Insect-killing Fungi for Management of Hemlock Woolly Adelgid: A Review of Progress

William Reid,[1] Bruce L. Parker,[1] Margaret Skinner,[1] Svetlana Gouli,[1] and H. Brenton Teillon[2]

[1]Entomology Research Laboratory, University of Vermont
661 Spear Street, Burlington, VT 05405-0105

[2] Vermont Department of Forests, Parks and Recreation
103 South Main St., Building 10, Waterbury, VT 054676

Abstract

Entomopathogenic fungi are viable options to supplement and enhance existing strategies for management of the hemlock woolly adelgid (HWA). Exploratory activities to isolate insect-killing fungi from HWA were done in Massachusetts, Connecticut, New Jersey, and Virginia. Foreign exploration was done in China where this pest is suspected to have originated. From this work, 68 fungal strains were recovered, cultured, identified, and placed in long-term storage. The isolates commonly recovered were members of the following genera: *Beauveria*, *Verticillium*, and *Paecilomyces*. Laboratory bioassays were conducted against the green peach aphid and field-collected HWA to confirm isolate virulence. Several were highly pathogenic and worthy of further development. Field testing is underway.

Keywords:

Entomopathogenic fungi, *Beauveria*, *Verticillium*, *Paecilomyces*, insect-killing fungi.

Introduction

The hemlock woolly adelgid (HWA)**,** *Adelges tsugae* Annand, is an exotic pest of eastern and Carolina hemlocks that is expanding its range steadily. This insect is killing trees throughout its current range along the eastern seaboard, from Massachusetts in the north to the Carolinas in the south and west to West Virginia. Isolated infestations have been discovered recently in Maine and New Hampshire (D. Souto, personal communication). Efforts are underway to develop biological controls for this serious pest (McClure 1995a, 1995b; Montgomery and Lyon, 1996; Gouli et al. 1997; Cheah and McClure 1998; Wallace and Hain 2000; Yu et al. 2000; Lu and Montgomery 2001). Management of forest pests such as HWA in natural areas relies on natural controls that are simple to use and of low cost (Coulson and Witter 1984). To date the major emphasis of research in this area has been on the rearing and release of exotic coccinelid predators. However, rarely will one biological control organism—a "silver bullet"—effectively suppress serious exotic pest populations below damaging levels (R. Reardon, personal communication). More realistic is a multifaceted approach using several compatible agents that together reduce pest populations.

Entomopathogenic fungi comprise a group of naturally occurring organisms that penetrate, multiply within, and ultimately kill their insect hosts. These represent a group of promising, but as yet under-utilized biological control agents for management of HWA and other exotic insect pests. Fungi are particularly promising for HWA management for several reasons. They have been found infecting HWA naturally in the eastern United States and in low-level adelgid populations in China. Many species of these fungi are relatively easy and inexpensive to mass-produce, and most have little or no negative impact on the environment, humans, or non-target organisms. Production is species and strain specific, and under ideal conditions, enough material for 1 ha. can be prepared for under $20.00 (Wraight et al. 2001). Naturally occurring epizootics caused by fungi have been observed in populations of scales and various aphids demonstrating the potential for their use. An additional benefit of entomopathogenic fungi is their potential to persist in an infected population, providing an ongoing chronic fungal infection. Such conditions may cause an overall reduction in health and fecundity of the pest species. This stress may sufficiently reduce the pest population to a more manageable level—a level perhaps that coccinelid predators could reduce even further.

Developing fungi for integrated pest management (IPM) is a multiphase process beginning with searching for isolates infecting HWA in infested areas. Through the research reported herein, we initiated development of entomopathogenic fungi against HWA, a viable component in the arsenal for management of this devastating pest.

Exploratory Activities

Preliminary surveys to collect entomopathogenic fungi from HWA were begun in 1996. Five dominant eastern hemlock trees with heavy infestations of HWA located at Mt. Tom, Massachusetts were sampled. Between January and March, 600 apical twig samples were taken, from which 6,000 HWA were collected, and 2,882 appeared symptomatic (potentially infected with a fungus). After completing several microbiological procedures on the symptomatic adelgids, as described in Gouli et al. (1997), 17 fungal genera were recovered, of which three are recognized entomopathogens and worthy of further assessment (Table 1).

