

ABSTRACT

WALKER-LANE, LAURA NEWMAN. The Effect of Hemlock Woolly Adelgid Infestation on Water Relations of Carolina and Eastern Hemlock. (Under the direction of John Frampton.)

In North America, hemlock woolly adelgid (HWA; *Adelges tsugae* Annand) is an exotic insect pest from Asia that is causing severe decimation of native eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.). Extensive research has been committed to the ecological impacts and potential control measures of HWA, but the exact physiological mechanisms that cause tree decline and mortality are not known. Eastern and Carolina hemlock may be reacting to infestation in a manner similar to the response of Fraser fir (*Abies fraseri* (Pursh.) Poir.) to infestation by balsam woolly adelgid (BWA; *Adelges picea* Ratz.). It is known that Fraser fir produces abnormal xylem in response to BWA feeding. This abnormal xylem obstructs water movement within the trees, causing Fraser fir to die of water-stress. In this study, water relations within 15 eastern and Carolina hemlock were evaluated to determine if infestation by HWA was causing water-stress. Water potential, carbon-13 isotope ratio, stem conductivity, and stomatal conductance measurements were conducted on samples derived from those trees. In addition, branch samples were analyzed for possible wood anatomy alterations as a result of infestation.

Pre-dawn branch water potential (Ψ) measurements were more negative in infested hemlock than in non-infested trees. Carbon isotope ratios (normalized $\delta^{13}\text{C}$ vs. VPDB) of the branches were more positive for infested trees, while stomatal conductance (g_s) was lower in infested trees. These results indicate that infested eastern and Carolina hemlock are experiencing drought-like symptoms. Wood anatomy of the branches

provided evidence that infested hemlocks are experiencing abnormal wood production in the xylem.

The Effect of Hemlock Woolly Adelgid Infestation on Water
Relations of Carolina and Eastern Hemlock

by
Laura Newman Walker-Lane

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Forestry

Raleigh, North Carolina

2009

APPROVED BY:

John Frampton
Chair of Advisory Committee

Fred P. Hain

John S. King

Jean-Christophe Domec

BIOGRAPHY

Laura Newman Walker-Lane was born in Charlotte, North Carolina on November 28, 1981 to Leigh Barnett Walker of Virginia and John Vincent Walker of New Jersey. She is the younger sister of Edwin Bradley Walker. The family of four left North Carolina to live in Harford County, Maryland shortly after Laura's birth, where Laura remained until she moved to Baltimore, Maryland in 2001.

Laura earned her undergraduate degree from Towson University in Towson, Maryland, several miles north of Baltimore City. She graduated Summa Cum Laude in May 2003 with a Bachelor of Science degree in Biology. During July 2003, she moved to Greensboro, North Carolina to eventually pursue further education in North Carolina. From December 2003 to October 2006, Laura worked as an oncology cytogenetics technologist for Laboratory Corporation of America, Inc.

In January 2007, Laura entered graduate school at North Carolina State University as a candidate for a Master of Science in Natural Resources. She developed a research project under the direction of Dr. John Frampton and consequently changed her major to Forestry, with a minor in Entomology.

ACKNOWLEDGEMENTS

I would like to thank Dr. John Frampton for his continued support and commitment in helping me complete this research. At times, I felt unbelievably frustrated during the construction and completion of this project. He was there to ground and reassure me, providing the encouragement that allowed me to continue focusing on my goals. I would also like to thank JC Domec for his help with the equipment and patience in explaining material that I had never previously encountered. Thanks also to the other members of my committee: John King and Fred Hain for their expertise in their respective fields and advice on experimental design.

I would like to give a special thank-you to Dr. Ilona Peszlen and her graduate students for facilitating my anatomy studies. Dr. Peszlen sacrificed a great amount of her time to help my analysis and understanding of wood anatomy. Thanks also go to Brian Joyce of Montreat College for allowing the use of his beautiful campus and its poor hemlock trees for my research.

The graduate experience has been worth the time and effort in large part because of the friends that I have made at NCSU. I would especially like to thank Haley Hibbert and Saul Garcia for the good times we had. It is always awesome to meet people that interact with the world in the manner that I do, even if some may be a bit more outspoken than others.

Lastly, my greatest appreciation goes to Cisco Rivera. His forgiving and adaptable nature has provided the environment I need in order to complete my Master's degree. He has helped me to become a stronger person, for which I will be forever grateful.

