adults emerged during the week of 4-11 June, although a few adults were observed flying on 28 May. Mean emergence dates for males and females were 25 and 26 June, respectively. The last adults emerged during the period 24-30 July. The sex ratio approximated 1:1. To determine adult activity periods, Tangle Trap-coated plastic bands were placed on boles of host trees about 2 m above the ground. A total of 130 trees in three plots, with different levels of damage, were banded. Bands were examined for adults at weekly intervals throughout the summer. These bands captured about 10,000 beetles. Mean activity periods in the three plots were 3, 10 and 13 July. Adults were active into the second week of August. Larval and pupal development

was tracked by dissecting host logs throughout the growing season.

In the laboratory, adult longevity, oviposition, fecundity and egg development were investigated under controlled conditions. Mean longevity for unmated males and females was 29.8 and 26.5 days at 24° C. Although fecundity was extremely variable, one female deposited 275 eggs over her lifetime. The mean number of days to first-observed mating for females was 23.3 days (range 12-53 days; n = 16) and the mean time to first oviposition was 23.9 days (range 13-56 days; n = 22) at 24° C. There was a strong positive correlation between first oviposition and first observed mating. Mean longevity of mated females was 56.4 days at 24° C, which was much longer than the period for unmated females. Females were observed mating with multiple partners. Eggs took an average of 19.4 days to hatch at 24° C and 38.5 days to hatch at 18° C. A preliminary estimate for a developmental threshold for egg development was 12.3° C. The goal of these investigations is to develop models for predicting the phenology of this insect.

# ASIAN LONGHORNED BEETLE COOPERATIVE ERADICATION PROGRAM

## PROGRAM STATUS REPORT FY 2003

Christine K. Markham

USDA APHIS, PPQ, ALB National Program Director, 20 Main Campus Dr., Ste. 200, Raleigh, NC 27606

### Significant Events

Shortfalls in funding had a severe negative impact on the program in 2003. Total appropriated dollars allocated to the eradication program, national survey, public outreach, and research was \$26 million. The allocation of emergency funds in the amount of \$7 million came very late in FY 2003, too late to support program activities. Funding for the 2003 program was about \$20 million less than the amount received in FY 2002. As a result, drastic cuts were made to operational activities.

### Program Cutbacks

- Tree climbing and bucket truck survey contracts were terminated in NY in May 2003. Since May, surveys continue at a much slower rate through ground inspection using federal and state program personnel.
- No restoration activities were funded in New York or Illinois.
- Chemical treatments in NY were severely cut from the planned activities of 143,000 trees. Only 17,570 trees were treated.
- The development of the emergency program database was stopped.
- Research activities were drastically reduced.

### New Jersey Accomplishments

- Full funding for program activities.
- All host trees located within the quarter-mile radius of the Jersey City, NJ infestation were removed and destroyed.
  - \* As a result of this aggressive control measure, 11 more very lightly infested trees were detected after take down which were not detected when these trees were surveyed previously with climbers.
  - \* Total trees removed in NJ were 461 (113 infested and 348 high risk exposed trees)
- Chemical treatments were applied to all non-infested host trees per protocol levels

\* All host trees located within half-mile radius of the infestation in Jersey City and Hoboken NJ were treated. Trees treated = 1,060.

- 100% of all surveys were completed to protocol levels. Bucket trucks and tree climbers were used exclusively for the delimiting and detection surveys.
- No infested trees found in 2003.
- Regulated area remained static at 3.7 square miles.
- 100% restoration was achieved with 395 trees replanted.
- Four companies are under compliance agreement. Seven site visits were made. No warnings or violations were issued.

### Illinois Accomplishments

- Full funding for program activities.
- The treatment area was expanded to half-mile radius around the Chicago infestation and the Park Ridge infestation. The remaining satellite infestations (Addison, Summit, and O'Hare) were treated to quarter-mile radius. More aggressive measures were taken in Chicago because of the size and age of that infestation and in Park Ridge because of the difficulty with inspecting a forested area. Total trees treated = 92,045 compared to 51,307 in 2002.
- 100% of all surveys were completed. Bucket trucks and tree climbers are used exclusively for the delimiting and detection surveys.
- In November 2003, three infested trees were found about half-mile south of the Chicago quarantine boundary in the Oz Park area of Chicago.
  - \* These trees were detected as a result of a live adult beetle which was discovered by a member of the public in October 2003. The infested trees were about two blocks away from where the beetle was found.

- \* This small pocket of infested trees was located 1.9 miles southeast of the closest known infested trees which were detected in April 2002.
- Three trees with signs of old infestation were detected within the infested core of Chicago Ravenswood Quarantine.
- Proposed deregulation of the satellite infestations of Addison and Summit.
  - \* Two years of negative survey were completed in 2003. As per ALB eradication protocol, an infested area may be considered for deregulation after 2 years of negative survey and may be declared eradicated after 4 years of negative survey.
- Proposed changes to regulated area:
  - \* 6.7 square mile expansion to the Chicago quarantine as a result of the infested trees found near Oz Park.
  - \* 1.74 square mile reduction as a result of the deregulation of Addison and Summit
    - o New regulated area = 34.49 square miles — (25.09 square miles in Chicago, 0.35 square miles in Park Ridge, 9.05 square miles in O’Hare).
    - o Previous regulated area = 29.52 square miles — (18.39 square miles in Chicago, 0.81 square miles in Addison, 0.92 square miles in Summit, 0.35 square miles in Park Ridge, and 9.05 square miles in O’Hare).
- There are 229 companies are under compliance agreement; 1,125 compliance visits were completed; 15 caution letters and two violations were issued.
- 29% of the Level 1/Level 2 surveys were completed; 190 infested trees were detected - (56 in Brooklyn, 126 in Queens and 8 in Central Long Island). No infested trees were detected in Manhattan or in Islip this year.
  - \* An additional 20 high risk trees were removed for control purposes.
  - \* The largest infestation was found in Mt. Olivet Cemetery in Queens with 81 infested trees detected and removed.
- 99% of the Level 4 survey was completed.
- 12 square miles was added into regulation due to the detection of infested trees just outside the Level 1 survey boundary along southern Brooklyn.
  - \* New regulated area = 132 square miles (16 square miles in Manhattan, 54 square miles in Brooklyn/Western Queens, 32 square miles in Eastern Queens, 23 square miles in Central Long Island, and 7 square miles in Islip, Long Island).
  - \* Previous regulated area = 120 square miles (16 square miles in Manhattan, 42 square miles in Brooklyn/Western Queens, 32 square miles in Eastern Queens, 23 square miles in Central Long Island, and 7 square miles in Islip Long Island).
- There are 1,240 companies under compliance agreement; 2,072 compliance visits were completed; 124 caution letters and two violations were issued.
- Public awareness activities:
  - \* Forest Service hired an employee for full-time support to the public awareness efforts in NY. NY Department of Environmental Conservation also hired a full-time employee to support restoration and also public awareness efforts.
  - \* As a result of the program’s summer advertising campaign “ALB Beetle Alert”, two adult beetles were reported to the program within the infested areas of Queens and Brooklyn and the source trees were identified and removed.

## **New York Accomplishments**

- Chemical Treatments were applied ONLY to the infested areas of Manhattan and Islip, Long Island for a total of 17,570 trees; 134,744 trees were treated in 2002 in all the infested areas (Manhattan, Brooklyn, Queens, Central Long Island, and Islip).
- All climbing contracts for survey were terminated in May 2003. Surveys are continuing using program personnel by ground survey.

	Number of Infested Trees Detected								Total
	1996	1997	1998	1999	2000	2001	2002	2003	
New York	812	841	978	1798	715	547	239	190	6120
Illinois	0	0	521	728	209	69	18	6	1551
New Jersey	0	0	0	0	0	0	113	0	113
Total	812	841	1499	2526	924	616	370	196	7784

\* The Department of Transportation reported a live beetle from an area they were tree trimming in Brooklyn.

\* One of the infestations found in central Long Island was the result of a homeowner reporting a possible infested tree on her property.

- Research Support

\* A full-time employee was hired by Otis Plant Methods Center for on ground support to the NY project.

### Program Wide Accomplishments

- Enhanced trunk injection technology

\* Otis Plant Methods Center continued with the evaluation of several enhanced trunk injection methods which offer pressurized injection of the chemical into the tree. This methodology will eliminate the need to safeguard the trees for 4 hours during treatment.

- Contract for the design and development of an ALB and emergency program database

\* Due to the reduced funding, the development of this database was stopped.

\* The development of the database, now named Emergency Response Information System (ERIS) resumed in 2004 with funding support from other emergency programs.

- The label for Mauget Imicide Hp is pending approval by USEPA for tree injection use in liquid loadable injectors. In addition, this label increases the dosage rate for larger trees treated under the USDA ALB eradication program.

- Otis Plant Methods Center perfected their techniques in aging infested trees.

- The ELISA technique for residue analysis of chemically treated trees was implemented into

the program this year. This technique allows for immediate and more sensitive readings of chemical concentrations. This process was also an accomplishment of the Otis Plant Methods Center.

- A pathway analysis of the risk of spread and establishment of ALB in municipal solid waste from the quarantine area of New York City to landfills outside of the quarantine area was completed by APHIS CPHST. The study shows that the collection and disposal practices of in place for NYC do not pose a large risk of ALB spread to landfills sites.
- All of the administrative hurdles have been overcome and the program will be hiring tree-climbers starting in early 2004.

### Infested Trees Detected

All surveys were completed in IL, and only three infested trees with active signs of infestation were detected. These trees were found about one-half mile outside of the delimiting area of the Chicago Ravenswood infestation. The detection was due to a member of the public finding a live adult beetle clinging to a light pole about two blocks away. Intensive surveys kicked in with climbers and bucket trucks. Aging of the infestation indicates that this population began no later than 2001.

Three additional trees with old signs of infestation were found in IL as a result of program surveys. All three of the trees were located within the core center of the Ravenswood infestation. Two of the trees had treatments applied in 2001 and 2002. The third tree was an Amur maple. This tree escaped treatment in error. The last viable infestation in this tree was aged at year 2000.

In NY, 29% of the detection and delimiting surveys were completed—45% in Manhattan, 18% in Brooklyn,

30% in Queens, 36% in Central Long Island, and 14% in Islip, Long Island. Total infested trees found: 56 in Brooklyn, 126 in Queens, and eight in central Long Island. Eighty-one of the infested trees in Queens were located at one site - in Mt. Olivet Cemetery, Maspeth, NY. Infested trees were not found in Manhattan or in Islip, Long Island.

In NJ, 100% of all surveys were completed and no infested trees were found.

### Chemical Treatments

This was the fourth year of chemical treatments in IL. To summarize the history of treatments: In 2000, all host trees within 1/8 of a mile radius of the infested core received treatment via trunk injection in the satellite infested areas of Summit, Addison, Park Ridge, and O'Hare. Also in 2000, treatments were applied within the outermost boundaries of the Chicago Ravenswood infestation. In 2001, trunk injection treatments were applied to all host trees within 1/8 mile radius of all the infested areas in Illinois. In 2002, chemical treatments were applied to all host trees within a ¼ mile radius of the infested core through out all the infested areas in Illinois. Soil injection was implemented operationally. There were over 51,000 trees treated in 2002 with over 31,000 receiving treatment via soil injection. In 2003, treatments were extended to ½ mile radius in Chicago and in Park Ridge. More aggressive measures were taken in Chicago because of the size and age of that infestation and in Park Ridge because of the difficulty with inspecting a forested area. A total of 92,045 trees were treated in 2003—48,400 by soil injection and 43,645 by trunk injection.

