Special Technology Development Program
Progress Report

PROJECT NUMBER: NA-2001-03

PROJECT TITLE: Entomopathogenic Fungi for IPM of Hemlock Woolly Adelgid

PROJECT STATUS: Continuing

EXPECTED PROJECT DURATION: 3 Years

ORIGINAL EXPECTED COMPLETION DATE OF THE PROJECT: January 2004

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SUBJECT: (mark all that apply using 1 for primary category and 2 for secondary categories: Biological Control __1__ Invasive Species __1__ Methyl Bromide Alternatives _____ Models _____ Monitoring _____ Organism Biology _____ Pesticides: Microbial __1__ Pesticides: Synthetic _____ PTIPS _____ Population _____ Risk and Hazard _____ Remote Sensing _____ Semiochemicals _____ Silvicultural Technology _____ Social Values __2__ Spray Technology __2__ Other: Non target organisms __1__.

STATUS OF SUBJECT SPECIES: non-native noxious

PROJECT OBJECTIVES (question to be answered by the project):

1. Field test select indigenous strains of entomopathogenic fungi and a commercial strain for efficacy against HWA to identify the best application time and most suitable dosage.
2. Determine the yearly carryover of entomopathogenic fungi and their impact on HWA populations.
3. Assess, in laboratory assays and forest trials, the impact of select fungi on *Pseudoschmnus tsugae,* a coccinellid predator being released for HWA suppression.

BRIEF DESCRIPTION OF PROJECT:

We are conducting research to develop entomopathogenic fungi for management of the hemlock woolly adelgid (HWA), *Adelges tsugae*, and to examine the effect of these fungi on a non-target HWA predator, *Pseudoscymnus tsugae*. In 2001, we established sites within HWA-infested forests in which to conduct field tests. Three indigenous isolates and a commercial fungal-based formulation are being tested against HWA when applied in the spring or fall. Indigenous fungi were selected for virulence based on previous laboratory assays. Trees selected for the tests will be used to assess the fungal carryover effect. For the spring trial, isolates were mass-produced on a solid substrate, and fresh spores were applied as a formulated suspension. For the fall application, techniques were developed to dry harvested spores yet maintain their viability and virulence. Once dried, this viable spore powder will remain viable for several months. Laboratory trials were conducted to develop assay procedures to test the susceptibility of *P. tsugae* to our test fungi. Additional trials will be conducted in Year 2 to further evaluate the fungal susceptibility of this predator. Results from this project will provide critical information on how best to include entomopathogenic fungi in a comprehensive IPM approach to HWA.

SUMMARY OF RESEARCH ACCOMPLISHMENTS:

Spring Field Application (Obj. 1). On 19 April 2001, we selected 68 hemlock trees at Mount Tom State Park, Holyoke, MA with active HWA infestations for the spring fungal application. Eight trees were used to monitor HWA development and determine the best time to apply the fungi. In the first two HWA population samples (20 April and 01 May), the number of live overwintering adults was 7.8 and 6.0 per twig, respectively. As per our original proposal, we planned to make a fungal application to target the overwintering adults. After further consideration, we instead chose to target the new generation of immature HWA, including crawlers and second instars. The overwintering adults are non-mobile and hidden beneath a woolly mass, which may be difficult to penetrate with fungi. In contrast, the crawlers are unprotected by wool and are mobile, moving over the surface of the branch where they could come in contact with spores. This maximized the potential for HWA to become infected through indirect as well as direct contact with the inoculum. Based on the 1 May sample, we found that the overwintering adult population produced 91.6 eggs (± 34.8) per sample twig or 28.7 (± 10.3) eggs/cm of twig.

The spring application was made on 25 May, at which time the HWA population was 24.7 (± 3.5) immature HWA per sample twig (average twig length 4.5 cm (± 0.6)) and 111 unhatched eggs per twig. Spray applications were made using a handheld, CO_2 pressurized (35 psi) sprayer (nozzle - SFC TG 0.3). Three experimental isolates (*Metarhizium anisopliae*; Ma-1080, *Beauveria bassiana*;GA-082 and *Paecilomyces farinosus*; AT-159) and a commercial standard (*B. bassiana*; GHA Strain, Mycotech) were mass produced on a solid grain substrate, from which the fresh spores were extracted by suspending them in liquid (Table 1). Each fungus was applied at concentrations representing a medium and high dosage, 5×10^7 and 1×10^8 spores/ml spray suspension (water w/ 0.0025% Silwet and 0.001 % Tween 80). Each treatment was replicated four times, with non-sprayed and blank-sprayed controls. Treatments were randomly assigned to individual trees (4 trees/treatment).

