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

AGENDA

Tuesday Afternoon, January 16

REGISTRATION
POSTER DISPLAY SESSION I

Wednesday Morning, January 17

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

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

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

PLENARY SESSION .... Moderator: Robert Mangold, USDA-FS

The National Council on Invasive Species
Lori Williams, Department of the Interior

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

Wednesday Afternoon, January 17

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

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

POSTER DISPLAY SESSION II

Thursday Morning, January 18

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

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

Thursday Afternoon, January 18

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

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

Friday Morning, January 19

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

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

Closing Remarks

CONTENTS

RECENT STUDIES ON MIDGUT BACTERIA OF THE GYPSY MOTH

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ABSTRACT

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

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

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ABSTRACT

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

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

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

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

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ABSTRACT

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

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

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

DISTANT EFFECT OF PHEROMONE USED FOR MATING DISRUPTION OF GYPSY MOTH

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ABSTRACT

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

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

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

ANOPLOPHORA GLABRIPENNIS ANTENNAL SENSORY RECEPTORS:

A RESEARCH UPDATE

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ABSTRACT

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

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

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

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

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

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

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

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ABSTRACT

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

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

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ABSTRACT

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

DISPERSAL POTENTIAL OF *ANOPLOPHORA GLABRIPENNIS* MOTSCH.

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INTRODUCTION

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

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

MATERIALS AND METHODS

These studies were conducted 1 km west of the town of Liu Hua, bordering the Yellow River in Gansu Province, north central China. This field site was selected because it possessed landscape characteristics similar to those of the urban infestations in the U.S., particularly site-specific factors that are thought to most likely influence dispersal distance. The general landscape is composed of both host (72.3%) and non-host (27.7%) tree species of mixed age classes. Known ALB hosts are dominated by *Populus nigra* L. var. *thevestina* (Dode) Bean, comprising ca. 87% of the ALB hosts, followed by *Salix* sp. and *Ulmus* sp., at 9% and 4%, respectively. The study site was composed of isolated trees and trees planted along paths amid dwellings, such as homes and greenhouses, as well as trees planted as wind-rows (generally 2 m spacing within rows and 50 m spacing among rows) bordering agricultural fields. Greenhouses and small dwellings were also commonly found within or adjoining agricultural fields.

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

RESULTS AND DISCUSSION

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

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

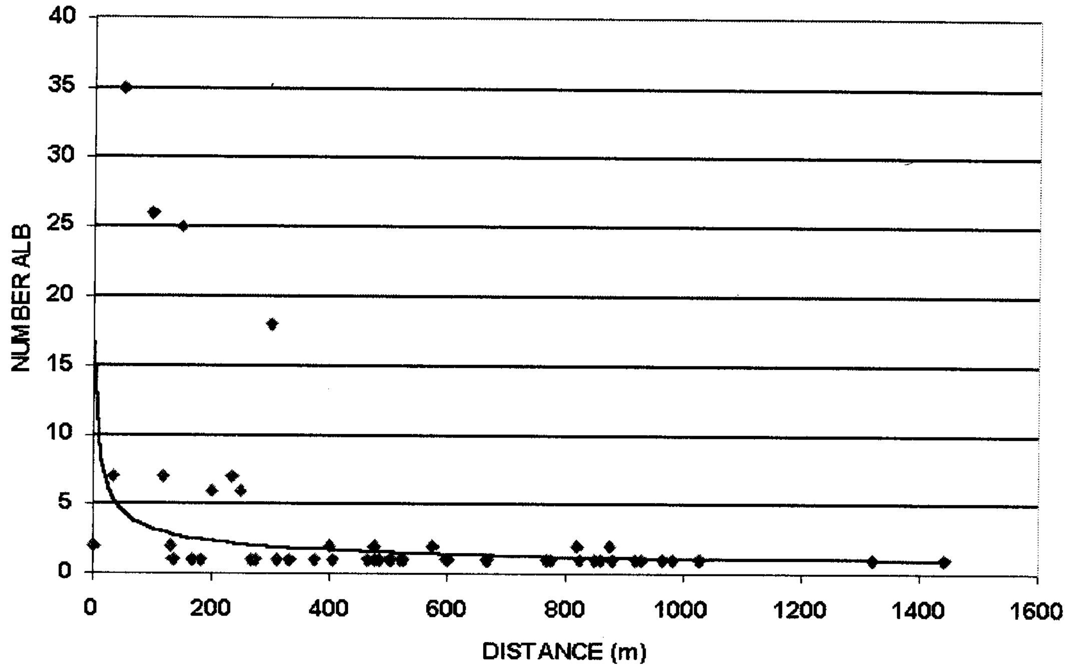

Figure 1. Dispersal distance of adult ALB (1999).

