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# PINE SHOOT BEETLE IN MICHIGAN FORESTS

Deborah G. McCullough[1,2], Nathan W. Siegert[1,2], and Amy A. Kennedy[1]

[1]Department of Entomology, Michigan State University,
243 Nat. Sci. Bldg., East Lansing, MI 48824

[2]Department of Forestry, Michigan State University,
126 Nat. Resources, East Lansing, MI 48824

## ABSTRACT

Pine shoot beetle (*Tomicus piniperda* L.) (Coleoptera: Scolytidae) is a recently introduced bark beetle that is known to be established in at least 220 counties in the north central region of the U.S., and in several counties in the Canadian province of Ontario. Our current research addresses ecological and economic impacts of this exotic pest in Michigan red, jack and Scotch pine forest stands.

Studies were conducted in red pine stands in 1996 and 1997 to determine phenological activity of pine shoot beetle, in relation to native phloem-boring insects and native natural enemies. Results indicate that pine shoot beetle adults begin to colonize brood material activity early in the spring. This activity occurred at least 4-8 weeks before *Ips pini*, an abundant and well-known native scolytid, became active. However, at least two other native phloem-feeding insects, including the scolytid *Hylurgop rugipennis rufipes* and a *Pissodes* sp. weevil, were also active early in the spring. Four native predators of bark beetles were active early in the spring, including the important clerid predator *Thanasimus dubius*.

In a second study, we are assessing host preference of pine shoot beetle adults to determine if they are likely to preferentially colonize Scotch pine logs over red or jack pine logs. Preliminary results from a wind tunnel experiment and from a large scale field experiment suggest that Scotch pine may be somewhat preferred, but additional studies are needed.

We are also evaluating levels of shoot-feeding damage in Scotch, red and jack pine stands. Surveys of stands in southern Lower Michigan in 1998 indicated that more shoots were injured by pine shoot beetle in Scotch pine stands than in red or jack pine stands. Damage by pine shoot beetle and other shoot-feeding insects was relatively light, however, never exceeding one damaged shoot per $m^2$ of ground area. No shoots injured by pine shoot beetle were encountered in stands surveyed in northern Lower Michigan. Most shoots that were found on the ground during the surveys in southern and northern stands were damaged by non-insect related factors such as wind, ice, and squirrels. Additional surveys are planned for 1999, and laboratory studies will be set up to assess the preference of progeny adults for Scotch, red and jack pine shoots.

# BIOLOGICAL CONTROLS FOR THE HEMLOCK WOOLLY ADELGID

Michael E. Montgomery

USDA Forest Service, Northeastern Research Station, Northeastern Center for Forest Health Research, 51 Mill Pond Rd., Hamden, CT 06514

## ABSTRACT

In this paper, I review the progress to obtain biological controls for the hemlock woolly adelgid, *Adelges tsugae* Annand. Adelgids have no known parasites and no significant diseases. Predators in several insect families attack adelgids and many of these are specialists on the Adelgidae.

Specialist predators of adelgids are known to occur in the families: Coccinellidae (lady beetles), Derodontidae (tooth-necked fungus beetles), and Chaemyiidae (aphid flies). The families Cecidomyiidae (gall midges), Anthocoridae (minute pirate bugs), and Syrphidae (flower flies) have species that are frequently found feeding on adelgids, but it is not known if any of these species feed only on adelgids.

Of the 35+ known species of adelgids in the world, only three are native to eastern North America. Because of the depauperate adelgid fauna in this region, there are few known native predators that specialize on adelgids. The derodontid beetle, *Laricobius rubidus* Leconte, may be the only known species native to eastern North America that specializes on adelgids. One or more chaemyiid flies of the genus *Leucopis* may be specific on adelgids, but additional work is needed on the host range and taxonomy of species in this genus before conclusions can be made. Although lady beetles are important members of the adelgid predator guild in Europe and Asia, there are no known native species of lady beetles in North America that specialize on adelgids.

For the past three years, I have been searching for natural enemies on the hemlock woolly adelgid in China. This research is done cooperatively with scientists from the Chinese Academy of Forestry in Beijing. We have focused on lady beetles and so far more than 50 species of lady beetles have been found in China on hemlocks infested with *A. tsugae*. Twenty-five of the species are new to science. Three of these have been imported and studied at the USDA Forest Service Quarantine Laboratory. One species, *Scymnus sinuanodulus* Yu and Yao, has been mass-reared and is ready for release. It differs from *P. tsugae* in that it is univoltine and is active very early in the season. Both lady beetles most likely will be efficient at maintaining the adelgid at low densities as they diversify the adelgid predator guild in North America. In China, the few patches of dense adelgids that I found usually were attacked by Diptera, particularly Cecidiomyiids. These and other species of the adelgid predator guild from China also should be considered for potential importation.

# 1998 SUMMARY OF LYMANTRIID MONITORING PROGRAM

# IN THE RUSSIAN FAR EAST

A. Steve Munson

USDA Forest Service, Forest Health Protection, R-4, 4746 S. 1900 E., Ogden, UT 84403

## ABSTRACT

A cooperative monitoring program began in 1993 to determine population intensity and flight periodicity for three Lymantriid species in Russia's Far East ports. Populations of the three species of Lymantria, Asian gypsy moth, *Lymantria dispar* (L.), Nun moth, *L. monacha* (L), and rosy or pink gypsy moth, *L. mathura* (Moore) were assessed using a variety of monitoring techniques. The information obtained from the monitoring program has been used to develop a database to determine levels of infestation and regulatory risk associated with ships and cargo within the ports.

The four federal agencies involved in the monitoring program include the Russian Federal Forest Service, State Plant Quarantine Inspection of Russian Federation, USDA Forest Service and the USDA Animal and Plant Health Inspection Service. Six Russian ports participate in the monitoring program; Vladivostok, Nakhodka, Vostochny, Slavyanka, Olga and Vanino. The latter three are smaller ports with only a limited monitoring network of 10 milk carton pheromone traps baited with disparlure and placed within port boundaries.

In the three larger ports of Vladivostok, Nakhodka and Vostochny, milk carton pheromone traps were used to monitor adult male flight of Asian gypsy moth (AGM) and the Nun moth. Black light traps were used in the forest and port areas to capture Lymantriid adults. Permanent plots were established in forested sites near the port areas to monitor larval stages and the incidence of natural enemies. Egg mass counts were conducted at all traps sites where adult Asian gypsy moth catches exceeded 100 moths.

Results of the 1998 monitoring program indicate populations of AGM continue to increase in all three of the larger ports. Following six years of flight periodicity data, the flight period begins around July 15 and ends near September 15. Peak flight generally occurs over a two week period from early to late August, depending on weather. The average number of male moths caught per trap have increased significantly since 1996, from 140 moths per trap to 385 in 1998. In 1996, egg mass numbers for the larger ports totaled 16. In 1998, total egg mass counts rose in all ports, particularly Vostochny. In this container port alone, egg mass numbers grew from 29 in 1997 to 391 in 1998. The highest egg mass density exceeded 2,000 egg masses/ha in a forested site near Vostochny. Nucleopolyhedrosis virus (NPV) accounted for most of the natural mortality of

AGM and the pink gypsy moth. Approximately 16 percent of the AGM and eight percent of the pink gypsy moth specimens died as a result of this viral infection in 1998. Hymenopteran and Dipteran parasites were the most frequently observed affecting larvae of *L. dispar* and *L. mathura*.

*L. mathura* populations began to increase in 1998 in all of the larger ports. The flight period of this forest defoliator peaked between late July and mid-August. Most of the *L. mathura* activity was observed near the ports of Nakhodka and Vostochny. *L. monacha* populations remain at endemic levels in all six ports and have not increased significantly since 1993. Flight periodicity for *L. monacha* closely resembles the flight period discussed previously for AGM. In the smaller ports, AGM populations have increased only near the port of Slavyanka. Populations of AGM are at endemic levels in Olga and Vanino. Populations of *L. mathura* are also at endemic levels in all three smaller ports.

In 1997, 82 ships were inspected by Russian Plant Quarantine. Of these, 11 ships were found to have egg masses. In 1998, 100 ships were inspected and 12 ships were infested with egg masses. Of the 23 infested ships, 19 were inspected during the high risk period of July 15 to September 15. Twelve of the 23 infested ships were inspected in the port of Vladivostok. Cooperative Agreement funding to continue the monitoring program has been extended through the year 2000.

# DENDROCHRONOLOGY AND GYPSY MOTH DEFOLIATION

Rose-Marie Muzika

School of Natural Resources, University of Missouri, Columbia, MO 65211

## ABSTRACT

Gypsy moth defoliation represents a form of disturbance that can result in tree growth loss of preferred species such as oak or aspen. Radial increment of host and non host species (1478 individual trees) from 1952 - 1976 were measured from samples collected in 6 sites located in Massachusetts, New Jersey and New York. During that period, the gypsy moth expanded its range through these areas and several outbreaks occurred. Defoliation levels were recorded at sample locations from 1972-1976; outbreaks occurred at each location during this period. Standardized chronologies for each species were averaged by year at each location. A difference series, i.e. subtraction of the non-host standardized chronology from the host standardized chronology, performed well as a measure of gypsy moth outbreak intensity. Examination of difference chronologies prior to 1972 indicated the occurrence of historical outbreaks in certain areas and these episodes appeared to coincide with historical outbreaks in the region. The use of difference chronologies appears to be a useful method for quantifying historical gypsy moth outbreaks when no other records exist. All oaks, but not all host tree species, exhibited a decrease in increment associated with defoliation levels recorded from 1972-1976. Below average radial increment occurred in all oaks in the year of defoliation and the year following defoliation. Other favorable hosts, e.g. paper birch, gray birch, basswood, and bigtooth aspen, were not affected by defoliation. Surprisingly, trembling aspen, appeared to have increased growth the year following defoliation. Compensatory growth was apparent in some, but not all non hosts. Stand level effects have been demonstrated by an increased increment in the immune species ash and yellow poplar, and the resistant species white pine and pitch pine.

# STAND AND LANDSCAPE LEVEL ANALYSIS OF HEMLOCK WOOLLY ADELGID OUTBREAKS IN SOUTHERN NEW ENGLAND

David A. Orwig, David R. Foster, and Matthew L. Kizlinski

Harvard Forest, Harvard University, Petersham, MA

## ABSTRACT

Hemlock woolly adelgid (HWA) (*Adelges tsugae*), an introduced aphid-like insect from Asia, is expanding across the northeastern United States through the range of *Tsuga canadensis* (eastern hemlock) and has the potential to severely reduce or eliminate this important late-successional species. As part of a large project investigating stand to landscape forest dynamics resulting from HWA, we examined the initial community response of eight *T. canadensis* stands in south-central Connecticut for three years following various levels of infestation. We assessed mortality patterns in *T. canadensis*, evaluated subsequent changes in stand environment, and related these and stand composition to patterns of regeneration, understory response, and community reorganization. The dominant vegetative response following crown thinning and tree mortality was a prolific establishment of *Betula lenta* (black birch).

At the landscape level, we have mapped the distribution of *T. canadensis* prior to HWA infestation in a 5900 $km^2$ transect through southern New England to characterize the temporal and spatial patterns of damage generated by HWA since the time of its arrival. Data from over 125 stands has been used to describe forest structure and composition and predict potential replacement species. Eighty percent of the stands contained HWA and over 70% had experienced *T. canadensis* mortality. The spatial pattern of HWA-induced damage currently exhibits a distinct south to north trend in decreasing damage and mortality consistent with HWA migration patterns.

