Enclosure 3

USDA FOREST SERVICE
SPECIAL TECHNOLOGY DEVELOPMENT PROGRAM
PROGRESS REPORT

PROJECT NUMBER: NA-99-0371 (Also known as NA-99-01).

PROJECT TITLE: Increasing the Success of the Gypsy Moth Pathogen *Entomophaga maimaiga* in Michigan and Other States in the Great Lakes Region

PROJECT STATUS: Continuing

EXPECTED PROJECT DURATION: 4 years

ORIGINAL EXPECTED COMPLETION DATE OF THE PROJECT: FY 2002

EXPECTED COMPLETION DATE OF THE PROJECT (fiscal year)**:** FY 2002

SUBJECT: Biological control, Insect management, Gypsy moth, *Entomophaga maimaiga*

STATUS OF SUBJECT SPECIES: non-native, invasive, noxious pest

PROJECT OBJECTIVES:
1. Develop *in vivo* methods to produce larval cadavers containing *E. maimaiga* resting spores for introduction or inoculation in areas with established gypsy moth (*Lymantria dispar* L.) populations;
2. Evaluate the suitability of Michigan weather conditions for *E. maimaiga*; compare weather in Michigan with weather in northeastern states where *E. maimaiga* has been important in gypsy moth regulation;
3. Identify site and stand-related factors that affect the establishment, persistence or infection rate of *E. maimaiga* in gypsy moth populations in Michigan forests;
4. Develop methods and recommendations to increase the effectiveness of *E. maimaiga* as a biological control for gypsy moth in forests in the Great Lakes region.

BRIEF DESCRIPTION OF PROJECT: A section briefly describing the background and context of this project is presented below. Methods and preliminary results for each objective begin on page 2.

Background: Populations of gypsy moth, a notorious defoliator of oaks, aspen and many other species of forest trees, continue to expand into areas of Wisconsin, Indiana, Ohio and Illinois. Severe defoliation and the nuisance resulting from gypsy moth outbreaks often lead to anxiety and frustration for residents, especially those not familiar with gypsy moth. Managing gypsy moth outbreaks is expensive; costs of suppressing gypsy moth in Michigan alone amounted to over $30 million from 1986-2001 (USDA Forest Service GMDigest). In addition, concerns about potential impacts of widespread Bt (*Bacillus thuringiensis* var. *kurstaki*) sprays on non-target Lepidoptera continue to be raised (Peacock et al. 1998, Sample et al. 1996, Wagner et al. 1996). Other undesirable impacts of gypsy moth outbreaks include the use, and often misuse, of conventional broad-spectrum insecticides by property owners. Enjoyment and use of recreation areas in affected forests typically decreases, reducing income in locations dependent on tourism (Goebl 1987). Regulations associated with state and federal gypsy moth quarantines result in economic hardship for plant-related industries, as well as regulatory agencies that must enforce the quarantine.

There is much interest in Michigan and other Great Lakes states in identifying effective biological controls that can help reduce the duration of gypsy moth outbreaks or increase the interval between outbreaks. *E. maimaiga* is an attractive biological control agent because it has relatively few impacts on non-target organisms (Hajek et al. 1996a, 1997, 2000), appears to be compatible with the gypsy moth virus and other natural enemies (Buss et al. 1999, Malakar et al. 1998), and effectively regulated gypsy moth populations in northeastern states for several years after its discovery in 1989 (Hajek et al. 1995, 1996b). Overwintering resting spores infect young gypsy moth larvae, which then produce conidia that infect mid- and late instar larvae. Late instar larval cadavers produce resting spores that remain in the soil until the following spring. Currently, there is no consistent source of *E. maimaiga* inoculum, which presents problems for managers interested in introducing this pathogen in new gypsy moth infestations.

Improved methods for *E. maimaiga* introduction and distribution are needed. To-date, *E. maimaiga* has been introduced by disseminating soil collected from areas with epizootics, or by collecting and re-distributing cadavers killed by *E. maimaiga*. Both methods are problematic (Hajek 1997). Soil introductions carry the risk of transferring undesirable insects, plant pathogens or nematodes, and the abundance of resting spores in the soil is typically unknown and difficult to quantify. Cadavers can be collected for only a limited time in the summer and this option is available only if epizootics can be readily located and accessed. Protocols for consistent production and distribution of viable *E. maimaiga* resting spores are needed, and will continue to be needed as the range of gypsy moth expands.