Table 1. Select entomopathogenic fungi for field testing

Fungal Species	Isolate #	Origin	Original Host	LC_{50} for HWA
M. anisopliae	MA 1080	Vermont	Maple leafcutter	7.9×10^5 spores/ml
B. bassiana	GA 082	Massachusetts	HWA	1.1×10^7 spores/ml
B. bassiana	GHA (Mycotrol)	Unknown	Grasshopper	1.2×10^{13} spores/ml
P. farinosus	AT 159	So. Connecticut	HWA	7.7×10^7 spores/ml

A third treatment concentration representing a low dosage was planned. However, because we were targeting mobile crawlers, we reduced the range of test dosages and instead used these trees to assess the suitability of enclosing treated branches within mesh bags. These bags would prevent movement of untreated crawlers onto treated branches, which could mask treatment effects. This was done for 4 additional trees each for those treated with the high MA 1080 concentration and a set of non-sprayed and blank controls. We also conducted a preliminary experiment (2 trees/trt) to assess the efficacy of applying blastospores, produced by liquid fermentation, rather than conidia produced on a solid substrate. Previous laboratory trials indicate that blastospores are sometimes more virulent than conidia, though they lose viability relatively rapidly and are hightly sensitive to UV light. Blastospore applications were made with the *M. anisoplia* and *B. bassiana* isolates and compared with a water control. These were applied on 22 May.

On 18 June and 14 August, samples were taken to determine treatment effects on the 1st and 2nd generation of HWA, respectively. Five HWA-infested twigs from each sample tree were examined microscopically, and the number of each selected life stage recorded. On 18 June, differences in the percent mortality of immature HWA or the total number of live crawlers per cm of twig among the treated and control trees were not significant ($\propto = 0.05$) for any of the experiments. The average percent mortality in the open and bagged branch experiments was 44.6% (± 2.2) and 41.3% (± 3.6), respectively. In the blastospore experiment, one treatment (GA 082 – *B. bassiana*) had appreciably greater mortality (72% ± 4.0) compared

with the controls (43.5% ± 4.0), but differences were not significant. The average number of live crawlers per cm twig was 3.6 (± 0.1), 4.7 (± 0.5) and 2.4 (± 0.4) for the open, bagged and blastospore experiments, respectively, with an overall average twig length of 4.4 cm (± 0.1). Few 1st generation progridian adults or 2nd generation egg masses were present on 18 June. Though several winged sexupare adults were observed on the samples, it was impossible to determine how many were formed and flew off the twigs.

On 14 August, live sistens (2nd generation HWA), the only living HWA life stage present, were counted. No attempt was made to quantify the number of dead HWA because it was impossible to ascribe them to the sisten, 1st spring or previous fall generation. Therefore, rather than determine percent mortality, we assessed the number of live sistens/twig as an indication of a fungal effect, which ultimately reflects the level of continuing HWA pressure. No significant effects from the fungal treatments were observed. The average number of live HWA over the three experiments was 2.2 (± 0.21)/cm of twig with an average twig length of 4.7 cm (± 0.1).

Several factors may have contributed to our inability to detect fungal activity. First, rain began to fall within hours of making the application, which continued for several days. A significant portion of the spray may have been washed away before spore germination on the insect could occur. We applied the fungi as a fresh spore suspension, which once prepared would not remain viable for >48 h. For this reason we had to make the application despite the threat of rain. As a result of this experience, we have now developed methods to prepare fungi as dry spore powders, which can be stored for months. This gives us more flexibility to make applications at the most optimal time with regard to weather. Another factor that made it difficult to detect a fungal effect was the high level of natural mortality among the HWA population. Although we believed we had selected trees with a newly established and apparently healthy, building HWA population, there is evidence from our subsequent sampling that in fact the population and tree health may have been in decline. For instance, we selected trees with fresh apparently live buds on the twigs, but on 19 June and 14 August only 7.1 & 17.1% of the twigs collected, respectively, had new growth on which the second generation HWA (sistens) could settle and feed. This may have led to the extensive mortality and low number of sistens relative to the previous overwintering population. For subsequent trials, we will take larger twig samples to ensure there are sufficient HWA populations for treatment evaluation.