We have recently initiated a project examining the timing, magnitude, and duration of nitrogen cycling changes associated with HWA infestation in a subset of *T. canadensis* forests varying in HWA density. All data from this multi-faceted project is being incorporated into a GIS analysis of the landscape-level, biological, edaphic, and historical factors that control the spread of mortality and stress observed in *T. canadensis*.

# ADAPTATION OF THE PEST RISK ASSESSMENT PROCESS FOR PINE SHOOT BEETLE SPREAD WITHIN THE UNITED STATES

Judith E. Pasek

USDA Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology, Raleigh Plant Protection Center, 1017 Main Campus Dr., Suite 2500, Raleigh, NC 27606-5202

## ABSTRACT

Pest risk assessments (PRA) are science-based evaluations that provide a link between scientific data and decision-makers considering changes in regulations. It is the first step in pest risk analysis, which also includes pest risk management and pest risk communication. The standard pest risk formula includes an evaluation of the likelihood of introduction plus consequences of introduction. Elements are typically rated as low, medium, or high and corresponding values are added to assign an overall rating for pest risk potential. Assessment of the consequences of introduction is based upon biological characteristics of the organism and is described by five rating elements: climate-host interaction, host range, dispersal potential, economic impact, and environmental impact. Elements typically evaluated to determine the likelihood of introduction for an organism traveling with a commodity include the quantity of commodity shipped, and likelihood of surviving post harvest treatment, surviving shipment, not being detected, being moved to a suitable environment, and contacting host material.

A PRA was conducted to evaluate potential risk for spread of pine shoot beetle (PSB), *Tomicus piniperda* (L.), from the currently quarantined counties in the Lake States and northeastern US to other regions of the continental US. Some modifications in the generic process were incorporated to assess likelihood of introduction for PSB to southern and western pine forests. Values for element ratings were multiplied rather than added to better reflect the probabilistic nature of the estimates and reduce the overestimation of risk that can result from equal weighting of elements through addition. Also, consideration was given to differences in population levels in the element for likelihood of contacting host material, which might be better described as likelihood of establishing in host material. Three main pathways (or commodities) were evaluated given that different life stages and habits of PSB are associated with each means of transport: logs and lumber (immatures under bark), Christmas trees (adults in trunk bases), and nursery stock (adults in shoots). Additionally, each pathway was evaluated given current regulations (i.e., quarantine, inspection, compliance management program, and treatment requirements) compared to the absence of regulations restricting movement of infested materials. Likelihood of introduction was found to be medium for all three pathways under current regulations. Without regulation, risk was determined to remain medium for Christmas trees, but

became high for logs and lumber and nursery stock. Evaluation of consequences of introduction resulted in a rating of medium risk of damage by PSB if it spread to southern or western pine forests. Resultant pest risk potentials for PSB ranged from medium to high. Information presented in the PRA will be considered by regulatory officials and plant board members in conjunction with pest risk management considerations to determine whether changes in PSB regulations are warranted.

# DISPERSAL OF PINE SHOOT BEETLES AFTER OVERWINTERING AND POTENTIAL DISRUPTANTS OF ATTRACTION

Therese M. Poland and Robert A. Haack

USDA Forest Service, North Central Research Station, Rm. 220,
1407 S. Harrison Rd., E. Lansing, MI 48823

## ABSTRACT

The pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae), is a native pest of pine trees in Europe and Asia. It was first discovered in North America in 1992 and is currently regulated by a federal quarantine that controls movement of pine material from infested counties to uninfested counties within the US. A National Compliance Program was initiated in 1997 to allow nursery and Christmas tree growers in regulated areas to ship pine trees to unregulated areas if they follow a series of strict management guidelines. Several sawmill managers in unregulated areas have asked to receive logs year-round from regulated areas. If reproductively - active *Tomicus* adults do not leave a millyard when surrounded by freshly cut pine logs, then such a change may pose little risk of spreading *Tomicus*. A field study was conducted in order to gain a greater understanding of the potential for *Tomicus* movement within and outside of millyards. Overwintering *Tomicus* adult beetles were self-marked and released from a central log pile containing infested logs coated with fluorescent powder. Their dispersal was evaluated by capturing beetles in multiple-funnel traps baited with alpha-pinene and by locating galleries of marked beetles on surrounding log piles set out at different distances. Beetles were captured in the funnel traps up to 230 m from the release log pile. A total of 481 *Tomicus* galleries were found in all of the logs from four simulated millyards. Most of the galleries (89%) were found on logs in the central log piles; however, galleries were found on logs up to 100 m from the release log piles. These results indicate that if overwintering *Tomicus* adults were transported to millyards in infested pine material, then when temperatures warm, these adult beetles will almost always reproduce in the same logs or in nearby logs within the millyard. However, some beetles will leave the millyard and may pose a significant threat of becoming established in nearby pine stands. The use of semiochemicals may enhance the efficacy of management tactics for the pine shoot beetle. For instance, disruptive semiochemicals may deter beetles from attacking susceptible hosts for brood production or maturation shoot-feeding. Green leaf volatiles (GLVs) are prevalent in herbaceous plants and deciduous trees and generally disrupt attraction of conifer-infesting bark beetles. Field trapping experiments were conducted in 1998 in infested scotch pine Christmas tree plantations in Michigan and Indiana to test GLVs against the pine shoot beetle. Twelve-unit multiple-funnel traps were baited with $\alpha$-pinene lures alone or combined with various combinations of common GLVs and specific volatiles from aspen bark and foliage which elicited antennal responses by the pine shoot beetle. The common GLV alcohols and alcohols from aspen bark and foliage disrupted pine shoot beetle attraction by 54% and 74%, respectively. Therefore, GLVs and non-host volatiles may have considerable potential for managing *Tomicus* by disrupting host location.

# OUTBREAK OF THE FOREST COCKCHAFER *MELOLONTHA HIPPOCASTANI* F. (COLEOPTERA: SCARABAEIDAE ) IN THE HESSIAN RHEIN-MAIN-PLAIN

Martin Rohde

Hessian Agency of Forest Management, Forest Research, and Forest Ecology,
Prof.-Oelkers-Str. 6, 34346 Hann. Münden, Germany

## ABSTRACT

In the Hessian Rhein-Main-Plain forestry has to face a natural focus of *Melolontha hippocastani* F. The four-year-lasting development from the egg to the adult is enclosed in a very long population cycle with culminations approximately every 30-40 years. Serious silvicultural damage during an outbreak can occur for more than 20 years. The last outbreak lasted from the end of the forties to the midth of the fifties. The actual one started in the midth of the eighties in the forest districts of Darmstadt, Bensheim, and Lampertheim. Up to now the infestation area is about 6500 hectares of which nearly 2500 hectares of young plantations, thickets, polewoods, and deciduous understory in pine stands are extremely endangered by feeding of the larvae (white grubs) on the roots of the trees (all tree species are accepted). Small root fibres are destroyed, stronger main roots are peeled so that the trees die.

Extension and development of the chafers and the white grubs are supported by natural and anthropogenous factors (dry and sandy soils, low precipitation, thinly stocked grass-covered stands, damage by windthrow, air pollution, soil acidification, high nitrogen inputs, lowering of the ground water level). In principle several mechanical, chemical, or biological control methods are possible, but all of them have some critical disadvantages: (1) Mechanical removal of live ground cover (food for the larvae) with a rotary hoe is effective only in young plantations and only for one flight period. Manual collecting of the adult beetles is ineffective and expensive. (2) Soil insecticides against white grubs are not registered or allowed for the use in forestry since 1988. Registered insecticides (pyrethroids) against adults are not very selective (side effects) and repeated treatment would be necessary on the whole infestation area with uncertain results. In addition the adult „may"-beetle is a too nice insect for airborne control measures due to its positive emotions in urban population and in German mythology (spring herald, fertility symbol). More selective means on the basis of natural substances (e. g. seed extracts from the neem-tree) are not yet registered as botanicals for application in forests. (3) The only suitable mean for the use as a biological insecticide in future could be the entomopathogenic deuteromycete *Beauveria brongniartii* (Sacc.) Petch. Several other pathogens up to now are effective only under laboratory conditions. Research is focused on improving more selective control methods (microcapsuled insecticides, biological insecticides), behavior of the cockchafer (chemical orientation and communication, habitat preferences) and silvicultural methods (fostering of natural regeneration and succession).

# CHRISTMAS TREE COMPLIANCE PROGRAM

Clifford S. Sadof

Department of Entomology, Purdue University, West Lafayette, IN 47907-1158

## ABSTRACT

We developed an integrated program to manage *Tomicus piniperda* in pine Christmas tree fields. Components of the program include sanitation to reduce availability of cut trees and stumps that serve as brood material, use of trap logs to attract parent beetles into cut trees which are subsequently destroyed, and an insecticide spray to control shoot-feeding beetles. Scouting throughout the production season is required with mechanical removal of infested shoots or insecticide sprays mandated upon beetle detection. This management program was formalized into a Compliance Program in cooperation with regulatory agencies and tested in 1995 and 1996 in a total of 48 fields in Michigan and Indiana. Indiana fields were conducted in heavily infested areas surrounded by Scotch Pine wind breaks. Indiana fields had an average of 79% of trap logs containing *T. piniperda* galleries before trap log destruction.

We periodically surveyed fields and growers to assess compliance with program requirements, levels of *T. piniperda* shoot damage, and feasibility of the management activities. In fields where all program requirements were completed, percentage of trees with *T. piniperda* shoot-feeding damage ranged from 0-4%. In comparison, shoot-feeding damage was observed on 28-67% of trees in unmanaged fields where brood material was available for colonization by parent beetles. Information provided by cooperating growers indicated that Compliance Program activities could be readily integrated into standard production practices.

After the national initiation of the Compliance Program in 1997, fields enrolled in the program are subject to less stringent inspections by state plant health regulatory officials than required before its initiation. Grower activity records are inspected to ensure that they have implemented all aspects of the compliance program. No infested trees have been reported by receiving states in the 1997 or 1998 growing season.

Still, as with any new program, growers can have some unexpected problems. Many growers enrolled in the Compliance Program were unable to ship trees because they failed the fall pre-harvest inspections. Warm temperatures in the first week of January 1998 caused many beetles to start their spring mating flight before growers had a chance to put out their trap logs. As such, logs placed in fields after January 7 trapped fewer beetles than in a normal year. Data taken from Plymouth, IN represent northern Indiana growing conditions.

*Number of Days above 53 °F in Plymouth, IN*

| Year | January | February 1-15 | Total |
|---|---|---|---|
| **1998** | 4 | 2 | 6 |
| **1997** | 3 | 0 | 3 |
| **1996** | 2 | 1 | 3 |
| **1995** | 3 | 0 | 3 |

Preliminary research on the pine shoot beetle indicates that adult beetles will begin to emerge from breeding logs after 450-500 degree days base 50 degrees F. Based on weather station information in Plymouth, IN it seems likely that adults began to fly before the May 20 deadline. Degree day accumulation by May 20 was almost double that in previous years.

*Base 50 Degree Day Accumulation, in Plymouth, IN from January 1*

| Date | 1998 | 1997 | 1996 | 1995 |
|---|---|---|---|---|
| **February 1** | 5 | 5 | 3 | 6 |
| **March 1** | 11 | 6 | 20 | 6 |
| **April 1** | 92 | 29 | 37 | 73 |
| **May 1** | 213 | 74 | 131 | 149 |
| **May 10** | 313 | 103 | 187 | 204 |
| **May 20** | 487 | 152 | 293 | 275 |

In 1999, growers are being instructed to monitor degree-days to ensure that trap logs are destroyed prior to a 400 $DD_{50}$ cutoff and emergence of new brood beetles.

