Insect-Killing Fungi as a Component of Hemlock Woolly Adelgid Integrated Pest Management

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ABSTRACT

The goal of this project is to develop formulations of insect-killing fungi for application to hemlock forests as part of a hemlock woolly adelgid (HWA) integrated pest management program. Previous studies have identified two strains of *Beauveria bassiana* and a single strain of *Verticillium lecanni* with potential for use against hemlock woolly adelgid. Laboratory and field studies were conducted to develop ultra-low volume (ULV) formulations for delivery of fungal conidia. These studies lead to the development of prototype formulations of conidia in oil- and whey-based carriers. The target density of 1×10^{10} conidia/ml formulation was achieved while maintaining sufficient fluidity for spray application. The formulations were used in a small scale forest trial and delivered with an ULV sprayer onto hemlock branches infested with hemlock woolly adelgid. Appropriate controls were included. No significant difference ($P > 0.05$) in droplet densities on upper and lower needle surfaces was found within spray treatments. Nearly 50% of the droplets were in the 100-125 micron size range with the conidia in whey formulation. An unexpected delay of field applications in fall 2004 allowed HWA to molt to a stage that contains a protective woolly coat, which prevented any significant demonstration of field efficacy. Conidia formulated in whey appeared to be persisting at nearly 5 weeks post-treatment, and indications of fungal outgrowth from whey droplets were observed. Fungal growth in whey droplets could recycle fungi in the environment and facilitate development of disease outbreaks. Future research is slated to optimize oil-and-whey based formulations for fungal delivery, persistence, and efficacy against HWA under field conditions.

INTRODUCTION

The hemlock woolly adelgid (HWA) is an invasive pest that is devastating hemlock forests in Eastern United States. A concerted effort is underway to develop insect-killing fungi and other biological agents for use in integrated pest management of HWA (Cheah et al. 2004). The dramatic declines in gypsy moth populations in North America due to the fungus *Entomophaga miamaiga* highlight the potential of insect-killing fungi for forest pest management (Hajek *et al.* 1990). Initially, we directed our research on insect-killing fungi toward collecting numerous isolates and then identifying those with the most insect killing activity (Reid 2003). We also examined their suitability for mass production in anticipation of producing enough fungi for widespread application. Subsequent research examined fungal efficacy against HWA in the field on single hemlock branches to assess the rate and timing of fungal application (Cheah *et al.* 2004). Lab and field trials examined the non-target effects of the fungi on *Sasajiscymnus tsugae*, an introduced predatory beetle of HWA.

The results to date are encouraging for the development of insect-killing fungi as a management tool for HWA. We are actively researching three isolates, a *Verticillium lecanni* and two of *Beauveria bassiana*, because of their positive profiles for efficacy, mass production potential, and compatibility with *S. tsugae*. Field trials between spring 2001 and fall 2003 indicated that significant reductions in adelgid populations occur with fall application of fungal conidia. These fungi, when applied at twice the field application rate, did not negatively affect the predatory beetle, *S. tsugae* (Cheah *et al.* 2004). Currently we are optimizing formulations for ultra-low volume (ULV) delivery, further studying non-target effects and examining fungal persistence. The ability of applied fungi to persist in the environment and have lasting effects on HWA population dynamics influences the selection of deployment strategies for widespread applications.

MATERIALS AND METHODS

A forest trial was conducted in late fall 2004 to examine the spray characteristics of oil- and whey-based formulations and assess their influence on the efficacy of insect-killing fungi against forest populations of HWA. Three fungi (*Beauveria bassiana*: CA-603 and GA082; *Verticillium lecanii*: arsef-6010) were tested in oil formulations and CA-603 was also incorporated into whey (exact components of these formulations are not currently being released). All treatments were delivered using ULV applications of formulated fungal conidia ($1x10^{10}$ condia/ml). There were "no spray" and blank spray (oil and whey) controls. In a hemlock forest in central Massachusetts (Mount Tom Reservation, Holyoke), 1-meter-long branches with greater than 20 branchlets infested with HWA were selected and tagged for treatment. A completely randomized design was used. A pre-spray count of the density, survival, and life stages of HWA sistens was made. This was accomplished by randomly selecting five branchlets/branch that were positive for the presence of HWA and returning the specimens to the lab for microscopic (20-40x) examination.