TABLE OF CONTENTS

| | |
|---|-----|
| List of Tables | vi |
| List of Figures | vii |
| Chapter I | |
| Thesis Introduction | 1 |
| Eastern United States Hemlock Species | 2 |
| Hemlock Woolly Adelgid | 3 |
| Hemlock Woolly Adelgid Impact | 5 |
| Control Efforts | 6 |
| Hemlock Responses to Hemlock Woolly Adelgid Infestation | 7 |
| Water Relations | 9 |
| Chapter II | |
| Introduction | 14 |
| Materials and Methods | 17 |
| Results | 25 |
| Discussion | 30 |
| Conclusions | 35 |
| Literature Cited | 37 |
| Tables and Figures | 45 |
| Appendix | 58 |

LIST OF TABLES

| | |
|--|----|
| Table 1. Results of analysis of variance for effects of species, vigor, and species-by-vigor interaction for healthy and medium vigors only, and vigor for analysis of species separately. P-value significance shown for pre-dawn and mid-day water potentials (Ψ) from June and August 2007, and leaf conductance (g_s)..... | 45 |
| Table 2. June 2007 pre-dawn and mid-day water potential vigor class means..... | 46 |
| Table 3. Significance results (P-value) for analysis of variance of the June 2008 branch water potential of species, time, and species-by-time interaction for trees of healthy and medium vigor classes only, and effects of time, vigor, and time-by-vigor interaction for species separately..... | 47 |
| Table 4. Significance results (P-value) for effects of vigor, tissue, and vigor-by-tissue interactions for separate species in August 2007 and January 2008, with added effect of branch health for January 2008..... | 48 |
| Table 5. Means (\pm S.E.) of carbon isotope ratio ($\delta^{13}\text{C}$) results from August 2007 and January 2008. Units are normalized $\delta^{13}\text{C}$ vs. VPDB. | 49 |
| Table 6. Means (\pm S.D.) of stem radii, leaf area (A_L), leaf area-to-sapwood area ratio ($A_L:A_S$), specific conductivity (K_s), leaf conductance (K_L), and leaf specific conductivity (LSC) for healthy and poor health branches from both species combined, Carolina hemlock, and eastern hemlock separately. Means in the same column followed by an * are significantly different at the < 0.05 level. | 50 |
| Table 7. Means (\pm S.E.) of stomatal conductance (g_s) for species combined and separate, by tree condition. Units in $\text{mmol m}^{-2} \text{s}^{-1}$ | 51 |

LIST OF FIGURES

Chapter II

Figure 1. August 2007 Simple linear regression equation, and r^2 of Carolina (A) and eastern (B) hemlock pre-dawn water potentials (Ψ_p), by branch health (1 = healthy branch, 5 = most unhealthy branch)..... 52

Figure 2. June 2007 water potential (Ψ) diurnal curves of a healthy and medium health Carolina (A) hemlock, and a healthy and poor health eastern (B) hemlock. 53

Figure 3. June 2008 water potential (Ψ) diurnal curves of Carolina (A) and eastern (B) hemlock. Carolina hemlock has two medium health and two healthy trees represented, while eastern hemlock has two poor health and two healthy trees measured. 54

Figure 4. Leaf area-to-sapwood area of healthy and poor health branches from both species and one tree of each vigor class. 55

Figure 5. Wood anatomy photographs; Eastern hemlock; clockwise from the top left: healthy hemlock cross-section, poor health hemlock cross-section (arrow A displays false-ring structure, arrow B displays increased longitudinal parenchyma), poor health hemlock tangential section, healthy hemlock tangential section. Scale indicates $1\mu\text{m}$ 56

Figure 6. Wood anatomy photographs; Carolina hemlock; from left to right: healthy hemlock cross-section, poor health hemlock cross-section. Scale indicates $1\mu\text{m}$ 57

Appendix

Figure 1. Low-magnification wood anatomy (4x); Eastern hemlock; from left to right: healthy hemlock cross-section, poor health hemlock cross-section (A indicates false-ring structures). Scale indicates $1\mu\text{m}$ 59

Figure 2. Low-magnification wood anatomy (10x); Eastern hemlock; from left to right: healthy hemlock tangential section, poor health hemlock tangential section (arrows indicate abnormal wood). Scale indicates $1\mu\text{m}$ 60

Figure 3. High-magnification wood anatomy of poor health eastern hemlock cross-section (40x). Scale indicates $1\mu\text{m}$ 61