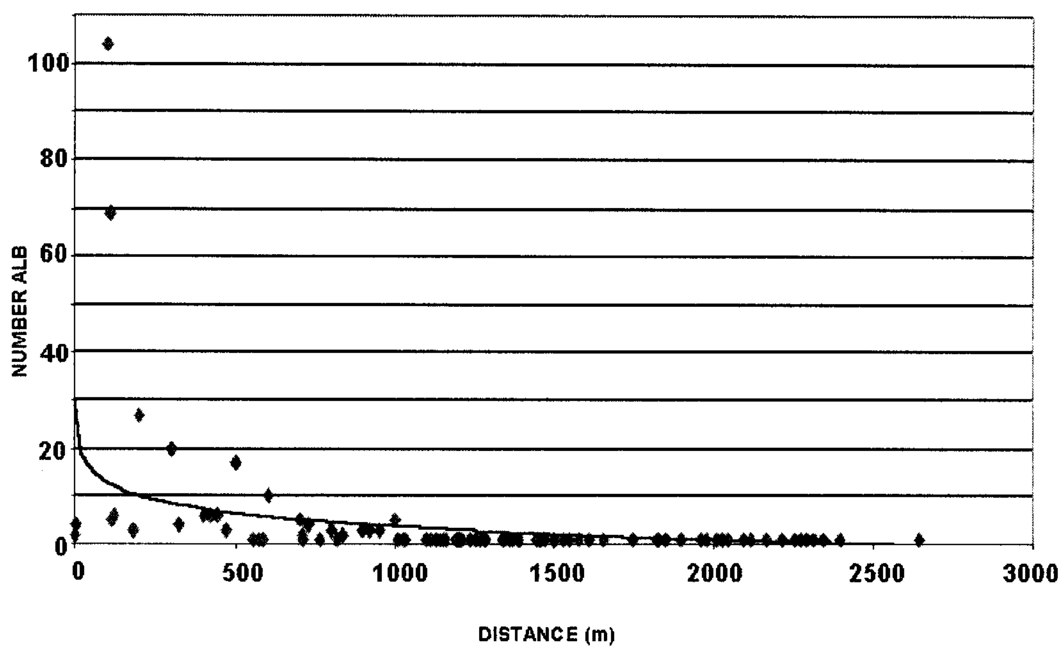

Figure 2. Dispersal distance of adult ALB (2000).

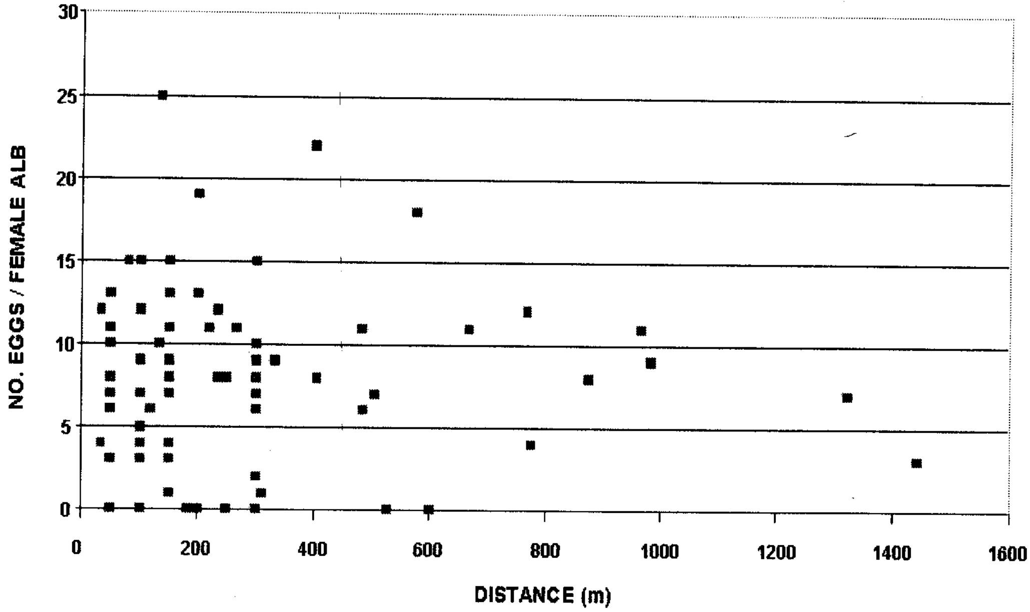

Figure 3. Distance ALB disperse eggs (1999).