# VARIABILITY IN ALLOZYME LOCI AND FLIGHT CAPABILITY IN LABORATORY STRAINS OF *LYMANTRIA DISPAR* (LEPIDOPTERA: LYMANTRIIDAE)

Vicente Sánchez

USDA Forest Service, Northeastern Research Station, Northeastern Center for Forest Health Research, 51 Mill Pond Rd., Hamden, CT 06514-1777

## ABSTRACT

Flight by female gypsy moths, *Lymantria dispar* L., follows a broad clinal pattern along its Palearctic range with full flight in Eastern populations and no flying in the West. Increased global movement resulted in the inadvertent introductions of gypsy moth with flying females into areas of Eurasia and North America. Identifying such new introductions requires not only inventories of extant populations but also understanding how characters facilitating dispersal are associated among these populations. This study compares measures of genetic diversity with female flight capability to investigate their possible linkage. Laboratory strains of *L. dispar* that had been assayed for flight potential were selected. The egg masses were collected in eastern to western Eurasia from Russia-Mineralni, Russia-Black Lake, Lithuania-Juodkrante, and Germany-Lampertheim, and in North America from the United States-Connecticut. Genetic variability of each strain was determined using enzyme electrophoresis at 9 allozyme loci. The average of "no sustained" flight frequency from free flight studies (Wallner, Grinberg, and Keena, personal communication) and "no flip" frequency tests of a female moth's ability to right herself (Keena, personal communication) were used in calculating an index of female flight capability. This index, derived as the difference from unity of the average of the two frequencies, was graphically compared to the genetic variability for each strain. Genetic diversity was highest in the Russian-Mineralni strain, and polymorphism decreased in the more westerly populations, with the Connecticut strain having the lowest number of alleles per locus. Variability within strains was low and no single locus provided sufficient information to be considered diagnostic. An unpaired group mean weighted analysis of Nei distances affirmed that genetic diversity among gypsy moth increased toward the eastern Palearctic. A graphical analysis using scatter plots of genetic heterozygosity and flight capability suggests some positive association between increasing female flight and higher genetic variability. Genetic diversity in *L. dispar* has been shown to increase in the western Palearctic. However, earlier electrophoretic studies of the gypsy moth did not evaluate female flight capability as a crucial character. Although genetic variation increases for eastern Palearctic populations of gypsy moth, loci have not been identified that link with female flight capability. Genetic diversity describes characteristics of population and not of individuals, consequently hierarchical analyses at many levels of resolution are needed to tease out any possible linkages between female flight and allozyme loci.

# BIOCHEMISTRY OF *TSUGA* TAXA IN RELATION TO THE HEMLOCK WOOLLY ADELGID

Frank S. Santamour, Jr.

USDA Agricultural Research Service, U.S. National Arboretum,
3501 New York Ave., N. E., Washington, DC 20002

## ABSTRACT

On 29 January 1997, I initiated biochemical studies in *Tsuga* (hemlock) to determine whether such research would prove useful in verifying putative interspecific hybrids or in establishing a chemical basis for resistance or susceptibility to the hemlock woolly adelgid (HWA; *Adelges tsugae* Annand). Although this early work concentrated on the needles produced during the previous growing season on mature specimens used as parents by Susan E. Bentz and A. M. Townsend (USNA) in their hybridization project, young plants of some other hemlock taxa and some older cultivars of *T. canadensis* (L.) Carr. were also included. By 30 February 1997, it was obvious that the needle chemistry of *T. canadensis* was significantly different from that of all the other taxa that were analyzed: *T. caroliniana* Engelm., *T. chinensis* (Franch.) Pritz., *T. diversifolia* (Maxim.) Mast., *T. dumosa* (D. Don) Eichl., *T. heterophylla* (Raf.) Sarg., *T. mertensiana* (Bong.) Carr., and *T. sieboldii* Carr. One compound (chlorogenic acid) appeared to be unique to *T. canadensis* and another (delphinidin) seemed to be absent from *T. canadensis* but present in all of the other species. This situation appeared to be ideal for the verification of hybrids involving *T. canadensis* if these compounds were inherited in a codominant fashion and if the juvenility of the supposed hybrid seedlings was not a problem.

On the other hand, there was a question of whether the compound unique to the needles of *T. canadensis*, which is highly susceptible to HWA, might be related to this susceptibility. In fact, it was uncertain whether chemicals in the needles would even be encountered by the adelgid nymphs during stylet insertion and feeding. The literature was somewhat ambiguous on this point. Young et al. (Ann. Entomol. Soc. Amer. 88:827-835, 1995) stated that "the adelgids always inserted their stylets into the needle petiole distal to the point of attachment to the stem" and that "this region contains a vascular bundle with secondary xylem and rays consisting predominately (sic) of ray parenchyma." Further, they stated that "the tissue on which *A. tsugae* fed was almost always the xylem ray parenchyma."

Following discussions with Dr. Kathleen S. Shields (USDA-FS) in March 1997, it was determined that the site of stylet insertion was nearly always in the "leaf cushion," a woody peglike projection or pulvinus, which is actually a part of the stem. Such leaf cushions are more pronounced in *Picea* (spruce) than in hemlock. Although vascular bundles are present in the

needles of conifers, they do not contain secondary xylem or ray parenchyma (or pith). Thus, Fig. 5 in Young et al. (1995) represents a cross-section of a leaf cushion and illustrates the xylem ray parenchyma cells that are the adelgid feeding sites.

Inasmuch as the observations on stylet penetration reported by Young et al. (1995) had been made on only *T. canadensis*, it was important to determine adelgid behavior on other susceptible species. On 20 May 1997, I shipped infested branches of *T. caroliniana* and *T. sieboldii* to Dr. Shields. Since no new shoot growth has yet taken place on these trees, the adelgid nymphs were located on shoots produced in 1996. It was determined that stylet insertion on these hemlock taxa was also predominantly in the leaf cushion.

Thus, although the differences in needle chemistry that had been found among hemlock taxa might be useful in the verification of certain hybrids, they are probably of no significance in HWA resistance or susceptibility. The leaf cushions are, as noted earlier, part of the stem, but even on fairly stout 1-year-old twigs they protrude only about 1 mm from the twig surface. Fortunately, the chemistry of the leaf cushions is the same as that of the stem, and we are currently analyzing 1-year-old stems, concentrating on the condensed tannins, or more properly, the condensed proanthocyanidins. One of the major properties of such compounds is their astringency, which translates into their ability to precipitate proteins. Thus, the abundance or nature of the condensed proanthocyanidins in bark and wood could be related to HWA resistance/susceptibility by interfering with normal adelgid development. One sideline to this research is the discovery of a totally novel compound in *T. canadensis*, although it may only be peripherally related to our general objectives.

# PINE SHOOT BEETLE DAMAGE IN ONTARIO

Taylor A. Scarr[1], Ed J. Czerwinski[1], and Gordon M. Howse[2]

[1]Ontario Ministry of Natural Resources, Forest Health and Silviculture Section, Forest Management Branch, 70 Foster Dr., Sault Ste. Marie, Ontario, Canada P6A 6V5

[2]Canadian Forest Service, Great Lakes Forestry Centre, 1219 Queen St. East, Sault Ste. Marie, Ontario, Canada P6A 5M7

## ABSTRACT

Pine shoot beetle (*Tomicus piniperda* (L.)) was first discovered in Ontario in 1992. Subsequent surveys by the Canadian Food Inspection Agency (CFIA), Canadian Forest Service (CFS), and Ontario Ministry of Natural Resources (OMNR) have found the beetle in 24 counties. The CFIA has imposed a quarantine on the movement of pine roundwood, bark, and Christmas trees out of infested counties. Until 1998, no appreciable damage by pine shoot beetle had been reported. In August 1998, surveys conducted under the OMNR-CFS Partnership in Forest Health Monitoring detected high population levels of pine shoot beetle in southwestern Ontario. Scots pine, as well as jack, red, and white pine were attacked. Stands of attacked trees turned red because of shoot or tree mortality. These stands were detectable during aerial surveys. In some stands, sources of brood material (e.g. dead trees, piled logs) were evident, or other contributing factors (notably Diplodia tip blight) were present. In other stands, no obvious sources of brood material were present. In December 1998, OMNR conducted detailed assessments of tree mortality and shoot attack rates in eight stands. One Scots pine stand showed almost 100% mortality. In the other 7 stands, mortality caused by pine shoot beetle ranged from 0% in a white pine stand adjacent to a Scots pine stand, to 28% in stands of Scots pine or Scots pine mixed with white pine or jack pine. In the mixed stands, mortality was roughly proportional to host species composition. Mortality caused by other factors ranged from 2% to 8%. Counts of dropped shoots on the ground ranged from 38 shoots/10m$^2$ to 118 shoots/10m$^2$. Age of shoot attacked varied by host species. Percentage of attack on current, 1 year old and 2 year old shoots were: Scots pine, 50/48/2; white pine 45/50/5; jack pine 28/63/9. Shoot counts were not necessarily correlated with tree mortality. Stands with high tree mortality actually showed reduced shoot counts, because tree death prevented any more shoot attack. Further studies are planned to investigate this damage. If this beetle does not require Scots pine to be present in the stand for it to cause these high levels of tree mortality, it will pose a serious threat to managed forests of native pines. Situations providing brood material (e.g harvest operations, stand thinnings or spacings, block cuts, fires, blowdown) or resulting in stressed trees (e.g. defoliation by other insects) could then contribute to serious pine shoot beetle problems.

# GEOGRAPHIC VARIATION IN THE PROCESS OF GYPSY MOTH RANGE EXPANSION

Alexei A. Sharov [1], Andrew M. Liebhold [2], E. A. Roberts [1], and Nick Clemens [3]

[1]Department of Entomology, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061-0319

[2]USDA Forest Service, Northeastern Research Station, 180 Canfield St., Morgantown, WV 26505

[3]Wisconsin Department of Agriculture, P.O. Box 8911, Madison, WI 53708

## ABSTRACT

The model of spread suggests that two factors have a major effect on the rate of population expansion: (1) the maximum distance at which isolated colonies become established, $x_{max}$, and (2) the rate of population increase in each colony, $r$. The objective was to test which of these factors can explain the geographic variation in the rate of spread of gypsy moth in the US.

The analysis was done individually in the mountains, piedmont, and coastal plain of Virginia, West Virginia, and North Carolina, and in 5 zones in Wisconsin separated by latitude. The rate of spread, rate of colony establishment, and rate of population increase was measured from moth captures in pheromone traps. Traps that caught $N$ or more moths ($N$ = 1, 2, 3, 5) were selected and then grouped if they were separated by <4 km. Each group of traps was considered a colony, and these groups were counted at various distances from the population front.

The rate of spread was highest in southern Wisconsin and in the Appalachian Mountains before the start of management of isolated colonies, and lowest in the coastal plain of Virginia and North Carolina and in northern Wisconsin. Colonies detected using the threshold of 5 moth/trap were found mostly within 250 km from the population front in all areas. But moth captures that exceeded the thresholds of 1-2 moth/trap, were found much farther from the 10-moth line in Wisconsin (up to 500 km) than in the Appalachian Mountains (up to 250 km). Apparently, the majority of traps that caught 1-2 moths in Wisconsin did not represent reproducing populations and resulted from long-range dispersal of male moths. The conclusion is that most gypsy moth colonies become established within 250 km from the defoliation front in all study areas. Thus, regional variations in the rate of spread can not be explained by the distance at which colonization occurs. Dispersal of male moths obscured annual measurements of the rate of population growth and expansion in several geographic areas; thus we used time averages in the analysis. The rate of population increase and the rate of spread, averaged over time in each geographic area, were strongly correlated. Thus, the rate of population increase appears to be an important factor explaining geographic variations in the rate of gypsy moth spread.

