Hemlock Woolly Adelgid Suppression with Aerial Application of Mycotal Fungus in a Microfactory Formulation: Can It Work?

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Abstract

The question is 'can we impact forest populations of hemlock woolly adelgid by aerially applying a commercial insect-killing fungus that is enhanced with a microfactory formulation additive'. Results from the 2009 Pilot Study, where 1.25 acre plots of hemlock forest in TN were aerially treated with *Lecanicillium muscarium* in Mycotal, a commercial biopesticide from The Netherlands, found HWA population growth reduced by >50%; but the reduction occurred only when the tank mix was augmented with a fungal enhancer based on whey microfactory technology. The ultimate answer to the "can it work" question will depend on a variety of factors, some biological and ecological, while others are practical, economic and regulatory. Notwithstanding, evidence from the achieved impact on HWA populations combined with an understanding of biotic and abiotic influences on HWA population dynamics suggests that *L. muscarium* can help bring HWA under management.

Keywords

Biopesticide, fungal enhancer, entomopathogen, *Lecanicillium muscarium*

Introduction

In developing a biopesticide to protect hemlock trees from insect pests certain other questions will also arise, such as: will the fungus integrate without harming beneficial predators, will there be a commercial product available, will it be cost-effective, are State and Federal regulatory hurdles surmountable, and who will fund management implementation. These ancillary factors must be kept in mind throughout the process of selecting and testing insect-killing fungi for implementation. In previous research, the insect-killing fungus *Lecanicillium muscarium* contained in the commercial product 'Mycotal' emerged as the best candidate for development (Reardon *et al.* 2004). We report on a pilot study to examine the efficacy of Mycotal for suppression of HWA when applied aerially to hemlock stands.

Lecanicillium muscarium (=*Verticillium lecanii*) is an insect-killing fungus that has been isolated from most terrestrial zones. Its host range is relatively narrow, infecting such insects as adelgids, thrips, aphids and whiteflies (Humber and Hansen 2005). In fact, HWA in the Eastern US were found to be infected by a fungus identified as *V. lecanii,* among other insect-killing fungi (Reid 2003). Fungi kill insects by growing profusely within their body cavity, after they germinate and penetrate from the outside, which requires suitable temperature and relative humidity. When several species of insect-killing fungi were tested in small-scale field trials, *L. muscarium*, then called *V. lecanii*, proved to be most consistently effective against HWA sistens (Reardon *et al*. 2004). Additionally, no negative impact of *V. lecanii* to *Sasajiscymnus tsugae*, an introduced predatory beetle, was found in a bagged foliage study (Reardon *et al*. 2004).

For an insect-killing fungus to be successfully deployed for forest pest management it must first be registered by the US EPA as a biopesticide, which can be time consuming and costly for fungi recently isolated and with no commercial history. Mycotal is a commercial biopesticide produced in The Netherlands that contains the insect-killing fungus *Lecanicillium muscarium*. Although not yet registered in the US, this biopesticide has a completed registration package for its European registration that will accelerate the timeline for its availability in the US. With the support of the manufacturer, Koppert Biological Systems, Inc., permits were obtained from USDA-APHIS-PPQ to allow Mycotal's importation and release into the environment for field testing. This allowed us to treat up to 10 acres/yr of hemlock forest with the fungus.

Fungal microfactory technology was developed to improve the effectiveness and economic feasibility of fungi applied over large acreage for pest management. Microfactory technology relies on the nutritive value of cheese whey for fungal growth and reproduction, which occurs after the fungus is applied in the field (Grassano 2008). MycoMax™ fungal enhancer is a formulation adjuvant based on this technology that was optimized for use with Mycotal against HWA. In laboratory experiments with the enhanced fungal formations, the number of fungal spores increased 10-100 fold and extensive fungal growth was observed on foliage collected from the field.