In NY, 2003 was the third year for treatment. In 2001, treatments were applied to all host trees within a one-eighth mile radius of the infested core in Manhattan, Bayside, Flushing, and Flushing Meadows Corona Park in New York City and in Islip, LI. In the infested area of Brooklyn and Western Queens, all the host trees located within a one-half mile wide band bordering the East River received treatment. Also in 2000, the outermost boundaries of the Central Long Island infestation were treated. In 2002, treatments were applied through out all

	Trees Chemically Treated			
	2000	2001	2002	2003
New York	0	23,740	134,744	17,570
Illinois	11,400	35,490	51,307	92,045
New Jersey	0	0	0	1,060

the infested areas to all host trees within a quarter mile radius of the infested cores. Over 134,000 trees were treated using trunk injection. In 2003, treatments were drastically reduced. Treatments were concentrated in the out lying areas of the infestation (Manhattan, NYC and Islip, Long Island) to half-mile mile radius. A total of 17,570 trees were treated—10,481 in Manhattan and 7,089 in Islip. All treatments were applied by trunk injection.

In NJ, control measures were applied to all host trees within half-mile radius of the infestation. All host trees located within a quarter-mile radius were removed and destroyed. Chemical treatments were applied to the host trees extending out to the half-mile radius. A total of 1,060 trees were treated—982 via soil injection and 78 through trunk injection.

### Regulated Area

The New York regulated area increased by 12 square miles with the expansion of the southern boundary in Brooklyn.

The Illinois regulated area is proposed for expansion by 5 square miles. Two events contributed to this pending change. 1) The Chicago regulated area extended along the southern boundary by 6.7 square miles with the detection of the infested trees near Oz Park; and 2) Two years of negative survey were achieved in the satellite infestations of Addison and Summit. The regulated area for the two infestations totaled 1.74 square miles.

	Square Miles of Regulated Area	
	2002	2003
New York	120.0	132.0
Illinois	29.5	34.5
New Jersey	3.7	3.7

# COMPARISON OF THE PALATABILITY OF GYPSY MOTH ACROSS SEVERAL WOODY SPECIES NATIVE TO NORTHERN JAPAN

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## Abstract

### Introduction

From 2000 to 2003, there was the outbreak of gypsy moth (*Lymantria dispar*) at several parts of Hokkaido, northern part of Japan. Gypsy moth is the representative herbivore feeding deciduous trees including several birch species. Although the outbreak of gypsy moth occurs cyclically (about once per 10 years) at extensive region in Japan, death of trees because of the outbreak was hardly reported.

In the forest, we could find specific variation in the intensity of feeding by gypsy moth. To clarify the specific difference of palatability, bioassay test, which can exclude several environmental factors, is efficient. In addition, chemical analysis of leaves is needed to investigate the factor affecting the palatability.

### Materials and Methods

Larvae of gypsy moth were grown from 1<sup>st</sup> instar to pupa with leaves of eight woody seedlings (*Alnus hirsuta*, *Betula maximowicziana*, *Betula platyphylla* var. *japonica*, *Betula ermanii*, *Ostrya japonica*, *Carpinus cordata*, *Acer mono* and *Quercus mongolica* var. *grosseserrata*). First bioassay experiment was done with larvae hatched on 13 May and second bioassay experiment was done with larvae hatched on 20 May. Total phenolics (Folin-Ciocalteu method),

condensed tannin (Proanthocyanidin method), toughness (Push-pull gage), water content, nitrogen and carbon content (NC analyzer) of each leaves harvested on 13, 29 May and 22 June were measured.

### Results and Discussion

Although survival rates were relatively high both in first and second bioassay, survival rate and growth of larvae growing with *O. japonica* and *B. maximowicziana* were regulated in second than in first bioassay. We could find significant difference in pupa masses among species. Regardless of the same genus (*Betula*), pupa masses of larvae growing with *B. ermanii* were largest and those with *B. maximowicziana* were smallest of all. However, we could not find any correlation between pupa mass and each parameter of leaves, phenolic compounds, toughness, nitrogen and carbon content. Only water content was positively correlated to masses of female pupa in first bioassay. It was reported that leaves containing high phenolic compounds were not palatable. However, growth of larvae in *B. ermanii* was largest, regardless of that *B. ermanii* had highest total phenolic compounds of all. These results suggested that it is difficult to compare the strength of defense against Gypsy moth by the amount of phenolics among species.

# EMERALD ASH BORER CANADIAN PROGRAM UPDATE

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## Abstract

The emerald ash borer (*Agrilus planipennis* Fairmaire) is an invasive alien pest native to eastern Asia and was first confirmed to be present in Canada in August of 2002. Based on a pest risk assessment completed by the Canadian Food Inspection Agency in August 2002, the emerald ash borer was identified as a pest which could have a major impact on ash trees in both urban and forested settings throughout their range in Canada and the U.S.

Ash trees are very valuable both from an economic and an environmental perspective. They are commonly planted in urban settings, reforestation projects, shelter belts and are used in the manufacture of furniture and sporting goods, such as baseball bats and hockey sticks. They are a major component of southern Ontario forests.

The emerald ash borer is a primary tree killer, i.e. it attacks and kills healthy trees. Trees are killed when the larval stage of the beetle girdles the vascular area of the tree and cuts off the flow of nutrients. Most ash trees that are infested with the emerald ash borer will die within a year.

On September 17, 2002, a Ministerial Order was enacted to prevent the spread of the emerald ash borer from the Windsor, Ontario area. The order prohibits or restricts the movement of nursery stock, trees, logs, lumber and wood with bark attached, wood chips or bark chips in the genus *Fraxinus* (commonly known as Ash) and on firewood of all tree species. Surveys conducted by the Canadian Food Inspection Agency in 2003 indicated the beetle is now distributed throughout Essex County and it would be necessary to expand the area under quarantine. The Emerald Ash Borer Infested Places Order has been amended in November 2003 to include all of Essex County to ensure that newly discovered infested areas are regulated.

The Canadian Food Inspection Agency has the legal authority, under the Plant Protection Act, to take control

actions to manage invasive quarantine pests, including the emerald ash borer, that are threats to Canada's agricultural and forestry sectors. In the fall of 2002, the Agency brought together a Science and Risk Mitigation Committee to evaluate the known science, identify research priorities and to develop strategies and policies to contain the emerald ash borer. The Committee consists of scientists and regulatory experts from Natural Resources Canada - Canadian Forest Service, Ontario Ministry of Natural Resources, Ontario Ministry of Agriculture and Food, the United States Department of Agriculture and the Canadian Food Inspection Agency.

Both delimitation and detection surveys were conducted by the Canadian Food Inspection Agency throughout 2003 to determine the extent and severity of the emerald ash borer infestation in Ontario. To date, the pest has been detected in Essex County and in three isolated locations in the western end of the Municipality of Chatham-Kent. An estimated 200,000 ash trees in Essex County are believed to be infested and likely to die over the next year. The emerald ash borer has already killed over 7 millions ash trees in Michigan and has spread to Ohio, Maryland and Virginia.

The strategy for 2002-03 called for intensive surveys and strategic tree cutting of infested ash trees along the leading edge of the infestation. To date, the Canadian Food Inspection Agency has authorized removal of approximately 10,000 trees at the Agency's expense. Much of the infested ash material has either been reduced to chips or burned. Targeted surveys are conducted at other high-risk areas in Ontario to which the emerald ash borer may have been artificially introduced such as parks, campgrounds and sawmills.

The Canadian Food Inspection Agency has accepted, in November of 2003, the recommendations of the Science and Risk Mitigation Committee that an ash-free zone would be effective in preventing or slowing the spread

of the emerald ash borer to those areas east of Essex County that are more heavily forested. The ash-free zone is located along the leading edge of the infestation on the west end of the Municipality of Chatham-Kent. The area selected for the zone is 272.34 square kilometres of which only 2.16 square kilometres or 0.8% is forested. A recent census completed by the agency indicates that 64,000 trees (estimated at 25% of the total tree population) are ash. This location was chosen for the zone because it is on the leading edge of the infestation and is relatively treeless. Only ash trees will be removed prior to the emergence of emerald ash borer adults next spring, and the zone would have to be maintained to prevent any regrowth of ash trees over the next few years or until such time as the beetle population is controlled. In addition to the ash-free zone, an active suppression zone on its western perimeter and an intensive survey zone on its eastern perimeter, to detect any outlier populations that may have been established already through natural spread or through the movement of infested firewood or nursery stock in past years, have been established and will greatly enhance the effectiveness of the zone.

The Canadian Food Inspection Agency is working with the scientific community and regulatory experts in both Canada and the U.S. to obtain the most current information on the biology, behaviour and potential

control of the emerald ash borer. At present, a Research Committee, has prioritized a list of potential research projects that would be deemed essential in support of the emerald ash borer management plan. The only proven method to combat this pest is to remove and destroy host trees infested, or suspected to be infested, by the emerald ash borer. Currently, there are no chemical or other controls available that are totally effective in controlling the emerald ash borer. Processes such as burning, chipping and grinding, which guarantee total control of the pest, are the only means acceptable to the agency and its regulatory counterparts in other countries.

Since the discovery of the emerald ash borer in Canada, the agency has implemented a public awareness campaign in Essex County to gain public support and to emphasize the dangers of moving wood outside of the quarantine area. These efforts have included the development of several posters and information brochures for distribution to the public, presentations to stakeholders and mailouts of information packages to local residents and all Ontario municipalities. The agency web site is updated regularly to provide current information on the emerald ash borer. In addition, signs have been placed on all roads leading into and out of the quarantine zone including Highway 401 and at all Canada Customs entry points from Sarnia to Pelee Island.

# ETHANOL AND $\alpha$ -PINENE—NATIONWIDE SURVEY OF RESPONSES OF BARK AND WOOD BORING BEETLES

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## Abstract

Validation of ethanol and (-)- $\alpha$ -pinene in traps for monitoring native bark and wood boring beetles at overseas ports-of-entry, silvicultural treatments in U.S., and further assessment of the occurrence and abundance of exotic species of bark and wood boring beetles in the United States. At each site, 32 traps are grouped into eight replicates of four treatments per replicate with traps spaced 10-15 m within a replicate and replicates spaced 10-100 m apart. We select sites with recent history of thinning or prescribed burns in the past 6-9 months. The following treatments are randomly assigned to one of four traps within each replicate: (1) blank; (2) ethanol alone; (3) (-)- $\alpha$ -pinene alone; and (4) ethanol with (-)- $\alpha$ -pinene (each released at about 2-3 grams/day). Collection cups contain an aqueous solution of propylene glycol, formaldehyde and soap. Trap catches are collected at 3-week intervals from early May to mid-July, with the glycol solution each occasion. Catches are placed into individual Whirl-Pak Bags and shipped back to Athens GA for processing. Catches, transformed by log function, are analysed by 2-way full-factorial ANOVA for each test separately. To date, tests have been conducted in six National Forests. Contingent upon new funding, tests are planned for sites in 30 National or State Forests throughout the United States. In initial tests in Florida and Georgia, we found that traps baited with ethanol and/or (-)- $\alpha$ -pinene caught significant numbers of Cerambycidae, Buprestidae, Scolytidae, Platypodidae, Curculionidae, Elateridae, Cleridae and Trogositidae. Some species demonstrate some specificity in attractants but no evidence of repellency or interruption.