Epizootics of *E. maimaiga* have occurred regularly in gypsy moth populations in northeastern states, resulting in substantially lower costs for suppression programs. However, the pathogen has been less consistent in Michigan. It was first introduced into northwestern Lower Michigan in 1991 (Smitley et al. 1995), and additional introductions were subsequently made by way of either spore-bearing cadavers or soil assumed to contain *E. maimaiga* resting spores (Buss 1997, D.R. Smitley and T. Davis, pers. comm.). There were only a few attempts to quantify the amount of *E. maimaiga* released or evaluate the success of introductions. Localized epizootics were observed frequently in 1996 (M. Mott and D. Smitley, MSU, unpubl. data) and anecdotal observations indicate that the pathogen is well-distributed throughout Lower Michigan. However, gypsy moth defoliation increased dramatically between 1996, when moderate defoliation was mapped on only 3600 acres, to 1998 when moderate to severe defoliation exceeded 300,000 acres. Defoliation ranged from 96,500 to 123,000 acres in 1998-2000 (GMDigest).

Climate, soils, forest cover types, and other factors obviously vary between the northeastern and Great Lakes regions of the U.S., and could account for the somewhat disappointing activity of *E. maimaiga* in Michigan compared with northeastern states. These factors may affect resting spore persistence or germination, dispersal of conidia, or amplification of the pathogen in gypsy moth populations. A better understanding of the conditions that contribute to successful *E. maimaiga* establishment in the Great Lakes region is needed, as gypsy moth populations continue to expand and build in this area. Forest health managers and pest specialists have expressed much interest in *E. maimaiga* introductions, especially in newly infested areas. Currently, however, there are no sources that can be consistently expected to provide adequate *E. maimaiga* inoculum. No guidelines are available to help managers in this region predict the probability of *E. maimaiga* effectiveness.

Work proposed here specifically addresses Items No. 1 and No. 4 in the FHP STDP list of priorities. Further, if we can identify site or stand factors related to *E. maimaiga* success, we may ultimately be able to assist managers and incorporate this information into risk-rating systems, indirectly addressing Item No. 2. For example, managers could prioritize stands in a given area for pre-salvage or other silvicultural actions, or for Bt treatment, using a combination of factors such as cover type, egg mass density and likelihood of *E. maimaiga* epizootics.

Summary of Accomplishments and Results To-Date:

Objective 1: Our goal is to develop methods to produce larval cadavers with viable *E. maimaiga* resting spores. These cadavers could then be used for introduction or augmentation in areas with established gypsy moth populations. If resting spores of *E. maimaiga* are to be distributed for biological control purposes, it is important that resting spores survive and that dormancy requirements be satisfied. Therefore, we have conducted experiments along both these interacting lines. Our progress during the past year is briefly summarized here.

We are pleased to report that in 2001, we were able to produce resting spores in gypsy moth larvae in the laboratory for release in the field.

Dormancy initiation. Our studies identified three stages of wall development and five stages of cytoplasmic development as resting spores matured after production. Contact with moisture was necessary for progression to the mature state, characterized by thick double walls and few-to-one internal oil droplets. Studies demonstrated that after larvae die, under optimal conditions, most resting spores had matured between 7-14 days later.

Resting spore survival during dormancy. Studies funded by this proposal investigated different ways to store resting spores and ensure their survival. Storage studies testing different humidity levels demonstrated that an intermediate humidity level was optimal. At present, based on the conditions we have tested, storage was optimal when resting

spores remained in cadavers placed on water agar. Based on ourresults,resting spores that dry or are formed without sufficient hydration will not germinate.

Fungal strain/Methods for infecting larvae. We have now conducted a study comparing whether production of resting spores *in vitro* is associated with production of resting spores *in vivo* for individual fungal strains and results are being analyzed. Based on our studies of resting spore production by different isolates, we used ARSEF 5378, isolated from Maryland in 1996, because we knew this isolate made plentiful resting spores *in vitro*. Approximately 900 fourth instar larvae individually injected with protoplasts January 15-16, 2001 were maintained at 20 C and checked daily for mortality. After death, cadavers were individually placed on water agar and continued to be incubated at 20 C. On February 5, 21 days after injection and allowing a sufficient period for resting spore production and maturation, resting spores still in cadavers on water agar were placed at 4 C. In early April, after ca. 2 months at 4 C, resting spores that were still in individual cadavers were mailed to Michigan for release (see Objective 3 below).

We monitored a subsample of the cadavers that had been sent to Michigan for release and found that 59% of the cadavers contained resting spores (mean = 5.3×10^5 resting spores/cadaver). Resting spore-bearing cadavers were crumbled over sterile soil and maintained in the laboratory or field to confirm infectivity after cold storage. Only 16.7% of resting spore-bearing cadavers maintained under optimal conditions in the laboratory at 15 C and 14:10 (L:D) demonstrated infectivity while 83.3% of resting spore-bearing cadavers maintained in the field with occasional watering demonstrated infectivity.

Objective 2: Our goal is to evaluate the relative suitability of weather in Michigan and the Lake States region for *E. maimaiga* resting spore germination or persistence.

