Dynamics of Naturally-Occurring Fungal-Induced Epizootics of Hemlock Woolly Adelgid (HWA)

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- The insect-killing fungus, *Myriangium* sp., is highly pathogenic to HWA when applied on infested branches, and appeared to have a carryover effect the following year.
- A broad spectrum of naturally-occurring fungi, predators and other mortality factors (e.g., cold winter temperatures) contribute to declines in HWA populations in nature, and a multi-pronged approach is essential to achieve sustained management.

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Project Summary

Eastern hemlock is a valued component of New England's forest, annually adding ~$100 million to the economy in New York and New England. It also contributes significantly to the biodiversity of the Northern Forest, playing a key role in erosion control along streams, and providing food, shelter and shade for a wide variety of wildlife and fish. Hemlock woolly adelgid, *Adelges tsugae* (HWA), is an exotic invasive pest that is ravaging hemlock forests along most of the eastern seaboard and continues to expand its distribution, threatening the entire range of eastern hemlock. It was recently found in Maine, New Hampshire and Vermont. In 2008, a significant natural decline in HWA populations in NH was observed by State foresters. HWA in these areas showed signs of infection, from which fungi were isolated. Through morphological and molecular analyses UVM scientists confirmed that the fungus from this epizootic of HWA was *Myriangium* sp., an insect-killing fungus of scale insects they had found in past field studies.

This project investigated the dynamics of fungal-induced epizootics of HWA in the hope of initiating one. Cooperators searched for evidence of epizootics in several HWA-infested sites, and samples were sent to the UVM Entomology Research Laboratory for inspection. No indication of a fungal epizootic caused by *Myriangium* sp. was detected. However another fungus, identified as being in the class Dothideomycetes was commonly isolated, which was associated with high mortality levels in some years. Several spray trials were conducted during the project at different HWA-infested sites in southern Vermont, with no previous history of fungal epizootics. These trials were designed to demonstrate the virulence of *Myriangium* sp. against HWA and assess the persistence and spread of the fungus. In general 74-92% mortality of HWA settleds was observed one month after treatment. In one trial, a carryover effect with lower numbers of eggs in the spring and greater mortality among the settled one year after treatment, was observed. However, a significant impact on HWA on untreated branches was not observed. In other field trials high levels of HWA mortality was observed that was caused by a *Phoma*-like fungus, which is usually considered a plant rather than insect pathogen. The overall results demonstrated that a broad spectrum of naturally-occurring fungi, predators and other mortality factors (e.g., cold winter temperatures) contribute to declines in HWA populations in nature, and a multi-pronged approach is essential to achieve sustained management.

Background and Justification

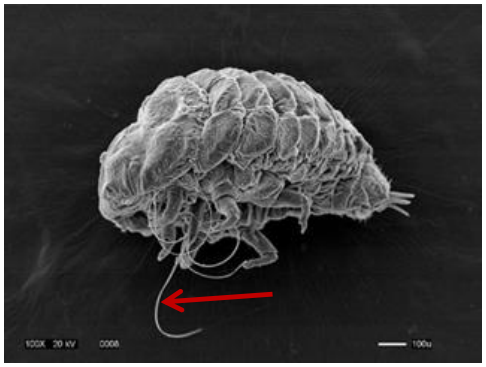
Why is Hemlock an Important Tree worth Protecting?

1. Important component of Northern and Southeastern forests, and urban and suburban landscapes.
2. An annual economic value in New York and New England of ~$100 million.
3. Provides critical habitat and food for wildlife (e.g., deer, birds, and small mammals).
4. Thrives in poorly drained and riparian zones, and minimizes stream bank erosion.
5. Shade-tolerant species that inhibits invasive plant growth.

Background and Justification

What is Hemlock Woolly Adelgid and Why is it an Important Pest?

1. Scientific name: *Adelges tsugae* Annand
2. Feeding habits: Sucks on sap, like with aphids, scales and whiteflies
3. Host species: Eastern and Carolina Hemlock
4. Area of origin: Asia--Japan, China
5. Introduced/detected into the US:
   - 1920s on West Coast
   - 1950s on East Coast
6. Currently infests 45% of the Eastern hemlock range.



HWA, showing stylet (indicated with red arrow) with which it sucks sap (100 x).

Mixed hardwood forest interspersed with hemlocks, killed by HWA.

Underside of hemlock twig heavily infested with HWA. HWA produce the white woolly masses for protection.

