The effects of hemlock woolly adelgid and imidacloprid on the abundance and diversity of eastern hemlock ectomycorrhizal fungi

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Abstract

Invasive insects like hemlock woolly adelgid (HWA) may compromise belowground functionality of infested hosts. Studies show that HWA affects belowground functionality of hemlocks by reducing ectomycorrhizal fungal associations (ECM). ECM benefit hemlock hosts by expanding the root system and increasing resource uptake. Defoliation from HWA herbivory halts photosynthate production, reducing ECM food supplies. To combat this, forest managers treat with the insecticide imidacloprid. Limited research exists on how imidacloprid may affect ECM colonies. This study aims to determine if imidacloprid affects ECM colonies. PCR amplification and Sanger sequencing will be used to compare the ECM community structure of treated hemlock trees, untreated healthy trees, and untreated infested trees. Results will reveal whether fungal communities diminish in the presence of imidacloprid treatments. We hypothesized that ECM colonies decline around imidacloprid-treated hemlocks.

Background

Hemlock woolly adelgid (HWA), which is a tiny insect, threatens to kill mature hemlock trees in roughly 1 million hectares of mature woodland, from the eastern United States to the southern Appalachians and from southern Canada to the central Great Lake states.

Invasive pests, such as HWA affect the aboveground structure and composition of ecosystems, but also may affect belowground functions, including root associations with beneficial fungi (ECM) and bacteria. What is not known is how treatments for HWA, such as the systemic insecticide imidacloprid, affect the fungal associations with hemlock trees.

Research Questions

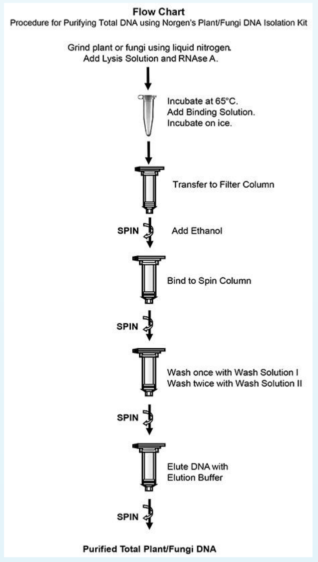
- Is HWA affecting the ECM colonies of hemlock trees in Western Michigan?
- Is the insecticide imidacloprid intensifying ECM colony loss?

Methodology

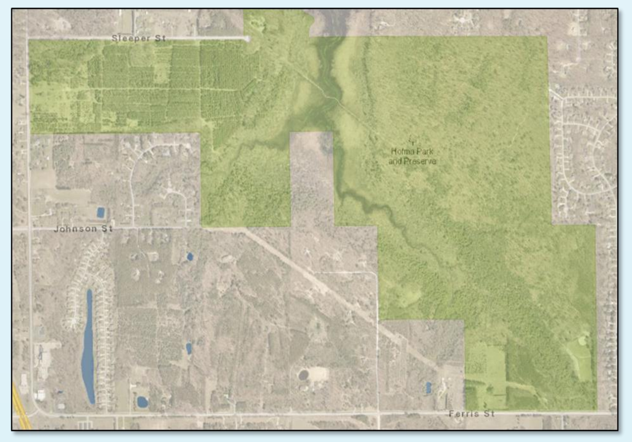
Research protocol:
- Mapping of site locations and infected trees
- Soil sampling and classification
- Microscopic ID of fungi presence on root tips)
- Analysis of soil chemistry (sent to Michigan State)
- Molecular identification of ECM (currently under way)

Photo by Ellen Larson. Root-tip mycelia

PCR amplification of the Internal Transcribed Spacer (ITS) of fungal rDNA allows for successful genetic identification of various ECM species. Environmental DNA will be extracted using the plant/fungal DNA extraction kit from Norgen Biotek.



Study Area



Hofma Park, Grand Haven, Michigan

Mulligan's Hollow, Grand Haven, Michigan

Study areas were chosen using these criterion:
- Had a canopy dominated by hemlock trees
- Had been infested for at least a full year

Two additional counties will be surveyed, sampled, and analyzed; Allegan and Muskegon counties.

Results

Soil physical characteristics were found to be relatively uniform throughout all sample locations. Within Mulligan's Hollow and Hofma park, all locations had the soil textures of loamy sand and sand (Table 1). Dry soil color ranged from 5YR 2.5/2 to 10YR 4/3 (Table 1). Wet soil color was the same for every sampled location, 10YR 2/2 (Table 1). Slight variations between sites was seen for Phosphorus content, but it hovered around 15 ppm (Table 1). All but one site, Mulligan's #2, was classified as mineral soil; Mulligan's #2 was classified as organic. Every location was sited as being nitrogen deficient (Table 1). The pH level for each location tested as being acidic (pH <5) with the exception of the control, Hofma Park (pH 5.7) (Table1).

Table 1 Soil nutrient content such as the classification, pH, nutrient levels and need, and cation exchange capacity are listed. Soil samples were collected in Grand Haven, Michigan July 2019. Processing procedures were conducted by the Michigan State University Soil and Plant Nutrient Laboratory in August 2019.

	Sample Site			
	Experimental			Control
	Mulligan's #1	Mulligan's #2	Mulligan's #3	Hofma
Soil Type	Mineral	Organic	Mineral	Mineral
Texture	Loamy Sand/Sand	Loamy Sand/Sand	Loamy Sand/Sand	Loamy Sand/Sand
Color	W: 10YR 2/2 D: 5YR 2.5/2	W: 10YR 2/2 D: 7.5YR 4/3	W: 10YR 2/2 D: 7.5YR 5/4	W: 10YR 2/2 D: 10YR 4/3
Soil pH	4.8	3.8	4.7	5.7
Phosphorus (ppm)	16	15	16	13
Potassium (ppm)	40	66	53	42
Magnesium (ppm)	119	102	116	134
Cation Exchange Capacity (meq/100g)	14.6	N/A	18.6	10.7
Nutrient Need	Nitrogen	Nitrogen	Nitrogen	Nitrogen

Discussion

It was interesting to find that sites tested for an increased need in nitrogen. In areas of heavy HWA infestation, we typically see higher nitrogen content due to increased hemlock mortality. While typically sandy soils are lacking in nitrogen content due to leaching, I was expecting the nitrogen and nitrate content to be above the norm. It is difficult to assess this relationship, however. Soil test results did not quantify nitrogen and nitrate levels. Currently, additional soil samples are being collected to be re-processed by Michigan State University for nitrogen and nitrate content.

At this time it is not possible to make concrete conclusions regarding whether imidacloprid and HWA affect ECM communities. Genetic processing is still currently underway to determine the diversity of ECM that exist in the presence of HWA and imidacloprid.

Research Application

Restoration efforts are currently underway in regard to HWA. This research will put another tool in the toolkit for all resource managers and ecologists. Overall, the data collected serves to inform forest ecosystem managers on the effects that imidacloprid may have on the ECM associated with hemlock trees in west Michigan. The results can better prepare managers by:
- Having a more robust understanding of imidacloprid and how it affects the soil mycobiome
- Provide evidence for the increased need for ECM re-inoculation post-HWA infestation and hemlock mortality.

Expected Results

It is expected that ECM colonies will be reduced in both abundance and diversity in the presence of imidacloprid and HWA. This hypothesis is based off previous studies that have found that imidacloprid has a negative effect on assorted soil arthropods, aquatic life, and honey bees (Peck 2009).

Additionally, in the presence of herbicides Glyphosate and Terbuthylazine, the arbuscular fungus *Glomus mosseae* showed inhibited growth rates (Giovannetti et al. 2006).

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Literature Cited

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