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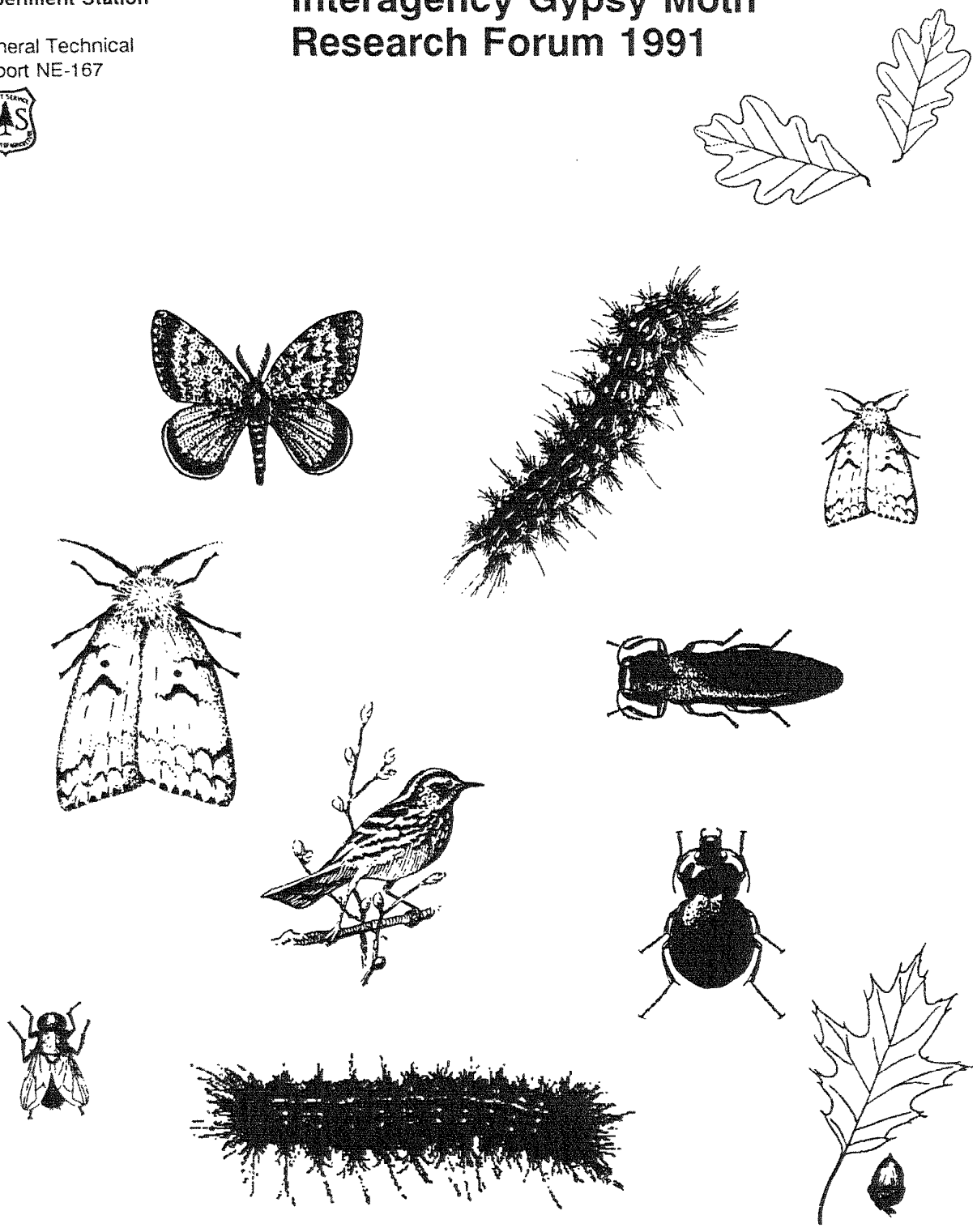

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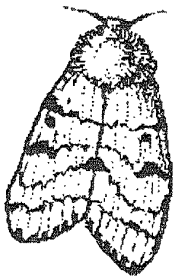

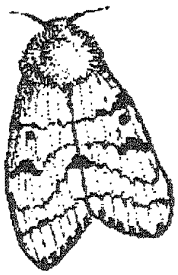

January 14-17, 1991
Loews Annapolis Hotel
Annapolis, Maryland

Edited by
Kurt W. Gottschalk and Mark J. Twery

Sponsored by:

Forest Service Research

Forest Service State and Private Forestry

Agricultural Research Service

Animal and Plant Health Inspection Service

Cooperative State Research Service

FOREWORD

This meeting was the second in a series of annual USDA Interagency Gypsy Moth Research Forums that are sponsored by the USDA Gypsy Moth Research and Development Coordinating Group. The Committee's original goal of fostering communication and an overview of ongoing research has been continued and accomplished in this meeting.

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

USDA Gypsy Moth Research and Development Coordinating Group

R. Bram, Agricultural Research Service (ARS)
C. Schwalbe, Animal and Plant Health Inspection Service (APHIS)
R. Riley, Cooperative State Research Service (CSRS)
T. Hofacker, Forest Service-State and Private Forestry (FS-S&PF)
M. McFadden, Forest Service-Research (FS-R), Chairperson

USDA Interagency Gypsy Moth Research Forum
January 14-17, 1991
Loews Annapolis Hotel
Annapolis, Maryland

AGENDA

Monday, January 14

REGISTRATION
WELCOME MIXER

Tuesday, January 15

OPENING SESSION . . . . . Moderator: Max McFadden, FS-R

Forestry Research, A Mandate for Change
Clifford Gabriel, CSRS

Forestry Research and the Appropriation Process
Deborah A. Weatherly, Administrative Assistant for Appropriations to Congressman McDade (R-PA)

OPENING SESSION, continued . . . . . Moderator: Thomas Hofacker, FS-S&PF

Agency Research and Development Objectives
Forest Service Research - Max McFadden
Agricultural Research Service - Ralph Bram
Cooperative State Research Service - Alan Cameron, Penn. State Univ.
Forest Service State & Private Forestry - Kenneth Knauer
Animal and Plant Health Inspection Service - Norman Leppla
Open Discussion

CONCURRENT SESSION I . . . . . Moderator: P. Schaefer, ARS
Population Dynamics: Natural Enemies
Presenters: R. Weseloh, Conn. Agric. Exp. Sta.; J. Elkinton, Univ. Mass.

CONCURRENT SESSION II . . . . . Moderator: R. Ridgway, ARS
Suppression Tactics
Presenters: C. Schwalbe, APHIS; J. Ghent, FS-S&PF; N. Schneeberger, FS-S&PF

POSTER SESSION

Wednesday, January 16

CONCURRENT SESSION I . . . . . Moderator: T. Kelly, ARS
Fundamental Biology
Presenters: J. Giebultowicz, Univ. MD; A. Valaitis, FS-R; C. Yin, Univ. Mass.

CONCURRENT SESSION II . . . . . Moderator: W.Wallner, FS-R
Sampling/Monitoring
Presenters: S. Fleischer, VPI; K. Thorpe, ARS

CONCURRENT SESSION I . . . . . Moderator: A. Wood, BTI
Molecular Biology
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CONCURRENT SESSION II . . . . . Moderator: W. Wallner, FS-R
Predicting Defoliation
Presenters: M. Montgomery, FS-R; D. Williams, ARS

CONCURRENT SESSION I . . . . . Moderator: M. Montgomery, FS-R
Population Dynamics: Insect/Host Interactions
Presenters: J. Witter, Univ. Mich.; J. Schultz, Penn. State Univ.

CONCURRENT SESSION II . . . . . Moderator: M. Shapiro, ARS
Microbial Control
Presenters: J. Podgwaite, FS-R; R. Webb, ARS; N. Dubois, FS-R

CONCURRENT SESSION I, continued
Population Dynamics: Insect/Host Interactions

CONCURRENT SESSION II . . . . . Moderator: A. Bullard, FS-S&PF
Application Technology
Presenters: K. Mierzejewski, Penn. State Univ.; D. Miller, Univ. Conn.

SPECIAL FORUM ON CONTAINMENT . . . . . Moderator: M. McFadden, FS-R
Presenters: W. Dickerson, NC Dept. Agric.; C. Schwalbe, APHIS

Thursday, January 17

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Rearing/Abnormal Performance Syndrome (APS)
Presenters: F. Proshold, ARS; M. Keena, FS-R; T. O'Dell, FS-R

CONCURRENT SESSION II . . . . . Moderator: K. Gottschalk, FS-R
Gypsy Moth Effects
Presenters: D. Fosbroke, West Va. Univ.; E. Corbett, FS-R

GENERAL SESSION . . . . . Moderator: D. Twardus, FS-S&PF
Integration
Presenters: J. Logan, VPI; W. Ravlin, VPI

CLOSING SESSION . . . . . Moderator: C. Schwalbe, APHIS
Research and Development Needs and Opportunities
R. Bridges, FS-R
W. Dickerson, NC Dept. Agric.
A. Bullard, FS-S&PF
K. Knauer, FS-S&PF replaced by T. Tigner, VA Dept. For.
F. Hain, NC State Univ.
Open Discussion

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ABSTRACTS OF PRESENTATIONS AND POSTERS

SUPPRESSION TACTICS

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WORKSHOP SUMMARY

INTRODUCTION

Development and practical use of suppression tactics for suppression of gypsy moth populations is a major challenge because of the complexities associated with the various tactics involved and the many variables associated with natural population dynamics of the gypsy moth and its hosts. The workshop was designed to review the current status of two suppression tactics, sterile insect technique and mating disruption, that are most appropriate for use against low-density populations, and two suppression tactics, diflubenzuron (Dimilin) and *Bacillus thuringiensis* (*B.t.*), that are most often used against high-density populations. Introductory presentations on each tactic were followed by short presentations and additional comments. The principal contributors are identified following the discussion of each tactic. This summary was prepared by Richard L. Ridgway and May N. Inscoe, USDA Agricultural Research Service.

LOW-DENSITY POPULATIONS

Sterile Insect Technique

The sterile insect technique is an autocidal technique that uses laboratory-reared, sterilized insects for population suppression. The technique relies on the interaction (*i.e.*, mating) of released sterile insects with the target population, thereby reducing the numbers of fertile matings which take place. Three release strategies have been developed for treatment of gypsy moth populations:

1) Release of fully sterile males, as pupae,
2) Release of substerile males (pupae), and
3) Release of sterile F_1 progeny (*i.e.*, eggs) of irradiated males and normal females.

A model for comparisons among the three release techniques suggests that strategy number 2, *i.e.*, release of males that have received a substerilizing dose, has a numerical advantage. There are, however, logistic and economic advantages to release of F_1 sterile progeny (eggs).

In competitiveness comparisons of hatch synchrony, larval development and survival, and adult mating, there are potential and known differences between F_1 sterile and normal insects. The degree of F_1 competitiveness has not been completely quantified. Releases of partially sterilized insects raised questions of differential larval mortality from predation. Results of studies to compare larval behavior have not demonstrated any large differences. This and the other areas of competitiveness comparisons require further study and corroboration.

Methods for characterizing feral low-level populations have not been developed in the generally infested area. Lack of such precise information makes it difficult to project overflooding ratios and to calculate the numbers of insects which must be released. Intrinsic rates of increase for feral populations are unknown; currently no techniques are available to estimate these. Better techniques must be developed for characterizing the target population and the impact of sterile releases.