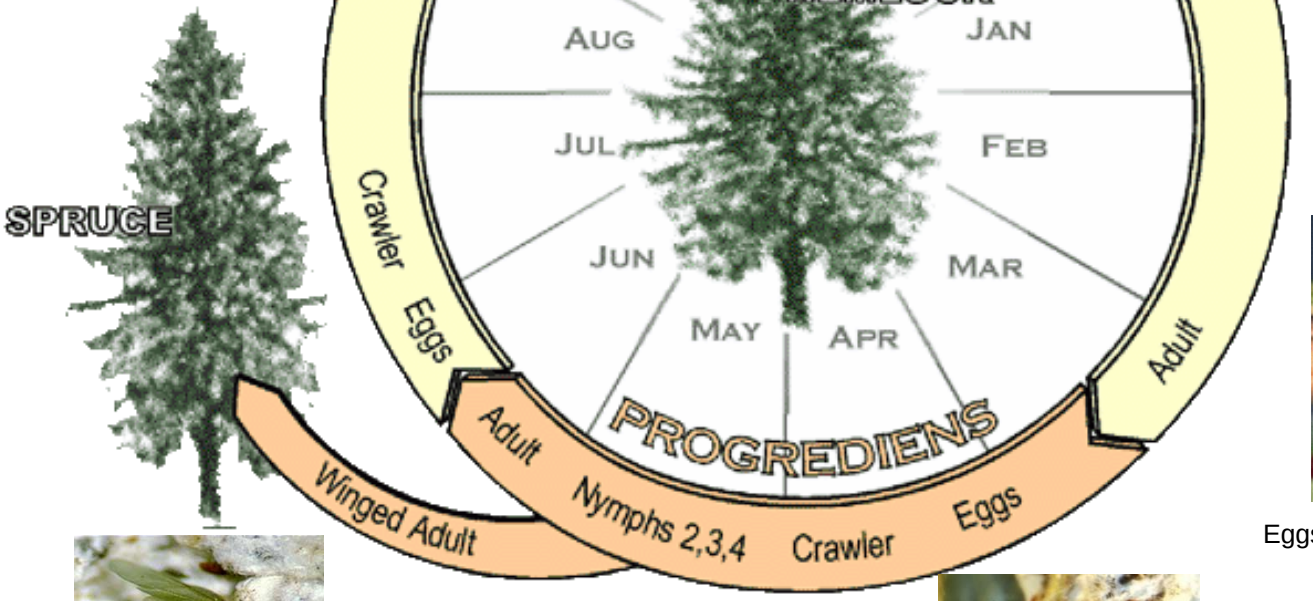
Background and Justification

Effective management can only be achieved with knowledge of the life cycle of the target pest and its natural enemies.

Settled HWA, the stationary nymphal feeding stage also called a settled.

Adult (covered with woolly mass), and settled with halo of white hairs.

Eggs protected under woolly mass made by female



Winged male

Crawlers, the first immature stage. This is the primary mobile stage.

Background and Justification

Factors that Affect HWA Survival

1. Cold temperatures: 95% mortality after exposure to -22 °F (-30 °C); 60% mortality at -13 °F (-25 °C)
2. Exotic predatory insects
3. Native predatory insects
4. Insect-killing fungi

Exotic predatory beetles released to attack HWA.

HWA immatures infected with fungi (fungal outgrowth indicated with red arrows).

Maggot of a native fly known to predate on HWA.

There is NO silver bullet!

Background and Justification

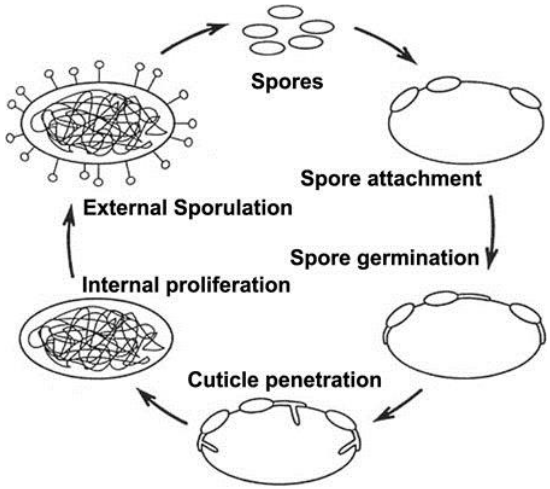
Insect-killing Fungi: A promising Option for HWA Biocontrol

1. What are they: Fungi that infect and kill insects, NOT plants or humans.
2. What are their benefits:

   Occur naturally in the environment.

   Many are easy and inexpensive to mass-produce.

   Many are fairly host-specific and non-toxic to other non-targets.



Typical life cycle of an insect-killing fungus.

Gypsy moth infected with a fungus.

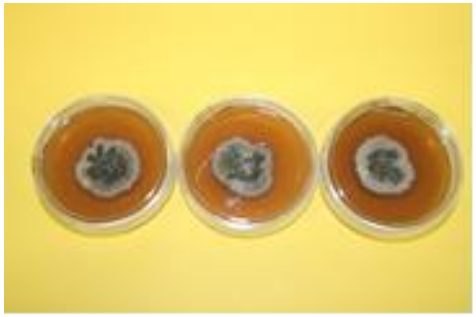
Fly infected with a fungus.

Background and Justification

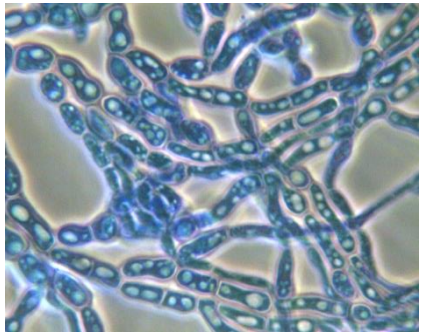
Harnessing the Potential of Fungi for HWA

1. Forest pest specialists in New Hampshire observed a naturally-occurring epizootic where all the HWA were killed, apparently by a fungus.
2. *Myriangium* sp., a known insect-killing fungus, was isolated from HWA in this area.

Dead , blackish HWA immatures, from which *Myriangium* sp. was isolated.



Petri plates in which *Myriangium* sp. isolated from HWA is growing.



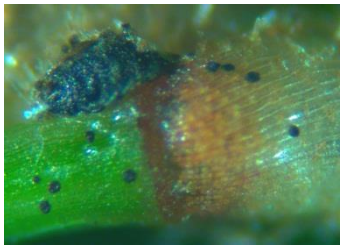
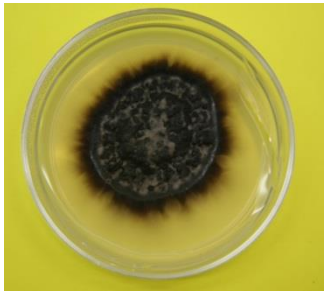
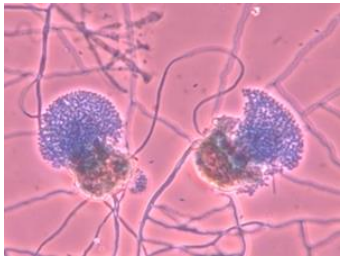
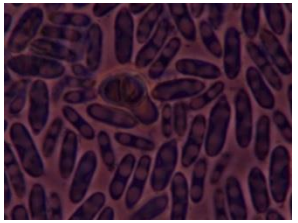
Spores of *Myriangium* sp. (greatly magnified).

The goal of this project was to further investigate the dynamics of fungal-induced epizootics of HWA in the hope of initiating one.

Methods

Obj. 1. Identify incidence of fungal epizootics within HWA infestations in NH, ME and VT, and determine the incidence of *Myriangium* sp. associated with these epizootics.