# SCYMNUS NINGSHANENSIS YU ET YAO (COLEOPTERA: COCCINELLIDAE) FOR BIOLOGICAL CONTROL OF ADELGES TSUGAE (HOMOPTERA: ADELGIDAE)

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## Abstract

*Scymnus ningshanensis* is one of three Chinese lady beetles in the subgenus *Neopullus* imported into the USDA Forest Service Quarantine Laboratory, Ansonia, CT, as a biological control agent for the hemlock woolly adelgid (HWA), *Adelges tsugae*. These lady beetles are found separately in three provinces along the eastern edge of the Tibetan Plateau (Yunnan, Sichuan, and Shaanxi). *Scymnus ningshanensis* was collected near Ningshan in Shaanxi Province from *Tsuga chinensis*.

In China, *S. ningshanensis* adults became active in early April when the 10-day average temperature reached 7° C. Eggs are laid from April to June when HWA progrediens generation eggs are present. *Scymnus ningshanensis* was found only on hemlock; it was not on nearby *Pinus armandii* that was infested with another adelgid species.

In the laboratory, oviposition by *S. ningshanensis* began within a week after removal from cold storage (5° C). Oviposition in the laboratory lasted for 5 weeks, declined as the abundance of HWA eggs declined on foliage brought from the field, but reinitiated and continued for another 8 weeks when beetles were provided abundant HWA eggs on foliage held in cold storage. Oviposition occurred singly in concealed locations such as bud scales

and under the edge of ovisacs and averaged 1.6 eggs per day. Larvae consumed an average of 23 ovisacs, which averaged 35 eggs/sac. In feeding preference tests, *S. ningshanensis* adults preferred HWA to aphids but there is no preference between HWA and other adelgid species. Response to odor from HWA infested foliage was not observed in olfactometer tests; visual and tactile cues seemed more important.

A positive numerical response to prey density is an attribute of effective biological controls. We compared the oviposition of *S. ningshanensis* with that of another lady beetle, *Pseudoscymnus tsugae*, at different prey densities in the laboratory. *S. ningshanensis* responded to increasing prey densities by laying more eggs, but *P. tsugae* did not. We compared the impact of these two lady beetles on the net per-capita increase of HWA from one generation to the next. In early spring, the adult HWA (sistens generation) on a hemlock branch were counted. The branches were enclosed in bags containing either a male and female of *S. ningshanensis* or *P. tsugae*, or no beetle (control). The adelgids of the next generation (progrediens) were counted 10 weeks later. The population of adelgids in the bags containing *S. ningshanensis* lady beetles decreased, whereas adelgids increased in the bags with no beetles or *P. tsugae*.

# EVIDENCE FOR HOST TREE RESISTANCE AGAINST THE ASIAN LONGHORNED BEETLE

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## Abstract

We have been screening hardwood tree species for susceptibility to / resistance against the Asian longhorned beetle [*Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae)] and have discovered evidence for three different forms of resistance in three different tree species.

**1) Antixenosis:** When offered a choice of gray birch (*Betula populifolia*), cockspur hawthorn (*Crataegus crus-galli*), littleleaf linden (*Tilia cordata*), and honeylocust (*Gleditsia triacanthos*) trees, honeylocust was avoided. Beetles were rarely seen, almost no feeding damage was found, and no sign of oviposition was found on honeylocust. We interpret these results as indicating possible antixenosis (lack of cues required for recognition as a potential host) in honeylocust for the Asian longhorned beetle. We plan to further investigate this possibility with a no-choice test for adults and possibly also with artificial insertion of larvae into honeylocust trees.

**2) Physical antibiosis:** When offered a choice of golden-rain tree (*Koelreuteria paniculata*), river birch (*Betula nigra*), London planetree (*Platanus x acerifolia*), and callery pear (*Pyrus calleryana*), adults showed a strong preference for golden-rain tree for feeding and oviposition; however, larvae showed slow growth and many larvae were found drowned in sap. Given a choice

between sugar maple (*Acer saccharum*) and golden-rain trees, adults were seen more frequently on sugar maple, but fed on the two different tree species with equal frequency and actually laid more eggs into golden-rain tree than into sugar maple. We plan to determine whether the Asian longhorned beetle can complete development in golden-rain tree. If not, this tree species might be used as a “trap tree” to attract adults and prevent their successful reproduction.

**3) Chemical antibiosis:** During the choice test outlined in 2 above, there was unexpectedly high mortality of adults, and a subsequent twig-feeding experiment pointed to callery pear as the most likely cause. Further, viable eggs were laid into callery pear trees but the resulting larvae established only very small galleries and none survived beyond their first stadium. Both longevity and fecundity were reduced in beetles placed on callery pear trees after maturation feeding on sugar maple twigs, compared to beetles placed on sugar maple trees, while beetles that had callery pear as their only source of food showed a further reduction in longevity and failed to produce any eggs. Larval insertion and artificial diet experiments confirmed resistance against larval establishment and also indicated a constitutive phytochemical basis for this resistance. We are currently planning chemical extractions and further bioassays to isolate and identify the active phytochemical(s) in callery pear.

# EVALUATION OF POTENTIAL HOST TREES FOR *ANOPLOPHORA GLABRIPENNIS* (COLEOPTERA: CERAMBYCIDAE)

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## Abstract

We have been evaluating oviposition by *Anoplophora glabripennis* (Motschulsky) offered a selection hardwood tree species, four species at a time, and performance of the resulting larvae, using living trees under greenhouse conditions.

Significantly greater numbers of oviposition sites were found on sugar maple (*Acer saccharum* Marshall) than on red maple (*Acer rubrum* L.), green ash (*Fraxinus pennsylvanica* Marshall), or red oak (*Quercus rubra* L.), with no significant differences among the latter three tree species. Similarly, significantly greater numbers of living larvae were found in sugar maple than in the other tree species; however, mean mass of living larvae did not differ significantly among the four tree species 90 days after removal of the adult beetles.

Significantly greater numbers of oviposition sites were found on golden-rain tree (*Koelreuteria paniculata* Laxmann) than on London planetree (*Platanus x acerifolia* (Aiton) Willdenow) or on callery pear (*Pyrus calleryana* Decaisne), with river birch (*Betula nigra* L.) intermediate. Similarly, significantly greater numbers of living larvae were found in golden-rain tree and in river birch than in callery pear, with London planetree

intermediate. No larvae were found alive in callery pear and, despite the high rate of oviposition in golden-rain tree, mean mass of living larvae was significantly lower in golden-rain tree than in London planetree, which was in turn significantly lower than in river birch.

Significantly greater numbers of oviposition sites were found on gray birch (*Betula populifolia* Marshall) than on honeylocust (*Gleditsia triacanthos* L.), with cockspur hawthorn (*Crataegus crus-galli* L.) and littleleaf linden (*Tilia cordata* Miller) intermediate. No evidence of oviposition was found on honeylocust and no living larvae were found in either honeylocust or littleleaf linden. Numbers of living larvae did not differ significantly between gray birch and cockspur hawthorn; however, mean mass of living larvae was significantly greater in gray birch than in cockspur hawthorn.

Numbers of oviposition sites and of living larvae did not differ significantly among silver linden (*Tilia tomentosa* Moench), common hackberry (*Celtis occidentalis* L.), Japanese zelkova (*Zelkova serrata* (Thunberg) Makino), and European hornbeam (*Carpinus betulus* L.). Mean mass of living larvae also did not differ significantly among these four tree species and was very low in all cases.

# DEVELOPING ATTRACTANTS AND TRAPPING TECHNIQUES FOR THE EMERALD ASH BORER

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## Abstract

Shortly after the 2002 discovery of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in southeastern Michigan and Windsor, Ontario, quarantines regulating the movement of ash logs, firewood, and nursery stock were established to reduce the risk of human-assisted spread of this exotic forest insect pest. Accurate delimitation of the infested area is critical to establish the quarantine boundaries and implement control measures. Potentially infested trees are identified by survey crews using signs and symptoms such as adult exit holes, bark splits over galleries, epicormic shoots and canopy dieback. However, it is extremely difficult to detect newly infested trees at the leading edge of the infestation because they typically demonstrate no external symptoms. Methods to attract and trap adult beetles would substantially increase our ability to determine the extent of the EAB distribution.

We collected volatiles from ash leaves using solid phase micro-extraction (SPME) and prepared ash extracts by crushing host tissues in hexane. Host compounds were identified by gas chromatography (GC) and mass spectrometry (MS). Antennal responses by adult EABs were determined using coupled gas chromatographic electro-antennal detection (GC-EAD). Compounds that elicited antennal responses were tested in a walking olfactometer bioassay and those with the highest percentage of positive responders were then selected for field-testing.

Three types of field experiments were conducted to compare different trapping techniques for EAB. The first experiment used a single lure comprised of a blend of the most active ash volatiles. The lure was tested in four different types of traps: multiple funnel traps, Intercept panel traps, Japanese beetle traps, and yellow sticky traps. Of the trap types tested, the multiple funnel traps caught significantly more EABs than the panel traps or yellow sticky traps. Japanese beetle trap catches were intermediate. Multiple funnel traps also captured more EABs when raised in the tree canopy as opposed to being placed at ground level. The second field experiment used only multiple funnel traps and compared different combinations of ash volatiles. There were no significant differences in the number of EABs captured with the different types of lures. The third field experiment compared the number of EABs captured on sticky bands on trap trees (healthy, girdled, or herbicide-treated green ash trees) or vertically placed trap logs (2 m-long logs cut from healthy green, white, or black ash trees). The girdled trees captured significantly more EABs than the other types of trap trees or trap logs. We plan to analyze volatiles from girdled trees and from EABs to identify other potential attractants. Compounds will be tested in the laboratory in a vertical wind tunnel and then in the field using several different trap designs.

# EXOTIC SCOLYTIDS IN NORTH AMERICA: WHO'S HERE AND WHAT ARE THE IMPACTS

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## Abstract

There are currently 50 exotic species of Scolytidae recorded from North America. The first exotic species reported from North America was *Xyleborus dispar* in 1817. Between 1817 and 1980, there were 25 species of exotic scolytids reported in the U.S. and Canada; however, since 1980 there have been an additional 25 species reported. Most of the species reported pre-1980 were true bark beetles from Europe. Since 1980, the majority of species established in North America are ambrosia beetles native to Asia. In the ambrosia beetle tribe Xyleborini, more than 50% of the species currently in North America are non-natives. Beetles in this tribe are easily transported to new areas and readily established because of their broad host range, skewed sex-ratio, and extreme inbred sib-mating system. Like many ambrosia beetles they are well adapted to warm-humid climates, as in the southeastern U.S. In fact, the majority of exotic scolytids, especially ambrosia beetles, in North America are found in the Southeast. A recent survey of 10 southeastern states found 66% of specimens collected were non-native species.

A recent U.S. Forest Service pilot project for the rapid detection of exotic scolytids was initiated in 2001.

Objectives of this project are to: identify potential exotic invasive species and provide guidance for their detection, detect and monitor populations of newly introduced species, provide APHIS and the Forest Service with current distribution information, and identify gaps in detection protocols and taxonomic skills. As protocols have developed, trapping is now concentrated in the urban forest/wildland-urban interface. During the 3 years trapping has taken place, four species of scolytids new to North America were identified: *Hylurgops palliatus*, *Xyleborus similis*, *Xyleborus glabratus* and *Scolytus schevyrewi*.

The USDA APHIS Pest Information Network (PIN) database has records of more than 600,000 pest interceptions at U.S. ports between 1985-2000. Scolytidae is the most commonly intercepted beetle family, with more than 68,000 records in 49 genera. Despite many of these records being aggressive species in the genera *Ips*, *Pityogenes*, *Tomicus* and *Hylurgus*, very few of these species have become established. Most of the established species are those associated with fungi, either as ambrosial associates or as a species vectored by the beetle, such as the *Scolytus* species.