CHAPTER I

THESIS INTRODUCTION

Humans have imparted a severe ecological impact on the ecosystems of North America by introducing exotic pests. This interaction has been all too severe in the past few centuries, as the populations of many dominant tree species have been decimated due to introduced organisms (Ellison et al. 2005). Before the introduction of the chestnut blight, *Cryphonectria parasitica* (Murrill) Barr, from Asia, American chestnut, *Castanea dentata* (Marsh.) Borkh. was a dominant tree in eastern United States forests. These trees served as an important food source for many animals, including humans, but the chestnut blight reduced the presence of American chestnut to a sparse shrub in the time span of fifty years (Anagnostakis 1987). Similarly, Fraser fir, *Abies fraseri* (Pursh) Poir., was a dominant species of the spruce-fir forests of the high-altitude southern Appalachians. In early nineteenth century, the balsam woolly adelgid, *Adelges piceae* Ratzeburg, was introduced to North America from Europe. By the middle of the last century, it had reached the southern Appalachians and proceeded to demolish the native Fraser fir stands within thirty years (Smith and Nicholas 1998). North America is now faced with a new formidable pest, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), that threatens to eradicate the native eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.) forests.

EASTERN UNITED STATES HEMLOCK SPECIES

Eastern hemlock is found from northern Georgia, north through the Appalachians, spreading into most of Pennsylvania, New York, New England, the northern Great Lakes states, and southeast Canada (Godman and Lancaster 1990).

It is a late successional species due to its high shade tolerance and long-lived nature (Quimby 1996). Although it has a history of economic uses, it is now a species of greater ecological importance (Godman and Lancaster 1990).

Requiring moist, well-drained soil, the dense canopy of eastern hemlock that forms in the forest creates a sheltered habitat and microclimate suitable for many animals and plants (Godman and Lancaster 1990, Evans et al 1996, Quimby 1996). Eastern hemlock is commonly associated with various hardwoods such as birch (*Betula* spp.), oak (*Quercus* spp.), and maple (*Acer* spp.), as well as white pine (*Pinus strobus* L.) in the North and rhododendron (*Rhododendron* spp.) in the southern Appalachians (Godman and Lancaster 1990).

Carolina hemlock is an endemic species of the southern Appalachians, growing in isolated patches from southwestern Virginia to northern Georgia (Harrar and Harrar 1962). Unlike eastern hemlock, it is a slower growing, stress-tolerant species that can grow on rocky, shallow soils above 914 m (3000 ft) in elevation. It is generally a climax species in sites of low species richness, commonly associated with oaks, maples and rhododendron (Humphrey 1989,

Rentch et al. 2000). However, it can be an early successional species on rocky sites due to the unsuitable nature of the habitat for other plant species (Humphrey 1989).

HEMLOCK WOOLLY ADELGID

HWA was first observed in Oregon in the early twentieth century (Annand 1924). In the Pacific Northwest, it was found to occasionally kill ornamental western hemlock, *T. heterophylla* (Raf.) Sarg., but it never had a severe impact on the native trees (McClure 1987). In the 1950s, it was first discovered on ornamental eastern hemlock in Richmond, Virginia. It continued to spread throughout the Mid-Atlantic States, restricting infestation to ornamental hemlocks, for the following thirty years (Souto et al. 1996). HWA was easily treated with insecticides on ornamental hemlocks, to prevent those trees from death. Unfortunately, the pest was not eradicated; as a result, HWA was first discovered causing widespread mortality in the natural hemlock stands of Connecticut in the 1980s (McClure 1987). Its presence in natural stands was observed earlier in the Blue Ridge Mountains of Virginia, but a cold snap eradicated the pest from the southern Appalachians until the 1990s (Souto et al. 1996). Since its introduction, HWA has spread to cause mortality of hemlocks in at least 17 states (USDA Forest Service 2007). Mitochondrial DNA studies on HWA identified the area of origin as southern Japan (Havill et al 2006). In its

native habitat, HWA infrequently causes mortality due to the presence of native predators and plant host resistance (McClure 1996).

