Special Technology Development Program
New Project Proposal

PROJECT NUMBER: NA-2001-03

PROJECT TITLE: Entomopathogenic Fungi for IPM of Hemlock Woolly Adelgid

PROJECT STATUS: New

EXPECTED PROJECT DURATION: 3 Years

EXPECTED COMPLETION DATE OF THE PROJECT: January, 2004

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):

Overall Goal: Evaluate the efficacy of entomopathogenic fungi as a component of a hemlock woolly adelgid (HWA) management strategy.

Specific Objectives:

1. Field test select indigenous strains of entomopathogenic fungi and a commercial strain for efficacy against hemlock woolly adelgid 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:

Indigenous entomopathogenic fungi, isolated from HWA and other forest insects in the Eastern US will be mass-produced and formulated. They will be tested against HWA in replicated forest trials. The isolates selected for testing represent fungi demonstrating the highest pathogenicity in laboratory bioassays. Each was chosen for their mass production potential and ability to germinate and grow rapidly at temperatures that exist when field applications are proposed. In addition, a commercially available fungal-based product, Mycotrol™, (Mycotech Corp.) will be tested. Three dosages (low, medium and high) will be tested at two application times (Fall and Spring). The carryover effect of the fungi at different dosages will be evaluated twelve months following application. We envision that fungi should be used as a component of a total IPM program that includes natural enemies. Therefore the test isolates will be tested against *P. tsugae*, the most promising natural enemy currently under development. Adult beetles will be bioassayed in the laboratory and caged field trials will be conducted to determine direct and indirect impacts of fungi on this predator.

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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%

JUSTIFICATION (How does the project strengthen FHP program delivery/capability? What is the potential advantage over existing technology?):

Why are effective management strategies for HWA needed?

Eastern hemlock, *Tsuga canadensis,* is an important component of the northern forest ecosystem, about 1.3 million hectares of this species occur in the eastern US (Rhea 1995). In this region, 4.16 million cubic meters are harvested annually, worth an estimated $207 million (Rhea 1995; D. Tobi, Univ. of VT, pers. comm.) In 1995, the nursery industry of North Carolina and Tennessee maintained $34 million in hemlock growing stock (Rhea 1995), and an equal value is likely to exist in other eastern states where ornamentals are produced. Because it can grow in moist, poorly drained locations, hemlock is an essential tree species in riparian zones, providing critical protection from erosion along stream banks. Without shade from this tree, the water temperature in streams increases, negatively impacting fish populations. In addition hemlock provides shelter in the winter for deer and is a significant source of food for birds and other wildlife. HWA is an exotic species that is expanding its range, at an estimated 20 miles annually. The entire range of eastern and Carolina hemlock is at risk within the next 20-30 years (Rhea, 1995). Hemlocks die within 3-6 years after infestation if HWA populations are not suppressed (Orwig and Foster, 1998). The recently detected infestations in Maine and New Hampshire highlight the need for immediate action. These represent a significant expansion of the area of infestation into a region where an abundance of the host occurs. Effective methods to manage HWA are lacking. Though some chemical insecticides have been found to suppress HWA on ornamental hemlocks, they are ineffectual and impractical in forest settings. Environmental and economic constraints limit the long-term feasibility of chemical controls. Biological control remains the most suitable and logical strategy for HWA management.

Why are entomopathogenic fungi promising?

Entomopathogenic fungi comprise a group of naturally occurring organisms that penetrate, multiply within and kill their insect hosts. This complex includes species in several genera, including *Beauveria*, *Verticillium*, *Paecilomyces* and *Metarhizium*. The biology of fungi is uniquely developed to enable them to remain dormant when host material is unavailable or when climatic conditions are unfavorable for fungal survival, making them ideal organisms for a sustainable management strategy. A recent stunning example of the effectiveness of fungi for sustained management of insect pests is *Entomophaga maimaiga*. This fungus has largely eliminated the widespread defoliation caused by gypsy moth in the Northeast.

We believe fungi are particularly promising for HWA biocontrol for several reasons. We have found entomopathogenic fungi infecting field populations of HWA throughout its eastern range in the US, and in low-level populations in China, the suspected origin of HWA. Many of these fungal species are relatively easy and inexpensive to mass-produce, and have little or no negative impact on the environment, humans or non-target organisms. Compared with other natural enemies, an effective fungal agent can impact the pest population relatively quickly (in 10-14 d), thereby avoiding the delay, for example, that is needed while a predator population builds up.