Finally, a comparison of both economics and the efficacy of the substerile male and the F_1 egg release techniques needs to be completed. There is an open question concerning the likelihood that a population which averages five egg masses per acre would expand, remain innocuous, or simply disappear. Recognizing clumped dispersion of individuals in the field suggests that a "population level of five egg masses per acre" may have limited utility. There is a need to test the assumption that a population would expand by a factor of five from generation to generation. The suggestion was made that release of adults is likely to be more effective than egg release. This is based on experience with an agricultural pest. Questions were asked about the likelihood and the frequency of multiple matings by feral males, compared with irradiated males. The answer is apparently unavailable. These questions as well as parts of the proceeding discussion identify specific research needs.

Principal contributors on the sterile insect technique were Charles Schwalbe, USDA Animal and Plant Health Inspection Service; Alan Cameron, Pennsylvania State University; and Vic Mastro, USDA Animal and Plant Health Inspection Service.

Mating Disruption

Disruption of gypsy moth mating communication by means of aerial dissemination of disparlure, the synthetic pheromone of the gypsy moth, was suggested as a means of control about twenty years ago. Early trials, using extremely low rates of 50 mg AI/ha effectively reduced male trap catch for a portion of the flight period. In subsequent trials involving a variety of controlledrelease formulations and increased dose rates, reduced trap captures and, in some cases, suppression of mating have been demonstrated. Results from the most recent trials, conducted by Ralph Webb of the USDA Agricultural Research Service and others demonstrated positive correlation between incidence of mating disruption and dose rate.

As part of a gypsy moth eradication effort, a treatment for a low-level population was needed in a region in Giles County, VA, which included an area where a National Science Foundation study on the behavior of the dark-eyed junco was being conducted. Applications of either *B.t.* or Dimilin were precluded because of possible adverse effects on lepidopteran larvae that make up the major component of this bird's diet, and a gypsy-moth-specific tactic was required. The use of Gypchek was not feasible because the amount of Gypchek available was insufficient for the area to be treated (about 1000 ha). Accordingly, a decision was made to pursue aerial application of racemic disparlure as a control tactic. An *ad hoc* team was formed, consisting of representatives from Appalachian Integrated Pest Management Demonstration Project (AIPM), USDA Animal and Plant Health Inspection Service (APHIS), USDA Agricultural Research Service (ARS), USDA Forest Service (USFS), and Virginia Department of Agriculture and Consumer Services (VDACS).

In 1989, in the Giles County treatment area, disparlure was applied from a fixed-wing aircraft at a rate of 75 g AI/ha. The formulation used was the only product registered for aerial application, Disrupt II, a plastic laminated flake formulation from Hercon containing at most 18.5% AI. The sticker, Gelva RA 1990 (Monsanto), was applied neat to the flakes during application with the necessary special delivery system. Sampling during application established an average of 41 flakes/sq. meter, but the pattern within the swath was uneven, with peaks and valleys. Flakes were deposited in all layers of the canopy, and 10% of the flakes penetrated all levels of foliage and were deposited on the forest floor. Over the 6-week study period, the sticker performed well and only an additional 6% of the flakes fell to the forest floor.

An area outside the treatment area was reserved as a control block; pretreatment egg mass surveys were negative in both the treatment and control blocks, and the areas had similar trap catches. In post-treatment grid trapping in both 1989 and 1990, no moths were caught in the treatment area, while traps in the control area continued to catch moths. Sterile lab-reared females were placed in the treated area to document the degree of mating disruption achieved; none of these were mated.

In 1990, aerial application of disparlure was used operationally in another eradication project in Tennessee as a joint effort between the Tennessee Department of Agriculture and APHIS. The first year trapping results are encouraging.

Eradication projects are not generally suitable as study sites for demonstrating efficacy of a control tactic, since maintaining untreated areas as controls is counterproductive to the objective of eradication. For this reason, a study site along the leading edge of the gypsy moth infestation in the northern portion of Rockbridge County, VA, was selected as the 1990 study site. The 1989 grid trapping results showed moth catches averaging 34 moths /trap over the proposed study area.

In planning the Rockbridge County study, a commitment was made to continue treatment and evaluation until a substantial change in the gypsy moth population could be demonstrated.

Efficacy evaluations were to include both indirect methods (*e.g.*, grid trapping and the use of wild monitor females in escape-resistant mating stations) and direct methods (intensive monitoring for life stages to document population trends). Application procedures, deposition, and release rates of all formulations used were also to be evaluated. Untreated blocks were established for comparison with the treated blocks. Within the blocks, more than a thousand 1/40-acre plots were established at 50-m intervals for pre- and post-treatment life-stage surveys.

Disrupt II was applied at two different rates — one application of 75 g AI/ha applied just prior to moth flight, and two applications of 75 g AI/ha, one applied just prior to moth flight and the other 2-3 weeks later, during peak flight. Post-treatment trapping caught fewer than 10 moths in the treated blocks and more than 600 moths in the control areas. In the control blocks, 7% of the monitor females were mated, while none were mated in any of the treatment blocks. Posttreatment egg mass surveys located an average of 4.1 egg masses/acre in the control blocks and no egg masses/acre in the treated blocks. In 1991, blocks that received a single application of Disrupt II in 1990 will be re-treated. Monitoring of all treated blocks will continue.

A product that can be applied with conventional spray equipment would be most desirable as an alternative to the laminate flakes. A flowable bead formulation made by AgriSense is still in the developmental stages. The formulation contains 40% AI and is designed to be applied with conventional spray equipment. A small amount of formulation was available at the time of the 1990 tests, but difficulties in application could not be resolved in time to compare and evaluate application procedures, deposition, and release rate profiles of the bead formulation and Disrupt II. Aging studies with the two formulations indicated that the patterns of disparlure emission from the two formulations over time were quite different.

The results obtained over the past two years are encouraging. Work will continue in Rockbridge County on efficacy evaluations using new products and on refinements in timing of application with all products. Research and development needs include sprayable formulations with predetermined release rates and improved methods for measuring actual mating disruption.

Principal contributors on mating disruption were Donna Leonard and John Ghent, U.S. Forest Service; and Vic Mastro, USDA Animal and Plant Health Inspection Service.

HIGH-DENSITY POPULATIONS

Diflubenzuron (Dimilin)

Data were presented from replicated aerially applied field trials conducted in 1990 with Dimilin in which population change in treated areas, measured by decrease in egg masses, was 94% and 99% with two different formulations. However, substantial decreases in egg masses also occurred in the control so that the effect of the Dimilin was unclear. Studies in

Maryland using early instar larval counts before and after aerial application of Dimilin resulted in corrected mortalities of 93% and 87% in 1989 and 1990, respectively. Use of Dimilin in Maryland in the years 1986 through 1988, as determined in the U.S. Forest Service treatment monitoring base, reduced populations by 83%. Comparative data with *B.t.* indicated that, when only efficacy is considered, Dimilin is the material of choice. Quantitative data on the efficacy of Dimilin throughout the season and the effects on subsequent generations are needed in order to develop comparative costs of gypsy moth management tactics.

Principal contributors on Dimilin were Charles Schwalbe and Win McLane, USDA Animal and Plant Health Inspection Service; and Robert Tichenor, Maryland Department of Agriculture.

Bacillus thuringiensis

The National Parks Service as a matter of policy does not use Dimilin in the National Capital Region because of concern about the non-target effects. Therefore, when intervention for the gypsy moth is practiced, *Bacillus thuringiensis* (*B.t.*) is the material of choice. Results in Greenbelt National Park in 1989 using one application of *B.t.* were very poor; however, it rained soon after application. Two applications were used in 1990 with excellent results. Two applications of *B.t.* are preferred for use, at least in the National Capital Region. Because of the short residual effects of *B.t.*, adequate biological activity from one application does not remain present over the period of time that gypsy moth eggs hatch.

Early instar larval counts before and after aerial applications of *B.t.* indicated mortalities after one application of 58%, 71%, and 64% in 1988, 1989, and 1990, respectively, and mortalities after two applications of 81%, 87%, and 80%, for those same years. These results indicated that two applications of *B.t.* were more efficacious than one.

Reports of experience by private gypsy moth managers indicated that two applications of *B.t.* might not be more effective than one application. These managers emphasized that to obtain desired results with *B.t.* low level populations probably should be treated and suppressed before they increase. One application of *B.t.* in Maryland in the years 1986 through 1988, as determined in the U.S. Forest Service treatment monitoring base, reduced populations only by 46%.

A number of areas need to be addressed in order to increase the probability of obtaining high levels of control with *B.t.*, such as: (1) more efficacious strains or increased dosages of *B.t.*; (2) more accurate sampling methods; (3) electronic guidance systems for aircraft to improve spray coverage; (4) increased size of spray blocks to reduce edge effects; (5) treatment of increasing rather than peak populations.

Principal contributors on *B.t.* were Christine Haggerty, US National Park Service; Ralph Webb, USDA Agricultural Research Service; Mark Tichehurst, National Gypsy Moth Management Group; and Robert Tichenor, Maryland Department of Agriculture.

FUNDAMENTAL BIOLOGY OF THE GYPSY MOTH:

A SUMMARY OF RECENT RESEARCH RESULTS

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ABSTRACT

Within the past several years significant progress has been made in developing information on the fundamental biology of the gypsy moth. Areas of progress include: regulation of sex pheromone production and sperm release, characterization of regulating enzymes, blood proteins and egg development, and isolation and characterization of hormones and neurohormones. Development of novel methods of pest control will depend on future developments in some of these areas with the addition of molecular biological techniques. Recent progress in these areas is discussed.

INTRODUCTION

Fundamental biology encompasses an enormous area of the biological sciences including most, if not all, the non-theoretical or non-applied disciplines. Disciplines such as chemistry, biochemistry, physics, genetics, endocrinology, neurobiology, virology, parasitology, and many others, contribute to or are encompassed by fundamental biology. Nearly ten years ago, a leading group of government, industry and academic scientists outlined the following areas as research priorities in insect fundamental biology (Klassen, 1983): (1) devise new methods for modifying germplasm of insect pests and their natural enemies; (2) genetics and genetic engineering of arthropods and their natural enemies; (3) insect metabolism, nutrition, and quality; (4) biochemical and environmental regulation of life processes in insects; (5) behaviors of insects and their natural enemies; (6) insect population processes and ecology; (7) reduce transmission of plant pathogens by insects; (8) fundamental aspects of insect control with synthetic chemicals. Among these, the category receiving the top priority was (4) biochemical and environmental regulation of life processes which included the subcategories (a) insect neurobiology and (b) hormone action, secretion and metabolism -- first and second priorities of all subcategories, respectively. Although not all of these categories have relevance to gypsy moth biology, many remain top priorities, today.

The 1991 USDA Interagency Gypsy Moth Research Review was convened for the second year in a row to provide and disseminate current information on research related to the gypsy moth. Many areas were covered, some of which fall under the broad definition of

fundamental biology. Summaries of these areas can be found in other chapters of these proceedings and will not be considered here. The present summary is limited to those areas presented by speakers invited to the Fundamental Biology Workshop and to recently published results from related areas on gypsy moth fundamental biology. These areas include neurobiology, endocrinology, reproductive physiology, behavioral physiology and anatomy. It is fitting that the invited speakers were from locations where most of the current research on these aspects of gypsy moth fundamental biology is being carried out. That is: (1) the USDA-ARS group at Beltsville, Maryland (recently consolidated as the Insect Neurobiology and Hormone Laboratory) and collaborators at the University of Maryland, College Park; (2) the University of Massachusetts, Amherst; and (3) the newly established USDA-FS gypsy moth research group at Delaware, Ohio.