# IMPACTS OF IMIDACLOPRID ON TARGET AND NON-TARGET SPECIES ON HEMLOCK

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## Abstract

Hemlock woolly adelgid, *Adelges tsugae* (Annand), is a very serious pest of hemlocks, *Tsuga* spp., in the eastern United States. Although a variety of insecticides are capable of controlling hemlock woolly adelgid, the systemic insecticide, imidacloprid has gained widespread acceptance and use in the plant care industry. While several studies demonstrate the efficacy of imidacloprid in reducing adelgid populations, none have examined how hemlocks recover following imidacloprid therapy. Specimen trees in a residential landscape were treated with soil drenches of imidacloprid in March of 1999 or left untreated. Trees were in three stages of canopy appearance at the time of the application, healthy, poor, or with dieback. On the day of application and at 434 and 816 days after application the abundance of hemlock woolly adelgid was determined. Furthermore, the amount of new growth, DBH, and appearance of the plants were also quantified. Imidacloprid provided remarkable and long lasting levels of control on all categories of trees. Adelgids were virtually absent on all treated trees, even trees with appreciable dieback more than 2 years following the application despite the fact that adelgids were present in the same landscape on trees nearby. Hemlocks recovered dramatically once the pressure of the adelgids was removed following an application of imidacloprid. The amount of new growth increased dramatically as did the appearance of the trees. We could not detect a significant difference in the increase in caliper of trees in the different health categories following the application of imidacloprid. However, there was a clear trend for trees released from adelgid pressure to increase in caliper. Trees in the best health generally experienced the largest gains. More importantly, the response of trees to imidacloprid therapy differed in relation to their condition at the onset of the experiment. With respect to the amount of new growth and appearance, trees with the healthiest canopy at the onset of the experiment improved the least following

the reduction in adelgid populations. Trees in poor condition, that is, with little new growth but no dieback, recovered the quickest and most dramatically. Trees in the poorest condition at the onset recovered impressively but more slowly. Trees left untreated remained ugly and in poor health. These results confirm the value of imidacloprid in reducing adelgid populations and improving the quality of hemlocks under attack by the hemlock woolly adelgid in urban forests (Webb et al. 2003).

By the end of the 1990s scientists at the University of Maryland (Raupp), Beltsville Agricultural Research Center (Webb), and Bartlett Tree Experts (Booth) received reports from commercial arborists and landscape managers that mites and their injury seemed to be more common on hemlocks treated with imidacloprid. Due to the widespread use of imidacloprid for control of hemlock woolly adelgid and growing concerns raised over increased mite problems following the application of imidacloprid, two independent studies were conducted. The first by Webb and Raupp examined the effects of imidacloprid on the abundance of spruce spider mite, *Oligonychus ununguis* (Jacobi), and the hemlock rust mite, *Nalepella tsugifolia* Keifer and their damage on Canadian hemlocks, *T. canadensis* (L.) Carrière, in a residential landscape. The second by Raupp, Szczepaniec, Booth, and Ahern compared hemlock rust mite abundance and spruce spider mite injury on treated and untreated Canadian hemlocks in parks, gardens, and residential landscapes in the Washington-Baltimore metropolitan area. The objectives of the studies were twofold. First, we wanted to know if mites were more or less abundant on imidacloprid treated trees compared to untreated ones. Second, we wanted to know if mite injury was greater or less on trees treated with imidacloprid compared to untreated controls.

Hemlocks were treated with soil drenches or soil injections of imidacloprid. In a residential landscape we found populations of spruce spider mites, hemlock rust mites, and their injury to be greater on hemlocks treated with imidacloprid than on untreated trees. A survey of hemlocks in gardens, parks, and residential landscapes revealed that hemlocks treated with imidacloprid were more likely to be infested with spider mites but not rust mites. Moreover, terminals on imidacloprid treated hemlocks were approximately nine times more likely to have severe needle damage than untreated trees. Arborists and landscape managers applying imidacloprid to hemlocks should carefully monitor mite populations on treated trees and be prepared to intervene should mite populations increase (Raupp et al. in press).

We are currently investigating the reasons for increased mite abundance and injury of hemlocks treated with imidacloprid. Three hypotheses have been suggested to explain greater mite abundance on plants treated with imidacloprid. One suggests that sublethal doses of imidacloprid may stimulate the reproduction of these pests. This phenomenon is known as hormoligosis (Luckey 1968). A second mechanism to explain increased mite abundance on imidacloprid treated plants is the elimination of natural enemies or suppression of their activities. We have preliminary evidence that woody plants treated with imidacloprid can be lethal to predatory omnivores such as minute pirate bugs. Sclar et al. (1998) suggested that suppression of natural enemies such as minute pirate bugs might be a factor contributing to greater levels of spider mites and their

injury on honeylocust trees treated with imidacloprid. A third hypothesis for increased mite populations on imidacloprid treated plants has been proposed by researchers at the Bayer Corporation (Royalty 2003). This hypothesis suggests that imidacloprid alters the physiology of the plant in a way beneficial to phytophagous mites. Improved nutritional quality of leaves may result in enhanced mite performance such as increased fecundity.

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# ANALYSIS OF THE ASIAN LONGHORNED BEETLE INFESTATION IN JERSEY CITY, NJ

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## Abstract

In October 2002 an adult Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae) was discovered on a tree in Jersey City, NJ. A survey of the area by APHIS and NJDA personnel revealed an infestation involving over 100 trees, mostly Norway maples, concentrated in a 3 hectare urban area. Because the infestation was well isolated from previous finds (Manhattan, > 3.2 km away), this site provided a unique opportunity to study the history and dynamics of colonization by the ALB. Detailed information on the numbers and spatial distribution (between-tree and within-tree) of ALB egg sites, tunnels and exit holes was collected from the infested trees before and during removal. Infested material from selected trees was taken to the quarantine facility at Otis where it was dissected to collect ALB life table data and to estimate the ages of exit holes from growth rings. A total of 5,698 oviposition sites and 446 exit holes were found on 113 infested trees. Most trees had no exit holes or very few. Ten trees had 10 or more exit holes, including four having 40, 45, 56 and 104. Only the latter two trees were found to have exit holes pre-dating 2002 (1998-2001). Some exit holes were completely closed by new growth in a single growing season. Based on excavation of >1200 oviposition pits and tunnel systems in Norway maples, approximately 95% actually had eggs laid in them. Survival rates through the egg, neonate, phloem-feeding larval and pupal stages were about 85% each, while survival of xylem-feeding larvae was only 60%. Overall, 30% of observed oviposition pits produced adults. Intraspecific competition or cannibalism appeared to be the principal cause of the relatively high mortality rate seen among large larvae in the heavily infested trees from which most of these sites were collected. A simple population model (with conservative assumptions: sex ratio = 0.5, mating success = 0.8, oviposition pits chewed per female = 28 and adults emerging per initial pit = 0.25) indicates that the number of egg sites and exit holes observed in January 2003 could easily have resulted from a colonizing event of one gravid female arriving on site in the summer of 1997. The distance from the likely epicenter of this infestation to the most distant tree bearing an egg site in January 2003 was only 250 m.

# THE ASIAN GYPSY MOTH SITUATION IN MONGOLIA

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## Abstract

In the last several summers, the Asian gypsy moth (AGM), *Lymantria dispar asiatica* (Lepidoptera: Lymantriidae) in northern Mongolia has exhibited signs of a widespread, devastating outbreak. Siberian larch, *Larix sibiricus*, was impacted most, however, some *Populus* spp., *Betula* spp and *Salix* spp. also served as suitable food species. From a base camp in Dalbay Valley (N 51° 1.4', E 100° 45.3'), on the northeastern shore of Lake Hovsgol (elevation 1650 m (5200 ft)), we investigated the biology, behavior and ecology of AGM during the summers of 2002 (BAT) and 2003 (PWS & BAT).

Field evidence indicated that female AGM behavior very definitely concentrated vast quantities of egg masses on rock outcroppings, often on the higher ridges that delineated the river drainage in each valley that drains into Lake Hovsgol. In spring, the AGM neonate larvae on the exposed rocks, and vegetation near the ridges, were so abundant and they spun so much silk that most everything was festooned with thick layers of silk—giving the appearance of ice and snow. Many larvae failed to disperse by ballooning and were killed by entanglement. Those larvae that were successful drifted into the nearest larch trees and commenced feeding. This process left

a clear “edge effect” in that trees closest to the rock outcroppings were most severely defoliated, and therefore suffered greatest mortality, some in part do to secondary invasion by *Ips cembrae* (Coleoptera: Scolytidae). By mid-July, larvae had reached the 4th instar. Levels of parasitism proved to be uncharacteristically low. At the same time we noted that many egg masses showed no sign of hatch and many possessed high levels of non-embryonated (infertile) eggs. We expect to investigate this peculiarity in coming summer seasons. Unseasonably cool weather and precipitation caused larval development to slow and many larvae failed to reach maturity.

In late August, we experienced the adult flight period (ca. 100 miles south of Dalbay Valley) and we witnessed a massive nocturnal flight of AGM (96% ♀♀, 4% ♂♂) coming to a mercury vapor light illuminating a hotel front in Moron. Many thousands of females descended upon this light between ca. 10 PM and 1 AM. During daylight hours (ca. 10 AM to 3 PM), we observed many individual females flying high up onto the rock ridges to select sites for oviposition. We commenced experiments designed to clarify which came first, female flight or mating during both of the recognized active diurnal and nocturnal flight periods. Results are expected in 2004.

# 2002-2003 WINTER MORTALITY OF HEMLOCK WOOLLY ADELGID IN THE NORTHEASTERN UNITED STATES

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## Abstract

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is a nonnative pest responsible for extensive mortality and decline of hemlock trees in the eastern United States. Its range currently includes counties in 16 states from the Smoky Mountains to southern Maine. However, to date, infestations have not been reported in any area with minimum temperatures lower than that in USDA plant hardiness zone 5a (minimum low temperature is -26.5 to -28.8° C). Thus, cold winter temperatures may be a factor in reducing this insect's northward rate of spread. We assessed the mortality of selected HWA populations in the Northeast after the winter of 2002-03, the coldest winter in 9 years according to the National Weather Service.

Between 9 March and 24 April 2003, branch tips were examined from 10 HWA-infested eastern hemlocks, *Tsuga canadensis* (L.) Carrière, at each of 36 sites in NH, MA, CT, NY, PA, and NC. Where possible, at least 100 HWA sistens, nymphal stage 2 or older, were examined on each tree at each site (total of at least 1,000 HWA/site). Latitude, longitude, and elevation were recorded

at each site. Highest and lowest daily temperatures for the period November 2002 through March 2003 were obtained from the National Climate Data Center for the weather station closest to each site. Data were analyzed using the Spearman rank correlation test; values of  $P < 0.05$  were considered significant.

Adelgid mortality ranged from 11.2% at the NC site to 99.4% at a NH site. The lowest temperature reported was -27.2 ° C at a site in MA where mortality of HWA was 83.2%. At the NH site with 99.4% mortality, a minimum temperature of -24.5° C was recorded. Initial analysis indicates that mortality was positively correlated with degrees of latitude ( $r=0.422$ ,  $P=0.010$ ) even when the outlying NC site was excluded ( $r=0.371$ ,  $P=0.028$ ). There were no significant correlations among percent mortality and plant hardiness zone, elevation, or mean low temperature, but there was a slight negative correlation between percent mortality and the minimum temperature recorded at each site ( $r=-0.333$ ,  $P=0.047$ ). Comparative studies are planned for subsequent years.