What are the advantages of fungi over other available management tools?

In North America there are two methods of managing HWA, chemical insecticides and predators. Insecticides are expensive, demonstrate limited efficacy on small trees, and little or no efficacy on mature trees (McClure 1987). Because of their negative impact on fish and water quality, they cannot be used in riparian zones, where hemlock commonly grows and plays a critical role in reducing erosion and flooding. There are also environmental and human health issues associated with their use in suburban and forest areas where hemlock is often found. The two insect predators that are currently under development show potential, but have provided several challenges. They are difficult and expensive to mass rear (R. Chinaise, NJ Dept. of Agric., pers. comm.). Their capacity to effectively reduce HWA populations is as yet uncertain

as is their ability to become established following release (B. Onken, US For. Serv., pers. comm.). In contrast, entomopathogenic fungi are relatively easy and inexpensive to mass-produce, and standard methods for this purpose have been developed. Standard spray equipment such as that used for chemical pesticides is suitable for applying these materials in forest and suburban settings. Based on previous research the impact of these fungi on the environment is significantly less than that of many chemical pesticides. Our previous research demonstrates that several indigenous strains are highly pathogenic to HWA (Gouli et al. 1997; Parker et al. 1997, 1998).

We recognize that no biological agent or chemical compound can be expected to provide the "silver bullet" that will eliminate the devastation of HWA (Onken 1998). Nor can quarantines, silvicultural techniques or genetic selection completely solve the HWA problem. Rather, a multi-faceted IPM package must be devised that incorporates all of these potentially compatible tools into an effective long-term approach to suppress HWA and protect the hemlock resource.

URGENCY (Does the project address a crisis situation? Would delay result in irreversible loss?): ***Yes.***

This project was prepared in direct response to needs identified by state forest protection personnel in northern New England (W. Archey, MA State Forester, pers. comm.). HWA is now established in 11 eastern states, from North Carolina to Massachusetts, and has caused widespread mortality of hemlocks in forest and suburban settings. The latest reports indicate that 25% of hemlock in southern areas of this region are infested with HWA, and tree mortality is rapid (McClure et al.1996, Orwig & Foster 1998). Effective management strategies, particularly non-chemical approaches, do not currently exist. Every effort must be made now to develop management methods that work to protect the hemlock resource.

Judging from the infested areas in Connecticut, Massachusetts, New Jersey and Virginia, the natural ecosystem in HWA infested areas is being dramatically changed as a result of mortality of Eastern hemlock. Invasive alien plant species are replacing hemlock and outcompeting native vegetation. If this trend is not stopped, the ecosystem will be altered and valuable plant groups will be lost. In addition, several spot infestations of HWA have been detected in New Hampshire and Maine, and within 10 miles of Vermont's border. Hemlock comprises 1/6th of the forest in these states. It is the third most abundant tree in Vermont. If HWA is not stopped now and becomes established in forested regions of these states, the ecological degradation that has already occurred in states farther south will take place in these newly infested regions.

NATIONAL FHP TECHNOLOGY DEVELOPMENT PRIORITY (check one):

Priority 1: __X__ Priority 2:_____ Priority 3:_____ Priority 4:_____.

TECHNICAL COMMITTEE DEVELOPMENT PRIORITY (check one):

Priority 1:_____ Priority 2:_____ Priority 3:_____ Priority 4:_____.
Priority 5:_____ Priority 6:_____ Priority 7:_____ Priority 8:__X__.

SCOPE OF APPLICATION:

This project targets HWA, which is currently a pest of several hemlock species in the eastern US. The results of this work will apply to the entire range of eastern hemlock, including eastern and mid-western US, the Appalachian Mountains and parts of southern Canada. Also affected by HWA is Carolina hemlock, which is confined to the lower slopes and rocky stream beds of the Blue Ridge Mountains and Appalachian Mountains of North and South Carolina. Though HWA is the focus of this project, results will also apply to balsam woolly adelgid, a closely related species, which impacts fir trees in the southern states, and is a recurrent pest problem for Christmas tree growers. Results from this work will accelerate the further evaluation and future registration and usage of commercially available fungal-based products. These products have not been fully assessed under field conditions nor are application strategies perfected.