One milliliter of formulated material was applied with a hand-held ULV sprayer to each of five branches per treatment on October 28, 2004. Post-treatment samples taken as described above were collected five weeks after treatment for comparison to pre-treatment counts to ascertain treatment effects on survival and population density. The data were analyzed for treatment effects using GLM-ANOVA in SAS (SAS 2002) (± = 0.05 for all analysis).

Hemlock foliage was collected directly after spray treatment and examined microscopically to determine the number of droplets on upper and lower leaf surfaces and size distributions of droplet deposits. This was done for the CA-603 treatments formulated in oil and whey and for their respective blank controls. Ten needles were examined per replication within a treatment for the number of droplets within a 0.625 mm^2 microscopic field. Droplet size was examined on a single needle from each replication within a treatment. Droplets sizes were classified in 25 mm increments ranging from 25 to 300 mm by counting the number of droplets within each class. The data on the number of droplets were analyzed for treatment effects using GLM-ANOVA, whereas the distribution of in each size class was examined using Chi-square analysis (SAS 2002). Preliminary examinations of persistence were made from foliage during the post-treatment HWA assessment. This was accomplished by examination of fungi isolated from hemlock needles onto nutritive agar and visual examination of leaf imprints taken using adhesive tape.

RESULTS AND DISCUSSION

No significant differences (P > 0.05) in droplet densities on upper and lower needle surfaces were found within spray treatments (Figure 1). This is a critical finding as HWA are typically located on lower surfaces and therefore difficult to reach with standard spray applications. The total number of droplets found was influenced by the formulation applied, with the oil without conidia having the highest number of droplets. There were significant differences in the distribution of droplet size classes among oil and whey formulations and their controls (Figure 2). For instance, oil without conidia produced a larger proportion of small droplets that probably accounts for the higher number of droplets overall in this treatment. In the case of both oil and whey formulations, when conidia were added, the size of droplets tended to increase. With conidia in whey, nearly 50% of the droplets were in the 100-125 micron size class, and there were none of the larger droplets (>225 microns) found with the oil and conidia formulation, which are indicative of clumping.

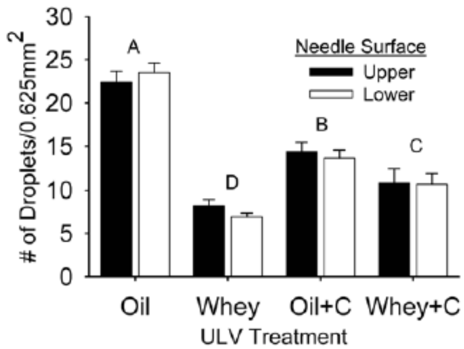

Figure 1. The number of droplets observed on the upper and lower surfaces of hemlock needles after ULV applications of oil and whey formulations with and without conidia of *Beauveria bassiana*. Oil+C and Whey+C are the formulations with conidia. Capital letters indicate significant differences in total counts among formulations. No significant difference was found between needle surfaces.