Cooperators: We are continuing to collaborate with Dr. Robert C. Venette (USDA APHIS and University of Minnesota), who has expertise in modeling and climatic analysis, as well as ecological risk analysis. He is an expert in the application of CLIMEX software to estimate suitability of locations for exotic organisms based on climatic conditions. In 2001, we also began cooperating with Dr. Jeffrey Andresen, MSU Meteorologist, Dept. of Geography, to collect weather data from additional sites in Michigan (see below).

In 1999-2000, we worked with Dr. Venette to collect 30-year averages for temperature and precipitation variables from 100 weather stations distributed across North America and 63 weather stations in Michigan. The same variables were acquired for 4 sites in New York and eastern Pennsylvania known to be climatically suitable for *E. maimaiga* and to have experienced frequent epizootics – e.g "optimal" sites for *E. maimaiga*. CLIMEX software was used to calculate the similarity of weather in other locations to the 4 northeastern sites. Results were imported into ArcView and GIS maps were produced using the CLIMEX results and spatial interpolation. Separate maps were developed for temperature and precipitation, and then all variables were overlaid on a single map. Maps generated for North America indicate a relatively high degree of similarity (> 70%), indicative of suitable conditions for *E. maimaiga*, through much of the midwestern region of the U.S., with the exception of northern areas of the Lake States (e.g. Upper Michigan, northern WI and MN). In other regions, the success of *E. maimaiga* could be limited by lower than optimal precipitation, cold winter temperatures or warm temperatures. A similar procedure was used with data from 63 weather stations in Michigan. Results suggest that the southern half of lower Michigan is highly suitable for *E. maimaiga*, but areas of northern lower Michigan and much of the Upper Peninsula have different weather conditions (e.g. 60-70% similarity) and the activity of *E. maimaiga* could be different there as well. Similarity indices in 2 areas of the western Upper Peninsula were less than 50%, suggesting that activity of *E. maimaiga* could be different than activity in NY and PA. While these analyses are useful, we all agree that they represent a relatively coarse examination of climatic influences on *E. maimaiga*.

In 2001, therefore, we cooperated with Dr. Andresen to collect weather data from additional sites in Michigan and other states in the north central region. These data include annual temperature and precipitation variables, as well as long-term averages. Our intent is to assess temporal variability, as well as spatial variability, in the suitability of climatic conditions for *E. maimaiga*. We also acquired data for 1901-1945 for 58 weather stations in Japan, where the *E. maimaiga* fungus is native. These data will enhance our ability to compare conditions in Michigan and the north central region to conditions in Japan, as well as conditions in northeastern states where the fungus is known to persist. We are continuing to work with Venette to use climate modeling to identify geographic locations where *E. maimaiga* may be limited by weather conditions and to assess the proportion of years when conditions in this region

would favor *E. maimaiga* epizootics.

Objective 3: We have been working in 33 oak-dominated stands since 1999 to evaluate the distribution of *E. maimaiga*, and effects of site and stand-related factors on *E. maimaiga* persistence and efficacy. To be included in the project, stands must have been previously inoculated with *E. maimaiga* by MSU researchers or MI Dept. of Natural Resource (MDNR) entomologists, or have sustained a documented *E. maimaiga* epizootic. All stands are at least 1.5 ha in size and dominated by oak (minimum of 70% of the overstory basal area). Permits to conduct this research were granted by the Huron-Manistee National Forest and MDNR. Most stands are located in the northwestern or north central Lower Peninsula because of the relatively long history of gypsy moth and *E. maimaiga* research in these areas. However, stand locations extend from the eastern Upper Peninsula to southwestern Lower Michigan. Stands were stratified into 3 classes based on location: Lake Effect (within 35 miles of Lake Michigan or Lake Huron – subject to lake effect weather); Frost Pocket (stands in north central Lower Michigan that experience relatively cold winter temperatures) and Inland (other stands).

Within each stand, we located a plot center, then selected dominant oak trees at 10, 25 and 50 m in each cardinal direction (total of 12 trees per stand). Personnel wear disposable booties and vinyl gloves at all times to prevent inadvertent transfer of *E. maimaiga* spores among stands. Gypsy moth population density is estimated annually based on egg mass counts in four 0.01 ha circular plots located systematically in each plot (Kolodny-Hirsch 1986). In 2000, gypsy moth populations were at low densities in 15 stands, moderate in 13 stands, high in 4 stands and negligible in the Upper Peninsula stand. In 2001, gypsy moth density was low in all but 2 stands, where populations were moderate to high.

