



United States
Department of
Agriculture

Forest Service

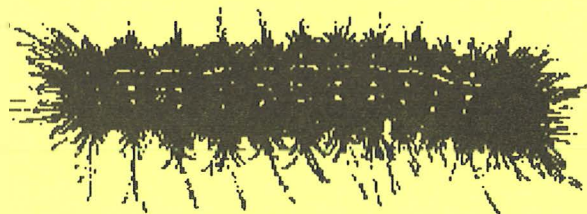
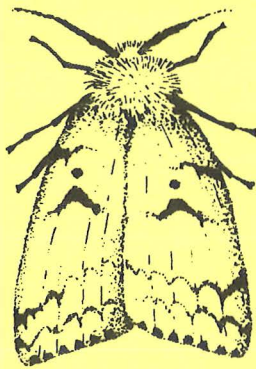
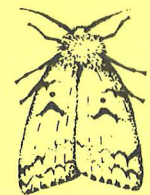
Northeastern Forest
Experiment Station

General Technical
Report NE-213



PROCEEDINGS

U. S. Department of Agriculture Interagency Gypsy Moth Research Forum 1995



Most of the abstracts were submitted on floppy disk and were edited to achieve a uniform format and type face. Each contributor is responsible for the accuracy and content of his or her own paper. Statements of the contributors from outside the U. S. Department of Agriculture may not necessarily reflect the policy of the Department. Some participants did not submit abstracts, so they have not been included.

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U. S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

Remarks about pesticides appear in some technical papers contained in these proceedings. Publication of these statements does not constitute endorsement or recommendation of them by the conference sponsors, nor does it imply that uses discussed have been registered. Use of most pesticides is regulated by State and Federal Law. Applicable regulations must be obtained from the appropriate regulatory agencies.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish and other wildlife--if they are not handled and applied properly. Use all pesticides selectively and carefully. Follow recommended practices given on the label for use and disposal of pesticides and pesticide containers.

ACKNOWLEDGMENTS

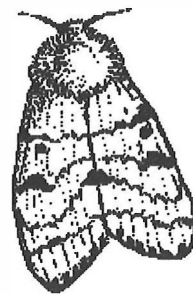
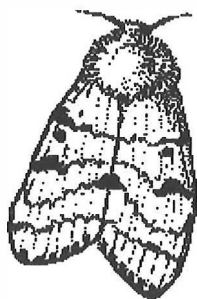
Thanks to Dr. Mark J. Twery for providing the cover and title page design.

Proceedings

U.S. Department of Agriculture

Interagency Gypsy Moth Research Forum

1995



January 17-20, 1995
Loews Annapolis Hotel
Annapolis, Maryland

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

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 sixth 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. Faust, Agricultural Research Service (ARS)
N. Leppla, 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 17-20, 1995
Loews Annapolis Hotel
Annapolis, Maryland

AGENDA

Tuesday Afternoon, January 17

REGISTRATION
POSTER DISPLAY SESSION I
WELCOME MIXER

Wednesday Morning, January 18

PLENARY SESSION Moderator: M. McFadden, USDA-FS

Changes in Funding and Direction of Science

Welcome
Michael McManus, USDA-FS

Research and Service Programs of the USDA-ARS European Biological Control Laboratory in
Montpellier, France
Lloyd Knutson, USDA-ARS

Agricultural Research Service Program Strategies and Priorities
Edward Knipling, USDA-ARS

Inside the Animal and Plant Health Inspection Service
Charles Schwalbe, USDA-APHIS

Changes in Forest Service Research: What Lies Ahead?
Robert Lewis, Jr., USDA-FS

Wednesday Afternoon, January 18

CONCURRENT SESSION A Moderator: E. Delfosse, USDA-APHIS

Concerns About Biological Control Agents and Non-Target Lepidoptera
Presenters: A. Hajek, Cornell University; L. Solter, Illinois Natural History Survey; J. M.
Scriber, Michigan State University; J. Miller, Oregon State University; J. Maddox, Illinois
Natural History Survey

CONCURRENT SESSION B Moderator: R.M. Muzika, USDA-FS

Potpourri

Presenters: D. Leonard, USDA-FS and A. Sharov, Virginia Polytechnic Institute & State University; R. Hicks, Jr., West Virginia University; D. Gray, Virginia Polytechnic Institute & State University; A. Liebhold, USDA-FS; R. Whitmore, West Virginia University; R. A. Smith, Abbott Laboratories

POSTER DISPLAY SESSION II

Thursday Morning, January 19

GENERAL SESSION Moderator: V. Mastro, USDA-APHIS

Asian Gypsy Moth

Presenters: K. Garner, D. Schreiber and J. Slavicek, USDA-FS; D. Prasher, USDA-APHIS; M. Keena, USDA-FS; R. Cardé, University of Massachusetts; W. Wallner, USDA-FS; P. Schaefer, USDA-ARS; T. McGovern, USDA-APHIS

Thursday Afternoon, January 19

GENERAL SESSION Moderator: K. Thorpe, USDA-ARS

Entomophaga maimaiga: A Fungus Among Us

Presenters: A. Hajek, Cornell University; R. Weseloh, Connecticut Agricultural Experiment Station; J. Elkinton, University of Massachusetts; L. Bauer, USDA-FS; S. Walsh, University of Toronto

GENERAL SESSION Moderator: R. Fuester, USDA-ARS

Gypsy Moth Biological Control Activities in Europe

Presenters: E. A. Cameron, Pennsylvania State University; J. Novotný, Forest Research Institute, Slovak Republic; M. McManus, USDA-FS

Friday Morning, January 20

GENERAL SESSION Moderator: N. Leppla, USDA-APHIS

The Increasing Significance of Biological Control and an Overview of Regulations Governing Biological Control Organisms

Presenters: E. Delfosse, USDA-APHIS; K. Lakin, USDA-APHIS; L. Turner, US-EPA; J. Brooks, USFWS

GENERAL SESSION Moderator: R. Reardon, USDA-FS

Future Directions in Virus Research

Presenters: V. D'Amico, University of Massachusetts; J. Podgwaite, USDA-FS; J. Slavicek, USDA-FS; M. McFadden, USDA-FS

CONTENTS

PLENARY PRESENTATIONS

Programs of the European Biological Control Laboratory, USDA/ARS,
Montpellier, France 1
L. Knutson

WORKSHOP SUMMARY

Introduction to the Session: "The Increasing Significance of Biological Control and
an Overview of Regulations Governing Biological Control Organisms" 9
N. C. Leppla and E. S. Delfosse

Guidelines for obtaining a plant pest permit from the U.S. Department
of Agriculture 20
K. R. Lakin

Obtaining EPA approval to test or commercialize microbial and/or
biochemical pesticides 28
M. L. Mendelsohn and P. O. Hutton

U.S. Fish and Wildlife Service regulations governing the collection, possession, and
transportation of wildlife and plants, as related to the scientific community 31
U.S. Fish and Wildlife Service, Division of Law Enforcement

ABSTRACTS AND PAPERS OF PRESENTATIONS AND POSTERS

The Russian and Ukrainian literature on the gypsy moth 48
Y. N. Baranchikov, G. N. Nikitenko, and M. E. Montgomery

Suitability of foreign tree species for *Lymantria mathura* Moore 49
Y. N. Baranchikov, T. Vshivkova, and M. E. Montgomery

Studies on the transmission of an exotic microsporidium that infects the gypsy moth 50
L. S. Bauer, D. L. Miller, D. W. Onstad, J. V. Maddox, and M. L. McManus

Dynamics and impact of <i>Entomophaga maimaiga</i> introduced into gypsy moth populations in Michigan	52
L. S. Bauer, D. R. Smitley, A. E. Hajek, F. J. Sapio, and R. A. Humber	
Status of mass-reared gypsy moths: protocol	54
G. L. Bernon	
Identification of the <i>Lymantria dispar</i> nuclear polyhedrosis virus 25K gene	55
D. S. Bischoff and J. M. Slavicek	
Sequence characterization and temporal expression of an early gene in the <i>Lymantria dispar</i> nuclear polyhedrosis virus	56
D. S. Bischoff and J. M. Slavicek	
Corsica, gypsy moth, and parasitoids: challenges and opportunities	57
E. A. Cameron and F. Hérard	
A field assessment of the effects of <i>Bacillus thuringiensis</i> on non-target Lepidoptera: light trap sampling	59
J. L. Carter, J. W. Peacock, L. Neale, and S. E. Talley	
Gypsy moth management in non-forest settings: 1994 field and laboratory studies	60
S. P. Cook, R. E. Webb, and K. W. Thorpe	
A field test of genetically engineered gypsy moth NPV	61
V. D'Amico, J. S. Elkinton, H. A. Wood, J. D. Podgwaite, M. L. McManus, J. Slavicek, and J. P. Burand	
Gypsy moth defoliation in Coastal Plain pine-hardwood stands	62
C. B. Davidson and J. E. Johnson	
Litterfall dynamics in gypsy moth defoliated pine-hardwood stands	63
C. B. Davidson and J. E. Johnson	
Effect of repeated treatments of <i>Bacillus thuringiensis</i> against gypsy moth populations: initial survey	64
N. R. Dubois, M. A. Keena, P. Huntley, and D. Newman	
Impact of <i>Entomophaga maimaiga</i> on gypsy moth population dynamics	65
J. Elkinton, R. Malakar, and G. Dwyer	
Fate of F ₁ -sterile gypsy moths released in Florida in 1994	66
J. L. Foltz, W. N. Dixon, and J. R. Meeker	

Status of the introduced gypsy moth pupal parasite <i>Coccygomimus disparis</i> (Viereck) on the Del-Mar-Va Peninsula	67
R. Fuester, R. Peiffer, P. Sandridge, N. Dill, J. M. McLaughlin, L. Kershaw, and J. Sigmond	
Trends in parasitism and host density affinities in Pennsylvania populations of <i>Lymantria dispar</i> (Lepidoptera: Lymantriidae)	68
R. W. Fuester, E. E. Simons, L. D. Rhoads, and R. P. Kling	
Status of nuclear DNA markers	69
K. J. Garner, D. E. Schreiber, and J. M. Slavicek	
Geographic robustness of a three-phase model of gypsy moth egg phenology	70
D. R. Gray and F. W. Ravlin	
Effects of silvicultural management on rates of predation on gypsy moth larvae and pupae	72
S. Grushecky, R. Greer, A. Liebhold, R. Muzika, and R. Smith	
Persistence of <i>Entomophaga maimaiga</i> in the environment	73
A. E. Hajek	
Effect of <i>Entomophaga maimaiga</i> on non-target Lepidoptera	74
A. E. Hajek, L. Butler, S. R. A. Walsh, and J. C. Silver	
Natural enemies of the gypsy moth at the leading edge of its invasion into the southern U.S.	76
F. L. Hastings, F. P. Hain, H. R. Smith, and T. M. O'Dell	
Predicting susceptibility of forest stands to gypsy moth defoliation	77
R. R. Hicks, Jr. and D. E. Fosbroke	
Complete nucleotide sequencing of a genomic clone encoding the large subunit of vitellogenin from the gypsy moth	78
S. Hiremath, K. Lehtoma, and R. Prasad	
The parasitoid complex of the gypsy moth in high and low level populations in Eastern Austria and Slovakia	79
G. Hoch and M. Zúbrik	
Asian gypsy moth genetics: biological consequences of hybridization	80
M. A. Keena, P. S. Grinberg, and W. E. Wallner	

Mixing experiments between CryIAa and CryIAC insecticidal crystal proteins suggest oligomerization of toxins	81
M. K. Lee, A. Curtiss, N. R. Dubois, and D. H. Dean	
Slow the Spread Project update: developing a process for evaluation	82
D. S. Leonard and A. A. Sharov	
Gypsy moth population suppression with pesticides: how often is suppression realized? ..	86
A. M. Liebhold	
Forest type affects predation on gypsy moth (Lepidoptera: Lymantriidae) pupae in Japan	87
A. M. Liebhold, Y. Higashiura, and A. Unno	
A computer program to predict gypsy moth larval mortality in sprayed forests	88
S. Maczuga and K. Mierzejewski	
Future Gypchek production	89
M. W. McFadden	
APHIS Asian gypsy moth policy	90
T. McGovern	
Distribution of microsporidia isolated from gypsy moth populations in Europe	94
M. McManus, J. Maddox, L. Solter, and M. Jeffords	
Assessing the impact of <i>Bacillus thuringiensis</i> <i>Kurstaki</i> on field populations of non-target Lepidoptera	96
J. C. Miller	
Comparison of performance on several tree species of gypsy moth from Central Asia, North America, and their hybrids	97
M. E. Montgomery and Y. N. Baranchikov	
Effects of defoliation and thinning on herbaceous ground flora	98
R. M. Muzika, D. L. Feicht, and S. L. C. Fosbroke	
Seedling dynamics in oak forests managed for gypsy moth	99
R. M. Muzika and M. J. Twery	
Maternal effects revisited	100
J. Myers, R. Malakar, J. Elkinton, and G. Boettner	

The use of <i>Bt</i> FORAY 48 FC (<i>NOVO NORDISK</i>) for control of gypsy moth in the forests of the Slovak Republic	101
J. Novotný	
Mortality agents affecting gypsy moth populations in Slovakia	102
J. Novotný and M. Zúbrik	
Development of the endophagous parasitoid <i>Glyptapanteles porthetriae</i> (Hym., Braconidae) in its host <i>Lymantria dispar</i>	103
C. Nussbaumer	
Gypsy moth nutritional ecology: the importance of iron bioavailability during various parental larval growth periods on the development and survival of their progeny	104
T. M. ODell, D. R. Mikus, M. A. Keena, and R. B. Willis	
Molecular markers for the <i>Lymantriae</i>	105
T. A. Pfeifer, A. P. Bokova, L. M. Humble, and T. A. Grigliatti	
Production and formulation of Gypchek	106
J. D. Podgwaite and R. C. Reardon	
The Asian genotype in the 1994 gypsy moth port survey	107
D. C. Prasher	
Biocontrol perspective of parasitoids of the <i>Lymantriids</i> , <i>Lymantria obfuscata</i> Wlk. and <i>L. ampla</i> in India	108
G. Ramaseshiah	
Some of the other species of <i>Lymantria</i> (<i>Lymantriidae</i>)	109
P. W. Schaefer	
Larval gypsy moth dorsal abdominal glands: histology, ultrastructure and preliminary chemical identification of exudate*	110
P. W. Schaefer, K. S. Shields, and J. R. Aldrich	
(* presented at the 1994 U.S. Department of Agriculture Interagency Gypsy Moth Research Forum)	
Peritrophic membrane: site of action for LdMNPV/optical brightener in gypsy moth larvae	112
K. S. Shields and J. D. Podgwaite	
Development of enhanced viral strains for cell culture production	113
J. Slavicek	

Interaction of exotic microsporidia with forest Lepidoptera	114
L. F. Solter	
Infectivity of non-indigenous gypsy moth microsporidia to native non-target forest Lepidoptera	115
L. Solter, M. McManus, J. Maddox, and M. Jeffords	
Identification of a <i>Lymantria dispar</i> nuclear polyhedrosis virus host range gene	116
S. M. Thiem, X. Du, M. Berner, M. Quentin, and C. Chilcote	
GypsES: a decision support system for gypsy moth management	117
S. Thomas, L. Selmon, D. Twardus, J. Ghent, M. Twery, and K. Gottschalk	
<i>Bacillus thuringiensis</i> CryIA insecticidal toxins effect rapid release of gypsy moth midgut epithelium aminopeptidase	118
A. P. Valaitis	
Identification of the <i>Bacillus thuringiensis</i> CryIAC toxin binding protein in the gypsy moth midgut	119
A. P. Valaitis, M. K. Lee, F. Rajamohan, and D. H. Dean	
Asian/Siberian/European gypsy moth research: are further efforts necessary?	120
W. E. Wallner	
Characterization of a species-specific DNA probe for the identification of <i>Entomophaga maimaiga</i>	126
S. R. A. Walsh, A. E. Hajek, D. Tyrrell, and J. C. Silver	
Development and use of molecular markers for the identification of <i>Entomophaga maimaiga</i>	127
S. R. A. Walsh, A. E. Hajek, D. Tyrrell, and J. C. Silver	
How <i>E. maimaiga</i> infects hosts: a tale of two spores	128
R. M. Weseloh	
<i>Calosoma sycophanta</i> does affect gypsy moth populations	129
R. Weseloh, G. Bernon, L. Butler, R. Fuester, D. McCullough, and F. Stehr	
Influence of weather on the synchrony of gypsy moth outbreaks in New England	130
D. W. Williams and A. M. Liebhold	
LIST OF ATTENDEES	131

PROGRAMS OF THE EUROPEAN BIOLOGICAL CONTROL LABORATORY,
USDA/ARS, MONTPELLIER, FRANCE

Lloyd Knutson

USDA, ARS, European Biocontrol Laboratory, c/o Amembassy Paris
PSC 116 (EBCL) APO AE 09777

The Biological Control of Weeds Laboratory - Europe, established in Italy in 1958 and the European Parasite Laboratory, established in France in 1919, were combined in 1991 as the European Biological Control Laboratory in Montpellier, France. This laboratory is one of three overseas biological control laboratories in the Office of International Research Programs of the Agricultural Research Service (ARS), United States Department of Agriculture, R. S. Soper, Assistant Administrator.

ARS is a mission-oriented agency responsible for developing new knowledge and technology to meet the needs of American agriculture. Research on biological control systems, the complex of pest/natural enemy interactions that can be manipulated for practical, economical, effective, energy-conservant, and safe, environmentally sound pest management is a prime priority of the Agency.

Many of the insect pests and weeds in the United States are of Eurasian origin, and most were accidentally introduced free of the natural enemies that control them in their homeland. Many have become problems of national importance, insect pests attacking many crops, ornamentals, forests, and domestic animals, and weeds infesting millions of acres of range, pasture, crop lands and natural areas. Millions of dollars of losses, annually, are caused by immigrant pests.

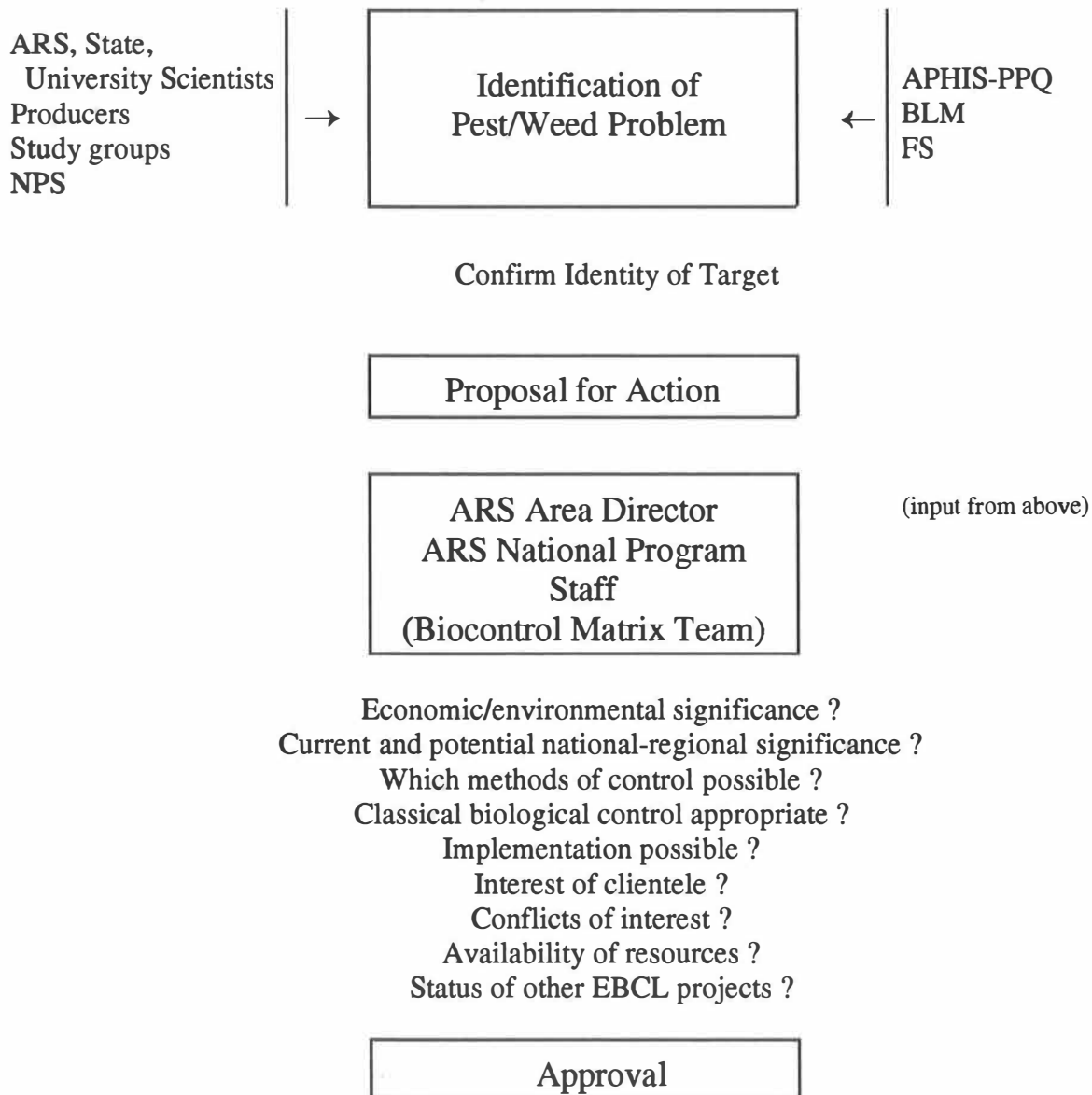
The mission of the Laboratory is to discover, conduct research on, and introduce safe natural enemies (insects, mites and pathogens) into the United States to abate these insect pests and weeds.

The Laboratory's research and service program is in support of stateside ARS and university laboratories and state and federal agencies such as the Forest Service, Animal and Plant Health Inspection Service, Bureau of Land Management, etc. The laboratory cooperates extensively with biological control specialists in state agencies and universities throughout the United States, and with other biological control workers throughout the world. The Laboratory serves as the focal point of ARS exploration in Eurasia, the Middle East, and North Africa, and as a source of information on biological control activities.

Protocol for Establishment of EBCL Projects

EBCL research and technology transfer ("collect-and-ship") projects are carried out in close collaboration with U.S. stateside scientists. The general procedure for establishing a new project in EBCL is described in the flowchart, below.

Phase 1: Target Proposal - Approval - Planning (year 1)



Office of International Research Programs
European Biological Control Laboratory

1. Re-confirm identity of target
2. What is needed ? Collection of known natural enemies, exploration for new natural enemies, research
3. Time - frame for project ?
4. Priority relative to current EBCL projects ?
5. Resources available ?
6. Stateside research and implementation coordinator(s) available ?
7. Nature of cooperation ?
8. Quarantine available ?
9. Integration with other control methods ?
10. Taxonomic support available ?
11. Value as a basic research - learning subject ?
12. Relationship to EBCL long-term research objectives ?
13. Cooperation with other European labs useful (IIBC, CSIRO, ENEA, Montpellier, etc.) ?

Research plan developed with stateside cooperators

(Weeds)

TAG BCW

Research and Technology Transfer Program

Our research approaches are basic biology; host specificity testing; biotype, microhabitat, and host characterization; laboratory and field evaluation of efficacy; population dynamics; and pathogen, parasitoid, predator interactions. Future research at EBCL will include: competition among natural enemies, computerized climate matching, habitat and microhabitat matching, and quality control of natural enemies. The current research and service projects, with indication of the lead scientist for each and year the project was initiated, is shown below.

Weed research targets include: CRIS 1- **Leafy Spurge** (*Euphorbia esula*) (Campobasso, Fornasari, Kashefi, Sobhian, 1980). CRIS 2 - **Saltcedar** (*Tamarix ramosissima*) (Fornasari, Sobhian 1991-); **Common Crupina** (*Crupina vulgaris*) (Sobhian, Knutson 1992-); **Hawkweed** (*Pilosella pratense*) (Fornasari, 1993-); **Russian Thistle** (*Salsola kali*) (Knutson, 1994-).

Weed research has been completed on the following and we are collecting and shipping natural enemies of these weeds to U.S. collaborators for establishment : - **Yellow Starthistle** (*Centaurea solstitialis*) (Kashefi); **Diffuse and Spotted Knapweeds** (*Centaurea diffusa*, *C. maculosa*) (Campobasso, Kashefi); **Musk Thistle** (*Carduus nutans*) (Campobasso); **Field Bindweed** (*Convolvulus arvensis*) (Campobasso, Kashefi, Sobhian); **Puncture Vine** (*Tribulus terrestris*) (Campobasso); and **Common Toadflax** (*Linaria vulgaris*) (Campobasso).

Target insect pests include: CRIS 3 - **SWEETPOTATO AND SILVERLEAF WHITEFLIES** (*Bemisia tabaci* and *B. argentifolii*) (Kirk, Lacey 1991-). CRIS 4 - **INSECT PESTS OF CEREALS AND OTHER CROPS** including: **Cereal Leaf Beetle** (*Oulema melanopus*) (Dysart *et al.* 1993-); **Russian Wheat Aphid** (*Diuraphis noxia*) (Lacey 1991-); **Pine Shoot Beetle** (*Tomicus piniperda*) (Dysart 1995 -); **Wheat-Stem Sawfly** (*Cephus cinctus*) (Dysart 1995-). CRIS 5 - **ORCHARD/URBAN TREES PESTS** including: **Gypsy Moth** (*Lymantria dispar*) (Hérard & Lacey 1992-).

Visiting Scientists

The Laboratory welcomes visiting scientists; a brief sketch of the current facilities follows.

1. **Montpellier**

The environment around Montpellier is very diverse, from coastal marshes to mesic forests at elevations to 1,300 m about 50 km north of the lab, and is a rich resource for exploration for natural enemies. The lab is currently situated in the Parc Scientifique, village of Montferrier, 10 minutes north of downtown Montpellier, near CIRAD (Centre Cooperative International de Recherche Agronomique pour Développement), ORSTOM (Institut Français de la Recherche Scientifique pour le Développement et Coopération), ENSA (Ecole Nationale Supérieure Agronomique) and other scientific organizations. With CSIRO and INRA biological control laboratories, EBCL is a member of the Centre International Lutte Biologique, AGROPOLIS.

The lab is well equipped for work on arthropod and pathogen natural enemies of insects and weeds, although space is limited.

Most of the Rome and Behoust staff moved into the current rented facilities in Science Park, Montpellier during September, 1991. These consist of 400 sq. m. in the main building, including 49 sq. m. of quarantine space; 140 sq. m. in a nearby building (insect pathology lab, library, visitor and student offices), and 32 sq. m. in another building. A quarantine module is situated at the nearly ORSTOM lab; greenhouses, quarantine garden, storage, and a shop are situated at the Lavalette Campus, nearby. (Please communicate with us in advance if you will need quarantine space.). The laboratory maintains a library of specialized books and reprints, extensive map collections, and insect and herbarium collections. A new laboratory of about 1,000 sq. m. will be constructed in 1995-96 on a 2-hectare plot next to the CSIRO laboratory at the Baillarguet Science Park, a few kilometers north of Montpellier.

Address: AmeEmbassy Paris
PSC 116 (EBCL)
APO AE 09777
Tel: (33)-67-04-56-00, fax: (33)-67-04-56-20

2. Thessaloniki

Beginning in 1981, the work in Thermi, near Thessaloniki, northern Greece, was carried out in the Plant Protection Institute for two years and then in facilities of the University of Thessaloniki Farm Campus. In 1989, a 40 sq. m. building was constructed on the farm campus. The facility, consisting of one large lab and two small offices is fairly well equipped and is the base of operations for J. Kashefi. It is used extensively during the field season by the EBCL staff and visiting scientists.

Address: 59 Nikis Avenue
54622 Thessaloniki
Greece
Tel. and fax: (30)-31-473-272

3. Rome

Since many biocontrol of weeds projects were in midstream when the laboratories were consolidated in 1991, and since Italy is a rich resource for natural enemies of many of the EBCL targets, the decision was made to maintain a one-man capability in Rome. An excellent, fully equipped laboratory was developed. Gaetano Campobasso, who is a permanent, full-time employee of EBCL is in charge. The laboratory is used extensively by other EBCL staff and visiting scientists.

Address: Via Colle Trugli No. 9
00132 Rome
Italy
Tel.: (39)-6-2060-93-46, fax: (39)-6-207-90-86

Types of Cooperation. Cooperative activities range from long-term projects with mission (TDY) researchers from ARS and other stateside laboratories and universities and from other countries, to brief research visits. Although we must give priority to scientists cooperating on lab targets and USDA supported projects, our interest is to host everyone we can. Depending on prior commitments and resources, the laboratory also is pleased to be of assistance to scientists working in biological control and related areas who are passing through the area and need assistance.

ARS has an established procedure for initiating cooperative research projects and for requesting collection and shipment of natural enemies from its overseas laboratories. Further information in this regard should be requested from Dr. R. S. Soper or the ARS National Program Leader for Pest Management Systems.

Contacts. Depending on the nature of the visit, initial contact is usually made with the ARS National Program Staff Leader for Pest Management Systems, Assistant Administrator for International Research Programs (Dr. R. S. Soper), or the Director of the European Biological Control Laboratory (Dr. L. Knutson). Subsequently, the visitor is usually in communication with the appropriate laboratory scientist to make arrangements, but the Laboratory Director should be kept informed of planning for the visit. The laboratory does not charge "bench " fees. Budgets for long-term cooperative work are established in advance of the work. Visitors should communicate in advance about their needs for space, supplies, and equipment. The cheapest way to ship material to EBCL is by our APO address.

Students:

With the consolidation in Montpellier, EBCL is in a much better position to have students work in the laboratory. Emphasis is on Master's and Ph. D. level students working on specific aspects of EBCL CRIS projects. The EBCL project supervisor serves on the student's official University Committee. Individual and/or joint publications with the EBCL project supervisor is an expected result. The laboratory appreciates the opportunity to contribute to the Montpellier scientific/academic community in this manner. We expect training/cooperative research to be a continuing strong element in the EBCL program, and specific allowance is being made for space for students in the new facilities. The laboratory recently developed an International Internship program for U.S. college students to work for 3-month periods with EBCL scientists.

Research and Service Activities on Gypsy Moth

Research on and technology transfer of parasitoids and predators of gypsy moth is led by Dr. Franck Hérard, and on pathogens is led by Dr. Lerry Lacey. Following is a brief description of their research program on gypsy moth.

Parasitoids and Predators

(F. Hérard)

1994 - Corsica

- A study of population dynamics to identify promising natural enemies, by contrasting high density and low density host populations, was made in cork oak stands near Porto Vecchio, (Corsica) France.
- To increase knowledge of alternate host utilization by gypsy moth parasitoids, we determined the temporal pattern of oviposition by its parasitoids by exposing laboratory reared larvae for 5-day periods during April through September.
- Data analysis is in progress. The species and numbers of parasitoids reared from the collected hosts were recorded for each time period, age class, host density, and tree. Possible effects/comparisons include: differences among age classes, low versus high host density, and differences among sites.
- Shipments were made to the U.S. of braconids (*Glyptapanteles porthetriae*, *Glyptapanteles liparidis*, *Cotesia melanoscela*, *Meteorus pulchricornis*) and tachinids (*Blepharipa pratensis* and *Parasetigena silvestris*) attacking, respectively, early and late instar gypsy moth larvae in Corsica.

1995 - Alsace

Objectives:

- Study gypsy moth population dynamics to identify promising natural enemies, by contrasting non-outbreak and outbreak populations in stands of mixed oaks, beeches, and hornbeams in Alsace (northeastern France).
- Examine factors that influence effectiveness of natural enemies (biology, behavior, hyperparasitoids, interspecific competition, host range, host quality, and habitat preference association).
- Investigate host-parasitoid relationships between *L. dispar* and the two tachinids, *Blepharipa schineri* and *Ceranthia samarensis*, because introduction into the U.S. of highly specific

univoltine parasitoids, effective at low levels of population density, are needed to slow the spread of the pest on its leading edge.

- Ship to the U.S. the two above mentioned tachinids.
- Establish a colony of *B. schineri* at the laboratory to ultimately study its host foraging behavior and fecundity, during 1996.

Pathogens

(L. Lacey)

1994 - Corsica

- Surveys for patently diseased larvae conducted in June. Diseased larvae were very few in the high density host populations of this region and were infected with virus. Diseased larvae from Alsace were infected with Hyphomycetes. An unidentified fungus was also isolated from egg masses in Alsace. Several shipments were made to the ARS microbial germplasm repository in Ithaca, NY.

1995 - Alsace

Objectives:

- Survey and collection of diseased gypsy moth eggs and larvae, to inventory the native pathogens with emphasis on fungi occurring in the Alsace area.
- Investigate potential for using pheromone traps for contaminating adult male gypsy moth with spores of various fungi.
- In conjunction with A. Hajek and F. Hérard, study the effects of *Entomophthora maimaiga* on gypsy moth and its natural enemies (contingent upon funding).
- With summer student (Ms. Franklin) assay of several Hyphomycetes against *L. dispar* larvae.

INTRODUCTION TO THE SESSION: "THE INCREASING SIGNIFICANCE OF
BIOLOGICAL CONTROL AND AN OVERVIEW OF REGULATIONS GOVERNING
BIOLOGICAL CONTROL ORGANISMS"

Norman C. Leppla and Ernest S. Delfosse

United States Department of Agriculture, Animal and Plant Health Inspection Service, Office of the
Administrator, National Biological Control Institute, 4700 River Rd.,
Unit 5, Riverdale, MD 20737

Advancement of Biological Control

National and local commitments to increasing the use of biological control, the use of live natural enemies to reduce populations of pest species to levels below which would occur in the absence of the natural enemies, in integrated pest management are codified in official policy statements (Table 1), as exemplified by the following policy statement from Robert Melland, former Administrator, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS): "APHIS believes that modern biological control, appropriately applied and monitored, is an environmentally safe and desirable form of long-term management of pest species. It is neither a panacea nor a solution for all pest problems. APHIS believes that biological control is preferable when applicable; however, we also recognize that biological control has limited application to emergency eradication programs. Wherever possible, biological control should replace chemical control as the base strategy for integrated pest management. In support of this philosophy, APHIS will develop regulations that facilitate the release of safe biological control agents, while maintaining adequate protection for American agriculture and the environment. The regulations will give clear and appropriate guidance to permit applicants, including specific types of data needed for review and environmental analysis and specific time limits for Agency review. They will be updated as the science progresses. APHIS believes that public input on procedures to approve the release of biological control agents is a desirable and necessary step, and will strive to gather input from scientists, industry, and the public."

The development of new biological control technologies is justified by the multi-billion dollar costs of damage caused by pests and their control (Schwartz and Klassan 1991; U.S. Congress, OTA 1993; Cate and Hinkle 1993). The total preharvest losses of food to pests in the U.S. is about 37%, 12% being due to weeds (Pimentel 1991). These losses occur consistently each year despite the widespread use of modern pest control technologies and application of about 500 million kilograms of pesticides. Pesticide use in the U.S. has been essentially stable during the past 17 years, with atrazine and metolachlor being the most widely used; both are herbicides (Anonymous 1995). The estimated average annual loss due to weeds from 1975 to 1979 in field crops, vegetables, fruits and nuts, forage seed crops, hay, and pasture and rangelands was \$9 billion (Chandler 1991).

Table 1. Important Recent Developments in Policy for Biological Control and Integrated Pest Management.

- o U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) announces a “Biological Control Philosophy” (August 1992) and initiates policy changes now being implemented.
- o USDA Forest Service (FS) and Department of Interior announce major policy changes to “ecosystem management” (1992-3).
- o USDA FS establishes the “National Center for Forest Health Management” in Morgantown, WV (April 1993).
- o Major pest management policy change announced by Clinton Administration: U.S. will reduce pesticides by increasing biological and cultural control (USDA, Environmental Protection Agency and Food and Drug Administration, June 1993).
- o *Pesticides in the Diets of Infants and Children* report released by National Research Council (National Academy Press, Washington, D.C., 386 p., August 1993).
- o Office of Technology Assessment (OTA), the research arm of Congress, releases a major report on *Harmful Non-Indigenous Species in the United States* (September 1993).
- o Charge given to OTA by the House Committee on Agriculture to investigate biological pest control and suggest policy options (September 1993).
- o USDA Cooperative State Research Service announces a biological control section of the National Research Initiative (1994).
- o National Academy of Science conducting a major study into biologically-based pest management in natural systems (report due mid-1994).
- o Department of Defense (DoD) issues pest management standards for DoD installations.
- o North American Plant Protection Organization announces a “Biological Control Philosophy” (October 1994) based on APHIS’ philosophy.