# A MUTATION IN THE Ld*M*NPV POLYHEDRAL ENVELOPE PROTEIN GENE REGION CAUSES ABNORMAL POLYHEDRON FORMATION

J. M. Slavicek, M. E. Kelly, M. Mercer, and N. Hayes-Plazolles

USDA Forest Service, Forestry Sciences Laboratory,
359 Main Rd., Delaware, OH 43015

## ABSTRACT

During investigations on the formation of Ld*M*NPV few polyhedra mutants a virus exhibiting an abnormal polyhedron morphology was identified and designated as isolate PFM-2. Ld*M*NPV isolate PFM-2 generates abnormally large polyhedra that range from approximately 2-8 micrometers in diameter, either lack or possess a fragmented polyhedron envelope, and contain virions. The shape of PFM-2 polyhedra was often irregular, and sometimes exhibited protrusions or holes. Isolate PFM-2 produces approximately three fold fewer polyhedra compared to wild type (WT) virus. A greater proportion of Ld652Y cells infected with isolate PFM-2 contained polyhedra in comparison to cells infected with WT virus. Marker rescue studies localized the region containing the mutated gene(s) in isolate PFM-2 to the genomic area of approximately 130.7 to 134.0 kbp. This genomic region contains the *polyhedral envelope protein* (*pep*) gene and a number of other open reading frames. Marker rescue experiments are in progress to identify the mutated gene(s) responsible for the abnormal polyhedron formation phenotype of isolate PFM-2. Rescued PFM-2 virus generated WT polyhedra. However, a greater percentage of Ld652Y cells infected with rescued PFM-2 virus contained polyhedra compared to cells infected with WT virus. This result suggests that the abnormal polyhedron phenotype and the trait of an increased percentage of infected cells containing polyhedra exhibited by isolate PFM-2 are the consequence of different gene products.

# DELETION OF THE Ld*M*NPV ECDYSTEROID UDP-GLUCOSYL TRANSFERASE GENE ENHANCES VIRAL KILLING SPEED IN THE ULTIMATE LARVAL INSTAR OF INFECTED *LYMANTRIA DISPAR*

James M. Slavicek, Holly J. R. Popham, and Christopher I. Riegel

USDA Forest Service, Forestry Sciences Laboratory,
359 Main Rd., Delaware, OH 43015

## ABSTRACT

The *Lymantria dispar* multicapsid nucleopolyhedrovirus (Ld*M*NPV) is used on a limited basis as a gypsy moth (*L. dispar*) control agent. In an effort to improve the efficacy (i.e., killing speed) of the Ld*M*NPV we generated a recombinant viral strain (vEGT-) that does not produce the enzyme ecdysteroid UDP-glucosyltransferase (EGT). Previous studies have shown that deletion of the *egt* gene from the Ac*M*NPV genome generates a viral strain that causes a reduction in the time of death and in feeding of infected larvae. In this study we compared the potency and efficacy of vEGT- to wild-type virus, and the impact on larval weight gain in larvae infected with vEGT- and wild-type virus. The biological activity of vEGT- was determined through bioassay on *L. dispar* 1st, 4th, and 5th instar larvae. No significant difference was found in the LC50 values in larvae infected with vEGT- and control virus. The LT50 values of 1st and 4th instar larvae infected with vEGT- and wild-type virus were similar. In contrast, there was a significant decrease in the LT50 of approximately 33% in 5th instar larvae infected with vEGT- compared to larvae infected with wild-type virus. Female 4th and 5th instar larvae infected with vEGT- gained significantly less weight than larvae infected with wild-type virus. There was no significant difference in weight gain in 4th and 5th instar male larvae infected with either virus. Significantly more polyhedra were produced in 5th instar larvae infected with wild-type virus compared to larvae infected with vEGT-. Larval weight gain was used as an indicator of larval feeding activity through generation of FT50 values. Fifth instar larvae infected with vEGT- exhibited a decrease in the FT50 of approximately 32% compared to larvae infected with wild-type virus. These results suggest that larvae infected with vEGT- stopped gaining weight, and by inference stopped feeding earlier than larvae infected with wild-type virus. Deletion of the Ld*M*NPV *egt* gene generated an improved virus strain since the killing speed of vEGT- is significantly faster than wild-type virus in 5th instar *L. dispar* larvae, and larvae infected with vEGT- stopped gaining weight earlier than larvae infected with wild-type virus. In addition, since the majority of foliage consumption by *L. dispar* larvae occurs in the 5th instar the use of vEGT- may offer better foliage protection than wild-type virus in the field.

# *ANOPLOPHORA GLABRIPENNIS* (MOTSCHULSKY):

# FIELD BEHAVIOR AND NATURAL ENEMIES IN CHINA

Michael T. Smith[1], Ruitong Gao[2], Yang Zhong-qi[2], and Baode Wang[3]

[1]USDA Agricultural Research Service, Beneficial Insects Introduction Research Unit, 501 S. Chapel St., Newark, DE 19713

[2]Institute of Forest Protection, Chinese Academy of Forestry, Beijing, CHINA 100091

[3]USDA Animal and Plant Health Inspection Service, Plant Protection Quarantine, Otis Plant Protection Center, Building #1398, Otis ANGB, MA 02542

## ABSTRACT

Development of management strategies for control of *A. glabripennis*, including eradication, biological control, bait trees, breeding and improved silvicultural practices, all require a fundamental understanding of its biology, behavior and ecology. *A. glabripennis* has been studied for at least the past four decades in China, during which time much has been published on its biology and its control, primarily with insecticides. While in-depth investigations of its host plant range are limited, some progress has been made in the areas of bait trees, host plant resistance and breeding, particularly within *Populus* species. However, very little is known about *A. glabripennis* behavior, ecology and host-plant interactions (i.e. preference). In regard to the natural enemies of *A. glabripennis*, only a few species have been identified. Furthermore, no systematic exploration for their natural enemies has been conducted, and investigations of their fundamental biology, behavior, ecology and efficacy are lacking. Therefore, in an effort to take a proactive approach towards the development of biological control of *A. glabripennis*, as well as to provide basic information on *A. glabripennis* which is essential for the other management strategies, investigations of its behavior and natural enemies in China were collectively undertaken during 1998.

Behavioral investigations of *A. glabripennis* were initiated in Hebei, Gansu and Ningxia Provinces of China. While such studies are needed at several ecological levels, i.e. within-tree, among tree and among infestation, as well as in different habitats (i.e. windrows, plantations, forests), emphasis was placed on the within-tree behavior of male and female adult beetles. The primary behaviors under consideration were adult feeding, courtship and mating, and oviposition. While the data collected to date are both descriptive and quantitative, they are preliminary in nature, and therefore require further replication. However, these studies will be expanded to include larval feeding behavior and adult emergence, both of which may play key roles in natural enemy efficacy (i.e. search behavior and ecological synchronicity). Investigations of *A.*

*glabripennis* dispersal among trees, also initiated in 1998, will be intensified. As such, the temporal and spatial aspects of tree colonization are being elucidated.

The natural enemies of *Anoplophora* in China and Japan reported to date are within the Eulophidae (egg parasitoid), Colydiidae (larval parasitoid) and Braconidae (larval parasitoid). Additionally, the natural enemies of other cerambycids in China and Japan are within the Encyrtidae (egg parasitoid), Bethylidae (larval parasitoid) and Ichneumonidae (larval parasitoid). However, given both the limited number of known natural enemies of *Anoplophora*, and the lack of detailed knowledge on these few known species, investigations of natural enemies for biological control of *A. glabripennis*, as well as other *Anoplophora* species, will include identified natural enemies of other cerambycid species which are native to Asia and the U.S. However, as efforts will focus on those species with the greatest probability of providing biological control of *Anoplophora*, selection of these natural enemies will be prioritized according to the relatedness of the respective cerambycid hosts to *Anoplophora* at three levels: (1) phylogenetic relatedness; (2) ecological relatedness; and (3) behavioral relatedness. Certain aspects of each of these likely play a major role in determining the potential efficacy of a given natural enemy to control *A. glabripennis*, as well as other *Anoplophora* species. The specific objectives of this research will be: (1) to explore, collect, identify and characterize natural enemies and other biological control agents of *Anoplophora* species and other similar cerambycid species in China and the U.S., respectively, including investigations of host-parasitoid/predator relationships, host specificity, host-finding cues, dispersal behavior, and bioecological studies; (2) to determine the potential non-target impacts of the promising natural enemies; (3) to develop mass rearing methodology for the most promising natural enemies; and (4) to release and evaluate the most promising natural enemies, including investigations of both inoculative releases and the classical biological control approach.

In closing, as little or nothing is known about the biology, behavior and ecology of *A. glabripennis* within the U.S., reciprocal investigations to those discussed above for China are sorely needed.

# INVADING FORESTRY PLANT PESTS AND APHIS

Jeffrey N. L. Stibick

USDA Animal and Plant Health Inspection Service, Plant Protection and Quarantine,
Room 4C73, 4700 River Rd., Riverdale, MD 20737

## ABSTRACT

The Animal and Plant Health Inspection Service (APHIS) is an agency of the United States Department of Agriculture (USDA). It is charged with "Protecting American Agriculture." APHIS provides leadership in ensuring the health and care of animals and plants. The agency improves agricultural productivity and competitiveness and contributes to the national economy and the public health. APHIS has five major program areas: Plant Protection and Quarantine (PPQ), International Services (IS), Veterinary Services (VS), Animal Care (AC) and Wildlife Services (WS). PPQ is the program area which deals with plant pests, and consequently with exotic pests and pests of limited distribution in the United States. Some of these pests are pests of forests in whole or in part. Because these pests threaten the well-being of agriculture and/or of the national economy, they are matters of concern to APHIS. Some of the more prominent forestry pests with which APHIS has been involved with over the years are gypsy moth, pine shoot beetle and now the Asian longhorned beetle. In cooperation with other federal and state agencies, APHIS has attempted to do its share in controlling these pests and slowing their spread. Such assistance includes research in state of the art technology and methodologies that improve the effectiveness of plant health programs. APHIS also lays plans for pests not yet in the country or which have been repeatedly eradicated from the country. For forestry pests, such plans include those for (1) Pink Hibiscus Mealybug, which may attack many kinds of trees, including mulberry, locust, fig, and many kinds of fruit trees and ornamental bushes as well as a range of other plants; (2) Plum Pox Virus, which attacks many species of *Prunus*, including wild or cultivated sour cherry, blackthorn, and plum as well as a range of other plants; (3) Economic species of Tussock Moths (in preparation), which, of course, cover many different species of trees, including oak, birch, and poplar, to name just a few. APHIS has an open door information policy. We encourage people to learn about our activities, and we try to share as much information as possible. The USDA visitor center and outreach program at Riverside is located in the APHIS headquarters building in Riverdale, Maryland. The visitor program helps people to better understand USDA's role in an expanded global trade environment. The center is a branch of the USDA visitor center located in Washington, D.C. It is open during normal business hours and is the central contact point to receive domestic and international visitors to the Riverside complex. The staff provides assistance with information exchange, training programs, meetings, conferences, and other learning experiences. For additional information on APHIS, visit our Web page at http://www.aphis.usda.gov or write to USDA, APHIS, Unit 1, Distribution Center, 4700 River Rd., Riverdale, MD 20737-1232.