# CONTROL OF THE BROWNTAIL MOTH IN MAINE WITH A BACULOVIRUS

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The browntail moth, *Euproctis chrysorrhoea*, was introduced into the United States at Somerville, MA near Boston in 1867. By 1913 it was present in all of New England as well as Nova Scotia and Brunswick, Canada. From 1913 until the 1960s the range of the browntail moth decreased until it was present only on Cape Cod, MA and in the Casco Bay area of Maine from Portland to Bath. Population densities have fluctuated in these locations over the years but recently rose to levels that caused severe defoliation in 1998-2002. High levels of mortality caused by *Entomophaga aulicae* occurred in 2002 and 2003 in Maine. In contrast *E. aulicae* was not active in Massachusetts and the browntail moth population has built up to a high level.

A baculovirus (*Euproctis chrysorrhoea* nucleopolyhedrovirus, EcNPV) that infects the browntail larvae has been found in browntail moth populations England, central Europe, Russia, and in the United States. We performed studies in the spring and fall of 2003 to determine whether the EcNPV could be used as an effective browntail moth control agent in populations present in Maine. EcNPV produced in browntail moth larvae during the summer of 2002 was used for these studies. EcNPV was added to a lignosulfonate-based formulation to a final concentration of  $2.5 \times 10^6$  polyhedra/ml and applied to test trees at a rate of approximately  $5 \times 10^{12}$  polyhedra/ha. Larvae were collected from 10 test apple trees and two control apple trees prior to virus application, and were reared until death or pupation. Five test trees were sprayed on May 7, 2003 and an additional five trees were treated on May 20, 2003. The same formulation and application rate were used in an application performed on September 8, 2003 to branch tips on oak, cherry, and hawthorn trees containing newly formed winter webs. Larvae were collected 1, 2, 3, and 4 weeks after the May 7 and May 20 applications and reared until death or pupation. Larvae were collected 1 and 2 weeks after the fall virus application and reared until death or the reformation of the winter web. Webs were collected 7 weeks after the fall application, opened, the number

live and dead larvae counted, and the dead larvae were inspected microscopically for the presence of EcNPV.

No virus mortality was observed in the pretreatment larvae collected prior to the May 7th and May 20th virus applications. EcNPV mortalities ranging from 75-85 % were found in larvae collected from trees treated on May 7, and EcNPV mortalities ranging from 82-88 % were found in larvae collected 1-3 weeks after the May 20th virus treatment. An average mortality of 50% was found in larvae collected 4 weeks after the May 20th virus application; however, these data were compromised by a high level of *E. aulicae* present in the larvae. Mortality levels of an average of 62% and 55% were found in larvae collected 1 and 2 weeks, respectively after the September 8th virus application. Mortality on larvae collected from oak and cherry trees were similar ranging from 70% to 82% and 60% to 80%, respectively. In contrast, mortality in larvae collected from hawthorn was less, ranging from 30% to 35%. An average of 94% of the larvae were alive in the control webs collected 7 weeks after the September 8th virus application, and no virus was found in the dead larvae. In virus-treated nests an average of 60% of the larvae were alive, and 77% of the dead larvae contained EcNPV. However, the virus-treated webs contained 68% fewer larvae compared to the control webs. If the reduction in the number of larvae was due to virus mortality outside of the web and that number was included with the larvae found with EcNPV then the total virus induced mortality would be approximately 78%. Additional webs will be collected in the winter of 2004 and the larvae reared in the spring to assess levels of EcNPV mortality in the surviving larvae.

Overall, these results suggest that the EcNPV could be an effective browntail moth control agent. Spring application of EcNPV gave very high levels (85% mortality) of browntail moth control. Fall application of EcNPV gave good (40%) levels of control; however, once the final results are obtained in 2004 the fall application may prove to be the most effective time for treatment.

# RESEARCH ON MICROSPORIDIA AS POTENTIAL CLASSICAL AND AUGMENTATIVE BIOLOGICAL CONTROL AGENTS OF THE GYPSY MOTH

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Three genera of microsporidia infect European gypsy moth larval populations and have been documented to reduce the intensity and duration of outbreaks. In order to introduce isolates of these pathogens into North American gypsy moth populations as classical biological control agents, the taxonomy and biology of the pathogens must be elucidated. Taxonomic relationships have been particularly difficult to evaluate and historical reports are inadequate to satisfy taxonomic guidelines for introduction. In addition, host specificity has become an increasingly important issue for introduction. The following completed and ongoing projects were designed to elucidate taxonomic relationships and variability between closely related isolates; evaluate physiological and ecological host specificity; and investigate basic host-pathogen interactions with a goal of introducing microsporidia into North American gypsy moth populations.

**Host Specificity:** Field studies of the non-target effects of applying ULV sprays ( $10^9$  spores/500 m<sup>2</sup>), a method for inoculating gypsy moth populations with microsporidia, are indicating that the *Nosema* sp. microsporidia are host specific for the gypsy moth. One species, *Vairimorpha* sp., infected 7 of 80 non-target species (18/640 total individuals), but was not found infecting non-target species in the same plots the following year (0/708). *Nosema* sp. [Levishte, Bulgaria isolate] was not infective to nontarget species (0/591) when sprayed. *Endoreticulatus* species appear to be generalist pathogens and are not being considered for release.

**Competition between Microsporidia:** When microsporidian infections were mixed in individual host

larvae, certain species dominated in laboratory tests based on sequence of infection, but some combinations of the pathogens allowed maturation of both species. Horizontal transmission, however, may be a factor in competition among microsporidian species infecting the gypsy moth.

**Taxonomy and Isolate Variability:** The *Nosema* and *Vairimorpha* microsporidian pathogens of the gypsy moth represent a closely related group with variable characteristics. The redescription of one species, *Vairimorpha* (*Thelohania*) *disparis* (Timofeeva) will set the stage for taxonomic evaluation of the entire group and will serve as a basis for either accepting or redescribing the named species within the group. A clearly defined taxonomy is important in order to pursue permission to release these naturally occurring pathogens in North American gypsy moth populations. PCR-RAPDs and proteomics studies using 2-D PAGE analysis are being used to fingerprint and differentiate closely related gypsy moth microsporidia. In addition, relationships and genetic plasticity between isolated populations are being evaluated.

**Transmission:** Most terrestrial microsporidia are horizontally transmitted between conspecific hosts during the larval stages via feces, cadavers or contaminated silk from infected silk glands. Laboratory tests in diet cup bioassays show that the gypsy moth microsporidia are no exception, but field studies will show the extent to which horizontal transmission occurs in a more ecologically complex arena.

**Immune Responses:** Gypsy moth larvae fed spores of microsporidia that are natural pathogens in other forest

Lepidoptera (*Hyphantria cunea*, *Malacosoma americanum*) or spores of a virulent noctuid microsporidium, *Vairimorpha necatrix*, mounted some cellular immune response but not significantly stronger than responses elicited by *L. dispar* microsporidia. Nor was the phenoloxidase cascade that produces melanin elicited more strongly than for naturally occurring microsporidia. The strongest responses were elicited by microsporidia, both naturally occurring species and those from other hosts that heavily infected the fat body tissues.

**Effects of Parasitism:** Parasitism and, thus, release of immune suppressant polydnviruses (PDV) by *Glyptapanteles liparidis* promoted microsporidian infections in the gypsy moth. Parasitism, as well as PDV

+ venom, led to increased spore load in infected hosts. However, PDV + venom did not increase permissiveness of *L. dispar* larvae to microsporidia that were otherwise unable to cause patent infections.

**Effects of Dimilin on Microsporidia:** The chitinsynthetase inhibitor Dimilin, used extensively in Europe for gypsy moth control, does not affect environmental spores of *Nosema* sp. [Schweinfurt, Germany] directly, but appears to interfere with the formation of both the autoinfective primary spores and the environmentally resistant spores in the infected gypsy moth host. Microsporidian spores produced in larvae fed sublethal concentrations of Dimilin were not infective.

# RECENT IMPROVEMENTS IN THE EFFICACY OF TRAPPING THE BROWN SPRUCE LONGHORN BEETLE, *TETROPIUM FUSCUM* (F.), USING HOST VOLATILE-BAITED TRAPS

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## Abstract

The brown spruce longhorn beetle, *Tetropium fuscum* (F.), native to Europe, has been the target of an eradication program led by the Canadian Food Inspection Agency in Halifax, Nova Scotia, since June 2000. In field trapping experiments in 2001 and 2002, we showed that a synthetic lure ('spruce blend') that simulated the blend of monoterpenes emitted from stems of infested red spruce trees was attractive to *T. fuscum*, and that the addition of an ethanol lure synergized attraction of both *T. fuscum* and *T. castaneum* (L.). In 2003, our objectives were to determine the effect of release rate on attraction of *Tetropium* spp. to spruce blend and ethanol, and to compare the efficacy of different trap designs with wet vs. dry preservatives. Trapping experiments were conducted from May to July 2003 on McNabs Island, Halifax, N.S., and in the Białowieża Forest, Poland. Traps baited with high release rate lures of spruce blend plus ethanol caught significantly more *T. fuscum* and *T. castaneum* than any

other treatment, a two to sevenfold increase in catch over traps baited with low release rate lures of spruce blend plus ethanol. Trap design and type of preservative in the collecting cup significantly affected mean catch of *T. fuscum*. Colossus traps caught about twice as many beetles as the IPM-Intercept traps, and "wet" traps (with 50% propylene glycol in the collecting bucket) caught more beetles than "dry" traps (with a dichlorvos strip). Colossus-wet traps, baited with high release rate lures of spruce blend and ethanol, are recommended for surveys in 2004 as their use will increase the chances of detecting *T. fuscum* where it is present, compared to the low release rate lures used in 2002 and 2003. In future studies we plan to determine the relationship between trap catch and density of *T. fuscum* infestation in host material and investigate the use of pheromones for long distance attraction in *Tetropium* spp.

# EFFICACY OF GYPSY MOTH MATING DISRUPTION TREATMENTS IN THE SLOW-THE-SPREAD (STS) PROJECT

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## Abstract

The National Slow-the-Spread of the Gypsy Moth Project (STS) is a coordinated effort by the USDA (Forest Service and APHIS) and 10 state governments (NC, VA, WV, KY, OH, IN, MI, WI, MN) to slow the rate of spread of gypsy moth, *Lymantria dispar* (L.), populations into uninfested areas. Using an extensive grid of pheromone traps, gypsy moth populations along the leading edge are detected and delimited, and if certain criteria are met they are treated with insecticides or mating disruptants. The project was pilot tested from 1993 to 1998 and was fully implemented in 1999. Initially, most acres were treated using *Bacillus thuringiensis* (B.t.). By 2000, mating disruption using the gypsy moth sex pheromone, Disparlure, became the primary treatment tactic. Application rates for mating disruption of 30 g active ingredient per acre were used exclusively until 2000. Based on field tests showing high levels of efficacy at lower doses, mating disruptants were applied at 15 g/acre starting in 2001, and at both 15 and 6 g/acre starting in 2002. Based on criteria developed for use in the STS decision support system, an analysis of the treatment results for the years 1993 to 2001 (prior to the use of the 6 g/acre rate) showed that the success rate for blocks treated with mating disruption was greater than for blocks treated with B.t. (Sharov et al. 2002, J. Econ. Entomol. 95: 1205-1215). The objectives of the analyses reported here are: (1) to extend the evaluation of STS treatment success through the year 2003; (2) to evaluate treatment success in blocks treated with different mating disruption application rates; and (3) to identify factors (e.g. block size, pre-treatment moth density, population growth trends, etc.) associated with reduced treatment success.