Exploratory investigations for entomopathogenic fungi were expanded to locate isolates in other areas of the eastern range of HWA, with samples taken in Massachusetts, Connecticut, New Jersey, and Virginia. In addition, foreign exploration was done in several areas of China where HWA exists in fairly low numbers. These efforts produced additional strains and a sizeable bank of fungal material is presently in long-term storage at the USDA, ARS Collection of Entomopathogenic Fungi (ARSEF) located at Cornell University, Ithaca, New York and at the Entomology Research Laboratory, University of Vermont, Burlington, Vermont. Following isolation from HWA, all fungal identifications were verified by Dr. Richard A. Humber, USDA, ARS, at Cornell University prior to placement in permanent storage. The following recognized entomopathogens were commonly recovered: *Beauveria bassiana* (Balsamo) Vuillemin, *Verticillium lecanii* (Zimmerman) Viegas and *Paecilomyces farinosus* (Holm ex. SF Gray) Brown and Smith (Table 2).

Table 1. Fungi Isolated From HWA Collected at Mt. Tom, Massachusetts, January to March 1996.[1]

Fungal Species	No. of HWA Infected	% of Total HWA Collected
Trichoderma Lignorum (Tode) Harz	289	10.30
Trichoderma sp.	157	5.45
Fusarium sp.	49	1.70
Gliocladium sp.	27	0.94
Paecilomyces sp.	11	0.38
Beauveria bassiana (Balsamo) Vuillemin	6	0.21
Verticillium lecanii (Zimmerman) Viegas	5	0.17
Scopulariopsis sp.	1	0.04

[1]Extracted from Gouli et al. (1997).

Table 2. Summary of Numbers of Entomopathogenic Fungal Isolates Recovered From HWA Collected Along the Eastern Seaboard of the United States and From China.

Identification	Number recovered	
	US	China
Beauveria sp.	7	1
Verticillium sp.	15	5
Paecilomyces sp.	18	12
Fusarium sp.[1]	10	0

[1]Classified as a weak to moderate entomopathogen.

To establish that fungi recovered from HWA were indeed pathogenic, preliminary bioassays were conducted using field-collected HWA and laboratory-reared green peach aphid (GPA) (dosage 1 x 10^8 spores/ml). Mortality was assessed 6 days after treatment. Mortality rates of 42 to 90% were obtained among the HWA and 25 to 90% among the aphids (Table 3). These results confirmed that entomopathogenic fungi occurred naturally in HWA populations, and that several of these isolates exhibited significant activity against the host, which warranted further investigation.

Further bioassays were conducted to determine which of the isolates had the most potential. Sixty-two isolates, most of them collected from HWA, were first assayed at a single concentration (5 by 10^6 conidia/ml) against GPA, which is easily reared under laboratory conditions. The 10 most promising isolates, based on their pathogenicity to GPA, were selected for further testing against HWA. For these tests, HWA-infested twigs were field collected in Lovingston, Virginia and treated by spraying them with an airbrush sprayer. Assays were conducted at the Virginia Department of Forestry, Charlottesville, Virginia. Six test concentrations were assayed.

Table 3. Bioassay of Select Isolates Against HWA and Green Peach Aphid (GPA).[1]

ID No.	Species	% Mortality	
		HWA[2]	GPA
5165	*V. lecanii*	82	80
5167	-do-	72	90
5164	*Scopulariopsis* sp.	90	48
5170	*B. bassiana*	58	60
5178	-do-	64	52
5180	*P. farinosus*	ND	44
5177	*Gliogladium*	ND	27
None	*Fusarium* sp.	42	25

[1]Extracted from Gouli et al., (1997).
[2]HWA were sprayed with an aqueous solution of 1 by 10^8 spores/ml; GPA with 1 by 10^6 spores/ml. ND = no data, assay not conducted.

Interestingly, the strain found to be the most effective was MA1080 (*Metarhizium anisopliae),* which was originally isolated from maple leaf cutter in Vermont. It killed 84% of HWA at 1 by 10^8 conidia/ml. The LC_{50} value of this isolate was 7.9 by 10^5 conidia/ml, which was 200 times lower than the typical field application rate. Among the strains isolated from HWA, HWA 304 (*V. lecanii)*

(Figure 1) and GA 82 (*B. bassiana)* (Figure 2) produced high mortalities. GA 82 had an LC_{50} value about comparable to a typical field application rate of 10^{11} conidia/ha. The strain HWA 304, which was the second most virulent isolate among the 10 tested had an LC_{50} slightly higher than one-half that of a normal field application rate. Mortality rates such as this again demonstrate the potential for management of HWA with an inundative release of fungus. If these strains prove to be good spore producers they may be suitable for field testing in the future.