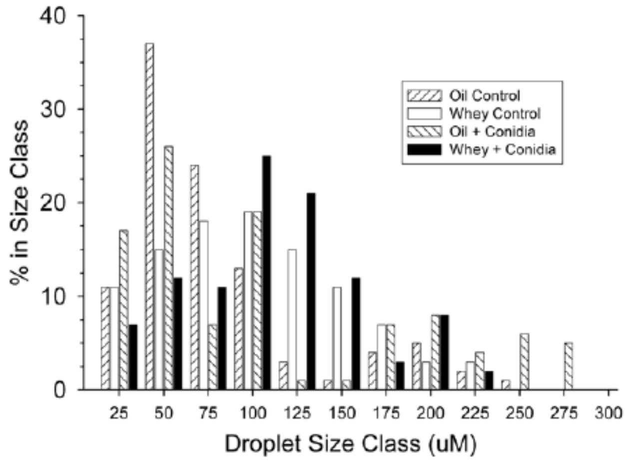

Figure 2. The Frequency of size class distributions of droplets on hemlock needles after ULV applications of oil and whey formulations with and without conidia of *Beauveria bassiana*. Data from both surfaces are combined. There is significant difference in the distribution of size classes among the formulation treatments.

Data on HWA density and survival taken before treatment applications found no significant differences (P > 0.05) in either average density (overall avg. 4.36 live HWA/cm, SE ± 0.24) or mortality (overall avg. 11.8%, SE ± 1.2) of HWA field populations among groups of trees slated for treatment This indicates the overall uniformity of HWA populations within the study site before treatment. However, an unexpected delay of field applications in fall 2004 allowed nearly the entire HWA population (98.4%, SE ± 0.5) to break aestivation and molt to a stage that develops a woolly coat. Nearly five weeks post-treatment, there were no significant differences (P > 0.05) in either average density (overall avg. 4.17 live HWA/cm, SE ± 0.31) or mortality (overall avg. 12.1%, SE ± 1.7) of HWA populations among fungal treatments and the controls. Our previous studies found that fungal applications made with higher-volume formulations during periods when HWA contain a woolly coat were ineffective (unpublished data). This circumstance may have precluded any significant demonstration of field efficacy. Our current strategy is to shift applications six weeks earlier in the year to better avoid the resumption of HWA development in late fall and take advantage of temperatures more favorable for fungal infection.

Conidia formulated in whey appeared to be persisting at nearly five weeks post–treatment and indications of fungal outgrowth from whey droplets were observed (Figure 3). No similar outgrowth was observed on needles treated with oil. Fungal growth in whey droplets could recycle fungi in the environment and facilitate development of disease outbreaks. Future research is slated to optimize whey based formulations for fungal delivery, persistence and efficacy against HWA under field conditions. The compatibility of oil- and whey-based fungal formulations with predatory beetles, adult *Sasajiscymnus tsugae*, will also be examined.

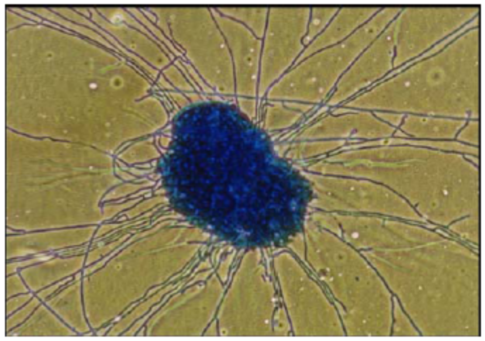

Figure 3. An example of fungal outgrowth found on hemlock needles five weeks after treatment with a whey-based formulation containing conidia of *Beauveria bassiana*. No similar outgrowth was observed on needles treated with oil.

Currently, we are testing fungi against HWA using a methodology in which we select healthy insect populations, apply a dose of fungi selected to allow discrimination between fungi and test formulations, and then examine for treatment effects – commonly referred to as the spray-and-count method. This approach does not reflect the full potential of insect-killing fungi that is often observed under natural conditions. The ability of insect-killing fungi to cause a massive disease outbreak or epizootic is dependent on more than the number of fungal spores in the insects environment. Epizootic potential is also a function of suitable environmental conditions (mostly temperature and water) and insect susceptibility to infection. Insect susceptibility to infection is not static. Developmental changes or response to various stressors, such as low temperature, insect density, and host condition, may cause increased susceptibility to infection. Greater realization of insect-killing potential for impacting HWA populations will likely occur as operational formulations become available for testing under more natural field conditions.

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