SEX PHEROMONE PRODUCTION

The first speaker, Jaga Giebultowicz (Dept. of Zoology, Univ. of Maryland, and ARS-Insect Neurobiology and Hormone Laboratory, Beltsville, Maryland), presented recent work on the control of pheromone synthesis, followed by work on the mechanism and control of testis sperm release (Proceedings' abstract). Virgin female gypsy moths produce a sex pheromone, disparlure, or cis-2-decyl-3-(5-methylhexyl)oxirane (Bierl *et al.*, 1970). In *Helicoverpa (Heliothis) virescens* (Raina *et al.*, 1989), production of female sex pheromone is under the control of pheromone biosynthesis activating neuropeptide (PBAN), which is probably true for gypsy moths, as well, and is currently under investigation. The regulation of disparlure production has recently been examined in mated females (Giebultowicz *et al.*, 1990c, 1991) and found to be regulated by a two-step process. Initially, pheromone production is suppressed by the introduction of male genitalia into the bursa copulatrix. Permanent suppression requires the presence of spermatheca that have an adequate supply of sperm. This mechanism of pheromone suppression clearly differs from *Heliothis* where sperm play no essential role in the termination of pheromone production following mating (Raina, 1989). Thus the necessity of studying the gypsy moth, specifically, is demonstrated. Giebultowicz *et al.* (1990b) have also shown that mated-like behavior, including pheromone suppression, occurs in senescent virgin females, although the spermatheca is not required, as it is in mated females.

Another speaker, Chih-Ming Yin (Department of Entomology, University of Massachusetts, Amherst) presented recent work on the ultrastructure of the sex pheromone gland in gypsy moth females. Researchers from this group have studied the regulation of pheromone production in gypsy moths for some time (for references see Tang *et al.*, 1987; Crnjar *et al.*, 1988). The dorsal sex pheromone glands are located on the intersegmental membrane between the eighth and ninth abdominal segments (Proceedings' abstract). They appear externally as areas of convoluted cuticle. Dramatic ultrastructural changes occur specifically in the glandular cells between the pharate adult stage and egg laying, and seem to be correlated with pheromone production. These changes include increases in the number of basal infolds, alignment of microtubules from apical to basal ends (possibly related to cellular

transport of pheromone), and the presence of dense granules that are unique to pheromone gland cells.

REGULATION OF SPERM RELEASE

Another area presented by Dr. Giebultowicz is the regulation of sperm release in gypsy moth males. It was originally studied by Giebultowicz *et al.* (1988). They determined that the rhythm of sperm release from the testes, which is initiated on pupal day 8 at 25°C, has an endogenous circadian nature. That is, it free-runs in constant darkness, undergoes rapid phase-shifts and is temperature compensated. In addition, the circadian-pacemaker (i.e. clock) was shown by *in vitro* studies to be localized in the testis-seminal ducts complex (Giebultowicz *et al.*, 1989; Giebultowicz and Riemann, 1990). This rhythm can be suppressed by rearing males in constant light through adult eclosion (Giebultowicz *et al.*, 1990d), but can be reversed by switching to normal LD 16:8 h conditions by day 9 after pupation. In light-suppressed males, approximately half the normal sperm are released from the testis into the vas deferens, and of the few sperm bundles that are transferred to the bursa copulatrix during mating, few disperse their sperm so that no sperm reach the spermatheca. Spermatheca filled with sperm are required for egg laying. Further studies into the regulation of sperm release in gypsy moth males have revealed a requirement for a normal decline in hemolymph 20-hydroxyecdysone titer to allow initiation of sperm release (Giebultowicz *et al.*, 1990a) and cyclical secretory activity in the upper vas deferens that correlates with sperm release (Riemann and Giebultowicz, 1991). Future studies will attempt to locate the circadian clock and light receptors and to identify specific proteins and other factors involved in cyclical release of sperm.

REGULATORY ENZYMES

The third speaker, Al Valaitis (FS-Northeastern Forest Experiment Station, Delaware, Ohio) presented recent work on the isolation and characterization of three regulatory enzymes important in insect metabolism (Proceedings' abstract): trehalase (THA), 6-phosphofructo-1-kinase (PFK) and juvenile hormone esterase (JHE). One of the major means of energy storage in insects is in the form of trehalose which is hydrolyzed by THA. Disruption of the normal trehalose titer might be expected to result in changes in blood volume, hyperactivity and compensation by release of multiple neurohormones which would be detrimental. Gypsy moth THA appears to be a glycoprotein with a molecular size of approximately 60 kD. Further characterization is underway.

A key regulatory enzyme in glycolysis is PFK. Gypsy moth PFK is allosterically regulated by many of the same activators and inhibitors as mammalian PFK, although it does differ in some respects, such as its poor inhibitory response to citrate (Valaitis, 1991; Proceedings' abstract). Gypsy moth PFK has a molecular size of 330 kD, as determined by gel filtration,

indicating a likely tetrameric structure since mammalian PFKs are approximately 84 kD and tryptic digests of gypsy moth PFK show similar sequences to the human muscle enzyme.

The third enzyme under study, JHE, shows considerable promise for development as a biological control agent. It regulates the hemolymph titer of insect juvenile hormone, a hormone present in femto- to picogram levels per microliter of hemolymph. Proper regulation of juvenile hormone is critical to normal insect development and metamorphosis. JHE has been purified from gypsy moth hemolymph (Valaitis, 1990, 1991; Valaitis and Jolliff, 1990; Proceedings' abstract). There are two probable genetic variants, undistinguishable by N-terminal amino acid sequence and HPLC tryptic peptide mapping, with an apparent molecular size of 62 kD. Sequencing and immunological results suggest the unexpected possibility that different JHEs are present in different insect species. JHE titers have been determined for the gypsy moth, showing three peaks in last instar (sixth) females and (fifth) males with the major peak occurring in the middle of the instar when JH titers are rapidly declining (Tanaka *et al.*, 1989). One peak of JHE activity was observed in day 3 pupae. Although this research on the gypsy moth has just begun, it has the potential for developing biologically-based insect control agents, as evidenced by a recent study demonstrating feeding and growth inhibition of *Trichoplusia ni* larvae by a baculovirus, *Autographa californica*, engineered with a *Heliothis virescens* gene for JHE (Hammock *et al.*, 1990).

JUVENILE HORMONE

Although it was not discussed at the Workshop, Yin's group has recently developed a bioassay for juvenile hormone (JH) biosynthesis in *L. dispar* (Jones and Yin, 1989). This radiochemical assay in conjunction with high pressure liquid chromatography has demonstrated that JH III is the primary biosynthetic product of gypsy moth female CC-CA complexes *in vitro*, with possibly a little JH II produced in fourth instar female larvae. This finding that JH III is the primary JH in *L. dispar* is unusual for Lepidoptera and likens it to non-Lepidoptera where JH III predominates. In *L. dispar* synthesis of JH was maximal during the beginning of the last (sixth) larval instar, dropped dramatically on day 6 and decreased to negligible levels by day 10 (Jones and Yin, 1989). Tanaka *et al.* (1989) have demonstrated similar hemolymph titer changes for JH using the *Galleria mellonella* bioassay.

NEUROHORMONES

Most of the research in this area has been done within the last five years at the ARS-Insect Reproduction Laboratory, Beltsville, Maryland (now consolidated into the Insect Neurobiology and Hormone Laboratory) and has focused on the ecdysiotropins: prothoracicotropic hormone (PTTH) and testis ecdysiotropin (TE). With regard to PTTH, the neurohormone that promotes insect molting and metamorphosis by stimulating ecdysone synthesis by the prothoracic glands (PG's), much of this work on the gypsy moth has been

recently reviewed by Masler *et al.* (1989), Kelly *et al.* (1991) and was recently described in Agricultural Research Magazine (Silva, 1991). To briefly summarize: *in vitro* and *in vivo* assays for PTTH have been developed, larval and pupal PTTHs have been partially purified and characterized to give molecular size ranges for the two forms of 4-6 kD (small PTTH or bombyxin) and 11-15 kD (large PTTH), and gene isolation studies have begun. Research on the *L. dispar* large PTTH gene was presented in a poster by Davis *et al.* (Proceeding's abstract). Other recent research, not included in these reviews, is the progress in the purification of *L. dispar* egg PTTH (Masler *et al.*, 1991). This PTTH appears to be small (< 5 kD; Kelly *et al.*, 1990b) and may be involved in embryonic diapause (Bell *et al.*, 1990). Other areas include the demonstrations of 3-dehydroecdysone production by gypsy moth PGs (Kelly *et al.*, 1990c), the insect gut as a new source of ecdysiotropic peptides (Gelman *et al.*, 1991; Proceeding's abstract), and evidence by Thyagaraja *et al.* for a non-cerebral neurohemal center that controls development (Proceedings' abstract). With regard to TE, Loeb *et al.* (1990) have partially purified this brain factor(s) which stimulates ecdysteroid production by gypsy moth testes, and have demonstrated multiple forms with molecular sizes ranging from approximately 1-2 kD to greater than 29 kD. In another neuropeptide-regulated area, a proctolin-like neuropeptide has been demonstrated in the nervous system of *L. dispar* (Davis *et al.*, 1989). As with JHEs, insect neuropeptides hold promise for development as biologically-based control agents by insertion through baculoviruses (Maeda, 1989; Kelly *et al.*, 1990a).

HEMOLYMPH PROTEINS AND EGG DEVELOPMENT

Within the past few years considerable progress has been made in characterizing some of the hemolymph (i.e. blood) and egg proteins of *L. dispar* and in describing their developmental variation. Hemolymph vitellogenin (Vg) and egg vitellin (Vt) have been partially characterized by Davis *et al.* (1990b), showing similarity in molecular size for pupal Vg and Vt (>500 kD). Vg and Vt have two subunits of 180 and 165 kD. Vg appears in the hemolymph after day 2 of the last larval instar, but is not accumulated by the ovaries until day 3 of the pupal stage. Vg synthesis and ovarian uptake are thus temporally and developmentally separate events in the gypsy moth. Davis *et al.* (1990a) have shown that juvenile hormone inhibits the initiation of Vg synthesis in *L. dispar*, a novel role for juvenile hormone in insect species. The decline in inhibitory sensitivity to JH correlates with the drop in JH synthesis and hemolymph JH titers, as previously described above.

Another *L. dispar* hemolymph protein that has been well characterized by Karpells *et al.* (1990) is arylphorin (Ap). *L. dispar* Ap has a molecular size of 440 kD with two subunits of 73 and 80 kD suggesting a hexameric structure. As in other insect species, its concentration cycles with each molting cycle, showing an increase during feeding, followed by a sharp decrease between apolysis and ecdysis. Unlike other lepidopterans, no larval serum protein (LSP) could be identified in *L. dispar* hemolymph.