Based on criteria established in the STS decision support system, the success rate of all treatments combined (including blocks rated as partially successful) ranged

from 85 to 95% from 1994 to 2003. Using the same criteria, treatment success ranged from 88% for B.t. to 100% for mating disruption at 6 g/acre. Treatment success was 93% and 95% for mating disruption at 15 g and 30 g/acre, respectively. However, it is important to note that B.t. tends to be used more often in STS on smaller blocks and on those blocks that have a higher pre-treatment moth density, so the comparison may be biased. Of the blocks considered successful or partially successful by the STS decision support system, average moth density was reduced in 98% of the mating disruption blocks treated at 30 g/acre, in 90% of the blocks treated at 15 g/acre, and in 69% of the blocks treated at 6 g/acre. Moth density declined in 80% of the blocks that were treated with B.t. and were considered successful. While this may be an indication that lower mating disruption application rates result in reduced efficacy, there are many potential sources of bias that could also account for the differences among the treatments. For instance, the 6 g/acre rate for mating disruption has been used only in the past two years. The majority of treatment acres during the past two years have been in the extreme north of the STS project area. Therefore, it may be that gypsy moth populations are responding differently to treatments in this newly-invaded area. There is some evidence to support this idea. In Wisconsin, 32% of the successful or partially successful blocks experienced increases in moth density, compared to only 3% in states other than Wisconsin. Interestingly, there also appear to be differences in the growth of surrounding untreated populations. In Wisconsin, 82% of the successful or partially successful blocks experienced increases in moth density in areas adjacent to the blocks, while this number was 40% for all blocks in other states. Subsequent analyses will focus on comparisons of gypsy moth population dynamics over larger areas in Wisconsin versus other regions.

# THE SPREAD OF GYPSY MOTH IN WISCONSIN: A NEW PARADIGM FOR THE MIDWEST INVASION?

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## Abstract

The gypsy moth, *Lymantria dispar* (L.), has been gradually expanding its range in North America since its introduction into Massachusetts approximately 150 years ago. Gypsy moth is a highly polyphagous herbivore that can exploit over 300 species of deciduous and coniferous hosts (Elkinton and Liebhold 1990). The management of gypsy moth can be divided into three components: (1) eradication, which is done in regions located away the expanding population front; (2) suppression, which is done in regions that are generally infested; and (3) management, which is done in transition zone between the uninfested and infested areas and which is currently realized through the Slow-the-Spread program. All three require an understanding of the population boundaries that delimit these areas, and past work has identified methods for generating boundaries based on the monitoring of male moths through pheromone-baited traps (Sharov et al. 1995). In parallel, additional interest is often placed on the prediction of the future boundaries of the population so that the appropriate state and federal agencies, as well as landowners, can prepare adequately for the gypsy moth advance in these newer areas to minimize its impact (Gottschalk 1993, McFadden and McManus 1991).

A model of population boundaries of gypsy moth requires an understanding of the rate of spread, which is more likely than not to be specific to the region of interest. Topography would most likely play a role; for example, hilly regions could limit the dispersal of individuals more so that those regions that are relatively flat. Overwintering temperatures have previously been observed to play a role in spread rates. Liebhold et al. (1992) analyzed historical data on the movement of gypsy moth populations from the initial source of infestation near Boston, Massachusetts. They observed that when the average minimum January temperatures  $> 7^{\circ}\text{C}$ , then spread rates were roughly 20.8 km/yr, whereas for the inverse condition ( $< 7^{\circ}\text{C}$ ), spread rates were only 7.6 km/yr.

Until the invasion of gypsy moth into, first, the upper peninsula of Michigan, and then Wisconsin, this paradigm has generally held to be true. Sharov et al. (1999), for example, observed that temperatures in Michigan were actually inversely related to gypsy moth spread: spread rates were higher in the northern Lower Peninsula than in the southern Lower Peninsula despite the former having the lower minimum January temperatures. They also demonstrated that forest composition played a definite role in the spread of gypsy moth. Higher than previously expected rates of spread (Liebhold et al. 1992) in Midwest poses new challenges to the management of gypsy moth. Tcheslavkaia et al. (2002) contended that these higher rates of spread may be due, in part, to decreased predation on females with the consequence of higher female mating success, which in turn could be furthermore enhanced by increased male moth dispersal. Recent empirical and theoretical evaluations of the range expansion of exotic species (Shigesada et al. 1995), in which the “range of the founder population expands, new colonies created by [dispersers] increase in number to cause an accelerating range expansion...”.

In Wisconsin, we have observed (1) higher rates of spread (relative to the rest of the Slow-the-Spread areas); (2) trap catch data that are dominated by a continuum of low—and often single—catches of male moths without clear core populations to treat; and (3) trap catch data appearing as “bulges” that are in different places in different years. In this paper, I will address these issues and present a case for the development of a new paradigm for the management of the spread of gypsy moth in Wisconsin, which may have ramifications for its movement and consequent management in other Midwestern states.

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# DISPERSAL AND SPATIOTEMPORAL DYNAMICS OF ASIAN LONGHORNED BEETLE IN CHINA

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## Abstract

We studied dispersal and spatiotemporal dynamics of the Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae), in Gansu Province of north central China. We used mass mark-recapture methods and observed that 98% of beetles were recaptured within 920 m from a release point, while the median dispersal rate for all recaptured adults was 30 m/d. Dispersal potential within the course of a season for males and gravid females was 2,394 and 2,644 m, respectively; however, more work is needed to evaluate the potential of long distance dispersal events to initiate new colonies outside current U.S. quarantine boundary guidelines. Peak population abundance of unmarked beetles (i.e., background populations) in both 1999 and 2000 occurred between 800-900 degree days (base threshold = 10° C) from 1 January. Background populations exhibited local spatial autocorrelation during peak abundance, with ranges of spatial dependence of 229-543 m.

# UPDATE ON THE EFFICACY OF SELECTED SYSTEMIC INSECTICIDES FOR THE CONTROL OF THE ASIAN LONGHORNED BEETLE

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## Abstract

This is an update of the study we have been doing in China to evaluate insecticides and delivery techniques for the control of the Asian longhorned beetle (ALB), *Anoplophora glabripennis*. The following people and institutions have also contributed or participated in the study: Phillip A. Lewis, David R. Lance, and David M. Cowan from USDA APHIS CPHST, Otis Laboratory, and Baiying (city) and Jingtai (county) Forest Protection Station, Gansu Province, China.

Our studies prior to 2003 involved cover insecticides and systemic insecticides. Different cover insecticides were tested for efficacy and persistency at different doses. Various systemic insecticides were tested for their efficacy for ALB control at different doses, application times and delivery methods. Several cover insecticides were tested in two different years with different application methods. In one test, the twigs of poplar trees were dipped into insecticide solutions and then provided to ALB adults, while in another test; twigs were collected from treated poplar trees and then provided to ALB adults. In both cases, generally, the tested insecticides showed good short term efficacy, but poor long term efficacy. Among the tested systemic insecticides (i.e., methamidophos, disyston, acephate and metasystox-r, imidacloprid, acetamiprid, thiamethoxam, and thiacloprid), imidacloprid and possibly thiamethoxam and acetamiprid can be used effectively to kill adult ALB beetles through soil and tree trunk injection. Significant mortality of adult beetles was observed 2 years after application with imidacloprid treatments. Mortality of ALB larvae, especially, late instars, however, was low. Residue levels of imidacloprid in treated trees and efficacy were limited by a number of factors such as delivery method, timing of application, tree species, and tree size. To establish the response of adult beetles to doses of imidacloprid, we also conducted

two tests. In one test, twigs of silver maple were dipped in different concentrations of imidacloprid solutions. While in another test, twigs were freshly cut from treated host trees. In both cases, these twigs were provided to caged adults and the beetles were checked regularly for their mortality. The levels of imidacloprid in twigs were determined through chemical analysis. The  $LC_{50}$  of imidacloprid for adult ALB is 1.9 ppm for a 3-day exposure.

In 2003, to minimize the time that takes to inject and guard injected trees when imidacloprid is applied with the standard Mauget micro injection technique, and to compare efficacy of imidacloprid with other neonicotinyl systemic insecticides, we evaluated dinotefuran and thiamethoxam, and imidacloprid through different delivery methods for their efficacy for ALB and the standard Mauget Imicide. The three insecticides were compared using the delivery methods (checked) listed in Table 1.

Mortality was checked for ALB adults caged with treated trees, and for wild beetles that migrated to and fed freely on treated trees. Five to 10 trees in each treatment group were dissected in October of the same year to check status of different stages such as egg, larva, pupa, and adult. The number of egg sites and exit holes were recorded.

Mortality of adult beetles caged with treated trees was greater than the control for all treatments, except for soil injected and trunk implanted dinotefuran, and soil injected thiamethoxam. A possible reason for their lower adult mortality in treatments of tree implant and soil injection might be that beetles were caged soon after insecticide application. We know that translocation of imidacloprid is faster where trees are trunk injected versus trunk implanting and soil injecting. Speed of uptake

**Table 1.—Delivery methods for the three neonicotinyl insecticides in the 2003 study**

Delivery Method (Treatment Name)	imidacloprid	dinotefuran	thiamethoxam
Imicide by J. J. Mauget Co. (Imicide)	x		
SW100 Injector Gun by ArborSystems, Modified & with USDA Tip (Modified Wedgle)	x	x	
Manually Operated Device by ArborJet, Inc., Tip replaced with USDA Tip (Modified Arborjet Device)	x	x	
Soil Injection, Kioritz (Soil Injection)		x	x
Tree (Trunk) Implants by Creative Sales (Trunk Implant)		x	

might also be similar for dinotefuran and thiamethoxam using these techniques. The number of dead beetles found on the ground under the canopy of treated trees was higher than the control except for dinotefuran when it was trunk implanted. The highest mortality was found in the treatment using the Modified Wedgle, which was not statistically different from several other treatments, such as Imicide, as well as imidacloprid and dinotefuran applied through Modified Arborjet. Treated trees were dissected in October to assess the efficacy of different treatments. All stages of beetles, whether dead or alive, were recorded. In terms of the combined mortality of different stages of the beetle found inside of the tree, Mauget Imicide treatment was significantly greater than others. Dinotefuran applied using Trunk Implant and the control was among the lowest.

Samples were collected from treated trees to determine the level of insecticide in treated trees using different

delivery methods, but chemical analysis has not completed and additional samples will be collected.

To establish a dose-response curve for ALB to dinotefuran and thiamethoxam, caged beetles were provided with fresh twigs cut from trees treated with these insecticides. Beetles were checked for mortality and consumption of the bark of twigs at least 7 days starting 24 hours after the introduction of adults. Levels of insecticides in twigs were determined through chemical analyses using HPLC, and LC<sub>50</sub> was determined through regression using the SAS Probit procedure. Generally, mortality increased as the level of dinotefuran and thiamethoxam increased, but mortality increase leveled off after mortality reached around 80%. The 3-day LC<sub>50</sub> value for dinotefuran was close to that for imidacloprid, but higher than that for thiamethoxam. But the 3-day LC<sub>90</sub> values for dinotefuran and for thiamethoxam were both much higher than that for imidacloprid.