Little was known about the insect until an extensive study in 1989 determined the lifecycle. McClure (1989) found that HWA in the Eastern United States has three generations that develop on hemlock, all of which are parthenogenetic. Two of the generations are wingless: the sistens and the progrediens. The sistens is active from July through early spring, when it produces an ovisac of approximately 50 eggs. About half of these eggs hatch a month later into progrediens. The progrediens develop through four instars to maturity by June and then produce an ovisac of about 25 eggs on the hemlock. All of these eggs hatch into sistens, which enter diapause until October. The other half of the sistens' eggs develop into the winged sexupara, which leave the hemlock in search of a suitable spruce (*Picea* spp.) host. The sexupara produce about ten eggs that result in the sexual generation, the sexuales. In North America, there are not any suitable spruce hosts, so the sexuales die before developing beyond the first instar (McClure 1987, 1989).

HWA is a very small insect (<2mm) that is covered by a secreted woolly mass for most of its lifespan (McClure 1987, 1989). One study found that the wingless stage of the adelgid passively travels by wind, birds and mammals (McClure 1990) and can travel more than 1350 m by wind. Most of the birds caught in the study were carrying HWA, including ground-dwelling birds, which

suggests that HWA crawlers also reside on the ground. It was observed that browsing deer spread the pest, as well as human activity in forests impacted by HWA.

HEMLOCK WOOLLY ADELGID IMPACT

In southern Connecticut, a study in 1997-98 found that ninety percent of eastern hemlock stands examined had HWA present, with mortality present in two-thirds of the stands. In terms of locations susceptible to infestation and tree decline, no concrete relationship has been found between size, age, aspect, slope, and location, but a relationship has been shown to exist with latitude (Royle and Lathrop 2000, Orwig and Foster 1998, 2000, Kizlinski et al. 2002, Orwig et al. 2002). In general, the forests of southern latitudes are much more likely to experience decline from infestation, since many of these latitudes do not experience the critical lethal temperature of -25°C (Skinner et al. 2003, Ford and Vose 2007). It was noted that after four years of observation in Connecticut, there was no sign of recovery after initial impact of HWA (Orwig and Foster 2000). In fact, mortality of hemlocks in the area increased, on average, by about 10 percent each year.

Hemlock decline has led to many observations that the species are quickly replaced by birch, maple, oak, shrubs, and herbaceous species due to the open canopy and increased sun exposure on the forest floor (Orwig and Foster 1998, Kizlinski et al. 2002, Orwig and Kizlinski 2002, Ford and Vose 2007). The

outlook for regeneration of hemlock is even more dismal since the seedlings are facing additional obstacles such as deer browsing and increased leaf litter cover (Mladenoff and Stearns 1993, Lei et al. 2002). Hemlock seeds are only viable for two to four years (Orwig and Foster 2000). If the seeds do germinate, HWA infests and kills the seedlings, so the trees cannot reach sexual maturity.

Aside from aesthetic value, response on the landscape scale results in severe ecological impacts, including the loss of nesting habitat for many birds (Evans et al. 1996). In the Appalachians, eastern hemlock serves as an important riparian buffer. Once the forest canopy has been diminished, creeks will warm due to greater sun exposure, resulting in loss of aquatic habitat. Hemlock serves as an irreplaceable source of transpiration in the early spring and winter months (Ford and Vose 2007). Loss of the species results in drastic alteration of carbon, nitrogen, and water cycling. Studies have found that soil nitrogen levels are greatly increased in sites that have been impacted by HWA (Jenkins et al. 1999, Stadler et al. 2006). These studies found that because of increased solar radiation on the forest floor, organic decomposition and N turnover is higher, resulting in a decline of organic matter quality.

CONTROL EFFORTS

Mortality is increasing on a large scale, and hemlocks are expected to be eradicated from many areas in the near future, if a successful control is not implemented (Souto et al. 1996, Orwig and Foster 2000). While chemical control

has proven to be effective, it is expensive, it does not last long (up to 2 years), and it cannot be used on a large scale or near streams (McClure 1987, Cheah et al. 2004, Cowles et al. 2006). Many believe that biological control is the best option for long term management of HWA. There are North American native predators of HWA, but they are generalists and do not kill HWA in great enough numbers to keep the impact at low levels (Wallace and Hain 2000). Non-native species are now being analyzed to determine if they can have a more prevailing effect. One species is *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) collected from the Osaka prefecture of Japan (Sasaji and McClure 1997). This insect is a predator of adelgids that feeds on all the life stages of HWA and it is amenable to lab-rearing (Cheah et al. 2004). Another predator being examined is *Laricobius nigrinus* Fender (Coleoptera: Derodontidae). It is suited to prey on HWA because it prefers the cold temperatures in which HWA is active (Cheah et al. 2004). These species of beetles have sometimes been found to colonize the immediate areas in which they are released, but have not yet had any impact on a large scale.