- Cooperating foresters from New Hampshire, Maine and Vermont collected samples from HWA-infested sites throughout the project period.
- Sites for sampling included areas where signs of an epizootic has been observed in the past and other infested areas.
- Specimens were inspected, and live and dead HWA were surface sterilized and placed on a selective medium.
- Pure cultures of individual fungi were prepared and identified based on culture characteristics and other morphological traits. In some cases it was necessary to send specimens to specialists to confirm identification.
- Select cultures were placed in permanent storage in the UVM Worldwide Collection of Entomopathogenic Fungi.

Methods

Obj. 2. Characterize the spatial relationships among live, dead, and fungal infected HWA in hemlocks within and outside epizootics.

Because a *Myriangium*-induced epizootic of HWA could not be found, this aspect of the project could not be investigated.

Methods

Obj. 3a. Initiate a *Myriangium*-induced epizootic of HWA and determine its persistence and rate of spread.

- Several different spray trials were conducted at various HWA-infested sites in southern VT with no evidence or history of fungal epizootics .
- The goal of these experiments were
  - To demonstrate the virulence of *Myriangium* sp. against HWA
  - To determine the extent of *Myriangium* sp. spray persistence and spread to adjacent unsprayed branches causing HWA mortality.
- Each trial was designed to adjust for conditions at the specific site.
  - Some were conducted on lower branches of mature hemlocks (>100 ft tall).
  - Some were conducted on branches of young hemlocks (~25 ft tall).
  - One was done on a hemlock hedge where trees at one end were infested and at the other end were not.
- *Myriangium* sp. from HWA or elongate hemlock scale were grown on agar medium and prepared as suspensions of ~5 x 10^7propagules(fresh)/ml distilled water.
- Twig samples were taken from treated and untreated branches before treatment and at various times after treatment. They were held in the laboratory under quarantine conditions and inspected to determine mortality levels over time.
- Fungal isolations were made from dead insects, as described in Obj. 1.

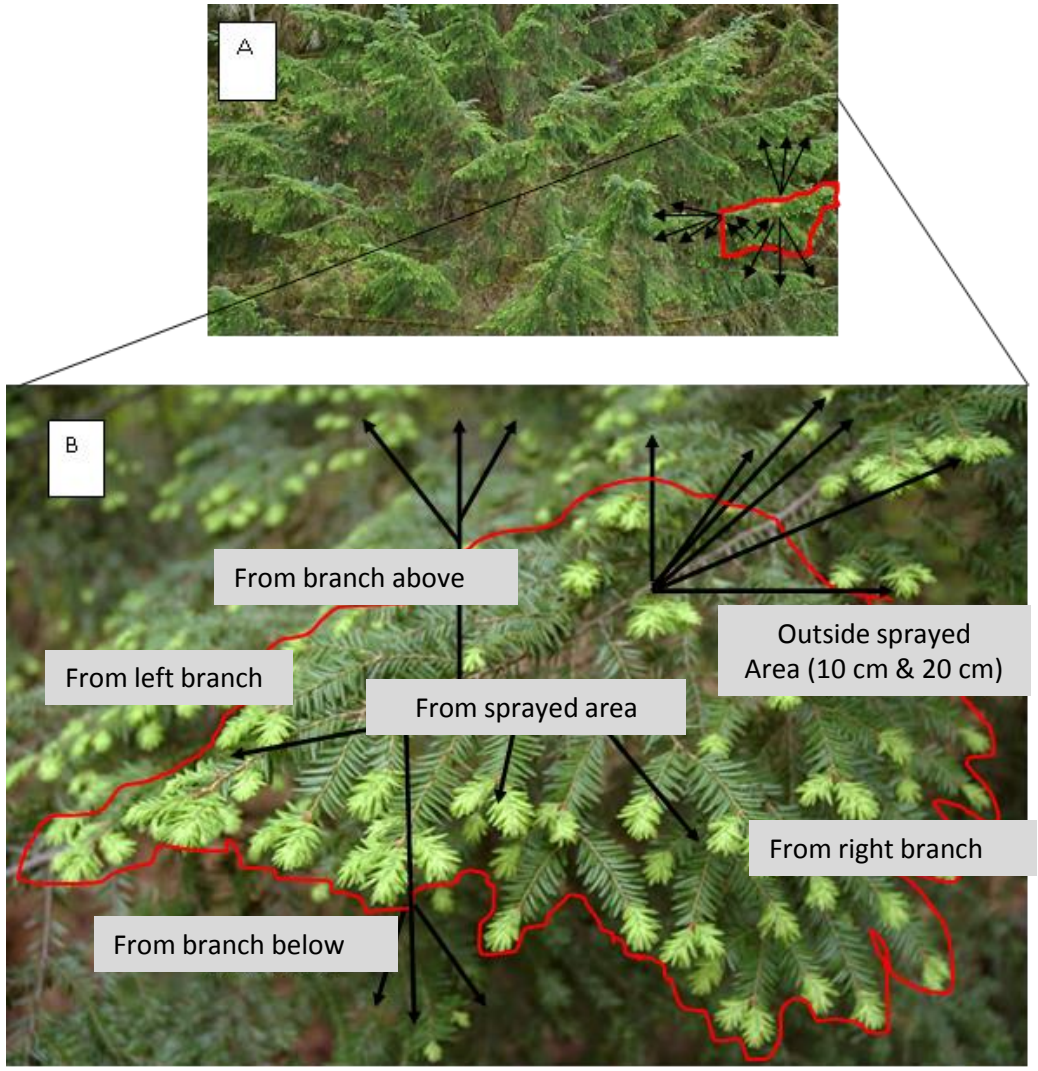
Methods: Obj. 3a, continued

Spray application for one of the trials of mature trees. Select branches to be sprayed were placed within a cloth sleeve to prevent drift to other unsprayed branches.

Water sensitive spray card used to assess coverage

Methods: Obj. 3a, continued

Sampling design for 2012 spray trial on mature trees. Samples were taken pre spray, 1 hr post spray, and 5 and 15 wk post pray.