Summary 2009 Pilot Study Tennessee Wildlife and Recreation Area - Titus Creek

On the evening of May 28 and again on the morning of May 29, 2009 replicate 1.25 acre plots of hemlocks infested with adelgid crawlers were treated *via* helicopter (10 liters/acre). To account for density-dependent population effects a total of 12 plots were grouped into 4 blocks on the basis of pre-treatment HWA population counts. Four plots, one from each block, served as 'No Spray' control, 4 were treated with *L. muscarium* alone (1×10^8 spores/ml: Mycotal, Koppert Biological Systems) and 4 received *L. muscarium* enhanced with the microfactory formulation (5% w/v MycoMax); the oil adjuvant Addit (0.25% v/v : Koppert Biological Systems) and the sticker Hyperactive (0.05% v/v : Helena Chemical) were also added to both fungal treatments. Treatments were applied aerially using a Bell Jet-Ranger helicopter with mounted AU6539 Micronair atomizers. Targeting of treatments was accomplished with an on-board navigational system and ground-truthed plot locations. Temperature, rainfall, relative humidity and leaf wetness were collected within the study area. Weather conditions immediately post- treatment were generally favorable for the germination and growth of *L. muscarium*, with RH from 85-100% and temperatures nearing 20°C.

A total of 3000 foliage samples were collected to assess HWA population status pretreatment in spring 2009, in summer 5 weeks post-treatment and again in March 2010.

Both lower and upper (~ 15 meters) canopy foliage was collected and examined during the 2009 and 2010 spring evaluations, but only the lower canopy was sampled during summer 2009. At each tree elevation, 5 sample branches (~25 cm) were collected from each of 10 pre-selected and tagged trees. HWA were quantified on the spring (2009 and 2010) samples using a modification of the 'Rich Cowles' method where only up to 20 HWA woolly masses are counted/branch (Cowles and Montgomery 2006). The difference between post and pre-treatment counts were considered to assess treatment impact. Minute HWA sistens are present during summer sampling period and each of these must be counted microscopically and punctured to determine its survival status; alive insects are indicated by body turgor and burgundy colored blood; the HWA on five branchletes/branch were examined (~12,000 individuals). Data were log transformed when necessary and analyzed using GLM is SAS (SAS Institute 2008) (Alpha=0.05); P-diff was used for pre-planned means separation (SAS Institute 2008).

When foliage was collected 5-weeks post-treatment to assess populations of aestivating sistens the number of alive HWA tended to be lower in fungal treated plots, and more so when the fungal enhancer was included, 73% and 43% of control populations, respectively (Figure 1), although the differences were not significant (P>0.05). The latter post-treatment sample, taken in spring 2010, revealed an anticipated density-dependent crash of HWA populations in the lower elevation of block (4) where the HWA density was extremely high the previous year. The change in HWA population from the preceding spring reflected this crash in a significant interaction between block (based on initial population) and fungal treatment, which masked the overall affect of the fungus in the analysis (Figure 2). The crash was most prominent in the block 4 control plot, whereas the dramatic reduction was not as evident in the plot of the corresponding block receiving enhanced fungus. It is unclear whether this failure to completely crash was the result of the enhanced fungal treatment or that initial HWA populations were somewhat lower.

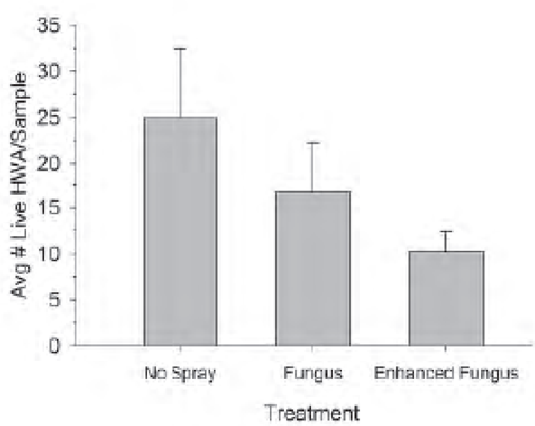

Figure 1. The number of live HWA sistens found in the lower hemlock canopy 5 weeks post-treatment for plots receiving no spray or aerial treatment of *L. muscarium* fungus (Mycotal) with or without fungal enhancer (MycoMax). Vertical lines indicate the standard error of means. There were no significant difference (P>0.05) among treatments.