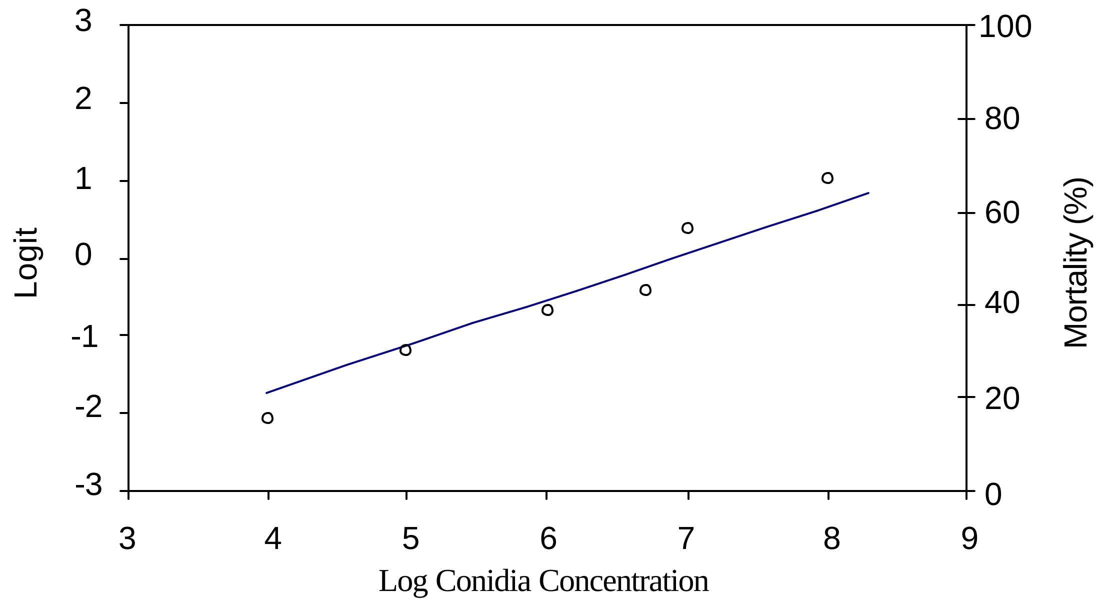

Figure 1. HWA mortality for isolate HWA 304 (*Verticillium lecanii*).

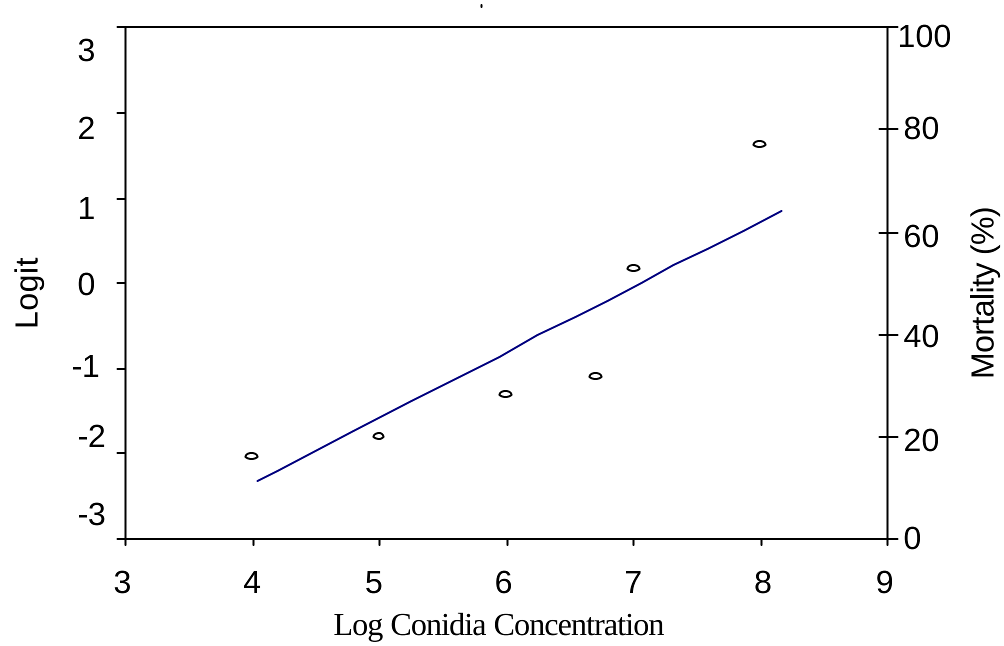

Figure 2. HWA mortality for isolate GA 82 (*Beauveria bassiana*).