We found the 2012 sampling design too complicated and time consuming. In subsequent trials we sampled the spray branch and adjacent unsprayed branches to determine spread. We eliminated samples in each direction (e.g., left, right, above and below).

Methods

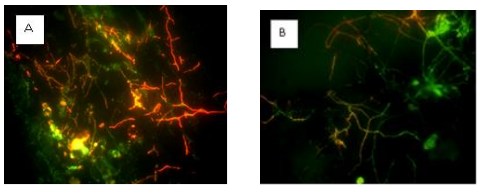
Obj. 3b. Determine if there is an impact on growth and longevity of hemlock seedlings as a result of a single application of *Myriangium* sp.

- 20 hemlock seedlings (<2 in. DBH, 1-2 m tall) that showed no signs of HWA infestation were selected at a forested area of the UVM Horticultural Research Center.
- 10 seedlings were sprayed to runoff with a suspension of *Myriangium* sp. (5×10^7 propagules/ml) suspended in sterile distilled water.
- 10 seedlings were sprayed to runoff with sterile distilled water.
- Seedlings were inspected 1,2,3 and 4 wk after treatment and monthly for 2 years.
- The following information was collected on each seedling: Annual new growth (including 2011 based on previous growth), 2013, and 2014, and evidence of insect or disease damage.

Methods

Obj. 3c. Determine the persistence of *Myriangium* sp. after spraying on branches.

- Because there is no selective medium for *Myriangium* sp., it was necessary to develop other methods to determine persistence.
- One method used scotch tape pressed to hemlock needles to remove spores from sprayed branches and then treat the sample with acridine stain. This stain causes live fungal spores to show up as orange under a luminescent scope.
- Another method used glass cover slips placed on a pipe cleaner and suspended to the spray branch.
  - Ten branches and the suspended cover slip devices were sprayed to runoff with the fungal suspension, 10 others were sprayed with sterile distilled water. Half of the cover slip devices were brought to the lab to compare environmental effects of persistence.
  - One cover slip was removed at each sample period and the fungal propagules were inspected under the microscope after staining.



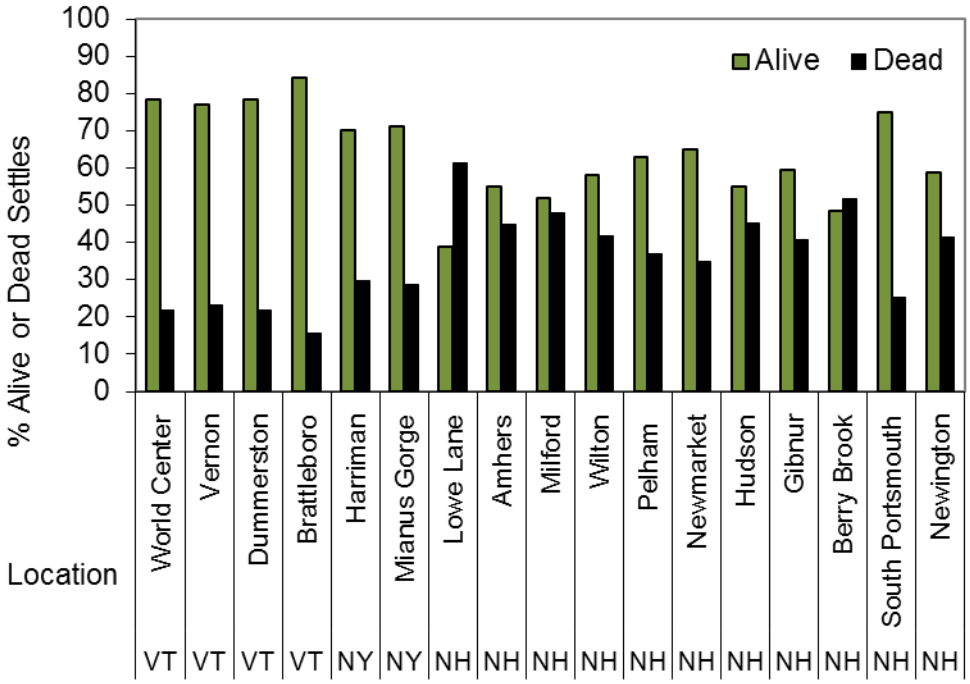
Scotch tape imprints from the upper (A) and lower (B) surfaces of hemlock twigs 24 hours after spray of *Myriangium* sp.; acridine orange (1:50 000), objective x40.

Glass coverslips held in place on spray branch with pipe cleaner.

Results

Obj. 1. Identify incidence of fungal epizootics within HWA infestations in NH, ME and VT, and determine incidence of *Myriangium* sp. associated with these epizootics.

The process of isolating fungi from HWA was extremely challenging and time consuming. It was particularly difficult to isolate *Myriangium* because no selective medium exists for this fungal species. It is a slow-growing fungus that is often overgrown by other saprophytic fungi.