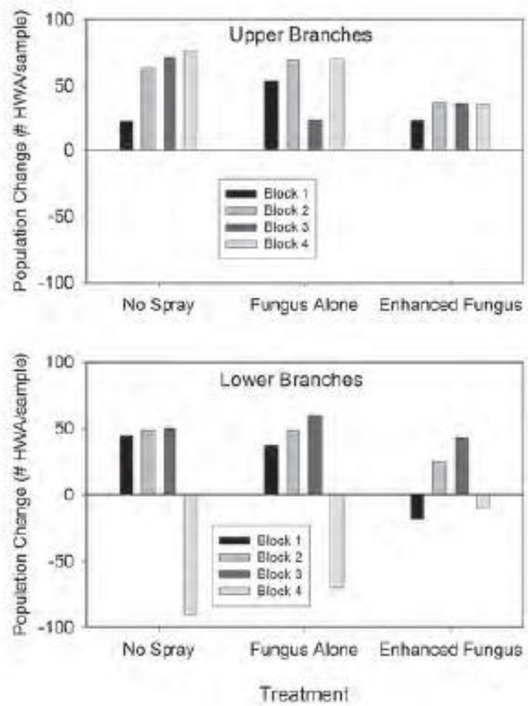

Figure 2. The change in HWA populations between pre- and post-treatment samples taken from the lower and upper branches (~ 15 meters). The sum of 5 samples/elevation/tree was used to calculate population change. In most plots HWA populations were continuing to expand, except that a density dependent crash in HWA populations occurred in the lower canopy of block 4, which is characterized by a significant ($P<0.05$) Block (density) X Treatment interaction.

Regardless of the cause, the presence of a residual HWA population may be of some benefit by providing food for continued survival of HWA predators, instead of the dearth caused by the density-dependent tumult in unregulated HWA populations.

The population change data was re-analyzed without block 4 to obtain a more precise evaluation of treatment effects. In this case, there was a significant ($P<0.05$) reduction in HWA population growth when hemlocks were treated with fungus enhanced in the whey microfactory formulation (Figure 3). Although HWA population in the enhanced fungus plots still continued to grow, they expanded at a rate less than half that found in control plots. HWA population expansion in plots treated with fungi, but without the fungal enhancer, tended to be lower than the untreated control although the difference was not significant. These results reflect that observed in summer (Figure 1) and suggest HWA populations were impacted by aerial application of the insect-killing fungus when formulated with the fungal enhancer.

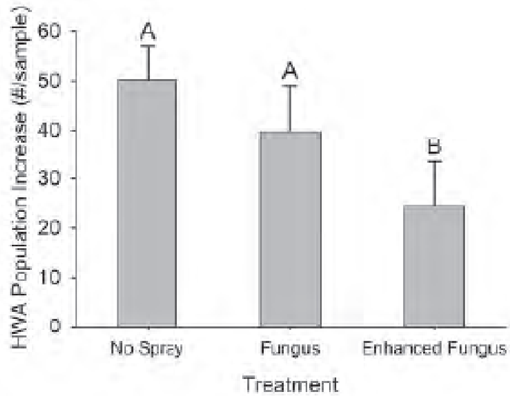

Figure 3. The change in HWA populations between pre- and post-treatment samples taken from the lower upper branches (~ 15 meters), combined over elevation. Results from block 4 were excluded because of an anticipated density dependant crash in HWA population. Plots were either not treated (No Spray) or treated with *L. muscarium* fungus (Mycotal) with or without fungal enhancer (MycoMax). The growth of HWA populations was significantly ($P<0.05$) reduced in plots receiving the enhanced fungal treatment, as indicated by different capital letters over standard error bars.

The influence of reducing population growth on longer-term HWA population dynamics are uncertain. To explore possible outcomes, a preliminary assessment was made using a HWA Population Simulator developed by Trotter (2010) to model, among other factors, impacts of cold induced, over-wintering mortality; mortality to the summer and/or overwintering sisten population is analogous to the fungal impact reported herein. Nominal settings were made in the model to reflect potential impacts of other biotic and abiotic factors on the progridiens and sistens population: egg production=50% of maximum, egg mortality=50%, crawler mortality=90%. The mortality of juvenile and adults progridiens was set to 50% to suggest predator influences and overwintering mortality of sistens was held at 0%; sisten summer mortality was adjusted to capture different levels of fungal impact, i.e., 0, 25%, 75% yearly mortality.