The lack of discernable differences in HWA populations between the bagged and unbagged branches suggests that this technique may not be necessary for assessing fungal efficacy in the spring. However, because overall HWA populations were low, it may not have been possible to detect differences among these two treatments. This experiment provided critical information for work projected for the 3rd year in Obj. 3, when *P. tsugae* will be enclosed on branches and treated with fungi. Blastospores showed promise for HWA management and we will consider conducting additional trials with this fungal stage in the future.

Year 1 Conclusion. Spring applications of entomopathogenic fungi should be timed to target maximum numbers of newly hatched immatures (May 20- June 1). The most suitable fungal depends on the isolate and should be ≥1 x 10^8 spores/ml spray suspension.

Fall Fungal Application (Obj. 1). We selected 44 trees having branches with new growth and an apparently recent HWA infestation. As for the spring application, high and medium concentrations were tested, but the high concentration was increased from 1 x 10^8 to 2 x 10^8. Two experimental isolates (Ma-1080, GA-082) and a commercial standard (Mycotech GHA Strain) were applied at 5 x 10^7 and 2 x 10^8 spores/ml spray suspension (water w/ 0.0025% silwet and 0.001% Tween 80). Testing of the *P. farinosus* isolate was discontinued because dry spores could not be readily harvested. This would make it an unsuitable candidate for future widespread use. We increased the replication from 4 to 5 trees per treatment.

When taking spore deposition data for our spring trial, we noted an abundance of epiphytic fungi present on the hemlock needles that may be antagonistic to our fungi. The fermentation broth residue from

fungal production may contain secondary metabolites that could suppress these epiphytes. We conducted an experiment (2 trees/trt) in which the fermentation broth was used as the carrier for the GA-082 fungus (2 x 10^8 spores/ml). This was compared with a broth only control. On 17 September a pre-treatment sample was taken and spray applications were made. A post-spray sample will be taken 16 October, after which data from these trials will be compiled.

Year 1 Conclusion. Fall applications of entomopathogenic fungi should be timed to target maximum numbers of overwintering sistens before they develop a protective woolly covering (15 Sept. - 1 Oct.). The most suitable fungal dosage depends on the isolate but should be between 1 x 10^8 and 2 x 10^8 spores/ml suspension.

Fungal Carry-over (Obj. 2). The applications made in the spring and fall 2001 forms the basis for assessing the yearly carry-over of entomopathogenic fungi and their impact on HWA. Trees treated in 2001 will be sampled in the spring of 2002 for this objective.

Year 1 Conclusion. Spring and fall applications of fungi have been made and will be assessed for persistence in year 2.

Predator Susceptibility (Obj. 3). A total of 1,000 adult *P. tsugae* were supplied by the N. J. Dept. of Agric., Alampi Beneficial Insect Lab for our laboratory assays. Experiments were conducted to evaluate 44 different combinations of assay containers, moisture levels and food source to develop a standardized assay protocol that encouraged beetle survival in the controls. The most suitable bioassay container was found to be a 35 x 10 mm Petri dish lined with a sterile filter paper. A thin bead of honey (42.6 mg ± 3.6) was streaked along the centerline of the filter paper as a food source. A Potter spray tower was used to apply (15 psi) 4 ml of spray liquid to individual dishes. Moisture applied during spraying was sufficient to keep the insects alive. Each dish was covered with a fitted lid immediately after treatment and sealed with parafilm to retain moisture and prevent escape. They were held at 22°C in the dark. An average of 83% survival after 7 d at was obtained using these assay methods.

A dose titration assay was conducted using the commercial fungus, *B. bassiana* (GHA Strain, Mycotech Corp). The fungus was tested at six concentrations, ranging from 4.1 x 10^5 to 1.0 x 10^8 spores/ml of water containing 0.0025% Silwet, and water and formulation blank treated controls. Each trial contained 10 beetles/dish/treatment and was replicated five times. Mortality was assessed on days 4, 7 and 10. On day 4, mortality was <2% in the controls and 16% at the highest fungal concentration. By day 7, mortality of 68 and 48% was obtained in the 2 highest concentration treatments and 26% had died in the control blanks. After day 7, mortality increased in all treatments and controls.

