

# **ELONGATE HEMLOCK SCALE AS THE PRIMARY DRIVING FORCE OF HEMLOCK DECLINE IN THE PRESENCE OF HEMLOCK WOOLLY ADELGID AND FUNGAL ENTOMOPATHOGEN *MYRIANGIUM DURIAEI***

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*Abstract.* Invasive forest pests have become an increasing problem in forests globally. In the eastern United States, much focus has been towards addressing the problem of hemlock woolly adelgid (*Adelges tsugae*) and its damage to eastern hemlock (*Tsuga canadensis*) stands. However, another invasive pest, elongate hemlock scale (*Fiorina externa*), has established in the eastern United States and has also been impacting hemlock stands. Due to the presence of both pests, it is important to understand how these pests interact with hemlock health when both are present. Furthermore, how do combinations of these hemlock woolly adelgid and elongate hemlock scale interact while also in the presence of naturally occurring fungal entomopathogen black scale fungus, *Myriangium duriaei*. To study these phenomena, 98 understory hemlock trees were assessed across ten 1-acre plots at Mohonk Preserve in New Paltz, NY for infestation levels of elongate hemlock scale, hemlock woolly adelgid, and measures of tree health. The presence of a fungal pathogen (*Myriangium duriaei*) of elongate hemlock scale was also measured. Across the preserve, density of elongate hemlock scale was found to be the primary factor in declining hemlock trees. The fungal pathogen of elongate hemlock scale was not found to be directly correlated with density of the insect, suggesting that environmental conditions may be more influential on prevalence of the disease than host density. Interestingly, this pathogen was negatively associated with hemlock woolly adelgid suggesting that conditions favoring hemlock woolly adelgid may in fact be similar conditions that the fungus struggles to survive. At Mohonk Preserve, elongate hemlock scale seems to be the primary contributor to hemlock decline rather than hemlock woolly adelgid, suggesting that in areas where both pests are present management decisions should focus on both pests for the greatest chance at success.

## **INTRODUCTION**

Invasive forest pests have caused severe damage to forest communities across the US becoming one of forest managers' upmost concerns. The decline in eastern hemlock as a result of invasive pests has resulted in replacement by black birch in hemlock dominated stands (Orwig et al. 2002). In mixed stands, hardwoods such as red oak have replaced the hemlock in the canopy resulting in a more homogenous forest with fewer habitats for organisms that rely on hemlock for habitat (Lovett et al. 2016). The eastern hemlock provides a unique habitat for a variety of organisms as its relative shade tolerance allows for the creation of dense vegetation beneath the canopy (Tingley et al. 2002). In riparian zones, the presence of hemlock allows for greater canopy coverage of streams, resulting in colder temperatures which support many organisms including temperature sensitive trout (Lovett et al. 2016). In the forest, deer rely on the thick hemlock canopy for shelter during the winter months, while other organisms such as porcupines also depend on hemlock stands as a vital part of their habitat (Tingley et al. 2002).

Hemlock woolly adelgid (*Adelges tsugae*, HWA) has been widely proclaimed as the primary driver of hemlock decline (McClure 1980). HWA has proved especially damaging to *Tsugae canadensis* and in some stands has resulted in mortality of 95% of infected hemlock trees, with little to no regeneration (Bair 2002). HWA was unintentionally introduced during the 1950's from Japan. Since then, HWA has spread across the East Coast causing widespread hemlock stand decline and continues to spread across the range of eastern hemlock (Lovett et al. 2016). A wide range of efforts have been undertaken to limit its damage including the implementation of a variety of biological controls and insecticide campaigns (McClure 1987).