Ovarian development has been characterized by Davis *et al.* (1990b) for the last larval instar and pupa where egg development progresses from germarial separation in the early last instar, through yolk deposition in the early pupa, to chorion formation in the late pupal stage. Ballarino *et al.* (1991a,b) have characterized ovarian development in male *L. dispar* showing that yolk uptake and chorion formation proceed in the absence of Vg, but at a rate slower than in normal females. Work on characterization of *L. dispar* chorion proteins and structural genes was presented in discussion by Robert Leclerc (working with Jerome Regier, Center for Agricultural Biotechnology, Univ. of Maryland, College Park). Dr. Leclerc described the unusually thick eggshell of *L. dispar* (approx. 50 lamellae vs. several lamellae for *H. zea*) which may account for some of the success of *L. dispar* in establishing itself as a serious pest in the United States. Forty choriogenic cDNA clones have been selected from three hundred as a start in characterization of the chorion gene family. Future studies will involve characterization of the chorion gene regulatory regions and their associated transcription factors.

SUMMARY

Significant progress has been made in acquiring fundamental information about the development and behavior of the gypsy moth. Some of this information has a clear potential for development of novel biologically-based control methods. Further research is necessary in the areas of protein and peptide sequence identification and the isolation and sequencing of structural genes for these moieties.

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MOLECULAR BIOLOGY

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WORKSHOP SUMMARY

Alan Wood, moderator, welcomed the approximately 50 participants. He introduced the speakers and the subject areas to be covered by them.

The first presentation by Jim Slavicek dealt with the functional gene map of LdNPV. Using cloned DNAs corresponding to various genes of another baculovirus, *Autographa californica* nuclear polyhedrosis virus (AcNPV), as probes, he checked for the presence and location of similar genes in LdNPV. the AcNPV genes used as probes included immediate early 1 (IE-1), immediate early N (IE-N), p26, p10, 6.9k core protein, 39k capsid protein, proliferating cell nuclear antigen (PCNA), DNA polymerase, Ecdysteroid UDP-glucosyl transferase (EGT), and superonide dismutase (SOD) genes. He was able to detect several of these genes in LdNPV. However, their arrangement in LdNPV genome was different from that found in AcNPV genome. He also described the work in progress on isolation and characterization of IE-1 and EGT genes from LdNPV (see abstract in this proceedings).

Dwight Lynn reviewed the research on development of gypsy moth cell lines. Initial works by Quiot, Goodwin, and Lynn *et al.* resulted in cell lines that could replicate LdNPV. However, none of them matched other insect cell/virus systems in their efficiency. Lynn described his work on production and improvement of Ld-fat body cell line. The new LdFb cell line could replicate LdNPV about 50-fold better than the best of Goodwin's cell lines. Clones that could replicate the Abington-LdNPV at a higher rate were obtained. He is selecting Ab-LdNPV strains that are suitable for higher *in vitro* production (in LdFb cell line) and have low LC_{50} and/or LT_{50} (see abstract in this proceedings). Answering questions during the discussion following the presentation, he mentioned that LdFb cell lines will be available to other researchers soon.

Continuing on the subject of *in vitro* production of LdNPV for use in biological control, Ed Doughtery reviewed positive and negative aspects of *in vitro* production of virus. Advantages are:

a) it is not labor intensive
b) it gives a better quality, cleaner final product
c) the final product has no contaminants
d) it is favored by biotech companies

However, the *in vitro* method has disadvantages such as:

a) it is not economical due to high cost of equipment, media, and other supplies
b) the problem of FP mutation which will result in reduced yields of OBs
c) the need for regular bioassay because of FP mutations
d) there is a possibility of gypsy moth developing resistance to the homogeneous virus population

Ed Doughtery informed the group that the technology transfer arrangement with the American Cyanamid Corporation has enabled scale up of *in vitro* production of LdNPV (Abington strain) using the LdFb cell line (see abstract in this proceedings).

Following Doughtery's presentation, issues of FP mutation and insect-resistance were discussed. Alan Wood suggested whether FP problems can be avoided by using Gypchek® instead of Abington strain. He also mentioned that in Europe resistance to homogeneous strains of baculovirus has been observed. In some cases, the resistance was only during early instars and in some only during late instars.

There was more discussion about sharing information and materials among the scientists participating in gypsy moth research. Everyone felt that lack of interaction will hurt the progress of the research. It was agreed that in most part, the technology transfer arrangements and involvement of industries was responsible for this. The session ended with Alan Wood's comments that increased co-operation among scientists is necessary to expedite research on biological control of gypsy moth.

APPLICATION TECHNOLOGY

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WORKSHOP SUMMARY

This session dealt with the current state of aerial application technology, subject areas presently under investigation and perceived needs for future study. Approximately 20 persons were in attendance.

Karl Mierzejewski presented an overview of the Northeastern Forest Aerial Application Technology Group (NEFAAT) and a summary of some of the work this group has done during the past three years. NEFAAT is comprised of representatives from Penn State University, USDA APHIS (both the Otis Methods Development Group and Aircraft Operations) and the USDA Forest Service including the Northeastern Forest Experiment Station, Forest Health Protection and the AIPM Project. The group was formed in 1987 to look into causes of treatment failures and has expanded to the identification and investigation of methods of improving aerial application technology. It has carried out a number of evaluations since then dealing with development of new atomizers, tightening of droplet spectra and ensuring even distribution of materials over the spray swath. Work to date suggests that treatment failures result from poor application procedures or assorted application problems. NEFAAT suggests that gypsy moth control could be greatly improved through better training and the development and use of application guidelines.

While a number of studies have been conducted in the recent past on the improvement of aerial application technology, much work remains to be done. Suggested areas include:

Cross wind application versus with wind applications

Improving "evenness" of effective swaths

Improvements to the Swath Kit

Improving aircraft guidance and flight recording

Control of pesticidal drift and the improvement of predictive models

Continued evaluation of droplet size and improved deposit analysis

Continued development and evaluation of new atomizers

Improvement of insecticide formulations and amounts of active ingredient

Questions from the floor following the presentation suggested these additional areas of study:

Accountability of material released from the aircraft

Determination of the number of crystals/droplet for Bt

Evaluation of the palatability of various droplet sizes

Matching aircraft, materials and appropriate dispersers

Better definition of appropriate wind speeds at application time

More study of application timing relative to larval and foliage development

The overall conclusion of this presentation was that aerial application is and will continue to be an essential part of gypsy moth management programs and improvement in this technology will result in greater efficiency, effectiveness and increased environmental safety and reduced costs.

Dave Miller indicated that the real question being dealt with in improvements to aerial application technology is very simple--how to hit the target at the proper time with the minimum amount of material necessary to do the job. In looking at this question, he reported on his work aimed at developing micrometeorological criteria for use during aerial spray operations. Once developed, these will become one component of the operational guidelines suggested by Mierzejewski.

Two different studies have been conducted to describe spray movement and deposition under specific meteorologic conditions. Results indicate that it may be possible to determine drift and dispersion based upon atmospheric stability. Miller is working with the US Forest Service FSCBG Aerial Spray Model and is using the results of his studies to verify this model for use in the eastern hardwood forest. He suggests that improved characterization of the hardwood canopy component of the model along with increase sensitivity to the turbulence, stability and humidity regimes found in these areas will greatly improve the predictive ability of this tool.

Discussion following Dave's presentation helped clarify that spray drift is a function of wind speed and direction, turbulence and atmospheric stability; that in the presence of sunlight, the upper canopy tends to be unstable while the lower canopy is stable; and the best time to spray for gypsy moth control is in the early morning.

WHY NORTH CAROLINA IS INTERESTED IN SLOWING THE SPREAD OF THE GYPSY MOTH

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ABSTRACT

The south and westward spread of the gypsy moth is occurring at a rate of ten to fifteen miles per year. The current advancing front is represented by a line running approximately from Lake Erie through eastern Ohio to Roanoke, Virginia to Kill Devil Hills, North Carolina. North Carolina has a comprehensive detection system consisting of approximately 16,000 traps located throughout the state. Despite this intensive effort of detection and eradication, a small area of northeastern North Carolina is generally infested with the gypsy moth and under quarantine. The expected impact of the spread of this pest into North Carolina includes economic and environmental factors.

Economic: North Carolina is the tenth most populous state in the United States with over 8,000,000 residents and 18,500,000 acres of timberland. Hardwoods make up 53% of this total. The value of harvested saw timber is approximately $300,000,000.00 per year. The value of harvested cordwood exceeds $150,000,000.00 annually. The payroll for the timber industry as a whole is approximately $475,000,000.00 per year. The retail value of furniture produced in this state is approximately $4,200,000,000.00 per year with a corresponding manufacturing payroll of $110,000,000.00. Approximately 3,750,000 Christmas trees are harvested each year, generating a farm sales of $75,000,000.00. The plant nursery industry is a rapidly expanding segment of the North Carolina economy with over $100,000,000.00 in annual sales. Tourism, with a large portion based on out-of-doors attractions, is a $7,000,000,000.00 per year industry. It is estimated that North Carolina homeowners will spend over $11,000,000.00 per year to protect residential trees from this pest.

Environmental: North Carolina is an environmentally conscious state. The impact of defoliation caused by the gypsy moth will result in tree mortality, deterioration of watersheds, and changes in wildlife composition. The increased use of insecticides, both rural and urban, will also increase the problems associated with chemical application, runoff, contamination of groundwater, non target impact, and disposal of pesticide containers.

The people of North Carolina have a history of being proactive in dealing with agricultural pest problems. The experience gained by the North Carolina Department of Agriculture in gypsy moth monitoring and treatment activities provides optimism that a coordinated, comprehensive approach to gypsy moth management can slow the spread of this pest. By

slowing the spread, residents of North Carolina and other uninfested areas will delay the occurrence of adverse environmental factors and making expenditures for controls. Slowing the spread will also give more time for the development of more effective treatments for controlling this insect.

SCOPE OF A CONTAINMENT PROGRAM

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ABSTRACT

The purpose of this presentation is not to convey my opinions on how containment of the gypsy moth can actually be accomplished but rather to provide a perspective on the scope of what such a program might entail. I also hope that these comments will provide a platform from which important policy and scientific questions can be considered.

Current gypsy moth distribution in the United States is not perfectly defined. While maps are published that illustrate gypsy moth infestation from a regulatory standpoint, much greater detail on the nature of populations along the "leading edge" is revealed by the four zones that have been defined through the Appalachian Integrated Pest Management Program. An understanding of these zones and an appreciation of how they can fit into the development of an operational containment project are of important conceptual value.

A potential containment zone may be envisioned as a 50 kilometer-wide border along the generally infested area. While this description is certainly oversimplified, it gives an idea of the scale on which a containment project would take place. Different pathways for spread and varying rates of spread along the front suggest considerable complexity of intervention activities that might be mobilized. For example, natural spread is likely to occur at a much faster rate along mountainous routes whereas short-range artificial spread might be the most important component of movement in flatter terrain.

The Appalachian Integrated Pest Management project is certainly the biggest program that has been tried thus far, and the general belief is that it is "manageable" in scale. A 50 kilometer-wide containment area along the front of the generally infested area would be only slightly larger than the current Appalachian Integrated Pest Management program. Admittedly, there would be more States, political jurisdictions, and land use types involved which would greatly increase the complexity of operations. Nonetheless, such a program would not be out of the realm of our experience in terms of scale of geographic area involved.

The major questions that need to be addressed are technical and political: Does technology exist or can it be developed that will enable us to meet the objective of slowing the rate of natural spread, and can the resources be maintained that will be necessary to keep such a program effective over the long term? After all, a containment program is an initiative which should not be started unless economic and biological assessments are favorable.

GYPSY MOTH EFFECTS

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WORKSHOP SUMMARY

This session dealt with the current state of research into the effects of gypsy moth on forest vegetation and water quantity and quality. The presenters were David E. Fosbroke of West Virginia University and Edward S. Corbett of USDA Forest Service, Northeastern Forest Experiment Station.