# APPLIED BACULOVIRUS AND *ENTOMOPHAGA MAIMAIGA* PROVIDE OUTSTANDING CONTROL OF GYPSY MOTH

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## Abstract

Gypchek® (USDA, Forest Service, Washington, DC), a product with the *Lymantria dispar* multienveloped nuclear polyhedrosis virus (LdNPV) as the active ingredient, is registered by the USDA Forest Service with the U.S. Environmental Protection Agency as a general use bioinsecticide under the watchful eye of John Podgwaite, USDA Forest Service, Hamden, CT. Strain 203 is an isolate of LdNPV with improved characteristics being developed by James Slavicek, USDA Forest Service, Delaware, OH. In 2003, a considerable area of U.S. government land in Prince Georges County, MD, was infested with gypsy moths and was scheduled to be sprayed with Gypchek. This area included the Beltsville Agricultural Research Center (BARC), represented by Kevin Thorpe, and Greenbelt National Park (GNP), represented by Jil Swearingen. It was decided to use this program to compare Strain 203 against the standard Gypchek. (Strain 203 had been found comparable to Gypchek in lab bioassays and limited ground studies, but the two had not previously been compared by aerial application.) The operational goal of the study was to

compare Gypchek and Strain 203 from the air. The test is considered successful if 203 performs as well as Gypchek. Experimental goals are (1) to compare the second wave levels of LdNPV at sites treated with Gypchek vs those treated with Strain 203; and (2) to delineate the combined impacts of LdNPV and *Entomophaga maimaiga* in the various plots.

The two products were sprayed, each at the rate of  $1 \times 10^{12}$  occlusion bodies per ha, by a rotary-winged aircraft equipped with a Differentially Global Positioning System on 30 April, 2003 under the supervision of Brad Onken, Rod Whiteman, and Karen Felton. The virus preparations were mixed by Podgwaite and his crew. Strain 203 was applied to 153 ha at BARC, while Gypchek was applied to 172 ha at BARC and GNP. The two products gave equivalent levels of control, and a similar "second wave" of virus was observed at evaluation sites in both areas. Late season *E. maimaiga* outbreaks combined with the virus application to cause a combined 99% mortality of collected larvae at Gypchek and 203 sites.

# APPLICATION OF HYPERSPECTRAL IMAGING TO SURVEY FOR EMERALD ASH BORER

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## Abstract

The emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) was first discovered in the Detroit metropolitan area in the summer of 2002 and has been found subsequently throughout southern Michigan. As a killer of ash trees, the borer is already having severe impacts on urban and natural forests. Because conventional ground survey is laborious and time-consuming and the early stages of infestation are difficult to detect, survey for new infestations using remote sensing technology—especially, hyperspectral imaging (HSI)—holds great promise. Because of its wide spectral range and narrow resolution, HSI has the potential to detect the subtle spectral differences that may characterize individual tree species and trees exhibiting stress symptoms resulting from insect damage. We are exploring the twofold application of HSI to mapping distributions of ash trees and locations of EAB-infested hosts in various stages of decline over wide areas. Our work consists of four components: image acquisition, collection of spectral signatures for ashes and other tree species, collection of ground truth information on ashes and other species, and image analysis and classification of land cover.

The imaging flight was made on 17 September 2003. Images were acquired at 4-m resolution. The study area encompassed about 5 km by 2 km around Brooklyn, MI. The Probe 1 hyperspectral sensor recorded 128 bands spanning the EM spectrum from visible light through infrared. At the same time, reflectance characteristics of ash trees in various states of health were collected on the ground with a handheld spectrometer. Readings were made above tree crowns around midday so as to approximate the conditions recorded by the airborne sensor. Additional ash trees in various states of health and trees of other species were examined and georeferenced on the ground under the flight path of the imaging aircraft to provide ground truth information. Image analysis is under way at Clark University in Worcester, MA. Spectrometer readings and reflectance characteristics of ground truth trees will be used to develop spectral signatures—combinations of several spectral bands that characterize individual tree species and health classes—which in turn may be used to classify the hyperspectral images so as to produce maps of EAB host trees for use in the survey program.

## POSTER DISPLAYS 2004

**Invasive.org: A web-based image archive and database system focused on North American Exotic and Invasive Species.** G.K. Douce, D.J. Moorhead, C.T. Barger and R.C. Reardon.

**Development of Survey Tools for the Emerald Ash Borer, *Agrilus planipennis*.** J. A. Francese, V.C. Mastro, D.R. Lance, S. G. Lavalley, J.B. Oliver and N. Youseff.

**Effects of parental age at mating on sex ratios of the gypsy moth parasitoid *Glyptapanteles flavicoxis* (Hymenoptera: Braconidae).** R.W. Fuester.

**A New Species of *Laricobius* (Coleoptera: Derodontidae) from China, a Predator of Hemlock Woolly Adelgid.** H. Gattton.

**Analysis of Japanese Oak Wilt Spread Using Aerial Photography and GIS.** R. Komura.

**Influence of light conditions on the spatial distribution of an ambrosia beetle *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) flying in a natural secondary broad-leafed forest.** Y. Igeta.

**Use of an artificial inoculation technique to identify American beech trees with resistance to the beech scale insect.** J. Koch

**Ethanol and alpha-pinene: Nationwide survey of responses by bark and wood boring beetles.** D.R. Miller and Cooperators.

**2002-2003 Winter Mortality of Hemlock Woolly Adelgid in the Northeastern U.S.** K.S. Shields and C. Cheah.

***Scymnus ningshanensis* Yu et Yao (Coleoptera: Coccinellidae) for biological control of *Adelges tsugae* (Homoptera: Adelgidae).** M. Montgomery, H. Wang, E. Butin, D. Yao, W. Lu, and N. Havill.

**Assessment of the Control of Pine Processionary (*Thaumetopoea pityocampa*) By the Exosex Auto-Confusion System.** J.C. Martin, F. Pouvillon and K. Underwood.

**Imidacloprid Distribution Over Time Within a Norway Maple Tree Canopy.** P. Lewis.

**Comparison of Damage and Residue Levels in Green Ash and Norway Maple Between Six Trunk Injection Systems.** P. Lewis.

**Invasive Plants of the Eastern United States: Identification and Control.** C.T. Barger, D.J. Moorhead, G.K. Douce, R.C. Reardon and A. E. Miller.

**Development of Insect-Killing Fungi for Management of Hemlock Woolly Adelgid.** S.D. Costa, M. Skinner, S. Gouli, M. Brownbridge, V. Gouli, W. Reid and B.L. Parker.

**Comparing Systemic Imidacloprid Application Methods for Controlling Hemlock Woolly Adelgid.** R. S. Cowles, C. Cheah, and M. Montgomery.

**Information System on 100 Invasive Plants from Asia.** Z. Hao, D. Jianqing, Y. Wu, D. Binion, and R. Reardon.

**Evaluation of potential host trees for *Anoplophora glabripennis*.** D. Morewood, J. Sellmer, P. Neiner, and K. Hoover.

**Evidence for host tree resistance against the Asian longhorned beetle.** D. Morewood, J. Sellmer, P. Neiner, and K. Hoover.

**Feasibility of Noncontact Ultrasound as a Method to Detect Insects in Solid Wood Packing Material.** M. Fleming, M. Bhardwaj, J. Janowiak, J. Shield, R. Roy, D. Agrawal, L. Bauer, D. Miller, and K. Hoover.

**Efficacy and Treatment Parameters for 2.45 GHz Microwave Irradiation of Cerambycids in Laboratory-size Wood Samples.** M. Fleming, Y. Fang, Y. Wang, L. Zu, B. Kappes, D. Whitmore, J. Janowiak, J. Shield, J. Kearns, R. Roy, D. Agrawal, L. Bauer, D. Miller and K. Hoover.

**Host Range of the Emerald Ash Borer (*Agrilus planipennis*) in North America: Preliminary Results from Laboratory and Field Tests.** A. Agius, D.G. McCullough, D.L. Cappaert, T. Poland, D. Miller, L. Bauer.

**Dispersal of the exotic emerald ash borer from a point source.** D.G. McCullough, T. Poland, D. Cappaert.

- The Effect of Placement Height, Color and Release Rates on Trap Catches of the Asian Longhorned Beetle. *Anoplophora glabripennis*.** J. Lund, J. A. Francese, S. A. Teale.
- Measuring Responses of Southern Appalachian Ecosystems to Hemlock Woolly Adelgid at the Coweeta Watershed.** M. Hunter, H. Keys, P. Spaine, S.V. Lumpkin, and J. Vose
- CFS-Atlantic Exotic Beetles and Associated Fungi Project.** J.E. Hurley, G.A. Smith, K.J. Harrison, A.W. MacKay, A.S. Doane, D.S. O'Brien, and T.J. Walsh.
- Ecological Niche Modeling of the Potential Distribution of Emerald Ash Borer (*Agrilus planipennis*).** D. Kluza.
- Dispersal and Spatiotemporal Dynamics of Asian Longhorned Beetle in China.** P.C. Tobin, M.T. Smith, G. Li, and R. Gao.
- Arborjet Methodology: Assaying New Systemic Formulations for Trunk Injection and Micro-infusion Technologies.** J.J. Docola, P.W. Wild, C. Taylor, and E. Bristol.
- Mating Requirements to Maintain Fertility in *Anoplophora glabripennis* (Coleoptera: Cerambycidae).** M. Keena and V. Sanchez.
- Alternate Iron Sources for Use in Gypsy Moth (Lepidoptera: Lymantriidae) Artificial Diet.** M. Keena.
- Recent improvements in efficacy of trapping the brown spruce longhorn beetle, *Tetropium fuscum*, using host volatile-baited traps.** J. Sweeney, P. de Groot, L. MacDonald, J.E. Hurley, and J. Gutowski.
- Reproductive strategies of *Scymnus* ladybeetle predators of HWA.** C.A. Cheah, K.S. Shields, P.M. Moore, J.E. Ogrodnick and G.G. Bradford.
- Hemlock Woolly Adelgid (*Adelges tsugae* Annand) Phenology and Predators in the Pacific Northwest.** M.K. Byrkit and D. W. Ross.
- USDA Forest Service Research Efforts on Non-Native Invasive Pathogens in the North Central Region.** J. Juzwik.
- Biology of *Scymnus ningshanensis*.** M. Montgomery et al.
- Monitoring Hemlock Woolly Adelgid Damage to Hemlock Crowns in the Delaware Water Gap Using Ground Plots and Satellite Imagery.** M. Montgomery, D. Royle, B. Onken and A. Evans.
- Halyomorpha halys*, (Heteroptera: Pentatomidae), The Brown Marmorated Stink Bug; are Trees the Primary or Reservoir Hosts for this New Invasive Pest?** G. Bernon, K.M. Bernhard, E.R. Hoebeke, M. Carter and L. Beanland.
- Research on Microsporidia as Potential Classical and Augmentative Biological Control Agents of the Gypsy Moth** L. Solter, V. D'Amico, D. Goertz, G. Hoch, A. Linde, M. McManus, J. Novotny, D. Pilarska, P. Solter, J. Vavra, J. Weiser, & M. Zubrik.
- USDA/APHIS/PPQ New Pest Advisory Group (NPAG).** S. Scott
- Natural Enemies of Emerald Ash Borer.** L. Bauer.
- Microbial Control of Emerald Ash Borer.** L. Bauer.
- Emerald Ash Borer Studies: Host Range, Dispersal, and Survival in Firewood.** R.A. Haack, T.R. Petrice, D. L. Miller, and L. Bauer.
- Entomophthorales Infecting the Green Spruce Aphid *Elatobium abietinum* Walker in Iceland and Arizona, USA.** C. Nielsen, J. Eilenberg, G. Halldorsson, S. Harding, A.M. Lynch and E. Oddsdottir.
- Preliminary Analysis of Mitochondrial DNA Sequence Data for *Anoplophora*: Implications for Source Population Determination of Asian Longhorned Beetle (*A. glabripennis*) in the United States.** M.E. Carter, E.R. Hoebeke, R.G. Harrison, S.M. Bogdanowicz, M. Keena and A. Sawyer.
- The National Exotic Woodborer/Bark Beetle Survey: Non-target Trap Captures in Illinois.** C. Helm, I. Pearse, and K. Kruse.
- NAPPO Phytosanitary Alert System.** C. Hurt