HWA has been the major hemlock pest upon which research and management efforts have been focused (Orwig and Foster 1996). Elongate hemlock scale (*Fiorina externa*, EHS) however, is also an important contributor in the pest load that affects hemlock stands. Of particular interest, are any interactions between the two pests – which are commonly found together – that could inform management and control of HWA? EHS was first detected in the United States in 1905 in New York City where it was accidentally introduced from Japan. EHS has spread across the range of eastern hemlock but densities typically remain low (McClure 1978). The highest densities of EHS are present within a 300 km radius of New York City (Danoff-Burg and Bird 2002). EHS is widely considered the lesser of two evils relative to HWA as at high EHS densities mortality often occurs in 10 years, as opposed to HWA which can cause mortality in as little as 4-15 years, although there are trees that live with infestation for over 30 years (McClure 1991).

Control methods for both insects are widely needed and many efforts are currently being employed. In an attempt to control HWA, several natural predators have been periodically released in addition to the implementation of insecticide campaigns (Chea et al. 2004). These controls have not yet shown any drastic declines in HWA. EHS, on the other hand, has not had the same sort of effort dedicated to its control. Although some natural predators of EHS have been observed, they have not been seen to demonstrate any significant reduction in their populations (Miller-Pierce and Preisser 2012). Previous research on *F. externa* has found several fungal infections of EHS. One of those fungal entomopathogens produces macroscopic black sclerotia masses on the surface of EHS and was identified as *Myriangium duriaei* (Gouli et al. 2013). In researching this fungal infection, a wide variety of mortality rates were found (Gouli et al. 2013).

As HWA and EHS are commonly found together, several studies have been performed to better characterize their interaction. One study suggested the existence of a priority effect between the two insects. The study found that co-infested trees with both pests differed in that their response to the total pest load. In particular, trees which were first infested with EHS and later by HWA suffered significantly less damage than the trees infested solely with HWA and trees infested first with HWA and then with EHS (Miller-Pierce and Preisser 2012). Furthermore, several studies have demonstrated that EHS is not the driving factor of hemlock decline but rather HWA (Miller-Pierce and Preisser 2012, McClure 1980). This finding has been widely assumed by forest managers and almost all attempts at managing hemlocks center around HWA.

This study aims to assess the interaction between both of these pests and also to determine the relationship between observed entomopathogenic fungal infection and tree health. By assessing trees of the same size class across 30 sites with different levels of infestation by HWA and EHS, the goal is to quantify the impact of each pest and the fungal entomopathogen have on tree health. A better

understanding of these interactions will hopefully inform management decisions in combating widespread hemlock decline.

## METHODS

### *Study Site*

The study was performed at the Mohonk Preserve in Ulster County, New York. The roughly 8000 acre preserve is located on the Shawangunk Ridge of the Appalachian Mountains and contains a mix of hemlock dominated and mixed hardwood tree stands (Abrams and Sands 2010). Within this preserve, 21 1-acre plots had already been established across the preserve in hemlock dominated tree stands (at least 50% basal area of hemlock) as part of a broader research effort of which this study forms a component. Of the 21 pre-existing plots, 10 were chosen for further analysis based on unpublished data of EHS and HWA prevalence. From this long term data, plots were chosen that had varied levels of the two pests' prevalence and that would provide a good representation of the varied habitats in the preserve. The black scale fungus (*Myriangium duriaei*) on EHS had been previously identified at the Mohonk Preserve by Skinner et al. (2009). I identified the fungal infection based on macroscopically visible sclerotia masses (see Fig 1b in Gouli et al. 2013) using a stereo microscope.

### *Study Design*

Within the 10 selected plots, 10 trees with branches at most 2.5 meters from the forest floor and of diameter at breast height (DBH) less than 12 cm were selected. This class of trees was chosen for logistical reasons as these trees have branches easily accessible. Three branch samples were collected by cutting approximately 30 cm segments from three different branches on each selected tree.