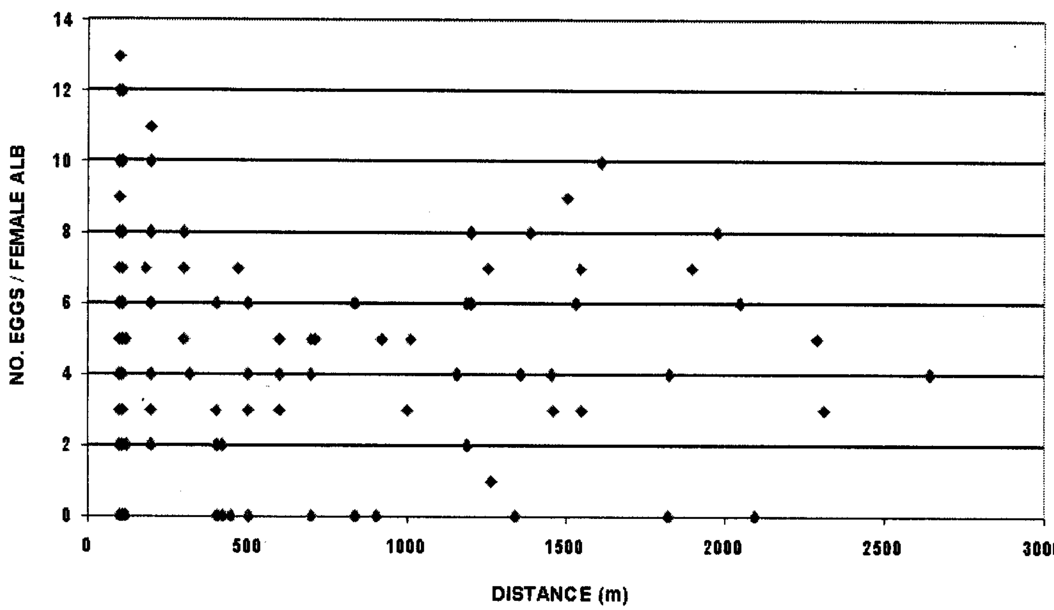

Figure 4. Distance ALB disperse eggs (2000).

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

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

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

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

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

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INTRODUCTION

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

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

MATERIALS AND METHODS

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

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

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

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

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

RESULTS

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

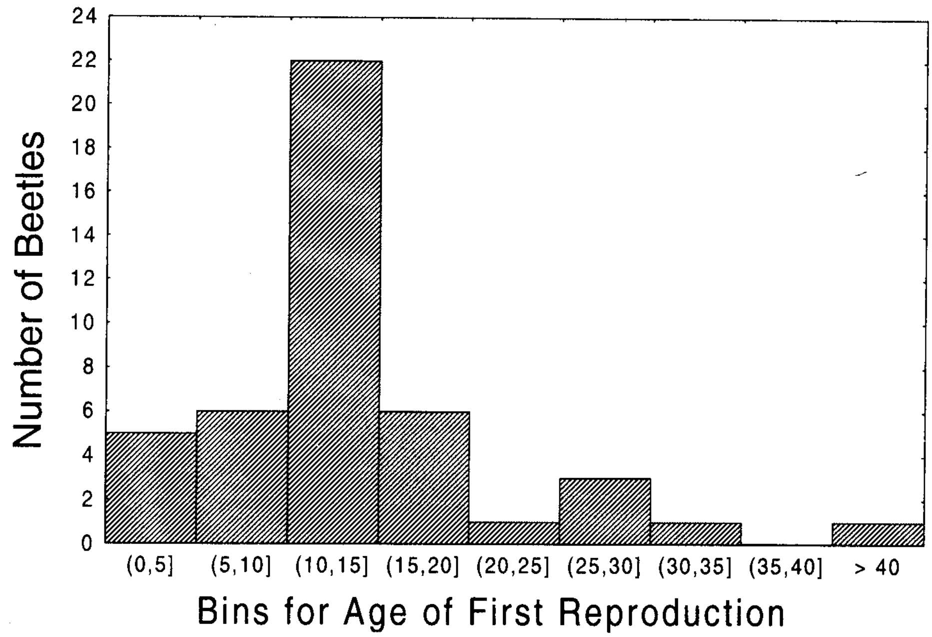

Figure 1. Preovipositional period.