Combining the cost of losses with expenditures for control, the total cost per year was about \$15.1 billion. Biological control, particularly the classical approach, can reduce these losses with returns sometimes in excess of 100 times the cost of their development (Tisdell 1990, Williams and Leppla 1992, Leppla *et al.* 1995). Moreover, there is very minimal risk in the use of relatively host-specific natural enemies that have passed the regulatory scrutiny required for their release into the environment (Bruzzese 1990, Harris 1990 and 1993, Hill 1990, Laird *et al.* 1990, Lima 1990, Hopper 1995, Storey 1992, Wapshere *et al.* 1989).

The USDA maintains a substantial biological control program, including overseas laboratories in France, Argentina and Australia, to discover new natural enemies, gain firsthand understanding of the ecological context in which they operate, and selectively ship potentially useful species to the U.S. For this purpose, efforts are being made to improve both domestic and foreign laboratories of the USDA. Specifically targeted pests currently include Russian wheat aphid, *Diuraphis noxia* (Mordvilko); codling moth, *Cydia pomonella* (L.); salt cedar, *Tamarix* sp.; melaleuca, *Melaleuca quinquenervia* (Cav.) S. T. Blake; European corn borer, *Ostrinia nubilalis* (Hubner); sweetpotato whitefly, *Bemisia tabaci* (Gennadius); brown citrus aphid, *Toxoptera citricida* (Kirkaldy); Japanese beetle, *Popillia japonica* Newman; gypsy moth, *Lymantria dispar* (L.); hydrilla, *Hydrilla verticillata* (L. F.) Royle; common crupina, *Cuprina vulgaris* Cassini; diffuse knapweed, *Centaurea diffusa* Lam.; spotted knapweed, *C. maculosa* Lam.; leafy spurge, *Euphorbia esula* L. and purple loostrife, *Lythrum salicaria* L.. Natural enemies are collected overseas, shipped under permit to one of about 40 USDA certified containment facilities in the U.S., screened for identity and purity, moved to the receiving states and released under conditions specified on another federal permit (Coulson and Soper 1989, Coulson *et al.* 1991, Van Driesche and Bellows 1993). USDA biological control projects are conducted by APHIS; the Agricultural Research Service; Cooperative State Research, Education and Extension Service; and Forest Service with a combined departmental investment of about \$60 million per year (Anonymous 1992a). The U.S. Army Corps of Engineers and U.S. Department of Interior, Bureau of Land Management, Bureau of Reclamation and National Park Service, and about 14 state departments of agriculture also conduct biological control programs.

Biological control will progress at a reasonable pace and its potential will be realized only if pest management needs are accurately assessed relative to biological control options. International, national and regional partnerships, involving a wide variety of private and public institutions, can then be formed to establish common target systems, set priorities and measure success (Klassan 1993). Funding and personnel will be increased to conduct interdisciplinary research and form "implementation teams". Emphasis will be on applied projects that solve or prevent pest populations from expanding to outbreak levels, particularly pilot testing potential biological control technologies (Knipling 1992, Klassan 1993). This approach incorporates ecosystem-based management with its integration of research, consultation, planning, implementation and monitoring of all the social, economic and environmental factors that are of concern over large geographical areas (Slocombe 1993, Gregory *et al.* 1995).

Regulation of Biological Control

APHIS, the U.S. Environmental Protection Agency (EPA) and U.S. Department of Interior, Fish and Wildlife Service (FWS) are the primary U.S. federal agencies involved in issuing permits for the importation, interstate movement, and release into the environment of biological control agents (Abrams 1990, Charudattan and Browning 1992, Delfosse 1994, Mendelsohn *et al.* 1995) (Figure 1). Regulatory authorities of the federal and state institutions are coordinated through a process of independently reviewing the same permit applications and any cooperating state institution can also deny a permit. Federal approval of a permit is an action that usually does not “trigger” the National Environmental Policy Act, 1969 (NEPA). NEPA can be triggered by an unprecedented release into the environment of a non-indigenous organism. In the absence of an existing environmental assessment that addresses the release, a new one must be prepared or a more rigorous environmental impact statement substituted. Implementation of NEPA is costly and time-consuming, particularly because there is a wide range of interpretations as to what constitutes compliance.

The major goal of NEPA is “to create and maintain conditions under which man and nature can exist in productive harmony, and fulfill the social, economic, and other requirements of present and future generations of Americans.” It “endeavors to secure that goal through integrating the entire range of environmental values meaningfully into society’s pursuit of other important policies and values in a variety of ways, including open, thought-provoking governmental decisionmaking procedures, education, research, and data gathering.” Its purpose is to prevent damage to the environment and associated human health risks, and to mediate the inevitable conflicts of interest that arise from any significant federal action (Bausch 1991a, b). NEPA thus establishes a fair means of arbitration that encourages wide input and assures that their interests are protected by monitoring pest control practices for efficacy and environmental safety (Miller and Aplet 1993, Aplet 1994, Maddox 1994, Reagan *et al.* 1994).

If an organism is regulated as a direct or “indirect” (a legal, not biological, term) plant pest, or a Federal Noxious Weed, a permit application is submitted by the importer to the regulatory officials of the state that is the intended destination and, after it is reviewed at that level, it is forwarded to the USDA, APHIS, Plant Protection and Quarantine, Organism Permitting and Risk Analysis group to assure compliance with federal regulations. APHIS grants or denies permits for the importation, interstate movement and release into the environment of biological control agents, and microbial and multicellular organisms. Importation of a biological control agent into a certified containment facility is considered to be essentially risk-free; however, interstate movement requires additional pest risk analysis and state approval. Policies and procedures for assuring adequate regulation of biological control agents by APHIS have evolved case-by-case as needs occurred. Biological control containment facilities are certified by APHIS and operated according to local requirements, although uniform specifications and standards have been developed. APHIS regularly convenes a Technical Advisory Group to review permit applications for weed biological control agents and a similar peer review procedure has been considered for entomophages. Release into the environment triggers the NEPA process, specifying either an environmental assessment or a full environmental impact statement (Klingman and Coulson 1983, Anonymous 1992b, Delfosse 1993).

National Environmental Policy Act

EPA	USDA, APHIS	DOI, FWS
Chemical pesticides	Non-indigenous species	Threatened and endangered species
Microbial pesticides	Biological control agents	
FIFRA	PQA FPPA FNWA	ESA Lacey Act

Figure 1. Principal federal agencies responsible for implementing biological control regulations, policies and procedures in the United States. The U.S. Environmental Protection Agency (EPA); U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS); and U.S. Department of Interior (DoI), Fish and Wildlife Service (FWS) regulate the indicated aspects of biological control by using corresponding legislative authorities and associated regulations. Acronyms: FIFRA, 1947 (Federal Insecticide, Fungicide and Rodenticide Act); PQA , 1912 (Plant Quarantine Act); FPPA, 1957 (Federal Plant Pest Act); FNWA, 1974 (Federal Noxious Weed Act); and ESA, 1973 (Endangered Species Act). The Lacey Act was enacted in 1900 and amended in 1981 (from Leppla *et al.* 1995).

This procedure is considerably less rigorous for precededented organisms, those that already exist in the release environment, or for organisms included in an existing environmental assessment.

An organism to be used as a pesticide, under the definition of the Federal Insecticide, Fungicide, and Rodenticide Act, must be registered and granted a label by the EPA. The EPA was created in 1970 to protect and preserve the quality of the environment, in order to protect human health and the productivity of natural resources. Consequently, the EPA regulates certain microbial entities such as bacteria, fungi, viruses, and protozoans as “microbial” pesticides. Potential non-target environmental effects are of concern, particularly toxicity and host-specificity. Once an organism is classified as a pesticide, use becomes an issue. For example, urban and industrial pest control uses are under the jurisdiction of the U.S. Food and Drug Administration, and vectors of human and animal diseases are regulated by the Public Health Service, U.S. Department of Health and Human Services.

Additionally, the FWS requires documentation from the country of origin indicating that an organism is not a threatened or endangered species and that approval has been granted for its collection and export. The FWS is charged with enforcing the Endangered Species Act, 1973 and Lacey Act, 1900 and 1981 amendments, the latter being legislation to support CITES, the Convention on the International Trade in Endangered Species. Regulations based on the Lacey Act are currently being revised and the scientific leadership in systematics and biological control is attempting to have most research activities exempted. They are arguing that biological control, especially host-specificity testing, is adequately regulated by the USDA, APHIS with authority from the Federal Plant Pest Act and Plant Quarantine Act, and by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (Anonymous 1991). Moreover, scientists operating in affiliation with “bona fide” research institutions are easily identified and do not require federal regulation. It has never been the intention of FWS to impede scientific research in the enforcement of legislation designed to protect biodiversity. FWS regulators and scientists worldwide have the same goal: protecting the world's flora and fauna.

Significant progress has been made within the allied U.S. federal agencies in implementing workable procedures for regulating biological control but there is no formal structure for harmonizing their actions. Consequently, someone attempting to develop, implement and especially market a biological control technology must work with several agencies independently, often requiring duplicate information and providing somewhat different guidance (Harris 1993). This can impede investment by both the private and public sectors in alternatives to chemical pesticides for managing pests and leads to a continuation of the curative rather than preventative approach. However, due largely to the traditional, informal networking of the biological control community, and to the diligence of the federal agencies involved, the field has advanced safely and at a reasonable pace (Hopper 1995, Maddox 1994).

The goal of this session was to inform an audience that is not familiar with federal regulations that affect biological control about procedures that must be followed to obtain permits. Therefore, we asked knowledgeable representatives from APHIS, EPA and FWS to briefly describe the federal regulations that provide regulatory authority to their respective agencies, diagram and explain the

process for granting permits under these regulations, and discuss the procedures for assuring that these regulations are implemented. Related information was also requested, along with pertinent literature. We hope to provide an efficient and uniform means of obtaining and sharing information, assuring a systematic and expeditious review of applications, enhancing the scientific basis for decision-making, establishing consistent risk assessment protocols, and specifying uniform data and review standards. Additionally, science-based enabling legislation for biological control has been proposed to ensure that safe and effective agents are approved and released efficiently (Delfosse 1992). We are moving toward a coordinated permitting and approval system that incorporates all of the federal regulatory requirements into an efficient process that works for our customers.

Acknowledgments

The authors gratefully acknowledge Michael McManus, the Program Chairman, for providing an opportunity for this unique session to be presented and Sandy Fosbroke for helping to make the information available in the proceedings. Without their support, it would not have been possible to take this first step in harmonizing federal procedures for evaluating biological control permits.

References

- Abrams, J. C. 1990. Biological control agents in integrated pest management: are they regulated? *Pace Environmental Law Review* 8:91-114.
- Anonymous. 1991. United States Code of Federal Regulations, 40 CFR Ch. 1 (7-1-92 Edition) Environmental Protection Agency, 152.20 Subpart B, Exemptions for Pesticides Regulated by Another Federal Agency. U.S. Government Printing Office. 621 p.
- Anonymous. 1992a. The National Biological Control Program, A Plan for Accelerating and Coordinating USDA Biological Control Research and Implementation. Unpublished report developed by the USDA Interagency Biological Control Coordinating Committee. 21 p.
- Anonymous. 1992b. Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act, 40 CFR, Parts 1500-1508. U.S. Government Printing Office. Washington D.C. 46 p.
- Anonymous. 1995. Pesticide Industry Sales and Usage - 1992 and 1993 Market Estimates. Unpublished Environmental Protection Agency Report. 34 p.
- Aplet, G. H. 1994. Ecosystem integrity, alien species, and biological control. pp. 1-3 *In*: Fosbroke, S. L. C. and K. W. Gottschalk, Eds. Proceedings USDA Interagency Gypsy Moth Research Forum. USDA-Forest Service, General Technical Report NE-188. 97 p.
- Bausch, C. 1991a. Achieving NEPA's purpose in the 1990's. *The Environmental Professional* 13:95-99.

- Bausch, C. 1991b. NEPA Integration: Effective, Efficient Environmental Compliance in the 1990's. Unpublished summary of the proceedings of a workshop. 9 p.
- Bruzzese, E. K. 1990. Protocols for biological control of weeds and current Victorian priorities. *Plant Protection Quarterly* 5:98-99.
- Cate, J. R. and M. K. Hinkle. 1993. Integrated Pest Management: The Path of a Paradigm. The National Audubon Society Special Report. Washington D.C. 43 p.
- Chandler, J. M. 1991. Estimated losses of crops to weeds. pp. 53-68 *In*: Pimentel, D., Ed. *CRC Handbook of Pest Management in Agriculture*, Vol. 1., 2nd Edition. CRC Press. Boca Raton, Florida. 765 p.
- Charudattan, R. and H. W. Browning. 1992. Regulations and Guidelines: Critical Issues in Biological Control. Proceedings of a USDA/CSRS National Workshop. IFAS, University of Florida, Gainesville. 205 p.
- Coulson, J. R. and R. S. Soper. 1989. Protocols for the introduction of biological control agents in the U.S.. pp. 1-35 *In*: Kahn R. P., Ed. *Plant Protection and Quarantine*, Vol. III, Special Topics. CRC Press. Boca Raton, Florida. 215 p.
- Coulson, J. R., R. S. Soper, and D. W. Williams. 1991. Biological Control Quarantine: Needs and Procedures. United States Department of Agriculture, Agriculture Research Service, ARS-99. 336 p.
- Delfosse, E. S. 1992. The biological control regulatory process in Australia. pp. 135-141 *In*: R. Charudattan and H. W. Browning, Eds., *Regulations and Guidelines: Critical Issues in Biological Control*. Proceedings of a USDA/CSRS National Workshop, Vienna, Virginia.
- Delfosse, E. S. 1993. User's Guide for Obtaining a Permit from APHIS for Importation, Interstate Movement, or Field Release of a Biological Control Agent. Unpublished draft document. 25 p.
- Delfosse, E. S. 1994. "Facilitators vs. Gatekeepers:" The NBCI Conceptual Model of Biological Control Regulation. Unpublished report. 13 p.
- FAO. 1995. International Standards for Phytosanitary Measures, Code of Conduct for the Import and Release of Exotic Biological Control Agents. Secretariat of the International Plant Protection Convention, Food and Agriculture Organization, United Nations. 14 p.
- Gregory, R., J. Flynn, and P. Slovic. 1995. Technological stigma. *American Scientist* 83:220-223.

- Harris, P. 1990. Environmental impact of introduced biological control agents. pp. 289-300 *In*: Mackauer, M., L. E. Ehler, and J. Roland, Eds. Critical Issues in Biological Control. Intercept. Andover, Hants, UK. 330 p.
- Harris, P. 1993. Effects, constraints and the future of weed biocontrol. *Agriculture, Ecosystems and Environment* 46:289-303.
- Hill, R. L. 1990. Environmental protection procedures and the biological control programme against gorse in New Zealand. pp. 127-133 *In*: E. S. Delfosse, Ed. Proceedings, VII International Symposium on Biological Control of Weeds. CSIRO Publications, East Melbourne, Victoria, Australia. 701 p.
- Hopper, K. R. 1995. Potential impacts on threatened and endangered insect species in the continental United States from introductions of parasitic Hymenoptera for the control of insect pests. *In*: H. M. T. Hokkanen and J. M. Lynch, Eds., Benefits and Risks of Biological Control. Cambridge University Press, London, in press.
- Klassan, W. 1989. Eradication of Arthropods: Theory and Historical Practice. Entomological Society of America, Miscellaneous Publications, 73, 29 p.
- Klassan, W. 1993. Pest management and biologically based technologies: a look to the future. pp. 410-422 *In*: R. D. Lumsden and J. L. Vaughn, Eds., Pest Management: Biologically Based Technologies. Beltsville Symposium XVIII, Agricultural Research Service, U.S. Department of Agriculture. American Chemical Society, Washington, D.C.
- Klingman, D. L. and J. R. Coulson. 1983. Guidelines for introducing foreign organisms into the United States for the biological control of weeds. *Bull. Entomol. Soc. Amer.* Fall:55-61.
- Knipling, E. F. 1992. Principles of Insect Parasitism Analyzed from New Perspectives: Practical Implications for Regulating Insect Populations by Biological Means. USDA, Agriculture Handbook No. 693. 337 p.
- Laird, M., L. A. Lacey, and E. W. Davidson. 1990. Safety of microbial insecticides. CRC Press. Boca Raton, Florida. 259 p.
- Leppla, N. C., E. S. Delfosse, and R. S. Soper. 1995. Technical and regulatory constraints to international cooperation in biological control. *In*: Gerling, D., Ed. Proceedings of a BARD-Sponsored International Workshop on *Bemisia tabaci*, Israel. *Phytoparasitica*. In press.
- Lima, P. J. 1990. United States Department of Agriculture (USDA) safeguards for introducing natural enemies for biological control of weeds. pp. 109-115 *In*: Delfosse, E. S., Ed. Proceedings, VII International Symposium on Biological Control of Weeds. CSIRO Publications, East Melbourne, Victoria, Australia. 701 p.

- Maddox, J. V. 1994. An evaluation of opposing viewpoints of classical biological control. pp. 43-51 In: S. L. C. Fosbroke and K. W. Gottschalk, Eds., Proceedings, U.S. Department of Agriculture Interagency Gypsy Moth Research Forum 1994. United States Department of Agriculture, Forest Service, General Technical Report NE-188.
- Melland, R. 1992. The APHIS Biological Control Philosophy. Formal approval, August 7, 1992.
- Mendelsohn, M., E. S. Delfosse, C. Grable, J. Kough, D. Bays, and P. Hutton. 1995. Commercialization, facilitation, and implementation of biological control agents: a government perspective. pp. 123-133 In: C. L. Wilson and M. E. Wisniewski, Eds., Biological Control of Postharvest Diseases - Theory and Practice. CRC Press, Boca Raton, Florida.
- Meyerdirk, D. E. 1992. International opportunities for classical biological control. pp. 7-14 In: W. C. Kauffman and J. E. Nichols, Eds., Selection Criteria and Ecological Consequences of Importing Natural Enemies. Entomological Society of America, Thomas Say Publications in Entomology, Lanham, Maryland.
- Miller, M. and G. Aplet. 1993. Biological control: a little knowledge is a dangerous thing. Rutgers Law Review 45:285-334.
- OTA. 1993. Harmful Non-Indigenous Species in the United States. U.S. Congress, Office of Technology Assessment. U.S. Government Printing Office. Washington D.C. 379 p.
- Pimentel, D. 1991. Introduction. pp. 3-11 In: Pimentel, D., Ed. CRC Handbook of Pest Management in Agriculture, Vol. 1, 2nd Edition. Boca Raton, Florida. 765 p.
- Reagan, D. P., M. Firko, and F. B. Taub. 1994. The role of ecologists in ecological risk assessments. Bull. Ecological Soc. Amer. June 1994:96-99.
- Schwartz, P. H. and W. Klassan. 1991. Estimate of losses caused by insects and mites to agricultural crops. pp. 15-77 In: Pimentel, D., Ed. CRC Handbook of Pest Management in Agriculture, Vol. 1, 2nd Edition. CRC Press. Boca Raton, Florida. 765 p.
- Slocombe, D. S. 1993. Implementing ecosystem-based management, development of theory, practice, and research for planning and managing a region. BioScience 43:612-622.
- Storey, J. M. 1992. Biological control of weeds: selective, economical and safe. Western Wildlands 18:18-23.
- Tisdell, C. A. 1990. Economic impact of biological control of weeds and insects. pp. 301-316 In: Mackauer, M., L. E. Ehler, and J. Roland, Eds. Critical Issues in Biological Control. VCH Publishers, New York. 330 p.

- Van Driesche, R. G. and T. S. Bellows. 1993. Steps in Classical Arthropod Biological Control. Proceedings, Thomas Say Publ. in Entomol., Entomol. Soc. Amer., Lanham, Maryland. 88 p.
- Wapshere, A. J., E. S. Delfosse, and J. M. Cullen. 1989. Recent developments in biological control of weeds. Crop Protection 8:227-250.
- Williams, D. W. and N. C. Leppla. 1992. The future of augmentation of beneficial arthropods. pp. 87-102 *In*: W. C. Kauffman and J. E. Nichols, Eds., Selection Criteria and Ecological Consequences of Importing Natural Enemies. Entomol. Soc. Amer., Thomas Say Publications in Entomology, Lanham, Maryland.

GUIDELINES FOR OBTAINING A PLANT PEST PERMIT
FROM THE U.S. DEPARTMENT OF AGRICULTURE

Kenneth R. Lakin, Ph.D.
Branch Chief
USDA-APHIS-PPQ-BATS-OPRA

United States Department of Agriculture, Animal and Plant Health Inspection Service,
Plant Protection and Quarantine, Biological Assessment and Taxonomic Support,
Organism Permitting and Risk Analysis (USDA-APHIS-PPQ-BATS-OPRA),
4700 River Rd., Riverdale, MD 20737-1236

Introduction

Three Federal statutes, the Plant Quarantine Act of 1912, the Federal Plant Pest Act (FPPA) of 1957, and the Federal Noxious Weed Act (FNWA) of 1974, provide authority for the Animal and Plant Health Inspection Service (APHIS) to regulate the movement of live plant pests into and through the United States.

The U.S. Department of Agriculture (USDA) is also required to comply with the regulations of other Federal Agencies. The Endangered Species Act of 1973 requires “consultation with Fish and Wildlife Service when a federal action may affect endangered or threatened species.” Applicants should repeatedly consider throughout their projects potential impacts on non-target species, especially endangered and threatened species, when the proposed action is to release an organism into the environment. This consideration must address the eventual spread of the organism throughout the environment.

The National Environmental Policy Act (NEPA) of 1970 requires Federal Agencies to prepare a “detailed statement by the responsible official” on the environmental impact of every major Federal action significantly affecting the quality of the human environment, i.e. an environmental impact statement. To determine whether or not the issuance of a permit by APHIS for the release of plant pests or potential plant pests into the environment constitutes a major Federal action for the purpose of NEPA, an environmental assessment (EA) must be prepared. However, the importation and containment of plant pests are not subject to NEPA.

General Steps for a Permit Application

The following six steps are the same whether the permit application is for importing organisms into containment, moving them interstate between containment facilities, or releasing them into the environment:

1. Apply for a separate permit in each category.
 - a. Import a plant pest into the U.S.
 - b. Movement of a plant pest between States.
 - c. Release a plant pest into the environment.
2. Obtain a PPQ Form 526. The application form and instructions on its completion can be either mailed or sent by facsimile.
 - a. Telephone Barbara Jenkins, Permit Assistant at (301) 734-5609, or
 - b. Download from the World Wide Web @
<http://www.aphis.usda.gov/ppq/bats/permits.html>
 - c. Write to Deborah Knott, USDA-APHIS-PPQ-BATS-OPRA, 4700 River Rd., Unit 133, Riverdale, MD 20737-1236.
3. Complete Section A of Form 526, and submit a signed copy to the appropriate regulatory official for each affected State. The names and addresses of these State Plant Regulatory Officials (SPRO's), available on the World Wide Web @
<http://www.aphis.usda.gov/ppq/bats/permits.html>, will be supplied along with Form 526, if the request is made in writing or by telephone.
4. The SPRO will review and approve or deny the permit application and forward it to BATS. Once BATS receives it from the State official, a notification card will be sent. Almost invariably, if a State regulatory official denies an application, BATS will concur (Fig. 1).
5. BATS will evaluate the application to determine whether it falls under its regulatory authority. For organisms within BATS' authority, the evaluation will include an assessment of risk associated with the action requested on the permit application.

HOW TO APPLY FOR A PPQ PLANT PEST PERMIT

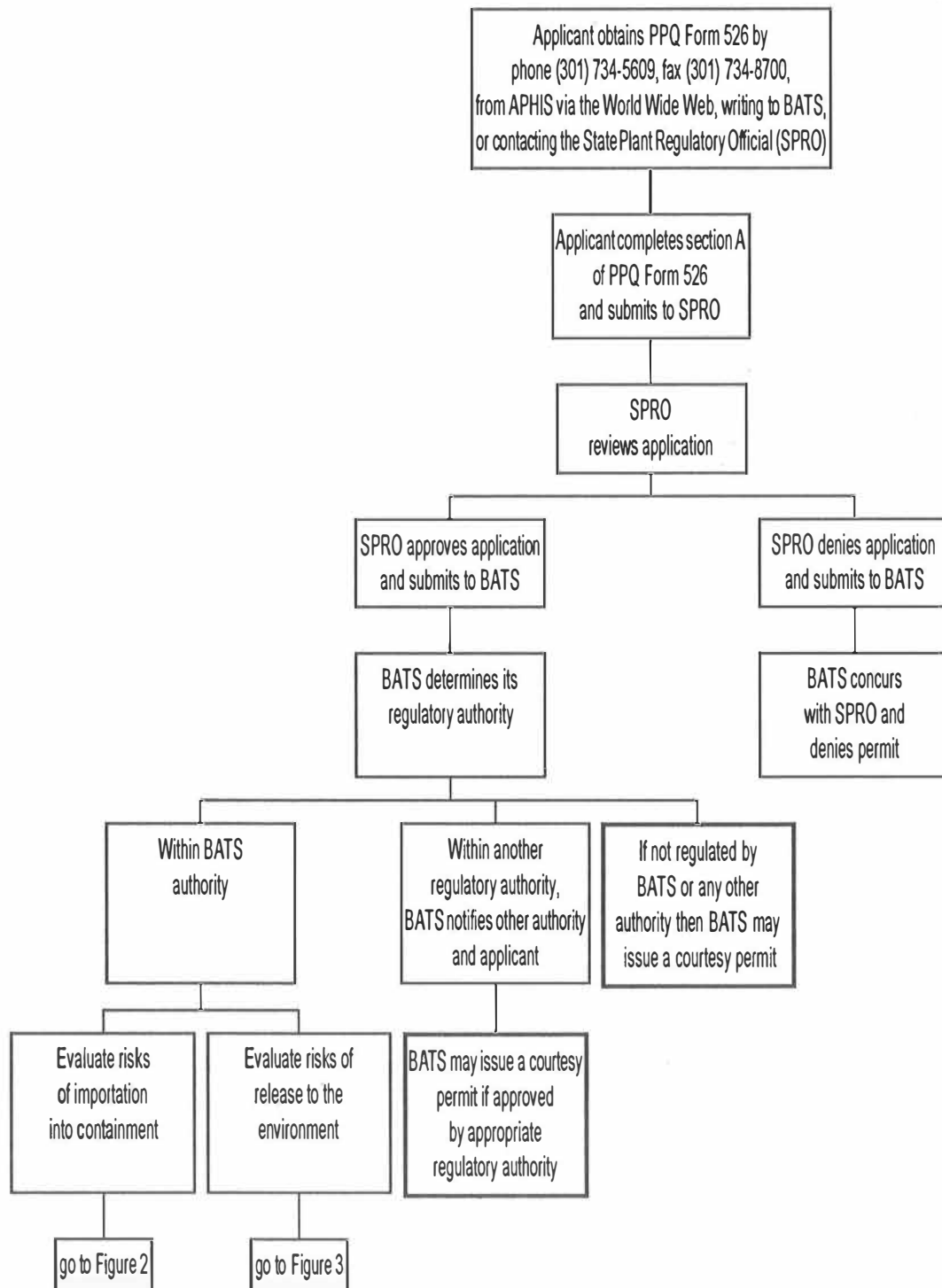


FIGURE 1.

6. If the application does not fall within APHIS' authority, BATS may:
- a. Forward the application to the appropriate regulatory authority, such as the APHIS-Biotechnology Permit Unit of Biotechnology, Biologics, and Environmental Protection; APHIS-PPQ Port Operations Permit Unit; APHIS Veterinary Services; National Center for Import-Export (Animal Program, and Products Service); Food and Drug Administration; etc., and provide notification to the applicant that this action was taken.
 - b. In some instances BATS may issue a courtesy permit to facilitate movement when movement might otherwise be impeded because of the similarity of the organisms to others regulated under the FPPA.

PERMITS TO IMPORT ORGANISMS INTO CONTAINMENT FACILITIES OR TO MOVE ORGANISMS INTERSTATE BETWEEN CONTAINMENT FACILITIES

Applications for movement of organisms between containment facilities do not involve the preparation of EA's. BATS will evaluate the risks and the physical and operating features of the containment facility where organisms are to be housed, if they have not been inspected and approved for this purpose (Fig 2). BATS will initiate a facility inspection and provide the PPQ officer with guidelines for conducting it. The officer will report the inspection findings to BATS which will either approve the facility and issue a permit or not. If BATS determines the facility is inadequate, the reasons will be communicated to the applicant and mitigative measures may be undertaken. An applicant may either comply with the measures specified by BATS and request another inspection or withdraw the application.

An application for study of an organism in a containment facility will be reviewed initially to determine whether or not the facility has been pre-approved by BATS. The containment capabilities of a pre-approved facility will be evaluated to ascertain whether the facility design and operating procedures can reliably contain the requested organism. Permits will be granted for facilities that are secured appropriately. If facilities are not secure, they can be remodeled in consultation with BATS. Approval of a new or remodeled facility will enable the applicant to be granted a permit by BATS-OPRA for containment of the organism for which the facility was constructed.

PERMITS FOR RELEASE TO THE ENVIRONMENT

Permits will be issued (1) for organisms previously reviewed and determined not to present a risk, or (2) for organisms having an existing EA that resulted in a Finding Of No Significant Impact (FONSI). The permits will be accompanied by lists of conditions designated to mitigate pest risk to plants and the environment.

If it is necessary to prepare an EA prior to the issuance of a permit, then applicants will be encouraged to expedite the application process by preparing a draft EA and submitting it to BATS for finalization and scientific review (Fig. 3). Upon receiving a phoned or written request, BATS

IMPORTATION OF ORGANISM INTO A CONTAINMENT FACILITY

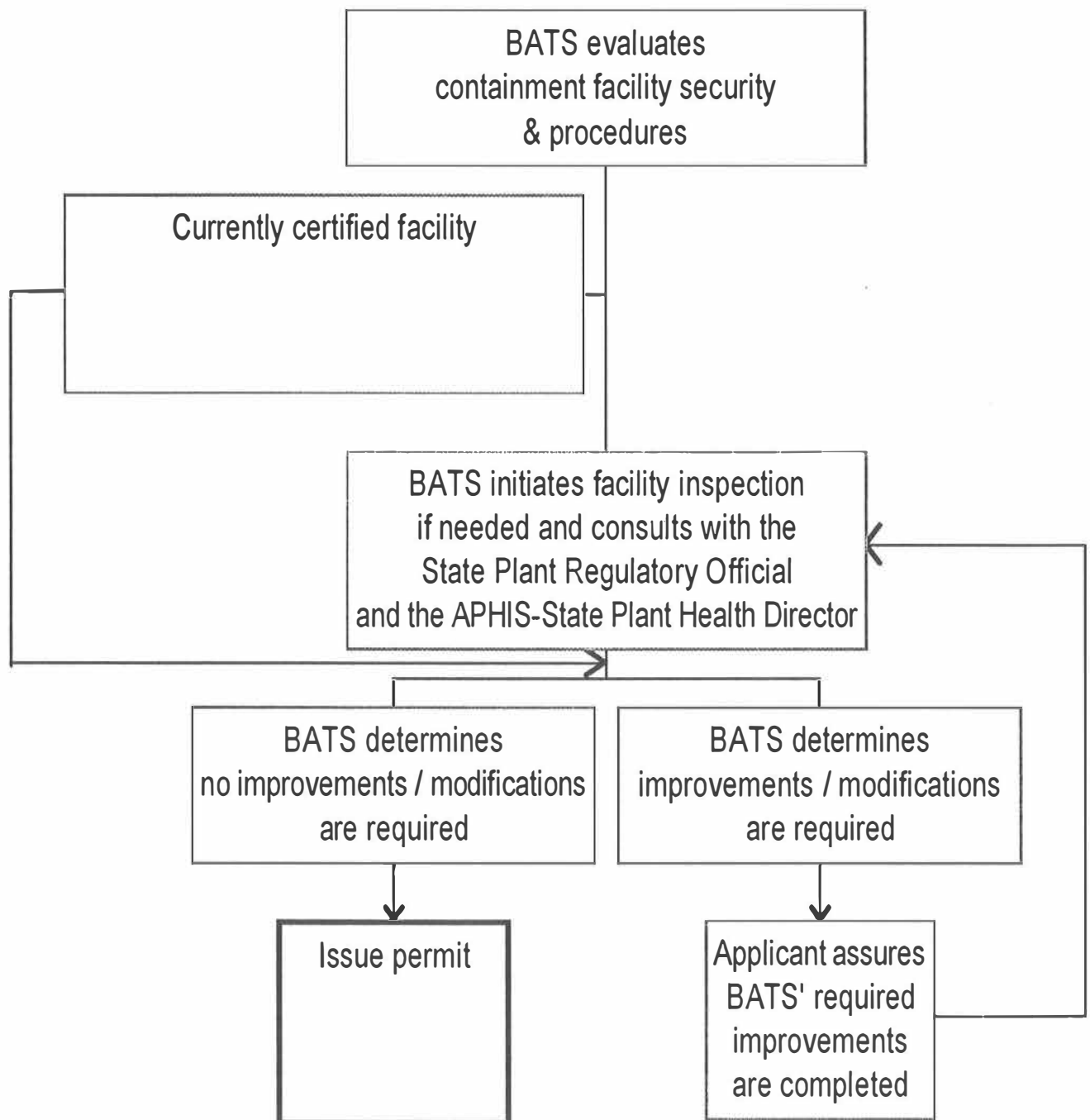


FIGURE 2.

RELEASE OF ORGANISMS TO THE ENVIRONMENT

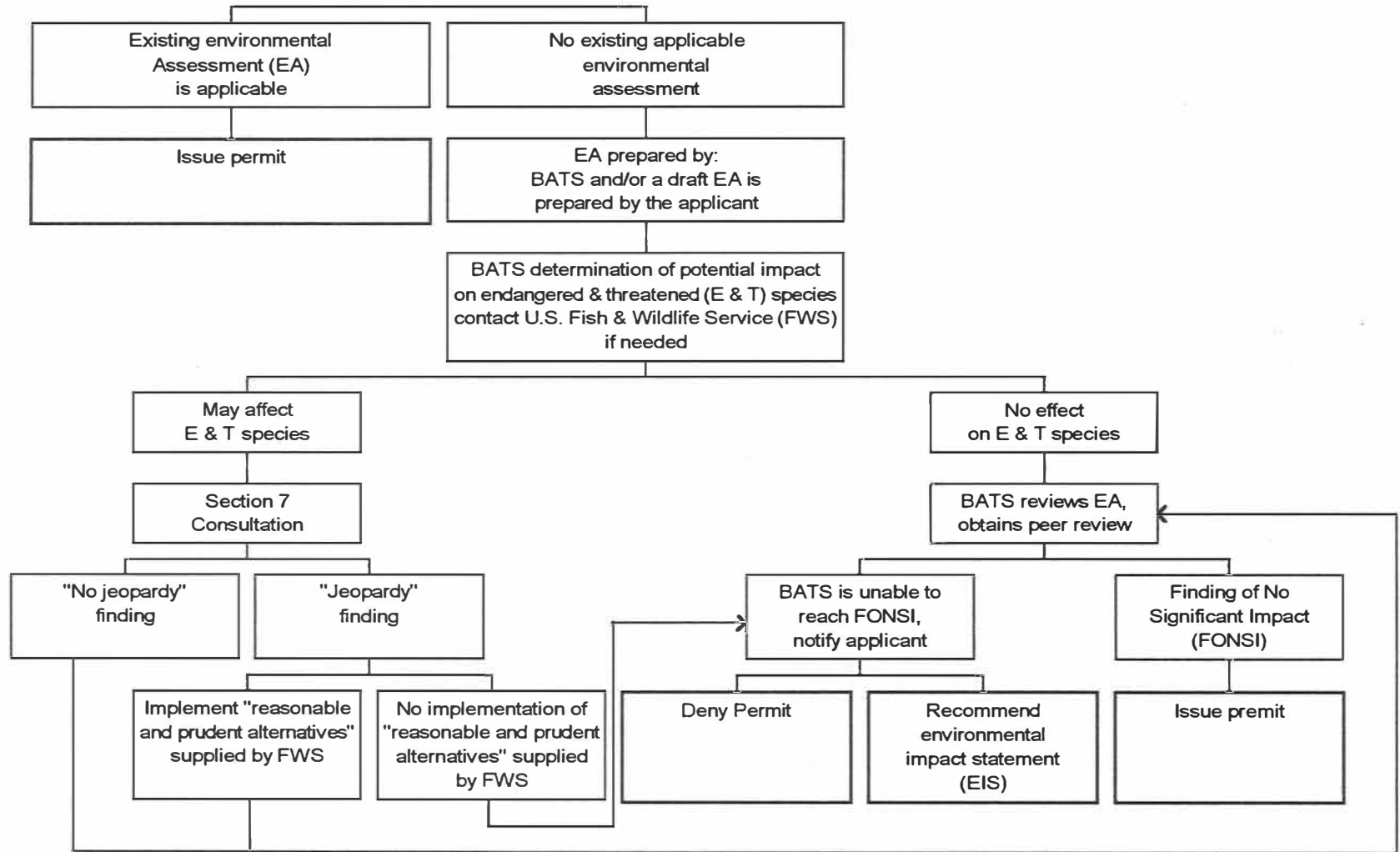


FIGURE 3.

will provide a template EA and example EA's, *both of which are soon to be available on the APHIS GOPHER*. Also, applicants must consider endangered and threatened species at an early stage in the preparation of an EA. The applicant should contact: Division of Endangered Species (ARLSQ452), U.S. Fish and Wildlife Service, 4401 N. Fairfax Drive, Arlington, VA 22203, telephone: 703-358-2106. BATS-OPRA will evaluate the EA for areas of concern such as potential effects on endangered and threatened species (E & T) and potential impacts on the environment.