Each individual branch was assessed for health status, hemlock woolly adelgid and elongate hemlock scale infestation, percent of scales that had fungal infection, and new growth. Health Status was based on needle loss, coloration, and amount of new growth (Table 1). This health matrix was created for use in this project; however some elements were adapted from Danoff-Burg and Bird (2002). Hemlock woolly adelgid infestation was assessed in terms of the amount of the branch covered by the adelgid. The categories were 0,  $\leq 1\%$ ,  $\leq 10\%$ ,  $\leq 25\%$ ,  $\leq 50\%$ ,  $\leq 75\%$ , and  $\leq 100\%$  of branch covered. Branch new growth was measured by randomly selecting 10 end branchlets per sample and measuring the new growth in centimeters. Similarly, tree new growth was measured by randomly selecting 10 branchlets on parts of the tree that was not sampled for infestation and measuring in centimeters. Ten measurements were taken from each sample collected. New growth in eastern hemlock trees is easily determined as it is a brighter/lighter shade of green than older growth. Lastly, scale infestation was measured in terms of scale insects per 100 needles (McClure 1980). This scale infestation metric was assessed by randomly selecting 25 needles on each branchlet of a total of one third of the total number of extensions from the primary stem. Fungal infection was determined as the percent of scale insects that were showing black sclerotia masses.

### *Statistical Analysis*

Because three branch samples were taken per tree, data gained from each of the three branch samples per tree were combined to create a representative value for the tree as the whole. For both tree new growth and branch new growth this was performed by taking the mean of the measured new growth of each

sample and then taking a mean of those means. For fungal infection an arcsine transformation was performed because measurements were in percentages. This transformation allowed for an averaging of fungal infection measurements of each sample. This transformation was also used in analyzing infestation data for HWA. For characterizing branch health status, four categories were used ranging from “4”, which represented the healthiest class, down to “1” which represented severe stress. In order to characterize the branch health status for the whole tree from the three sampled branches, the median tree health value was used to characterize the tree health for each tree.

A multivariate analysis using a constrained redundancy analysis (RDA) was performed using the Canoco 5 program. This analysis was used to determine the amount of variation of multiple indicators of tree health (response variable) in relation to elongate hemlock scale, hemlock woolly adelgid, and the fungal pathogen of EHS (explanatory variables). The analysis also used dbh as a covariate. Linear regression was also performed (using R 3.3.1) used to determine relationships between single variables.

## RESULTS

An RDA multivariate analysis was performed with the EHS, HWA, and fungal infestations explaining a total of 13.2% of the variation in the following tree health metrics: live crown ratio, tree new growth, branch new growth, and branch health status. EHS on axis one explained the greatest amount of variation (10.95%) in the tree health metrics (see Fig. 1). EHS density had a negative relationship with both tree live crown ratio and branch new growth. EHS, however, was not correlated with tree new growth, HWA, or fungal presence. Furthermore, as EHS density increased branch health decreased. HWA was observed to be negatively correlated with both fungal infection and tree new growth. HWA presence was also found to be positively correlated with the branch health status of severe stress. Additionally, fungal presence was positively correlated with tree new growth, but was not correlated with any other response variable or tree health metric.

When comparing individual health metrics with each of the observed pests using linear regression, a similar story is seen (see Tab. 2). EHS remains the primary driving factor of hemlock decline, even in the absence of HWA. EHS is negatively correlated with live crown cration, branch new growth, and branch health status when including all trees as well as those that were only infested with EHS. When comparing the presence of EHS to the other pests, there was no relationship between presence of HWA and EHS, however in the absence of HWA, EHS and the fungal infection were positively correlated. Similar tests excluding the presence of the other insect pest were not possible for HWA as all trees tested had some level of EHS infestation.

A linear regression was performed between EHS scale density and average branch new growth, revealing a significant negative relationship between them, showing that as EHS density increases branch new growth decreases (Fig. 3:  $P < .001$ ,  $r^2 = .20$ ). This is also reflected in the partial RDA in Figure 1. Additionally, a singular linear regression was performed that demonstrated a trend where HWA decreased as black scale infection increased (Fig. 2:  $p = 0.063$ ,  $r^2 = .041$ )

## DISCUSSION

At Mohonk Preserve, it is evident that elongate hemlock scale is the primary driver in the decline of hemlock trees. This trend is seen from small-scale health metrics such as branch health to more holistic

health metrics such as live crown ratio. This finding is of special importance due to the fact that the pest is largely assumed to be a non-concern, thus highlighting a need for further research on the impact of EHS. Adding to this finding, HWA was seen to contribute to a decline only in branch health but not any other measurement of tree health. Since this effect on branch health status is a more local attribute, it further supports the idea that EHS is the primary pest; and HWA is a secondary pest affecting the trees on the preserve at this time since infestation. Both HWA and EHS arrived to the preserve in the early 1990's. Branch health is the least representative assessment of health of the total tree as it reflects the health of only localized portion of the tree. This showing that in this system that any affect HWA may have in tree decline is much less than EHS.