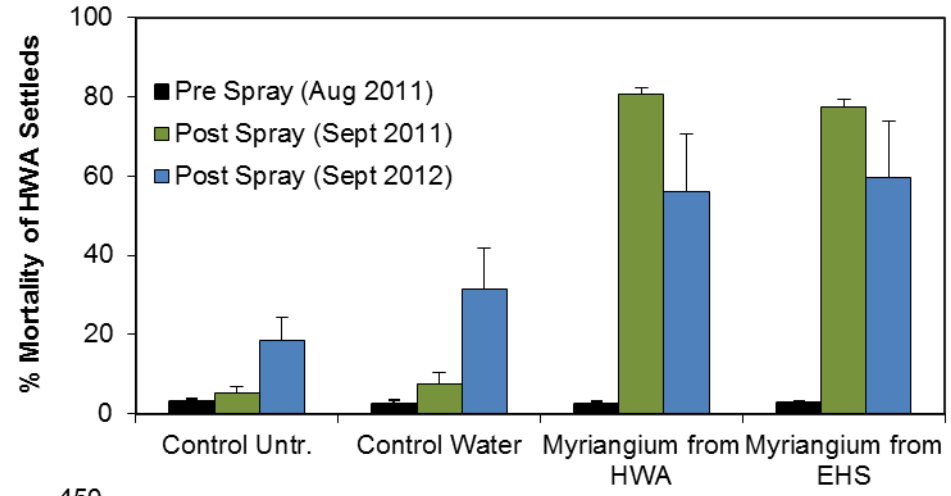


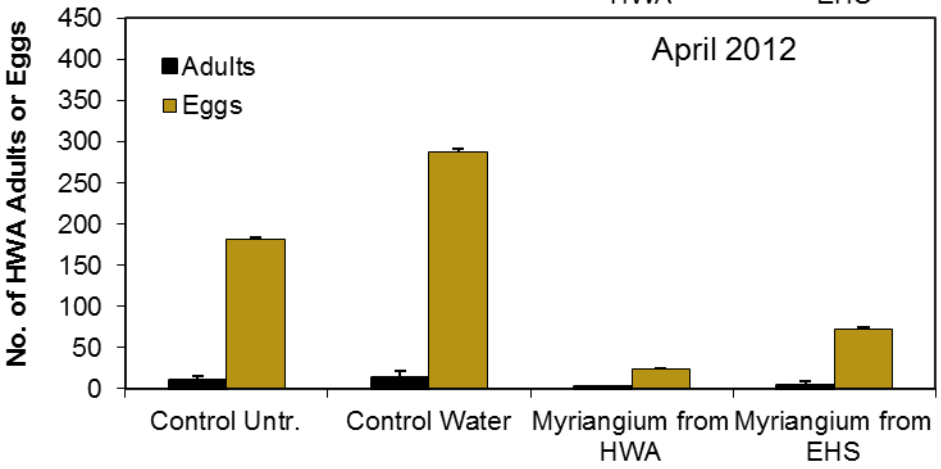
Results from 2011 sampling

- In general natural HWA mortality ranged from 10-30%.
- *Myriangium* sp. isolated from HWA at one site only, in Brattleboro, VT.
- *Myriangium* sp. isolated from elongate hemlock scale in NY.

Results

Obj. 3a. Initiate a *Myriangium*-induced epizootic of HWA and determine its persistence and rate of spread.





Greater mortality of HWA was observed on treated than control branches after 5 wks.

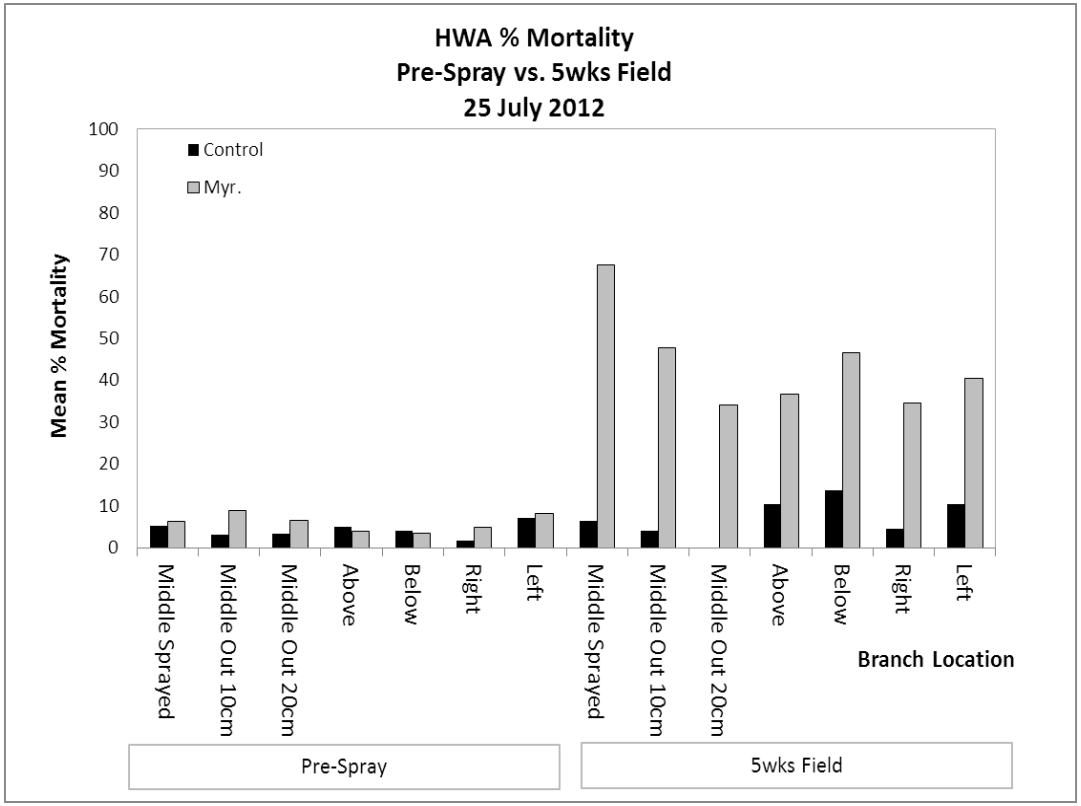
Fewer HWA eggs were found on treated than control branches in the spring after treatment.

Greater mortality of HWA settleds were detected on treated than control branches after 1 yr, suggesting a carryover effect .

There was no difference in mortality between the two *Myriangium* sp. (one from HWA and one from elongate hemlock scale).

These results demonstrate the ability of *Myriangium* to kill HWA, and provide some population suppression for 1 year after treatment.

Results: Obj. 3a, continued



Significantly more HWA mortality was observed 5 wk after treatment than before treatment on the samples taken from the spray branches, and branches adjacent to the spray branch, suggesting that the fungus quickly spread from branch to branch.