We began to measure and describe site and stand variables that could affect *E. maimaiga* persistence or germination in 1999 and most of this work was completed in 2001. These variables included quantification of dominant overstory and understory vegetation, litter depth and canopy closure. Additional analysis of soil composition and pH, and litter pH will be completed in 2001-2002, with the assistance of Dr. David Rothstein, a Forest Soil Ecologist in the Dept. of Forestry at MSU. Dr. Rothstein will provide the necessary equipment and supervise the analyses.

Our studies to address Objective 3 include a combination of field and laboratory bioassays, counts of resting spores in soil and dissection of larval cadavers from wild (e.g. natural) gypsy moth populations. In addition, we are evaluating the effectiveness of *E. maimaiga* augmentation using larval cadavers collected from wild gypsy moth populations and cadavers produced using *in vivo* methods in Hajek's lab. We also conducted an additional field bioassay in 2001for 6 weeks in 3 stands where precipitation, soil temperature and soil moisture were continuously recorded. A brief description of the methods and progress of each of these projects is presented below.

Field bioassay: We conducted field bioassays from 1999-2001 using methods developed in 1999 with minor modifications. All gypsy moth larvae used for the field bioassays were reared on artificial diet by USDA APHIS PPQ personnel at the Biological Control facility in Niles, MI. These bioassays began in early June and corresponded phenologically with development of wild gypsy moth populations. Over a 22 day period, freshly molted 4th instar larvae were collected early each morning by Niles technicians. Twenty larvae and 3 cubes of artificial diet were placed into a 15 x 20 cm cagelet, constructed of window screening. Cagelets were transported to the oak stands daily (a major logistical operation given the distances between stands). In each stand, leaf litter was brushed away from the bases of the selected trees. A cagelet was laid on the soil surface near the base of the tree on the north and south aspects (cagelets were placed in all 4 cardinal directions in 1999). Therefore, there were 2 cagelets per tree, 24 cagelets per stand and 15,840 larvae assessed annually in 2000 and 2001 (4 cagelets/tree, 48 cagelets/stand and 30,996 larvae in 1999). Cagelets remained in the stands for 4 days to allow contact between infective spores of *E. maimaiga* and larvae. Cagelets were then retrieved, individually bagged to prevent cross-contamination, placed into coolers and returned to the USDA APHIS Biological Control facility in Niles. Personnel at the Niles reared larvae individually on diet until they died or pupated. At least 10 cadavers from each cagelet (selected at random) were dissected and examined under a microscope to determine whether NPV (nuclear polyhedrosis virus) or *E. maimaiga* caused the mortality.

Results have consistently indicated that *E. maimaiga* infection rates in the field were surprisingly low regardless of stand location. Larvae are usually co-infected with *E. maimaiga* and NPV; few larvae are infected only by *E. maimaiga*. The appearance of co-infected cadavers is usually consistent with mortality caused by *E. maimaiga*, rather than the soft, liquid appearance of NPV cadavers. We have not observed strong, consistent effects of stand

location on infection rates.

In 1999, 21,769 larvae were returned to Niles and reared to pupation (some larvae are lost each year to raccoons, skunks and ant predation). Over 5000 larvae developed secondary infections and were not included in further analysis. Roughly 4000 larvae survived to pupate, while the remainder died from NPV, *E. maimaiga* or both pathogens. On average, in 1999, roughly 25-33% of larvae in each stand died from *E. maimaiga* infection (most were co-infected with NPV), compared with 29 to 51% larval mortality from NPV alone.

In 2000, we returned 11,885 larvae to Niles for rearing. Roughly 2200 survived to pupation, while 2920 larvae developed secondary infections and were not examined further. Of the remaining 6761 cadavers, 14% were co-infected by *E. maimaiga* and NPV and 86% were infected only by NPV.

Dissections of cadavers from the 2001 field bioassays are still continuing, but preliminary data are presented here. A total of 9919 larvae have been processed as of Oct. 5, 2001. Of these larvae, 3472 survived to pupation and 2125 were lost to secondary infections or did not die from a pathogen. Of the remaining 4322 cadavers, 8% were infected with *E. maimaiga* and 92% were infected by NPV.

Cadavers from wild gypsy moth populations: While the *E. maimaiga* infection rates in the 4-day field bioassays have remained low in all 3 years, infection rates in wild populations have been much greater. Burlap bands were placed around 4 of the trees on each site in June of each year from 1999-2000. In July, when late instar larvae in wild populations were dying from NPV and *E. maimaiga*, we returned to the sites. If cadavers were present, we collected up to 40 cadavers per site from the banded or nearby trees, returned them to the lab, recorded the appearance of each cadaver, then dissected each one to determine if they had been infected by *E. maimaiga*.