NATURAL ENEMIES IN LOW DENSITY GYPSY MOTH POPULATIONS

IN THE BIEBRZA NATIONAL PARK, POLAND

Lidia Sukovata

Forest Research Institute, Department of Forest Protection,
3 ul.Bitwy Warszawskiej 1920 r., 00-973 Warsaw, Poland

ABSTRACT

Studies of natural enemies in low density gypsy moth (*Lymantria dispar* L.) populations were started in Poland (in the Biebrza National Park) in 1998. The preliminary results by stage of gypsy moth development are presented. **Eggs:** So far no egg parasitoid was discovered in the studied area. Predation has been investigated by exposure of the egg masses obtained in the laboratory. They were exposed in early September 1998 at three sites. The rate of predation, which was estimated as the percentage of the egg mass removed by the time of survey (28 October), varied much between sites and within sites. The mean percentage of an individual egg mass destroyed ranged from 14 to 49%. The patterns of egg-mass damages let us suppose that in most cases the damages were made by birds, although no animals feeding on the egg masses were observed. **Larvae:** Parasitism was estimated by both sampling the larvae from the natural population (20 May, 3 and 24 June) and exposures (21 May-3 June and 9 June-24 June) of the trap-host larvae (Hérard, pers. comm.). The species composition of parasitoids and the peak sample percentage parasitism for each species are shown below. *Ceranthia samarensis* Vill. was found for the first time in Poland.

| Parasitoid Species | Larvae Sampled from | Trap-Host |
|---|---|---|
| *Apanteles liparidis* Bouché | 2.9 | 1.0 |
| *Apanteles melanoscelus* Ratz. | 2.1 | - |
| *Phobocampe unicincta* Grav. | 20.4 | 0.7 |
| *Lymantrichneumon disparis* Poda | 1.4 | - |
| *Hyposoter* sp. | 1.0 | - |
| *Parasetigena silvestris* R.-D. | 48.6 | 7.0 |
| *Blepharipa* sp. | 2.9 | 0.7 |
| *Ceranthia samarensis* Vill. | 1.0 | 14.2 |
| *Compsilura concinnata* Meigen | - | 27.5 |

Pathogens (*Nosema* sp. and NPV) and other, undetermined yet, agents caused 54.8% mortality in the first sample of larvae and this percentage decreased to 22.9% in the last sample. Only two specimens of nematodes emerged during the larvae rearings. The predation on larvae was recorded accidentally - 98% of exposed larvae in the cage not protected from birds just disappeared. We suppose that birds fed on them. **Pupae:** One hundred pupae had been exposed in the studied area for the period from 30 June-11 July. Most of them completely disappeared (68%) or were damaged (21%). Parasitoids (*P. silvestris*) emerged from only two pupae.

# GYPSY MOTH MATING DISRUPTION USING HERCON FLAKES WITH AND WITHOUT A STICKER

K. W. Thorpe[1], D. S. Leonard[2], V. C. Mastro[3], W. McLane[3], R. C. Reardon[4], P. Sellers[5], S. M. Talley[6], and R. E. Webb[1]

[1]USDA Agricultural Research Center, Insect Biocontrol Laboratory, Beltsville, MD 20705

[2]USDA Forest Service, Asheville, NC 28802

[3]USDA Animal and Plant Health Inspection Service, Otis Plant Protection Center, Otis Air National Guard Base, MA 02542

[4]USDA Forest Service, State and Private Forestry, 180 Canfield St., Morgantown, WV 26505

[5]USDA Forest Service, State and Private Forestry, Bridgewater, VA 22812

[6]Rockbridge County Gypsy Moth Program, Lexington, VA 24450

## ABSTRACT

Multi-year field plot studies have demonstrated that gypsy moth mating can be effectively disrupted, and subsequent populations reduced, by aerial applications of gypsy moth sex pheromone in a plastic flake formulation (Disrupt II, Hercon Laboratories, Emigsville, PA) (Leonhardt et al. 1996). Based on the results of early tests with hand-applied pheromone dispensers (Kolodny-Hirsch et al. 1990, Kolodny-Hirsch and Webb 1993), it was concluded that pheromone dispensers must be distributed throughout the forest canopy to be effective. To accomplish this with aerial applications of pheromone flakes, it is necessary to mix the flakes with a sticking agent prior to application. This process requires specialized equipment, and sometimes results in application problems. If the flakes could function effectively when applied without a sticker, the application process would be greatly simplified and costs reduced.

To determine if the addition of sticker increases the efficacy of Disrupt II applications, studies were conducted in Rockbridge County, VA in 1997 and 1998. Aerial applications of Disrupt II with and without sticker were made to isolated woodlots (1997) or plots established within forests (1998). Biological efficacy was determined by monitoring gypsy moth trap catch and mating success within each plot. Because earlier research indicated that pheromone dispensers that were distributed only near the ground did not disrupt mating in the canopies of trees, and since it is expected that the flakes without sticker would mostly fall to the ground, mating success was measured both at the ground level and in the canopy.

In 1997, twelve blocks consisting of isolated woodlots ranging in size from 10 to 60 ha were established in the northern part of Rockbridge County, VA. In 1998, twelve blocks ranging in size from 100 to 160 ha were established within larger forested areas in Augusta County, VA. An 8.1 ha sampling area was established in the center of each woodlot/plot. Each year, there were four replicate woodlots for each treatment (Disrupt II with sticker (Gelva 2333, Monsanto Corp., St. Louis, MO), Disrupt II without sticker, and untreated control), arranged in a randomized block design. The flakes were applied with fixed-wing aircraft fitted with specialized flake application pods. The flake treatments were calibrated prior to application to deliver a dose of 75 g a.i. per ha. In 1997, the study area had very low gypsy moth populations. To ensure that numbers of male moths were adequate to measure efficacy, laboratory-reared male moths were released at a target rate of 150 males per plot every three days for the 9 week evaluation period. Fifteen (1997) or 10 (1998) pulley systems were erected in each core sampling area so that pheromone traps and female monitoring stations could be hoisted into the canopy to a height of up to 25 meters. Standard USDA milk carton traps containing plastic laminate dispensers loaded with 500 $\mu$g of (+)-disparlure were placed at 5 of the pulley systems uniformly spaced throughout each plot. At each of the 5 systems, one trap was deployed in the canopy and one was deployed at breast height. When not deployed, the traps were sealed in a plastic bag. Mating success was measured from monitor females deployed in mating stations. Mating stations consisted of modified delta traps placed at the ground and canopy level at each of the pulley systems in each plot. One-day old laboratory-reared females obtained from USDA, APHIS, Otis ANGB, MA were placed into the mating stations. Females were collected, along with any egg masses, 24 hours after they were deployed and held an additional 48 h. All egg masses obtained were held 30 days in an outdoor insectary under ambient conditions and then examined for embryonation. The number of embryonated eggs was recorded for each egg mass.

Both the wet and dry flake treatments reduced male trap catch compared to the untreated plots. Male trap catch was reduced most in the plots treated with wet flakes. Mating success was reduced in both the wet and dry flake treatments, but efficacy was greatest with the wet flake treatment. Females were scored as mated if one or more of their eggs was embryonated. However, the percentage of eggs in the mass that was embryonated varied with treatment. In 1997, the percentage of egg masses with less than 5% embryonated eggs varied from 20% in the control plots to 75% in the dry flake treatment and nearly 100% in the plots treated with wet flakes. In 1998, this percentage varied from 50% in the controls to 80% in the dry flake treatment to 100% in the wet flake treatment. This effect has been noted previously in the literature (Webb et al. 1981). Any egg mass with less than 5% of its eggs embryonated will contribute a relatively small number of larvae to the next generation, and therefore it seems reasonable to filter out these egg masses for the purposes of calculating mating success. When this is done, mating success was reduced 93% under the dry flake treatment and more than 99% under the wet flake treatment compared to the controls in 1997. In 1998, mating success was reduced 95% under the dry flake treatment and 100% under the wet flake treatment compared to the controls. Based on these results, the wet flake treatment appears slightly more effective than the dry flake treatment, and both treatments should be highly effective at reducing subsequent gypsy moth population growth.

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# FIELD-BASED ESTIMATES OF DOSE RESPONSES OF THREE GYPSY MOTH VIRUS STRAINS WITH AND WITHOUT THE VIRUS ENHANCER, BLANKOPHOR BBH

K. W. Thorpe[1], J. D. Podgwaite[2], J. M. Slavicek[3], R. E. Webb[1], R. W. Fuester[4],
R. A. Peiffer[5], M. A. Valenti[6], and P. B. Taylor[4]

[1]USDA Agricultural Research Center, Insect Biocontrol Laboratory, Beltsville, MD 20705

[2]USDA Forest Service, Northeastern Center for Forest Health Research,
51 Mill Pond Rd., Hamden CT 06514

[3]USDA Forest Service, Forestry Sciences Laboratory,
359 Main Rd., Delaware, OH 43015

[4]USDA Agricultural Research Service, Beneficial Insects Introduction Research Unit,
501 Chapel St., Newark, DE 19713

[5]Delaware State University, Department of Agriculture and Natural Resources, Dover, DE 19901

[6]Delaware Department of Agriculture, Dover, DE 19901

## ABSTRACT

The gypsy moth nucleopolyhedrosis virus (LdMNPV) product, Gypchek, is registered with the United States Environmental Protection Agency (EPA) as a general use insecticide for aerial and ground application to control gypsy moth (Reardon et al. 1996). Because it kills only gypsy moths, Gypchek is in demand for use in areas where there is concern over non-target organisms. A 1997 survey of gypsy moth managers (Podgwaite et al. 1997) revealed that demand for the product is expected to rise, but that its current cost could be prohibitive. Furthermore, the successful transfer of gypsy moth virus production from the public to the private sector may require a reduction in production costs. *In vitro* production is one way costs could be reduced. In 1996 and 1997, an *in vitro*-produced gypsy moth virus strain developed by the USDA, Forest Service at Delaware, OH was field tested using ground-based application equipment (Thorpe et al. 1998). While the *in vitro* strain prevented defoliation, the viral occlusion bodies were smaller than those of virus produced in living insects, and they contained only 20% as many virions. Therefore, this study was conducted to provide information on the dose responses of two novel gypsy moth virus strains, including this *in vitro*-produced strain, compared to Gypchek. The test was conducted under field conditions to produce results that are more applicable to operational conditions.

The study was conducted at the Cedar Swamp Wildlife Area in New Castle County, DE. Three gypsy moth virus strains were tested: (1) Gypchek, produced at USDA, Forest Service, Hamden, CT from gypsy moth larvae inoculated with virus; (2) viral strain 2031a1c ("203"), produced at USDA, Forest Service, Delaware, OH from gypsy moth larvae; and (3) viral strain 122b1a ("122"), produced in tissue culture at USDA, Forest Service, Delaware, OH. Strain 203 was tested because it routinely exhibits 2-fold greater activity compared to Gypchek when tested in laboratory diet-incorporated bioassays. Strain 122 is the same *in vitro*-produced virus strain that was tested in Thorpe et al. (1998). Each strain was mixed in an aqueous solution from which the following concentrations of polyhedral inclusion bodies (PIBs) were prepared by serial dilution:

$10^9$, $10^{10}$, $10^{11}$, $10^{12}$, $10^{13}$ PIB per 100 gallons of water [without Blankophor BBH]
$10^7$, $10^8$, $10^9$, $10^{10}$, $10^{11}$ PIB per 100 gallons of water [with Blankophor BBH]

The viral enhancer, Blankophor BBH, was added at 0.5% (W:V). Each treatment, plus a water control, was sprayed on oak branch tips to runoff using a hand-held sprayer. When dry, 10 gypsy moth second instars were added to each branch tip, and the tip was covered by a nylon organza sleeve cage. After one week, the cages were removed and the larvae were placed in 30 ml plastic cups with artificial diet (Bell et al. 1981) in an outdoor insectary at Beltsville, MD. The cups were then checked every 2-3 days to record larval mortality. All dead larvae were examined microscopically to confirm that the cause of death was virus. The study was arranged in a randomized complete block design with 8 blocks. Each block consisted of 2 branch tips for each of the treatments and controls.