In the event that BATS-OPRA determines that a proposed permitted action may affect an E & T species, consultation with FWS will be initiated. In addition to the EA, the FWS may request additional information to assist them in determining whether the proposed action would be likely to jeopardize the continued existence of the species of concern. A “jeopardy” finding would be accompanied by “reasonable and prudent alternatives” supplied by FWS which would be required of the applicant to avoid jeopardizing the E & T species of concern. A finding of “no jeopardy” or a finding of “jeopardy” followed by implementation of the “reasonable and prudent alternatives” (50 CFR 402.02) would be followed by BATS' sending the EA for scientific review (Figure 3).

If BATS' evaluation of the EA results in a FONSI, APHIS will publish a notice of availability of the EA in the Federal Register and issue a permit for the application. If BATS-OPRA determines that there is a potentially significant impact of the proposed release that cannot be mitigated, they will notify the applicant of the denial and provide the reasons for the denial. The applicant may appeal the denial, either in person or in writing, to the Deputy Administrator of APHIS, PPQ. The applicant may also supply additional information in support of the original application. Alternatively, BATS-OPRA may retain the application until an EIS has been completed.

Definitions

Containment Facility: A structure where physical and operational characteristics are designed so that the risk of the enclosed organisms' escaping to the environment is minimized.

Movement: “Moved” and “movement” mean ship, deposit for transmission in the mail, otherwise offer for shipment, offer for entry, import, receive for transportation, carry or otherwise transport or move, or allow to be moved, by mail or otherwise (FPPA).

Plant Pest: Any insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof, viruses, or any organisms similar to or allied with any of the foregoing, or any infectious substances of the aforementioned which are not genetically engineered as defined in 7 CFR 340.1 which can directly or indirectly injure or cause disease or damage in any plants or parts thereof or any processed, manufactured or other products of plants (FPPA).

Biological Assessment and Taxonomic Support (BATS) recognizes that a vast majority of plant pests cause direct injury to plants through their phytophagous or disease-causing nature. However, instances may occur in which an organism may be considered a pest through indirect action, *e.g.* a hyperparasitoid of an established and successful biological control agent. BATS evaluates

non-target effects of potential plant pests, especially effects on plants and organisms listed as endangered or threatened by the FWS.

Noxious Weed: Any living stage, including but not limited to, seeds and reproductive parts, of any parasitic or other plant of a kind, or subdivision of a kind, which is of foreign origin, is new to or not widely prevalent in the United States, and can directly or indirectly injure crops, other useful plants, livestock, or poultry or other interests of agriculture, including irrigation, or navigation or the fish or wildlife resources of the United States or the public health (FNWA).

References

- Anonymous. 1912. Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act, 40 CFR, Parts 1500-1508. U.S. Government Printing Office. Washington, D.C.
- Coulson, J. R. and R. S. Soper. 1989. Protocols for the introduction of biological control agents in the U.S.. pp. 1-35 *In*: R. P. Kahn, Ed., Plant Protection and Quarantine. CRC Press. Boca Raton, Florida.
- Coulson, J. R., R. S. Soper, and D. W. Williams. 1991. Biological Control Quarantine: Needs and Procedures. USDA, ARS, ARS-99.
- Foster, J. A. 1991. Exclusion of plant pests by inspections, certifications and quarantines. pp. 311-338 *In*: Pimentel, D., Ed., CRC Handbook of Pest Management in Agriculture, Vol. 1, 2nd Edition. CRC Press. Boca Raton, Florida.
- Imai, E. 1992. Some insights on biological assessment. pp. 41-43 *In*: R. Charudattan and H. W. Browning, Eds., Regulations and Guidelines: Critical Issues in Biological Control. Proc. USDA/CSRS National Workshop, Vienna, Virginia.
- Klingman, D. L. and J. R. Coulson. 1983. Guidelines for introducing foreign organisms into the United States for the biological control of weeds. Bull. ESA, Fall 1983:55-61.
- Lima, P. J. 1989. United States Department of Agriculture (USDA) Safeguards for Introducing Natural Enemies for Biological Control of Weeds. pp. 109-115 *In*: E. S. Delfosse, Ed., Proc. VII Int. Symp. Biol. Contr. Weeds, Rome, Italy.

OBTAINING EPA APPROVAL TO TEST OR COMMERCIALIZE MICROBIAL AND/OR BIOCHEMICAL PESTICIDES

Michael L. Mendelsohn and Phillip O. Hutton

Biopesticides and Pollution Prevention Division,
Office of Pesticide Programs, U.S. Environmental Protection Agency,
401 M Street S.W., Washington, DC 20460

Introduction

U.S. Environmental Protection Agency (EPA) approval is required prior to full commercial and certain experimental use of most pesticides. Pesticides are defined in § 2(u) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as “(1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest and (2) any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant” except those articles considered to be new animal drugs or animal feeds bearing or containing a new animal drug.

Among the various types of pesticides currently regulated by EPA are the microbial and biochemical pesticides. Microbial pesticides include the following microorganisms when they act as pesticides per FIFRA § 2(u): protozoa, algae, fungi, bacteria, and viruses. Biochemical pesticides are distinguished from conventional chemical pesticides by their nontoxic or indirect mode of action toward target organisms and by their natural occurrence or structural similarity and functional equivalence to naturally occurring compounds, e.g., insect pheromones and certain growth regulators.

EPA is committed to encouraging the development and use of environmentally acceptable biological pesticides as alternatives to more toxic conventional chemical pesticides. The Agency recognizes that these pesticides are often different in their mode of action and has employed numerous measures to facilitate the application process. These include distinct data requirements, consolidation of biological pesticide application processing within a single group, [Biopesticides and Pollution Prevention Division (BPPD)], a soon to be available “Guide to the Registration of Biological Pesticides”, and regulatory relief activities in the area of lepidopteran pheromones.

EPA Contact Points

Inquiries regarding biochemical and microbial pesticides should be directed to the following individuals:

Phil Hutton
Regulatory Action Team Leader
Microbial and Plant Pesticides
Phone: (703) 308-8260
Fax: (703) 308-7026
email: Hutton.Phil@epamail.epa.gov.

Robert Torla
Regulatory Action Team Leader
Biochemical Pesticides
Phone: (703) 308-8098
Fax: (703) 308-7026
email: Torla.Robert@epamail.epa.gov.

For information on joining BPPD's Pesticide Environmental Stewardship Program (a voluntary public/private partnership dedicated to protecting human health and preserving the environment by reducing the use of pesticides and the risks associated with pesticide use), interested parties should call 1-800-972-7717.

Approvals Needed for Experimental Work

I. Experimental use permits are required for all field testing of pesticides except for the following:

A) A small-scale test involving use of a particular pesticide that is conducted on a cumulative total of no more than 10 acres of land or 1 surface acre of water per pest, provided that: 1) when testing for more than one target pest occurs at the same time and in the same locality, the appropriate 10 or 1 acre limitations shall encompass all of the target pests, 2) any food or feed crops involved in, or affected by such tests shall be destroyed or consumed by experimental animals unless an appropriate tolerance or exemption from a tolerance has been established under the Federal Food, Drug, and Cosmetic Act (FFDCA) for residues of the pesticide, 3) waters which are involved in or affected by such aquatic tests are not used for irrigation purposes, drinking water supplies, or body contact recreational activities, and 4) aquatic testing shall not be conducted in any waters which contain or affect fish, shellfish, plants or animals taken for recreational or commercial purposes and used for food or feed, unless an appropriate tolerance or exemption from a tolerance has been established under the FFDCA for residues of the pesticide.

B) For certain microbial pesticides, a notification to the EPA for a determination as to whether testing requires an experimental use permit is needed before any small-scale testing in the environment takes place. These microbial pesticides include: 1) microbial pesticides whose pesticidal properties have been imparted or enhanced by the introduction of genetic material that has been deliberately modified (except microbial pesticides resulting from deletions or rearrangements within a single genome that are brought about by the introduction of genetic material that has been deliberately modified), and 2) nonindigenous microbial pesticides that have not been acted upon by the USDA.

C) Non-aquatic experimental use (including food and feed use) of lepidopteran pheromones, regardless of formulation, when applied at a maximum use rate of 150 grams active ingredient per acre per year do not require an experimental use permit when tested up to 250 acres. However, all inert ingredients in these product formulations must be exempt from the requirement of a tolerance under the FFDCA.

II. Food or feed items treated in experimental testing require a tolerance or exemption from the requirement of a tolerance under the FFDCA. Certain generic tolerance exemptions exist for biochemical pesticides, including the following:

A) 40 CFR Part 180.1122 exempts inert ingredients in semiochemical dispensers.

B) 40 CFR Part 180.1153 exempts lepidopteran pheromones applied to growing crops at a rate not exceeding 150 grams per acre per year from the requirement of a tolerance.

Approvals Needed for Full Commercial Use

I. Registration of the pesticide product under FIFRA.

II. Establishment of a tolerance or exemption from tolerance under the FFDCA for uses involving food or feed. The generic tolerances mentioned above also apply here.

U.S. FISH AND WILDLIFE SERVICE REGULATIONS GOVERNING THE COLLECTION,
POSSESSION, AND TRANSPORTATION OF WILDLIFE AND PLANTS, AS RELATED
TO THE SCIENTIFIC COMMUNITY¹

United States Department of the Interior, Fish and Wildlife Service, Division of
Law Enforcement, P. O. Box 3247, Arlington, VA 22203-3247

- I. Introduction - Those individuals or institutions who have dealings with "fish or wildlife" as defined by the Lacey Act Amendments of 1981 and Title 50, Code of Federal Regulations (CFR), part 10, must be aware of the pertinent Statutes and Regulations with which they are required to comply.
- A. Laws and Regulations
1. 16 USC 1531, Endangered Species Act of 1973(ESA) and 50 CFR 17.
 2. 16 USC 1538(c), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and 50 CFR 23.
 3. 16 USC 1361, Marine Mammal Protection Act(MMA) and 50 CFR 18.
 4. 16 USC 703, Migratory Bird Treaty Act(MBTA) and 50 CFR 10 and 21.
 5. 16 USC 668, Bald and Golden Eagle Protection Act (EPA) and 50 CFR 22.
 6. 18 USC 42, Lacey Act (Injurious Wildlife) and 50 CFR 16.
 7. 16 USC 3371, Lacey Act Amendments of 1981.
 8. 16 USC 4901, Wild Bird Conservation Act of 1992 (WBCA) and 50 CFR 15.
 9. 16 USC 4201, African Elephant Conservation Act (AEC).

¹For further information, please contact Sheila Einsweiler at 703-358-1949.

B. Problems involving scientific collecting, possession, and transportation.

1. The collection by individuals and/or institutions, of wildlife and plants regulated by any of the above cited laws, without the required permits authorizing such taking.
2. The possession by individuals and/or institutions, of wildlife and plants regulated by any of the above cited laws, without the required permits authorizing such possession.
3. The receiving by individuals and/or institutions, of wildlife and plants regulated by any of the above cited laws, when such wildlife or plants were not legally acquired/possessed by the individual or institution from whom they are being received.
4. The importation, exportation, and interstate transportation by individuals and/or scientific institutions, of wildlife and plants, contrary to any of the above cited laws and regulations.
5. The activities conducted by individuals and/or institutions, related to regulated wildlife and plants, that is not authorized, or is contrary to, the conditions set forth on their Service permit.

C. General Importation and Exportation Requirements:

1. All wildlife shipments must leave and enter this country through Customs ports designated by the U.S. Fish and Wildlife Service (50 CFR 14.12). Currently there are twelve designated ports : New York, NY; Miami, FL; Baltimore, MD; Boston, MA; New Orleans, LA; Dallas/Ft. Worth, TX; Los Angeles, and San Francisco, CA; Chicago, IL; Portland, OR; Seattle, WA, and Honolulu, HI. If there are special circumstances that preclude the importer or exporter from using one of these designated ports, an Exception to Designated Port permit is required and must be obtained from the Regional Director's office. In addition, there are several border and special ports through which wildlife may be imported or exported (50 CFR 14.16 and 14.19).
2. All plant shipments must be made through ports designated by U.S. Department of Agriculture (50 CFR 24.12) and must comply with other USDA requirements.
3. Special port exemption permits may be issued for scientific purposes (50 CFR 14.31), to minimize deterioration or loss (50 CFR 14.32) or for economic hardship (50 CFR 14.33). These permits are obtained from the Regional Director's Office.

4. Inspection and clearance requirements including declaration (Form 3-177) requirements are found in 50 CFR 14. Section 14.62 (c) provides an extension period for the filing of an amended declaration form to scientific specimens imported for scientific institutions. Public museums and scientific or educational institutions are exempted from the import/export licensing requirements.
5. Prior notice (72 hours recommended) to the FWS inspection office of all wildlife imports, exports and re-exports is strongly recommended, particularly in the case of live wildlife. Be prepared to provide both the common and scientific name, as inspectors may not be familiar with every species of wildlife.

II. Definitions

- A. All definitions under this section must be understood to have a full comprehension of the Laws and Regulations.
- B. Definitions under the Endangered Species Act (16 USC 1532), and CITES (50 CFR 23.3), that are of particular importance to the scientific community:
 1. The term "fish or wildlife" means any member of the animal kingdom, including without limitation any mammal, fish, bird (including any migratory, nonmigratory, or endangered bird for which protection is also afforded by treaty or other international agreement), amphibian, reptile, mollusk, crustacean, arthropod or other invertebrate, and includes any part, product, egg, or offspring thereof, or the dead body or parts thereof.
 2. The term "plant" means any member of the plant kingdom, including seeds, roots, and other parts thereof.
 3. The term "commercial activity" means all activities of industry and trade, including, but not limited to, the buying or selling of commodities and activities conducted for the purpose of facilitating such buying and selling: Provided, however, that it does not include exhibition of commodities by museums or similar cultural or historical organizations.
 4. The term "import" means to land on, bring into, or introduce into, or attempt to land on, bring into, or introduce into, any place subject to the jurisdiction of the United States, whether or not such landing, bringing, or introduction constitutes an importation within the meaning of the customs laws of the United States.
 5. The term "re-export" means to export wildlife or plants that have previously been imported.

6. The term "take" means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct.
- C. Definitions under the Marine Mammal Protection Act (16 USC 1362), that are of particular importance to the scientific community:
1. The term "Marine Mammal" means any mammal which (A) is morphologically adapted to the marine environment (including sea otters and members of the orders Sirenia, Pinnipedia and Cetacea), or (B) primarily inhabits the marine environment (such as the polar bear); and, for the purpose of this Act, includes any part of any such marine mammal, including its raw, dressed, or dyed fur or skin.
 2. The term "moratorium" means a complete cessation of the taking of marine mammals and a complete ban on the importation into the United States of marine mammals and marine mammal products, except as provided in this Act.
 3. The term "take" means to harass, hunt, capture, or kill, or to attempt to harass, hunt, capture, or kill any marine mammal.
- D. Definitions under the Migratory Bird Treaty Act (16 USC 703), 50 CFR 10.12 and 50 CFR 20.11, that are of particular importance to the scientific community:
1. The term "migratory bird" means any bird, whatever its origin and whether or not raised in captivity, which belongs to a species listed in 50 CFR 10.13, or which is a mutation or a hybrid of any such species, including any part, nest, or egg of any such bird, or any product, whether or not manufactured, which consists, or is composed in whole or part, of any such bird or any part, nest, or egg thereof.
 2. The term "migratory game bird" means those migratory birds included in the terms of conventions between the United States and any foreign country for the protection of migratory birds, for which open seasons are prescribed in 50 CFR part 20, and belong to the following families:
 - a. Anatidae (ducks, geese [including brant], and swans);
 - b. Columbidae (doves and pigeons);
 - c. Gruidae (cranes);
 - d. Rallidae (rails, coots, and gallinules); and
 - e. Scolopacidae (woodcock and snipes).
 3. The term "take" means to pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to pursue, hunt, shoot, wound, kill, trap, capture, or collect.

4. The term "transportation" means to ship, convey, carry or transport by any means whatever, and deliver or receive for such shipment, conveyance, carriage, or transportation.
- E. Definitions under the Eagle Protection Act (16 USC 668c), that are of particular importance to the scientific community:
1. The term "take" includes also pursue, shoot, shoot at, poison, wound, kill, capture, trap, collect, molest or disturb.
 2. The term "transport" includes also ship, convey, carry, or transport by any means whatever, and deliver or receive or cause to be delivered or received for such shipment, conveyance, carriage, or transportation.
- F. Definitions under the Lacey Act Amendments of 1981 (16 USC 3371), that are of particular importance to the scientific community:
1. The term "taken" means captured, killed, or collected.
 2. The term "transport" means to move, convey, carry, or ship by any means, or to deliver or receive for the purpose of movement, conveyance, carriage, or shipment.
 3. The term "fish or wildlife" means any wild animal, whether alive or dead, including without limitation any wild mammal, bird, reptile, amphibian, fish, mollusk, crustacean, arthropod, coelenterate, or other invertebrate, whether or not bred, hatched, or born in captivity, and includes any part, product, egg, or offspring thereof.
- G. Definitions under the Wild Exotic Bird Conservation Act (WBCA) of 1992 (50 CFR 15) that are of particular importance to the scientific community:
1. The term "exotic bird" means (a) any live or dead member of the class Aves that is not indigenous to the 50 States or the District of Columbia, including any egg or offspring thereof; and (b) does not include domestic poultry, dead sport-hunted birds, dead museum specimens, dead scientific specimens, or products manufactured from such birds; and birds in the following families: Phasianidae, Numididae, Cracidae, Meleagrididae, Megapodiidae, Anatidae, Struthionidae, Rheidae, Dromaiinae, and Gruidae.
 2. The term "qualifying facility" means an exotic bird breeding facility that is included in a list published by the Secretary of Interior.

III. Prohibited Acts/Penalties

A. Statutory Prohibitions and Penalties under the Endangered Species Act, 16 USC 1538:

1. Importation or exportation of endangered species of fish, wildlife or plants.
2. Taking endangered species of fish or wildlife within the United States or the territorial sea of the United States; including endangered species of plants if removed and reduced to possession from areas under Federal jurisdiction.
3. Possession, sale, delivery, transportation, carriage, or shipment of illegally taken endangered species of fish, wildlife or plants.
4. Delivery, receipt, carriage, transportation, or shipment of endangered species of fish, wildlife or plants, in interstate or foreign commerce in the course of a commercial activity.
5. Sale or offer for sale of endangered species of fish, wildlife or plants, in interstate or foreign commerce.
6. Violation of any regulation pertaining to endangered or threatened species of fish, wildlife or plants.
7. Violation of the terms of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). (1538[c])
8. Engaging in business of importing or exporting fish, wildlife or plants, without permission of the Secretary. (1538[d])
9. Failure to file the proper declaration upon importing or exporting fish, wildlife or plants. Note: The U.S. Fish & Wildlife Service is not currently requiring a 3-177 declaration form for plants. However, the U.S. Department of Agriculture requires all plants to be declared via the U.S. Customs form, and the applicable CITES permits are required.
10. Importation or exportation of fish, wildlife or plants at a non-designated port.
11. Attempting to commit, soliciting another to commit, or causing an offense defined in this section to be committed.
12. There are no criminal felony provisions under the Endangered Species Act. Criminal misdemeanor charges may be assessed up to \$100,000 and one year imprisonment for an individual (or \$200,000 for an organization) who knowingly violates the Act, including any provision of any permit or

certificate. Civil penalties of not more than \$10,000 can be assessed, and forfeiture of all fish, wildlife or plants may be required.

The list of endangered and threatened wildlife and plants can be found in 50 CFR 17.11 and 17.12. The list of wildlife and plants covered by the Convention on International Trade in Endangered Species (CITES) can be found in 50 CFR 23.23.

B. Statutory Prohibitions and Penalties under the Marine Mammal Protection Act, 16 USC 1372:

1. Take of a marine mammal:
 - a. It is unlawful for any person, vessel or other conveyance subject to U.S. jurisdiction take any marine mammal on the high seas.
 - b. It is unlawful for any person, vessel or other conveyance to take any marine mammal in waters or on lands under U.S. jurisdiction.
 - c. It is unlawful for any person to use any port, harbor, or other place under U.S. jurisdiction to take or import marine mammals or marine mammal products.
2. To possess any marine mammal or product which has been taken in violation of the Act.
3. To transport, purchase, sell, export, or offer to purchase, sell, or export any marine mammal or marine mammal product.
4. To import into the United States, any marine mammal or marine mammal product. Note: While this prohibition is not found in 16 USC 1372, it is expressly stated in 16 USC 1371.
5. There are no criminal felony provisions under the Marine Mammal Protection Act. Misdemeanor charges may be assessed up to \$100,000 for an individual (\$200,000 for an organization) and up to one year imprisonment for knowingly violating the Act including any provisions of any permit or certificate. Civil penalties of up to \$25,000 can be assessed and forfeiture of all marine mammals or products may be required.

C. Statutory Prohibitions and Penalties under the Migratory Bird Treaty Act, 16 USC 703 and 705:

1. It is unlawful at any time, by any means or in any manner, to [pursue, hunt, take, capture, kill, attempt to take, capture, or kill, possess, offer for sale, sell, offer to barter, barter, offer to purchase, purchase, deliver for shipment,

ship, export, import, cause to be shipped, exported, or imported, deliver for transportation, transport or cause to be transported, carry or cause to be carried, or receive for shipment, transportation, carriage, or export], any migratory bird, any part, nest, or egg of any such bird, or any product, whether or not manufactured, which consists, or is composed in whole or part, of any such bird or any part, nest, or egg thereof, included in the terms of the conventions between the United States and Great Britain, the United Mexican States, the Government of Japan and the Union of Soviet Socialist Republics.

2. To ship, transport, or carry, by any means whatever, from one State, Territory, or district to or through another State, Territory, or district, or to or through a foreign country, any bird, or any part, nest, or egg thereof, captured, killed, taken, shipped, transported, or carried at any time contrary to the laws of the State, Territory, or district in which it was captured, killed, or taken, or from which it was shipped, transported, or carried.
3. To import any bird, or any part, nest, or egg thereof, captured, killed, taken, shipped, transported, or carried contrary to the laws of any Province of the Dominion of Canada in which the same was captured, killed, or taken, or from which it was shipped, transported, or carried.
4. Criminal felony charges for sale or barter may be assessed up to \$250,000 for an individual (\$500,000 for an organization) and up to two years imprisonment. Criminal misdemeanor charges may be assessed up to \$5,000 for an individual (\$10,000 for an organization) and not more than 6 months imprisonment for knowingly violating the Act including any provisions of any certificates or permits. There are no civil penalties under the Migratory Bird Treaty Act. Forfeiture of equipment and vehicles/transportation may be required.

D. Statutory Prohibitions and Penalties under the Eagle Protection Act, 16 USC 668:

1. It is unlawful for any person within U.S. jurisdiction to take, possess, sell, purchase, barter, offer to sell, purchase or barter, transport, export or import, at any time or in any manner, any bald eagle (*Haliaeetus leucocephalus*), or any golden eagle (*Aquila chrysaetos*), alive or dead, or any parts, nests, or eggs of such birds.
2. Criminal felony charges of not more than two years and \$250,000 for an individual (\$500,000 for an organization) will be assessed for a second conviction. Criminal misdemeanor charges of up to one year and \$100,000 for an individual (\$200,000 for an organization) may be assessed for knowingly violating the Act, including any permit provisions.

E. Statutory Prohibitions and Penalties under the Lacey Act Amendments of 1981, 16 USC 3372:

1. To import, export, transport, sell, receive, acquire, or purchase any fish, wildlife or plant taken or possessed in violation of any law, treaty, or regulation of the United States, or in violation of any Indian tribal law.
2. To import, export, transport, sell, receive, or purchase in interstate or foreign commerce (a) any fish or wildlife taken, possessed, transported, or sold in violation of any law or regulation of any state or in violation of any foreign law, or (b) any plant taken, possessed, transported, or sold in violation of any law or regulation of any state.
3. Within the special maritime and territorial jurisdiction of the United States to possess (a) any fish or wildlife taken, possessed, transported, or sold in violation of any law or regulation of any state or in violation of any foreign law or Indian tribal law, or (b) any plant taken, possessed, transported, or sold in violation of any law or regulation of any state.
4. To make or submit any false record, account, label, or identification.
5. To attempt to commit any of these prohibitions.
6. To import, export, or transport in interstate or foreign commerce any container or package containing any fish or wildlife unless the container or package has previously been plainly marked, labelled, or tagged in accordance with specific regulations.
7. To cause or permit any wild animal or bird to be transported to the U.S. under inhumane or unhealthful conditions.
8. Criminal felony charges for sale or purchase may be assessed up to \$250,000 for an individual (\$500,000 for an organization) and up to five years imprisonment. Criminal misdemeanor charges of up to \$100,000 for an individual (\$200,000 for an organization) and up to one year imprisonment may be assessed for knowingly violating the Act. Criminal convictions may require forfeiture of equipment and vehicles. Civil penalties of up to \$10,000 may be assessed and forfeiture of all fish, wildlife or plants may be required.

F. Statutory Prohibitions and Penalties under the Wild Bird Conservation Act (16 USC 4901):

1. To import any exotic bird in violation of any prohibition, suspension, or quota on importation.

2. To import any exotic bird listed in the Appendices of CITES that is not part of an approved list, if the bird was not bred at a qualifying facility.
3. To violate any provision of any permit issued.
4. Criminal felony charges may be assessed up to \$250,000 for an individual (\$500,000 for an organization) and up to two years imprisonment. Criminal misdemeanor charges may be assessed up to \$5,000 for an individual (\$10,000 for an organization) and not more than six months imprisonment for knowingly violating the Act including any provisions of any certificate or permit. Civil penalties of up to \$25,000 may be assessed.

G. Statutory Prohibitions and Penalties under the African Elephant Conservation Act (16 USC 4201):

1. To import raw ivory from any country other than an ivory producing country.
2. To export raw ivory from the United States.
3. To export raw or worked ivory that was exported from an ivory producing country in violation of that country's laws or of the CITES Ivory Control System.
4. To import worked ivory, other than personal effects, from any country unless that country has certified that such ivory was derived from legal sources.
5. To import raw or worked ivory from a country for which a moratorium is in effect.
6. There are no criminal felony provisions under the African Elephant Conservation Act. Misdemeanor charges may be assessed up to \$100,000 for an individual (\$200,000 for an organization) and up to one year imprisonment for knowingly violating the Act including any provisions or any permit or certificate. Civil penalties of up to \$5,000 can be assessed.

IV. Exceptions/Permits - Those individuals or institutions related to the scientific community, may qualify for exceptions and/or permits to conduct activities contrary to pertinent Statutes and Regulations.

A. Statutory exceptions under the Endangered Species Act, 16 USC 1539:

1. Permits may be issued for scientific purposes or to enhance the propagation or survival of the affected species.

2. Pre-Act endangered species parts consisting of any sperm whale oil, including derivatives, or any finished scrimshaw product (any art form which involves the substantial etching or engraving of designs upon, or the substantial carving of figures, patterns, or designs from, any bone or tooth of any marine mammal of the order Cetacea), may be exempted from the prohibition, (if such exemption is not in violation of the Convention,) involving:
 - a. Importation or exportation [1538 (a)(1)(A)]
 - b. Transportation in interstate or foreign commerce, and sale or offer for sale in interstate or foreign commerce; provided, they were lawfully held within the United States on December 28, 1973, in the course of a commercial activity [1538 (E)or(F)].
3. Certain Antique Articles shall not be in violation of protective regulations for threatened species [1533(d)], general prohibitions for endangered species [1538(a)] or violations of CITES if the article [1538(c)]:
 - a. is 100 years of age; or
 - b. is composed in whole or in part of any endangered species or threatened species listed under Section 1533; or
 - c. has not been repaired or modified with any part of any such species on or after November 10, 1978;
 - d. is entered at a designated customs port (19 CFR 12).Note: Pre-Convention Certificates are required for CITES listed wildlife.

B. Exceptions by Permit for Endangered and Threatened Wildlife and Plants under the Endangered Species Act (50 CFR 17):

Permits may be issued for scientific purposes related to endangered or threatened wildlife or plants.

Each application (Form 3-200) must be submitted to the permit office located in Arlington, Virginia. The following summarized information regarding wildlife is required in addition to the standard application form:

1. The common/scientific name of the species, including number, age and sex, and the activity to be conducted.
2. Information as to whether the species is still in the wild, already removed, or was born in captivity including country and place.
3. Information on any attempts to obtain the wildlife in a manner which would not cause death or removal from the wild.

4. A description and address where the wildlife will be used, displayed or maintained.
5. For live wildlife, a complete description of the facilities, and experience of caretakers.
6. Complete justification as to why a permit should be issued.
7. For propagation enhancement, a statement of willingness to participate in breeding programs and maintain or contribute to studbook data.

Refer to 50 CFR 17.22, 17.32, and 17.62, or contact the Office of Management Authority, (1-800-358-2104) for more detailed permit application information on plants and wildlife.

C. Scientific Exceptions under CITES (50 CFR 23):

1. The prohibitions concerning import, export, and re-exportation shall not apply to herbarium specimens, other preserved, dried or embedded museum specimens, and live plant material when they are imported, exported or re-exported as a non-commercial loan, donation or exchange between scientists or scientific institutions that have been registered by a management authority of their country, and when a label issued or approved by such management authority is clearly affixed to the package or container [50 CFR 23.13(g)]. The Office of Management Authority maintains a current list of registered institutions.

D. Permit requirements under CITES (50 CFR 23):

1. Permits or certificates to import, export or re-export wildlife or plants listed in Appendix I, II, or III are required. Appendix I requires both an import and export (re-export) permit. Appendix II requires an export (re-export) permit. Appendix III requires an export (re-export) permit if it is from the country that listed the wildlife or plant, or a certificate of origin if it is from a country that did not list it. All living or dead animals and plants and all readily recognizable parts and derivatives are subject to the regulations. Note that there are some exceptions for plant parts and derivatives. Also note that tissues, blood, blood products and DNA are regulated by CITES. Synthetic DNA, however, does not require any permit.
2. To import, export, or re-export wildlife or plants listed in Appendix I, II, or III, that are also listed as endangered or threatened under the Endangered Species regulations (50 CFR 17), requirements under both 50 CFR 17 and 23 must be met.
3. To import wildlife in Appendix I, II, or III that are marine mammals listed in

the Marine Mammal Protection Act, the requirements in both 50 CFR 18 and 23 must be met.

4. Shipments of CITES live wildlife must be shipped in accordance with the International Airline Transport Association (IATA) regulations. The requirements can be obtained from the Office of Management Authority or any wildlife inspection office.

Applications (Form 3-200) for CITES permits are generally submitted to the Management Authority in Arlington, Virginia. In addition to the general application requirements, the following summarized information is required:

1. The scientific/common name of species, number, and activity (import/export/re-export).
2. Information as to whether the species is living in the wild, living but not in the wild, or is dead.
3. Description of the species including size, sex, and type of goods for parts and derivatives.
4. Container description and care arrangements during transport for live species.
5. Name/Address of persons involved in U.S. and foreign country.
6. Country and place where species is to be taken from the wild.
7. For Appendix I species- the purpose and details of the activity, expertise of caregivers, facility description, mortality information at facility involving same species (or genus or family).
8. All documentation showing:
 - a. Pre-convention
 - b. Captive-bred or Artificially Propagated
 - c. Scientific loan/donation/exchange of herbarium/museum specimens between scientists or scientific institutions.

Refer to 50 CFR 23.15 or the Office of Management Authority for more detailed application requirements.

E. Statutory exceptions under the Marine Mammal Protection Act, 16 USC 1371 and 1372:

1. Permits may be issued for scientific research or display purposes related to taking or importation.
2. The Marine Mammal Protection Act shall not apply to any marine mammal taken prior to December 21, 1972, or to any marine mammal product consisting of, or composed in whole or in part of, any marine mammal taken

before such date. Requires the submission of an affidavit to the Director prior to, or at the time of importation.

F. Permit/Registration requirements under the Marine Mammal Protection Act (50 CFR 18):

1. The collection of certain dead marine mammal parts may be authorized, provided the following conditions are met:
 - a. The bones, teeth, or ivory of any dead marine mammal may only be collected from a beach or from land within 1/4 mile of the ocean - includes bays and estuaries.
 - b. Parts so collected may be retained if registered within 30 days with an agent of the National Marine Fisheries Service or the U.S. Fish and Wildlife Service.
 - c. Registration information shall be supplied.
 - d. Title to any marine mammal parts collected under this section shall not be transferred, unless consented to in writing by the agent referred to in this section.
2. Permits for scientific research and public display, may be issued by the Director for the taking and importing of marine mammals. In addition to the general permit application requirements (Form 3-200), the following additional summarized information is required:
 - a. The purpose, date, location and manner of taking or importation.
 - b. Description including species/subspecies, population stock, number and anticipated age, size, sex, condition of animals involved.
 - c. Description of transport, care and maintenance including qualifications of personnel involved and veterinary approval.
 - d. Detailed description of scientific research project including copy of proposal with names/addresses of those involved.
 - e. Justification of scientific need and possible alternatives for endangered or threatened species.
 - f. Detailed description of proposed use for public display, including information on enterprise seeking permit.

All applications for marine mammal permits are reviewed by the Commission and Committee of Scientific Advisors on Marine Mammals, and the Office of Management Authority.

G. Exceptions under the Migratory Bird Treaty Act (50 CFR 21):

1. Public museums, public zoological parks, accredited members of (AZA), and public scientific or educational institutions may acquire by gift or purchase, possess, transport, and by gift or sale dispose of lawfully acquired migratory birds or their progeny, parts, nests, or eggs without a permit. Conditions that must be met in order to qualify for this exemption are summarized as follows:

Birds may be acquired only from persons authorized in 50 CFR 21.12 including those mentioned above; by a issued possession or disposal permit; or from Federal/State Game Authorities. Detailed records must be kept and maintained for five years. Any such birds or their progeny may be disposed of only to persons authorized to acquire birds without a permit. Refer to 50 CFR 21.12 for more detailed information regarding these exceptions.

H. Permit requirements under the Migratory Bird Treaty Act (50 CFR 21):

1. Permits to import and export migratory birds, their parts, nests, or eggs, are required. This does not include migratory game birds (Doves, pigeons, waterfowl). 50 CFR 20 Subpart G details import exceptions relating to migratory game birds including importation limits, and requirements for species identification, foreign permits, processing, and marking. In addition this subpart does not allow the import of migratory game birds belonging to another.
2. A banding or marking permit is required before any person may capture migratory birds for banding or marking purposes.
3. A scientific collecting permit is required before any person may take, transport, or possess migratory birds, their parts, nests, or eggs for scientific research or educational purposes. Scientific collecting permits are also subject to additional permit conditions. Refer to 50 CFR 21.23 for detailed application information and additional permit conditions. Migratory bird permit applications, other than banding, shall be submitted to the Regional Director's office in the region where the applicant resides. Banding permits shall be submitted to the Bird Banding Laboratory in Laurel, Maryland.

I. Statutory exceptions under the Eagle Protection Act, 16 USC 668a:

1. Authorization may be given for the taking, possession, and transportation of specimens for scientific or exhibition purposes of public museums, scientific societies and zoological parks.

J. Permit requirements under Eagle Protection Act (50 CFR 22):

1. Permits to take, possess, or transport bald or golden eagles, their parts, nests, or eggs for scientific or exhibition purposes, may be issued. Application should be submitted to the Regional Director's office in the region where the applicant resides.

K. The Lacey Act prohibits the importation or transportation of injurious wildlife. The restrictions apply to:

1. Live mammal specimens of fruit bat (*Pteropus*), mongoose or meerkat (*Atilax*, *Cynictis*, *Helogale*, *Herpestes*, *Ichneumia*, *Mungos*, *Suricata*), European rabbit (*Oryctolagus*), Indian wild dog (*Cuon*), multimammate rat or mouse (*Mastomys*), or raccoon dog (*Nyctereutes*);
2. Live bird specimen or egg of starling (*Sturnus roseus*), dioch (*Quelea quelea*), java sparrow (*Padra oryzivora*), or bul-bul (*Pycnonotus jocosus*);
3. Live or viable eggs of Family Clariidae, mitten crabs (*Eriocheir*), or zebra mussels (*Dreissema*);
4. In addition all live or dead fish or eggs of salmonids (Salmonidae) are prohibited entry unless under direct shipment with disease certificates (refer to 50 CFR 16.12 for more specific disease certificate requirements).