Similar findings of EHS being the primary driver of needle loss and hemlock decline have also been made at Black Rock Forest in West Point, NY, approximately 65 km to the south. Both Mohonk Preserve and Black Rock Forest are located in the region of highest density of EHS, with a 300 km radius around New York City (Danoff-Burg and Bird 2002). As both sites are in the area of highest EHS density, the observed impact of EHS may be related to the high density of EHS. Another of the shared factors between these sites is both being near the northern range of HWA. Of the two insects, HWA is cold sensitive relative to EHS (Preisser et al. 2008). As the sites are located in the northern range of HWA, the observation that it is not the primary pest may reflect the stress it faces in the more northern region of its range due to more severe winters.

Various relationships between HWA and EHS have been reported including antagonistic relationships manifesting in increased rates of hemlock mortality in co-infested trees (McClure 1980). Additionally, there has also been evidence that an asymmetric priority effect exists between HWA and EHS (Miller-Pierce and Preisser 2012) in which infestations of HWA on a tree previously infested with EHS is limited in its ability to damage the tree, without HWA having this effect on subsequent EHS infestation. Evidence of this priority effect is difficult to discern in this study without knowing mortality rates. However, the fact that EHS seems to be the driver of the decline at Mohonk as opposed to HWA, which is largely considered more severe, could be evidence in itself.

When considering the black scale fungus (*Myriangium duriaei*), its presence was seen to be positively correlated with tree new growth. Although no relationships were seen with other measurements of tree health, its positive correlation with tree new growth suggests that presence of the entomopathogen may be aiding in the health of the tree. The fact that its presence was not related to all measures of tree health suggests that this fungus may not be able to eliminate the pest, but an increase in long-term survival is possible. It has been previously suggested that this fungus may be limited in controlling EHS, as females have been observed laying eggs while infected (Gouli et al. 2013), suggesting that it would be unable to curtail the number of pests feeding on the host plant. Interestingly, a negative relationship was seen between HWA and the fungal infection of EHS. Although *Myriangium* genus affects only scale insects like EHS, the conditions needed for the fungus may be ones that are not favorable for HWA. Additionally, there is a possibility that the presence of the fungus may make the hemlock host less palatable for HWA. Thus, this finding is important to note in any potential consideration of the pathogen to be used as a biocontrol agent for EHS.

At Mohonk Preserve, the finding that EHS has a greater impact on tree health than HWA is very important when considering management decisions (Fig. 1). Not only is this contrary to popular

consensus, but it also can have a practical impact in combating tree mortality from invasive pests. In particular, releasing specialized insect predators of HWA in this region, may not affect hemlock mortality if it is largely attributable to EHS. Furthermore, chemical controls used for treating HWA may not be effective on EHS. Any management decisions should focus on both pests for maximum benefit. Additionally in forests where both pests are present, control methods should be directed at both insects as decline in HWA may result in only a temporary relief from damage before EHS inflicts further damage on the trees.