The outputs from the modeling exercise when the initial population is set to 500 individuals are depicted in Figure 4. Note that models are designed to explore possible outcomes and results should be considered with that in mind. When no (0%) fungal impact was incorporated the HWA population spiked regularly twice each year as crawlers emerged, escalated in between and went off the chart after 2 years, much like what occurs naturally. At 25% yearly impact, the severe escalation was delayed for 1 year. Incorporating the fungal impact obtained in the pilot study (~50% suppression in expansion), the spikes in crawler numbers were still evident but the general escalation of the HWA population is appreciably inhibited. Surprisingly, when the fungal impact was increased to 75%, crawler spikes began to subside and overall populations decreased over time! However, it must be reiterated that results from this modeling exercise can only be used to help understand interactions of potential mortality factors and cannot be substituted for real world evaluation of longer-term impacts.

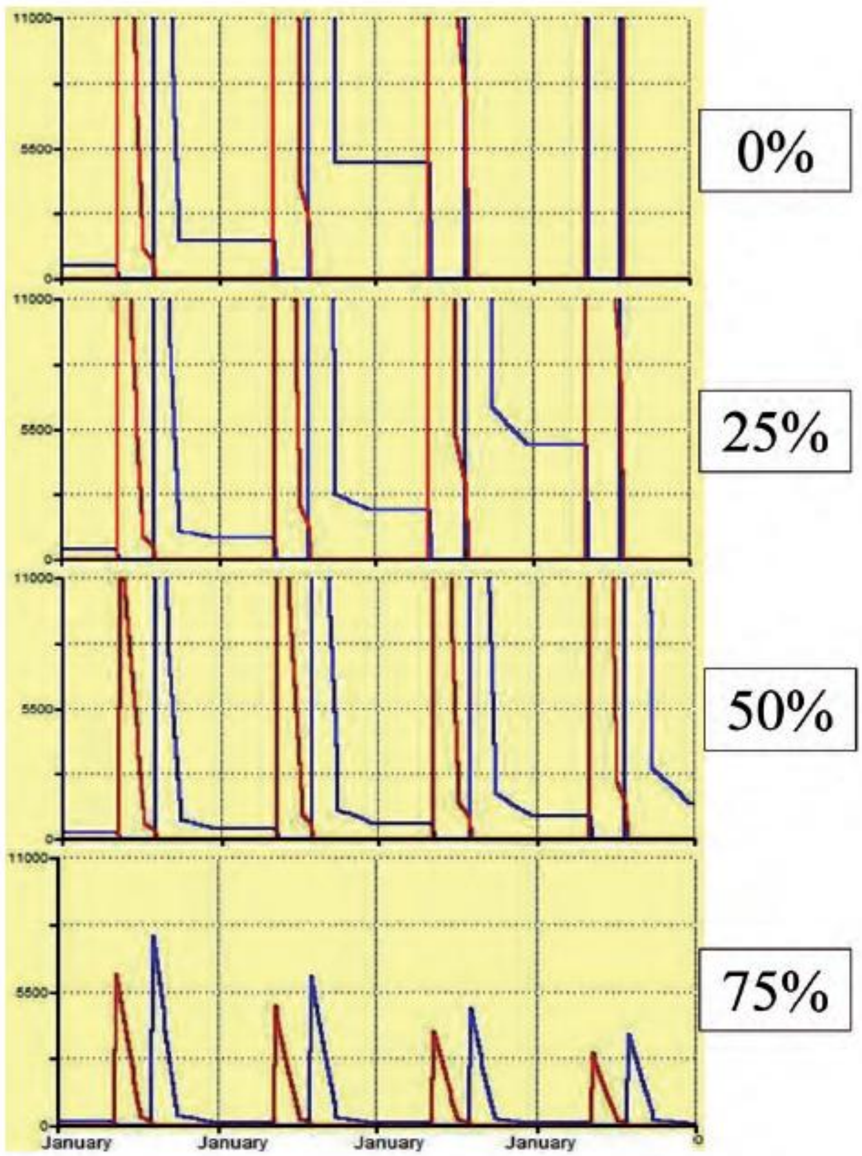

Figure 4. Results from the hemlock woolly adelgid population simulation model (Trotter 2010) at different levels of projected yearly impact by insect-killing fungus, i.e., 0, 25, 50, and 75% reduction in summer sisten populations. Repeating pairs of large spikes represent yearly populations of progridien and sisten crawlers, respectively, followed by a trough indicating the sisten population that would summer aestivate and overwinter. The Y-axis ranges from 0-11,000 individuals and crawler spikes go off-scale. The model is started with 500 individuals and other parameters are defined in the text.