Dave Fosbroke presented data on changes in forest vegetation: the differential mortality and growth of overstory trees due to differential defoliation and the changes in regeneration and woody shrubs and herbaceous plants that occur in the understory following defoliation and overstory mortality. Studies under way by Ray Hicks and Dave Fosbroke at WVU and by Kurt Gottschalk and Mark Twery of the Forest Service were presented. A key point that Dave made was the large degree of variability that exists in the forest response to defoliation and mortality. Another important point involved the shift in composition of the regeneration toward species that are less susceptible to gypsy moth.

Following Dave's presentation, a number of questions and lively discussion occurred. Some of the major points of the discussion were:

> Is the large degree of variability in response of mortality and growth in part due to genetic variation within the oaks? Has this been looked at? How important is it?
>
> What are the differences between feeding preferences and stand susceptibility? What are the important ecological implications of these differences?
>
> What is the relative importance of defoliation levels versus site factors in determining mortality?

Are the differences in mortality seen on good sites versus poor sites primarily influenced by defoliation differences, or are other factors important?

A suggestion was made that an appropriate management approach to minimizing impacts in southern hardwood stands would be to shorten rotations to 30 to 50 years.

A question was raised whether the ecological parameter, importance value, could be modified to include a multiplier for management importance as well as the abundance importance.

Ed Corbett reviewed the previous work that has been done relative to defoliation effects on water quantity and quality and nutrient cycling. There are no active funded projects in this area at the current time. The defoliation upsets the normal nutrient cycle with an increase in the amount of nitrogen in the litterfall of 65 percent. This increase in nitrogen in the litterfall and feces of the insect results in an increase in the amount of nitrate in stream water. However, nitrogen was the only nutrient in the stream water to increase. The increased nitrogen levels have contributed to an increase in coliform and streptococcus bacteria in the stream water as well. This bacterial flush can create problems for surface drinking water sources. Defoliation does increase the amount of water coming out of a defoliated area compared to an undefoliated area.

Discussion following Ed's presentation centered around the potential health effects of contaminated drinking water.

USDA INTERAGENCY GYPSY MOTH RESEARCH FORUM:

ANALYSIS AND CRITIQUE

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The January 1991 USDA Interagency Gypsy Moth Research Review differed in format from all other previous reviews. Because of this, a small team was formed and asked to evaluate the effectiveness of the session. We were asked to:

1. Determine overall effectiveness in terms of the meeting objective

2. Assess the meeting format and concept

3. Assess the "quality" of the research discussed and provide our opinions on what is needed for the future

The evaluation team consisted of Bob Bridges (FS/FIDR), Allan Bullard (FS/AIPM), Bill Dickerson (NCDA), Fred Hain (NCSU) and Ken Knauer (FS/FPM). Ken Knauer was asked to head the group. Unfortunately, Ken was unable to attend the meeting and was replaced on the team by Tim Tigner from Virginia.

RESULTS

General

The conference organizers and arrangers are commended for their sincere effort to improve the format and character of the session. All team members felt the informal workshop format stimulated discussion and tended to identify more areas of collateral research needs than has been historically the case with previous research reviews.

The team also felt the opening session dealing with the the R&D objectives of the various agencies was excellent, both in concept and in delivery. These presentations helped promote understanding and, ultimately, good relations between the various groups represented. It is the consensus of the team that this type of interagency awareness should be continued and stressed.

Objective

A specific statement of the overall objective of the meeting would have been extremely beneficial. As our group met to discuss and evaluate the session, we found that each member had a different feeling for what the overall objective was. We ultimately agreed that the true objective was to encourage and facilitate communication between researchers and between researchers and managers/others. We likewise agreed that this objective was met very well by the revised meeting format.

Format and Concept

The consensus of the team was that the format and concept of the meeting was a refreshing change from the more traditional "show and tell" type presentations. Although as a group we felt the informal workshop format stimulated discussion and led to beneficial interchanges, one team member felt the more traditional approach would have afforded the opportunity for a more comprehensive review of the research currently being conducted than having 2-3 speakers summarize current activities. Suggestions for future meetings:

1. Several moderators indicated that they were not entirely sure of their role. We suggest that better instruction be given to the session moderators.

2. Expand the time (and space) given to poster session. This would encourage more scientist-to-scientist interaction.

3. Modify the seating arrangements into a circle or semicircle to provide less of a "speaker-audience" setting.

4. Several recommendations were received relative to having future sessions at a continuing education center at a university.

5. Reduce introductions and discussion of methodologies to a minimum.

"Quality" and Future Needs

Between the five team members, all sessions were attended and evaluated. Rather than list a blow-by-blow analysis of each session, the following comments common to all sessions are reported:

1. All sessions were extremely informative.

2. All speakers were well prepared and made excellent presentations and subject matter summaries (a difficult task when reporting on methods/findings other than your own!).

3. There was no consensus on "needs". Not surprisingly, the team (and other meeting participants who shared thoughts with us) felt that future research should emphasize either basic or applied research! As with all other meetings of this sort, this is not an unusual conclusion. In certain areas, such as biotechnology, a great deal of basic work still needs to be done while in other areas, work is necessary to define the most appropriate way to apply the developed technology.

4. No lines or specific directions of research were identified as being complete or as being something that should be dropped.

5. One consensus reached was that long term studies are needed on gypsy moth effects on host, stands, watersheds and other organisms.

6. We must constantly strive for better communication, both within our own community and between ourselves and others who may either benefit from our work or have input to us regarding important areas requiring our attention.

7. One line of possible new research is in the area of economic impacts of quarantine activities on long and short range movement of gypsy moth. The

specific question becomes one of "Are our resources best spent on quarantine or on detection and controls?"

CONCLUSION

This was a successful and informative session. This format represents an excellent start toward improving communications within the research and research/user community. The informal workshop approach facilitated this improved communication and should be continued at future sessions of this type.

COMMENTS FROM THE FLOOR

After presentation of the above at the final session of the meeting, the following comments were received from the floor and are reported here for information and possible future action:

- Posters should be available during the entire meeting with specific times set aside for them to be staffed.

- Perhaps both "interactive" and "passive" posters could be presented ("interactive" posters would be those with people available to discuss the research reported while "passive" posters would be those which were more stand alone).

- The visual aids for this meeting represent a significant improvement over past meetings.

- Some time should be made available for "unplanned" sessions with postings of topics and signups on a bulletin board.

- Better attention should be paid to starting times and break times of individual sessions.

- Keep the research review session separate from the national review.

- The session should be held somewhere where informative side trips could be made (a suggestion was to hold the session at Woods Hole with a side trip to the APHIS Otis Development Center).

- Each workshop (as well as the overall session) should have specific objectives developed and articulated in the agenda and to the moderators and speakers.

MEASUREMENTS OF THE MICROMETEOROLOGY AFFECTING ABOVE AND WITHIN CANOPY DEPOSITION OF SPRAY FROM AIRCRAFT IN MOUNTAINOUS TERRAIN

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ABSTRACT

Effective use of spray involves selection of times in which meteorological conditions will be most favorable. A beginning step in this regard is the observation of deposition patterns and the corresponding atmospheric conditions above and within the canopy affecting them.

A 40 m tower was erected and outfitted with fast response, three dimensional turbulence sensors, heat flux, radiation flux and an array of energy balance sensors at 6 different levels down through the canopy. Micrometeorological data was taken during 18 aerial spray trials. The site was located in the Black Moshannon State Forest in central Penn. Spray deposition was sampled along line perpendicular to the airplane flight path both above and below the canopy. The samples were analyzed in the Pesticides Research Lab at PSU.

Spray deposition patterns were influenced by directional shear and thermal stability of the surface layer. In stable conditions with considerable directional shear through the canopy the deposition patterns were skewed, indicating considerable drift taking place. In unstable convective conditions the patterns were broader with less skew.

FSCBG MODEL EVALUATION OVER AN OAK FOREST

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ABSTRACT

A formulation of *Bacillus thuringiensis* isolate and tracer to quantify the spray residue was applied over a fully leafed oak forest in central Pennsylvania. A total of 18 applications were made with TeeJet flat fan nozzles on a Cessna AgTruck over a three day period under various atmospheric conditions. Spray pattern was measured from washoff of samplers oriented perpendicular to the flight path. Samplers were spaced a 1 m intervals along a line at tree top and at ground level. An extensive array of meteorological instrumentation measured the above and within canopy environment on a 40 m tower at the time of spraying.

Predictions of spray patterns were made with the FSCBG model using the array of meteorological data. Comparisons are made between this prediction and that measured. In this study the model, on average underestimated the total deposition by 5 to 15%. But the root mean square error averaged about half the typical peak values forecasted by the model. Thus the point to point discrepancies in any given run were quite large, but the average deposition in all 17 runs was quite close to the average prediction.

INHIBITION OF LdNPV BY LEAF TANNINS: OXIDATIVE ACTIVATION REQUIRED

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ABSTRACT

Leaf tannins inhibit the activity of LdNPV. Mortality of third-instar gypsy moth larvae is negatively correlated with the hydrolyzable tannin content of foliage of different tree species, resulting in five- to eight-fold differences in mortality between aspens and oaks. Even within oak species, LD_{50}'s are negatively correlated with foliar hydrolyzable tannin content. In artificial diet tannins can reduce mortality due to LdNPV by one half at concentrations commonly found in oak foliage.

The ability of tannins to inhibit LdNPV requires enzymatic oxidation. Polyphenol oxidases (PPOs) of plant and insect origin convert tannins from the phenol form to the more reactive quinone form which can bind covalently and irreversibly to protein and lipids. We have shown that inhibition of PPO activity releases the inhibition of LdNPV by tannins in artificial diet. Experiments are planned for this spring to determine whether inhibition of PPO activity has the same effect on foliar tannins.

Activated tannins may inhibit LdNPV by several mechanisms. Tannins may block infection indirectly by altering the morphological or physicochemical environment of the gut to prevent exit from the peritrophic membrane or virion release, or to prevent recognition at the epithelial receptor site. Tannins may also block infection directly by binding to the polyhedral protein and/or the virion. Experiments are underway to distinguish between these mechanisms.

Understanding of the mechanism(s) of tannin inhibition of LdNPV will permit more efficient design of adjuvants to improve LdNPV efficacy and resistance management. Widespread use of LdNPV is limited by its slow kill (17 days) and the high volume application necessary for effective control. While the latter is is in part due to its environmental instability (esp. w.r.t. UV light), foliar inhibition is responsible for significant increases in LD_{50}s on preferred hosts. We believe significant advances in LdNPV efficacy will be made with knowledge of how foliar inhibition works. For example, our basic research on the gut physiology and mechanisms of action of tannins in caterpillars led us to predict that tannin oxidation had to be important in the gypsy moth, which led to our discovery of endogenous PPOs and their inhibitors. If experiments this spring also demonstrate PPO inhibitors can reduce foliar inhibition of LdNPV, then we have the potential to significantly lower LD_{50}s in the field.