RESEARCH BASIS (strength of research basis, including publication citations):

Development of fungi for IPM is a multi-phase process. Scientists at the Univ. of VT Entomology Research Lab have been working on it as funds were available. In 1996-97 we conducted a search for isolates in HWA in four states throughout its current eastern range (VA, NJ, CT, MA). Over 150 isolates were recovered (Gouli et. al. 1997; Parker et. al. 1997). These have been assayed in the laboratory on field-collected HWA to determine pathogenicity. The most pathogenic strains were then characterized to determine which produced the most spores and at what temperature they germinated and grew the fastest, (Parker et. al. 1998). This information was critical for selecting strains that have the greatest potential for efficacy under field conditions and would be the easiest to mass-produce. Based on this research, three indigenous isolates were chosen. The next critical step is to test them under forest conditions. In addition, we will include a commercially available fungal-based product in our trials. Though this product was not specifically developed to target HWA, if effective, it will enable rapid use of these biological control tools.

Through this project we will determine the best time of year to apply fungi and at what dosage to maximize control. Fungi can be used either as an inoculative release, much like a predator, or as an inundative treatment, like a pesticide spray. This project will provide direction in selecting the strategy that is the most appropriate and effective. Rarely does one biological control agent alone suppress pest populations sufficiently. A complex of compatible agents must be developed. Therefore, it is critical to evaluate the impact of our test fungi on *P. tsugae*, which currently is being mass reared and released in a number of states for HWA control. Results from this project will pave the way for developing a comprehensive strategy for long-term management of the most serious forest pest threatening the northern forest resource today.

METHODS (project design, hypothesis, statistical approach, and QA/QC procedures):

Obj. 1. Field test select indigenous strains of entomopathogenic fungi and a commercial strain for efficacy against hemlock woolly adelgid to identify the best application time and most suitable dosage.

Three fungi have been selected for field testing based on their high virulence to HWA and potential for mass-production (Table 1) (Parker et al. 1998). The fungi will be produced on a solid substrate (eg., millet or rice) using methods developed at our laboratory and formulated with an ES carrier from Mycotech Corp. (Butte, MT) (unpublished data). A commercial fungal-based product, Mycotrol™, (Mycotech Corp.) will be included in the tests. It is not as virulent as our isolates based on the LC_{50} value, but because it is registered, it could be used against HWA now based on our results (Table 1).

The fungi will be tested for viability before application, and only those with >90% germination after 24 h will be used. The field tests will be conducted in Massachusetts on hemlocks in the early stages of infestation by HWA. Each isolate will be tested at three concentrations ($5x10^7$, $1x10^8$ and $2x10^8$ spores/ml suspension), which represent a low, medium and high dosage, based on our past pathogenicity trials. Treatments made with the emulsifiable suspension (ES) formulation alone will serve as a control. Applications will be made in the Spring and Fall. The Fall application will target aestivating sistens, who at this time are not protected with a woolly coating. The Spring application will target reproductive adults, which may be more susceptible to infection because of winter stress. Field tests will be replicated twice, in Year 1 and 2.

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

In each year, 112 dominant or co-dominant eastern hemlocks, having a relatively homogenous, moderate level of HWA infestation, will be selected. Fifty-six trees will be used for the Fall application and 56 trees for the Spring application. Four trees will be reserved as unsprayed controls. These will provide valuable data on natural mortality. Four of the 56 trees will be sprayed with the ES blank. Of the remaining 48 trees (56-8), 12 trees will be used for each test isolate, four trees per dosage. Treatments and the time of application (Fall vs. Spring) will be randomly assigned to individual trees.

The terminal 1.5 m on a single branch of each tree will be sprayed to run-off using a pressurized backpack sprayer. Three weeks after treatment we will remove 5 branchlets (5 cm long) from each sprayed branch and bag each one separately. They will be evaluated microscopically within 48 hr to determine the number of live and dead HWA in each development stage. HWA that do not respond to slight probing, appear off-color, or have a negative hemolymph pressure when probed will be considered dead (Parker et al. 1999). The data will be analyzed using the general linear model of analysis of variance (SAS 1996) to examine for significant (alpha=0.05) effects of treatment, concentration and application season. The fungal treatments will first be contrasted with the control and then analyzed separately based on the factorial structure. The error terms outlined by Zar (1974) will be employed to analyze the mixed models as fixed (fungi, concentration, season) and random (application year) effects. The P-diff procedure in SAS will be used for pre-planned comparison of least-significant means where appropriate.

Obj. 2. Determine the yearly carryover of entomopathogenic fungi and their impact on HWA populations.