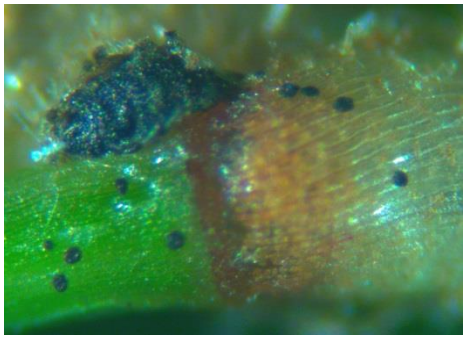
However, overall HWA populations were not reduced on the infested trees as a result of the treatment.

We continued to monitor HWA populations in 2013 at the test sites. HWA populations in the summer were very low at all of the sites. Therefore it was not possible to detect differences in treated and control samples.

Among the many spray trials conducted for this project, in general 74-92% mortality of HWA settleds was observed for the fungal treatments one month after the spray.

Results: Obj. 3a, continued

A new fungus, a *Phoma*-like sp. in class Dothideomycetes, was isolated from HWA from ALL NH and VT sites. *Phoma* is generally considered a plant pathogen rather than an entomopathogen, but this fungus was found infecting HWA in several years of the study.

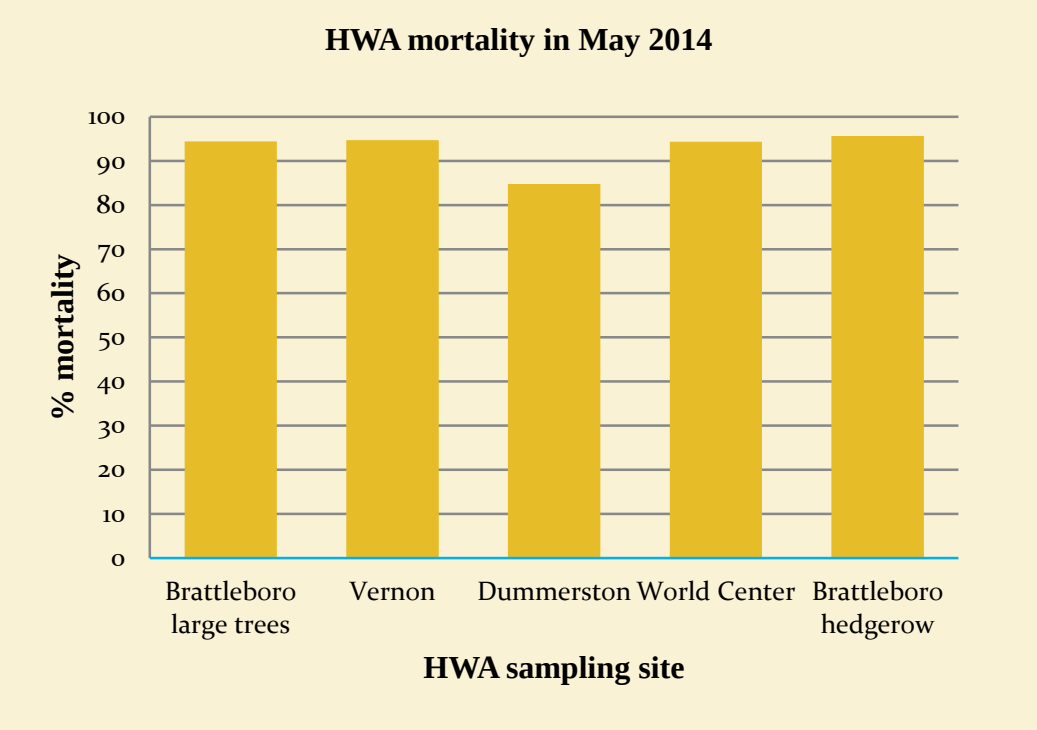


Growth of Dothideomycetes fungus at the margin of an HWA settled collected in NH (left image); HWA cadaver with fungal outgrowth and sporulation (right image).

Some plant pathogens demonstrate entomopathogenic capabilities. Therefore, they should not be excluded from consideration as a biological control tool.

Results: Obj. 3a, continued

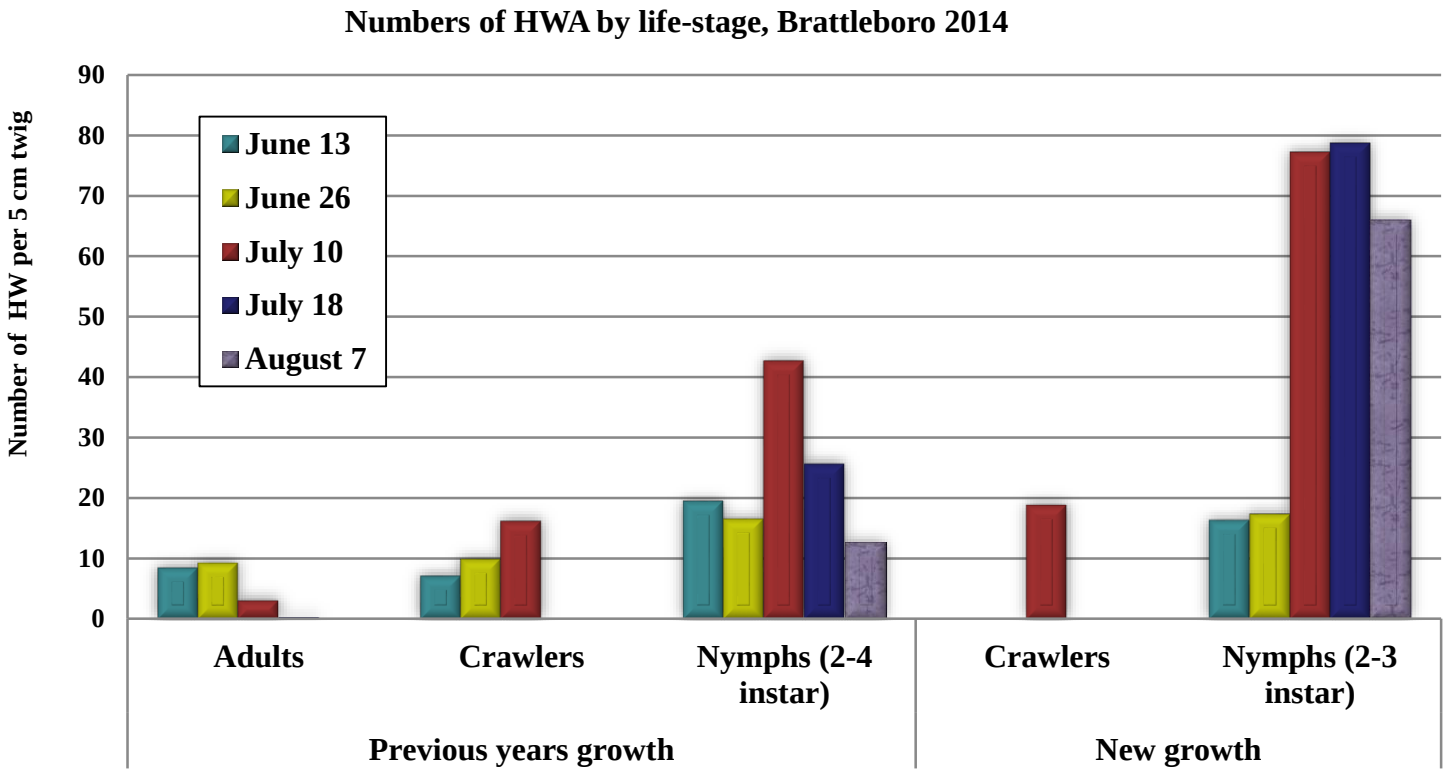
What Happened to HWA in 2014?