ASSESSMENT OF POTENTIAL IMPACTS OF GYPSY MOTH INFESTATIONS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

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ABSTRACT

The gypsy moth, Lymantriidae: *Lymantria dispar*, is an introduced insect pest which in a peak year (1981) defoliated 5 1/4 million hectares of hardwood forests in the US. Although the approaching front of the moth population now exists in northern Virginia, small numbers of moths have been trapped in and around the Great Smoky Mountain National Park (GRSM). In order to assess the areas sensitive to defoliation in the park, we evaluated factors including: vegetation type, disturbance history, elevation, topography, and proximity to roads and campgrounds. In a preliminary analysis utilizing a park-wid vegetation map (90m2 pixel resolution) based upon Landsat imagery, we identified 5 out of 13 vegetation types (72% of the park area) to be at some risk to defoliation. Two types, Xeric Oak and Mesic Oak (each about 10% of the total park area which is 207,000 ha) are likely to be at high risk. Almost 47,000 ha of the park has been identified as "undisturbed" or old growth. Within the undisturbed areas, two major vegetation components of undisturbed areas at GRSM, Cove Hardwood and Mixed Mesic Hardwood, are at some risk to defoliation and a smaller component, Mesic Oak, is at high risk.

PRODUCTION OF GYPSY MOTH NUCLEAR POLYHEDROSIS VIRUS (NPV)

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ABSTRACT

Through a cooperative USDA project between Animal and Plant Health Inspection Service, (APHIS), and Forest Service, over 3,500 acre equivalents of the nuclear polyhedrosis virus, (NPV) were produced. The host larvae were reared and inoculated by APHIS (1,000 larvae per acre equivalent). A new procedure was developed to inoculate larvae using a semi-automated machine. A pneumatic ram holding five hypodermic needles simultaneously inoculated five rearing cups. The ram device was driven by a diastolic pump that pushed the needles through the closed lids as the rearing cups were moved along a conveyor belt.

The frozen cadavers were processed by the Forest Service. The formulation will be used in ongoing projects. A technology transfer program with private industry is in progress which will result in Espro, Inc., becoming a potential producer of NPV.

An alternate production strain of the gypsy moth was also produced by APHIS on a weekly basis. The new strain was the result of a three year selection process.

GYPSY MOTH DEFOLIATION IMPACTS ON WATER QUALITY AND QUANTITY

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ABSTRACT

The reduction in interception and transpiration losses due to forest defoliation can lead to increased soil moisture and streamflow and the opportunity for altered nutrient leaching. Water yield has increased by as much as 5.4 inches on heavily defoliated watersheds. This is equivalent to a yield of 146,000 gal./acre defoliated. Defoliation by the gypsy moth can speed the transfer of nutrients from vegetation to the soil surface. However the nutrients are in organic form and may not be readily available to plants. In one central Pennsylvania study nitrogen return on defoliated areas was 68 percent greater than on undefoliated areas; potassium was 82 percent greater; phosphorus was 21 percent greater; and magnesium showed trends of a slight increase. The return of calcium was 27 percent lower on the defoliated areas. In another study, of the nutrients monitored only slight increases in streamwater nitrate were observed, and this occurred during the second year after the initial watershed defoliation.

Increased densities of indicator organisms have been found on test watersheds. Fecal streptococci densities as high as 25,000/100 ml were observed in stream samples during periods of active defoliation, while fecal coliform densities exceeded 90/100 ml on occasion. Total coliform densities frequently exceeded 150/100 ml. The fecal coliform/fecal streptococci ratios indicated that these indicator organisms were from nonhuman sources. The new EPA Surface Water Treatment Rule that went into effect December 31, 1990 requires water systems that wish to avoid filtration must comply with limits on fecal or total coliform for source water (fecal coliform <20/100 ml or total coliform <100/100 ml in 90 percent of the samples taken for the 6 previous months that the system served water to the public). Quantitative profiles of pollution-indicator bacteria densities and general heterotrophic bacteria densities in relation to the onset, duration, and decline of gypsy moth infestations need to be developed. Speciation of specific bacterial strains should be made to evaluate potential health implications.

GYPSY MOTH PROTHORACICOTROPIC HORMONE (PTTH) PARTIAL SEQUENCE

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ABSTRACT

Development of new, environmentally safe methods of gypsy moth (*Lymantria dispar*, L.) control requires further knowledge of the biochemical and endocrinological pathways controlling growth and development. Disruption of many of these pathways would result in growth inhibition and death. One major pathway involves the regulation of ecdysteroid hormone production by the prothoracic gland under the influence of the neuropeptide hormone PTTH.

We have designed mixed oligonucleotide primers for the amplification of PTTH-specific sequences from *L. dispar* brain cDNA and genomic DNA. Primers were designed based on previously published amino acid sequence information for *Bombyx mori* PTTH (Kawakami *et al.*, 1990, *Science, 247*: 1333). Initially, a modification of a PCR method (mixed oligonucleotide primed amplification of cDNA, MOPAC, Lee *et al.*, 1988, *Science, 239*: 1288) was used to amplify and sequence a 221 b.p. fragment. In order to determine the nucleic acid sequence for *L. dispar* PTTH external to the 221 b.p. MOPAC amplified and sequenced region, an anchored PCR method (single site PCR or ssPCR, Roux and Dhanarajan, 1990, *Biotechniques, 8*: 48) was utilized. Anchor-adaptor ligated *L. dispar* DNA templates were amplified with an anchor primer and internal sense or anti-sense primers in order to amplify the 3' and 5' regions of PTTH, respectively. PTTH-specific amplification products were identified by hybridization with a PTTH probe and isolated for direct sequencing and/or subcloning. Using single site PCR, we have isolated DNA fragments which potentially encode for the entire gypsy moth PTTH polypeptide sequence and most of the PTTH gene, demonstrating the potential of PCR-based methods for sequencing insect neuropeptide hormones once a small amount of sequencing information is available. Another PCR-based method (RACE, rapid amplification of cDNA ends, Frohman *et al.*, 1988, *P.N.A.S. USA, 85*: 8998) is currently being utilized to extend this sequence information.

The homology between the partial *L. dispar* nucleotide sequence and the corresponding region of the *B. mori* nucleotide sequence is very high (>96% for the 221 b.p. MOPAC fragment). The deduced amino acid sequence for this portion of the PTTH polypeptide is also nearly

identical to the *B. mori* sequence, suggesting that a high degree of homology exists between the PTTH neurohormones of these species.

A COMMERCIAL SYSTEM FOR IN VITRO PRODUCTION OF THE ABINGTON STRAIN OF A NUCLEAR POLYHEDROSIS VIRUS OF THE GYPSY MOTH

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ABSTRACT

A nuclear polyhedrosis virus is the only naturally occurring biological organism which regulates gypsy moth populations in North America. A significant research effort has been made by government, industry and academia to adapt *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) for commercial development as a biological control agent. The culmination of this effort was achieved by the Forest Service (USDA) in 1978. The Forest Service successfully registered the Hamden isolate of LdNPV as GYPCHEK with the Environmental Protection Agency. Production methods were originally limited to *in vivo* methods however an *in vitro* research effort conducted jointly by ARS and FS attempted to use cell systems for virus production (Forest Service Science and Education Agency Technical Bulletin 1584 1981). Several pioneering discoveries were made concerning *L. dispar in vitro* systems however commercial feasibility was not attained.

More recently new cell lines, virus strains (Lynn et al. Proceedings USDA Interagency Gypsy Moth Research Review, 1991) and media formulations have been developed which make *in vitro* virus production feasible. To date only one insect virus-cell system (AcNPV-IPLB-21AE) produces cells and virus in both quantity and quality capable of biological control of an insect pest. The development of the IPLB-LdFB cell line and virus strain a624 has produced a *L. dispar* cell virus system comparable in efficacy to the existing AcNPV system.

During the last two years a technology transfer agreement between the authors and IGB Ltd., San Leandro, Ca (now American Cyanamid) has enabled scale-up of the *L. dispar* system to pilot plant production using serum free medium and air lift bioreactors. Yields of virus make this system commercially feasible. Virus produced in this system has comparable efficacy to *in vivo* produced virus in both laboratory bioassays and field trials. Yields of virus also make this system attractive for creating a baculovirus expression system comparable to the existing AcNPV/IPLB-21AE-Sf9 system.

REGULATORY MECHANISMS IN REPRODUCTIVE PHYSIOLOGY OF THE GYPSY MOTH

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ABSTRACT

Regulation of sex pheromone production was studied in virgin females. We have found that photoperiod, female age, and temperature affect the pattern of pheromone production. In 16:8 light-dark cycles and 25°C, pheromone content extracted from the pheromone gland was highest in the evening and the peak value increased with female age. Temperature of 33°C and higher strongly inhibited pheromone production in laboratory females. Pheromone titer was also monitored in the wild females kept outdoors in Beltsville. Pheromone production was very low in the evening, when temperatures reached 35°C, and the peak production was observed in the early morning. Interestingly, trapping experiments performed on the same dates have shown very low flight activity of the males in the early morning which suggests that high outdoor temperatures might interfere with pheromonal communication in the gypsy moth.

Regulation of sperm release from the testis was studies in gypsy moth males. It was found previously that timing of sperm release is controlled by a circadian pacemaker located in the reproductive system itself. We have determined recently that this pacemaker develops during adult differentiation, approximately 7 days after pupation. We plan to study changes in cellular protein pattern correlated with the development of the circadian pacemaker and the beginning of sperm release.

PURIFICATION, CHARACTERIZATION AND REGULATION BY JUVENILE HORMONE OF VITELLOGENIN IN THE GYPSY MOTH.

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ABSTRACT

Vitellogenin (Vg), precursor of the major yolk protein vitellin, is synthesized during the last larval instar in the gypsy moth. Its synthesis and accumulation in the hemolymph is regulated by the JH. Unlike in the other insect systems studied, where JH has been shown to induce synthesis of Vg, in the gypsy moth JH suppresses synthesis and accumulation of Vg. This unique physiological property, coupled with the fact that Vg is a sex-specific protein synthesized in abundant quantities, and a major component of insect eggs, makes it an ideal candidate for studying JH action in the gypsy moth. Knowledge of the molecular mechanism(s) of action of JH has a great potential to be applied towards designing biological control agents.

Using FPLC techniques coupled with ammonium sulfate fractionation, nearly homogeneous preparations of Vg were obtained. The Vg was shown to consist of three types of subunits of 190 kD, 165 kD, and 36 kD. The larger subunits were purified and used to raise polyclonal antibodies. Western blotting experiments indicated that the larger subunits were immunologically related.

Using a potent JH analog (JHA), fenoxycarb, Vg was shown to be regulated by JH. Vitellogenin could be detected by SDS-PAGE in the hemolymph on day 4th of 5th instar female larvae. Treatment with 100 nmol fenoxycarb on L4DL delayed appearance of Vg until day 8 and its levels were considerably less than those of controls even on the last day of 5th instar.

Analysis of poly(A) RNAs from fat bodies of L5D7 male, female and female larvae treated with JHA as above showed the presence of a 5.8 kb RNA only in untreated female larvae. It could not be detected in males (L5D7), L5D1 females and L5D7 females treated with JHA on L4DL. Untreated L5D6, L5D7 and L5D10 female larvae had abundant quantites of this RNA. Correlative evidence suggested that the 5.8 kb RNA codes for Vg-subunits.

In vitro translation of the RNAs indicated the presence of a 175kD product only in samples which contained the 5.8 kb RNA. This 175 kD product was immunologically related to the

suggest that chestnut oak would be a much less suitable host for the gypsy moth, but bioassay results indicate that chestnut oak is a better host plant than red oak.

When gypsy moth larvae were reared on chestnut oak and red oak foliage, pupal weights and fecundity were 20-35% greater on chestnut oak foliage than red oak foliage from both xeric and mesic sites. Indeed, female pupal weights and fecundities were positively correlated with chestnut oak hydrolyzable tannins. In contrast, female pupal weights and fecundities were negatively correlated with red oak hydrolyzable tannins.