The fungi we are testing are facultative saprophytes, and can exist in the absence of an insect host. This enables them to persist in the environment, which could enhance their biological control potential by eliminating or reducing the need for repeated sprays. Control and treated branches from Obj. 1 will be used to assess the on-going effect of the fungal applications. These will be examined 12 mo after the initial efficacy evaluation to determine HWA mortality and fungal impact on population changes. To limit re-infestation of the treated branches, a sticky barrier will be put around the stem leading to the treated section of each branch. Adjacent branches coming into contact with the treated branch will be trimmed back. The sampling procedure outlined in Obj. 1 will be used. The total number of HWA, dead and live, will be recorded. These data will be analyzed in combination with the Obj. 1 data. We consider the sample taken destructively from the same branch during the first and second year to be independent samples. The sampling time will be considered as a fixed variable and analyzed on a within branch/tree basis.

Obj. 3. Assess the impact of promising strains of entomopathogenic fungi on *Pseudoschmnus tsugae* in lab bioassays and caged field trials.

Lab Bioassays. *P. tsugae* used in the laboratory bioassays and field cage trials will be obtained from the NJ Department of Agriculture Beneficial Insect Laboratory. Dr. Brad Onken, USDA Forest Service, who manages the distribution of the *P. tsugae* available for release and research, has committed to provide insects for the research outlined herein. The pathogenicity of a water suspension of the four test fungi will be evaluated at the three dosages used for the field trials in Obj. 1. Test fungi will be grown on an agar medium.

Because of the small size of *P. tsugae*, we anticipate that the standardized lab bioassay systems we use to evaluate the pathogenicity of fungi to thrips (a very small insect) will be appropriate. Any minor modifications to the assay to accommodate *P. tsugae* will be done in Year 1. The assay method will test both the effect of direct and residual contact with the fungi simultaneously. Five adult *P. tsugae* will be placed on a filter paper disk at the bottom of a clear 30-ml plastic cup. Using a Potter Spray Tower, 2 ml of fungal suspension will be sprayed onto the insects in the cup. A thin line of honey across the filter paper will serve as a food source during the bioassay (M.S. McClure, pers. comm.). The cup will be covered with parafilm. Assay containers will be held at 22°C with a 14:10 (L:D) photoperiod. Controls will be treated with sterile distilled water only. Mortality will be evaluated daily for 14 d. Each treatment will be replicated 5 times

within a bioassay and three trials will be conducted. The data will be analyzed separately in SAS using the general linear model of analysis of variance model. The P-diff procedure in SAS will be used for pre-planned comparison of least significant means as appropriate.

Field cage trials. This trial will be conducted in the spring, the only time when *P. tsugae* adults are available. HWA-infested branches (0.5 m long) will be sprayed with the 4 test fungi, as described in Obj. 1. However, more then one branch on a given tree will be used because our previous research suggests that the potential for cross contamination of fungi between branches is minimal in trials of short duration. Each tree will serve as a block with every treatment represented on a single tree, including the control. To avoid cross contamination, branches will be selected at the same level in the canopy. Treatments will be randomly assigned to branches having a homogenous, moderate population of HWA. Two test concentrations will be selected based on results from the lab assays. There will be 6 replicates for each treatment and the ES formulation blank control. After spraying, each branch will be enclosed in a screen bag to which 20 *P. tsugae* adults are added. After 3 wk the branch will be excised and the number of live and dead *P. tsugae* and HWA will be counted. Results will provide us with information under field conditions on the following: a) the predator effect alone on HWA, b) the combined effects of the predator and fungus on HWA; c) the secondary effect of the fungus on the predator. The data will be analyzed as a randomized complete block design in SAS using the procedure for the general linear model of analysis of variance. The P-diff procedure in SAS will be used for pre-planned comparison of least significant means where appropriate.

MEASURES OF SUCCESS:

Standard of Success: The efficacy of select entomopathogenic fungi will have been demonstrated in field infestations of HWA with statistically sound data and their compatibility with an important predator assessed. This information will lay the foundation for future studies that focus on large-scale implementation of this technology.

Expected Outcomes:

Forest trials: Because HWA lacks a protective waxy wool covering in the Fall, we expect that applications made at this time will be more effective than those made in Spring. With regard to dosages, at this time we cannot anticipate which rate will be most efficacious, but believe the ES formulation we use will maximize fungal effectiveness. It is expected that some carryover effect will be evident. Because we have included multiple fungal species with different epizootic potentials, we expect variability in their persistence. This information will enable us to design strategies tailored to the unique characteristics of each fungal species, such as how often to make applications and at what rate. This will provide insights into the long-term costs and benefits of a fungal-based management technology.

