INVESTIGATING THE EFFECTS OF NITROGEN AND DROUGHT ON HEMLOCK WOOLLY ADELGIDS AND HEMLOCK TREES

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Abstract. Hemlock woolly adelgid (HWA, *Adelges tsugae*) is an invasive pest that is causing widespread mortality of its obligate host species, Carolina and eastern hemlocks in the United States. However, some of the adelgid infested hemlock stands remain living more than a decade after infestation. To date, this has been attributed to site and climatic variables that control HWA population growth. This study will be examining how HWA respond to physiological attributes of hemlock trees under experimental conditions that simulate drought and varying availability of nitrogen. We will monitor HWA population dynamics and its long-term infestation effect on hemlocks in these experimental treatments. Assuming that HWA populations respond to nitrogen (N) and starch in hemlock twigs then we hypothesize that: increasing N availability should increase N in twigs and increase starch reserves, decreasing water availability should decrease N in twigs and decrease starch reserves. Increased N and/or starch in the twig should increase the HWA population.

INTRODUCTION

Invasion of exotic pathogens and pests, and the resulting decline in native species is an important ecological, economic and evolutionary process that may alter ecosystem structure and function, and cause devastating impacts on natural resources and aesthetic conditions (Castello et al. 1995; Enserink 1999; Everett 2000). The hemlock woolly adelgid (HWA) is an exotic pest from Asia that was introduced in the eastern United States in the 1950's. This invasive has the potential to dramatically change forest composition, structure, and microenvironments; alter critical ecosystem processes such as nutrient retention and cycling; and increase an ecosystem's susceptibility to other invasives (Vitousek 1986).

Research studies have shown that the HWA will infest Carolina and eastern hemlocks and that high HWA populations on these species are associated with tree mortality (Orwig and Foster 1998). In the eastern U.S., HWA populations consist mainly of asexually reproducing females, each of which has the ability to lay 50-300 eggs. It also has a complicated two part life cycle that includes an active generation in late winter and a dormant period during the summer. This pest has the potential to spread at a rate of 15km/yr (Orwig and Foster 1998). The HWA feed off of the tree's ray parenchyma cells by inserting a style into the base of needles in search of the carbohydrate reserves. Once infestation has occurred the trees are expected to survive 4-8 years (Braun 1950).

Hemlocks are an extremely important part of northeastern forests not only because of their aesthetic qualities, but because they provide a deep shade and deep, acidic forest floor. This unique habitat promotes biodiversity in the forest. The hemlocks can live over 300 years and are often the most important conifer (soft wood) in our northern hardwood forests. Discoloration, needle desiccation, and dead branches are early symptoms of a HWA attack (McClure et al. 2001). Logging of infested trees, development of insecticides both oil and soap based, and the use of bio-control for example, the black lady beetle has been used to attempt to stop or slow the spread of the pest. None of these methods has yet been successful. Developing an effective method against the adelgids will require a deeper understanding of the physiological interactions between the adelgid and the tree.

The main goal of this study is to investigate the effects of nitrogen and moisture on the hemlock woolly adelgid (HWA). Research has suggested, but has not proven that hemlocks containing high levels of nitrogen are more susceptible to infestation by HWA. (McClure 1991). Also, little research on drought has been done, but it is thought to weaken hemlocks enough that it may enhance adelgid population growth and lead to tree mortality (McClure et al. 2001). We specifically examine survival and growth of HWA populations as we alter nitrogen levels and water availability in hemlocks and evaluate the physiological attributes of hemlock trees that support this change.

Herbivorous insects require carbohydrates from the plant for energy, and their population growth is often limited by nitrogen. (Hopkins 2004). We therefore assume that the hemlock physiological attributes most important to the adelgids are nitrogen (N) and starch. We hypothesize that increasing N availability should increase N in twigs and also increase starch reserves, because when a tree has more N available it tends to photosynthesize more and produce extra carbohydrates for storage. Decreasing water availability should decrease N in twigs and decrease starch reserves, because when a tree experiences drought it tend to photosynthesize less, closes up its stomata and uses up its storage supply. Increasing the amount of nitrogen and the amount of starch reserves available should increase the HWA population because both N and carbohydrates are essential nutrients for the insects.