In 1999, we collected 342 cadavers from 23 sites and 77% had *E. maimaiga*. In 2000, we collected 436 cadavers from 29 sites and 89% were infected by *E. maimaiga*. In 2001, we collected 140 cadavers from 12 sites and 87% had *E. maimaiga* infection. Most cadavers in each year were co-infected, with at least some NPV occlusion bodies, but when both pathogens are present, *E. maimaiga* typically causes mortality more rapidly than NPV. Over the long-term, this pattern of co-infection could alter NPV dynamics if *E. maimaiga* substantially reduces deposition of NPV occlusion bodies.

Lab bioassay: Each year we collect samples of the upper 3 cm of soil near the bases of the trees for lab bioassays and resting spore quantification. Soil is collected at the same time that the cagelets are set out during the field bioassays in June. In 1999, we collected soil in 4 cardinal directions from each tree. Based on 1999 results, in 2000 and 2001 we collected soil only from the northern and southern aspects of the trees. Soil samples were immediately placed in plastic bags, kept in coolers, then transported back to the lab. In 1999 and 2000, we measured soil temperature and soil moisture where each sample was collected using an Aquaterr soil probe. We can eventually relate these variables to larval infection rates or resting spore density (see below). Sampling equipment was rinsed with 95% EtOH and distilled water between each sample to prevent contamination. Soil samples were kept cool at all times and shipped overnight in insulated coolers to Hajek's lab for larval bioassays.

At Cornell, soil is held at 4 C for 4 months, then brought to room temperature and ca. 35 g (at 200% moisture) of soil is placed in each of 3 polypropylene containers with lids (4.5 cm height x 10.5 cm diameter). Soil cups are placed at 15 C, 14:10 (L:D) for one week before bioassays were conducted to allow time for resting spores to acclimate. After 7 days, 10 early 4th instar laboratory colony gypsy moth larvae are placed in each cup for four days. Larvae are then transferred to individual 29.6 ml plastic cups containing ca. 10 ml wheat germ artificial diet and are monitored daily for mortality and production of conidia for 10 days. This process enables us to quantify *E. maimaiga* infection rates under optimal temperature and moisture conditions.

In 1999, lab bioassays indicated that larvae were infected by *E. maimaiga* when exposed to soil from 12 of the 32 sites. On average, infection rates ranged from 0 to 84% per stand, but overall infection averaged 14.4%. The lab bioassays revealed that there was some microsite effect; infection rates of larvae exposed to soil from the southern and western aspects were low (19 and 17%, respectively) while larvae exposed to soil from the northern aspect had the highest infection rates (27%). This is perhaps indicative of somewhat drier conditions on the aspects that experience high solar radiation.

In 2000, *E. maimaiga* infections occurred with soil from 32 out of 33 stands. The highest level of infection was found in Lake County (stand 12=85.0% infection) and 12 stands had >50 % infection during laboratory bioassays. The average level of infection in 2000 was 43.7 $\pm$ 4.4% (mean $\pm$ SE), a rate that was substantially higher than the infection rate during the field bioassay. Larval mortality again tended to be lower when exposed to soil on the southern aspect than soil from the northern aspect. Bioassays using soil collected in 2001 were initiated in October and are currently in progress.

Resting spore counts in soil: Resting spore abundance/g soil was quantified using the composite soil samples collected for the lab bioassays. Methods involve wet sieving followed by density gradient centrifugation using Percoll, (Hajek and Wheeler 1994). This is an extremely time and labor intensive process. In 1999, resting spore density was quantified in 15 of the 33 stands. Resting spores were predominantly quantified for stands that had relatively high levels of infection in the 1999 lab bioassays because we know the procedure for resting spore quantification is more precise at higher resting spore densities. Samples were pooled by aspect, resulting in a total of 4 samples per stand. Results indicated that there were 80 to 623 spores/g soil on average in each stand. To put these data into context, 250 resting spores/g soil can cause 90% larval mortality under optimal conditions but counts of 5000 resting spores/g are not unusual after epizootics (Hajek and Roberts 1991, Hajek et al. 1998). In 13 of the 15 stands, less than 250 resting spores/g soil were present.

In 2000, soil samples were pooled for northern and southern aspects. The sites where resting spores were quantified were the same as 1999 (although 1 additional stand was added) so that comparisons across years can be made. Resting spores were present in all 16 stands and densities ranged from 37 $\pm$ 6 to 648 $\pm$ 259 resting spores/g dry soil, with an average of 253 $\pm$ 36 resting spores/g dry soil. There appeared to be a trend toward more resting spores in samples from the northern sides of trees but this awaits statistical analysis. We used regression analysis to evaluate the relationship between larval infection rates and the number of resting spores/g dry soil and a publication is in preparation.