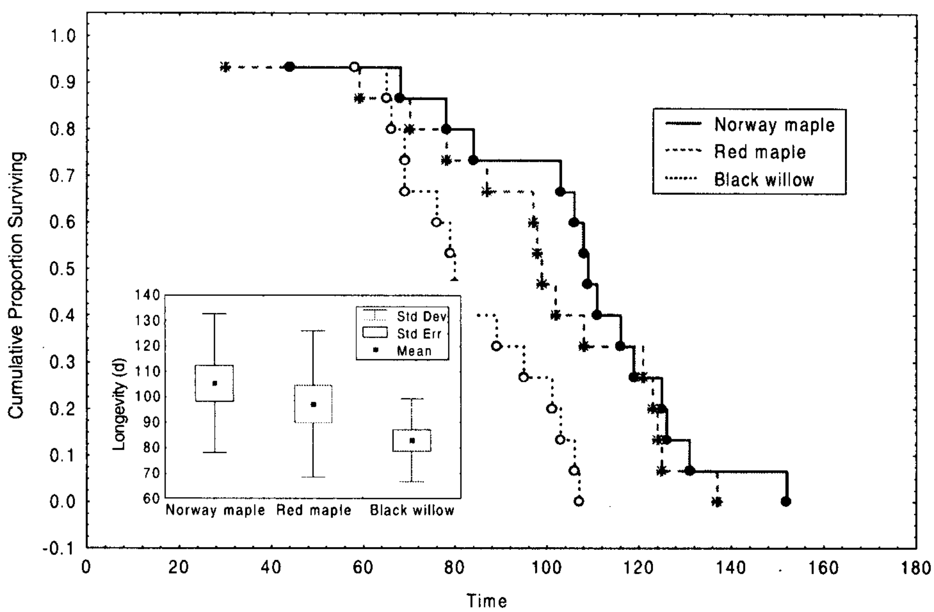

Figure 2. Survival of ALB.

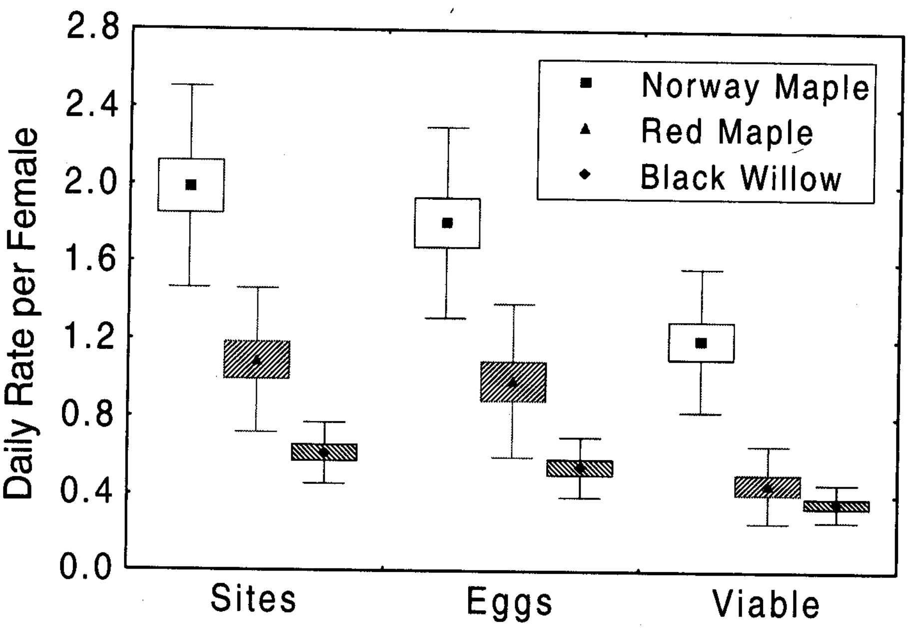

Figure 3. Daily reproductive rate.