Without enhancer, $10^{12}$ PIB/100 gallons of the *in vivo*-produced strains were required to produce >90% mortality with the *in vivo*-produced strains. Mortality was only 66% at $10^{13}$ PIB/100 gallons with the *in vitro*-produced strain without enhancer. With enhancer, mortality was 98% for Gypchek and 87% for 203, the other *in vivo*-produced strain, at $10^9$ PIB/100 gallons. Mortality exceeded 95% with both of the *in vivo*-produced strains at $10^{10}$ PIB/100 gallons. With enhancer, mortality from the *in vitro*-produced strain was 82% at $10^{10}$ and 94% at $10^{11}$ PIB/100 gallons. The presence of Blankophor BBH decreased the $LD_{50}$ values of the *in vivo*-produced strains by about 3 logs (= 1000-fold) and that of the *in vitro*-produced strain by 3.75 logs (= 5,600-fold). In the absence of enhancer, 203 killed larvae faster than Gypchek and 122, at doses of both $10^{11}$ and $10^{12}$ PIB/100 gallons. ($10^{12}$ is the currently recommended dose.) The enhancer greatly increased the speed of kill of the *in vivo*-produced strains, but not that of the *in vitro*-produced strain, at $10^{11}$ PIBs/100 gallons. ($10^{11}$ is likely to be the recommended dose for virus when used in conjunction with enhancer.)

One reason for the reduced efficacy of the *in vitro*-produced virus is that the PIBs of the 122 strain are smaller and contain about 5-fold fewer virions. Another possible reason is that this strain may have lost some activity due to the way it was formulated and stored. This is currently being investigated experimentally.

The currently recommended dose for the application of Gypchek through ground-based hydraulic equipment is $10^{12}$ PIBs per 100 gallons. Research to date indicates that this dose can be reduced to $10^{11}$ PIBs per 100 gallons with the addition of the enhancer, Blankophor BBH, with no loss of activity. Both *in vivo*-produced strains exhibited greater mortality and faster kill with the addition of the enhancer at $10^{11}$ PIBs per 100 gallons. The *in vitro*-produced strain caused greater mortality, but not faster kill at this dose in the presence of enhancer. It appears that a higher dose of *in vitro*-produced virus may be required to provide the desired levels of mortality in field applications. Since it will presumably be possible to produce virus more economically *in vitro*, the dose increase will hopefully be offset by lower production costs. The dose-response relationships developed here should help in determining the appropriate doses required for the two new strains tested.

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# THE ROLE OF GYPSY MOTH PREDATORS IN POPULATION DYNAMICS IN RELATION TO ITS POPULATION DENSITY IN SLOVAKIA - FIRST EXPERIENCE

Marek Turcani

Forest Research Institute Zvolen, Research Station Banska Stiavnica,
Lesnicka 11, SK - 96923 Banska Stiavnica, The Slovak Republic

## ABSTRACT

The gypsy moth is the most important pest of oak stands in Slovakia. Outbreaks of this pest repeat in a period of 6 - 10 years. In our study, its population density has been monitored on 12 monitoring points every year since 1990 (Table 1).

Table 1. Abundance of gypsy moth in Slovakia in the period 1990-98 (egg masses/tree)

| **Place/Year** | **1990** | **1991** | **1992** | **1993** | **1994** | **1995** | **1996** | **1997** | **1998** |
|---|---|---|---|---|---|---|---|---|---|
| Busince | 0.000 | 0.002 | 0.010 | 0.008 | 0.031 | 0.002 | 0.002 | 0.004 | 0.013 |
| Pata | 0.005 | 0.050 | 5.640 | 0.040 | 0.002 | 0.008 | 0.071 | 0.002 | 0.004 |
| T. Mlynany | 0.012 | 0.169 | 30.938 | 0.000 | 0.000 | 0.010 | 0.008 | 0.006 | 0.021 |
| Tehla | 0.001 | 0.079 | 3.719 | 0.096 | 0.000 | 0.008 | 0050 | 0.000 | 0.033 |
| Kovacova | 0.060 | 0.090 | 0.140 | 0.280 | 0.054 | 0.041 | 0.035 | 0.016 | 0.017 |
| Zvolenak | 0.000 | 0.040 | 0.290 | 3.070 | 0.000 | 0.002 | 0.002 | 0.004 | 0.019 |
| Vojnice | - | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.006 | 0.000 |
| Kurinec | 0.000 | 0.015 | 0.002 | 0.110 | 0.108 | 0.006 | 0.002 | 0.000 | 0.008 |
| Par. Haje | - | 0.402 | 21.413 | 0.221 | - | 0.008 | 0.019 | 0.006 | 0.027 |
| V. Zaluzie | - | 0.133 | 0.181 | 0.152 | 0.002 | 0.004 | 0.000 | 0.008 | 0.008 |
| Casta | - | - | - | - | - | - | 0.006 | 0.000 | 0.013 |
| Trebisov | - | - | - | - | - | - | 0.000 | 0.002 | 0.002 |

Big attention was paid to the research of pathogens and parasitoids during the last 10 years. In spite of this, the role of predators in gypsy moth population dynamics is almost unknown in the territory of Slovakia. The only information we have is about the role of birds as predators during a gypsy moth outbreak. We have also tried to obtain basic information about the role of other predators during the last 2 years. Our research was focused on predation of egg masses and pupae. Population density is low now, but started to increase in the last year and it is possible to expect the next outbreak in the following 2 - 3 years, depending on weather conditions.

We found that the role of predators was very important when population density was low. During the winter of 1997-98, 100% of the egg masses were damaged, and in 1998-99 it was 77%. The predators appeared to be mainly birds and beetles (in some cases we found Dermestiidae as predators). Although all egg masses were damaged in the winter of 1997-98, 77% of them showed the average proportion of destroyed eggs less than 30%. Next year, 23% of the egg masses were not damaged, 51% were damaged slightly (less than 30% destroyed eggs) and 26% were damaged strongly (over 30% destroyed eggs). In the winter of 1997-98, the average population density was 0.004 egg masses per tree; in the winter of 1998-99, there were 0.014 egg masses per tree. It seems to be that the value 0.01 (or lower) of egg masses per tree is the threshold, below which predators of egg masses may strongly affect the population density of gypsy moth. In Slovakia, this status (population density below 0.01 egg masses per tree) takes a long period (6-8 years) between outbreaks. There is thus probably a possibility to support predators in order to reduce population density of the pest's eggs.

Pupae predation was studied at 3 plots in southwestern Slovakia during the summer of 1998. The artificially reared pupae were exposed on the ground in the quantity of 100 specimens in every plot. Predation was recorded on the 1st, 2nd, 6th and 7th day after exposition. The results are presented in Table 2.

Table 2. Predation of gypsy moth pupae in Slovakia (Summer 1998)

| **Plot** | **Zvolenak** | | | | **Pata** | | | | **Kovacova** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | **1st** | **2nd** | **6th** | **7th** | **1st** | **2nd** | **6th** | **7th** | **1st** | **2nd** | **6th** | **7th** |
| Nondamaged | 86 | 77 | 35 | 18 | 83 | 35 | 0 | 0 | 85 | 69 | 32 | 22 |
| Damaged | 13 | 17 | 21 | 25 | 6 | 6 | 4 | 4 | 11 | 13 | 15 | 13 |
| Disappeared | 1 | 6 | 44 | 57 | 11 | 59 | 96 | 96 | 4 | 18 | 53 | 65 |
| Mortality % | 14 | 23 | 65 | 82 | 17 | 65 | 100 | 100 | 15 | 31 | 68 | 78 |

Average mortality reached 86.7%. The predators could be small mammals, wild boar (observations based on its traces and trails near the exposed pupae in the cases when pupae disappeared). From among insects, there were - following direct observation - beetles (Dermestiidae, Staphylinidae), ants, wasps and earwigs (Japygidae).

Research of the predators complex was omitted for a long period in Slovakia. It seems to be, however, that predators play an important role in population dynamics of gypsy moth all over Central Europe as well. There is a good chance to study the role of predators in gypsy moth population dynamics now, when abundance starts to increase and outbreak of the pest is close.

# ISOLATION AND KINETIC ANALYSIS OF THE PURIFIED GYPSY MOTH CRY1Aa/b *BACILLUS THURINGIENSIS* TOXIN RECEPTOR

Algimantas P. Valaitis[1], Mi Kyong Lee[2], and Donald H. Dean[2]

[1]USDA Forest Service, Northeastern Research Station, 359 Main Rd., Delaware, OH 43015

[2]Department of Biochemistry, The Ohio State University, 48 West 12th St., Columbus, OH 43210

## ABSTRACT

*Bacillus thuringiensis* (Bt) crystal proteins (Cry proteins) are toxic to a number of insect larvae. The Cry1A class of Bt toxins are active only against certain lepidopteran insects such as the gypsy moth. The target of these toxins is the brush border membrane of larval midgut cells where the toxins bind to specific receptors. Binding of toxin to its receptor is believed to be a key factor in eliciting the insecticidal activity, and in determining the specificity of different Bt toxins. The precise mechanism of toxic action is not known. Understanding the receptor binding properties could guide the development of more potent toxins and new Bt-based biopesticides with increased specificity towards target insect pests. A 270 kDa Cry1Aa/b toxin-binding molecule identified in gypsy moth was purified by preparatory gel electrophoresis using reversible zinc chloride staining. Rabbit antibody was raised against the purified receptor (BtR-270). Attempts to label BtR-270 using protein-directed techniques were unsuccessful. However, after oxidation of BtR-270 with periodate reactive aldehydes were generated, and the oxidized receptor was coupled to digoxigenin or biotin by reaction with hydrazide derivatives of these reagents. Biotin-labeled receptor provided a sensitive probe in development of an assay to detect toxins that bind with high affinity. The oxidative modification and aldehyde coupling method was also applied to immobilize purified BtR-270 to the surface of a BIAcore sensor chip for kinetic analysis of the toxin-receptor interaction without compromising the receptor function. Kinetic binding properties of Cry1Aa, Cry1Ab and Cry1Ac toxins to the purified 270 kDa molecule were analyzed using a surface plasmon resonance (SPR) based optical sensor. All three toxins bound to the 270 kDa with high binding affinities. Two-site model or conformational change model fit better than a single binding site model. The two-site model demonstrated that for the first site, both Cry1Aa and Cry1Ab showed higher binding affinity ($K_{D1}$=11 nM) compared to Cry1Ac ($K_{D1}$=4870 nM). For the second site, Cry1Aa and Cry1Ab exhibited extraordinary tight binding affinities with $K_{D2}$ of $10^{-15}$ and $10^{-14}$ M, respectively. Cry1Ac toxin also showed tighter binding to the second site than the first site with $K_{D2}$ of 141 nM. GalNAc inhibited Cry1Ac binding but not Cry1Aa and Cry1Ab binding. These results suggest that Cry1Aa/b toxins bind to a different site than Cry1Ac on BtR-270. Other studies to examine the detailed binding properties and binding site relationships among these toxins are in progress.