We continued to evaluate HWA populations after our fungal treatments in several sites. The 2014 winter was extremely cold, and was thought to have been responsible for the high mortality observed region wide in the spring. However we had observed high mortality of HWA in the summer and fall of 2013 due largely to infection by the *Dothideomycetes* fungus. The drop in HWA populations over the summer allowed the hemlocks to rebound, producing an abundance of new growth in the spring of 2014.

Results: Obj. 3a, continued

HWA Population Trends in 2014



We tracked HWA population changes over the summer of 2014 and found that though populations were very low in the spring, because HWA has 2 generations, and because there was an abundance of succulent new growth, the HWA population rebounded within one year.

Nature is complicated! Many factors affect the population dynamics of HWA. To accurately determine the effect of these factors on HWA infestation levels, ongoing sampling throughout the year is essential.

Results

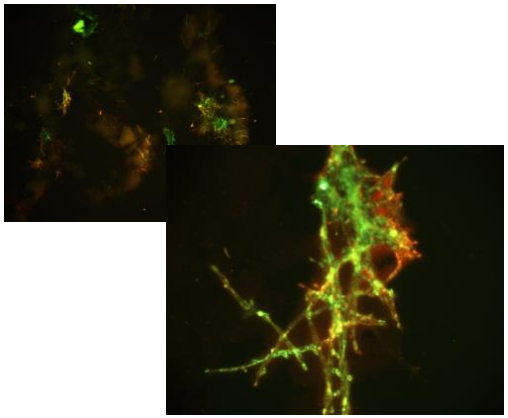
Obj. 3b. Determine if there is an impact on growth and longevity of hemlock seedlings as a result of a single application of *Myriangium* sp.

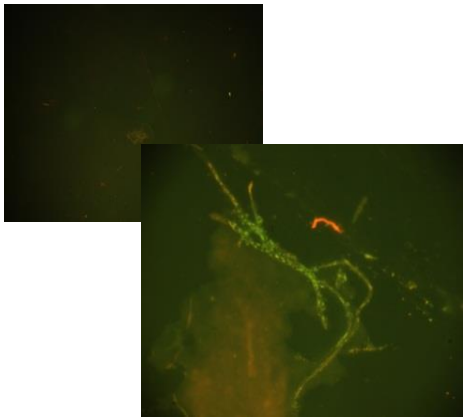
Hemlock seedlings sprayed with *Myriangium* sp. showed no evidence of fungal infection or decline as a result of the treatment throughout the entire research period (4 years).

Results

Obj. 3c. Determine the persistence of *Myriangium* sp. after spraying on branches.

Imprints taken from the glass cover slips after 4 weeks showed a decrease in the amount of viable propagules, but some persistence was observed on the lower surfaces. No evidence of persistence on the upper surface of the cover slips was detected 2 wk after treatment. We believe similar results would be obtained from the needle surface.





Scotch tape imprints from glass cover slips 4 wk after spray. Orange filaments visible on these images are live fungal propagules stained with an acridine dye. Images on the left are from slides held in the laboratory after spraying (protected from the elements), images on the right were from the field.

Results: Outreach Efforts

Five presentations and one webinar were given during the project, reaching over 300 people, including:

- Forest pest specialists
- First detectors
- Forest managers
- Researchers
- Landscapers
- Master gardeners
- General public

A list of the presentations is provided in the List of Products

Implications and Applications in the Northern Forest Region

- HWA remains a threat to the valuable hemlock resource in forest, rural and urban ecosystems in the Northern Forest region.
- Our research showed that *Myriangium* sp. is very pathogenic to HWA, and when sprayed on infested branches, high HWA mortality of the settleds consistently resulted.
- *Myriangium* sp. persisted in the HWA ecosystem after being sprayed, and a carry over effect was observed 1 year later.
- Other fungal species, some of them thought to be plant pathogens, play a significant, yet under-recognized role in reducing HWA populations.
- Cold winter temperatures alone will not stop the spread of HWA, though they can reduce populations in some years.
- Because HWA has 2 generations per year, population levels can rebound within 1 year following a decline either from a cold winter or a fungal outbreak. Therefore, management tools that provide sustained suppression are critical.