It has become apparent that quantification of resting spore density in all stands is a critical part of this project. Therefore, MSU has agreed to take on the responsibility for conducting the resting spore quantification for the remaining samples from 1999 and 2000. Nathan Siegert, the MSU graduate student working on this project, learned techniques from Hajek and her technician at Cornell University in August 2001. Soil samples from 1999 and 2000 that had not been processed were returned to MSU in August. Soil samples from 2001 will be quantified by Cornell between Nov. 2001 and April 2002; any remaining stands will be completed by MSU. We are currently setting up equipment at MSU to run the analyses. Major equipment such as a centrifuge and oven are available at MSU, and we have acquired most of the supplies needed for the sieving process. We expect this process to require several months, but these data are essential if we are to understand the influence of microclimate, weather and stand factors on resting spore persistence and germination.

Cadaver release: In July 1999, we collected late instar gypsy moth cadavers that appeared to have died from *E. maimaiga* from wild populations in Michigan. In 9 randomly selected stands, we released 3-4 cadavers at each cardinal direction around the base of the 4 trees located 25 m from the plot center, after all gypsy moth larvae had pupated. In May 2001, cadavers produced using *in vivo* methods at Cornell (Objective 1) were released in 9 other stands, using similar methods, prior to gypsy moth egg hatch. Additional soil samples were collected from the base of the 25 m trees in all stands and we intend to quantify resting spore density in these samples. In 2002, we will, therefore, be able to compare resting spore density and larval infection rates between stands augmented with wild cadavers, stands augmented with lab-produced cadavers and unaugmented stands.

New in 2001 – 6 week bioassay and intensive soil monitoring: We recognized that the relatively low larval infection rate in our field bioassays compared to lab bioassays in 1999 and 2000 was likely due to sub-optimal moisture or temperature conditions in the field that prevented resting spore germination. In 2001, we initiated a new study designed to identify specific soil temperature and moisture conditions associated with resting spore germination and larval infection. In 3 of our stands, we set cagelets containing freshly molted 4th instar gypsy moth larvae at the north and south aspect of the four trees located 25 m from plot center. Cagelets were retrieved at four day intervals and replaced with new cagelets with fresh larvae. After each 4-day period, cagelets were returned to the Niles lab and larvae were reared to pupation or death as described above. This bioassay ran from May 25 (when most egg hatch occurred in these areas) to July 4 (when pupation was nearly complete). A total of 4800 larvae were used in the bioassay. Dissection of these larvae is currently underway and we will determine larval infection rates for each

4-day period.

During this 6 week bioassay period, we also continuously monitored precipitation, min. and max. air and soil temperatures, min. and max. humidity and volumetric soil moisture at the north and south aspects of representative oak trees in each of the 3 stands. These data were collected with Campbell Scientific dataloggers (loaned by Dr. Bob Haack and Dr. Therese Poland, USDA Forest Service, North Central Research Station) and sensors provided by Dr. Jeff Andresen (MSU). We are excited about these data because we will be able to relate larval infection by *E. maimaiga* to specific temperature and moisture conditions in each 4 day period at each of the 3 sites. This information will also be useful for Objective 2, where we need to identify climatic factors associated with optimal conditions for *E. maimaiga* to assess the potential efficacy of the fungus across the region.

Plans for 2002: The lab bioassay and cadaver dissections from the 2001 field season are in progress and should be completed this winter. Quantification of resting spores in soil samples collected in 2001 and samples remaining from 1999-2000 will begin in October and will require several months to complete.

A major goal in 2002 will be to compare resting spore abundance and germination rates among (a) stands with *E. maimaiga* augmentation using wild cadavers; (b) stands augmented with lab-produced cadavers; and (c) stands that received no additional *E. maimaiga* cadavers. To accomplish this, we will collect soil from the 33 stands for laboratory bioassays and for quantification of resting spores in June, 2002. During the 2002 field season, we will also repeat the 6-week field bioassay/soil temperature and moisture study in 3 of the Michigan stands. MSU and Cornell will both work on quantification of resting spore density in the soil samples collected in 2002, but this process is expected to take several weeks. Analysis of data is underway and will continue in FY 2002.

Objective 4: Recommendations and guidelines for enhancing or introducing *E. maimaiga* will be developed once data collection and analysis have been completed. Preliminary results of our work-to-date, however, have been shared with federal, state and private forest managers, gypsy moth program managers, and other scientists in Michigan and surrounding states (see *Accomplishments To Date*).

CHANGES TO ORIGINAL PROJECT SCOPE OR OBJECTIVES: The scope and objectives of our project have not changed, but we have been able to expand our work well beyond what we initially envisioned. Our collaboration with Dr. Venette and our work with climate modeling have greatly enhanced our ability to address Objective 2. Collaboration with Dr. Andresen will increase the quality of our work in addressing Objective 2 and has been instrumental in enabling us to monitor soil and weather conditions during our intensive 6-week bioassay which will contribute to Objective 3. Our project has been improved by equipment and expertise provided by Dr. Haack, Dr. Poland and Dr. Rothstein. Virtually all aspects of the project are progressing well.