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**APPENDIX**

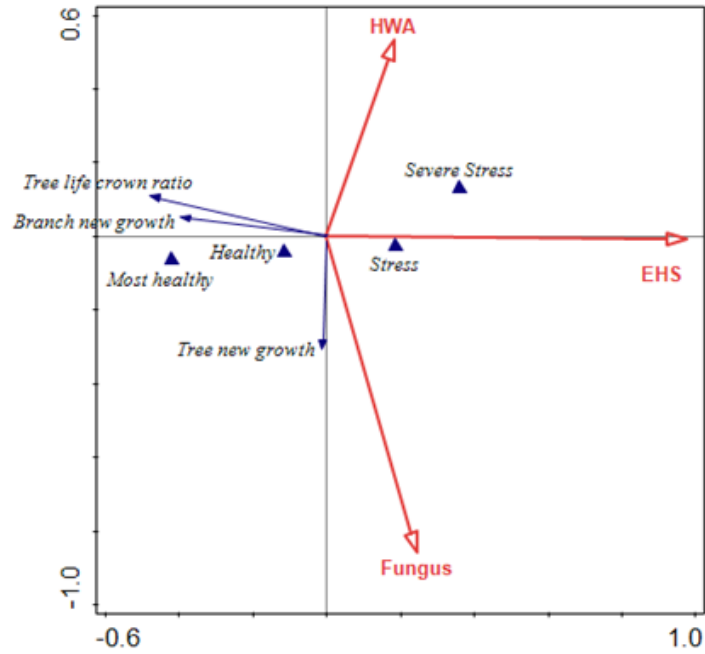
**TABLE 1.** A table describing the characteristics used to determine branch health.

<b>Characteristic</b>	<b>Most Healthy</b>	<b>Healthy</b>	<b>Visually Stressed</b>	<b>Severe stress</b>
Needle Coverage	>90%	>75%	>50%	<50% branch coverage
Needle Color	Dark green	Dark green, localized slight yellowing	Widespread yellowing, dark, pale green needles, blue tintage	Widespread yellowing, dark, pale green needles, blue tintage
New Growth	>75%	>50%	<50%	<33%
Needles on primary stem	Needles present	Needles Present	No Needles	No Needles
Rust	Minimal	Minimal	Widespread	Widespread
Needle-less branchlets	No	No	1 or 2	More than 3

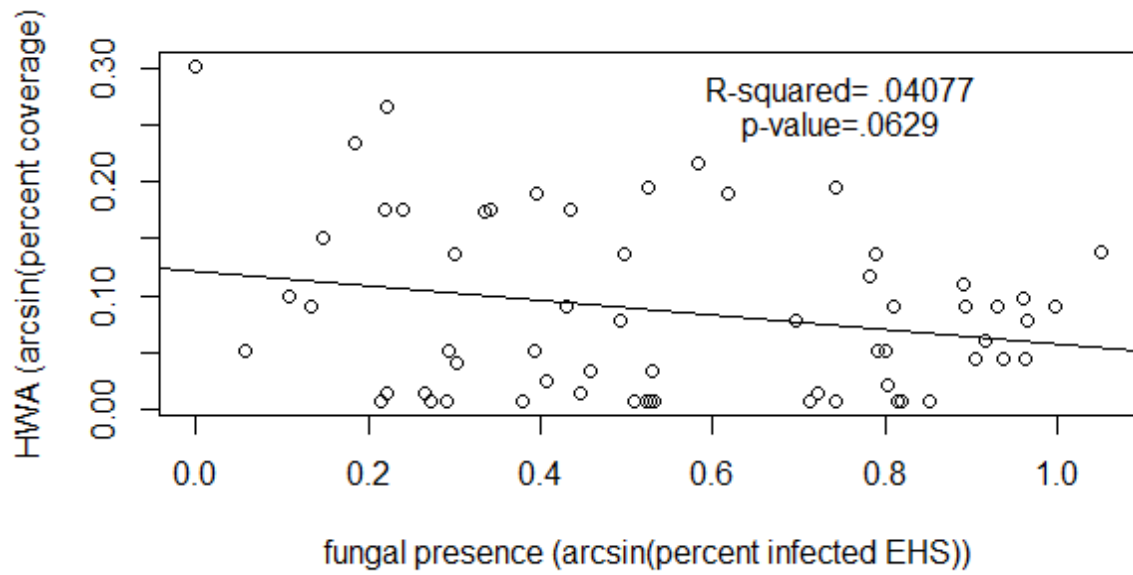
**TABLE 2.** Table presenting results from individual linear regressions in the presence and absence of each pest. Of the total 98 tree, 62 trees had HWA, while 35 trees did not. \*\*\* P<0.001 \*\* P<0.01 \* P<0.05 +p<0.1.