L. Exceptions for Injurious Wildlife (50 CFR 16):

1. Nothing shall restrict the importation and transportation, without a permit, of dead natural-history specimens of wildlife or their eggs for museum or scientific collection purposes: Provided that the provisions of this section shall not apply to dead migratory birds (50 CFR 20 & 21); to dead game mammals from Mexico (50 CFR 14); or to dead bald or golden eagles or their eggs (50 CFR 22).

M. Permit requirements for Injurious Wildlife (50 CFR 16):

1. Permits to import or ship injurious wildlife for zoological, educational, medical, or scientific purposes may be issued by the permit office in Arlington, Virginia. Injurious wildlife permits are also subject to additional permit conditions[16.22(b)]. In addition to the general application (Form 3-200) the following summarized information is required:

The number and common/scientific name of the wildlife; the purpose for the activity; address of the premises; and the qualifications/experience in care/handling.

Refer to 50 CFR 16.22 for additional special permit conditions.

N. Exceptions by permit under the Wild Bird Conservation Act (16 USC 4901) pertaining to the scientific community:

1. Permits may be issued if the importation is not detrimental to the survival of the species and if it is for (a) scientific research, (b) zoological breeding or display programs, or (c) cooperative breeding programs that meet all criteria. Refer to 50 CFR 15 Subpart C, and contact the Office of Management Authority for further details.

Permit applications are submitted to the Office of Management Authority in Arlington, Virginia.

O. Exceptions for the importation and exportation of African elephant ivory under the African Elephant Conservation Act, Endangered Species Act, CITES, and current Service policy that pertain to the scientific community:

1. Worked ivory may be imported for non-commercial purposes if the item was acquired prior to the date the Convention applied to the African elephant (Feb. 4, 1977), and is accompanied by a valid pre-CITES certificate.
2. Worked ivory may be imported as "personal effects" if the worked ivory was legally acquired/possessed in the U.S. for non-commercial purposes and was legally exported.
3. Worked ivory may be imported or exported for non-commercial purposes and for commercial purposes if the item is at least 100 years old. Proof of antiquity must be provided, the item must be composed of elephant ivory and the item cannot have been repaired or modified with elephant ivory after the date of listing (Feb. 4, 1977 for African elephant and July 1, 1975 for Asian elephant).

THE RUSSIAN AND UKRAINIAN LITERATURE ON THE GYPSY MOTH

Yuri N. Baranchikov¹, Galina N. Nikitenko², and Michael E. Montgomery³

¹V.N. Sukachev Institute of Forest, Siberian Branch, Russian Academy of Sciences,
Krasnoyarsk, Russia

²A.A. Shmalgausen Institute of Zoology, Ukrainian Academy of Science,
Kiev, Ukraine

³USDA Forest Service, Northeastern Forest Experiment Station, Northeastern Center
for Forest Health Research, 51 Mill Pond Rd., Hamden, CT 06514

ABSTRACT

The bibliography on the gypsy moth in the former territory of the USSR covers the period from 1837 to 1991. It contains publications on gypsy moth distribution, ecology, biocenotic relations, physiology, and biochemistry, as well as prognosis and pest control, both chemical and biological. Most of the 1,154 references are followed by an abstract, keywords, and the region of the former USSR where the study was conducted. The full scientific name of natural enemies is listed in a separate field. An index of keywords and natural enemies is cross-referenced. The references have been entered into Papyrus, a bibliographic database. Both a hard copy of output and the database will be available to users.

The introduction to the bibliography gives information needed to locate an original in Russian, including the main libraries and catalogues. The bibliographic description of literature in the former USSR was regulated by State Standards, which remain in effect in Russia. These standards have several peculiarities that make it difficult for the Western reader to recognize the different parts of the bibliographic description. The uniform style and the fact that all published material was deposited in central libraries and catalogued means that all the literature, theoretically, is retrievable. The source names of the citation records are in Russian or Ukrainian to facilitate location and retrieval of the publication.

SUITABILITY OF FOREIGN TREE SPECIES FOR *LYMANTRIA MATHURA* MOORE

Yuri Baranchikov¹, Tamara Vshivkova¹, and Michael Montgomery²

¹V.N. Sukachev Institute of Forest, Krasnoyarsk, Russia

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

ABSTRACT

The pink gypsy moth (*Lymantria mathura* Moore) is widely distributed over southeastern Asia from northwestern India to the Russian Far East. Only one publication documents outbreaks of this species in the Primorie region of the Russian Far East where hundreds of hectares of forests with *Quercus mongolica*, *Juglans mandjurica*, *Malus mandjurica* and species of *Ulmus*, *Betula*, and *Populus* were defoliated in different years. The biology of *L. mathura* is reported to be similar to that of gypsy moth (*Lymantria dispar*), but no special investigations were made. Since *L. mathura* has been found on ships from the Far East entering ports on the West Coast of North America, there is concern about its introduction to the North American continent. To assess its danger to North American forests, we examined its host plant range.

The study was done at a field laboratory in the arboretum of the Institute of Forest near the city of Krasnoyarsk located in Central Siberia, far from the natural distribution of *L. mathura*. *L. mathura* from the Russian Far East, the Asian race of *L. dispar* from south-central Siberia, and the North American race from Michigan (1993) and West Virginia (1994) were reared on the same hosts at the same time. This allowed comparisons to *L. dispar*, which is more familiar to forest pest workers on both continents. All tests were done in the laboratory with excised foliage fed to larvae in petri dishes.

When newly hatched larvae of each species were reared for 10 days, mortality was high for both species on *Fraxinus pennsylvanica*, *Lonicera edulis*, *Pinus sylvestris*, and *Parthenocissus quinquefolia*, but *L. mathura* also had high mortality on *Tilia cordata*, *Sorbus aucuparia*, and *Shepherdia argentea*. Hosts that were good for both species were: *Hippophae rhamnoides*, *Malus pruniflora*, *Quercus mongolica*, and *Rosa rugosa*. *Salix fragilis* was a good host for *L. mathura*, but only moderately good for *L. dispar*. *Larix siberica* was good for gypsy moth, but *L. mathura* grew poorly on it. Development during the entire larval period was about the same in terms of weight and head capsule width, until the last instar, which is longer in *L. mathura*. *L. mathura* pupae were 10 to 35 percent heavier than *L. dispar* pupae.

L. mathura grew well on several species of host plants that are foreign to it. Growth on species in the plant families of Betulaceae, Fagaceae, Rosaceae, and Salicaceae was comparable to that of the gypsy moth. On Pinaceae, though, *L. mathura* did not do as well as the gypsy moth.

STUDIES ON THE TRANSMISSION OF AN EXOTIC MICROSPORIDIUM THAT INFECTS THE GYPSY MOTH

Leah S. Bauer¹, Deborah L. Miller¹, David W. Onstad²,
Joseph V. Maddox², and Michael L. McManus³

¹USDA Forest Service, North Central Forest Experiment Station,
Pesticide Research Center, Michigan State University, East Lansing, MI 48824

²Illinois Natural History Survey, 1101 W. Peabody Drive, Urbana, IL 61801

³USDA Forest Service, Northeastern Forest Experiment Station,
Northeastern Center for Forest Health Research, 51 Mill Pond Rd., Hamden, CT 06514

ABSTRACT

Microsporidia are a diverse group of obligate, intracellular pathogens that are commonly found infecting invertebrates, but that are conspicuously absent from North American gypsy moth populations. They cause chronic and often debilitating disease in their hosts, characterized by egg, larval, and pupal mortality, and reductions in larval vigor, growth, adult fecundity and longevity, and egg fertility. Infected larvae release spores into the environment via cadavers, frass, silk, or regurgitate, transmitting the disease to other larvae within the same generation (horizontal transmission). Infected individuals that survive to the adult stage may transmit the pathogen to the next generation in or on eggs (vertical transmission). Thus, the prevalence of microsporidian disease increases throughout the season, between generations, and as host density increases. We are conducting studies on a microsporidium isolate from gypsy moth populations in Portugal in order to estimate the degree of transmission within this species; this microsporidium has a high degree of specificity to gypsy moth and is a good candidate for release as a classical biological control agent.

We determined that fecundity of infected females (mean = 507 eggs) was almost half that of healthy females (mean = 902 eggs). In addition, only 52% of the eggs from infected females hatched, whereas 69% of eggs from healthy females hatched. Infected females transmitted this microsporidium in the egg (transovarially) to F₁ progeny. Unhatched embryonated eggs, as well as successfully eclosing neonates, contained large numbers of mature spores. There was considerable variation in transmission efficiency, ranging from 9 to 100% infection from 23 different egg masses from infected females. This variation was correlated with maternal dose and level of disease. Significantly fewer progeny from infected females (68%) survived to adult stage, when compared to progeny from control females (97%). Infected males transferred microsporidian spores to female reproductive tracts during mating; however, transmission to F₁ progeny was not detected.

Estimates of horizontal transmission were ascertained by placing an equal number (25) of transovarially-infected neonates along with healthy neonates on 1-meter poplar saplings enclosed in a large mesh bag. Larvae were removed over a period of 20 days and placed on artificial diet for at least two weeks; larvae removed at day 15 and day 20 were reared to the adult stage. We detected a steady increase in prevalence of infection over the 20-day period, beginning with 44% at day 5, and increasing to 63, 86, and 92% infection at days 10, 15, and 20, respectively. The early initiation of horizontal transmission from transovarially-infected larvae to uninfected larvae results from the early dissemination of mature spores from infected neonates, particularly in the silk. The survival of individuals to adult stage reared from days 15 and 20 was similar (69 and 67%, respectively). The high survival of infected individuals to the adult stage supports this microsporidium's ability to successfully maintain itself in the population, even at low host density.

DYNAMICS AND IMPACT OF *ENTOMOPHAGA MAIMAIGA* INTRODUCED INTO GYPSY MOTH POPULATIONS IN MICHIGAN

Leah S. Bauer¹, David R. Smitley², Ann E. Hajek³, Frank J. Sapio⁴, and Richard A. Humber⁵

¹USDA Forest Service, North Central Forest Experiment Station,
Pesticide Research Center, Michigan State University, East Lansing, MI 48824

²Department of Entomology, Michigan State University, East Lansing, MI 48824

³Department of Entomology, Cornell University, Ithaca, NY 14853

⁴Michigan Department of Natural Resources,
Forestry Management Division, P.O. 30028, Lansing, MI 48820

⁵USDA Agricultural Research Service, Plant, Soil, & Nutrition Laboratory,
Ithaca, NY 14852

ABSTRACT

In 1989, researchers made the surprising discovery that gypsy moth larvae in the Northeast were succumbing in large numbers to the fungal pathogen *Entomophaga maimaiga*, which is native to Japan. Overt symptomology is similar to that of nuclear polyhedrosis virus (NPV), the only known causal agent of widespread epizootics throughout the range of gypsy moth in North America before 1989. There remains much uncertainty regarding the sudden and relatively widespread appearance of *E. maimaiga*, although early speculation presumed that the 1910-1911 efforts to introduce this pathogen in eastern Massachusetts were actually successful (Hajek *et al.* 1995, Amer. Entomol. 41:31-42).

By the fall of 1990, further survey of gypsy moth cadavers revealed that *E. maimaiga* was well established in North America, and that its distribution was expanding. In Michigan, there was considerable interest in this pathogen as a natural enemy of gypsy moth. Gypsy moth populations in Michigan were not surveyed in 1989-90 for *E. maimaiga* because they are disjunct from the northeastern populations, the epicenter of the fungal epizootic. Therefore, in the fall of 1990, we developed an *E. maimaiga* research project with the following objectives: 1) to survey the gypsy moth populations throughout Michigan for presence of *E. maimaiga* infections in 1991-1992 (10 counties); 2) to compare the establishment of *E. maimaiga* after introduction by either the application of soil contaminated with resting spores or the release of infected larvae (Crawford, Lake, and Grand Traverse Counties); 3) to monitor establishment and rate of spread over time from these release sites in relation to weather, NPV, population density; and lastly, 4) to determine the impact of *E. maimaiga* on gypsy moth infestations.

The pathogen survey did not detect larvae infected with *E. maimaiga* in over 1500 larvae sampled in 1991 from 50 sites, some of which have been infested with gypsy moths since 1984. In 1991, the two methods for introducing *E. maimaiga* showed low-level establishment only in plots that received the infected larvae. However, by 1992 establishment of *E. maimaiga* was confirmed for both inoculation methods, and there were no significant differences between the level of infection achieved by the two release methods, ranging from 9.3 to 11.7%. In addition, by 1992, infection was detected at low levels in the control plots (0.5 to 2.4%) which were located ca. 100 m away from the treatment plots. In 1992, we added a third research site to the study, using the method of relocating soil containing resting spores. Post-treatment sampling of that site in 1992 detected low levels of establishment at the epicenter only (5.5% infection).

Continued monitoring of fungal establishment during the 1993 season revealed that epizootics of *E. maimaiga* occurred at all three research sites, with the incidence of infection ranging from 29 to 99% in the plots. Differences in infection levels between sites were highly correlated with the precipitation totals or the relative humidity $\geq 90\%$ for the two weeks or 10 days before larval sampling, respectively. In 1993, the egg mass densities at the three research sites averaged 3- to 10-fold lower than in control plots. Our data also supports that *E. maimaiga* epizootics are less density dependent than gypsy moth NPV. In 1993, we also monitored the establishment and rate of spread of *E. maimaiga* at 20 release sites set up by the Michigan Department of Natural Resources in 1991 by the relocation of soil containing resting spores. *E. maimaiga* establishment was confirmed in six of the 20 release sites, and the rate of spread was estimated at ca. 1.0 km per year.

In 1994, sampling of live larvae and cadavers at the study sites in the three counties revealed 1) few larvae at the plot epicenters; 2) infection distributed at least 2000 m from original epicenters; and 3) seasonal amplification of fungal infection. In addition, egg mass counts at the center plot were also lower than in surrounding areas. The slow rate of spread of *E. maimaiga* in Michigan, when compared to that observed in the eastern states during the same time period, may be attributed to low rainfall during late May and early June when the fungus releases airborne conidia from cadavers of young caterpillars dying from *E. maimaiga* infection. As the season progresses, *E. maimaiga* switches to propagation of the large, overwintering resting spores that are eventually washed into the soil from cadavers adhering to tree boles. Two independent studies established research release sites in Ingham and Kalamazoo Counties using contaminated soil relocated from our 1991 study site in Lake Co. However, establishment of *E. maimaiga* was not confirmed during the 1994 season, and we speculate that the low numbers of resting spores present in the soil from Lake Co. were inadequate for establishment.

STATUS OF MASS-REARED GYPSY MOTHS: PROTOCOL

Gary L. Bernon

USDA, APHIS, Otis Methods Development Center, Building 1398, Otis ANGB, MA 02542

ABSTRACT

A laboratory strain of the gypsy moth, referred to as the New Jersey Standard Strain (NJSS), has been maintained at the USDA-APHIS Otis Methods Development Center for over 40 generations. Although the strain was originally developed for APHIS programs, it is now used for a variety of projects. APHIS provides this resource, primarily as diapaused egg masses, to any research group that might contribute to an overall integrated pest management program for the gypsy moth. In 1994, over 100,000 egg masses were provided to 50 individuals or groups working on the gypsy moth. An additional 6,000,000 larvae were mass-reared and inoculated with the gypsy moth nucleopolyhedrosis virus. This cooperative project with the Forest Service was the only source of the virus in 1994.

The NJSS is often cited as a resource in the literature but recent developments in rearing methodology remain unpublished. Each week, 6,000 larvae are reared, yielding approximately 2,500 egg masses after larval development (35 days) and oviposition/embryonation (42 days). Each weekly cohort of egg masses is then placed in chill mediated diapause for 170 days. The egg masses are stored and shipped in quart containers with egg masses attached to strips of paper removed from the oviposition containers.

Egg masses used to maintain each subcolony are surface sterilized in 3 percent formaldehyde, "dehaired" and seeded onto the artificial diet using a mechanical eggging machine. For production projects, the larvae are reared to pupation from eggs seeded directly into containers. However, to optimize colony, neonate larvae are counted and placed with a brush onto fresh diet. The lineage of each subcolony is maintained with 200 egg masses selected at random. Details of rearing procedures are available upon request (1994 Gypsy Moth Rearing Protocol).

The laboratory life cycle is 35 weeks. However, preliminary results from diapause experiments indicated that the chill mediated diapause can vary from 150 - 180 days with no significant differences in the next generation; this may allow reducing subcolonies to as few as 12 per year.

A problem of differential larval growth has been absent from the colony since 1989, thus allowing for a consistent and dependable supply of insects for both APHIS projects and cooperator needs. The APHIS gypsy moth mass-rearing program now plays an integral role in the entire spectrum of on-going research to better manage this pest.

IDENTIFICATION OF THE *LYMANTRIA DISPAR* NUCLEAR
POLYHEDROSIS VIRUS 25K GENE

David S. Bischoff and James M. Slavicek

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

ABSTRACT

The baculovirus life cycle has two morphologically distinct forms: a non-occluded budded virus that infects cells within the same larva, and an occluded form termed a polyhedron that ensures transmission from one larva to another. Passage of multinucleocapsid nuclear polyhedrosis viruses (MNPV) in cell culture results in the rapid generation of few polyhedra (FP) mutants with increased budded virus titre and reduced numbers of polyhedra. Polyhedra produced from FP mutants are virtually devoid of nucleocapsids. The phenotype associated with FP mutants of *Autographa californica* MNPV (AcMNPV) and *Galleria mellonella* MNPV (GmMNPV) have been shown to be due to the inactivation of a 25 kDa protein. Often these mutants have deletions or large (0.8 to 2.8 kB) insertions of host DNA into the 25K gene. This loci appears to be a "hot spot" for DNA mutation as these insertions and deletions occur at a higher frequency than mutations in other regions of the viral genome. The function of the 25K protein is unknown. It is not required for viral replication but is required for the production of wild type numbers of polyhedra. One possibility is that the 25K protein is a nucleocapsid envelope protein that interacts with the polyhedrin and initiates nucleation. FP mutants are also generated upon serial transfer of the *Lymantria dispar* MNPV (LdMNPV) in cell culture. Unlike AcMNPV and GmMNPV FP mutants, LdMNPV mutants do not appear to contain large deletions or insertions as determined by DNA restriction analysis. While mapping the genomic location of a group of FP mutations in LdMNPV, we have identified, sequenced, and characterized a gene encoding the LdMNPV 25K homologue.

SEQUENCE CHARACTERIZATION AND TEMPORAL EXPRESSION OF AN EARLY GENE IN THE *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS

David S. Bischoff and James M. Slavicek

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

ABSTRACT

Baculovirus expression studies indicate that viral genes are expressed in a temporal cascade in which early genes encode regulatory proteins that control the transcription of all other viral genes. Identification of these regulatory proteins is an important step in understanding the molecular aspects that govern viral potency. Many of the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus (AcMNPV) early genes have been identified and sequenced. These genes are transcribed by the host RNA polymerase and do not require other viral proteins for their expression, although transcription of these genes may be enhanced by *trans*-activation with other viral factors.

Temporal analysis of transcripts in the *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus (LdMNPV) had identified three putative early genes which were localized between 6.0 and 13.7 map units on the viral genome. One of the genes, designated G22 because it is located within the *EcoRV*-G fragment and is predicted to encode a 22 kDa protein, was chosen for further study. This region has been cloned and sequenced. A 24 kDa protein is seen when G22 is expressed in an *in vitro* translation and transcription system, which is in close agreement to the 22 kDa protein predicted from the DNA sequence. Comparison of the predicted amino acid sequence of G22 with other proteins in GenBank reveals no strong homology to any other known protein. Temporal expression studies indicate that G22 codes for a 0.85 kB transcript that can be detected immediately following the adsorption period of the virus even in the presence of the protein synthesis inhibitor cycloheximide. This transcript continues to be expressed throughout the time-course of infection with an increase in transcription from 4 to 8 hr post-infection. This enhanced transcription was not seen when cycloheximide was present, indicating that G22 can be *trans*-activated by itself or other viral proteins.

CORSICA, GYPSY MOTH, AND PARASITOIDS: CHALLENGES AND OPPORTUNITIES

E. Alan Cameron¹ and Franck Hérard²

¹Department of Entomology, Penn State University, 501 A.S.I. Building,
University Park, PA 16802-3508

²European Biological Control Laboratory, USDA, ARS, B.P. 4168, Agropolis
34092 Montpellier, Cedex 5, France

ABSTRACT

During the 1994 field season, twice-monthly trips to gypsy moth infestations on the island of Corsica were made to investigate the natural enemy complex associated with the current gypsy moth outbreak. Three plots were established in the southeastern portion of the island, one each chosen to represent sparse, intermediate, and high density populations based on pre-season egg mass counts. Primary hosts were cork oak, *Quercus suber*, and holly oak, *Q. ilex*. Corsica has a Mediterranean climate, with dry and hot summers; it has some climatic similarities to areas in the southern United States that may become infested with gypsy moth within the next decade. Parasitoids and/or predators from Corsica thus might be well adapted to and a priority for consideration for introduction into southern states as the gypsy moth continues to expand its North American range.

At each site, four trees (3-4 m in height) were selected. A 20-cm high sticky barrier was placed on the ground outside the canopy projection of each tree in an effort to minimize tree-to-tree movement of larvae across the ground. All larvae were individually picked off each tree (foliage and trunk); lab-reared larvae were placed on the foliage for 5-day exposure periods. At each site, two of the four trees received 200 L₁-L₂ larvae, plus 40 L₄-L₅ larvae ('high' density), and two received 40 L₁-L₂ larvae plus 10 L₄-L₅ larvae ('low' density); up to 10 pupae (as available) were also exposed per tree. Five days later, as many exposed larvae and pupae as possible were recovered, and returned to the laboratory to be reared for parasitoid emergence or until adult emergence (or earlier death). While we hoped to recover only those which we had placed, there was a lot of immigration to the trees from natural populations. Recoveries were supplemented by targeted and mass collections (up to 2,000 larvae per site per trip) which were reared for natural enemies.

Natural enemies of eggs included a suspected dermestid predator (evidence observed; no individuals were collected) and unidentified avian predation. *Calosoma sycophanta* was common as a larval predator. Large numbers of *Blepharipa* sp., and small numbers of *Parasetigena silvestris*, will be forwarded to USDA quarantine facilities later. Other species recovered, some in considerable numbers, included the egg parasitoid *Ooencyrtus kuvanae*, and larval parasitoids

Glyptapanteles porthetria, *G. liparidis*, *Cotesia melanoscelus*, and *Meteorus pulchricornis*. Evidence of the fungus, *Entomophaga maimaiga*, was not seen on the island.

Corsica is an area from which a number of species could be recovered for introduction into the United States. However, the logistical problems and costs associated with operations on the island impose constraints on easy utilization of the species available unless there is evidence that biotypes from Corsica possess special attributes desired for introductions to targeted areas.

A FIELD ASSESSMENT OF THE EFFECTS OF *BACILLUS THURINGIENSIS* ON NON-TARGET LEPIDOPTERA: LIGHT TRAP SAMPLING

Jane L. Carter¹, John W. Peacock², Laura Neale³, and Steve E. Talley³

¹USDA Forest Service, Northeastern Center for Forest Health Research,
51 Mill Pond Rd., Hamden, CT 06514

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

³County of Rockbridge, Lexington, VA 24450

ABSTRACT

Bacillus thuringiensis Berliner var. *kurstaki* (*Btk*) is one of the most commonly used pesticides against lepidopteran forest pests. Previously, we reported on the results of foliage and burlap sampling from a 3-year field study examining the impacts of a single application of *Btk* on non-target Lepidoptera. In addition to foliage and burlap sampling, our field study included light trap sampling and results from that sampling are reported here.

The study was conducted on 10 plots in the Goshen Wildlife Management Area, Rockbridge Co., Virginia. Five replications were obtained by pairing adjacent plots. One member of the pair was randomly selected to be treated with a single aerial application of *Btk* (Foray 48B, 14.4 BIU's/ha) and the other served as the control plot. A single light trap (modified BioQuip 2851A with 8-watt BL tube) was deployed in the approximate center of each plot at a height of 2 meters and set to operate for 1/2 hour every two hours. Trapping was conducted one to two times weekly from about 1 March to the end of September in 1991-93. Statistical analysis was conducted only on the trap samples from June 1992 through September 1993. The samples were divided into three groups: summer 1992, captures from June to September 1992; spring 1993, captures from March to April 1993 (adults that would have been larvae during summer of treatment); and summer 1993, captures from May to September 1993. Data were log transformed and a t-test performed on selected families (Arctiidae, Geometridae, Lasiocampidae, Limacodidae, Lymantriidae, collective group of Microlepidoptera, Noctuidae, Notodontidae, Saturniide, and Sphingidae).

Thirty-seven families representing 353 genera and 621 species were collected and identified. No significant differences were found between control and treatment areas for the total number of adults collected for the majority of the families and years analyzed. There was a significant difference between control and treatment areas in spring 1993 for the noctuids.

GYPSY MOTH MANAGEMENT IN NON-FOREST SETTINGS:

1994 FIELD AND LABORATORY STUDIES

Stephen P. Cook, Ralph E. Webb, and Kevin W. Thorpe

USDA, ARS, Insect Biocontrol Lab, Building 402, BARC-East, Beltsville, MD 20705

ABSTRACT

Two of the microbial agents currently being investigated for use in the management of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), populations in non-forest environments are a bacterium, *Bacillus thuringiensis* (*Bt*), and a baculovirus, the *L. dispar* nuclear polyhedrosis virus (NPV). During 1994, ground application of *Bt* in combination with sticky barrier bands was examined and compared with similar treatments of the chemical insecticide, cyfluthrin. Both insecticide treatments resulted in significant reductions in within-canopy gypsy moth populations and tree defoliation. The sticky barrier bands were also a significant population reduction factor, but had no influence on defoliation levels. No insecticide or barrier band treatment had a significant influence on egg mass replacement rate.

The gypsy moth NPV was examined using aerial applications. Several aspects of viral treatment efficacy testing were examined. Following treatment, no significant differences in overall larval mortality between understory and canopy collected larvae were detected. However, the data suggest that higher rates of parasitism may have occurred in the understory sample. Sample sizes were small and more work is planned for 1995.

The substrate on which the gypsy moth NPV is consumed can significantly impact viral efficacy. During 1994, we compared sweetgum and white oak as host substrate. Initial data analyses indicate no significant differences in larval infection rates between the two species, suggesting that sweetgum and white oak behave similarly as a host substrate for the NPV. Additional samples are currently being processed.

During 1994, our laboratory studies focused on examining potential viral enhancing agents for use in non-forest environments. The stilbene disulfonic acid Blankophor BBH was examined at various concentrations in formulations with the gypsy moth NPV. There appears to be an upper threshold beyond which the addition of more stilbene reduces the enhancement activity. This threshold varies with droplet size and possibly substrate. To better delineate this threshold and other properties of the adjuvants, additional tests are being conducted with this and other potential viral enhancing agents.

A FIELD TEST OF GENETICALLY ENGINEERED GYPSY MOTH NPV

Vincent D'Amico¹, Joseph S. Elkinton¹, H. Alan Wood², John D. Podgwaite³,
Michael L. McManus³, James Slavicek⁴, and John P. Burand¹

¹University of Massachusetts at Amherst, Department of Entomology,
Fernald Hall, Amherst, MA 01003

²Boyce Thompson Institute for Plant Research, Inc., Tower Rd., Ithaca, NY 14853

³USDA Forest Service, Northeastern Forest Experiment Station, Center for
Biological Control, 51 Mill Pond Rd., Hamden, CT 06514

⁴USDA Forest Service, Northeastern Forest Experiment Station,
359 Main Rd., Delaware, OH 43015

ABSTRACT

The gypsy moth (*Lymantria dispar* L.) nuclear polyhedrosis virus (LdNPV) was genetically engineered for non-persistence by removal of the gene coding for polyhedra production. A beta-galactosidase marker gene was inserted into this virus, so that larvae infected with the engineered virus could be easily tested for its presence using a chemical assay. In May 1993, field tests were established in two 4-ha plots in Otis Air Force Base on Cape Cod. Gypsy moth larvae were released in both plots to serve as test populations. The virus was released in the center of the test plot by confining infected gypsy moth eggs on oak foliage in mesh bags. These bags were not removed until larvae hatching from infected eggs were 2nd instar. Most of these larvae became infected with the engineered virus. No virus was released in the control plot. Weekly collections were made in both plots, beginning one week prior to virus release, and continuing for six weeks thereafter. Each week, several hundred larvae were collected at random from the control plot and 25 larvae were collected from each of the 33 sectors in the test plot. These larvae were reared individually and checked for mortality every second day. Parasitism by the wasp *Cotesia melanoscela* was high on both plots. Because we discovered that gypsy moth larvae infected with immature of *C. melanoscela* respond positively (turn blue) to the beta-galactosidase assay, the gypsy moth cadavers were tested for the presence of the engineered virus using both the chemical assay and restriction enzyme analysis. Throughout the seven collection weeks, the engineered virus was recovered in both early and late instars, but only from collection points within 50 m of the release point. No engineered virus was seen in the test plot in weeks 1 or 7. No virus was seen in the control plot at any time. In 1994, 18 million gypsy moth larvae were released in each of the plots to test for persistence of the engineered virus. Weekly collections were made as above. Larval mortality due to *C. melanoscela* was far less than in 1993, possibly due to the larger number of larvae released or the timing of the release. Some samples of virus-killed larvae have tentatively been identified as containing the genetically engineered virus.

GYPSY MOTH DEFOLIATION IN COASTAL PLAIN PINE-HARDWOOD STANDS

Christopher B. Davidson and James E. Johnson

Department of Forestry, College of Forestry and Wildlife Resources,
Virginia Tech, Blacksburg, VA 24061-0324

ABSTRACT

Despite numerous attempts at control, the European gypsy moth (*Lymantria dispar* L.) has continued to expand its range within the United States. Extensive investigations have been conducted in hardwood forests of the northeastern and north-central United States. However, the effect of defoliation on the growth of individual species in southeastern mixed pine-hardwood stands, and their vulnerability to mortality remains to be proven. We designed a study to examine the impact of gypsy moth defoliation on tree growth and mortality in mixed pine-hardwood stands. Two stand types were selected for study: loblolly pine (*Pinus taeda*) mixed with oak (*Quercus* sp.) and loblolly pine mixed with sweetgum (*Liquidambar styraciflua*). In 1992 and 1993, research plots were established in 47 stands in the Atlantic Coastal Plain physiographic province of Virginia and Maryland. The study has three objectives: 1) determine whether the pine component in mixed stands reduces stand susceptibility, or conversely, whether the presence of pines contributes to defoliation; 2) determine whether defoliation of the preferred hardwoods serves to promote pine growth, or only leads to subsequent pine defoliation; and 3) determine whether the proportion of pine (or hardwoods) in the stand is a useful predictor of growth loss and/or mortality of the hardwood species. In 1994 gypsy moth populations were active in 18 stands. These populations were substantially larger than in previous years which resulted in considerable defoliation. Mean total stand defoliation in 1994 ranged from 5.9% to 66.2%, and averaged almost 30% for all stands. In 1993, defoliation of all stands averaged only 15%. Susceptible species in both stand types suffered extensive defoliation in 1994. In pine-oak stands defoliation averaged 71%; in pine-sweetgum stands defoliation averaged 56%. Complete defoliation (100%) of numerous oak and sweetgum within the plots was not uncommon; these trees then refoliated later in the growing season. Refoliation has a negative effect on the physiological condition of individual trees, and when combined with other factors may increase vulnerability to mortality. For the first time, extensive defoliation of resistant species (mainly pines) was observed. The majority occurred in pine-oak stands, and was most likely due to the larger gypsy moth populations within these stands. Defoliation of resistant species in pine-oak stands averaged 19%, and 13% in pine-sweetgum stands. In previous studies, crown position has been related to defoliation intensity and subsequent mortality. In 1994, mean defoliation of susceptible species was relatively consistent across crown classes ($\approx 66.7\%$). Among resistant species, suppressed and intermediate trees were defoliated at a higher rate (38.9% and 25.0%) than dominants and codominants (13.1% and 16.5%). Prior research indicates that tree mortality rapidly increases two years after a severe defoliation episode. If mixed stands in the southeast respond similarly, we should begin to observe mortality in 1995 and 1996.

LITTERFALL DYNAMICS IN GYPSY MOTH DEFOLIATED PINE-HARDWOOD STANDS

Christopher B. Davidson and James E. Johnson

Department of Forestry, College of Forestry and Wildlife Resources,
Virginia Tech, Blacksburg, VA 24061-0324

ABSTRACT

While phytophagous insects are ubiquitous in forest ecosystems, the extensive defoliation that accompanies epidemic outbreaks results in dramatic changes in the normal cycle of litter fall. We designed a study to examine these changes in mixed pine-hardwood stands defoliated by *Lymantria dispar* (L.), the European gypsy moth. The study has two objectives: 1) determine the relationship between stand composition and the quantity of litter fall deposited in defoliated mixed stands, and 2) determine the relationship between stand composition and nutrient content of litter fall in defoliated mixed stands.

Sixteen mixed loblolly pine-hardwood stands were selected for study. Selection was based on stand composition (similarity in species composition and basal area) and defoliation activity. Each stand contains three 400 m² plots; these plots are part of an ongoing study to determine the effect of defoliation on tree growth and mortality. In May of 1994, three 1 m² litter traps were randomly located within each stand, each trap falling within the boundary of a single 400 m² plot. Litter was collected weekly during periods of gypsy moth larval activity. When larval activity ceased, collections were reduced to a monthly basis until the completion of leaf fall in the autumn. A single collection was taken during winter. Subsequent to collection, samples were sorted into six litter fall components, and dry weight of individual components and total sample dry weight were determined. Each litter fall component will also be tested to determine the concentrations of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (M). Prior to nutrient analysis, the samples from each stand will be assigned to one of seven seasonal periods based on date of collection: 1) winter, 2) gypsy moth activity periods (a total of four), 3) remainder of growing season, and 4) autumn. Within each period all samples for a single stand will be bulked by litter fall component.

Stands with active populations experienced heavy to severe defoliation in 1994. This was reflected in the dry weights of litter fall components collected during this period. Due to the quantity of litter collected, sorting and weighing of samples is incomplete. It is anticipated that this portion of the analysis will be completed in early 1995, prior to the resumption of gypsy moth larval activity and concurrent litter fall collection. Nutrient analysis of the 1994 data will occur during the summer of 1995. This study was designed as a complement to an existing investigation and will continue through 1995 with the completion of sample collections in February, 1996.

EFFECT OF REPEATED TREATMENTS OF *BACILLUS THURINGIENSIS*
AGAINST GYPSY MOTH POPULATIONS: INITIAL SURVEY

Normand R. Dubois, Melody A. Keena, Pamela Huntley, and DeAdra Newman

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

ABSTRACT

Gypsy moth populations located in the George Washington National Forest (GWNF) in Virginia (i.e. Sherando Lake, Trout Pond, Hawk, Little Fort, Powell's Fort and Elizabeth Furnace) and in the Allegheny National Forest (ANF) in Pennsylvania (i.e. PA #3708, #2430, #3724, and #5234) that had been treated with *Bacillus thuringiensis* (*Bt*) three to five times in the last three to six years, were analyzed for their susceptibility to *Bt*. Untreated populations adjacent to and associated with these sprayed populations were analyzed also for their susceptibility to *Bt*. Response to the graded doses of Foray 48B (BBN 6325) by the different untreated populations were parallel (no significant difference between their respective regression coefficients (slopes)). Based on the LC_{50} , there were three levels of susceptibility. The populations from untreated areas near Sherando Lake and Little Fort were the least susceptible and were not significantly different from the F_{40} N.J. gypsy moth standard routinely used in the laboratory. Populations from Hawk, Trout Pond, and the PA #3708 were equally the most susceptible. The differences between these two extreme groups appeared to be significant. In between and not significantly different from either group were the populations from Elizabeth Furnace and Powell's Fort. With the treated populations, regression coefficients indicated that dose response relationships were similar (i.e. parallel slopes), and except for the population from Little Fort, the LC_{50} estimates were not significantly different from each other nor from the most susceptible untreated populations (Hawk, Trout Pond, and PA #3708). Neither the response to graded doses (slope) nor susceptibility (LC_{50}) to *Bt* by population in PA #3708 after having been treated three times between 1987 and 1992 indicated that there was any treatment effect when compared to its associated untreated population. Response of the population in PA #3724 did not appear unusual and its susceptibility (LC_{50}) was not significantly different from most of the other populations that were analyzed. Response to graded doses of *Bt* in population PA #2430 was very variable resulting in unacceptably large confidence limits about the LC_{50} . Therefore, *Bt* treatment effects, if any, could not be assessed. Based on this preliminary analysis, it does not appear that gypsy moth populations treated up to five times within a six-year period show any indication of having developed any resistance to *Bacillus thuringiensis*.