ADDITIONS TO ORIGINAL PROJECT SCOPE OR OBJECTIVES: As noted above, we have not substantially altered our objectives. We were able to augment *E. maimaiga* populations with *in vivo* produced cadavers in 2001 and will evaluate the success of these efforts in 2002, which will fulfill Objective 1. In addition, challenges associated with developing methodology for the lab bioassays and development of *in vivo* resting spores for Objective 1 led to new knowledge about the way that *E. maimaiga* resting spores respond to moisture and temperature. This information will be valuable to others who may be interested in *E. maimaiga* development and production on a larger scale. We have, moreover, expanded our work related to Objectives 2 and 3.

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Hajek, McCullough and Katovich will cooperatively survey and quantify *E. maimaiga* establishment, persistence and infection rates in relation to other characteristics in Michigan forests. McCullough will oversee fieldwork and data collection in Michigan, including site and stand evaluations, bioassays, and comparison of weather data. Hajek will direct laboratory bioassays and *in vivo* spore production at Cornell. Katovich will serve as liaison with USDA Forest Service and assist McCullough in requesting experimental use permits as needed. Venette will cooperate with McCullough in assessing suitability of climatic variables in the Lake States region for *E. maimaiga*. Kaufmann will oversee gypsy moth rearing and cadaver processing at the USDA APHIS Biological Control Laboratory in Niles, Michigan. The Niles Laboratory has the facilities and provides the trained personnel necessary for a gypsy moth rearing project of this magnitude. Andresen will cooperate in analysis of microclimatic and weather-related data.

PRODUCTS AND DUE DATES:
We are continuing to provide reports on our preliminary results at state, regional and national meetings of gypsy moth managers, foresters and others involved in gypsy moth management (see Accomplishments below). Results including protocols for producing and distributing *E. maimaiga*, identification of regions and sites where *E. maimaiga* is likely to be successful, factors useful in predicting *E. maimaiga* success, etc. will be published in peer-reviewed scientific journals, technical publications, extension bulletins and other avenues as needed to reach appropriate audiences.

STATUS OF PRODUCTS/PRESENTATIONS:
We anticipate that all fieldwork will be completed in 2002. Several additional months will be required to complete cadaver and soil processing, analyze data and prepare final reports. Our FHP contact in the Northeastern Area (Katovich) considers our progress to be highly acceptable.

ACCOMPLISHMENTS TO DATE: (products, publications, technology transfer):
Results from several studies conducted by Hajek and her collaborators, including some of those funded under Objective 1 of this project, were described in a manuscript that was submitted for publication in Biocontrol Science and Technology. This manuscript, entitled "Storage of resting spores of the gypsy moth fungal pathogen *Entomophaga maimaiga*", was recently published (Oct. 2001, volume 11: 637-647)..

A second manuscript entitled "Using bioassays to predict resting spore abundance in the soil" is in preparation.

Two abstracts from presentations related to this project have been published:

McCullough, D.G., A.E. Hajek, N.W. Siegert, M. Wheeler, R.C. Venette, W.C. Kaufmann. 2001. Factors influencing success of the gypsy moth biological control *Entomophaga maimaiga*. Abstract. *In:* Proceedings, National Exotics Review. January 16-18, 2001, Annapolis, Maryland. USDA Forest Service, Northeastern Forest Experiment Station. *(In press)*.

McCullough, D.G., N.W. Siegert, A.E. Hajek, M. Wheeler, R.C. Venette and W.C. Kaufmann. 2000. Recent research on gypsy moth eggs and larval mortality in Michigan - implications for other states. Abstract. In: Proceedings, North Central Forest Pest Workshop. Sept. 25-28, Rhinelander, Wisconsin.

Work related to Objectives 2 and 3 is still in progress and we have not prepared manuscripts for scientific journals or technical reports. We have, however, presented preliminary results (oral presentations and posters) from studies associated with Objectives 2 and 3 at meetings of gypsy moth program managers, state and federal forest health managers, scientists and other audiences. These presentations included:

March, 2000: North Central Branch meeting, Entomological Society of America. Minneapolis, MN.
September, 2000: Gypsy Moth Coordinators Fall Training – Kellogg Biological Station, Gull Lake, MI.
October, 2000: North Central Forest Pest Workshop – Rhinelander, WI.
November 2000: National Gypsy Moth Review – Norfolk, VA.