# EFFECT OF NATURAL PREDATORS ON HEMLOCK WOOLLY ADELGID IN NORTH CAROLINA AND VIRGINIA

Matthew Wallace and Fred Hain

Department of Entomology, North Carolina State University,
Box 7626 Grinnells Lab, Raleigh, NC 27695-7626

## ABSTRACT

Exotic predators show promise for regulating the hemlock woolly adelgid (HWA) in the eastern United States. However, before mass releases are made, an extensive examination of the role of native predators in controlling HWA in North America is needed. Thus we will be better prepared to choose the most effective biological control agents and to study the relationships of exotic predators with native predators. The objectives of our two-year study were to survey for predators of HWA present within our research plots and to determine their effect on adelgid survivorship.

Field surveys for native predators were conducted in the spring, summer, and fall of 1997 and the late winter, spring, and summer of 1998 at 3 field sites in North Carolina and Virginia. Predators were collected in very low densities from beat samples and twig samples from all 3 sites during all sample dates in both 1997 and 1998. The most abundant predators collected during both years were *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), brown and green lacewings (Neuroptera: Chrysopidae and Hemerobiidae), and gall gnats (Diptera: Cecidomyiidae). In twig samples in 1997, larvae of *Aphidoletes abietis* Kieffer (Diptera: Cecidomyiidae) were found in relatively high densities (1 / 3 cm of twig). In 1998 field surveys, predators were found to be marginally synchronized with the spring generation of the adelgid. Cage exclusion experiments were conducted to determine the effect of predation on HWA survivorship. Results from 1997 revealed no predator effects at any site while only one site in 1998 revealed predator effects. However, it is believed that this effect was not significant enough to control adelgid populations due to the low densities of predators collected in the field surveys.

A small native enemy complex associated with HWA was discovered in our plots. However, due to low densities, lack of optimum synchronization, and evidence from the cage exclusion experiments, it is unlikely that this complex is having any control of HWA populations. Although there are a number of predators that feed on adelgids, many are considered generalists. The results of this study reveal an important question that future research needs to address. What impact will an aggressive generalist predator such as *H. axyridis* have on attempts to establish an exotic predator at innoculative levels?

COOPERATIVE RESEARCH ON THE ASIAN LONGHORNED BEETLE,

*ANOPLOPHORA GLABRIPENNIS* (COLEOPETERA: CERAMBYCIDAE) IN CHINA

Baode Wang[1], V. Mastro[1], R. Reardon[2], W. McLane[1], R. Gao[3], Y. Luo[4], D. Cowan[1], Y. Wu[2], G. Li[3], and Z. Xu[4]

[1]USDA Animal and Plant Health Inspection Service, PPQ, Otis Methods Development Center, Otis ANGB MA 02542

[2]USDA Forest Service, 180 Canfield St., Morgantown, WV 26505

[3]Chinese Academy of Forestry, Beijing, 100094 China

[4]Beijing Forest University, Beijing China

ABSTRACT

The Asian longhorned beetle (ALB), *Anoplophora glabripennis* Motschulsky, a serious pest of hardwood trees, was found in New York City and Long Island in 1996 and Chicago in 1998. A cooperative research project was set up in China in 1997 to develop reliable survey tools and effective techniques for the suppression and management of the beetle. We tested systemic insecticides through soil and trunk injections at two sites north of China in 1998. We also conducted field evaluations of monomers and compounds originating from the beetle and its host plants. In addition, we studied the cross breeding between *A. glabripennis* and *A. nobilis*, and observed and documented the behavior of the beetle under field conditions. Although it will take years for some of these studies to be finished, preliminary results are encouraging. The results of systemic insecticide tests suggest that Imidacloprid may have great impact on the mortality of the beetle. Field evaluation of plant extracts has led to the discovery of several promising monomers that may be used by the beetles as kairomones and may be developed into effective traps. The observation of the beetle's behavior in the field has added important information that may help us to manage the beetle. Results of the cross breeding between *A. glabripennis* and *A. nobilis* revealed that the two, which are morphologically similar, may essentially be different types or subspecies of a single species. We will continue these studies in the next few years.

# THE EURASIAN INVASION OF FUNGAL PLANT PATHOGENS

# PAST, PRESENT AND FUTURE: AN INTRODUCTION

Philip M. Wargo

USDA Forest Service, Northeastern Research Station,
51 Mill Pond Rd., Hamden, CT 06514

Though sometimes not as spectacular as insect infestations, introduced fungal tree pathogens have had a significant impact (biological and economic) on our Eastern forests. Beech bark disease by *Nectria* spp. and *Cryptocococcus fagisuga*, scale, ca 1890, chestnut blight caused by *Cryphonectria parasitica* ca 1900, white pine blister rust by *Cronartium ribicola* ca 1960, dutch elm disease by *Ophiostoma ulmi* and *nova-ulmi* ca 1930, butternut canker by *Sirococcus clavigignenti-julandacearum* ca 1960's, and dogwood anthracnose by *Discula destructiva* ca 1970's are the well known members of this notorious group of introduced tree fungal pathogens.

Our forests and cities attest to their former, especially, and current presence of them by the conspicuous absence or paucity of tree species from our forests and city streets. Legacies of these diseases are not only reflected in the trees that have died but also in the trees that have replaced them, to wit the preponderance of defect in the regeneration stands of beech, and the abundance and contiguousness of the oak forests in our Eastern forests that have replaced chestnut and which now are susceptible to the effects of the gypsy moth. I've arranged our speakers in a chronology relative to the recognition of the disease of their expertise and asked them to focus on the past, present, and future of their disease. I believe our presenters, who are my colleagues and close friends, will treat you with their stories on chestnut blight, Dr. William MacDonald, WVU, beech bark disease, Dr. David Houston, USFS retired, and butternut canker, Dr. Dale Bergdahl, UVM.

# HEMLOCK WOOLLY ADELGID: EVALUATION OF AESTIVAL DIAPAUSE AND POTENTIAL NATURAL ENEMIES

G. M. G. Zilahi-Balogh, S. M. Salom, and L. T. Kok

Department of Entomology, Virginia Polytechnic Institute & State University,
Blacksburg, VA 24061-0319

## ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* (Homoptera: Adelgidae) Annand, sistens enter diapause immediately after crawlers settle at the base of the hemlock needles. The duration of this stage has been documented to last in the field for approximately four months. We evaluated the factors responsible for initiating and maintaining aestival diapause. In the first of three experiments, 100% of sistens went into aestival diapause at a photoperiod of 16:8 (L:D). At 12:12, and 12°C, the percentage going into diapause was reduced to 60%. In a second experiment in which HWA from the previous lifestage (progrediens) were preconditioned at the same photoperiod and temperature conditions as the sistens were exposed (12:12, 11:10, and 10:14 and 12°C), the percentage going into diapause was reduced to 0% for all three photoperiods. In a third experiment, progrediens were again preconditioned at similar conditions to sistens (12:12, 13:11, and 14:10 and 14.5°C) and again 0% of sistens went into diapause at all photoperiods.

*Laricobius nigrinus* (Coleoptera: Derodontidae) has consistently been found feeding on HWA in British Columbia. In 1998, 400 *L. nigrinus* were imported to Virginia where they are now maintained at the Virginia Tech Quarantine facility. We have evaluated host suitability and egg development of *L. nigrinus* to assess its potential as a biological control agent for HWA. Thermal development requirements are being determined and will be related to HWA phenology. *L. nigrinus* reproduced and developed successfully on a diet of HWA. The life cycle of the insect is described here for the first time. Egg development was inversely proportional to temperature within the range of 13-18 °C. Current work involves sampling for *L. nigrinus* and HWA in western hemlock seed orchards in British Columbia to determine seasonal abundance of *L. nigrinus* and its phenological synchrony with HWA. Host preference tests are also being conducted.

# GYPSY MOTH OVIPOSITION SITE FINDING BEHAVIOR ON SURFACES WITH DIFFERENT SLOPE

Paulius Zolubas[1], Arturas Gedminas[1], Kathleen Shields[2]

[1]Lithuanian Forest Institute, Girionys, 4312 Kaunas, Lithuania

[2]USDA Forest Service, Northeastern Center for Forest Health Research, 51 Mill Pond Rd., Hamden, CT 06514 USA

## ABSTRACT

Gypsy moth females from Lithuanian populations have good capability for sustained flight and fly shortly after eclosion as well as after mating. However, the stimuli responsible for flight are unknown. In earlier studies we noticed that gypsy moth females attempt to escape from horizontal or slightly inclined surfaces and tend to move to a vertical position, but these trends had not previously been tested experimentally.

Immediately after eclosion, gypsy moth females were placed on the inner surface of a cylindrical mesh screen with 2 m radius at slopes of 70°, 100°, 150° and 180° (upside down) relative to the horizon. Copulation was allowed on the following day, after which mated females were randomly placed at 70°, 100°, 150° and 180°, without regard to previous placement. Female behavior was recorded prior to mating and after mating and three orders of behavior were designated in each phase: 1) inert - moved $< 10$ cm from initial location; 2) walking - walked $> 10$ cm from initial location; 3) flying - exhibited sustained flight. Each female was tested only once, and the highest order behavioral event was recorded for each female in each of two intervals: eclosion to mating, and mating to oviposition.

Before mating, almost all of the 323 females tested were moving, but in the group placed on the 180° slope (i.e. upside down), 26.5±7.6% of the individuals were inert, and this was significantly different ($p<0.05$) from other locations, where only 2.3±2.2% - 7.8±2.7% of the females remained at the spot where they were placed. Behavior of 116 females was recorded after mating. Of the females mated on a 70° slope, 53.8±8.0% exhibited sustained flight; this was not significantly different ($p>0.1$) from other groups where only 21.1±9.4% - 34.3±8.0% of the individuals exhibited sustained flight. On the other hand, significantly more females ($p<0.025$) placed on surfaces with 100-180° slope remained inert (42.1±11.3% - 56.5±10.3%) and initiated egg laying than females placed on a 70° slope where only 5.1±3.5% were inert.

**USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species**
**January 19-22, 1999**
**Annapolis, Maryland**

## List of Attendees

Lynn Alexander, Maryland Dept. Agric., 2107 N. Fountain Green Rd., Bel Air, MD 21015
Heather Apostolos, Delaware Dept. Agric., 2320 S. DuPont Hwy, Dover, DE 19901
John Bain, New Zealand Forest Research Inst., Private Bag 3020, Rotorua, New Zealand
Yuri Baranchikov, Sukachev Institute of Forest, Russian Academy of Sciences, Krasnoyarsk, Russia
Robert Bennett, USDA-ARS, BARC, PSI, Insect Biocontrol Lab., Beltsville, MD 20705
Susan Bentz, U.S. National Arboretum, 11601 Old Pond Dr., Glenn Dale, MD 20769
Dale Bergdahl, Dept. Forestry, Univ. of Vermont, Burlington, VT 05405
Ken Berger, 501 ASI Bldg., Dept. Entomology, Pennsylvania State Univ., University Park, PA 16865
E. Michael Blumenthal, Pennsylvania Bureau of Forestry, 34 Airport Dr., Middletown, PA 17057
A. Temple Bowen, Jr., Stillmeadow Farm, P.O. Box 669, Antrim, NH 03440-0669
J. Robert Bridges, USDA-FS, NERS, 100 Matsonford Rd., Radnor, PA 19087
John Burch, USDA-APHIS, PPQ, 12927 Stonecreek Dr. NW, Pickerington, OH 43147
Joe Cavey, USDA-APHIS, 4700 River Rd., Riverdale, MD 20737
Hong Chen, Food Inspection Service, 59 Camelot Dr., Nepean, Ontario K1A 0Y9 Canada
Gary Clement, USDA-APHIS, PPQ, One Credit Union Place, Ste. 310, Harrisburg, PA 17110
David Cohen, Maryland Dept. Agric., 3 Pershing St., Cumberland, MD 21502
György Csóka, Forest Research Institute, P.O. Box 2, 3232 Matrafured, Hungary
Vincent D'Amico, III, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
Dana DeWeese, USDA-APHIS, PPQ, 1442 Aaron Ct., Jefferson City, MO 65101
Andrea Diss, Wisconsin Dept. Natural Resources, 1125 N. Military Ave., Green Bay, WI 54307
Charles Divan, USDA-APHIS, PPQ, 4700 River Rd., Unit 149, Riverdale, MD 20737
Thomas Dubois, Dept. Entomology, Comstock Hall, Cornell Univ., Ithaca, NY 14853
Joseph S. Elkinton, Dept. Entomology, Univ. of Massachusetts, Amherst, MA 01003
Barry Emens, USDA-APHIS, PPQ, CN330, Room 202, Trenton, NJ 08625
Daniel Fieselmann, USDA-APHIS, PPQ, 1017 Main Campus Dr., Raleigh, NC 27606
Frank Finch, 12011 Government Center Pkwy, Ste. 518, Fairfax, VA 22035
Charlotta Ford, Maryland Dept. Agric., 3 Pershing St., Cumberland, MD 21502
Roger W. Fuester, USDA-ARS, BIIRL, 501 S. Chapel St., Newark, DE 19713
Robert Fusco, Abbott Labs., HC 63, Box 56, Mifflintown, PA 17605
Bruce Gill, Agriculture Canada, 960 Carling Ave., Ottawa, Ontario K1A 0C6 Canada
Hilary Gillock, Dept. Entomology, North Carolina State Univ., Raleigh, NC 27695
William Gimpel, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Mr. Godfrey, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Kurt Gottschalk, USDA-FS, NERS, 180 Canfield St., Morgantown, WV 26505
Steve Grossi, Maryland Dept. Agric., 2107 N. Fountain Green Rd., Bel Air, MD 21015
Michael Grove, Dept. Entomology, Pennsylvania State Univ., State College, PA 16801
Jan Hacker, WV Dept. Agric., 1900 Kanawha Blvd. E, Charleston, WV 25305
Fred Hain, Dept. Entomology, Box 7626, North Carolina State Univ., Raleigh, NC 27695-7626
Ann Hajek, Dept. Entomology, Cornell Univ., Ithaca, NY 14853
Laurence Hanks, Dept. Entomology, Univ. Illinois, 505 S. Goodwin Ave., Urbana, IL 61801
Lynn Harrison, Delaware Dept. Agric., 2320 S. DuPont Hwy, Dover, DE 19901
Felton Hastings, Dept. Entomology, North Carolina State Univ., Raleigh, NC 27695
Jim Heath, Maryland Dept. Agric., 27722 Nanticoke Rd., Unit 2, Salisbury, MD 21801
Franck Hérard, USDA-ARS, European Biocontrol Lab., P.O. Box 4168 Agropolis 34092 Montpellier Cedex 5, France

Robert Heyd, Dept. Natural Resources, 1990 US 41 South, Marquette, MI 49855
Gernot Hoch, Inst. Forest Entomology, Hasenauerstrasse 38, A 1190, Vienna, Austria
Gregory Hoover, Dept. Entomology, Pennsylvania State Univ., State College, PA 16801
Kelli Hoover, Dept. Entomology, Pennsylvania State Univ., State College, PA 16801
David Houston, USDA-FS, NERS, retired, 51 Mill Pond Rd., Hamden, CT 06514
Robin Huettel, USDA-CSREES, PAS, 901 D St., Aerospace Bldg., Washington, DC 20250
Ralf Johnson, P.O. Box 800, Aberdeen, MD 21001
Melody Keena, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
John Kegg, New Jersey Dept. Agric., P.O. Box 330, Trenton, NJ 08625
M. Kirby, Maryland Dept. Agric., 209 Forest Dr., Cumberland, MD 21502
Matthew Kizlinski, Harvard Forest, P.O. Box 68, Petersham, MA 01366
Craig Kuhn, Maryland Dept. Agric., 2107 N. Fountain Green Rd., Bel Air, MD 21015
Donna Leonard, USDA-FS, P.O. Box 2680, Asheville, NC 28802
Sandy Liebhold, USDA-FS, NERS, 180 Canfield St., Morgantown, WV 26505
Harri Liljalheto, Food Inspection Agency, 300 Water St., Peterborough, Ontario Canada
Steve Lingafelter, USDA SEL, National Museum of Natural History, MRC-168, Washington, DC
Marcia Lipetz, WPWR-TV Channel 50 Fndtn., 2151 N. Elston, Chicago, IL
William MacDonald, West Virginia Univ., P.O. Box 6057, Morgantown, WV 26506-6057
Priscilla MacLean, Hercon, P.O. Box 467, Emigsville, PA 17318-0467
Stephen Malan, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Christine Markham, USDA-APHIS, 505 S. Lenola Rd., Moorestown, NJ 08052
Horst Gossenhauer-Marohn, Forstlische Lnadesanstalt, Prof.-Oelkers Str. 6, 34346, Hann. Münden, Germany
Victor C. Mastro, USDA-APHIS, Bldg. 1398, Otis Methods Development Ctr., Otis ANGB, MA 02542
Gary McAninch, Virginia Dept. Agric., P.O. Box 1163, Richmond, VA 23218
Deborah McCullough, Michigan State Univ., 243 Natural Sciences Bldg., East Lansing, MI 48824-1115
Max W. McFadden, USDA-FS, NERS, retired, P.O. Box 741, Georgetown, DE 19947
Win McLane, USDA-APHIS, Bldg. 1398, Otis Methods Development Ctr., Otis ANGB, MA 02542
Joseph McLaughlin, Delaware Dept. Agric., 2320 S. DuPont Hwy, Dover, DE 19901-5515
Michael McManus, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
Michael E. Montgomery, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
Ben Moody, Canadian Forest Service, 580 Booth St., Ottawa, Onatrio K1A 0E4 Canada
Steve Munson, USDA-FS, FHP, 4746 S 1900 E, Ogden, UT 84403
Rose-Marie Muzika, Univ. Missouri, 203 Natural Resources Bldg., Columbia, MO 65211
Coanne O'Hern, USDA-APHIS, PPQ, 4700 River Rd., Unit 134, Riverdale, MD 20737-1236
Amy Onken, USDA-FS, FHP, 180 Canfield St., Morgantown, WV 26505
Brad Onken, USDA-FS, FHP, 180 Canfield St., Morgantown, WV 26505
Judith Pasek, USDA-APHIS, PPQ, 1017 Main Campus Dr., Raleigh, NC 27606-5202
Randy Peiffer, Biology Dept., Delaware State College, Dover, DE 19901
John Podgwaite, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
Therese Poland, USDA-FS, Room 220, 1407 S. Harrison Rd., East Lansing, MI 48823
Robert Rabaglia, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Kenneth Raffa, Univ. Wisconsin, 345 Russell Labs., Madison, WI 53706
Kenneth Ivan Rash, 400 St. Andrews Dr., Gun Barrel City, TX 75147
Richard Reardon, USDA-FS, FHTET, 180 Canfield St., Morgantown, WV 26505
David Roden, Canadian Forest Service, P.O. Box 490, Sault Ste. Marie, Ontario P6A5M7 Canada
Mark Rothschild, Maryland Dept. Agric., 27722 Nanticoke Rd., Unit 2, Salisbury, MD 21801
Cliff Sadof, Purdue Univ., 1158 Entomology Hall, West Lafayette, IN 47906
Vicente Sánchez, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
John Sanders, Abbott Laboratories, 3 Downes St., BRIDPORT, Dorset DT6 3JR, UK
Frank Santamour, Jr., U.S. National Arboretum, 3501 New York Ave., NE, Washington, DC 20002
Taylor Scarr, Food Inspection Agency, 70 Foster Dr., Sault Ste. Marie, Ontario P6A6V5 Canada

Paul Schaefer, USDA-ARS, 501 S. Chapel St., Newark, DE 19713
Noel Schneeberger, USDA-FS, FHP, 100 Matsonford Rd., Radnor, PA 19087
Shane Sela, Food Inspection Agency, 118-816 Government St., Victoria, BC V8W 1W9 Canada
Patricia Sellers, USDA-FS, FHP, 112 North River Rd., Bridgewater, VA 22812-1698
Alexei Sharov, Dept. Entomology, Virginia Polytechnic Institute, Blacksburg, VA 24061
Troy Shaw, 12011 Government Center Pkwy, Ste. 518, Fairfax, VA 22035
Kathleen Shields, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
James Slavicek, USDA-FS, NERS, 359 Main Rd., Delaware, OH 43015
Harvey Smith, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
I. Barton Smith, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Michael T. Smith, USDA-ARS, 501 S. Chapel St., Newark, DE 19713
Susan Smith, Dept. Entomology, Comstock Hall, Cornell Univ., Ithaca, NY 14853
Leellen Solter, 140 EASB, NSRL, 1101 W. Peabody Dr., Champaign, IL 61801
Bruce Steward, Bayer Corporation, 104 Davenport Rd., Kennett Square, PA 19348
Jeffrey Stibick, USDA-APHIS, 4700 River Rd., Riverdale, MD 20737
Tod Sukontarak, USDA-ARS, IBC, Bldg. 402, Biocontrol Rd., Beltsville, MD 20705
Lidia Sukovata, Forest Research Institute, ul. Bitwy Warszawskiej 1920R,N3, 00-973, Warsaw, Poland
Jil Swearingen, National Park Service, 4598 MacArthur Blvd. NW, Washington, DC 20007
Steve Talley, Rockbridge Co. Gypsy Moth Prg., 348 Sherwood Ave., Staunton, VA 24401
Ronald Tatman, Maryland Dept. Agric., 2107 N. Fountain Green Rd., Bel Air, MD 21015
Mark Taylor, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Stephen Thomas, Dept. Entomology, Univ. of Massachusetts, Amherst, MA 01003
Kevin Thorpe, USDA-ARS, Bldg. 402, BARC-E, Beltsville, MD 20705
Robert Tichenor, Maryland Dept. Agric., 50 Harry S. Truman Pkwy, Annapolis, MD 21401
Steve Tilley, Maryland Dept. Agric., c/o 912 Market St., Denton, MD 21629
Denny Townsend, USDA-ARS, 11601 Old Pond Dr., Glenn Dale, MD 20769
Marek Turcani, Forest Research Institute, Lesnicka 11, 96923, Banská Stiavnica, Slovak Republic
Daniel Twardus, USDA-FS, FHP, 180 Canfield St., Morgantown, WV 26505
Michael Valenti, Delaware Dept. Agric., 2320 S. DuPont Hwy, Dover, DE 19901
Baode Wang, USDA-APHIS, PPQ, Bldg. 1398, Otis Methods Development Ctr., Otis ANGB, MA 02542
Philip Wargo, USDA-FS, NERS, 51 Mill Pond Rd., Hamden, CT 06514
Ralph Webb, USDA-ARS, Bldg. 402, BARC-E, Beltsville, MD 20705
Geoff White, USDA-ARS, Insect Biocontrol, Bldg. 402, BARC-East, Beltsville, MD 20705
David Williams, USDA-FS, NERS, 100 Matsonford Rd., Radnor, PA 19087
Michael Wright, USDA-APHIS, PPQ, 1 Winners Circle, Ste. 203, Albany, NY 12009
Steve Yaninek, USDA-CSREES, 901 D. Street SW, Washington, DC 20250
Gabriella Zilahi-Balogh, Dept. Entomology, Virginia Polytechnic Institute, 216 Price Hall, Blacksburg, VA 24061
Paulius Zolubas, Forest Research Institute, 4312 Giornys, Kaunas, Lithuania