IMPACT OF *ENTOMOPHAGA MAIMAIGA* ON GYPSY MOTH POPULATION DYNAMICS

Joseph Elkinton, Rakish Malakar, and Greg Dwyer

Department of Entomology, University of Massachusetts, Amherst, MA 01003

ABSTRACT

There was a massive epizootic caused by *E. maimaiga* among gypsy moth populations in 1989 and 1990 throughout the northeastern United States. In subsequent years, however, we noted a steady decline in the mortality caused by this agent and we wondered if its impact would dwindle to the insignificant levels that existed prior to 1989. In 1994, however, we recorded high levels of mortality in several high and low density populations in western Massachusetts. These mortalities were nearly as great as those we observed in 1989 and were presumably caused by the higher levels of rainfall we had in May and June compared to 1992 and 1993. These findings support the view that *E. maimaiga* has caused a major shift in gypsy moth population dynamics and is not an ephemeral phenomenon.

We have also studied the interaction between *E. maimaiga* and nuclear polyhedrosis virus LdNPV. In previous laboratory studies we have shown that when larvae are inoculated simultaneously with LdNPV and *E. maimaiga*, both agents will develop, but that larvae usually die from *E. maimaiga* because of the shorter incubation time compared to LdNPV. When the larvae are inoculated with LdNPV first and several days later with *E. maimaiga*, they are equally likely to die of either agent. Our conclusion from these studies was that there is no evidence for direct antagonistic or synergistic interaction between the two pathogens within the same host individual. However, it is still likely that the two pathogens may influence one another at the population level by way of their mutual effects on population density. In particular, since LdNPV epizootics reach peak levels just before pupation, we hypothesized that mortality from *E. maimaiga* among early instars may suppress densities of larvae sufficiently to have an adverse impact on mortality from LdNPV. In 1994 we explored these population level interactions by collecting 100 gypsy moth larvae weekly during the larval stage from four plots in a high density population. We fit a host-pathogen model to the data and compared the levels of LdNPV and overall survival with and without *E. maimaiga*. The model fit the observed mortality in our 1994 field collections quite well. However, removal of *E. maimaiga* from the model had an insignificant effect on mortality from LdNPV. The biological explanation for this result is that both pathogens attain peak levels of mortality during the last larval instar. Incubation of LdNPV in gypsy moth is relatively long. Larvae dying at peak LdNPV mortality acquire their inoculum as fourth instars. Prior to the fourth instar, there have been insufficient declines in density caused by *E. maimaiga* to have any measurable effect on LdNPV transmission rates or resulting mortality from LdNPV.

FATE OF F₁-STERILE GYPSY MOTHS RELEASED IN FLORIDA IN 1994

John L. Foltz¹, Wayne N. Dixon², and James R. Meeker³

¹University of Florida, Gainesville, FL 32606-0620

²FDACS, Division of Plant Industry, Gainesville, FL 32614-7100

³FDACS, Division of Forestry, Gainesville, FL 32614-7100

ABSTRACT

Analysis of gypsy moth detection and delimitation data collected since 1981 indicates that the males trapped each year come from newly introduced eggs and pupae. This information suggests that Florida is beyond the southern limit for gypsy moth establishment in the United States. To learn more about factors affecting hatch-to-adult survival, we placed ready-to-hatch F₁-sterile eggs on eight plots in mid February, mid March, and mid April. Initial neonates/plot were ca. 175,000, 222,000, and 202,000, respectively. Weekly observations and pheromone traps provided information on cohort development and survival.

The presence/absence of newly flushed foliage of acceptable hosts appeared to be a key factor affecting neonate establishment. Water, swamp chestnut, and overcup oaks (*Quercus nigra*, *Q. michauxii*, and *Q. lyrata*) were present on the bottomland hardwood plot having the two highest survival rates (0.098, 0.089%). Water oak was also the principal hardwood on the two plots having the next three highest survival rates (0.074, 0.067, and 0.043%). Turkey oak (*Q. laevis*) on one plot was readily colonized by neonates of the March cohort, although overall survival fell to just 0.016%. The remaining species, including laurel, live, and sand live oaks (*Q. laurifolia*, *Q. virginiana*, *Q. geminata*), river birch (*Betula nigra*), and sweetgum (*Liquidambar styraciflua*), never showed signs of supporting neonate establishment.

Laboratory rearing of field collected larvae yielded low numbers of two tachinid parasitoids. Otherwise, we were unable to quantify or assess factors producing the obvious decline in numbers as each cohort matured. Pheromone trap collections for the 24 cohorts ranged from 0 to 73 male moths and survival rates ranged from 0 to 0.098%. Four of the February cohorts and five of the April cohorts produced no adults. The best survival occurred in three of the February cohorts and two of the March cohorts. For 1994 the "best" hatch period for neonates ranged from late February to the end of March. The ensuing flights of males began in mid April and were completed by early June.

Historical data and the results of this and other research indicate there is little likelihood of the North American strain of the gypsy moth becoming established in Central Florida, and probably North Florida as well. Detection and delimitation surveys can be safely reduced, and traps need be operated for only the April to June flight period.

STATUS OF THE INTRODUCED GYPSY MOTH PUPAL

PARASITE *COCCYGOMIMUS DISPARIS* (VIERECK)

ON THE DEL-MAR-VA PENINSULA

R. Fuester¹, R. Peiffer², P. Sandridge^{2,3}, N. Dill^{2,3}, J. M. McLaughlin²,
L. Kershaw², and J. Sigmond²

¹USDA, Agricultural Research Service, North Atlantic Area,
Beneficial Insects Introduction Research, 501 S. Chapel St., Newark, DE 19713

²Department of Agriculture & Natural Resources,
Delaware State University, Dover, DE 19901

³Department of Biological Sciences, Delaware State University, Dover, DE 19901

ABSTRACT

Collections of gypsy moth pupae were made on the Del-Mar-Va Peninsula during 1989-93, for the purpose of recovering the recently introduced pupal parasite *Coccygomimus disparis* (Viereck) [Hymenoptera: Ichneumonidae]. This species was recovered in all regions of the peninsula. Parasitism was usually low, less than 1%, but averaged 4.5% in 1990. Parasitism by this species did not seem to be affected by host density, habitat, or latitude. Discriminant analysis suggested that sample size, minimum temperature the previous December, and June rainfall were the most important factors affecting recoveries of this species.

A field experiment was conducted in which females of *C. disparis* were allowed to sting pupae of *L. dispar* developing in the field. The fate of stung pupae was compared with those not known to be stung. Even though only a few of the stung pupae yielded parasite progeny, overall mortality was much higher in stung hosts, with enhanced incidence of disease, desiccation, and attacks by another introduced parasite *Brachymeria intermedia*.

TRENDS IN PARASITISM AND HOST DENSITY AFFINITIES
IN PENNSYLVANIA POPULATIONS OF *LYMANTRIA DISPAR*
(LEPIDOPTERA: LYMANTRIIDAE)

R. W. Fuester¹, E. E. Simons², L. D. Rhoads², and R. P. Kling²

¹USDA, Agricultural Research Service, Beneficial Insects Introduction Research,
501 South Chapel St., Newark, DE 19713

²Pennsylvania Department of Environmental Resources,
34 Airport Dr., Middletown, PA 17057

ABSTRACT

Parasitism of gypsy moth, *Lymantria dispar* (L.), was monitored in 40 study plots in Pennsylvania during 1984-92. Parasitism of eggs by *Ooencyrtus kuvanae* (Howard) varied between regions, averaging ca. 30%. Parasitism by the larval parasite *Cotesia melanoscela* (Ratzeburg) was lowest in building populations. Parasitism by *Phobocampe uncinata* (Gravenhorst) was low during the outbreak, but rose during the host decline. Parasitism by *Compsilura concinnata* (Meigen) was highest in low declining and stable populations. *Parasetigena silvestris* (R.-D.) was the dominant larval parasite, and exerted highest parasitism in low stable host populations. Parasitism by *Blepharipa pratensis* (Meigen) and *Brachymeria intermedia* (Nees) remained low over most of the host population cycle, rising during the outbreak. *Blepharipa pratensis* and *B. intermedia* showed direct density dependence, whereas *C. melanoscela*, *P. uncinata*, *C. concinnata*, and *P. silvestris* showed inverse density dependence. Only *O. kuvanae*, *C. melanoscela*, and *B. pratensis* exhibited delayed density dependence. Usually, density dependent responses were strongest in declining populations, weakest in building populations, and intermediate in stable populations. The only exception, *B. pratensis*, showed a statistically significant response only in stable populations. This suggests that a breakdown in the density dependent responses of larval parasites could contribute to increases in gypsy moth density.

Parasitism by some species differed between physiographic regions, suggesting influences of climate and habitat suitability. Density dependent responses were strongest in those regions where mean parasitism was highest. Most species exhibited weak responses in the Unglaciaded Allegheny Plateau, the region most recently invaded by the gypsy moth.

The relationship of parasitism to changes in host population was examined through stepwise multiple regression analysis. The most important parasites were *C. melanoscela*, *P. silvestris*, and *B. intermedia*.

STATUS OF NUCLEAR DNA MARKERS

Karen J. Garner, David E. Schreiber, and James M. Slavicek

USDA Forest Service, Northeastern Forest Experiment Station,
359 Main Rd., Delaware, OH 43015

ABSTRACT

Because of the recent introductions of Asian gypsy moths into North America, in some regions it has become necessary to determine the origin of trapped male moths in order to plan control strategies. The identification of the trapped moths by morphology alone can be difficult, so methods of identification based on analysis of the specimen's DNA have been sought. Mitochondrial DNA markers for Asian and North American moths are presently in use but have the disadvantage of identifying only the maternal parent's origin when hybrid moths are analyzed.

Four nuclear DNA markers have now been found using the DNA amplification method known as random amplification of polymorphic DNA by the polymerase chain reaction (RAPD PCR). Results obtained with RAPD PCR can be difficult to reproduce, so further analyses of diagnostic DNA fragments have been done so that locus-specific primer sequences can be determined. Amplification using locus-specific primers should be much more reliable and sensitive when used on a variety of DNA samples, including those from small or poorly-preserved specimens.

The FS1 marker has been previously described (Garner and Slavicek, USDA Interagency Gypsy Moth Research Forum, 1994). This DNA region contains a size polymorphism diagnostic for the sample origin. Locus-specific primers are available, and these primers amplify a region which is 110 basepairs longer in Asian than in North American moths. The FS2 marker is an 800 basepair fragment which is amplified only in North American moths. Locus-specific primers are not yet available for this marker. The FS3 marker is a 600 basepair fragment also limited to North American specimens. Locus-specific primers are now being tested for this marker. The FS4 marker is a 700 basepair fragment seen only in Asian moths, and sequence information is now available and locus-specific primers will be designed and tested in the near future.

The success rate of these markers has been tested using known Asian samples from eastern Russia (Mineralni) and ships intercepted in the Pacific Northwest in 1991. Known North American samples came from Massachusetts, Michigan, North Carolina, Ohio, Pennsylvania, and West Virginia. At least 50 samples of each strain have been tested. FS1 correctly identified 100% of Asian and 96% of North American samples; FS2 correctly identified 96% of Asian and 100% of North American samples; FS3 correctly identified 91% of Asian and 94% of North American samples; and FS4 correctly identified 91% of Asian and 98% of North American samples.

GEOGRAPHIC ROBUSTNESS OF A THREE-PHASE MODEL OF GYPSY MOTH EGG PHENOLOGY

David R. Gray and F. William Ravlin

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

ABSTRACT

A model of a biological process is described as geographically robust if it accurately simulates the process regardless of the geographic location in which it is applied, without the need to alter parameter values. Geographic location can have a profound effect on temperature, the predominant driving variable in insect development. Thus, geographic location can have a profound effect on gypsy moth egg phenology.

A geographically robust model of gypsy moth egg phenology is of interest for various reasons. Complex landscapes on a county or state scale will result in significant differences in the timing of egg hatch. A robust model can accurately predict the timing across such a landscape, and can thereby assist in planning pest management activities. Climatic limits to the potential range of gypsy moth in North America are currently unknown. A geographically robust model can be used in an investigation of potential limits. The expected timing of egg hatch can be completely unknown in situations where egg masses are transported great distances to their final hatch site. Such situations can occur in North America with intra-continental transportation of North American egg masses, or with the inter-continental transportation of various strains of Asian egg masses (Figure 1). Existing models of gypsy moth egg phenology have failed to demonstrate geographic robustness.



Figure 1.

We are developing a unique model of gypsy moth egg development that encompasses three distinguishable phases of egg development: prediapause, diapause, and postdiapause. Phase-specific developmental responses to temperature have been empirically estimated for the prediapause (Gray *et al.* 1991) and postdiapause (Gray *et al.* 1995) phases (Figure 2).

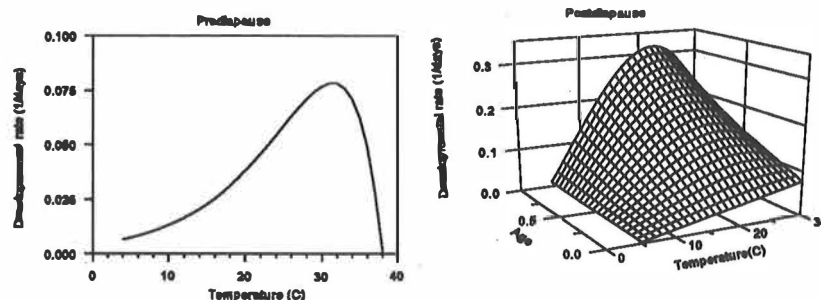


Figure 2.

A “best-guess” estimate of temperature-dependent, age-independent, diapause development is being used in the model until an ongoing experiment examining diapause is completed.

Our three-phase model has demonstrated good geographic robustness in simulations of temperature conditions in eastern North America from Quebec to Florida, and in British Columbia. Robustness is conferred on the model through the use of phase-specific, temperature- and age-dependent development responses. Warm winters in areas south of Georgia limit simulated egg hatch by failing to satisfy diapause requirements for a large proportion of the population. Simulated egg hatch can be limited in northern Canada where cool summers can be expected to retard larval development and therefore delay oviposition. Late oviposition results in limited simulated egg hatch the following year if prediapause cannot be completed before the onset of winter conditions. In locations between Maine and South Carolina, egg hatch is completely successful. Timing of simulated egg hatch in these locations is strongly dependent on the time of diapause completion, and the prevailing temperature conditions at the time of diapause completion. Final validation of the three-phase model will be undertaken upon completion of an ongoing diapause experiment.

Gray, D. R., J. A. Logan, F. W. Ravlin, and J. A. Carlson. 1991. Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature-time requirements of pre-diapause development. *Environ. Entomol.* 20(6):1645-1652.

Gray, D. R., F. W. Ravlin, J. Régnière, and J. A. Logan. 1995. Further advances toward a model of gypsy moth (*Lymantria dispar* (L.)) egg phenology: respiration rates and thermal responsiveness during diapause, and age-dependent developmental rates in postdiapause. *J. Insect Physiol.* 41(3):247-256.

EFFECTS OF SILVICULTURAL MANAGEMENT ON RATES OF PREDATION ON GYPSY MOTH LARVAE AND PUPAE

S. Grushecky¹, R. Greer², A. Liebhold³, R. Muzika³, and R. Smith¹

¹Division of Wildlife, West Virginia University, P. O. Box 6125, Morgantown, WV 26506

²Exxon Biomedical Sciences, Inc., East Millstone, NJ 08822

³USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

ABSTRACT

The gypsy moth, *Lymantria dispar*, has perhaps become the most important forest pest in North America. As an alternative to the application of pesticides, and as a long-term management strategy, silvicultural techniques have the potential of decreasing forest susceptibility or vulnerability to gypsy moth damage. Silvicultural thinnings may also benefit natural enemies of the gypsy moth, and thus indirectly increase predation rates on larvae and pupae. The effects of silvicultural management on predation rates of gypsy moth larvae and pupae were studied on the West Virginia University Forest from 1989-1992. Rates of predation on larvae and pupae were monitored in eight thinned and eight un-cut stands with the use of three types of exclosures placed at three heights in the forest strata. Small mammals were pitfall trapped within these stands so that any relationships between their abundance and predation rates could be ascertained. In the years following thinning, 37 percent of larvae and 25 percent of pupae were destroyed by predators. Silvicultural thinning did not influence the proportion of larvae or pupae killed; however, survival of larvae and pupae on the ground was significantly lower than that found on tree boles or in the foliage. It was found that invertebrates may have been the most important predators of larvae, whereas small mammals may have been the most important predators of pupae during the study period. Since silvicultural manipulations increased small mammal predator populations, but did not affect rates of predation on the gypsy moth, the vulnerability of larvae or pupae likely was not influenced. Vulnerability of gypsy moth larvae or pupae to predation may have declined due to an increase in the structural complexity of ground level vegetation and debris cover, or from an increase in alternate foods.

PERSISTENCE OF *ENTOMOPHAGA MAIMAIGA*
IN THE ENVIRONMENT

Ann E. Hajek

Department of Entomology, Cornell University,
Ithaca, NY 14853-0901

ABSTRACT

Since the gypsy moth fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper was first found in North America in 1989, the potential for use of this fungus as a biological control agent has been discussed. This pathogen was introduced as resting spores (RSP) in small plots in central New York in 1990 and in Maryland, Pennsylvania, Virginia, and West Virginia in 1991 and 1992. These releases resulted in establishment in most plots and epizootics in many. We have investigated the persistence of this fungus in 1991-92 release plots. At seven plots in George Washington National Forest, VA, where *E. maimaiga* was released in 1991-92, fungal infection levels found in 1994 ranged from 40.8-97.5% (mean $\pm$ SE; 72.9 ± 7.7). This constituted an increase in infection from the year of release for five of these seven plots. In plots in central New York, *E. maimaiga* caused epizootics in 1991 and 1992, leading to a collapse in the gypsy moth population. During 1993 and 1994, gypsy moth larvae were extremely difficult to find yet during experiments, the majority of gypsy moth larvae caged over the ground became infected throughout the season. These infections were due to the presence of germinating RSP in the soil. Presumably, these RSP had been formed during the 1991-92 epizootics.

Therefore, the RSP produced by this fungus are responsible for persistence of this disease in gypsy moth populations, as well as being the stage successfully used for introductions. RSP are formed by many species of insect-pathogenic fungi in the Entomophthorales, yet this stage is poorly understood. Thus, we have been studying the RSP stage produced by *E. maimaiga*. For *E. maimaiga*, RSP are predominantly formed within cadavers of later instars that become infected by the relatively short-lived, asexual conidial spores. When RSP germinate and infect larvae, the resulting cadavers produce only conidia. After a late instar dies, RSP require at least 2 d to mature, with the majority of RSP appearing mature by 4 d. RSP are produced in abundance, with approximately $1-2 \times 10^6$ within each late instar. Larvae frequently die from this disease while on tree boles where cadavers hang for some period but then fall to the ground and RSP are leached into the soil. We have found 1202-3895 RSP/g dry soil at the bases of trees that were covered with cadavers the previous spring. After being produced, RSP are constitutively dormant and will not germinate. Germination trials on RSP maintained in the field and brought to the lab on a monthly basis detected germination beginning only in April, with increased germination at 14:10 L:D compared with 13:11 or 12:12.

Preliminary studies demonstrated that counts of RSP overwintering in soil directly at the bases of five central trees did not predict levels of *E. maimaiga* infection in small plots the following spring. Unfortunately, our lack of knowledge regarding the distribution of RSP in the forest soil may have influenced the predictive ability of this technique. Experimentation using a simulation model of the *E. maimaiga*/*L. dispar* system suggests that conidial infections, as well as infections initiated by RSP, make significant contributions to the development of epizootics. The fact that infections initiated by RSP and conidia work in concert to produce epizootics may also explain why simple counts of RSP alone did not predict subsequent infection levels.

EFFECT OF *ENTOMOPHAGA MAIMAIGA* ON NON-TARGET LEPIDOPTERA

Ann E. Hajek¹, Linda Butler², Scott R. A. Walsh³, and Julie C. Silver³

¹Department of Entomology, Cornell University,
Ithaca, NY 14853-0901

²Division of Plant & Soil Sciences, West Virginia University,
Morgantown, WV 26505-6108

³Division of Life Sciences, University of Toronto,
Scarborough, Ontario M1C 1A4 Canada

ABSTRACT

The gypsy moth fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper was first reported in North America in 1989. Since then, this pathogen has repeatedly caused epizootics and has spread extensively. Further development of this fungus for biological control necessitates knowledge of its impact on species other than gypsy moth. Laboratory bioassays have never demonstrated infection in any organisms other than larval Lepidoptera. During 1992 and 1993, laboratory bioassays were conducted to test whether *E. maimaiga* could infect species of Lepidoptera native to West Virginia. Approximately one-third of the 78 species tested became infected although, in general, infection levels were very low. Infection in >50% of individuals tested was found only in three species of lymantriid and one species of sphingid. As a caveat, in the field of insect pathology, laboratory bioassays testing host specificity are well known to be poor indicators of naturally occurring host range.

During 1994, seven sites in the George Washington National Forest, VA, where *E. maimaiga* was released in 1991 and 1992, were chosen to sample non-target Lepidoptera (NTL). Throughout the field season, NTL were collected weekly under burlaps and on foliage, identified, and reared on foliage for 14 d. Simultaneously, gypsy moth larvae were sampled and reared to document the activity of *E. maimaiga*. For any NTL that died, cadavers were diagnosed microscopically and then DNA probes were used to identify infections by *E. maimaiga*. *E. maimaiga* was active in gypsy moth populations at all sites, causing epizootics at several. Of the 1421 NTL belonging to 53 species that were reared, *E. maimaiga* was found in only two individuals: *Malacosoma disstria* Hbn. and *Catocala ilia* Cramer. Comparisons of laboratory bioassay results with field sampling for 24 species demonstrated that all species not infected in the laboratory were not infected in the field. Of the six species infected in the laboratory, only one, *M. disstria*, was infected in the field but at a much lower level (lab 61.0%, field 0.4%). Our results confirm the disparity between host specificity data from the laboratory and field and suggest that evaluation of the potential impact of fungal pathogens in the field should take this discrepancy into account and should not be based solely on laboratory results, if at all possible.

NTL were also sampled in eastern Virginia, Michigan, and central New York at plots where *E. maimaiga* was active in gypsy moth populations. For the 300 larvae reared, no NTL were infected with *E. maimaiga*. In summary, sampling of NTL during 1994 in plots where *E. maimaiga* was simultaneously infecting gypsy moth yielded only two larvae (of two different species) that were infected with *E. maimaiga* out of 1721 larvae collected and reared from 62 different species.

Samples of NTL collected when opportunities occurred between 1989-1994 were tested for *E. maimaiga* or *Entomophaga aulicae* (Reich. in Bail) Humber. *E. aulicae* is a North American native fungal pathogen infecting Lepidoptera that is morphologically identical to *E. maimaiga* as well as sympatric but cannot infect gypsy moth. Results demonstrated *E. maimaiga* infection only in two species of the lymantriid genus *Dasychira* while *E. aulicae* was present in a notodontid, a geometrid, and two species of arctiid.

NATURAL ENEMIES OF THE GYPSY MOTH AT THE LEADING EDGE OF ITS INVASION INTO THE SOUTHERN U.S.

F. L. Hastings¹, F. P. Hain¹, H. R. Smith², and T. M. ODell²

¹Department of Entomology, North Carolina State University, Raleigh, NC 27695

²USDA Forest Service, Northeastern Forest Experiment Station,
51 Mill Pond Rd., Hamden, CT 06514

ABSTRACT

In a continuing effort to evaluate the impact of an exotic herbivore, the gypsy moth, on selected forest ecosystems of the South, small mammal live trap surveys were conducted in six sites, two each in the coastal plain (Currituck Co., NC and Northampton Co., VA), piedmont (Lake Anna State Park in Orange Co., VA) and mountains (George Washington National Forest in Amherst Co., VA) during 1992, 1993 and 1994. The predation of gypsy moth pupae by small mammals was examined between the live-trap surveys by placing freeze-dried pupae at trap stations, at the litter layer and at 0.25, 1.0, and 2.0 m heights on the bole. During 1992 total predation was high at all of the study sites, even where the mouse population was low. The lower predation by mice was at least partially compensated for by increased predation by invertebrates. In fact, our 1992 and 1993 data appeared to show an inverse relationship between vertebrate and invertebrate predation. During both years total predation remained about the same, even where low mouse density occurred. However, in 1994 predation was directly related to mouse density but again, invertebrates continued to contribute significantly to total predation. This constancy of total predation in these southern sites, despite radical reductions in small mammal populations, would be consistent with an hypothesis of greater biodiversity in these sites. This is unlike information from the northeastern U.S. (Bryant Mountain, VT, Cape Cod and Western MA) where there was a direct decrease in predation as small mammal populations decreased.

Trap-host populations using F₁-sterile gypsy moth eggs were created in three of six plots established in Virginia in 1993, one each in Northampton, Orange and Amherst Counties. An additional plot was established in Camden County, NC. Three collections of gypsy moth were made at each site; collections corresponded to instars 1-3, 4-5, and 5-pupa. Larvae were reared individually in plastic containers with artificial diet. Parasites were removed weekly. Dead larvae were autopsied for *Entomophaga maimaiga* and nuclear polyhedrosis virus (NPV). Significant habitat differences in parasitoid species diversity, within and between years, indicate the difficulty of using short-term data collection for making management decisions, and support the need for long-term permanent plot surveys to detect and quantify habitat specific trends in parasitoid diversity. The addition of a survey for *E. maimaiga* and NPV increased our knowledge of the diversity of compensatory natural biological control agents in the three habitat types.

PREDICTING SUSCEPTIBILITY OF FOREST STANDS TO GYPSY MOTH DEFOLIATION

Ray R. Hicks, Jr.¹ and David E. Fosbroke²

¹Division of Forestry, West Virginia University, P.O. Box 6125, Morgantown, WV 26506

²Division of Safety Research, NIOSH, 1095 Willowdale Rd., Morgantown, WV 26505

ABSTRACT

Gypsy moth hazard rating is based on the accurate prediction of forest stand susceptibility to defoliation. Herrick and Gansner (1979) have developed a model that predicts susceptibility of forests based on tree species and crown vigor. We utilized the data from approximately 400 0.1-acre plots in Pennsylvania and Maryland to test the accuracy of the Herrick and Gansner model. We measured the variables needed to input into the prediction model and monitored actual defoliation by gypsy moth over a three-year period. For the plots located in the Appalachian Plateau physiographic province, the model tended to predict a somewhat lower defoliation rate than actually occurred. The reverse was true for the Ridge and Valley province.

The prediction model was reasonably effective at predicting relative defoliation, but did not accurately predict the exact percent defoliation for a given stand. Additional testing of this model is recommended to delineate the useful range of the defoliation susceptibility model.

COMPLETE NUCLEOTIDE SEQUENCING OF A GENOMIC CLONE ENCODING THE LARGE SUBUNIT OF VITELLOGENIN FROM THE GYPSY MOTH

Shiv Hiremath, Kirsten Lehtoma, and Roopa Prasad

USDA Forest Service, Northeastern Forest Experiment Station,
359 Main Rd., Delaware, OH 43015

ABSTRACT

In the gypsy moth, vitellogenin (Vg) is synthesized and secreted by the fat body of female larvae during the last larval stadium. Expression of gene(s) coding for the Vg is developmentally regulated and is sex-specific. Furthermore, the Vg gene expression is suppressed by the juvenile hormone in the gypsy moth. This is in contrast to other insect systems, where the juvenile hormone either induces Vg gene expression or has no effect at all. Also, the oligomeric protein structure of gypsy moth Vg appears to be different from those of Vg's from other insects including lepidopterans. In order to understand more about the gypsy moth Vg and regulation of Vg gene expression by the juvenile hormone, we have attempted to isolate and characterize gene(s) coding for Vg.

A gypsy moth EcoRI subgenomic library was constructed in bacteriophage vector EMBL4 and screened using a cDNA clone encoding the large subunit of Vg (Vg190). One of the clones isolated, E4VgL, had an insert of ~12.5 kbp and appeared to contain the entire gene for Vg190. Initial characterization using Southern analyses and partial nucleotide sequencing identified the region corresponding to the N-terminus of the processed Vg190 protein.

In order to further characterize this clone and the gene, we have determined the nucleotide sequence of the entire clone in both orientations. At least one big intron was present just upstream of the region corresponding to the N-terminus of Vg190. Isolation and cloning (through 5'-RACE reactions), and sequencing of a cDNA clone representing the 5'- region of Vg mRNA indicated that the intron, 1335 nucleotides long, was present within the region encoding the signal peptide. The length (15 amino acids) of the signal peptide was consistent with those reported for Vg's from other insects and invertebrates. Partial sequence of Vg mRNA has indicated that the gene contains other introns. However, identification of the number and locations of these introns will have to wait until studies in progress to determine the complete sequence of Vg mRNA are completed.

THE PARASITOID COMPLEX OF THE GYPSY MOTH IN HIGH AND LOW LEVEL POPULATIONS IN EASTERN AUSTRIA AND SLOVAKIA

Gernot Hoch¹ and Milan Zúbrik²

¹Institut für Forstentomologie, Forstpathologie und Forstschutz der Universität für
Bodenkultur Wien, Hasenauerstraße 38, A-1190 Wien, Austria

²Výskumna stanica Bánska Stiavnica, LVÚ Zvolen Lesnicka 11, SK-96923
Bánska Stiavnica, Slovakia

ABSTRACT

Investigations on differences between the parasitoid complexes of the gypsy moth were carried out in eastern Austria and Slovakia in 1993 and 1994. In both countries two mixed oak stands with *Quercus cerris* and *Q. petraea* as the main tree species were chosen as latency and gradation sites. Gypsy moth egg masses, larvae and pupae were collected stage specific. The larvae were reared on oak foliage at the laboratory and checked daily for parasitoid emergence. At the latency site egg masses were exposed by attaching them to the stems of a group of small isolated oak trees.

The following egg, larval and pupal parasitoids were recovered in 1993 and 1994: *Glyptapanteles liparidis* (Bouché), *G. porhethriae* (Muesebeck) and *Cotesia melanoscelus* (Ratzeburg) (Braconidae), *Phobocampe unicincta* (Gravenhorst) (Ichneumonidae), *Para-setigena silvestris* (Robineau-Desvoidy), *Blepharipa pratensis* (Meigen) and *Zenillia libatrix* (Panzer) (Tachinidae) in Austria and Slovakia; *Cotesia ocneriae* Ivanov (Braconidae), *Brachymeria intermedia* (Nees) (Chalcididae), *Lymantrichneumon disparis* (Poda), *Theronia atalantae* (Poda) and *Pimpla hypochondriaca* (Ratzeburg) (Ichneumonidae) and *Para-sarcophaga uliginosa* (Kramer) (Sarcophagidae) in Austria only; and *Anastatus disparis* Ruschka (Eupelmidae), *Ooencyrtus kuvanae* (Howard) (Encyrtidae), *Monodontomerus* sp. (Chalcididae), *Compsilura concinnata* (Meigen), *Drino incospicua* (Meigen) and *Pales pavidus* (Meigen) (Tachinidae) in Slovakia only.

The laboratory rearings of field collected larvae showed that *P. silvestris* was the dominant parasitoid at the gradation sites. There, the rates of total parasitism were rather low and did not differ significantly between Austria and Slovakia.

At the latency site the exposed hosts were parasitized frequently, mainly by the specialized braconids. Parasitism was higher than at the outbreak sites, with *G. liparidis* as the dominant species in Austria and *C. melanoscelus* in Slovakia. The rate of total parasitism in Austria was significantly higher than in Slovakia.

ASIAN GYPSY MOTH GENETICS:
BIOLOGICAL CONSEQUENCES OF HYBRIDIZATION

Melody A. Keena, Phyllis S. Grinberg, and William E. Wallner

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

ABSTRACT

The following summarizes the results of research to determine the biological consequences of hybridization between gypsy moths of Asian and European origins. The research was conducted in the USDA Forest Service's Quarantine Laboratory in Ansonia, Connecticut.

Hybridization between a Far East Russian strain and a strain from North Carolina (collected in 1992) had the following biological consequences. Lower diapause chill requirements of Russian eggs were retained in their hybrids. The hybrids (F_1 and F_2) had similar chill requirements to that of the Russians and significantly less chill than those from North America. Faster larval growth was retained and accentuated with hybridization. The Far East Russian strain grew slower than the North Carolina strain. The growth of F_1 hybrid larvae was as fast or faster than that of the respective strain of the female parent. F_2 hybrid larvae grew as fast or faster than the original Far East Russian and North Carolina strains. The full range of larval color variation was present in hybrids. The Far East Russian strain had all color forms present (black, gray, yellow-gray, and yellow), but yellow predominated. The larvae of the North Carolina strain were predominantly the gray form and a few yellow-gray larvae were present. All larval color forms were present in the hybrids (F_1 and F_2), but yellow-gray and gray accounted for approximately 80 percent of the population. Some female flight capability is retained with hybridization, but the proportion of the population with strong directed flight is reduced. No females from the North Carolina strain attempted to fly; approximately 90 percent of the females from the Far East Russian strain were capable of strong directed flight. Virtually all of the F_1 hybrids were unable to gain altitude or sustain flight, but about half glided for a few meters while vigorously flapping their wings. In the F_2 generation, approximately 10 to 15 percent of the females were capable of strong directed flight. This suggests that the presence of hybrids in North America could substantially increase the ability of the gypsy moth to spread and could alter the timing of the presence of various life stages.

Gypsy moths collected from German populations in September 1993, where hybridization was suspected, were evaluated and compared to the hybrids produced in the laboratory. These populations had egg diapause chill requirements, larval color and growth rate, and adult female flight characteristics consistent with that of a population where hybridization between Asian and European gypsy moths has occurred for more than one generation.

MIXING EXPERIMENTS BETWEEN CRYIAA AND CRYIAC
INSECTICIDAL CRYSTAL PROTEINS SUGGEST OLIGOMERIZATION OF TOXINS

Mi Kyong Lee¹, A. Curtiss¹, N. R. Dubois², and D. H. Dean¹

¹Department of Biochemistry, Ohio State University, Columbus, OH 43210

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

ABSTRACT

Force-feeding bioassays were performed with CryIAa and CryIAC insecticidal crystal proteins (ICP) to gypsy moth larvae to test synergism among *Bacillus thuringiensis* (*Bt*) toxins. CryIAa ICP is about seven times more toxic than CryIAC ICP to gypsy moth larvae. From mixing experiments with these two ICPs, expected LD₅₀ values were calculated from individual toxins as described by Tabashnik (Appl. Environ. Microbiol. 58:3343, 1992), and observed LD₅₀ values were calculated by Probit analysis. Positive synergism was observed at all ratios tested. Observed LD₅₀ values were shown to be four to eight times lower than expected over the range from 1:1 to 1:8 ratio of CryIAa to CryIAC toxins. Synergism effects start getting lower at 1:12 ratio. These bioassay data support the model for oligomerization of *Bt* toxins.

SLOW THE SPREAD PROJECT UPDATE: DEVELOPING A PROCESS FOR EVALUATION

Donna S. Leonard¹ and Alexei A. Sharov²

¹USDA Forest Service, Forest Health, Asheville, NC 28802-2680

²Department of Entomology, Virginia Polytechnic Institute and State University,
Blacksburg, VA 24061

ABSTRACT

The Slow the Spread (STS) pilot project was initiated by the USDA Forest Service in 1993 in order to determine the feasibility of using IPM strategies to slow the spread of gypsy moth over large geographical areas. Three project areas have been established in the transition zone immediately ahead of the advancing front of gypsy moth populations. One is located in the upper peninsula of Michigan, one in the Central Appalachians in Virginia and West Virginia, and one in northeastern North Carolina.

Project objectives are to:

1. Demonstrate that new and current technology can slow the rate of gypsy moth spread.
2. Assess the technological, economic, ecological, and environmental viability of implementing an operational STS program.
3. Implement a plan for integration of STS technology into a national strategy for suppression of the gypsy moth, assuming the STS project is successful.

The project strategy is to detect and manage isolated gypsy moth populations located immediately ahead of the leading edge of gypsy moth spread (Fig. 1). Management of newly established low-level infestations can prevent their growth, coalescence, and subsequent contribution to gypsy moth spread. The previous Appalachian Integrated Pest Management (AIPM) program (1988-1992) conducted in Virginia and West Virginia was targeted both at high- and low-density populations. Reduction of gypsy moth spread rate was one of the AIPM objectives which was inherited by STS. However, STS is designed to slow gypsy moth spread with reduced pesticide use and management costs as compared to AIPM.