	No HWA	HWA present	No EHS	EHS present	No HWA	HWA present
<b>Tree health</b>	EHS as explanatory variable		HWA as explanatory variable ( <i>only cases with HWA</i> )		Fungus as explanatory variable	
Live crown ratio	declines**	declines***	<i>All trees had EHS</i>	no effect	declines**	no effect
Tree new growth	no effect	no effect		no effect	increases*	increases+
Branch new growth	declines**	declines***		no effect	declines*	no effect
Health status	declines**	declines***		declines*	no effect	no effect
<b>Fungus</b>	EHS as explanatory variable		HWA as explanatory variable			
Infestation rate	increases*	no effect	<i>Does not make sense biologically (so no tests here)</i>			
<b>HWA</b>	EHS as explanatory variable				Fungus as explanatory variable	
Infestation rate	-	no effect			-	declines+

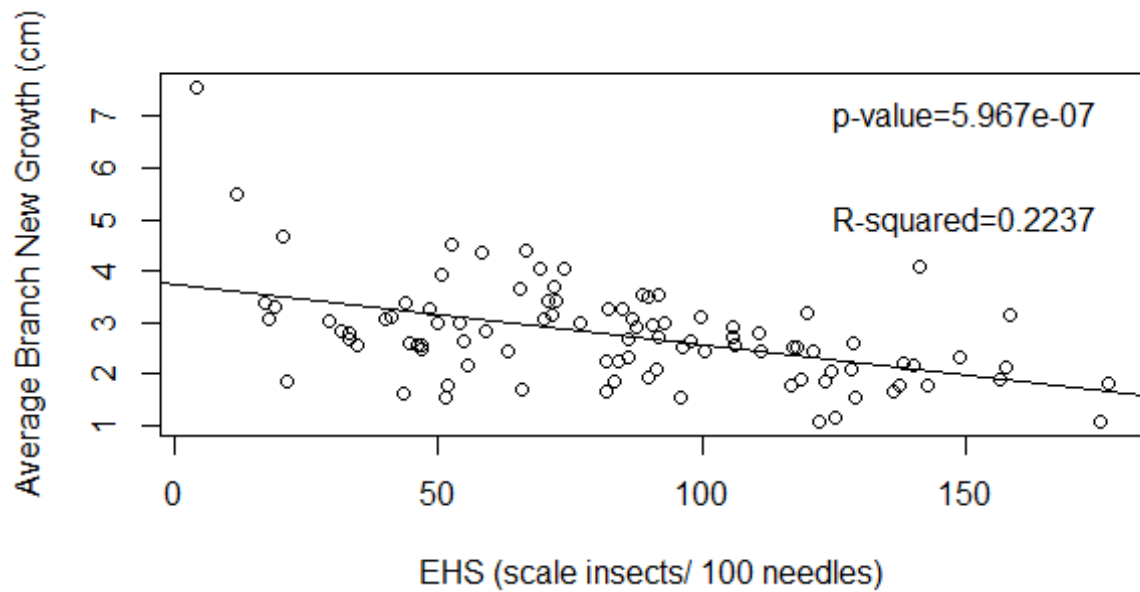




**FIGURE 1.** An RDA multivariate analysis produced by Canoco 5 software. Blue arrows describe response variables while red arrows describe explanatory variables. DBH is used as a covariate. Blue triangles describe branch health classes (p-value= .002, explanatory variables account for 13.2% of variation. Axis 1= 10.95, Axis 2= 12.83, Axis 3= 13.23).



**FIGURE 2.** A linear regression between amount of hemlock woolly adelgid and fungal presence. ( $r^2=0.0565$ ,  $p=.06285$ ).



**FIGURE 3.** A linear regression between EHS density and branch new growth.