Third instar gypsy moth larvae given a dose of 50,000 PIB/larvae had greater mortality rates when fed NPV with chestnut oak leaf discs than red oak leaf discs. In a 36 hour foliar consumption trial (2 nights), larvae presented with both chestnut and red oak leaf discs consumed nearly twice as much leaf area of chestnut oak as red oak. This suggests that the probability of gypsy moth larvae encountering a lethal dose of NPV are nearly twice as great on chestnut oak as red oak.

The mixture of chestnut and red oak on xeric sites presents a paradoxical situation for gypsy moth population dynamics. Both chestnut oak and red oak are considered to be favored host species largely on the basis of feeding preference, yet the two species have opposing effects on gypsy moth performance. Chestnut oak may contribute to population outbreaks through a combination of greater fecundity and enhanced survivorship at low NPV densities, but not provide enough resistance at high NPV densities, which occur at high gypsy moth densities. Performance results from chestnut oak and red oak indicate that even favored host species cannot be grouped together for modelling purposes without information on growth, NPV mortality and foliar consumption.

MICROMETEOROLOGY RESEARCH FOR AERIAL SPRAYING

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ABSTRACT

Our purpose is to develop micrometeorological criteria for aerial spray operations which will help the spray applicators hit specific target surfaces, in a specific time envelop with the minimum amount of material to do the job. The movement of spray material in the air over and inside a tree canopy depends on the wind and turbulence fields, the local and regional atmospheric stabilities, the evaporative power of the air and the canopy geometry and density. We have conducted two field experiments, in the Black Moshannon Penn. State Forest, to measure these elements while simultaneously measuring spray deposition patterns in cooperation with the pesticides lab (W. Yendol) group at PSU.

The 1988 data have been examined and the 1990 experimental data are currently being analyzed. We have also developed a technique to measure the vertical distribution of leaf area in hardwood forest stands for use in these analysis. The results of the experiments are also being used to verify the U.S. Forest Service, FSCBG Aerial Spray Model for use in hardwood forests.

Results to date demonstrate that the canopy decouples the air mass above from that below and the stability and wind conditions which control the drift are often opposite. But it appears that the different modes of drift and dispersion of spray material can be organized by atmospheric stability regimes. Thus we are now attempting to define criteria to detect these stability regimes and adjust the spray application techniques accordingly. These will be tested against the Blackmo90 data. It appears that the FSCBG model utility in hardwoods could be improved with a better description of the hardwood forest canopy, and more sensitivity to the turbulence, stability and humidity regimes.

GYPCHEK® PRODUCTION *IN VIVO*

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ABSTRACT

In the early 1960's, the U.S. Forest Service (FS) recognized the need to develop environmentally safe pesticides for gypsy moth management. The gypsy moth nucleopolyhedrosis virus (NPV) became a candidate because of its extremely narrow host range and its efficacy as a natural regulator in dense gypsy moth populations. During the research and development phase that culminated in the FS registration of the NPV product, Gypchek, it became clear that the road-block to the eventual commercialization of Gypchek would be the cost of production. In the decade following registration that was the case. Large pesticide-producing companies were not interested in bringing the product to the marketplace, and smaller "cottage industries" made unsuccessful attempts. FS policy has been to not compete with the private sector but, in the absence of a commercially available product, the FS has, in collaboration with the Animal Plant Health Inspection Service (APHIS), continued to produce limited quantities of Gypchek, primarily for laboratory and field research and small-scale operational tests.

Current FS-APHIS *in vivo* production technology is, with some modification, essentially that developed in collaborative efforts with ARS scientists in 1975-76, i.e., processing NPV from groups of 10-15 larvae that have been reared and infected in small 6 oz. cups. Private industry has determined that this technology is not cost-effective. The cost problem is exacerbated by reduced NPV yields that are associated with the sporadic appearance of Abnormal Performance Syndrome (APS), i.e., "straggling", in the New Jersey strain of the gypsy moth used for production. Further, recent field experiments have shown that product performance is acceptable only at dosages 2 to 5 times higher than originally thought necessary.

Recent FS technology transfer agreements with Espro, Inc., Columbia, MD have led to the development (by Espro) of large-scale gypsy moth rearing and NPV recovery technology that is expected to be cost-effective and bring significant quantities of Gypchek to the marketplace by 1992. While awaiting that, as well as the development of cost-effective *in vitro* production technology, FS-APHIS will continue to refine small-scale production technology and make available, on a priority basis, limited amounts of product.

PHENOLOGY PREDICTOR, DEFOLIATION PREDICTOR, AND MONITORING SYSTEM DESIGNER COMPONENT OF GYPSES

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ABSTRACT

GypsES (Gypsy moth Expert System) is a computer-based environment that will provide managers with an integrated set of decision making tools. It will assist managers in dealing with complex problems of gypsy moth control, resource allocation, and program implementation with state-of-the-art technologies including data base management systems, geographic information systems, knowledge based systems, and statistical and simulation models. The way problems can be solved is currently implemented in as simple, yet fully functional, a form as possible.

With temperature data from one NOAA weather station, an algorithm describing the elevation dependency of temperature and a simulation model a function between the timing of the target event and the elevation is constructed. USGS digital elevation model data and this function are used to predict the phenology in the landscape. Cells within the same target window are aggregated to provide a more comprehensible description of gypsy moth phenology.

The Defoliation Predictor produces maps of the estimated level of defoliation. The Hazard Component of GypsES needs defoliation projections to estimate risk rating. Currently the algorithm developed by Gansner et al. (1985) is used.

Forest managers need tools to assist in planning gypsy moth monitoring programs and interpreting monitoring results. The monitoring component of GypsES designs monitoring programs by interpreting data about gypsy moth populations, site/stand characteristics and management objectives. The system assists users in prioritizing management units and delimits areas to be monitored. The system suggests a sequential sampling plan for egg masses dependent on the hazard rating of the sampling areas. Dependent on the risk rating of the management unit male moth pheromone trap grids are proposed.

A FUNCTIONAL GENE MAP OF THE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS (LdNPV): THE ORGANIZATION OF THE LdNPV GENOME IS NOT COLINEAR TO THAT OF THE *AUTOGRAPHA CALIFORNICA* NUCLEAR POLYHEDROSIS VIRUS (AcNPV).

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ABSTRACT

Gene organization in the genome of LdNPV was investigated by hybridization analysis using gene probes from AcNPV. LdNPV isolate 5-6 genomic DNA was digested with the restriction endonucleases Hind III, Eco RI, Bam HI, Eco RV, Pst I, Nde I, and Cla I, and the fragments separated by agarose gel electrophoresis and transferred to nitrocellulose filters. The filters were hybridized, under low stringency conditions, with 32-P labeled AcNPV genes. The immediate early 1 (IE-1), immediate early N (IE-N), p26, p10, 6.9K core, 39K capsid, proliferating cell nuclear antigen (PCNA), DNA polymerase, Ecdysteriod UDP-glucosyl transferase (EGT), and super oxide dismutase (SOD) genes from AcNPV were used as probes in this analysis. Putative LdNPV homologs to the AcNPV IE-N, PCNA, DNA polymerase, EGT, and SOD were identified and mapped to the LdNPV genomic regions from approximately 5 to 10 and 85 to 92 map units (mu), 34 to 37 mu, 58 to 62 mu, 75 to 83 mu, 89 to 93 mu, respectively. Two LdNPV genomic regions were identified that contain limited sequence homology with the AcNPV IE-N gene. Hybridization analysis with the other AcNPV gene probes, IE-1, p26, p10, 6.9K core, and 39K capsid did not identify LdNPV gene homologs. The organization of the putative LdNPV IE-N, PCNA, DNA Polymerase, EGT, and SOD genes in the LdNPV genome is different than their organization in AcNPV, indicating a lack of colinearity in gene arrangement in the genomes of these viruses.

The putative LdNPV IE-N (LdNPV IE-I) and EGT (LdNPV EGT) gene homologs have been selected for further studies. The function of the IE-I and other immediate early (IE) viral genes are of interest since these genes often code for regulatory proteins which control the sequence of events occurring during viral replication. In addition, the IE gene promoters can be used to drive the expression of foreign genes inserted into the viral genome. The location of the IE-I gene has been mapped to a 1.2 kb Hind III/BamHI genomic fragment, and the direction of transcription determined. The sequencing of this gene is in progress.

The AcNPV EGT gene catalyzes the transfer of glucose from UDP-glucose to ecdysteriods, which are insect molting hormones. The glycosylated ecdysteriod is inactivated, thereby interfering with the ability of the larvae to advance through larval instars. Viral infected insects continue to feed during the early phase of viral infection, which may facilitate viral replication. Preliminary results suggest that infection of larvae with AcNPV lacking the EGT gene leads to a feeding cessation response. Our studies of the LdNPV EGT homolog are directed to the possible effect on gypsy moth larvae by infection with an LdNPV EGT minus isolate. As a prerequisite to the generation of a LdNPV EGT minus virus, the EGT gene will be cloned and characterized. Through hybridization analysis the location of the LdNPV EGT gene has been further defined to within a 4 kb region of a 8.6 kb genomic subclone. A 430 bp subclone from this region was generated and partially sequenced. The amino acid sequence from two regions of the subclone were determined and found to contain 40% and 48% amino acid sequence identity with the AcNPV EGT gene, thereby confirming the presence of an LdNPV EGT homolog. Expression analysis and complete gene sequencing are in progress.

ANALYSIS OF *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUSES (LdNPV) ISOLATED FROM GYPCHEK: PURIFICATION OF HIGH POTENCY LdNPV ISOLATES

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ABSTRACT

LdNPV isolates were obtained from Gypchek and several of their properties examined. Isolates were sought and identified that exhibited a range of potencies against the gypsy moth to provide viral isolates for studies aimed at discerning the basis for potency heterogeneities, and to provide a high potency LdNPV alternative to Gypchek for gypsy moth control. Viral isolates were generated through two or three rounds of *in vivo* purification, using an amount of polyhedra that resulted in the death of 2 to 5% of larvae. Polyhedra isolated from single larvae that died from 11, 14, and 18 days after infection were chosen for further purification. After one or two additional rounds of *in vivo* purification, isolate purity was assessed through genomic restriction endonuclease analysis. From this analysis fourteen isolates were selected for potency determinations. In addition, isolates A21 and B21 that were previously generated were also analyzed.

The LC-90 values of the isolates ranged from 2.4 x 10-3 to 648.9 x 10-3 polyhedra per ml of diet, with the LC-90 of Gypchek ranging from 31.5-65.6 x 10-3. At the LC-90 level, isolate 122 exhibited a potency 13.3 fold greater than Gypchek, suggesting its further development as a replacement for Gypchek. From the potency results, five isolates exhibiting potencies greater than Gypchek (122, 203, A21, 111, and 121 with potency ratios of 13.3, 4.6, 2.0, 1.5, and 1.5, respectively relative to Gypchek), three isolates that are comparable to it (121, 163, and 151 with potency ratios of 1.1, 1.1, and 0.9, respectively), and four isolates with potencies less than Gypchek (123, 141, B21, and B12 with potency ratios of 0.5, 0.2, 0.1, and 0.1, respectively) were chosen for further study.