Non-target assays: Based on previous results on non-target organisms conducted at our laboratory (Parker et al., pers. comm.), we expect there will be minimal impact of our isolates on *P. tsugae* and we will probably observe variability in virulence among isolates. We also expect that *P. tsugae* mortality will be less in the field trials than in the lab assays.

Implementation of Products/Methods: At the end of this research we expect to have one - two fungal isolates with data supporting their use in the IPM of hemlock. Information on the best time to make applications and at what rates will be obtained. We will also be able to specify its use in concert with *P. tsugae*. Because we are including a commercially available fungal product in our trials, the results will be immediately transferable for HWA management. Information from this project will hopefully lead to increased interest in the potential of fungi and funding to support larger-scale forest trials. If our other strains are more virulent than the commercial strain, our studies may stimulate interest in pursuing commercial support for future registration.

PRODUCTS AND DUE DATES:

October 15, 2001 – Complete Year 1 pilot test spray and sample collection and evaluation for Fall and Spring applications. Initiate lab bioassay of *P. tsugae.* Analysis of data for research completed. Submit progress report.

October 15, 2002 – Complete Year 2 pilot test spray and sample collection/evaluation for Fall and Spring application. Evaluate carryover effect for Year 1 applications. Complete lab bioassay of *P. tsugae.* Analysis of data. Prepare manuscript on pilot test results and progress report.

October 15, 2003 - Evaluate carryover effect for Year 2 pilot test applications. Complete caged assays with *P. tsugae*. Analysis of data. Prepare manuscripts on carryover effects of fungal application and results of caged assays. Submit final report.

PUBLICATIONS (how results will be reported):

We anticipate preparation of 2-3 manuscripts for submission to appropriate refereed publications (eg., J. of Economic Entomology, Biological Control, J. of Invertebrate Pathology). In addition, we will prepare several short articles and reports for non-refereed newsletters and publications, such as the Forest Service's HWA Newsletter, Tree Topics (publication produced by Bartlett Tree Experts), Northern Forests Magazine, and assorted regional newsletters for nursery personnel and foresters. Presentations will also be given at appropriate professional meetings, such as the Entomological Society of America and Society of American Foresters, Northeast Forest Pest Council, etc.

TECHNOLOGY TRANSFER: By including a currently registered fungal-based product in this project, we will provide forest pest managers with techniques for regulating HWA that may be immediately implemented. By presenting the results of this work at professional meetings attended by forestry professionals and pest specialists and in forest-related newsletters, information on the appropriate use of this product will be disseminated.

LONG-TERM BUDGET REQUEST (estimates by fiscal year and funding, both monetary and in-kind, excluding FHP base funding:

	Item	Requested FHP STDP Funding	Other-Source Funding[1]	Source
FIRST YEAR				
Administration	Salary	32,000	10,000	Univ. of VT, VT Dept. For. & Parks, MASS Div. of For.
	Overhead @34.6%	11,072	3,460	Same as above
	Travel	3,000		
Procurements	Contracting	3,000	1,000	Mycotech Inc.
	Equipment	1,000		
	Supplies	2,000		
Year Totals		**52,072**	**14,460**	
SECOND YEAR				
Administration	Salary	34,000	10,000	Univ. of VT, VT Dept. For. & Parks, MASS Div. of For.
	Overhead @34.5	11,730	3,450	Same as above
	Travel	3,000		
	Contracting	3,000	1,000	Mycotech, Inc.
	Equipment	0		
	Supplies	1,000		
Year Totals		**52,730**	**14,450**	
THIRD YEAR				
Administration	Salary	32,000	10,000	Univ. of VT, VT Dept. For. & Parks, MASS Div. of For.
	Overhead @35.2%	11,264	3,520	Same as above
	Travel	3,000		
Procurements	Contracting	2,000	1,000	Mycotech Corp.
	Equipment	0		
	Supplies	1,000		
Year Totals		**49,264**	**14,520**	
ALL YEARS				
Administration	Salary	98,000	30,000	
	Overhead	34,066	10,430	
	Travel	9,000		
Procurements	Contracting	8,000	3,000	
	Equipment	1,000		
	Supplies	4,000		
PROJECT TOTALS		**154,066**	**43,430**	

1. We assume that the Forest Service does not allow indirect charges for these cooperative research projects. Therefore this expense is not included as a budget item. However, the standard rate for indirect charges at the Univ. of Vermont is 20.4%. This would serve as additional cost sharing should this be funded.