METHODS

This work will experimentally manipulate hemlocks (*Tsuga canadensis)* in pots and naturally growing saplings in the forest. For the pot study, 28 hemlock saplings (~ 5 years old) will undergo drought and fertilization treatments. All saplings have grown in current containers with low-nutrient soil medium for at least one year. There will be four treatment groups, representing two levels of N availability and two levels of drought (Figure 1). There will be four replicate saplings per treatment group infested with HWA (Figure 1A), and 3 replicates per treatment group that will remain uninfested (Figure 1B) to examine the effects of the treatments on hemlock twig properties in the absence of the HWA. Drought will be manipulated by covering all saplings with a plastic wrap to avoid rain water from wetting the soil in pots; the saplings that will not experience drought will be watered on a weekly schedule. Nitrogen availability will be manipulated by adding fertilizer to the pots of the high-N groups of trees. Nitrogen and carbon will be measured in the needles/twigs of the saplings using mechanical and chemical techniques. Adelgid populations will be determined with the use of microscopy. For the purpose of this study we will not measure the immediate health of the saplings other than twig N and starch. The duration of this study makes monitoring adelgid effects on tree health unfeasible.

This study will also involve a parallel field experiment (Figures 2A and B) using understory trees, in a hemlock stand on Cary Institute property. Nitrogen will be manipulated by fertilizing the trees with ammonium nitrate. Drought experiments on adult trees are somewhat impractical and will not be attempted here. Eight replicate tree pairs will be used, with one tree of each pair fertilized and the other left unfertilized. Responses on infested and uninfested branches of the same tree will be compared.

DISCUSSION

Fertilization with nitrogen does not seem to make an eastern hemlock tree less susceptible to infestation by the hemlock woolly adelgids (HWA), the reverse effect occurs. Trees containing lower foliar nitrogen concentrations had high HWA mortality. The manipulation of nitrogen throughout the experiment on both the field and potted saplings show this. The monitoring of drought in this experiment became impractical because the roots of the potted saplings were bounded in the top soil layer of the pots and when drought conditions were administered, the saplings began to die. Also, storm water was also an issue despite having the saplings covered with plastic. For future studies we could starve future potted saplings from N when purchasing them so that they can take up nitrogen more efficiently during fertilization and given more time, carbohydrate analysis could allow us to monitor physiological changes within the tree's tissue in response to N fertilization, this allows us to monitor the response of HWA in response to the tree's physiological attributes.

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APPENDIX

A)

	High (N)	Low (N)
High H2O	4	4
Drought	4	4

B)

	High (N)	Low (N)
High H2O	3	3
Drought	3	3

FIGURE 1. Experimental design for the pot study. A) is the infested plot. The infested saplings, allow us to monitor the response of the adelgid populations to manipulation of water and N in the saplings. B) is the uninfested plot. The uninfested saplings, allow us to monitor physiological changes within the tree's tissue in response to fertilization with nitrogen and the manipulation of drought conditions, without the impact of the adelgid.

A)

High (N)	Low (N)
4	4

B)

High (N)	Low (N)
4	4

FIGURE 2. Experimental design for the field study. A) is the infested plot. The infested trees allow us to monitor the physiological changes in the tree's tissue in response to N manipulation and the impact of adelgid infestation. B) is the uninfested plot. The uninfested trees allow us to monitor the physiological changes in the tree's tissue in response to N manipulation without the impact of adelgid infestation.