The question remains, 'can it work', can we use aerial applications of insect-killing fungi as biopesticides to broadly suppress HWA populations and protect hemlock trees. A second aerial application at a higher fungal rate was made in 2010, for which the results were unavailable, and a larger trial is planned for 2011 in two climatic zones (TN-southern and PA-northern) to investigate interaction with low winter temperatures -- from these a clearer picture of fungal impacts is expected to emerge. However, the results obtained in the 2009 Pilot Study are a positive harbinger compared to other unsuccessful attempts at broad scale application of insect-killing fungi for pest suppression, and suggests the utility of using the fungal enhancer in the whey-based microfactory formulation. There is an understandably strong desire to observe rapid knockdown of HWA populations. In reality, massive disease outbreaks with insect-killing fungi are poorly understood and not usually captured through spray and count experimental approaches. While rapid knock-down is hoped for through making 'inundative' applications of fungal spores done in the 2009 and 2010 Pilot Studies, the benefits of broadly inoculating HWA populations to enhance prospects for future disease outbreaks should not be ignored.

Acknowledgments

The author is only the presenter and hopeful catalyst of this work, which would never have been accomplished without the steadfast intellectual and practical support of at least the following individuals: Brad Onken, Karen Felton. Dick Reardon, Rusty Rhea, Dennis Souto, Michael Bohne, Bob Tichenor, Brant Miller, Jerome Grant, Joe Elkins, Wes Pilley Justin Walden, Ashley Lamb, Bud Mayfield, Brad Register, and the Costa Boys. The author is also indebted to Helicopter Applicators Inc. and Koppert Biological Systems, Inc. for supply of Mycotal. The 2009 Pilot Study was funded under USFS Cooperative Agreement Number: 07-CA-11420004-117, Whey-Based, Fungal Micro-Factory Technology for Enhancement Mycopathogen Deployment in Forest Pest Management. COI Statement - Dr. Scott Costa is also President of Costa Enterprises, Ltd, which holds exclusive rights to whey-based fungal microfactory technology.

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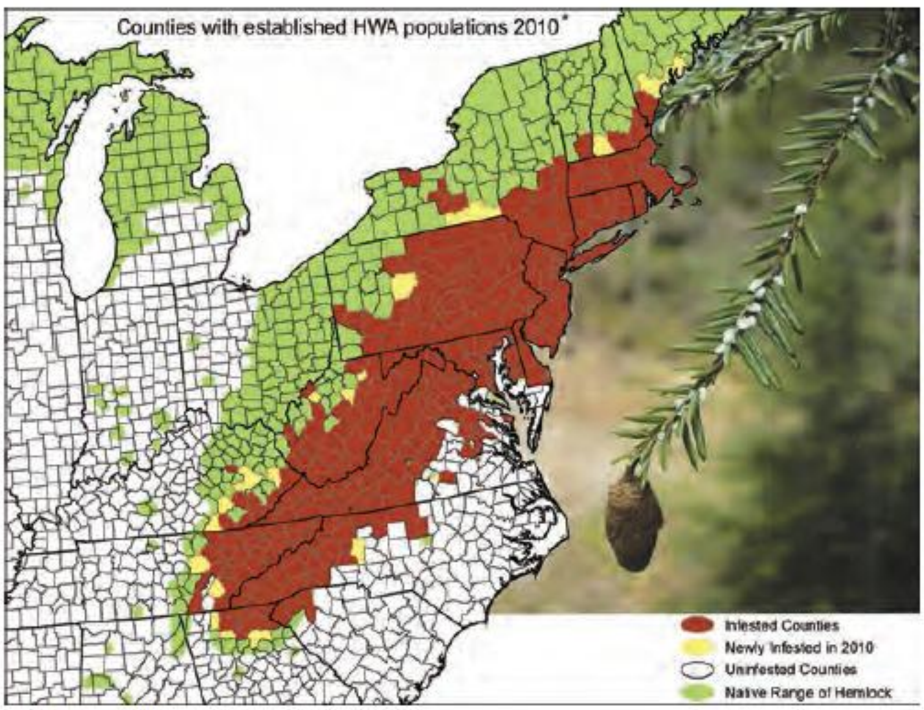

*See inside cover.

Compiled by Brad Onken and Richard Reardon

FHTET-2010-07
December 2010