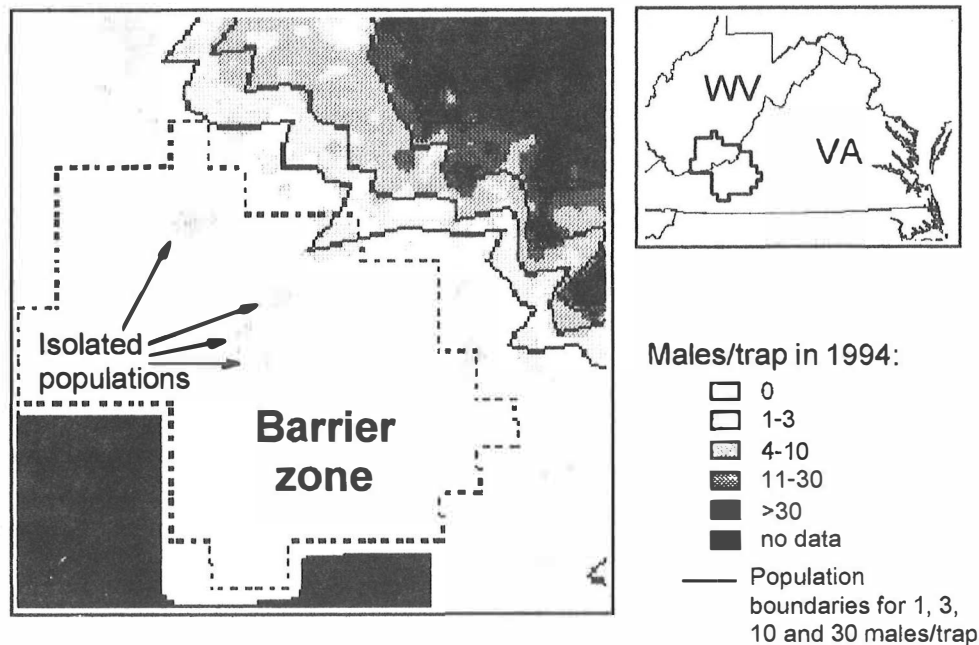


Figure 1. Barrier zone for slowing gypsy moth spread in the Central Appalachians.

Project components include:

1. **Monitoring System.** There are two monitoring zones within each STS area: the action zone where isolated populations are detected and managed, and the evaluation zone where the advance of the population front is estimated. Pheromone-baited traps are deployed at 1 km spacing in the action zone (805 m in Michigan), and at 3 km spacing in the evaluation zone. Monitoring results are used as the basis for most decision making in STS.
2. **Data Management.** The two databases (at Virginia Polytechnic Institute and State University and Michigan State University) are integrated with geographic information systems and are used for quality control in data collection as well as analysis of results.
3. **Control Tactics.** Emphasis is placed on the most environmentally safe tactics that will meet management objectives. Traditional methods for managing high density populations (diflubenzuron, *Bt* and Gypchek) are employed, as well as tactics designed to manage low-density populations such as mass trapping and mating disruption.
4. **Environmental Documentation.** All documents required for full compliance with the National Environmental Policy Act are produced for the project each year.
5. **Public Information and Education.**

The efficiency of the project will be evaluated using two criteria: 1) rate of population spread, and 2) boundary compression. The criteria will be compared among the STS area, historical data and non-managed areas. The rate of spread can be measured as a distance between population boundaries in two consecutive years. We developed several methods for estimation of regular population boundaries (a regular boundary has no “islands”, “lakes”, or folds) which are most convenient for estimation of both criteria. The Best Cell Classification method was shown to be one of the most accurate and robust methods. It minimizes the number of misclassified grid cells (e.g., occupied cells considered as non-occupied). Gypsy moth spread rate in 1988-1994 in the Central Appalachians ranged from 2.3 to 19.7 km/year, with the average of 10 km/year (Fig. 2). It was significantly lower than the average historical rate of spread in 1960-1990 of 20.78 km/year (Liebhold *et al.* 1992). However, no trend in spread rate change was detected within the period of 1988-1994.

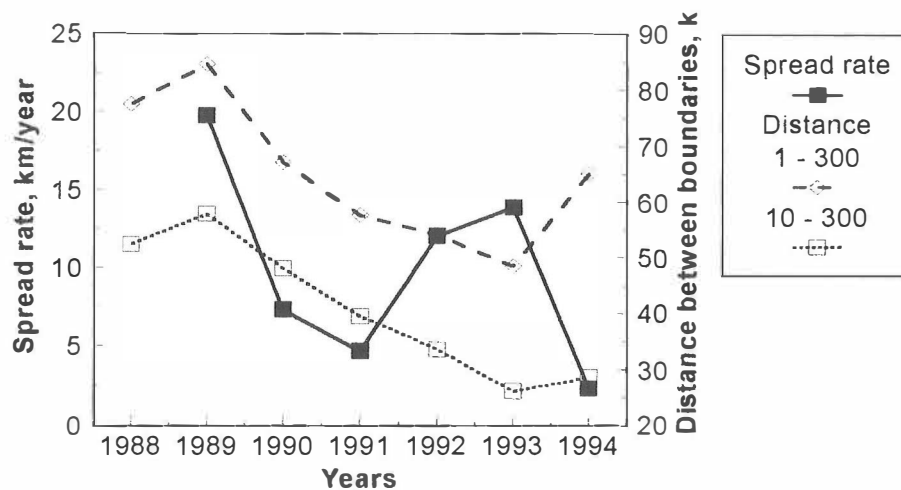


Figure 2. Gypsy moth spread rate and boundary “compression” (distance between population boundaries for 1, 10 and 300 moths/trap) in the Central Appalachians.

A simple model indicates that the distance between population boundaries for different male moth thresholds decreases as gypsy moth spread rate is reduced (boundary compression). If the intrinsic growth rate of established populations is constant, then the distance between boundaries is proportional to the rate of population spread. The distance between boundaries for 1 and 300 moths/trap, and between boundaries for 10 and 300 moths/trap in 1990-1994 in the Central Appalachians was significantly lower (by 31%) than in 1988-1989 (Fig. 2).

Thus, significant changes in the rate of population spread and in boundary compression have already been detected in the Central Appalachians. We expect that additional historical and future data which will be obtained for the STS project will increase the accuracy of the evaluation criteria. In Michigan and North Carolina, the evaluation of STS results may be problematic. In Michigan, migration of male moths has complicated the yearly interpretation of monitoring data; to date no control projects have been initiated. Therefore, there is no reason to expect any change in the spread rate. The North Carolina STS project area is located a greater distance ahead of the leading edge of gypsy moth populations (measured by moth boundaries). Thus, the changes in the rate of spread may be very limited. Also, the historical data in southern Virginia (evaluation zone for the NC STS) that can be used for comparison of spread rates and boundary compression are very limited.

Liebhold, A.M., J.A. Halverson, and G.A. Elmes. 1992. Gypsy moth invasion in North America: a quantitative analysis. *J. of Biogeography* 19: 513-520.

GYPSY MOTH POPULATION SUPPRESSION WITH PESTICIDES: HOW OFTEN IS SUPPRESSION REALIZED?

Andrew M. Liebhold

USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

ABSTRACT

The effectiveness of aerial applications of *Bacillus thuringiensis* (*Bt*) and diflubenzuron (Dimilin[®]) in the AIPM gypsy moth management program was evaluated using data compiled in a geographical information system. These data included counts of overwintering egg mass densities, defoliation maps, and treatment block boundaries collected by the Appalachian Integrated Pest Management Program in VA and WV from 1989-1992. Results indicated that Dimilin treatments resulted in a greater level of foliage protection and population reduction than did applications of *Bt* except when pretreatment egg mass densities were less than 1,000 egg masses per ha. Applications of both materials often did not provide foliage protection in the year following treatment, especially when treatment blocks were small and/or near to defoliating populations. Only application of Dimilin in large blocks appeared to provide population suppression adequate to protect foliage for several years. However, this strategy is undesirable because it probably causes the greatest impacts on non-target insects. Evaluation of increasing expenditures by the USDA Forest Service on both suppression and eradication suggests that eradication may be the most important focus of gypsy moth management in the future.

FOREST TYPE AFFECTS PREDATION ON GYPSY MOTH (LEPIDOPTERA:
LYMANTRIIDAE) PUPAE IN JAPAN

Andrew M. Liebhold¹, Yasutomo Higashiura², and Akira Unno²

¹USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

²Hokkaido Forestry Research Institute, Koshunai, Bibai, Hokkaido 079-01 Japan

ABSTRACT

A compilation of historical defoliation data from Hokkaido, Japan confirmed previous reports that plantations of *Larix leptolepis* are more susceptible to defoliation by the gypsy moth, *Lymantria dispar* (L.), than are natural forests dominated by *Quercus mongolica*. In this study, we compared levels of predation on laboratory-reared pupae deployed at a natural *Quercus* stand, a plantation of *Betula platyphylla*, and a plantation of *L. leptolepis*. Predation was highest in the natural *Quercus* stand, lowest in the *Larix* plantation and intermediate in the *Betula* plantation. Counts of small mammals in snap-traps indicated that populations of *Apodemus argenteus*, *Apodemus speciosus* *ainu*, and *Clethrionomys rufocanus bedfordiae* were most abundant in the oak forest, least abundant in the *Larix* plantation, and intermediate in the *Betula* plantation. We hypothesize that variation in the abundance of small mammals and their predation upon gypsy moth pupae during periods of low gypsy moth densities is the partial cause of the variation among stand types in their susceptibility to defoliation by the gypsy moth. We also argue that predation on gypsy moth pupae by small mammals in natural oak forests is more important to gypsy moth dynamics than previously considered and that considerable similarities may exist between gypsy moth dynamics in Japan and North America.

A COMPUTER PROGRAM TO PREDICT GYPSY MOTH LARVAL MORTALITY IN SPRAYED FORESTS

Steve Maczuga and Karl Mierzejewski

Department of Entomology, Pesticide Research Lab, University Park, PA 16802

ABSTRACT

A computer model "Deathguess" was created to aid in the prediction of gypsy moth larval mortality from aerially applied pesticides. The user inputs the material used, sampling results from deposit assessment of four leaves on 20 trees, larval age distribution, and the degree of larval control required. The output gives a spatially referenced map of likely larval mortality in the spray block, highlighting areas where the desired control level may not be achieved. The program integrates larval feeding, deposition and spread factor studies done at Penn State to construct mortality curves for gypsy moth larvae. Feeding studies initially looked at the effect of various droplet sizes and densities of Foray 48B on oak foliage when exposed to second, third and fourth instar caterpillars. The size and densities of Foray droplets were similar to deposits occurring during field spray projects. Subsequent studies were performed with NPV and Diflubenzuron. These last two materials have not yet been incorporated into Deathguess.

Deposition studies used as demonstration examples in the program were obtained from a NEFAAT study held on the George Washington National Forest (GWNF) in 1992. Foliage was sampled with shotguns from 20 oaks/plot with eight leaves/tree sampled. Data from two 50-acre plots were used in the program but due to space requirements, only data from only four leaves/tree were used.

A spread factor is a formula which defines the relationship between deposit stains and droplets. Spread factors used in Deathguess were determined under field conditions with aircraft or by using a 20-foot spray tower. The user enters deposit data containing only stain diameters. Deathguess calculates droplet size data from the stain size data using the spread factor in order to be able to relate the field deposit data to the results obtained with feeding studies.

Deathguess calculates mortality predictions based on larval numbers present at the time of spray. Deposition parameters that are used in this calculation are droplet diameter and leaf area. Data are stored in ASCII format and are compatible with most image analysis software packages. Output from the program is presented by tree by averaging predicted mortalities by leaf. The trees are drawn on a map based on their coordinates, so that it may quickly become apparent if any part of the spray block received an under-dosing of control material. The spatial reference aspect of the program has not yet been implemented at the time of writing. It is hoped that Deathguess can be used in field projects to quickly predict whether adequate deposition exists to ensure desired control.

FUTURE GYPCHEK PRODUCTION

Max W. McFadden

USDA Forest Service, Northeastern Forest Experiment Station,
100 Matsonford Rd., Radnor, PA 19802

ABSTRACT

Over the years, production of Gypchek by USDA scientists at the Otis Methods Development Center and the Northeastern Forest Experiment Station has been limited to approximately 2,000 acre equivalents/year. More recently, with support from State & Private Forestry, Forest Pest Management and with increased efficiencies in the purification process that amount has been increased to about 20,000 AE. Nevertheless, this amount does not begin to approach the perceived demand for this product. Cooperative efforts with several private companies to produce Gypchek on a commercial scale has met with repeated failure.

Last year, after the decision by American Cyanimid to drop its efforts to produce Gypchek in-vivo, the USDA Interagency Gypsy Moth Research and Development Coordination Committee met to determine the fate of Gypchek. Three alternatives were discussed: 1) drop all USDA sponsored work on Gypchek production; 2) maintain the current level of production through 1995 and then revert to pre-1988 levels of 2,000 AE; or 3) expand current production at a locality yet to be determined to a level that could be considered as operational (100,000 AE).

With concurrence of the USDA Gypsy Moth Working Group, the policy group for the Department of Agriculture, a decision was made to increment Gypchek production to approximately 30,000 AE. The Working Group also directed the Coordinating Committee to conduct a national needs assessment before deciding a future course of action. The needs assessment questionnaire is currently under development but will be distributed shortly. In the meantime, a new firm has expressed interest in producing Gypchek commercially and we are looking forward to working with them.

APHIS ASIAN GYPSY MOTH POLICY

Terry McGovern

USDA, APHIS, PPQ, 4700 River Rd., Unit 134,
Riverdale, MD 20737-1228

ABSTRACT

In response to a request from APHIS, PPQ, a Science Advisory Panel (SAP) was formed to address specific questions related to the Asian gypsy moth (AGM). These questions included what would be the likely consequences of an AGM introduction into the United States, and what would be an appropriate response should an introduction occur. On March 16, 1994 the SAP produced a report, based on current information available, that included the following recommendations:

- 1) In the event of a confirmed AGM introduction anywhere in the United States, treat the population with the most suitable materials to impart the greatest impact. If practical, the goal should be eradication.
- 2) Intensify efforts to exclude introductions of AGM into North America by lowering the risks at the point of origin and at the point of arrival.
- 3) Establish a monitoring program in ports and other high risk sites to rapidly detect introductions.
- 4) Support research efforts to allow informed decision making and provide the technology to support operational programs. Specifically this work should focus on the identification of precise diagnostic molecular markers and the examination of genetic and behavioral consequences of hybridization.

The SAP's report was reviewed at the April '94 PPQ Management Team/National Plant Board Council meeting. Examination of the recommendations resulted in the decision to develop a clearly defined policy to deal with future introductions of AGM. Of special concern was the identification of the appropriate course of action to take in the event AGM is introduced into an area already infested with North American gypsy moth (NAGM). Since the majority of GM control work is done in a cooperative manner, APHIS proposed the formation of a policy committee to develop the needed guidelines. Membership on this committee included the Forest Service, the National Plant Board, the National Association of State Departments of Agriculture, Agriculture Canada, and APHIS. The AGM Policy Committee (AGMPC) met on June 9, 1994, and developed the following recommendations for dealing with AGM both within and outside of the area considered to be generally infested with NAGM.

AGMPC Recommendations:

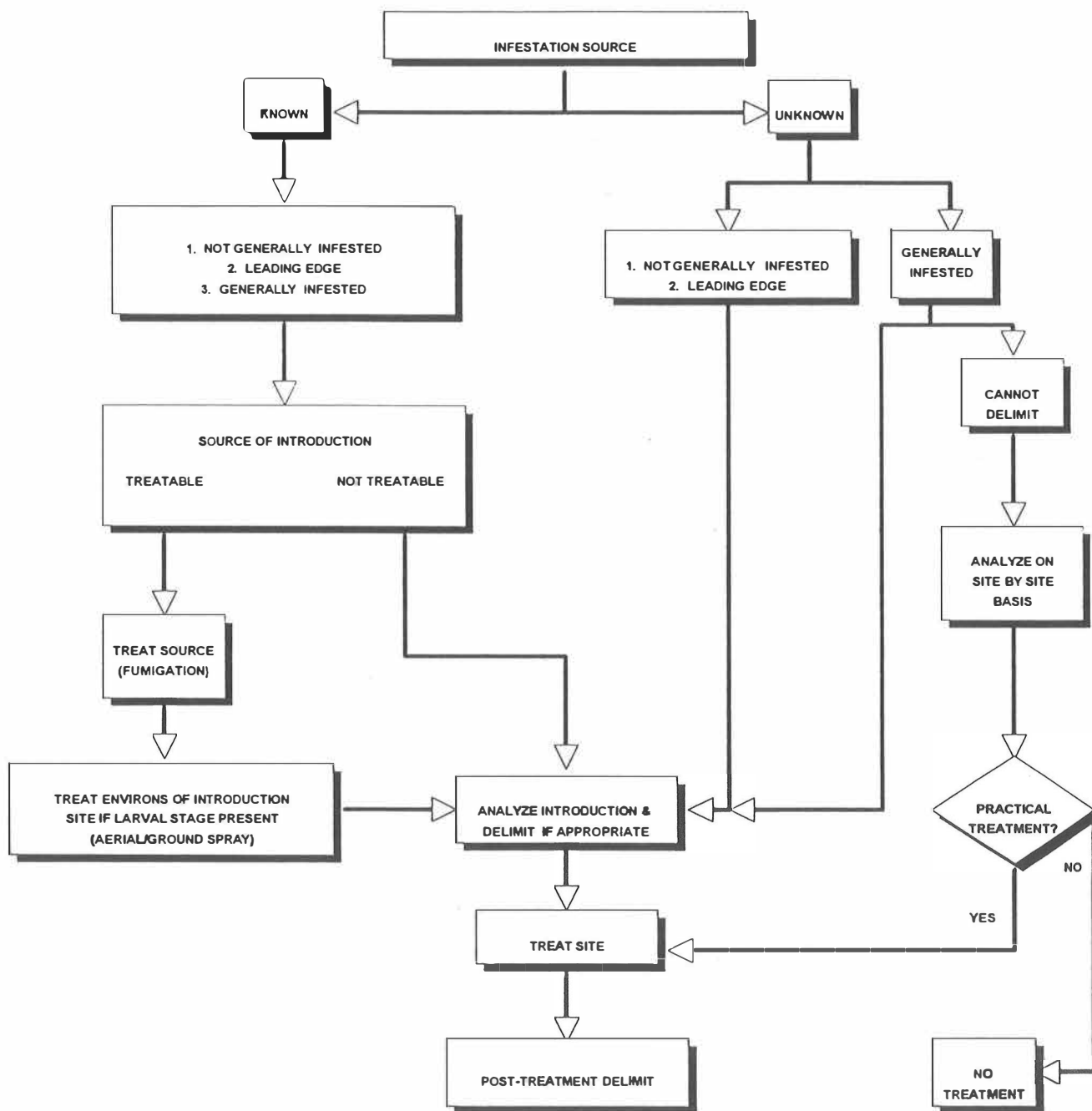
- 1) No major NAGM policy change is recommended at this time. However, the AGMPC concurs with the SAP that AGM possess certain behavioral characteristics that are significantly different from NAGM. Therefore, the AGMPC recommends that AGM should be regarded as a significant economic pest not known to be currently established in the United States.
- 2) While operational reactions to both NAGM and AGM introductions outside of the generally infested area are similar, the programmatic differences implemented to date are justified and should be continued.
- 3) Exclusionary efforts should be intensified and a regular detection survey should be carried out at all sites at high risk for introduction.
- 4) When the source of a new AGM introduction into the NAGM generally infested area is known, such as a specific infested cargo shipment, container, or vessel, aggressive efforts to eradicate the pest should be implemented.
- 5) Any future change in policy should be "triggered" by advances on the scientific front regarding such issues as DNA identification, population genetics, behavioral traits of hybrids and backcrosses, etc. The AGMPC recommends that work of this nature be supported with all the necessary means and resources required.

Recent interceptions of exotic GM in North Carolina, South Carolina, and Maryland indicate that the threat of introductions from places other than the Russian Far East is very real. In addition to ships, the possible pathways of these introductions has been expanded to include cargo and containers. In response to the pest risk posed by AGM, APHIS has implemented a nationwide port survey program to provide for the early detection of future introductions. The APHIS AGM policy has been developed to provide guidance on how to deal with any introductions, regardless of where they occur.

The APHIS AGM Policy Statement* is attached for reference.

*** UPDATE - Since the date of the Research Forum, the APHIS policy statement has been adopted without change by the Forest Service and is now considered to be Departmental policy. The revised USDA AGM Policy Statement is substituted here for the original APHIS version.**

OPERATIONAL RESPONSE TO ASIAN GYPSY MOTH



S. FORBES
6/2/94

USDA ASIAN GYPSY MOTH POLICY

It is well documented that gypsy moths (*Lymantria dispar*) display considerable variation in behavior throughout their range. Most Asian strains of gypsy moth are characterized by females capable of strong directed flight and host ranges broader than that of the gypsy moth strain currently established in North America (narrow genetic range based on isolation of population originally introduced from Europe in 1869; characterized by nonflying female moths). In recognition of these significant behavioral differences, it has been determined that Asian Gypsy Moth (AGM) warrants status as a significant, exotic pest of economic importance. Contrary to USDA's North American gypsy moth (NAGM) policy of not conducting eradication activities within the generally infested area, action will be taken against confirmed AGM infestations in the generally infested area when the source of the introduction is known. Knowledge of the time, location and extent of an Asian introduction will be required to trigger treatment activities. In cases where deductive, circumstantial or investigative information can be developed about an introduction of uncertain origin, appropriate action may also be recommended and taken. The goal of such treatments will be to eliminate all of the gypsy moths that exhibit traits characteristic of AGM.

USDA's current policy of excluding the introduction and preventing the establishment of exotic economic pests will be applied to AGM, regardless of whether an introduction occurs within or outside of the area generally infested with NAGM.

The consequences of an AGM introduction into the United States will be determined by several factors, the most important of which are: 1) source of the introduced AGM, 2) site of introduction, and 3) size of introduction relative to any resident North American population. Operational responses to mitigate these consequences will be based upon the specific circumstances of each occurrence to maximize the effectiveness of treatment strategies.

Recent studies indicate that several Asian strains are sexually compatible with NAGM, resulting in hybrid progeny that possess a mixture of behavioral characteristics and demonstrate observed hybrid vigor. While the exact effects of such hybridization are not well defined, the presence of NAGM in superior numbers is believed to dilute the expression of noxious Asian behaviors (i.e. female flight and a broader host range) in mixed populations. However, the possible retention of these traits at some low level requires mitigative measures where feasible to prevent or reduce the likelihood of introducing Asian genetic material into NAGM populations.

In recognition of the behavioral differences between AGM and NAGM, standard programmatic operations used outside of the generally infested area will be modified. Pretreatment delimiting surveys will not be conducted for AGM due to the potential increase in size and scope an AGM population can achieve in a single year. Control measures will commence as soon as possible after confirmation of an Asian introduction based upon the best information available, followed by extensive post-treatment delimiting surveys.

In order to reduce the likelihood of future introductions, USDA will conduct multifaceted exclusionary activities, supported by effective detection surveys at high risk introduction sites. These sites will include ports of entry, selected military bases, and other locations as needed.

Future policy changes will be determined by scientific advances that provide new information required for informed decision making and improved operational support.

DISTRIBUTION OF MICROSPORIDIA ISOLATED FROM GYPSY MOTH POPULATIONS IN EUROPE

Michael McManus¹, Joseph Maddox², Leellen Solter², and Michael Jeffords²

¹USDA Forest Service, Northeastern Center for Forest Health Research,
51 Mill Pond Rd., Hamden, CT 06514

²Illinois State Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820

ABSTRACT

Microsporidia are important entomopathogens of gypsy moth larval populations in Eastern Europe; however, these organisms have never been recovered from populations in North America. In 1985, foreign exploration for these organisms was conducted in Portugal, the former Czechoslovakia and Yugoslavia with assistance from insect pathologists in these countries. Two isolates were recovered from larvae on cork oak in Portugal, and one isolate obtained from a laboratory colony in Czechoslovakia. Over the next several years, extensive laboratory studies were conducted to characterize and identify these isolates and to evaluate their impact on gypsy moth larvae. It was determined that one isolate from Portugal is a new species, the other is a generalist that infects many lepidopteran species, and the isolate from Czechoslovakia had been mistakenly placed in the wrong genus because ultrastructural data were lacking at the time the species was described.

Because of the reported significance of microsporidia as mortality agents in Eurasian gypsy moth populations, we expanded our explorations in 1993-94 to include Siberia, Romania, Austria, Czech Republic, and the Slovak Republic. With assistance from cooperators, we also obtained single isolates from gypsy moth populations in northern Germany and Bulgaria. Preliminary results from laboratory studies indicate that we now have 8 to 10 distinct species of microsporidia that have been isolated from gypsy moth larval populations that occur over a broad geographical range and that occupy diverse habitats. We did not recover microsporidia from three geographically separated populations in Central Siberia, nor from sites in Bohemia and Moravia in the Czech

Republic; however, gypsy moth populations were at very low densities in the Czech Republic. The prevalence of microsporidia is affected greatly by both the density of host populations and the phase of the outbreak encountered.

Many of these isolates vary in their virulence to the gypsy moth, their infectivity to non-target Lepidoptera, and their ability to infect specific tissues of their host. This latter characteristic is a critical determinant to their horizontal and vertical transmission among larval populations in forested environments.

Based on our knowledge about the occurrence of microsporidia among species of forest Lepidoptera, it is unusual for one species, such as the gypsy moth, to serve as host for more than one or two species of microsporidia. After conducting the ultrastructural and life-cycle studies required to identify the isolates in our collection, we will describe these species and then publish a catalogue of microsporidia known to infect gypsy moth populations throughout its known range. In the meantime, we will continue to work with our network of cooperators to obtain additional isolates from gypsy moth populations in countries that have not yet been sampled, but where this pest is known to occur.

ASSESSING THE IMPACT OF *BACILLUS THURINGIENSIS KURSTAKI*
ON FIELD POPULATIONS OF NON-TARGET LEPIDOPTERA

Jeffrey C. Miller

Department of Entomology, Oregon State University,
Corvallis, OR 97331-2907

ABSTRACT

Large-scale application of *Bacillus thuringiensis* var. *kurstaki* [*Btk*] is an integral component of current forest pest management programs. The use of *Btk* avoids many of the non-target and environmental contamination problems inherent in the use of synthetic pesticides. However, concerns also exist regarding the large-scale application of *Btk* in woodlands and forests. These concerns focus on the effects of *Btk* on populations of non-target Lepidoptera. This is relevant to ecosystem processes because Lepidoptera are important components of certain food webs for other insects, vertebrates, and key to plant growth/competition relationships.

The impact of *Btk* may be assessed by sampling treated and untreated sites for: 1) the immature Lepidoptera (caterpillars) occurring on certain plants, or 2) the adult moths and butterflies in the general area. The technique, frequency and intensity of sampling is in part dependent upon whether caterpillars or adults are being collected. Also, as will be discussed, the interpretation of results is in part dependent upon which life stages are sampled. Ideally, both adults and immatures would be sampled during a non-target assessment project. Comments on monitoring protocol are presented to provide an outline for conducting a project focusing on the sampling of immatures and/or adults for an assessment of non-target impacts on populations of Lepidoptera. This discussion compares sampling with a beating sheet versus bagging foliage along transects. Samples should be assessed to determine species richness, abundance of individuals, and biomass of all Lepidoptera. The analysis of data should involve a comparison of values before and after the *Btk* application for each site and between each site by sample date. Typically, the data do not conform to the criteria of parametric statistical analysis. Thus, nonparametric tests might be most appropriate.

COMPARISON OF PERFORMANCE ON SEVERAL TREE SPECIES OF GYPSY MOTH FROM CENTRAL ASIA, NORTH AMERICA, AND THEIR HYBRIDS

Michael E. Montgomery¹ and Yuri. N Baranchikov²

¹USDA Forest Service, Northeastern Forest Experiment Station, Northeastern Center
for Forest Health Research, 51 Mill Pond Rd., Hamden, CT 06514

²V.N. Sukachev Institute of Forest, Siberian Branch, Russian Academy of Sciences,
Krasnoyarsk, Russia

ABSTRACT

Differences in the utilization of host plants by gypsy moth, *Lymantria dispar*, from Asia (Siberia, Russia) and North America (Massachusetts, West Virginia) have been examined during the past two years. These studies are being conducted at the USDA Forest Service Quarantine Laboratory in Ansonia, Connecticut, and at the arboretum of the V.N. Sukachev Institute of Forest, Krasnoyarsk, Russia. The trees selected for testing include species native to Asia and North America and species susceptible and resistant to the gypsy moth.

A comparison of newly hatched larvae found that the Asian larvae weighed more after a 10-day period for 39 of the 40 species where both biotypes survived. The Asian larvae also developed faster with more larvae in the second instar at the end of the 10 days. If survival of one race on a particular host was high (>85%), the other race also had high survival. Conversely, if one race had poor survival (<10%), the other race also had poor survival. The greatest differences in survival between the races occurred on intermediate hosts such as *Pseudotsugae menziesii*, *Robinia pseudoacacia*, *Acer rubrum*, and *Prunus serotina*. Tests on a more limited number of species were done with the pure strains and their hybrids reared from hatch to pupation. Performance of hybrids was more similar to the Asian than the North American race. At times, the hybrids outperformed the pure Asian race, but no general pattern of inheritance of faster growth or better survival was evident. Measurements made during instar IV larvae found no clear generalizations about instar IV growth being better for any group. The Asian race and hybrids produced heavier pupae and had shorter larval development times than the North American race. It seems that the differences among the races and their hybrids in pupal weight and duration of the larval period are primarily a reflection of patterns observed for the first 10 days of growth.

In summary, Asian (Siberian) populations of gypsy moth can be expected to survive and grow better than the gypsy moth generally established in North America, particularly on hosts such as Douglas-fir that are of intermediate suitability to gypsy moth. Hybrids between the Asian and North American gypsy moth can be expected to perform better than North American gypsy moth.

EFFECTS OF DEFOLIATION AND THINNING ON HERBACEOUS GROUND FLORA

R. M. Muzika, D. L. Feicht, and S. L. C. Fosbroke

USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

ABSTRACT

Herbaceous vegetation was inventoried in 1992 and 1993 in eight Appalachian mixed hardwood stands (< 50% basal area/acre in oak species) and eight oak stands (> 50% basal area/acre in oak species) in north central West Virginia. Vegetation was sampled on 20 6-foot radius plots per stand twice each growing season (once during late spring to sample spring ephemeral species, and later in mid summer). Stand disturbance prior to herbaceous sampling included 1) a silvicultural thinning (eight stands) in the winter/spring of 1990, and/or 2) moderate to heavy gypsy moth defoliation (six stands) in 1990 and 1991. Most overstory mortality occurred within three years of the first defoliation. By the fall of 1993, residual overstory basal area ranged from 30 - 74 ft² / acre for three thinned, defoliated stands; from 38 - 66 ft² / acre for three unthinned, defoliated stands; from 78 - 104 ft² / acre for five thinned, undefoliated stands; and from 117 - 132 ft² / acre for five unthinned, undefoliated stands.

In 1992, species richness ranged from 22 - 65 in mixed hardwood stands and from 19 - 38 in oak stands. In 1993, species richness ranged from 24 - 70 in mixed hardwood stands and from 23 - 42 in oak stands. Regardless of thinning or defoliation disturbance, the dominant herbaceous species (i.e. the species that had the highest average percent cover) consisted of common greenbrier (*Smilax rotundifolia*), Hayscented Fern (*Dennstaedtia punctilobula*), or New York Fern (*Thelypteris noveboracensis*); the one exception was stand #6, a mixed hardwood stand with the highest species richness values recorded in the study. Detrended Correspondence Analysis (DCA) was used to further examine relationships among herbaceous species and stand/site characteristics.

SEEDLING DYNAMICS IN OAK FORESTS MANAGED FOR GYPSY MOTH

R.M. Muzika and M. J. Twery

USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

ABSTRACT

In advance of defoliation by the gypsy moth (*Lymantria dispar* L.), eight stands (10-12 ha each) were thinned in 1989, with the objectives of reducing susceptibility and vulnerability. Eight comparable, nearby stands remained unthinned. Six stands (three unthinned and three thinned) were defoliated severely in 1990 and 1991. The composition and size structure of the woody species understory was assessed from 1989 to 1992. Total number of seedlings declined in 1990 and 1991 in all disturbed stands, but thinning appeared to moderate the defoliation effect. By 1992 the number of seedlings exceeded or equaled the 1989 values, except in the control stands. The previous dominant overstory species in the defoliated stands, primarily oak (*Quercus* spp.), accounted for a minor amount of regeneration. By 1992, red maple (*Acer rubrum* L.) was numerically dominant in defoliated stands, regardless of thinning. In terms of size structure, however, black cherry (*Prunus serotina*) dominated, for this species increased in all treatments from 1989 to 1992, in stems greater than 1.5 m tall.

MATERNAL EFFECTS REVISITED

Judy Myers¹, Raksha Malakar², Joseph Elkinton², and George Boettner²

¹ Department of Zoology, University of British Columbia, Vancouver, B.C. Canada V6T 1Z4

² Department of Entomology, University of Massachusetts, Amherst, MA 01003

ABSTRACT

Previous researchers have suggested that population density experienced by the female gypsy moth, *Lymantria dispar*, influences the development rate, body size, fecundity or sex ratio of her offspring. To test this, we collected egg masses from three high density and three low density populations from western Massachusetts. These egg masses were disinfected and then allowed to hatch. The larvae were reared individually on artificial diet. We recorded time to second instar, time to pupation, pupal weight, survival and sex ratio. Female pupal weight, time from egg to pupa and egg mass weight from the emerged adults did not vary between high and low density populations. However, more males were observed from high density populations. We concluded that on artificial diet, there is no maternal effect on growth, size or survivals. Therefore, we explored another mechanism - whether sublethal viral infection influences fecundity.

We inoculated 5-day old fifth instars with three different doses of LdNPV (nuclear polyhedrosis virus) @ 5×10^3 , 5×10^4 and 5×10^5 occlusion bodies/larva and reared them on artificial diet at 28°C. We observed that female pupal weight of the survivors of virus challenge was significantly lower than that of control pupae. The weight of pupae decreased as the viral dose increased. Egg mass weight from the virus exposed group was lower than from the control groups. Therefore, we concluded that the sublethal effects of the virus are more likely than the maternal effect to reduce fecundity or body weight in the following generations.

THE USE OF *Bt* FORAY 48 FC (*NOVO NORDISK*) FOR CONTROL OF GYPSY
MOTH IN THE FORESTS OF THE SLOVAK REPUBLIC

Július Novotný

Forest Research Institute, Research Station, Lesnicka 11,
969 23 Banská Štiavnica, Slovak Republic

ABSTRACT

Leaf-eating insects cause significant damage in forests of the Slovak Republic and it is often necessary to reduce their abundance below an economic threshold. The gypsy moth (*Lymantria dispar* L.) is a dominant pest with an outbreak period in oak forests every 5-7 years. Regulating populations of gypsy moth using broad spectrum chemical insecticides such as pyrethroids, carbamates and organophosphates is ecologically unacceptable. Therefore, attention has been focused on seeking ecologically acceptable, effective methods of insect pest control. One of the potential avenues is the use of biological agents. During the last decade, forest protection in Slovakia has focused on using entomopathogenic microorganisms for the bioregulation of defoliating caterpillars. Significant scientific resources concentrated on the application of several kinds of *Bt* preparations in field experiments of gypsy moth biocontrol. The best results were achieved with an ultra low volume (ULV) application of FORAY 48 FC.

Three doses of FORAY 48 FC (2 l/ha, 3 l/ha and 4 l/ha) were used in field trials, with two different types of application technologies - ultra low volume (2-4 l per ha) and high volume (HV) (100 l water suspension per ha). MICRONAIR 40000 atomizers were used in the ULV application; water nozzles were used in the HV application. The experiments were conducted in oak forests in southwestern Slovakia naturally infested with an outbreak of gypsy moth. Mortality of caterpillars was checked on the 2nd, 5th, 7th and 14th days post-spray, and was compared with mortality in an untreated check area. The efficacy of the applications was calculated using Abbott's formula.

In the field trials, there was no significant difference in the efficacy between the 3 and 4 liters per hectare application doses of FORAY 48 FC. However, the efficacy of the 2 l/ha dose was considerably lower. At 14 days post-spray, population reduction from the 2 l/ha dose was 82% compared to 95% for the 3 l/ha dose and 99% for the 4 l/ha dose of FORAY 48 FC. Therefore, the dose of 3 liters per hectare (not significantly different from 4 liters per hectare) was confirmed as the optimum dose for undiluted FORAY 48 FC for gypsy moth control in oak forests of Slovakia. At the high dose of 4 l/ha, post-spray mortality on day 2 after application was 75% and increased significantly to 85% by day 5 (i.e., 72 hours later); by day 7, post-spray efficacy was 98% mortality and, 7 days later, by day 14 post-spray, mortality continued to increase slightly to 98.8%. Generally, it appears that the crucial time for the most rapid increase in gypsy moth larval mortality from the *Bt* product, FORAY 48 FC, is between the second and fifth day post-spray.