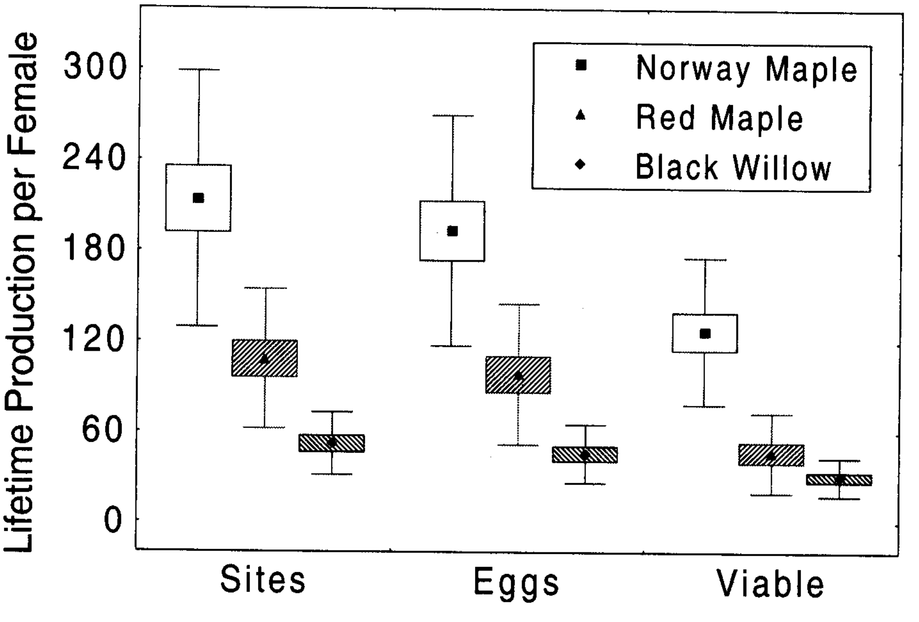

Figure 4. Lifetime reproductive rate.

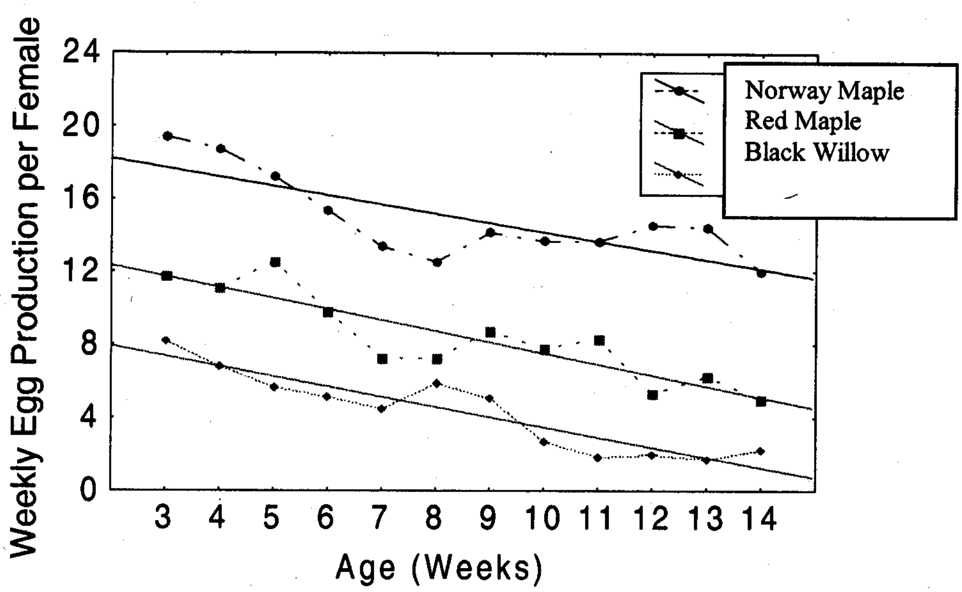

Figure 5. Age-specific fecundity.

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

DISCUSSION

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

ACKNOWLEDGMENTS

We thank USDA, APHIS, the State of Illinois, and City of Chicago officials, including Winn McLane (USDA, APHIS, PPQ, Otis Plant Protection Center, Otis ANGB, MA), Joseph J. McCarthy, Ken Kruse, and Joe Schaffer for assistance in the acquisition of *A. glabripennis*-infested logs from Chicago, IL. We also thank James Dobson (Forester for the Blackbird State Forest, DE) and Ken Swan (USDA, ARS, BIIR) for their assistance in acquiring trees used in this study.

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PATHOGEN-HOST AND PATHOGEN-PATHOGEN INTERACTIONS:

MICROSPORIDIA VS. THE GYPSY MOTH

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ABSTRACT

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

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

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

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

INFECTIVITY OF RHABDITOID NEMATODES TO THE ASIAN LONGHORNED BEETLE

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ABSTRACT

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

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

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

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ABSTRACT

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

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

TESTING MATING SUCCESS OF GYPSY MOTH FEMALES IN WISCONSIN

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ABSTRACT

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

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

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

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

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

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

TOXICITY AND EFFICACY OF IMIDACLOPRID TO

ANOPLOPHORA GLABRIPENNIS (COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT

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