These HWA bioassays were a compromise between a standard laboratory assay and a small-scale field test because they were performed in the laboratory on field-collected HWA, not laboratory-reared insects. Because the HWA could not be reared under sterile laboratory conditions, the HWA-infested twigs were contaminated with naturally occurring microflora of the target insect's environment. Some of these microorganisms can negatively impact the germination, survival, and ultimately its virulence. Several of the test strains displayed relatively high levels of virulence despite these factors, further suggesting their potential under forest conditions.

The ideal isolate for HWA management is one that exhibits a high level of virulence, rapid germination, a wide temperature range of activity, and ability to be mass produced as conidia. Formulation and tank mixes can enhance conidial resistance to UV radiation (Moore et al. 1993) and humectants such as glycerol can increase germination time and/or extend water availability from the spray (Wraight et al. 2001). Temperature range can be altered by adjusting the timing of application, which for the aestivating stage of HWA is July to November (McClure 1987). The remaining important characteristics (production of conidia and virulence) are more difficult to manipulate. The discovery of strains that show reasonable virulence at a typical field application rate is essential.

The next step in the process of developing fungi as a biological control is full characterization of the entire bank of entomopathogenic fungi of HWA found in the University collection. Information is needed on how each isolate responds to different temperatures and different levels of rH. Also, essential is information on how well they grow and sporulate. A small-scale pilot field test of select entomopathogenic fungi is being done and results will be available in the near future.

Acknowledgments

Assistance with sample site location was provided Charles Burnham and Kenneth Gooch, Massachusetts Department of Environmental Management; Dr. David Orwig, Harvard Forest; George Koeck, New Jersey Department Forest Health and Protection. We also thank Dr. Timothy Tigner, Virginia Department of Forestry, for providing laboratory space in which to conduct assays. This research was supported in part by the USDA Forest Service, Forest Health Technology Enterprise Team.

References

Cheah, C.A.S.-J. and M.S. McClure. 1998. Life history and development of *Pseudoscymnus tsugae* (Coleoptera: Coccinelidae), a new predator of the hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology* 27: 1531-1536.

Coulson, R.N. and J.A.Witter. 1984. *Forest Entomology: Ecology and Management*. John Wiley and Sons, New York.

Gouli, S., B.L. Parker, and M. Skinner. 1997. Isolation of fungi associated with hemlock woolly adelgid (Homoptera: Adelgidae). *Journal of Invertebrate Pathology* 70: 76-77.

Lu, W. and M.E. Montgomery. 2001. Oviposition, development and feeding of *Scymnus (Neopullus) sinuanodulus* (Coleoptera: Coccinellidae): a predator of *Adelges tsugae* (Homoptera: Adelgidae). *Annals of the Entomological Society of America* 94: 64-70.

McClure, M.S. 1995a. *Diapterobates humeralis* (Oribatida: Ceraatozetidae): an effective control agent of hemlock woolly adelgid (Homoptera: Adelgidae) in Japan. *Environmental Entomology* 24: 1207-1215.

McClure, M.S. 1995b. Using natural enemies from Japan to control hemlock woolly adelgid. *Frontier Plant Science* 47: 5-7.

Montgomery, M.E. and S.M. Lyon. 1996. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pp. 89-102. In: Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.). *First Hemlock Woolly Adelgid Review*, 12 October 1995, Charlottesville, Virginia. U.S. Department of Agriculture, Forest Service, Morgantown, West Virginia.

Moore, D., P.D. Bridge, P.M. Higgans, R.P. Bateman, and C. Prior. 1993. Ultra-violet radiation damage to *Metarhizium anisopliae* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Annals of Applied Biology* 122: 145-152.

Wallace, M.S. and F.P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. *Environmental Entomology*. 29: 638-644.

Wraight, S.P., M.A. Jackson, and S.L. de Kock. 2001. Production, stabilization and formulation of fungal biocontrol agents, pp. 253-288. In Butt, T.M., C.W. Jackson, and N. Magan (eds.). *Fungi as Biocontrol Agents, Progress, Problems and Potential*. pp. 253-288. CABI Publishing, New York.

Yu, G., M. Montgomery, and D. Yao. 2000. Lady beetles (Coleoptera: Coccinelidae) from Chinese hemlocks infested with hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). *The Coleopterists Bulletin* 54: 154-199.