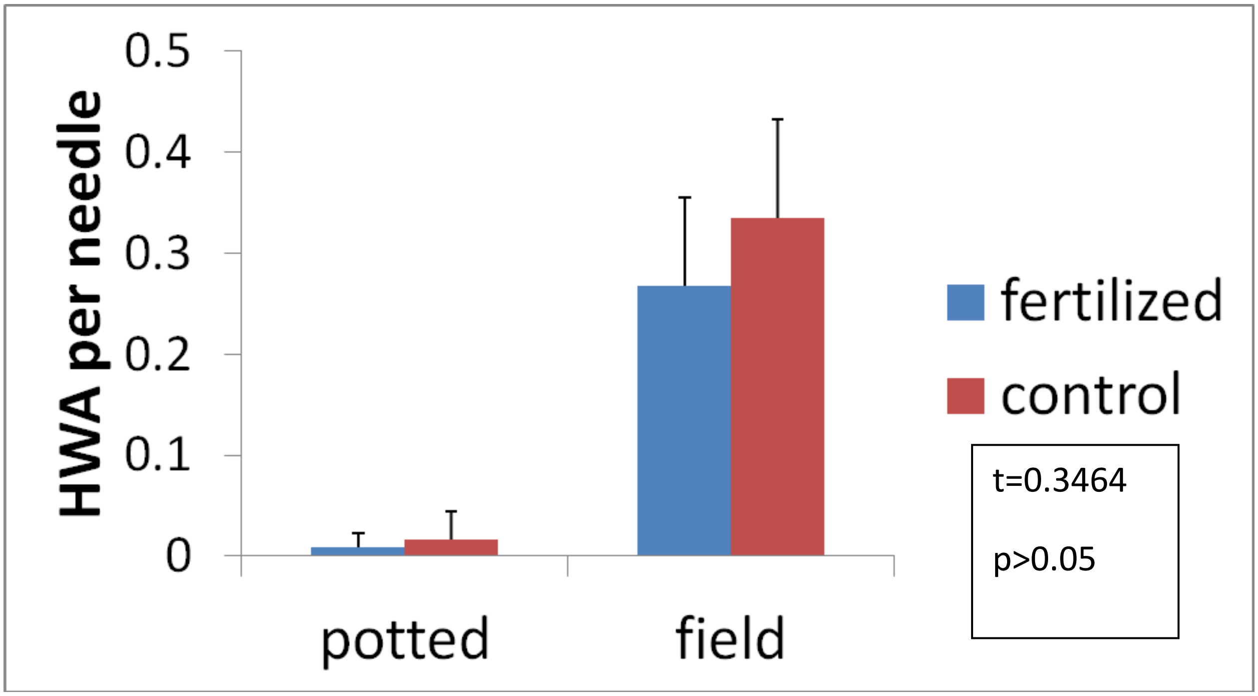

FIGURE 3 (not in text). Figure 1: HWA abundance was not different between N treatments. T-test showed that the means of these samples are not significantly different and that N treatment had no effect on the #HWA.

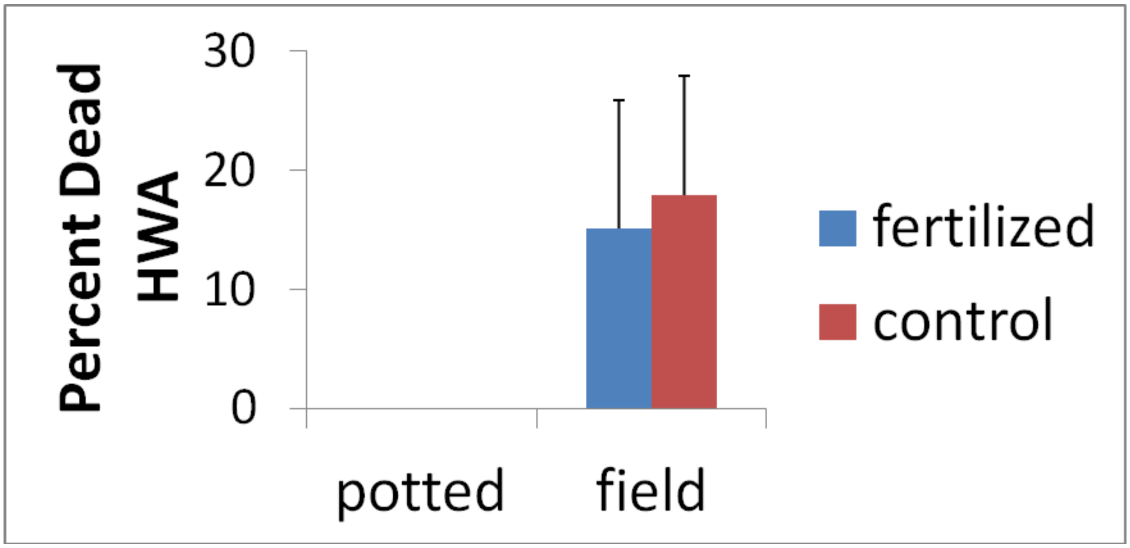

FIGURE 4 (not in text). HWA mortality was higher in field trees but no fertilization effect. There are no bars for the potted saplings because all of the adelgids were alive.