## MISSING PRESENTATION ABSTRACTS FROM THE 2004 USDA RESEARCH FORUM

- 1) **“Abating the threat of invasive species -- linking science and policy,”** Ann Bartuska, USDA-FS, Washington, DC
- 2) **“Economic analysis for invasive risk management: what makes a treatment strategy cost-effective?”** Lisa Wainger, University of Maryland Center for Environmental Science, Solomons, MD
- 3) **“Effects of invasive forest pests on nutrient cycling,”** Gary Lovett, Institute of Ecosystem Studies, Millbrook, NY
- 4) **“Sudden oak death (SOD) - where now, what next?”** Kerry Britton, USDA-FS, Arlington, VA
- 5) **“Pollinator visitation to the exotic Japanese honeysuckle: Impacts on fruit set and vegetative growth,”** Katherine Larson, University of Central Arkansas, Conway, AR
- 6) **“Japanese oak wilt as a newly emerged forest pest in Japan: Why does a symbiotic ambrosia fungus kill host trees?”** Naoto Kamata, Kanazawa University, Kanazawa, Ishikawa, Japan
- 7) **“Use of satellite imagery to monitor HWA impacts,”** Denise Royle, Rutgers University, New Brunswick, NJ
- 8) **“Role of terpenoids in hemlock resistance to HWA,”** Anthony Lagalante, Penn State University, Scranton, PA
- 9) **“Frequency and severity of spruce aphid, *Elatobium abietinum* (Walker) in western North America,”** Ann Lynch, USDA-FS, Flagstaff, AZ
- 10) **“The EPPPO project on quarantine pests for forestry: the current situation,”** Andrei Orlinski, European and Mediterranean Plant Protection Organization, Paris, France
- 11) **“Horizontal transmission of a non-liquefying strain of gypsy moth NPV,”** Vince D’Amico, USDA-FS, Newark DE
- 12) **“Behavior ecology of host selection by *Anoplophora glabripennis*: Implication for survey and detection,”** Michael Smith, USDA-ARS, Newark, DE
- 13) **“Tree stress and resistance to Asian longhorned beetle in the Chicago urban forest,”** Larry Hanks, University of Illinois, Urbana, IL
- 14) **“U.S. Program update,”** Craig Kellogg, USDA-APHIS, Brighton, MI
- 15) **“Research overview,”** Vic Mastro, USDA-APHIS, Otis ANGB, MA
- 16) **“Pesticide testing for emerald ash borer control,”** Deborah McCullough, Michigan State University, East Lansing, MI

## MISSING POSTER ABSTRACTS FROM THE 2004 USDA RESEARCH FORUM

- 1) **Assessment of the Control of Pine Processionary (*Thaumetopoea pityocampa*) By the Exosex Auto-Confusion System.** J.C. Martin, F. Pouvillon and K. Underwood.
- 2) **Imidacloprid Distribution Over Time Within a Norway Maple Tree Canopy.** P. Lewis.
- 3) **Comparison of Residue Levels in Green Ash and Norway Maple Between Six Trunk Injection Systems.** P.A. Lewis, D.M. Cowen, J.J. Molongowski.
- 4) **Information System on 100 Invasive Plants from Asia.** Z. Hao, D. Jianqing, Y. Wu, D. Binion, and R. Reardon.
- 5) **Feasibility of Noncontact Ultrasound as a Method to Detect Insects in Solid Wood Packing Material.** M. Fleming, M. Bhardwaj, J. Janowiak, J. Shield, R. Roy, D. Agrawal, L. Bauer, D. Miller, and K. Hoover.
- 6) **Efficacy and Treatment Parameters for 2.45 GHz Microwave Irradiation of Cerambycids in Laboratory-size Wood Samples.** M. Fleming, Y. Fang, Y. Wang, L. Zu, B. Kappes, D. Whitmore, J. Janowiak, J. Shield, J. Kearns, R. Roy, D. Agrawal, L. Bauer, D. Miller and K. Hoover.
- 7) **Host Range of the Emerald Ash Borer (*Agrilus planipennis*) in North America: Preliminary Results from Laboratory and Field Tests.** A. Agius, D.G. McCullough, D.L. Cappaert, T. Poland, D. Miller, L. Bauer.
- 8) **Dispersal of the exotic emerald ash borer from a point source.** D.G. McCullough, T. Poland, D. Cappaert.
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- 10) **Biology of *Scymnus ningshanensis*.** M. Montgomery et al.
- 11) **Monitoring Hemlock Woolly Adelgid Damage to Hemlock Crowns in the Delaware Water Gap Using Ground Plots and Satellite Imagery.** M. Montgomery, D. Royle, B. Onken and A. Evans.
- 12) **Entomophthorales Infecting the Green Spruce Aphid *Elatobium abietinum* Walker in Iceland and Arizona, USA.** C. Nielsen, J. Eilenberg, G. Halldorsson, S. Harding, A.M. Lynch and E. Oddsdottir.
- 13) **Preliminary Analysis of Mitochondrial DNA Sequence Data for *Anoplophora*: Implications for Source Population Determination of Asian Longhorned Beetle (*A. glabripennis*) in the United States.** M.E. Carter, E.R. Hoebeke, R.G. Harrison, S.M. Bogdanowicz, M. Keena and A. Sawyer.
- 14) **The National Exotic Wood borer/Bark Beetle Survey: Non-target Trap Captures in Illinois.** C. Helm, I. Pearse, and K. Kruse.
- 15) **NAPPO Phytosanitary Alert System.** C.Hurt

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# GENETIC ASPECTS OF INVASIVE LEAF MINERS—EXAMPLES OF THREE SPECIES INTRODUCED RECENTLY TO EUROPE

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## Abstract

Three invasive leaf miner moth species *Parectopa robiniella* Clemens 1863, *Phyllonorycter robiniella* Clemens 1859 and *Cameraria ohridella* Deschka et Dimic 1986 using mtDNA sequences and PCR-RAPD were investigated during this study. Populations were from North America and Europe.

All three, invasive species belong to the Gracillariidae (superfamily Gracillarioidea), a large, cosmopolitan family of over 2000 recognized species, with probably even greater number of species awaiting discovery. The genus *Parectopa* is a member of the largest, but far most diverse subfamily, Gracillariinae. *Phyllonorycter* and *Cameraria* are the largest genera within the Lithocolletinae, a subfamily currently including approximately 10 genera. *P. robiniella* and *Ph. robiniella* originated from North America. *C. ohridella* was first described in 1986 from the Ohrid lake region in Macedonia and in only a few years the moth dispersed over the entire European continent.

We used mitochondrial DNA markers for screening genetic diversity. 500-600 bp long fragments of the COI

gene were sequenced. A significant variation of 0.7% in the mtDNA was detected in *P. robiniella* and in that species the European and North American populations could be separated. The statistical analysis indicates that the Hungarian haplotypes are derived from the American ones. In the other two species, *Ph. robiniella* and *C. ohridella* no substitutions could be detected among the populations. The complement PCR-RAPD analysis in *C. ohridella* revealed differences in the genetic similarities according to their spreading. However, more populations and markers have to be analysed to obtain a more stable dataset.

The origin of *C. ohridella* is still not known. Thus it might be reasonable to investigate either the host tree or the origin of closely related species living on the same tree. Both in North America and in Asia (Japan) *Cameraria* species can be found on different *Aesculus* species. Regarding host switches from *C. ohridella*, species living on *Acer* (e.g. *A. rubrum* L.) like *Cameraria aceriella* Clem. should be taken into consideration for analysis.

# COMPARISON OF RESIDUE LEVELS IN GREEN ASH AND NORWAY MAPLE BETWEEN SIX TRUNK INJECTION SYSTEMS

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## Abstract

The objective of this research project is to develop an effective direct trunk injection technique for delivery of systemic insecticides for control of Asian longhorned beetle and emerald ash borer larvae. Comparative factors examined include injection method, ease of use of injection device, duration and pressure of injection, tree wounding and resultant pesticide residue levels in the injected trees. In September of 2002, the current method (Mauget capsules) was compared to five other injection systems in Norway maple and green ash. Several formulations of imidacloprid were also compared, using several of the injection systems: Imicide 10%, Imicide 25%, Pointer 10% and Imajet 5%. The wedgle device injected Pointer 20 and 40%. Injection dose was varied in order that a similar amount of active ingredient was delivered to each tree.

Systems were partially evaluated based on ELISA readings of tree sap insecticide residue after 1 month and 1 year post-injection. Imicide 25% failed to increase imidacloprid residue levels in either tree species as compared to the 10% formulation when using the Sidewinder and Arborjet systems. The Pointer 10% formulation, used in four of the systems, consistently resulted in very low values that were not significantly different from the control trees. Residue levels for the Quik-Inject System were similar to Mauget in maple, but much less in ash. Both the Wedgle, USDA

tip and Sidewinder systems had significantly lower residue levels as compared to Mauget. The Arborjet injection method resulted in similar residue levels to the Mauget. Within an injection system, residue data were combined when they did not differ as a result of formulation injected. Due to the high variability between samples, no injection system differed significantly from one another, though some trends are apparent.

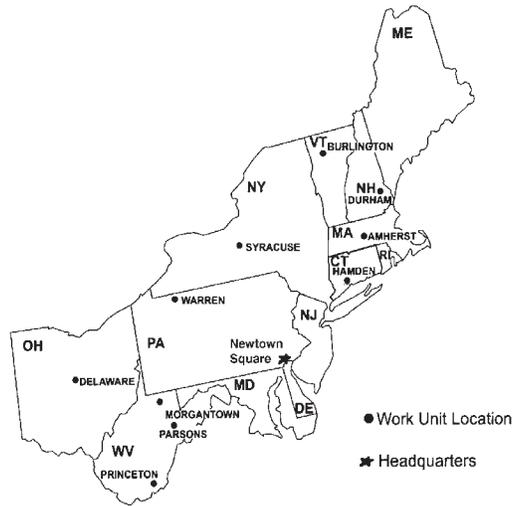
One year sampling was conducted in late August 2003. Three samples per tree were collected in order to reduce variability within treatments. Preliminary analysis of the results shows that residue levels have dropped off significantly from levels of the previous sampling. The Arborjet method resulted in significantly higher residue in ash with 10% Imicide and in maple with Pointer 10%. From these data, there is some indication that the Pointer formulations result in higher overall residue levels than other formulations after one year, though this is not consistent between treatments or tree species.

Damage to injection sites shows that Sidewinder and Arborjet injections cause significantly more vertical cracking in maple than the other systems. During injections the Wedgle method was observed to result in bark splitting and separation, though external damage is not evident.

Gottschalk, Kurt W., ed. 2005. **Proceedings, XV U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2004**; 2004 January 13-16; Annapolis, MD. Gen. Tech. Rep. NE-332. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 100 p.

Contains 61 abstracts and papers of oral and poster presentations on gypsy moth and other invasive species biology, molecular biology, ecology, impacts, and management presented at the annual U. S. Department of Agriculture Interagency Research Forum on Gypsy Moth and Other Invasive Species.





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