Because control mortality in the preliminary dose assay was relatively high, we will conduct additional tests to further refine the assay method and reduce control mortality. Our initial results suggest that *P. tsugae* may be somewhat susceptible to high dosages of the commercial isolate under laboratory conditions. Next year we will also assess the virulence of our experimental isolates to this predator. It should be noted that laboratory assays may not reflect results that occur in the field. Therefore, final assessment of the compatibility of fungi with *P. tsugae* can only be made after data from the laboratory and field-based trials are fully analyzed.

Year 1 Conclusion. Laboratory assay methods for determining impact of fungi on *P. tsugae* have been developed.

CHANGES TO ORIGINAL SCOPE OR OBJECTIVES:

- Tested 2 rather than 3 fungal concentrations.
- Targeted HWA immatures rather than overwintering adults for the spring treatment.
- Discontinued evaluation of one experimental isolate due to its poor mass-production characteristics.

ADDITIONS TO ORIGINAL PROJECT SCOPE OR OBJECTIVES:

- Tested the suitability of bagging branches.
- Tested blastospore suspensions as well as conidial formulations.
- Tested the value of including fermentation broth in fungal suspension to suppress antagonistic fungi.

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COOPERATORS (contributing to, but not leading the project):

Charles Burnham	Massachusetts Division of Forests and Parks
Allison Wright	Massachusetts Division of Forests and Parks
Michael Brownbridge	University of Vermont, Entomology Research Laboratory
Scott Costa	University of Vermont, Entomology Research Laboratory
Svetlana Gouli	University of Vermont, Entomology Research Laboratory
Mark S. McClure	CT Agric. Exp. Station

COOPERATOR INVOLVEMENT:

Charles Burhnam	Site selection and setup, HWA sampling	<5%
Allison Wright	Site selection and setup, HWA sampling	<5%
Michael Brownbridge	Conduct lab trials on non-targets, Assist with field applications and evaluation	15%
Scott Costa	Conduct research, spray application, data analysis HWA sampling	20%
Svetlana Gouli	Mass produce fungi for pilot test, HWA sampling pilot test evaluation	10%
Mark McClure	Consult on all matters relevant to the biology and ecology of *P. tsugae*	<5%

PRODUCTS AND DUE DATES:

2001: Year 1 pilot test spray and sample collection and evaluation of Fall and Spring applications. Initiate lab bioassay of *P. tsugae*. Analysis of data for research completed. Submit progress report.

STATUS OF PRODUCTS/PRESENTATIONS

- Pilot test sprays and sample collection for spring applications complete
- Pilot test sprays for fall application complete, final collection scheduled for 16 Oct.
- Laboratory bioassay for *P. tsugae* developed.
- Statistical analysis completed on data collected.
- Progress report submitted.

Products:

- Standard bioassay system for comparing the susceptibility of *P. tsugae* to insect-killing fungi.
- Standard method for mass-producing fungi on solid substrate and preparing viable dry spore powder.

Publications: None
Technology Transfer: None

FIRST FISCAL YEAR FUNDED: 2001

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH CURRENT FISCAL YEAR:

	Item	Requested Funding	Non-Federal Funding	Received Funding	Expended[1] Funding
Fiscal year 2001					
Administration	Salary	32,000	6,587	38,587	12,779
	Overhead	11,072	2,273	13,345	13,345
	Travel	3,000	0	3,000	1,213
Procurements	Contracting	0	1,000	0	1,000
	Equipment	1,000	0	1,000	0
	Supplies	5,000	0	2,000	100
Indirect Charges			12,466	12,466	12,466
Year Totals		**52,072**	**22,326**	**74,398**	**40,903**
Fiscal Year 2002					
Administration	Salary	34,000	10,000	44,000	
	Overhead	11,730	3,450	15,180	
	Travel	3,000		3,000	
	Contracting		1,000	1,000	
	Equipment	0	0	0	
	Supplies	4,000	0	4,000	
Indirect Charges			12,944	12,944	
Year Totals		**52,730**	**27,394**	**80,124**	

[1] A total of $33,495 remains unexpended in the FY 2001 budget. Though costs were incurred associated with this project, we were unable to use funds from this budget because the Univ. of VT Office of Grant & Contract Accounting did not set up the budget and assign a budget number until a month ago, despite our repeated requests. These funds will be used to replenish supplies in the next few months. This delay was beyond our control. In addition, fieldwork associated with this field season is still underway, for which FY 2001 funds will be needed.