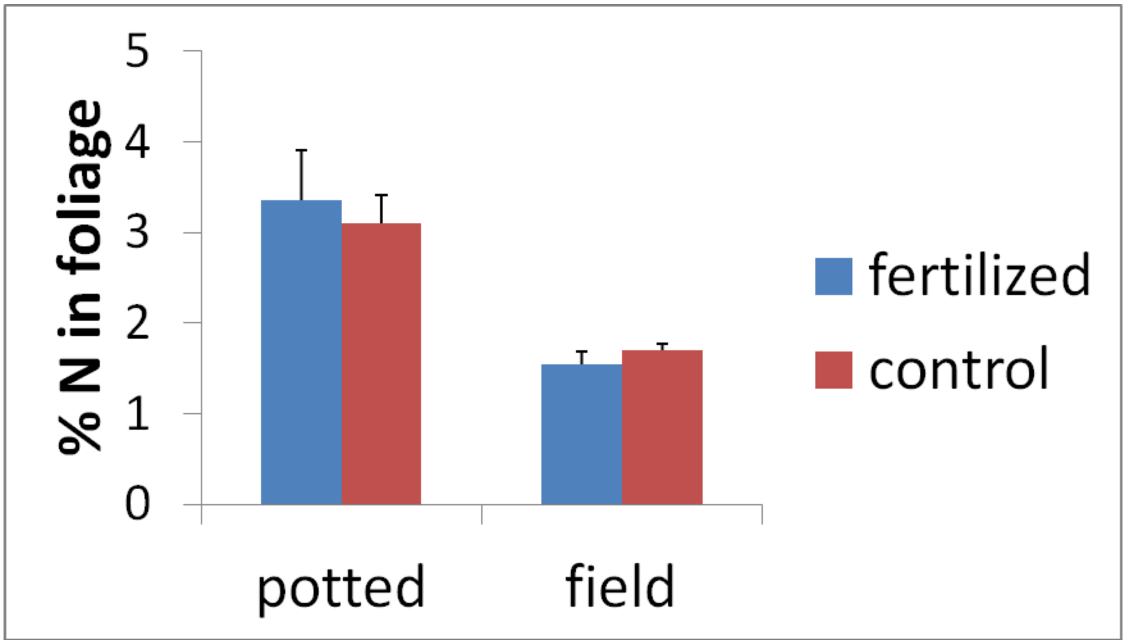

FIGURE 5 (not in text). Fertilization had no effect on foliar N. Experimental treatment did not effect %N, because plants were probably nitrogen sufficient.

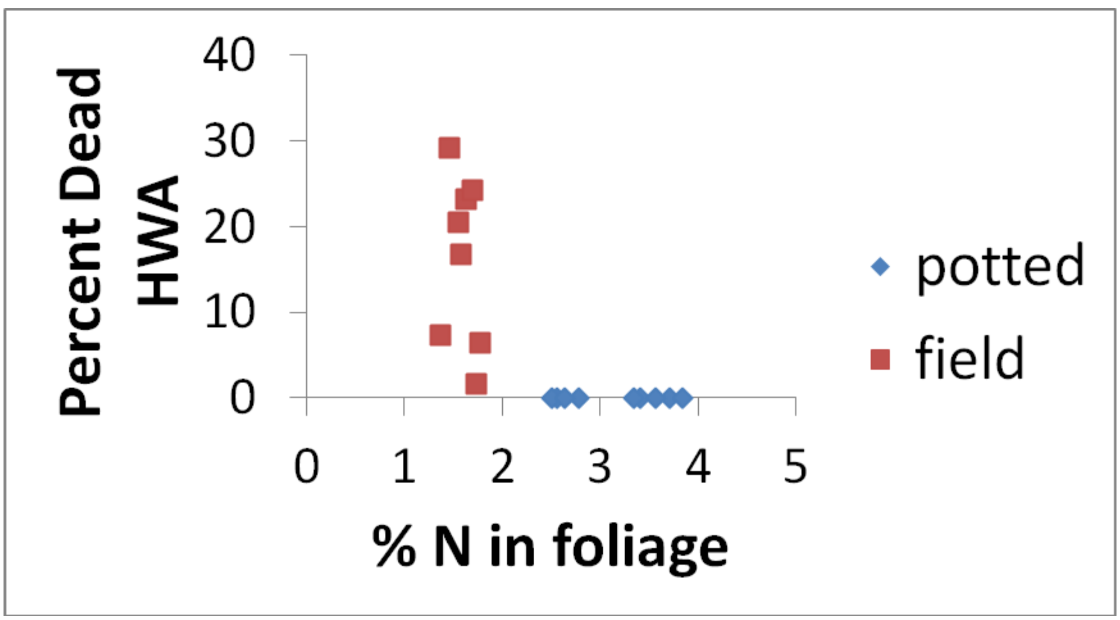

FIGURE 6 (not in text). Mortality was greater in field trees, which had lower N. HWA mortality ranged from 1-32% which could be due to the density of fewer HWA or the N treatment. We plotted the % of adelgids that were dead. This gives us an idea of mortality during this past generation (over this summer) – given all adelgid that hatched, how many died before laying eggs (we assume that the currently live adelgid will lay eggs). We could have plotted the % of the total that was alive too. The plotted samples would all be at 100% alive and the field trees would vary below 100%, this is the same result but with a different visual.