December, 2000: Entomological Society of America – Montreal, Quebec.
January, 2001: National Research Forum on Invasive Pests - Annapolis, MD.
June, 2001: North American Forest Insect Work Conference – Edmonton, Alberta
October, 2001: North Central Forest Pest Workshop – Big Bay, MI
October, 2001: MI Society of American Foresters state meeting – Newberry, MI
December, 2001 (accepted): Entomological Society of America – San Diego, CA

FIRST FISCAL YEAR FUNDED: FY 1999

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH CURRENT FISCAL YEAR

Please note that budgets and paperwork must be handled separately for McCullough at Michigan State University (MSU) and Hajek at Cornell University.

	Item	**Requested Funding**	**Expended Funding**	**Unused Funding**
PREVIOUS YEAR (FY 2001)		**MSU/Cornell**		
Administration	Salary	22,042/16,288		0/0
	Overhead			
	Travel	4500/0		0/0
Procurements	Contracting			
	Equipment			
	Supplies	4170/2000		0/0
YEAR TOTALS		30,712/18,288		0/0
CURRENT YEAR (FY 2002)		**MSU/Cornell**		
Administration	Salary	22,710/14,106		
	Overhead			
	Travel	2000/1000		
Procurements	Contracting			
	Equipment			
	Supplies	1500/1000		
YEAR TOTALS		26,210/16,106		
(FY 2003)		**Requested FHP STDP Funding**	**Other Source Funding**	**Source**
Administration	Salary			
	Overhead			
	Travel			
Procurements	Contracting			
	Equipment			
	Supplies			
Year Totals				
PROJECT TOTALS				

FUNDS NOT USED FROM PREVIOUS FISCAL YEAR:

All funds from FY 1999 and 2000 were used. Funds to cover FY 2001 expenses did not arrive until mid-summer but these funds are expected to be exhausted by the time that FY 2002 funding arrives.

Fiscal Year	**STDP Funding Allocated** MSU/Cornell	**Funds Obligated**	**Funds Unused**
1999	21,400/25,935		0/0
2000	28,188/20,879		0/0
2001	30,712/18,288		0/0

EXPECTED BUDGET FOR NEXT FISCAL YEAR:

FY 2003	**Item**	**Requested FHP STDP Funding** **MSU/Cornell**	**Other-Source Funding**	**Source**
Administration	Salary			
	Overhead			
	Travel			
Procurements	Contracting			
	Equipment			
	Supplies			
Totals				

DIFFERENCE BETWEEN ORIGINAL AND AMENDED REQUESTS AND JUSTIFICATION: The funding requested by MSU for FY 2002 is $1500 higher than what we anticipated when we submitted our report in October 2000. The slight increase in our funding request is needed to cover travel to the 33 field sites to collect soil for lab bioassays and to quantify resting spores in the soil samples. This will be necessary if we are to compare the success of the resting spore augmentation using laboratory cadavers versus wild cadavers, which is an integral part of Objectives 1 and 3. We will also need to travel to three stands repeatedly in 2002 to conduct the intensive 6 week bioassay/soil monitoring, which will contribute signficantly to our ability to accomplish Objectives 2 and 3. Additional supplies will be needed to cover to complete the resting spore analysis of remaining soil samples from 1999 and 2000.

The funding requested by Cornell University for FY 2002 is $1106 higher than what we anticipated in October 2000. The number of larvae needed for bioassays and the number of soil samples to be analyzed will be slightly greater in 2001 than we had anticipated. The funding designated for Cornell will pay for an experienced technician (M. M. Wheeler; 5 months time) to conduct the lab bioassays and quantify resting spores, as well as supplies for both bioassays and quantification. A change to quantification of resting spores is to quantify soil from trees where *in vivo*-produced resting spores were released in comparison with other trees in that stand. This will be done again in 2002. Funds for travel will partially cover costs of travel to meetings to present results from these studies.

Also, it should be noted here that we have received substantial in-kind support for this project from the USDA APHIS PPQ Biocontrol Lab in Niles, Michigan since 199 and this support is expected to continue in 2002. Each year, this lab has assigned up to 6 technicians to work on gypsy moth rearing and cadaver dissections for a period of roughly 4-5 months. Scientists and personnel at Niles also spend considerable amounts of time interacting with McCullough and Siegert at MSU to coordinate logistics and methodology. The Michigan Dept. of Agriculture (MDA) continues to be strongly interested in the results of this project and supports the interagency aspects of our

work. The MDA has provided at least some funding for the past 2 years to help cover costs of supplies for gypsy moth rearing at the Niles lab and related expenses. Additional in-kind support in the form of equipment or supplies has been provided by the USDA Forest Service North Central Research Station, MSU and Cornell.

STDP FUNDING NEEDED:

Fiscal Year	**STDP Funding** **MSU/Cornell**	**Other-Source Funding**	**Source**
2002	26,210/16,106		

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