MORTALITY AGENTS AFFECTING GYPSY MOTH POPULATIONS IN SLOVAKIA

Július Novotný and Milan Zúbrik

Forest Research Institute, Research Station, Lesnicka 11,
969 23 Banská Štiavnica, Slovak Republic

ABSTRACT

Gypsy moth, (*Lymantria dispar* L.), is the most important pest of oak forests in the Slovak Republic. There have been six outbreaks in the Slovak region since the end of the second World War, usually 5-8 years apart. The largest outbreak occurred during the period 1946-49 and 1993-95. In much of Central Europe, entomopathogenic organisms and parasitoids are very important in the bioregulation of gypsy moth populations.

Gypsy moth defoliation severely weakens trees in Slovak forests, which are then attacked by decay organisms. Therefore, it is often necessary to reduce the density of gypsy moth populations to below an economic threshold by using ecologically acceptable and effective methods of insect pest control. Biological control agents such as parasitoids and pathogens are promising candidates for our consideration.

Laboratory and field observations during two recent outbreak periods have confirmed that the natural enemy complex has been very important in reducing the abundance of gypsy moths; however, the intensity of this effect varies with the gradation of the pest. Mortality caused by natural enemies varied from 79.1 - 97.3% during the progression phase, 86.2-99.7% during the culmination, and 83.6-85.1% during the period of regression.

Entomopathogenic microorganisms and undetermined causes afflicted pest abundance most significantly. These factors caused 70.6% mortality during latency, 52.7% during progression, 66.8% during culmination, and 68.3% during the regression phase. Highest mortality was caused by the gypsy moth nucleopolyhedrosis virus. Several species of an unidentified entomopathogenic bacteria caused mortality in early larval stages of gypsy moth; however, spores of fungi were rarely observed. Microsporidia, specifically of the genera *Nosema* and *Vairimorpha*, were very common causes of mortality and these occurred frequently in mixed infections with the nucleopolyhedrosis virus. The effect of parasitoids was substantially lower especially during advanced phases of the gradation. Parasitoids caused 29.4% mortality during latency, 47.3% during progression, 33.2% during culmination, and 31.7% during regression. Parasitoids in the families *Braconidae* and *Tachinidae* were most important, though the braconids (*Cotesia melanoscelus* (Ratz.), *Glyptapanteles liparidis* (Bouché)) were most abundant during the latency and culmination phases. Tachinids, specifically *Parasetigena silvestris* (R-D) and *Blepharipa pratensis* (Meigen) were most abundant during the progression and regression phases of gypsy moth outbreaks.

DEVELOPMENT OF THE ENDOPHAGOUS PARASITOID *GLYPTAPANTELES*
PORTHETRIAE (HYM., BRACONIDAE) IN ITS HOST *LYMANTRIA DISPAR*

Christa Nussbaumer

Institut für Forstentomologie, Forstpathologie und Forstschutz
Hasenauerstr. 38, 1190 Wien, Austria

ABSTRACT

The solitary endoparasitic braconid *Glyptapanteles porthetriae* (Muesebeck) is an important natural controlling agent of the gypsy moth throughout its native range which is mainly Europe but also extends into Northern Africa. At the beginning of this century several attempts to establish this species in the USA have been made but they failed apparently due to the parasitoids' inability to find a suitable alternate host. Three native species were found in the USA in which *G. porthetriae* successfully developed but still there is a lack of knowledge on the biology of this wasp in general.

This study examined different developmental parameters of both the host and its parasitoid depending on time of parasitization. Host larvae during the premolt between first and second instar and second and third instar, respectively, were parasitized and reared on artificial diet. Duration of host's and parasitoid's instars, weight gain of host larvae as well as volume gain of parasitoid larvae and total developmental time of *G. porthetriae* were investigated and compared with unparasitized control larvae of the gypsy moth from the same egg clusters. Furthermore, parasitization efficiency and sex ratio were calculated.

The percentage of successfully developing parasitoids was much higher when first instar larvae were parasitized (62% compared to 36% when eggs were laid into second instar larvae). Older host larvae showed a high amount of pseudoparasitized larvae. They didn't release any parasitoids but contained teratocytes, which derive from the parasitoid's egg shell. Delayed larval growth and mortality even occurred in the absence of the parasitoid. Whether the parasitoid itself or other confounding factors like venom, teratocytes and/or calyx fluid are responsible for the developmental stop of the host is still to be investigated.

Glyptapanteles porthetriae who attack early instars of gypsy moth larvae are able to effectively diminish the damage of their host. Since under natural conditions gypsy moth larvae are parasitized mainly in first or late first instar, they die during their third instar before the massive weight gain starts.

GYPSY MOTH NUTRITIONAL ECOLOGY: THE IMPORTANCE OF IRON
BIOAVAILABILITY DURING VARIOUS PARENTAL LARVAL GROWTH PERIODS
ON THE DEVELOPMENT AND SURVIVAL OF THEIR PROGENY

Thomas M. ODell, David R. Mikus, Melody A. Keena, and Raymond B. Willis

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

ABSTRACT

Knowledge of insect nutritional ecology is central to proper interpretation of the effects of food quality upon physiology and behavior at the population and ecosystem levels. Iron (Fe) is an essential element for all organisms, and plays a central part in life processes as a constituent of oxygen carrier molecules and as a versatile biocatalyst. Laboratory studies have shown that trace amounts of Fe in the diet of gypsy moth parents can significantly affect both parent and progeny development depending on whether the Fe is in a form available (bioavailability) to the insect.

In preliminary tests, larvae (progeny) reared from field-collected eggs on a diet containing reduced Fe bioavailability grew significantly slower than siblings fed diet with available Fe. This suggests that bioavailability of Fe during parent development in the field was inadequate. High plant-fiber levels and dietary phenolics and tannins have been implicated in the reduced bioavailability of Fe. Variations in these plant variables occur due to phenological and ontogenetic changes, and to induced changes that occur following, for example, defoliation. Knowledge of when during the parent-feeding period Fe bioavailability affects gypsy moth maternal provisioning of the egg will increase our understanding of the importance of Fe in this plant/folivore relationship, and provide information that can be used to help predict the viability of the progeny generation. It also will increase our understanding of the effect of insect "physiological quality" on biological control organisms.

The purpose of this research was to evaluate the reduction of Fe bioavailability at various times (coinciding with each instar) during the parent-larval stage on parent and progeny development and survival. Iron was an essential element for development and survival of parent and progeny gypsy moth. When Fe bioavailability was reduced during early instar development of the parent generation, a significant portion of parent-female pupation was delayed. Reduction in the bioavailability of Fe beginning during any period of parent-larval development significantly reduced growth of progeny. The results of this study provide evidence that female deprivation of Fe, beginning at any stage of larval development including the penultimate instar, can significantly influence maternal provisioning of eggs and result in reduction in post-hatch development and survival of progeny.

MOLECULAR MARKERS FOR THE LYMANTRIAE

Tom A. Pfeifer¹, Anna P. Bokova¹, Lee M. Humble², and Tom A. Grigliatti¹

¹Department of Zoology, University of British Columbia, 6270 University Blvd.,
Vancouver, B.C. V6T 1Z4

²Forestry Canada, Pacific Forestry Station, 506 W. Burnside Rd.,
Victoria, B.C. V8Z 1M5

ABSTRACT

The genus *Lymantria* contains several species which are common in Asian Pacific countries and throughout Europe and other non Eurasian countries. The Russian/Asian subspecies of *L. dispar* has a number of undesirable characteristics which could hasten spread of an introduction throughout the Pacific Northwest. The accidental introduction of the Russian subspecies of *L. dispar* on both the East and West Coast of North America acutely precipitated the need to distinguish between these nearly identical subspecies in order to monitor introductions.

Two molecular markers are presented here. The first marker, BC-1, was initially developed as a nuclear marker to differentiate between the North American subspecies of *L. dispar*, the Russian Far East subspecies, and other *Lymantria* species. It consists of a PCR product identified by restriction site polymorphism and has been tested on over 100 samples with accurate results. A second marker, BC-2, was developed for the identification of offspring resulting from mixing of the two *L. dispar* subspecies ('hybrids'). This marker has been developed and modified to identify 'hybrids' resulting from a Russian by North American cross and also identifies other *Lymantria* species. This marker is also based on PCR amplification of a known restriction site polymorphism and has been tested with control crosses. It has correctly identified hybrid organisms when using egg, pupae or moths as the testing material. In addition, the genetic characteristics of BC-2 were determined by testing F1 backcrosses and intercrosses. Approximate Mendelian ratios were seen in both instances.

These tests have been developed into rapid diagnostics which contain internal controls and can be applied to field collected samples. Through monitoring and testing of field trapped samples, the presence of Russian/Asian *Lymantriae* can be confirmed by molecular markers.

PRODUCTION AND FORMULATION OF GYPCHEK

J. D. Podgwaite¹ and R. C. Reardon²

¹USDA Forest Service, Northeastern Center for Forest Health Research,
51 Mill Pond Rd., Hamden, CT 06514

²USDA Forest Service, National Center for Forest Health Management,
180 Canfield St., Morgantown, WV 26505-3101

ABSTRACT

The gypsy moth nucleopolyhedrosis virus (NPV) product Gypchek is produced by infecting laboratory-reared larvae and processing the cadavers to yield a technical powder that contains about 15% (by wt.) viral occlusion bodies (Obs). Though Gypchek is not currently available as a commercial product, 25,000- to 30,000-acre treatments of Gypchek are produced and made available for use each year through the collaborative efforts of the Forest Service (FS) and the Animal and Plant Health Inspection Service (APHIS). Current collaborative FS-APHIS studies are focused on optimizing *in vivo* production through the development of more efficient rearing and processing technology. In 1995, a market survey will be conducted to clearly define the national and international Gypchek markets for 1995 and beyond. Information gleaned from the survey will be used to formulate a Gypchek production strategy that will satisfy user needs for the foreseeable future.

Inconsistencies in Gypchek's performance as an operational pesticide have been addressed through numerous methods improvement projects and the development of a *ready-to-use* spray adjuvant [Carrier 038, Aqueous Flowable Carrier for Gypchek NPV (Novo Nordisk)] for use in operational Gypchek programs. Laboratory and field testing have indicated that, when applied at 1 gallon per acre, Carrier 038 provides UV-protection and foliar deposition superior to that of the standard FS molasses-lignosulfonate tank-mix applied at 2 gallons per acre. Novo Nordisk is continuing to refine the adhesive and flowability characteristics of Carrier 038 to improve performance. The FS will continue to work with any commercial interest toward providing a safe, efficacious, cost-effective Gypchek formulation for use in gypsy moth management programs.

THE ASIAN GENOTYPE IN THE 1994 GYPSY MOTH PORT SURVEY

Douglas C. Prasher

USDA, APHIS, Otis Methods Development Center, Otis ANGB, MA 02542

ABSTRACT

Plant Protection and Quarantine of APHIS expanded its monitoring activities in 1994 to include a nationwide AGM survey at all major U.S. ports to detect introductions of exotic moths. This followed interceptions at the ports of Baltimore and Charleston during the spring of 1994 and the introduction at the Sunny Point, NC military terminal in July 1993. The trapped specimens were sent to the Molecular Diagnostics Group at the Otis MDC for genetic analysis to distinguish the Asian strain from the North American strain. More than 60,000 specimens have been submitted and only a small fraction have been analyzed to date (~3800).

Two genetic markers, FS1 and mitochondrial DNA (mtDNA), out of the six under development, were used to analyze the port specimens. Two alleles (i.e. variations) of the nuclear marker FS1 can be detected, North American and Asian. FS1 was developed by Jim Slavicek's group of the Forest Service. Three variations (i.e. haplotypes) of the mtDNA were used: North American, Siberian, and Russian far eastern. The mtDNA marker was originally developed by Rick Harrison's group at Cornell. Since results of any port specimens had to be compared to the pre-existing situation in the North American population, a large number of specimens were collected far from U.S. ports. These specimens represent our N.A. 'control' population. Similar specimen collections, representing the Asian 'control' populations, were analyzed from Krasnoyarsk (central Siberia) and Khabarovsk and Vladivostok (Russian far east). Asian forms of FS1 and the mtDNA (Siberian only) were found in the N. American population at frequencies of 3.5% (gene frequency) and 2.6%, respectively. Thus, the N.A. and Asian populations were clearly distinct using the two genetic markers (Nei's genetic distance). However, because the error was so great (0.8) in this value, the level of confidence in this differentiation is very low.

Several sites were identified as potentially having the genes specific to the AGM. The most significant site was Puyallup near Tacoma, WA where a single specimen contained Asian forms of four of the six markers (FS and UBC aided in this analysis). The frequency of the Asian mtDNA was also found to be significantly elevated at the following sites: Sunny Point, NC (22.7%), Cleveland, OH (26%), Andover, OH (22%), and Detroit, MI (18%). Puyallup is adjacent to Fort Lewis which receives regular shipments directly from Germany, known to have flying female gypsy moths. The mtDNA was of the Siberian type, ruling out the possibility it was a descendant of the 1991 introduction from the far east. Andover also showed an elevated level of the Asian form of FS1 (8.3%); the nearest port is Ashtabula, 23 miles to the northwest. The explanation for the elevated levels of the markers at this site is not clear. Absent from the list is Baltimore, a site of known interception in the spring of 1994.

BIOCONTROL PERSPECTIVE OF PARASITIDS OF THE LYMANTRIIDS,
LYMANTRIA OBFUSCATA WLK. AND *L. AMPLA* IN INDIA

G. Ramaseshiah

377, Main 9, Cross 13
Vyalikaval, BANGALORE - 560003
INDIA

ABSTRACT

Lymantria obfuscata Wlk. (Lepidoptera: Lymantriidae), a voracious, polyphagous defoliator, and other lymantriids allied to *Lymantria dispar* (L.), localized in parts of Himachal Pradesh and Kashmir appear to have a good reservoir of promising natural enemies for trials to contain gypsy moth (*L. dispar*) in North America. Following requests for selected parasitoids, collections of *L. obfuscata* and its parasitoids were made in the Kullu Valley from April 22 to July 16, 1994. Ten airfreight shipments containing an ichneumonid, *Hyposoter lymantriae* Cushman; three braconids, *Rogas indiscretus* Reardon, *Glyptapanteles indiensis* Marsh, and *G. flavicoxis* Marsh; and several tachinids, *Compsilura concinnata* Meigen and *Pallexorista* spp., were sent to the USA for quarantine handling and eventual release in regions of the South Atlantic and Great Lakes recently infested by the gypsy moth. In addition, *G. indiensis* was obtained because it harbors an unusual virus capable of transforming several insect cell lines.

Observations made earlier indicated that the eupelmid *Anastatus disparis* Ruschka was the dominant egg parasitoid in the Shimla Hills and Kullu Valley, while *A. kashmirensis* was dominant in Kashmir. Numbers of *A. disparis* were positively and significantly correlated with the abundance of eggs of *L. obfuscata*. There was a significant positive correlation between males and females of this parasitoid. Percent parasitism was positively correlated with males of *A. disparis*. Additional parasitoid species newly recorded here include the braconids *Apanteles* sp. A (*ater* group), *Apanteles* sp. B (*ater* group), *Apanteles* sp. A (*ultor* group), *Apanteles* sp. B (*ultor* group), *Cotesia ruficrus* (Haliday), *Dolichogenidea lacteicolor* (Vier.), *D. hyposidrae* Wilkinson, *Glyptapanteles obliquae* Wilkinson; an ichneumonid, *Casinaria* sp. near *elegantula* Gupta & Maheshwary; and a tachinid, *Pallexorista subanajama* (Townsend). The need for specific determinations of several Indian *Apanteles* spp. is stressed, warranting further taxonomic studies.

Further search for lymantriid parasitoids suitable for control of gypsy moth is indicated. It is suggested that explorations be conducted in Nepal, a country hitherto unexplored for natural enemies of *L. obfuscata*.

SOME OF THE OTHER SPECIES OF *LYMANTRIA* (LYMANTRIIDAE)

Paul W. Schaefer

USDA, ARS, Beneficial Insects Research Lab., Newark, DE 19713

ABSTRACT

In recent years we have heard much about the Asian gypsy moth, *Lymantria dispar*, as it accidentally penetrated the North American continent (in the Pacific Northwest and more recently in North Carolina). The Asian gypsy moth (AGM) is that form of *L. dispar* in which the females are fully capable of flight, unlike the established North American (NAGM) or European form. It differs also in its overall size, development characteristics and food plant preferences. In addition, there are other species of *Lymantria* that deserve our concern and awareness as these too possess attributes that simulate the AGM and which could become established as new pests in North America. Several of these potential pests were reviewed and illustrated. These include the following which were compared to AGM, particularly in their traits that would make them potential invaders and unwanted pests:

Lymantria xyliana: Native to Southern Japan, Taiwan, China and India, *L. xyliana* is white in both sexes with a prominent brown band across the forewings (although this is quite variable) and entirely nocturnal. In Taiwan and China they are a pest in windbreak plantings of *Casuarina* and females readily fly to lights where they can oviposit like AGM in a mass covered with hairs on lights or ships. In this way, they could easily be transported into tropical and semi-tropical areas. Their highly polyphagous nature would make them ideal candidates for establishment. In Taiwan in 1994 we successfully trapped large numbers of males at standard gypsy moth milk-carton type traps baited with (+)-disparlure.

Lymantria monacha: The Nun moth of Eurasia is notorious as a serious defoliating pest of conifers and some deciduous trees. The sexes are similar, basically white with peppering of black spots, nocturnal, females flighted, attracted to lights and disparlure. In addition to possible invasion like AGM, in this species females insert their eggs under bark scales and any movement of raw (bark covered) logs could easily transport this pest to new areas.

Lymantria fumida: This exclusively conifer feeder from Japan and China is a brownish, non-distinctive species, in every other way identical to *L. monacha* and also could be unintentionally transported on raw logs.

Lymantria mathura: Found in Japan, eastern Siberia, China, India and Taiwan, this species is more sexually dimorphic like gypsy moth but with the female body pink (male yellow). It feeds mostly on deciduous trees but some conifers also. Nocturnally active, females fly to lights and oviposition is of the insertion under bark scales type and these two attributes make this species likely to be accidentally moved. In fact, viable eggs of this moth have been removed from ship superstructures in the Pacific Northwest and laboratory reared to confirm viability. Unlike all the above mentioned species, *L. mathura* is not readily trapped at (+)-disparlure baited pheromone

traps, although some specimens were captured in China. It appears that in this species, the pheromone is somewhat removed from (+)-disparlure. The potential for invasion by this species appears very real, either via movement of ships or raw logs.

By examining these congeners of the notorious gypsy moth, more particularly the AGM, we note that such traits as female flight, especially to artificial outdoor lighting sources (which might easily be ships at port facilities or shipping container transfer yards), and oviposition in masses, either under abdominal hairs or under tree bark scales, lend themselves well to potential invasion to other regions of the world. These species should, therefore, be watched for by those responsible for detection at port facilities everywhere. Should invasions be suspected anywhere, in most cases, the use of standard (+)-disparlure baited pheromone traps should be the first course of action to confirm an invading moth's presence.

LARVAL GYPSY MOTH DORSAL ABDOMINAL GLANDS: HISTOLOGY, ULTRASTRUCTURE AND PRELIMINARY CHEMICAL IDENTIFICATION OF EXUDATE¹

Paul W. Schaefer², Kathleen S. Shields³, and Jeffrey R. Aldrich⁴

²USDA, ARS, Beneficial Insects Introduction Research, 501 S. Chapel St., Newark, DE 19713

³USDA Forest Service, Northeastern Forest Experiment Station,
51 Mill Pond Rd., Hamden, CT 06514

⁴USDA, ARS, Insect Chemical Ecology Lab., Bldg. 005, BARC-West, Beltsville, MD 20705

ABSTRACT

Unique to larvae of the Lymantriidae (Lepidoptera) are dorsal abdominal glands (DAG) located on the midline of the 6th and 7th abdominal segments (some Eurasian species have but one gland). The DAG are often brightly colored, mainly reds, orange and yellow, while their function is not yet clear. Using gypsy moth larvae, *Lymantria dispar*, we examined these glands with LM, TEM and

¹Presented at the 1994 U.S. Department of Agriculture Interagency Gypsy Moth Research Forum.

SEM and we collected and chemically analyzed the exudate with gas chromatography (GC) and GC-mass spectrometry.

The DAG are composed of an often colored external papilla that is eversible, bears many minute pegs and bears several slits or pores at the apex. Internally, each papilla is fed by two enormous, single-celled secretory glands each lying dorsal and somewhat lateral of the dorsal vessel and each with associated ductule cells forming a channel from the glandular cells to the pores. Internally, the secretory cells show a central ramified nucleus and large elongate secretory vacuoles appear between the nucleus and cell periphery and appear to conduct glandular exudate toward the ductule channel. The exudate exits through the pits.

Chemical analyses reveal that both laboratory reared and field collected larvae produce similar exudate, and the exudate does not contain formic acid as has been previously reported. Instead, our preliminary analysis reveals the presence of the following (in order of appearance in the chromatogram): 2-hydroxy-2-methyl-propanoic acid; phosphoric acid; 4-(dimethylamino)-butanoic acid; succinic acid; 4-(methylamino)-butanoic acid; 4-aminobutanoic acid; 4-hydroxyquinoline; cisaconitic acid; citric acid; isocitric acid; and 4-hydroxy-2-quinoline-carboxylic acid. The function of this exudate with these numerous components remains unclear. We propose that a curious "back-arching" behavior, performed by larvae on occasions, is a concerted effort to spread exudate over greater surface areas (e.g. long setae) and presumably enhance the dissemination of exudate, as a benefit to the caterpillar. (Eversible nature of gypsy moth DAG shown.)

PERITROPHIC MEMBRANE: SITE OF ACTION FOR LDMNPV/OPTICAL BRIGHTENER IN GYPSY MOTH LARVAE

Kathleen S. Shields and John D. Podgwaite

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

ABSTRACT

A variety of stilbenedisulfonic acid derivatives, known as optical brighteners, have been shown to increase mortality of gypsy moth, *Lymantria dispar*, larvae when administered *per os* in combination with *L. dispar* nuclear polyhedrosis virus (LdMNPV). We tested the effect of the optical brightener Blankophor BBH (BBH) on potency of LdMNPV in gypsy moth larvae, and determined the consequences of ingesting BBH/LdMNPV combinations on the structural integrity of the larval peritrophic membrane.

Newly molted second instar larvae were fed high wheat germ diet (30 ml) overlaid with 1 ml of either water, LdMNPV (10^2 - 10^6 OBs/ml), BBH (0.5%), or LdMNPV + BBH. Five larvae from each group were dissected at intervals ranging from 30 minutes to 8 days post-ingestion of inoculum. Intact peritrophic membranes were removed, fixed, dehydrated, and critical-point dried in preparation for examination by scanning electron microscopy. Remaining larvae (50/dilution) were reared for 21 days.

The addition of BBH to LdMNPV resulted in enhanced potency of LdMNPV and decreased lethal times at all dose levels. The LC_{50} of LdMNPV + BBH was 2.7×10^2 OB/ml compared with 1.3×10^4 OB/ml for LdMNPV alone. The LC_{90} of LdMNPV + BBH was 2.7×10^3 OB/ml compared with 1.4×10^5 OB/ml for LdMNPV alone. This represents about a fiftyfold difference in potency at both the LC_{50} and LC_{90} values. The LT_{50} for LdMNPV + BBH was 6.0 days compared with 8.2 days for LdMNPV alone.

Our data indicate that peritrophic membrane is the site of action for BBH in gypsy moth larvae. LdMNPV treatment had little, if any, observable effect on peritrophic membrane, but BBH treatment resulted in aberrations in peritrophic membrane surface structure. BBH + LdMNPV treatment caused similar, but much more rapid and progressive changes, and ultimately resulted in complete disintegration of the peritrophic membrane. We suggest that BBH combined with viral proteins rapidly degrades structural elements of the peritrophic membrane, allowing greater numbers of virions to pass through the damaged peritrophic membrane into the ectoperitrophic space. The greater numbers of infectious particles available to enter and replicate in susceptible cells would account for the observed increase in the potency.

DEVELOPMENT OF ENHANCED VIRAL STRAINS FOR CELL CULTURE PRODUCTION

James Slavicek

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

ABSTRACT

The *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) is currently being produced in *L. dispar* larvae. Production of virus in cell culture is an alternative to larval production that has the advantages of large scale production in 10,000 or 50,000 liter fermentors, a high degree of process control, and a final product that is free from bacterial, fungal, and viral contaminants. However, wild type LdNPV exhibits a high frequency of mutation to form few polyhedra mutants during propagation in cell culture, and polyhedra generated in cell culture are often significantly less potent compared to polyhedra produced in *L. dispar* larvae. To overcome these problems we have been developing improved LdNPV strains for cell culture production.

LdNPV few polyhedra (FP) mutants exhibit the characteristics of production of very few polyhedra, synthesis of polyhedra that are essentially devoid of viral nucleocapsids, and production of increased amounts of budded virus (BV). Increased BV synthesis results in the conversion of the virus population from one exhibiting a wild type phenotype to one with an FP phenotype during viral production in cell culture. Consequently, the formation of FP mutants during viral propagation in cell culture is a significant impediment to development of cell culture virus production systems that would facilitate availability of these viruses for biocontrol purposes. We have isolated and characterized a strain of LdNPV that exhibits resistance to FP mutant formation. This viral strain was successfully produced in a cell culture pilot production system by a private sector company.

Comparisons of biological activities between polyhedra generated in cell culture and *L. dispar* larvae have shown that polyhedra produced in the Ld652Y cell line are approximately 50 to 100 fold less potent compared to polyhedra generated in *L. dispar* larvae. Polyhedra generated in cell culture were found to contain approximately half the number of viral nucleocapsids compared to polyhedra generated in cell culture. To determine if the decreased potency of polyhedra generated in cell culture is a consequence of decreased virion occlusion, we are attempting to isolate viral strains that occlude the same number of viral nucleocapsids when produced in cell culture as when produced in *L. dispar* larvae. Several LdNPV polyhedra formation mutants have been isolated that exhibit abnormal polyhedra synthesis. These mutants are being studied to gain an understanding of the processes of polyhedra formation and virion occlusion. Once these processes are understood, it may be possible to manipulate polyhedral traits to enhance viral potency.

INTERACTION OF EXOTIC MICROSPORIDIA WITH FOREST LEPIDOPTERA

Leellen F. Solter

Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820

ABSTRACT

Microsporidia are common obligate parasites in insects. They are horizontally transmitted when susceptible hosts feed on contaminated feces, silk, or on infected cadavers. Gregarious Lepidoptera such as fall webworms (*Hyphantria cunea*) and tent caterpillars (*Malacosoma* spp.) may be particularly suitable hosts for horizontal transmission, and populations of these species have reportedly been suppressed by microsporidia infections.

Several species of microsporidia have been recovered from gypsy moth populations in Europe but surveys of gypsy moth populations in the U.S. have revealed no microsporidian infections. Our dilemma is to predict the ecological host range of exotic microsporidia using results from laboratory studies before exotic microsporidia are released in the field. Non-target species are often infected by a microsporidium under ideal laboratory conditions. Nevertheless, unusual responses occur that assist in the interpretation of host specificity data. We are currently pursuing three strategies. We tested four species of gypsy moth microsporidia in 47 native non-target Lepidoptera species, all of which occur in areas invaded by the gypsy moth. We found a continuum of responses by non-target hosts to the microsporidia. We grouped the responses into three categories: 1. refractory, 2. light and/or atypical infections, and 3. heavy infections, in which sufficient infective spores were produced to predict possible horizontal transmission. We are also studying host and non-target host responses at the tissue level. Early results show that the hemolymph may be involved in the dissemination of infection to the target tissues. A better understanding of the development of infection in the natural host will help us evaluate abnormal infections in non-target hosts. In a third study, we are using a novel approach by designating the gypsy moth as a non-target host. The gypsy moth occurs sympatrically with many species of native Lepidoptera. Many of these species are infected with microsporidia. If the gypsy moth is susceptible to indigenous microsporidia from indigenous Lepidoptera, we can compare these results to the known ecological data - no known microsporidian infections in U.S. populations of the gypsy moth. Our most promising microsporidium for release as a biological control agent for the gypsy moth, an isolate of *Microsporidium* sp. from Portugal, produced patent infections in very few of the non-target hosts we tested in the laboratory. Laboratory host specificity experiments determine the physiological susceptibility but environmental factors also influence the transmission and dispersal of microsporidia. We predict that the Portugal isolate is very host specific in the field. Studies of tissue-level responses to microsporidian challenge may have value in predicting ecological host specificity, and may be useful in developing experimental guidelines for conducting host specificity studies on exotic biological control agents.

INFECTIVITY OF NON-INDIGENOUS GYPSY MOTH MICROSPORIDIA TO NATIVE NON-TARGET FOREST LEPIDOPTERA

Leellen Solter¹, Michael McManus², Joseph Maddox¹, and Michael Jeffords¹

¹Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820

²USDA Forest Service, Northeastern Forest Experiment Station,
51 Mill Pond Rd., Hamden, CT 06514

ABSTRACT

The physiological host specificity of a biological control organism, which is usually determined through laboratory experimentation, is used commonly to estimate the potential ecological host range of that organism. This information is critical to the assessment of any candidate biological control organism prior to its approval for release into the environment. Many non-target hosts that may be infected by an entomopathogen during laboratory challenges may not support infection in the field.

We studied the effects of four species of microsporidia, isolated from gypsy moth (*Lymantria dispar* L.) populations in Europe, on larvae of 40 species of native forest Lepidoptera. Larvae were fed both a high and a low dose of each isolate; observations were recorded over time on the tissues affected and whether typical or abnormal spores were produced. We observed a wide range of responses among the non-target larvae to the four isolates that were tested. *Endoreticulatis schubergi*, from Portugal, produced an "all or nothing" response; larvae were either completely refractive to this isolate or developed characteristic heavy infections in affected tissues. A *Vairimorpha* isolate from the Czech Republic produced infectious spores in several of the non-target species; however, all other species were either refractive to this microsporidium or developed atypical infections. We characterized infections to be atypical when abnormal spores were produced and/or the progression of infection was terminated at an early stage of development. The *Nosema*-like isolate from Portugal and Romania produced typical infections only in gypsy moth larvae and in a few species of non-targets, though the isolate from Portugal was the least infective of all the isolates that we studied.

We believe that horizontal transmission of microsporidia among larval populations can occur only when normal infectious spores are produced in susceptible tissues of hosts in relatively large numbers. When the isolates from Portugal, Romania, and the Czech Republic infected non-target hosts, the development of the microsporidium was altered and small numbers of mainly abnormal spores were produced. Therefore, little or no horizontal transmission should be realized and the ecological host range of these isolates is probably quite narrow. *E. schubergi* produced large numbers of normal infectious spores in many of the non-target species challenged and is, therefore, not a good candidate for release against the gypsy moth in North America.

IDENTIFICATION OF A *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS HOST RANGE GENE

Suzanne M. Thiem^{1,2,3,4}, Xianlin Du², Michelle Berner², Martha Quentin^{2,4}, and Charley Chilcote²

Departments of ¹Microbiology, ²Entomology, ³Program in Genetics, and
⁴Pesticide Research Center, S-12 Plant Biology, Michigan State University,
East Lansing, MI 48824-1115

ABSTRACT

Lymantria dispar nuclear polyhedrosis virus (LdMNPV) is a baculovirus used to control the gypsy moth. It has a narrow host range. Another baculovirus, *Autographa californica* nuclear polyhedrosis (AcMNPV), has a relatively broad host range, infecting approximately 30 lepidopteran species but not the gypsy moth. LdMNPV is propagated in cell culture using Ld652Y cells, a cell line that does not support the replication of AcMNPV. However, if AcMNPV-infected Ld652Y cells are superinfected with LdMNPV, AcMNPV is produced. We determined that transfected LdMNPV DNA was able to provide this "helper function". This allowed us to map the LdMNPV gene that promoted AcMNPV replication in Ld652Y cells. By transfecting AcMNPV DNA and various cloned fragments of LdMNPV DNA into Ld652Y cells, we identified a single 835 bp region of the LdMNPV genome that was sufficient to allow AcMNPV replication in this cell line. We sequenced the DNA fragment and found it to have an open reading frame encoding a polypeptide with a predicted molecular weight of 25.7 kDa. We then constructed recombinant AcMNPV that carried a segment of LdMNPV DNA including this region. The recombinant virus was able to replicate in Ld652Y cells and in gypsy moth larvae.

GypsES: A DECISION SUPPORT SYSTEM FOR GYPSY MOTH MANAGEMENT

Susan Thomas¹, Lisa Selmon¹, Daniel Twardus², John Ghent³,
Mark Twery¹, and Kurt Gottschalk¹

¹USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

²USDA Forest Service, State and Private Forestry, Northeastern Area, Forest Health Protection,
180 Canfield St., Morgantown, WV 26505

³USDA Forest Service, Forest Health, 200 Weaver Blvd., Asheville, NC 28804

ABSTRACT

GypsES is a decision support system for the management of gypsy moth. It provides tools which enable the user to develop and/or import GIS and database information about a particular location. It also allows the user to extract information and pass it to Forest Service developed models which address such issues as phenology, defoliation prediction, spray deposit, and the long-term effects of treatment decisions.

GypsES provides an interface between a geographic information system, a database, and a rule base interpreter. It utilizes the Unix X-Windows system to provide a user-friendly environment.

The system provides hazard and risk ratings based on site conditions and the user's management objectives. It supports both suppression (egg mass surveys, defoliation prediction) and eradication (trap placement and trap data management) applications. It will also support treatment operations. Users may draw their spray blocks within GypsES utilizing various types of images as a backdrop. The Phenology model helps in determining optimal spray timing, and the Spray Deposit model (FSCBG) will provide information on average deposition.

Plans for additional development include pre-flight analysis of probable spray deposit, analysis of actual flight lines recorded by GPS equipment, rule-based development of environmental risk factors, and expanded use of the stand damage model.

BACILLUS THURINGIENSIS CRYIA INSECTICIDAL TOXINS EFFECT RAPID RELEASE
OF GYPSY MOTH MIDGUT EPITHELIUM AMINOPEPTIDASE

Algimantas P. Valaitis

USDA Forest Service, Northeastern Forest Experiment Station,
359 Main Rd., Delaware, OH 43015-8640

ABSTRACT

The CryIA class of *Bacillus thuringiensis* insecticidal proteins are toxic to the larvae of the gypsy moth (*Lymantria dispar* L.), a major forest pest in the United States. However, members of the CryIA delta endotoxins show variation in their toxicity to the gypsy moth that may be attributed in large part to the presence of specific receptors in the midgut epithelium. The binding of a toxin to its receptor causes disruption of the epithelium membrane, leakage of ions and water into the cell, and eventually, lysis of the epithelial cells. Recently, the receptor for the CryIA(c) toxin in the midgut of the gypsy moth was identified as the brush border membrane aminopeptidase (APN). The receptor for another member of the CryIA delta endotoxins, CryIA(a), is a different binding protein with an apparent size of 210 kDa. In this study, gypsy moth larvae were force-fed various concentrations of CryIA(c) and CryIA(a) toxins to determine what effect the toxins may have on the brush border membrane APN and other gut enzyme activities.

Dose and time-course studies showed that even though CryIA(a) and CryIA(c) bind to different receptors in the brush border membrane of midgut epithelial cells, both induced a rapid release (solubilization) of the glycosyl-phosphatidylinositol (G-PI) anchored APN from the midgut epithelium. There was no change in trypsin and elastase activities in the luminal fluid. Brush border membrane vesicles (BBMV) from the intoxicated larvae treated with a bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) showed that APN associated with the BBMV was depleted. Force feeding AMP and GMP mimicked the effect of the toxins, and cyclic-AMP antagonized the effect of the toxins. These results suggest that one early event elicited by the CryIA toxins is the release of the membrane-bound APN possibly mediated by the activation of endogenous phospholipases. Since APN serves as the receptor for the CryIA(c) toxin, the release of APN from the cell surface offers an explanation as to why CryIA(c) is less toxic than CryIA(a) to the gypsy moth.