Viral isolate genomic DNA was digested with several restriction endonucleases to determine if genomic restriction fragment polymorphisms were present. If so, these heterogeneities could be used to distinguish the isolates at the molecular level and may be correlatable with phenotypic differences, e.g., potency. Restriction endonuclease digestion of isolates 203, 201, 163, 151, 141, 123, 122, 111, B12 with Bgl II generated one or more uniquely sized genomic fragments. Isolates A21 and 151, and 163 and 121 were distinguishable after digestion with the restriction endonuclease Eco RI. Isolates 141 and B21 gave the same restriction endonuclease digestion patterns with 12 enzymes, which could indicate that they are homologous.

Virion density in polyhedra cross sections was determined to assess the relationship of potency to polyhedra virion density. Polyhedra synthesized *in vivo* were prepared, cross sectioned, and viewed by electron microscopy. The LdNPV isolates exhibited very similar numbers of virions per square micrometer of polyhedra cross section, ranging from 12 to 16 virions. There is approximately a 130 fold difference in potency between isolates 122 and B21, yet their polyhedra contain essentially the same number of virions (16 and 15 virions/um-2, respectively).

This analysis has identified several viral isolates with distinct genotypes that exhibit greater potencies against the gypsy moth compared to Gypchek. LdNPV isolate 122 is greater than 10 fold more potent than Gypchek, making it an excellent candidate for development for field use.

GYPSY MOTH EGG MASS DISTRIBUTION AND SAMPLING IN A RESIDENTIAL SETTING

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ABSTRACT

The spatial distribution of gypsy moth, *Lymantria dispar* (L.), egg masses was determined in 60 developed lots in a residential community. The community was divided into low and high gypsy moth density sections, with average densities of 393.3 and 2656.3 egg masses/ha, respectively. In the high density lots, the proportion of egg masses found on trees, man made objects, and houses was 73.9, 21.6, and 4.5%, respectively. The distribution in the low density lots was similar. Oaks (*Quercus* spp.) received the highest proportion of egg masses out of all tree species subcategories at both low and high population densities. The cost effectiveness of a number of potential sampling units, including entire lots, back yards, fixed area plots, and individual trees, was evaluated. The entire lot sampling units required the fewest samples to achieve a predetermined level of precision. However, cost (= sampling time) for these sampling units was also the highest, resulting in relatively low cost effectiveness. Fixed area plot samples (0.01 ha in area) were in general the most cost effective sampling units, with 2 samples/lot appearing to be optimal. A binomial (presence/absence) sampling approach, in which the percentage of trees in each lot with more than 5 egg masses is determined, was approximately as cost effective as fixed area plots, but its usefulness is limited because it does not provide direct estimates of absolute gypsy moth population density.

EVIDENCE FOR A NON-CEREBRAL NEUROHEMAL CENTER WHICH CONTROLS DEVELOPMENT IN THE GYPSY MOTH, *LYMANTRIA DISPAR*

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ABSTRACT

Evidence for a novel pupation control mechanism comes from ligation studies done on *Lymantria dispar* female larvae. On day 5 to 8 of the last instar, females are individually ligated at abdominal segments 3 through 10. Pupation is completely prevented by ligature on day 5, while ligation on days 6 and 7 results in, respectively, 92% and 85% inhibition of pupation. Ligation on day 8 is much less effective (24% inhibition). These responses are observed for each ligature applied regardless of segment position, and suggest a posterior influence on pupation. Further, if the ventral nerve cord (VNC) alone is ligated on day 7 at thoracic segments 2 or 3, or at abdominal segments 3 through 10, pupation is prevented (89%) in each ligated group.

Radioimmunoassay of hemolymph from these larvae shows no peak of ecdysteroid activity, while the small percentage of day 7 ligated larvae which enters pupation shows, on day 4 after ligation, a peak of ecdysteroid activity similar to that of starved controls. The reduced hemolymph ecdysteroid titer in ligated larvae, and the failure of prothoracic glands (PG) from such ligated larvae to increase their ecdysteroid secretion *in vitro* when exposed to prothoracicotropic hormone (PTTH), suggest that a factor from the posterior abdominal area, specifically the anal papilla, influences the PG to become responsive to PTTH. We are attempting to determine the nature of this prothoracic gland preconditioning factor (PGPF) and whether it is humoral or neural.

Additionally, the anal papilla contains PTTH-like activity, detected *in vitro*, and the integrity of the papilla is essential for normal eclosion. Thus, the anal papilla may represent an important neurohemal center with at least two neurohemal activities, PGPF and PTTH-like, and influence upon a third, eclosion hormone. The presence of a neurosecretory center

outside the brain could provide new strategies for the development of endocrine-based control agents.

PURIFICATION AND PROPERTIES OF THREE REGULATORY ENZYMES FROM THE GYPSY MOTH

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ABSTRACT

The normal development of the gypsy moth may be disrupted by the manipulation of the insect's own enzymatic reactions. In pursuit of the development of a biological control agent, studies have been performed to isolate and to characterize three regulatory enzymes: trehalase, 6-phosphofructo-1-kinase and juvenile hormone esterase.

Trehalase (THA) hydrolyzes trehalose, the major form of energy storage in insects. Our current scheme for the purification of THA includes a heat treatment, gel filtration and conconavalin A-Sepharose chromatography. The purified enzyme appears to be a glycoprotein with a molecular size of approximately 60 kDa. The gypsy moth enzyme may be similar to the 65 kDa soluble THA purified from honey bee thorax.

Phosphofructokinase (PFK) is the key regulatory enzyme in glycolysis. Gypsy moth larval PFK was purified using affinity chromatography. A molecular size of 330 kDa was determined for the native enzyme by gel filtration studies. Gypsy moth PFK tryptic peptide sequences were found to be similar to the human muscle enzyme. The gypsy moth PFK displayed regulatory behavior with respect to both substrates. At pH 7.3 the enzyme was inhibited by high concentrations of ATP. AMP, ADP and cyclic-AMP were all activators of gypsy moth PFK with AMP being the greatest effector. Fructose-2,6-bisphosphate was the most potent activator of gypsy moth PFK, same as observed for PFK in higher organisms. Citrate was a very poor inhibitor of gypsy moth PFK.

Juvenile hormone esterase (JHE) catalyses the hydrolysis of JH to the physiologically inactive JH-acid. In the last larval instar of the gypsy moth there appears a major peak of JHE activity which contributes to the elimination of JH from circulation. The gypsy moth larval and pupal JHE has been purified using a classical approach. The gypsy moth enzyme is similar in size and in its properties to JHE purified from other Lepidoptera. A HPLC technique facilitated

the purification of JHE for antibody production and protein chemistry studies. The antibody cross-reacted with affinity-purified JHE from *Trichoplusia ni*. and indicated that the esterase in pupa is very similar if not identical to the JHE in larval hemolymph. A new method enabling the purification of JHE from hemolymph in three efficient steps is being developed.

FIELD COMPARISON OF DOSES AND STRAINS OF GYPSY MOTH NUCLEAR POLYHEDROSIS VIRUS AGAINST GYPSY MOTH IN WESTERN MARYLAND IN 1990

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ABSTRACT

Gypsy moth nuclear polyhedrosis virus from a 1990 *in vivo* production batch of Gypchek® (LDP226 strain) was compared with virus from a 1989 production batch of the Abington isolate (Pass 10), each at three doses, all with 2% Rhoplex B60A sticker, with 6% Orzan added as a sun screen, in replicated Garrett County, MD, small forest plots using ground equipment. Also included were one dose of virus produced in cell culture, one dose of Gypchek applied without sunscreen, and an untreated control. A dose-response was demonstrated for both materials in a bioassay of gypsy moth larvae taken from the plots 7 days after treatment and held for 21 days in individual diet cups in an environmental cabinet. In this bioassay, the Abington isolate killed significantly ($\partial = 0.05$) more rapidly than the Gypchek product, but no statistical difference was seen between the two products in LC_{50} potency. Virus produced in cell culture was found active in the bioassay of field collected larvae. The presence or absence of Orzan did not affect results. Treatment effects for late-season field parameters (larval mortality, defoliation, and egg mass population change) were obscured by forest-wide natural mortality factors.

IMPACT OF THE FUNGAL PATHOGEN, *ENTOMOPHAGA MAIMAIGA*, ON NORTH AMERICAN GYPSY MOTHS, *LYMANTRIA DISPAR*

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ABSTRACT

The effect of *Entomophaga maimaiga* on the gypsy moth was investigated in 1990 by determining the prevalence of disease in caterpillars collected in forest plots and exposed in wire cages. High rainfall in May appeared to be important for development of the fungus. Weekly infection rates were 5% or less in May and increased to 40% or more in June. Infective conidia were produced by dead, diseased caterpillars in May, while resting spores were increasingly common in larvae in June. Using a degree-day model, estimates of proportions of newly-infected and newly-dead fungus-infected caterpillars were obtained daily. These data were used to construct survival curves for larvae. Overall larval mortality ranged from about 50 to 100% in different plots. Reasons for the differences in survival are not known, but host density and tree composition may be factors. Variations in rainfall between the different plots did not appear to be important. A fuller understanding of the factors responsible for the variable impact of the fungus would help in predicting its effect on gypsy moth populations and could lead to greater control of the pest.

ULTRASTRUCTURE OF THE GYPSY MOTH PHEROMONE GLAND CELLS

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ABSTRACT

Ultrastructure of the dorsal sex pheromone gland cells of the female gypsy moth, *Lymantria dispar*, was studied for pharate adult (at day-10 to day-12 of the pupal stadium), as well as for calling, mated, and ovipositioning females. These females were injected with ice-cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) to protrude the terminal abdominal segments. Fifteen to twenty minutes later, the dorsal gland and an adjacent non-glandular control area were dissected from each female and transferred to a glass vial containing the

same cacodylate buffered fixative. After 4 h at 4°C, the glands and control areas were post-fixed at 4°C in 1% buffered OsO_4 for another 3 to 4 h. After dehydration, infiltration, and embedding, ultrathin sections (0.0 to 0.09 μm) were obtained using a Porter-Blum ultramicrotome and stained with uranyl acetate and lead citrate. Electron micrographs were taken using a Joel 100S operating at 80Kv. Sections of at least 4 moths were examined for each stage.

The dorsal sex pheromone glands appear externally as areas of convoluted cuticles. They are located on the intersegmental membrane between the 8th and 9th abdominal segments. In the vicinity of the convoluted glandular area, unspecialized epidermal cells were essentially squamous and comparable in ultrastructure to the epidermal cells of other lepidopterans.

The ultrastructure of the glandular cells changes tremendously from pharate adult stage to egg-laying stage. Most notable differences among these changes were found in the morphology and abundance of infolds of the basal plasma membrane, the degree of orientation of microtubules into one direction, and the appearance and disappearance of some conspicuously dark granules (of unknown identity) in the cytoplasm. These ultrastructural changes and their relation to the estimated pheromone biosynthesis are presented in Table 1.

Table 1. Prevalence and morphology of organelles found in the pheromone gland cells of *Lymantria dispar* of different stages[1].

	Organelles			
Stage	Basal infolds	Microtubule orientation	Dark granules	Estimated pheromone biosynthesis
Pharate	+[2]	somewhat	+++	+
Virgin	+++	somewhat	+++	+++
Calling	+++	highly	+++	+++
Mated	++	random	++, fused	+
Egg-laying	+/-	random	+/-	-

[1]Yin, unpublished.

[2] +++, ++, +: more, less, least abundant; +/-: sometimes absent; -: absent.

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