Implications and Applications in the Northern Forest Region

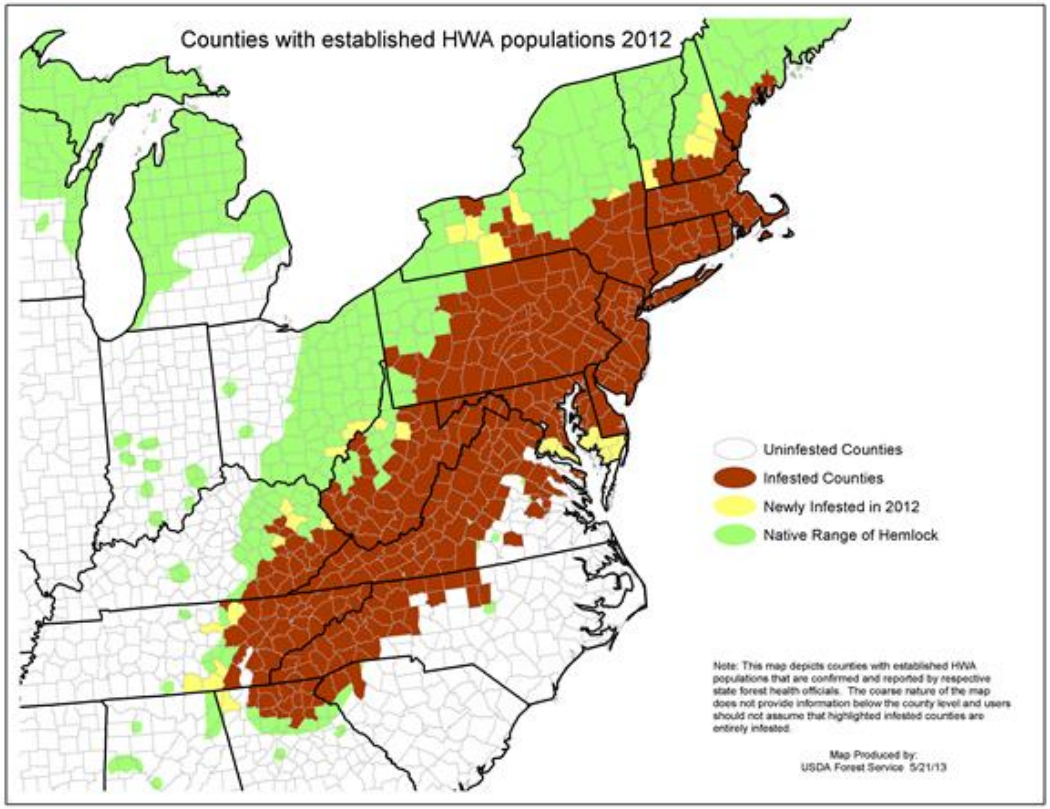
- Given the large forested areas where hemlock grows and their inaccessibility to traditional treatments, self-sustaining biological control agents with the ability to spread naturally must be found.
- HWA is a hard nut to crack. A multi-pronged approach, including fungi and other natural enemies, is needed to manage HWA effectively.
- Funding is needed to fully exploit the potential of fungi and as well as other traditional biological control agents.

Future Directions

- Options for harnessing the potential of all of the fungi in the hemlock ecosystem have yet to be fully evaluated. We have just begun to scratch the surface of this topic.
- Fungi growing as endophytes within hemlock needles have been shown to cause epizootics of elongate hemlock scale. There may be opportunities to exploit this tri-trophic plant/fungus/insect association for management of HWA.
- A broad assessment of the natural fungal diversity in hemlock forest ecosystems is needed. This would enhance our understanding of the role these microbial agents play in the population dynamics of HWA.
- Molecular analyses of fungi in and outside HWA-infested areas are critical to cost effectively evaluate the role of microbial organisms in the population dynamics of this pest. Fungal identification is a challenging task, esp. in hemlock forests, where limited study of the fungi that occur there has been done. Molecular tools could expedite an increase in our base of knowledge about existing diversity, as well as track future fungal-based management efforts.

Future Directions

Given the persistent nature of HWA and its expanding range in the eastern US, we will have to think outside the box and work collaboratively to develop multi-faceted solutions.



This!

Not This...

List of products

Presentations:

1. Skinner, M., B.L. Parker, V. Gouli & S. Gouli. 2015. Hemlock Woolly Adelgid: A Hard Nut to Crack. Oct. 2015. Webinar hosted by NE States Research Cooperative, Burlington, VT. https://vimeo.com/144122016 Invited speaker for broad audience including Mastergardeners, foresters, forest pest specialists, researchers and the public. Over 116 people took part in the webinar, the highest attendance ever for the NSRC. Usually they get only 10-25 people to sign up, another 51 viewed it online.

2. Skinner, M., B.L. Parker, S. Gouli & D. Tobi. 2014. Hemlock Woolly Adelgid: A Hard Nut to Crack. 18 Nov. 2014. Dummerston Conservation Comm., Dummerston, VT. http://www.brattleborotv.org/dummerston-conservation-commission/dcc-hemlock-woolly-adelgid-111814. Invited speaker for audience of 25 First Detectors, forestry personnel, members of the Conservation Board and the general public.

3. Skinner, M., B.L. Parker & S. Gouli. 2014. The Promise of Fungi for Sustained Management of Hemlock Woolly Adelgid. Apr., 2014. Dummerston, VT. VT Dept. of Forests, Parks & Recreation. Invited speaker for audience of 25 First Detector Pest Inspectors.

4. Skinner, M., S. Gouli, V. Gouli & B. L. Parker. 2013. Harnessing the potential of fungi associated with epizootics in hemlock woolly adelgid populations in Maine, New Hampshire and Vermont. 24th USDA Interagency Research Forum on Invasive Species. 8-11 Jan., 2013. Annapolis, MD. Poster presentation.

5. Skinner, M., B.L. Parker, & S. Gouli. 2012. Harnessing the Potential of Fungi Associated with Epizootics in Hemlock Woolly Adelgid Populations in Maine, New Hampshire and Vermont. Regional Foresters meeting. 24 Oct. 2012, Brattleboro, VT. Invited speaker for audience of 30 forestry professionals and researchers.

6. Skinner, M. & B.L. Parker. 2015. The Threat of Exotic Invasive Species. Northern New England Chapter of the Eastern Region of the Association of Physical Plant Administrations Spring Conference, 16 March, 2015. St. Michael's College, Colchester, VT. Invited speaker for audience of 15 administrators and landscapers.