IDENTIFICATION OF THE *BACILLUS THURINGIENSIS* CRYIAC TOXIN BINDING PROTEIN IN THE GYPSY MOTH MIDGUT

Algimantas P. Valaitis¹, Mi Kyong Lee², Francis Rajamohan², and Donald H. Dean²

¹USDA Forest Service, Northeastern Forest Experiment Station,
359 Main Rd., Delaware, OH 43015-8640

²Department of Biochemistry, The Ohio State University, 484 West 12th Ave.,
Columbus, OH 43210-1292

ABSTRACT

Aminopeptidase N (APN) was purified from gypsy moth (*Lymantria dispar* L.) brush border membrane vesicle (BBMV) proteins by mono-Q chromatography and Superdex-75 gel filtration in the presence of the zwitterionic detergent, CHAPS, using FPLC. The purified APN, identified by its enzymatic activity, had an apparent size of 100 kDa, and was identified as the unique *Bacillus thuringiensis* insecticidal toxin, CryIac, binding protein. Protein blots of the BBMV proteins probed with biotin-labeled and ¹²⁵I-labeled insecticidal proteins revealed that CryIac binds to a 120 kDa protein. The purified APN exhibited a slightly smaller size in comparison to the binding protein observed in blots of detergent-solubilized BBMV proteins. However, it clearly displayed the characteristic properties expected of the CryIac receptor, binding strongly to CryIac, exhibiting little or no binding to CryIa_a or CryIa_b, and showing no affinity for the coleopteran-specific toxin, CryIII_A. Antibodies raised against the gypsy moth APN demonstrated that the purified APN and the 120 kDa CryIac binding protein of total BBMV proteins are antigenically identical.

ASIAN/SIBERIAN/EUROPEAN GYPSY MOTH RESEARCH:

ARE FURTHER EFFORTS NECESSARY?

William E. Wallner

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

ABSTRACT

In November 1992, a comprehensive, 3-year research planning mission was completed for the Asian gypsy moth (AGM). At that time, the AGM had been introduced into North America on ships from the Russian Far East in the Pacific Northwest to load grain. While those introductions apparently have declined in numbers, new problems have arisen from a Siberian strain that has been introduced into western Europe and subsequently transported into the U.S. and Canada on military equipment and personal belongings of military personnel. Since the problem has changed in its complexity, the new research plan needed to be reformulated.

In December 1994, a preliminary AGM research outline consisting of seven major groupings was sent to more than 40 individuals at federal, state, and Provincial agencies and universities in the U.S. and Canada for comments and suggestions. This resulted in the following research/development document designed to address the problem of gypsy moth of Asian/Siberian/European origins. A detailed research schedule and priorities have not been ascribed to the various activities. The intent was to provide a comprehensive document for researchers, research administrators, regulatory personnel, and politicians in the U.S. and Canada that identifies needed research. Hopefully, the resources to confront this increasingly difficult situation can be found.

Most components are research tasks; others are regulatory or administrative issues reflecting the complex nature of this international problem. This complexity necessitates coordination, sharing research results, and harmonizing detection, eradication, and regulatory programs at state, federal and provincial levels. Given the modest budgets allocated for Asian gypsy moth research, the achievements both in basic findings and technology development are noteworthy. Clearly, the existence of an active research and development program for gypsy moth provided a substantive basis for focusing on gypsy moth of non-North American origins. However, reduction in program funding coupled with emerging global markets and increased international trade presents a daunting task for regulatory agencies to prevent additional introductions.

Detection and Delineation

Accomplishments

- attractancy of males to disparlure verified
- identified additional pheromone compounds from AGM
- AGM and NAGM male periodicity determined in the field in Russia and in Germany

Current Planned Activities

- observe and document pre-mating and post-mating behavior of adults in the field in Russia and in Germany

Research Needs/Development Activities

- relationship of male AGM captures to female location
- factors influencing female flight distance and direction in Russia and in Germany
- compile worldwide distribution records for hosts and outbreak frequency in proximity to shipping centers
- determine the feasibility of light trap monitoring for predicting population densities in foreign ports
- ascertain AGM male dispersal characteristics vs NAGM

Prevent AGM from Entering North America

Accomplishments

- lighting devices less attractive to AGM adults ascertained
- shipping schedules determined by female flight period - an accepted protocol by the industry
- developed pre-clearance inspection procedures for military goods returning from Europe

Current Planned Activities

- monitoring system operational for Russian ports for AGM as well as nun moth and pink gypsy moth

Research Needs/Development Activities

- highly attractive lighting devices for sampling females
- egg mass sampling procedure for containers
- suppression techniques (products, buffer treatments, etc.) for AGM populations around infested ports
- incorporate features imparted by filters that make lighting devices unattractive to adults into prototype lamps in cooperation with the lighting industry and field test

- expand monitoring to other Asian and European ports
- develop an international network to determine risk at port and cargo/container locations
- develop operational tracking system to detect at risk cargo/containers

Eradication of AGM from North America

Accomplishments

- effectiveness of *Bt* and NPV documented in laboratory bioassays for Far East and Russian strains
- utilized NAGM pheromone monitoring system for AGM
- evaluated microsporidia against AGM and pink gypsy moth

Current Planned Activities

- bioassaying *Bt* and NPV on German strains and its hybrids with NA strains
- effectiveness of *Bt* and NPV against hybrid populations
- effectiveness of *Bt* and NPV against central Siberian populations
- development of flying female light trap for delimitation activities
- comparison of electrophysiological characteristics of *Bt* ICP-receptor binding between North American and Asian gypsy moth (and hybrids)

Research Needs/Development Activities

- harmonize eradication/suppression activities with U.S./Canada, states and provinces
- assess impacts of eradication activities on non-target arthropods
- more precise delimitation of AGM infestations (e.g., trap densities over time)
- effectiveness of *Bt* and NPV against other Asian populations
- ascertain the effectiveness of different NPV genotypes against AGM and its NAGM hybrids
- ascertain infectivity of microsporidian isolates against AGM and its NAGM hybrids and related *Lymantria* spp.

Determining Susceptibility of Host Plants and Forests

Accomplishments

- in Russia, growth and survival highest for Siberian larvae followed by Germany and last, U.S.
- in U.S., AGM grew better than NAGM on 50 plant species
- greatest differences in growth rates were on conifers

Current Planned Activities

- susceptibility of host plants to different Asian populations (Far East Russian, Central Siberian, Japanese, Chinese)
- susceptibility of host plants to hybrid populations

Research Needs/Development Activities

- assess AGM and NAGM crosses on host utilization
- test additional U.S. tree and shrub species, especially southern pines and western species, including riparian types
- susceptibility of host plants to different Asian populations (Far East Russian, Central Siberian, Japanese, Chinese)
- susceptibility of host plants to hybrid populations

Consequences of Hybridization

Accomplishments

- AGM and NAGM sexually compatible
- lower diapause chill requirements of Russian eggs are retained in their hybrids
- faster larval growth is retained and accentuated with hybridization
- the full range of larval color variation is present in hybrids
- some female flight capability is retained with hybridization but the proportion of the population with strong directed flight is reduced
- behavioral and physiological traits of German gypsy moth are consistent with that of a population with multiple generations of hybridization

Current Planned Activities

- assess behavioral and biological characteristics of central Siberian x North American hybrids
- assess behavioral and biological characteristics of gypsy moths from other world areas (both Asian and European)
- correlate behavioral and biological characteristics with DNA marker results
- ascertain the behavioral and biological characteristics of individuals from North America whose DNA type is hybrid

Research Needs/Development Activities

- monitor female flight distribution in Europe for spread and effects of hybridization
- ascertain if mating between strains in the field (Germany or France) is random or assortative
- compare adult eclosion patterns for hybrids and their parent strains
- determine amount of outbreeding required to eliminate the capacity for female flight
- ascertain hybridization rates over time in Germany and France
- evaluate the influence of eclosion position within egg masses upon female flight propensity
- pheromone trap males in known AGM regions in Germany or France to determine seasonal phenology and verify by molecular diagnostic tools

Diagnostic Methods to Differentiate between Geographic/Behavioral Strains

Accomplishments

- three mtDNA haplotypes identified
- mtDNA procedure for batch samples developed
- six nuclear gene markers identified
- head capsule color discrimination developed
- selectivity of two nuclear markers determined

Current Planned Activities

- wing venation characterization for separating AGM/NAGM
- cuticular hydrocarbon separation of AGM/NAGM
- examine and describe female flight muscle anatomy and histology for AGM and EGM and their hybrids
- determine if head capsule color discrimination can identify AGM/NAGM hybrids
- correlate marker results with behavioral and biological characteristics

Research Needs/Development Activities

- develop additional nuclear gene markers
- identify nuclear chromosomal location marker for female flight and other phenotypic traits (e.g., host range/preferences)
- develop methodology for processing field samples
- clarify taxonomic relationships
- develop accuracy measurements for developed markers
- construct a phylogenetic tree for *Lymantria* spp. using ribosomal DNA sequencing
- determine the range of AGM in Germany
- test DNA markers for female flight in Europe with trading partners and military sites
- standardize the diagnostic procedure (i.e., which markers and interpretation of results)
- determine the specificity of genomic markers (i.e., the behavior with the same specimen)
- develop selectivity and sensitivity for candidate markers using "domestic" sample

Predicting Biological Events

Accomplishments

- model deployed for 1st instars dispersing from ships
- egg hatch model adapted to broad range of latitudes
- non-diapause AGM strain developed after first generation of selection
- Central Siberian strain develops faster than that from Far East
- bioassay for female flight developed

Current Planned Activities

- determine flight propensity for strains from the Russian Far East, Central Siberia, and Germany and hybrids with North American strain
- determine female flight propensity when food stressed, hatched from different portions of the egg mass, of different instar types (5 or 6 instars), early vs late hatch
- develop rapid technique for identifying female flight potential
- define diapause requirements for AGM and integrate into egg hatch model
- ascertain female flight propensity with flight mills

Research Needs/Development Activities

- compare female flight propensity using females reared on artificial diet with those reared on host foliage
- incorporate AGM developmental data into NAGM growth model
- determine population density and environmental effects on female flight
- determine the role of lipid metabolism in female flight assessment
- model female flight dispersal

The foregoing research/development listings were predicated on the concept that new strains could be prevented from establishing in North America. However, it is clear that with increasing international trade, military relocation, etc., that new or additional pathways of introduction will occur. The technology to manage new gypsy moth strains is untested, thus, the following activities are suggested in anticipation of future management needs.

Reducing the Impact of Established Asian/Siberian Infestations

Research Needs/Development Activities

- determine the risk of establishment and rate of spread in various North American ecosystems
- assess utility of NAGM technology
- monitor the potential AGM hot spots identified by molecular diagnostics
- evaluate management options in European infestations with scientific collaborators there

The Asian gypsy moth experience can serve as a model system for other Lymantriid moths that have similar habitats and pose comparable risks of introduction. An additional issue has been USDA-APHIS policy that frowns upon culturing potential pests within quarantine. Clearly, if this was not undertaken on Asian gypsy moth, the scientific advances made would not have been possible. Perhaps this will initiate a change in philosophy and regulations so that preemptive work can be conducted on other exotic pest Lymantriids such as pink gypsy moth and nun moth.

CHARACTERIZATION OF A SPECIES-SPECIFIC DNA PROBE FOR THE
IDENTIFICATION OF *ENTOMOPHAGA MAIMAIGA*

Scott R.A. Walsh¹, Ann E. Hajek², David Tyrrell³, and Julie C. Silver¹

¹Department of Microbiology and Division of Life Sciences, Scarborough Campus, University of Toronto, Scarborough, ON, Canada M1C 1A4

²Department of Entomology, Cornell University, Ithaca, NY 14853

³Forestry Canada, P.O. Box 490, Sault Ste. Marie, ON, Canada P6A 5M7

ABSTRACT

The zygomycete, *Entomophaga maimaiga* Humber, Shimazu & Soper, is the only member of the *Entomophaga aulicae* species complex to be taxonomically separated. A pathogen of the gypsy moth originally isolated in Japan, *E. maimaiga* has been identified as one agent responsible for the dramatic collapse of gypsy moth populations in the northeastern United States. This fungus is morphologically indistinguishable from other members of the *E. aulicae* species complex, but can be distinguished by isozymic composition, PCR-based techniques, and RFLP assays. We report here the identification of a DNA fragment cloned from a U.S. *E. maimaiga* isolate which hybridized to DNA of geographically diverse *E. maimaiga* isolates, but did not hybridize to DNA from other entomophthoralean fungi, including other isolates of the *E. aulicae* species complex. Sequence analyses revealed that the species-specific DNA fragment is 1962 base pairs in length, is 69.8% AT, and is composed of several simple sequence motifs including 5'-C(A_n)T-3' and 5'-T(A_n)C-3'. The DNA sequence contained several stop codons in all six reading frames, with the largest open reading frame potentially encoding a 63 amino acid protein. Although weak amino acid similarity was observed with a *Drosophila* LTR and a putative open reading frame terminating in the cloned sequence, no significant homologies at the nucleotide or amino acid levels were found in searches of existing databases. Quantitative dot blot hybridizations indicated that the sequence contained a repetitive element present at approximately 160±8 copies/*E. maimaiga* nucleus. The repetitive nature of this probe proved highly useful and allowed for the detection of an *E. maimaiga* infection within a single *L. dispar* larva. The development of this highly sensitive and rapid DNA-based assay for *E. maimaiga*-infected larvae has aided in the assessment of the role of alternative hosts in the production of epizootics in gypsy moth populations.

Supported by an NSERC Canada Strategic Grant (J.C.S.) and an NSERC Scholarship (S.R.A.W.).

DEVELOPMENT AND USE OF MOLECULAR MARKERS FOR THE IDENTIFICATION OF *ENTOMOPHAGA MAIMAIGA*

Scott R.A. Walsh¹, Ann E. Hajek², Dave Tyrrell³, and Julie C. Silver¹

¹Department of Microbiology and Division of Life Sciences, Scarborough Campus, University of Toronto, Scarborough, ON, Canada M1C 1A4

²Department of Entomology, Cornell University, Ithaca, NY 14853

³Forestry Canada, P.O. Box 490, Sault Ste. Marie, ON, Canada P6A 5M7

ABSTRACT

The fungal genus *Entomophaga* includes several insect pathogens which are currently under consideration for use as biological control agents. *Entomophaga aulicae* (Reichardt in Bail) Humber and *Entomophaga maimaiga* Humber, Shimazu & Soper, are important mycopathogens of Lepidoptera. *E. aulicae* has been isolated from 10 families of Lepidoptera, including Lymantriidae, while *E. maimaiga* has been isolated from only the Lymantriidae, and primarily from the gypsy moth, *Lymantria dispar* (L.). Although morphologically indistinguishable, laboratory assays have shown that *E. aulicae* isolates are unable to infect gypsy moth larvae, while *E. maimaiga* isolates are unable to infect typical *E. aulicae* hosts. Studies regarding the presence and/or importance of alternative hosts in the life cycle of these fungi have been hampered by the lack of rapid and unequivocal methods for the detection and differentiation of these two fungal species. Furthermore, without intra-specific markers, it is extremely difficult to assess the effectiveness of specific strains in the production of natural or induced epizootics.

To address these problems, we have investigated the use of various DNA-based techniques to assess variability among species of entomophthoralean fungi. We have developed both species-specific and strain-specific DNA markers useful in the identification of *Entomophaga* isolates. Southern analyses using *E. aulicae* rDNA sequences as probe (ribotyping) identified three distinct ribotypes among 28 isolates of *E. aulicae*. All eight isolates of *E. maimaiga* showed a distinct fourth ribotype pattern. Isolates of entomophthoralean fungi representative of other species and genera had ribotype patterns distinct to each different species examined. In order to assess intra-specific variability among isolates of *E. aulicae* or *E. maimaiga*, we isolated cloned DNA fragments from *E. aulicae* genomic and cDNA libraries, and *E. maimaiga* genomic libraries and tested these as probes in Southern analyses. Variability was detected among certain *E. aulicae* isolates, including in some cases variability among isolates from the same epizootic. However, no variation was detected among the geographically diverse isolates of *E. maimaiga*. Further analyses using PCR-RAPD assays identified some differences between the *E. maimaiga* isolates, but variability was low compared to that observed among isolates of *E. aulicae*.

Finally, highly repetitive DNA elements specific for *E. aulicae* and *E. maimaiga*, as well as for other entomophthoralean fungi, were identified. The combinatory use of these repetitive element probes was shown to provide a rapid and unequivocal technique for the differentiation of *E. aulicae*- and *E. maimaiga*-infected larvae.

Supported by an NSERC Canada Strategic Grant (J.C.S.) and an NSERC Scholarship (S.R.A.W.).

HOW *E. MAIMAIGA* INFECTS HOSTS: A TALE OF TWO SPORES

Ronald M. Weseloh

Department of Entomology, Connecticut Agricultural Experiment Station, New Haven, CT 06504

ABSTRACT

Entomophaga maimaiga, as is well known, produces two kinds of spores. Resting spores overwinter in the soil, germinating in the spring to infect gypsy moth larvae. Conidia are formed from fungal hyphae developing in hosts. Conidia released through the air can infect other larvae. Larger infected gypsy moth larvae also produce resting spores that are inactive until the next spring. To investigate the roles these spores play in infection of hosts, 2nd instar gypsy moths were exposed in cylindrical wire cages in a forest where gypsy moths and the fungus were prevalent. Cages were placed either on the soil to sample the germination of resting spores and subsequent infection of larvae, or about 2 m above the ground to detect infection due to conidia. Cages were exchanged daily throughout May and June, and exposed larvae reared out. Temperature and rainfall were also recorded daily at the site. Larvae in cages on the soil became infected with the fungus throughout the season, and usually one day after rainfall occurred. In contrast, larvae in aerial cages were infected mainly during June, and usually the same day that rainfall occurred. Infection rates of larvae collected weekly from the same site also mainly occurred in June. These results suggest that conidia produced by infected larvae were mainly responsible for the large mortality caused by the fungus at this site. However, the interaction between infection probability and rainfall, temperature, gypsy moth larval behavior, and host and pathogen phenology are complex, and this conclusion is probably simplistic. To obtain more information about these interactions, a computer model was developed (with the help of David Onstad) that was driven by temperature and rainfall values, and that predicted the dates when resting spores and conidia would have their greatest potential for infecting hosts. Runs using rainfall and temperature records for 1990 and 1991 gave infection times of larvae that were quite similar to field estimates. However, resting spores were largely responsible for infection of hosts. In general, results suggest that both resting spores and conidia are important for the successful suppression of gypsy moth populations caused by this pathogen.

CALOSOMA SYCOPHANTA DOES AFFECT GYPSY MOTH POPULATIONS

Ronald Weseloh¹, Gary Bernon², Linda Butler³, Roger Fuester⁴, Deborah McCullough⁵,
and Frederich Stehr⁵

¹Department of Entomology, Connecticut Agricultural Experiment Station,
New Haven, CT 06504-1106

²USDA, APHIS, Otis Methods Development Laboratory, Bldg. 1398, Otis ANGB, MA 02542

³Division of Plant and Soil Sciences, P.O. Box 6108, West Virginia University,
Morgantown, WV 26506-6108

⁴USDA, ARS, Beneficial Insects Introduction Research, 501 South Chapel St.,
Newark, DE 19713

⁵Department of Entomology, Michigan State University, East Lansing, MI 48824-1115

ABSTRACT

Calosoma sycophanta is a specific predator beetle of the gypsy moth that has the potential for controlling high populations of the pest. Its phenology is closely related to that of the gypsy moth, and adults are only obvious where there are outbreaks. Procedures have been developed to rear the beetle with some success using gypsy moths from USDA-APHIS at Otis, Massachusetts (Gary Bernon). Previous releases of adult beetles have been made in Connecticut with encouraging results. However, pupal mortality caused by the released beetles was only transitory because the beetle is already well established in Connecticut. An ideal way to assess the effectiveness of *C. sycophanta* would be to release the beetle in areas where the gypsy moth but not the beetle is present. Such areas occur in Michigan (McCullough, Stehr), West Virginia (Butler), and Delaware (Fuester), and this last summer releases were carried out in these states. Results showed that gypsy moth pupal survival was inversely related to estimates of the numbers of immature *C. sycophanta* present in each of the plots. This provides strong evidence that the predator was able to have an impact on gypsy moths in these areas, and suggests that additional releases of the beetle along the leading edge of gypsy moth infestations would be useful.

INFLUENCE OF WEATHER ON THE SYNCHRONY OF GYPSY MOTH OUTBREAKS IN NEW ENGLAND

David W. Williams¹ and Andrew M. Liebhold²

¹USDA Forest Service, Northeastern Forest Experiment Station,
P.O. Box 6775, Radnor, PA 19087-8775

²USDA Forest Service, Northeastern Forest Experiment Station,
180 Canfield St., Morgantown, WV 26505

ABSTRACT

Outbreaks of the gypsy moth were partially synchronous across the New England states from 1938 to 1992. To explain this synchrony, we investigated the Moran effect, a hypothesis that local population oscillations resulting from similar density dependent mechanisms operating at time lags may be synchronized over wide areas by exposure to common weather patterns. Time series analysis revealed defoliation series in two states as first-order autoregressive processes and the other two as periodic second-order autoregressive processes. Defoliation residuals series computed using the autoregressive models for each state were cross correlated with series of weather variables recorded in the respective states. Weather variables significantly correlated with defoliation residuals in all four states were minimum temperature and precipitation in mid-December in the same gypsy moth generation and minimum temperature in mid to late July of the previous generation. These weather variables also were correlated strongly among the four states. The analyses support the predictions of the Moran effect and suggest that common weather may synchronize local populations so as to produce gypsy moth outbreaks over wide areas.

USDA Interagency Gypsy Moth Research Forum
January 17-20, 1995
Annapolis, Maryland

List of Attendees

Linda Abbott, USDA-APHIS, Room 543, 6505 Belcrest Rd., Hyattsville, MD 20782
Jean R. Adams, USDA-ARS, IBL, BARC-West, Rm. 214, Beltsville, MD 20705
ONeil Albino, USDA-ARS, IBL, BARC-East, Bldg. 402, Beltsville, MD 20705
John Bain, Forest Research Institute, Private Bag 3020, Rotorua, New Zealand
Yuri Baranchikov, V.N. Sukachev Institute of Forest, Krasnoyarsk, 660036, Russian Federation
Jack Barry, USDA-FS, PSW, 2121 C 2nd St., Davis, CA 95616
Leah Bauer, USDA-FS, NCFES, 1407 S. Harrison Rd., East Lansing, MI 48824
Jon Bell, Agriculture Canada, 620 Royal Avenue, New Westminster, British Columbia V3L 5A8
Gary Bernon, USDA-APHIS, Bldg. 1398, Otis Methods Development Ctr., Otis ANGB, MA 02542
Liz Bills, Maryland Dept. Agriculture, 50 Harry S. Truman Pkwy., Annapolis, MD 21401
David Bischoff, USDA-FS, NEFES, 359 Main Rd., Delaware, OH 43015
E. Michael Blumenthal, Pennsylvania Bur. Forestry, 34 Airport Rd., Middletown, PA 17057
Steven Bogdanowicz, Cornell Univ., Ecology & Systematics, Ithaca, NY 14853
A. Temple Bowen, Jr., NOVO Nordisk Bioindustrials, 33 Turner Rd., Danbury, CT 06813-1907
J. Robert Bridges, USDA-FS, FIDR, P.O. Box 96090, Washington, D.C. 20090-6090
John Brooks, US Fish & Wildlife Service, Arlington, VA
E. Alan Cameron, Pennsylvania State Univ., Dept. Entomology, University Park, PA 16802
Ring T. Cardé, Dept. Entomology, Univ. Massachusetts, Amherst, MA 01003
Jane Carter, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
Ralph Charlton, Dept. Entomology, Kansas State Univ., Manhattan, KS 66506
Randy Ciurlino, Delaware Dept. Agriculture, 2320 S. DuPont Highway, Dover, DE 19901
Ed Clark, USDA-ARS, IBL, BARC, Beltsville, MD 20705
Jim Colbert, USDA-FS, NEFES, 180 Canfield St., Morgantown, WV 26505
John Lee Compton, AgriVirion, Inc., 460 West 25th St., New York, NY 10001
Stephen Cook, USDA-ARS, IBL, BARC-East, Bldg. 402, Beltsville, MD 20705
Marie-José Côté, Agriculture Canada, 3851 Fallowfield Rd., Nepean, Ontario K2H 8P9
John Cunningham, Forest Pest Management Institute, P.O. Box 490, Sault Ste. Marie, Ontario P6A 5M7
Sonia Dabulis, USDA-APHIS, PPQ, 505 S. Lenola Rd., Moorestown, NJ 08057
Vincent D'Amico, III, Dept. Entomology, Univ. of Massachusetts, Amherst, MA 01003
Ernest Dankwa, Abbott Laboratories, 1401 Sheridan Rd., N. Chicago, IL 60064
Christopher Davidson, Virginia Polytechnic Institute, 228 Cheatham Hall, Blacksburg, VA 24061
Ernest S. Delfosse, USDA-APHIS, NBCI, Federal Building, Room 538, 6505 Belcrest Rd., Hyattsville, MD 20705
Willard Dickerson, North Carolina Dept. Agric., P.O. Box 27647, Raleigh, NC 27611
Edward Dougherty, USDA-ARS, IBL, Bldg. 011A, BARC-W, Beltsville, MD 20705
Normand R. Dubois, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
Donald A. Eggen, Delaware Dept. Agric., 2320 S. DuPont Highway, Dover, DE 19901
Douglas C. Ferguson, USDA-Systematic Entomol. Lab., U.S. National Museum, Washington, D.C.
Chris Firmi, Maryland Dept. Agriculture, 50 Harry S. Truman Pkwy., Annapolis, MD 21401
John Foltz, Univ. of Florida, Dept. of Entomology, Gainesville, FL 32611-0620
Roger W. Fuester, USDA-ARS, BIIRL, 501 S. Chapel St., Newark, DE 19713
Robert Fusco, Hc 63, Box 56, Mifflintown, PA 17605
Karen J. Garner, USDA-FS, NEFES, 359 Main Rd., Delaware, OH 43015
Bruce Gill, Agriculture Canada, 960 Carling Avenue, Ottawa, Ontario K1A 0C6
William Gimpel, Maryland Dept. Agriculture, 50 Harry S. Truman Pkwy., Annapolis, MD 21401

Gregory Glovick, RD 2, Box 1198, Clayton, DE 19938
 Kurt Gottschalk, USDA-FS, NEFES, 180 Canfield St., Morgantown, WV 26505
 David Gray, Price Hall, Virginia Polytechnic Institute, Blacksburg, VA 24061
 Phyllis Grinberg, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Fred Hain, Dept. Entomology, Box 7626, North Carolina State Univ., Raleigh, NC 27695-7626
 Ann Hajek, Dept. of Entomology, Cornell Univ., Ithaca, NY 14853-0901
 Betsie Handley, Maryland Dept. Agriculture, 50 Harry S. Truman Pkwy., Annapolis, MD 21401
 Richard Harrison, Ecology & Systematics, Cornell Univ., Ithaca, NY 14853
 Felton Hastings, Dept. Entomology, North Carolina State Univ., Raleigh, NC 27695
 Ray Hicks, Jr., Division of Forestry, West Virginia Univ., Morgantown, WV 26506
 Shivanand Hiremath, USDA-FS, NEFES, 359 Main Rd., Delaware, OH 43105
 Gernot Hoch, Universität fuer Bodenkultur Wien, Institut Forstentomologie, Hasenauerstrasse 38, A-1190 Wien, Austria
 Robin Huettel, USDA-APHIS, PPQ, Room 816, 6505 Belcrest Rd., Hyattsville, MD 20782
 Pamela Huntley, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 William Kauffman, USDA-APHIS, 2534 S. 11th St., Niles, MI 49120
 Melody Keena, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Carson Kennard, 1455 Joshua-Clayton Rd., Dover, DE 19904
 Edward Knipling, USDA-ARS, Beltsville, MD 20705
 Lloyd Knutson, USDA-ARS, EBCL, AMEmbassy (Paris) PSC 116 (EBCL), APO AE 09777
 Vera Krischik, Univ. of Minnesota, 219 Hodson Hall, 1980 Folwell Avenue, St. Paul, MN 55108
 Kenneth Lakin, USDA-APHIS, PPQ, BATS, 6505 Belcrest Rd., Hyattsville, MD 20782
 David Laughlin, 5415 Connecticut Ave. NW, #500, Washington, D.C. 20015
 Donna Leonard, USDA-FS, P.O. Box 2680, Asheville, NC 28802
 Barbara Leonhardt, USDA-ARS, Insect Chemistry Lab., Beltsville, MD 20705
 Norman C. Leppla, USDA-APHIS, 6505 Belcrest Rd., Hyattsville, MD 20782
 Robert Lewis, Jr., USDA-FS, NEFES, 100 Matsonford Rd., Radnor, PA 19087
 Sandy Liebhold, USDA-FS, NEFES, 180 Canfield St., Morgantown, WV 26505
 Tom Lupp, Maryland Dept. Agriculture, 50 Harry S. Truman Pkwy., Annapolis, MD 21401
 Priscilla MacLean, Hercon Environmental, Aberdeen Rd., Emigsville, PA 17318
 Joseph Maddox, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820
 Raksha Malakar, Univ. of Massachusetts, Dept. Entomology, Amherst, MA 01003
 Philip Marshall, Vallonia State Nursery, 2782 W. County Rd. 540 S., Vallonia, IN 47281
 Victor C. Mastro, USDA-APHIS, Bldg. 1398, Otis Methods Develop. Cntr., Otis ANGB, MA 02542
 Max W. McFadden, USDA-FS, NEFES, 100 Matsonford Rd., Radnor, PA 19087
 Michael McManus, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Dale E. Meyerdirk, USDA-APHIS, PPQ, Federal Building, Room 648, Hyattsville, MD 20782
 Karl Mierzejewski, Pennsylvania State Univ., Pesticide Research Lab., University Park, PA 16802
 David Miller, Univ. of Connecticut, Nat. Res. Mgt. & Eng. Dept., Storrs, CT 06269-4087
 Jeffrey Miller, Dept. of Entomology, Oregon State Univ., Corvallis, OR 97331-2907
 Michael E. Montgomery, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Rose-Marie Muzika, USDA-FS, NEFES, 180 Canfield St., Morgantown, WV 26505
 Július Novotný, Forest Research Institute, Research Sta., Lesnicka 11, 969 23 Banská Štiavnica, Slovak Republic
 Christa Nussbaumer, Universität fuer Bodenkultur Wien, Institut Forstentomologie, Hasenauerstrasse 38, A-1190 Wien, Austria
 Thomas ODell, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Randy Peiffer, Delaware State College, Dover, DE 19901
 Tom Pfiefer, Dept. Zoology, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4
 John Podgwaite, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Douglas Prasher, USDA-APHIS, PPQ, Bldg. 1398, Otis Methods Development Cntr., Otis ANGB, MA 02542
 Robert Rabaglia, Maryland Dept. Agriculture, 50 Harry S. Truman Pkwy., Annapolis, MD 21401
 G. Ramaseshiah, 377 Front Upstairs, 9th Main, 13th Cross, Vyalikava, Bangalore, 560003, India

Kenneth Rash, NALCO, 2809 Tam O'Shanter, Richardson, TX 75080
 F. William Ravlin, Dept. Entomology, Virginia Polytechnic Inst., Blacksburg, VA 24061
 Richard Reardon, USDA-FS, NCFHM, 180 Canfield St., Morgantown, WV 26505
 Alain Roques, INRA, Station de Zoologie Forestière, Centre de Recherches d'Orléans, Orléans, France
 Paul Sandridge, Biology Dept., Delaware State College, Dover, DE 19901
 Paul Schaefer, USDA-ARS, 501 S. Chapel St., Newark, DE 19713
 Robert F.W. Schroder, USDA-ARS, IBL, Beltsville, MD 20705
 J. Mark Scriber, Dept. Entomology, Michigan State Univ., E. Lansing, MI 48824
 Martin Shapiro, USDA-ARS, Insect Pathology Lab., BARC-W, Bldg. 011A, Beltsville, MD 20705
 Alexei Sharov, Dept. of Entomology, Virginia Polytechnic Inst., Blacksburg, VA 24061
 Kathleen Shields, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 James Slavicek, USDA-FS, NEFES, 359 Main Rd., Delaware, OH 43015
 Harvey Smith, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Robert Smith, Abbott Labs., 6131 RFD Oakwood Rd., Long Grove, IL 60047
 Leellen Solter, 172 NRB, 607 E. Peabody Dr., Champaign, IL 61820
 Mike South, USDA-APHIS, P.O. Box 28, Goldsboro, NC 27533
 Jeff Stibick, USDA-APHIS, PPQ, 6505 Belcrest Rd., Hyattsville, MD 20782
 Tod Sukontarak, USDA-ARS, IBC, Bldg. 402, Biocontrol Rd., Beltsville, MD 20705
 Jil Swearingen, 8000 Meadowbrook Ln., Chevy Chase, MD 20815
 Steve Talley, Virginia Gypsy Moth Program, Count of Rockbridge, Lexington, VA 24401
 Suzanne Thiem, Dept. Entomology, Michigan State Univ., East Lansing, MI 48824
 Susan Thomas, USDA-FS, NEFES, 180 Canfield St., Morgantown, WV 26505
 Kevin Thorpe, USDA-ARS, Bldg. 402, BARC-E, Beltsville, MD 20705
 Robert Tichenor, Maryland Dept. Agric., 50 Harry S. Truman Pkwy., Annapolis, MD 21401
 Timothy Tigner, Virginia Dept. Forestry, P.O. Box 3758, Charlottesville, VA 22903
 Lawrence Turner, US-EPA, OPP, Washington, D.C.
 Al Valaitis, USDA-FS, NEFES, 359 Main Rd., Delaware, OH 43015
 James Vaughn, USDA-ARS, IBL, BARC-E, Beltsville, MD 20705
 Lee Venables, USDA-ARS, IBL, Bldg. 402, BARC-E, Beltsville, MD 20705
 David Wagner, Dept. Ecol. & Evol. Biol., Univ. of Connecticut, Storrs, CT 06269-3043
 William Wallner, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Scott Walsh, Univ. of Toronto, 1265 Military Trail, Scarborough, Ontario M1C 1A4
 Philip Wargo, USDA-FS, NEFES, 51 Mill Pond Rd., Hamden, CT 06514
 Ralph Webb, USDA-ARS, Bldg. 402, BARC-E, Beltsville, MD 20705
 Ronald Weseloh, Dept. Entomology, Conn. Agric. Expt. Sta., New Haven, CT 06504
 Geoff White, USDA-ARS, Insect Biocontrol, Bldg. 402, BARC-East, Beltsville, MD 20705
 Robert C. Whitmore, Division of Forestry, West Virginia Univ., Morgantown, WV 26506
 David Williams, USDA-FS, Global Change, 100 Matsonford Rd., Radnor, PA 19087
 Jeff Witcosky, George Washington National Forest, P.O. Box 233, Harrison Plaza, Harrisonburg, VA 22801
 Milan Zúbrik, Forest Research Institute, Research Sta., Lesnická 11, 969 23 Banská Štiavnica, Slovak Republic

Fosbroke, Sandra L. C.; Gottschalk, Kurt W., eds. 1995. **Proceedings, U.S. Department of Agriculture interagency gypsy moth research forum 1995**; 1995 January 17-20; Annapolis, MD. Gen. Tech. Rep. NE-213. Radnor, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 133 p. <https://doi.org/10.2737/NE-GTR-213>

Contains one workshop summary and 66 abstracts and papers of oral and poster presentations on gypsy moth biology, molecular biology, ecology, impacts, and management presented at the annual U.S. Department of Agriculture Interagency Gypsy Moth Research Forum.

Published by:
USDA FOREST SERVICE
5 RADNOR CORP CTR SUITE 200
RADNOR PA 19087-4585

For additional copies:
USDA Forest Service
Publications Distribution
359 Main Road
Delaware, OH 43015



Printed on Recycled Paper

Headquarters of the Northeastern Forest Experiment Station is in Radnor, Pennsylvania. Field laboratories are maintained at:

Amherst, Massachusetts, in cooperation with the University of Massachusetts

Burlington, Vermont, in cooperation with the University of Vermont

Delaware, Ohio

Durham, New Hampshire, in cooperation with the University of New Hampshire

Hamden, Connecticut, in cooperation with Yale University

Morgantown, West Virginia, in cooperation with West Virginia University

Orono, Maine, in cooperation with the University of Maine

Parsons, West Virginia

Princeton, West Virginia

**Syracuse, New York, in cooperation with the State University of New York,
College of Environmental Sciences and Forestry at Syracuse University**

Warren, Pennsylvania

The United States Department of Agriculture (USDA) Forest Service is a diverse organization committed to equal opportunity in employment and program delivery. USDA prohibits discrimination on the basis of race, color, national origin, sex, religion, age, disability, political affiliation, and familial status. Persons believing that they have been discriminated against should contact the Secretary, U.S. Department of Agriculture, Washington, DC 20250, or call 202-720-7327 (voice), or 202-720-1127 (TTY).

"Caring for the Land and Serving People Through Research"