HEMLOCK RESPONSES TO HEMLOCK WOOLLY ADELGID INFESTATION

HWA feeds on the nutrient-rich xylem ray parenchyma cells of hemlock. It inserts its stylet bundle into the intracellular area of the xylem, at the base of needles (Young et al. 1995). HWA prefers to feed on the current year's growth,

perhaps because of the more digestible compounds (McClure 1990, Young et al. 1995). The insect will feed on mature trees, saplings, and seedlings (McClure 1991).

The tree responds by ceasing new growth and dropping its needles (McClure 1987, 1991). One study found that the trees cease production of growth at densities lower than two adelgids per centimeter of branch (Broeckling and Salom 2003). HWA migrates to the next level of newest growth (the previous year's), but vigor of the insect is greatly reduced (McClure 1990, 1991). Greater numbers of sexupara are produced from HWA that feeds on older growth in an attempt to actively migrate to a more suitable host. McClure (1991) studied hemlocks infested by HWA for four years. After HWA populations drastically declined on hemlocks that ceased growth after infestation, the hemlocks resumed some growth the following year (year 3). HWA resumed feeding on the trees during that year, causing the hemlocks to halt needle production. In the fourth year, HWA populations were at their lowest level, and the trees finally succumbed to mortality.

Little is known about the mechanisms of decline of eastern and Carolina hemlocks response to infestation, but the prevailing hypothesis is that the hemlocks are suffering mortality from infestation because the HWA is extracting too much photosynthate from the trees (Broeckling and Salom 2003). Due to the rapid decline of the trees, suffering mortality in as little as four years, some have

proposed an alternative hypothesis that perhaps the adelgids are injecting salivary compounds that are perceived as toxic by the trees (McClure 1987, 1991, Young 1995). It has been found that many adelgid species can produce salivary compounds that cause modification in an infested plant's structure. A salivary compound such as this may cause the Fraser firs', *Abies fraseri* (Pursh.) Poir., alteration of the xylem which results in an alteration in water relations and prevents conductivity in trees infested with balsam woolly adelgid (BWA), *Adelges picea* Ratz. (Timell 1986, Hain 1988, Hollingsworth et al. 1991).

WATER RELATIONS

According to the Cohesion Theory of Sap Ascent, first described by Dixon and Joly (1894), water in the tree must maintain a pressure below the vapor pressure of water at the current environmental conditions in order for it to be transported within the xylem. Hydrogen bonds within water molecules allow for cohesion in the water column that occupies the xylem. As water is transpired from the leaves, tension increases in the water column and the negative pressure causes water to ascend from the soil through the tree (Tyree and Sperry 1989).

This negative pressure also causes cavitation in the conducting tissue. Cavitation starts when a microscopic air bubble forms at the intertracheid pit membranes, a process termed "air-seeding" by Zimmerman (1983). It can then progress to an embolism when air diffuses out of the surrounding cells to join the air bubble, causing that conduit of the xylem to fill with air. This prevents any

further conduction in that cell until the air is removed from the system (Zimmerman 1983). If embolism occurs on a massive scale, the xylem can be completely closed to water conduction resulting in death of the tree. To prevent cavitation, stomata may close when transpiration occurs at an accelerated rate, due to environmental factors such as high temperature and low humidity, or when soil water potential is very low (Jones and Sutherland 1991, Hubbard et al. 2001).

Jones and Sutherland (1991) have shown that the allowance of some embolism is advantageous to the tree because it prevents the loss of too much productivity in times of high transpiration. However, Tyree and Sperry (1988) warn that the formation of embolisms needs to be prevented as much as possible because the resulting reduced conductivity will cause a more negative water pressure. This will increase water-stress in the tree, resulting in the formation of more embolisms in a process they coined “runaway embolism.”

Water relations within the tree can be related with Ohm’s law analogue (van den Honert 1948):

$$\Delta P/\Delta x = E/LSC \quad (1)$$

where $\Delta P/\Delta x$ is the xylem pressure gradient, E is the transpiration (stomatal conductance (g_s)/vapor pressure deficit (VPD)), and LSC is the leaf-specific conductivity (Tyree and Sperry 1989).

Leaf specific conductivity (LSC) is a measure of conductivity that describes the ability of the xylem to efficiently provide water to the leaves. It is

calculated by stem hydraulic conductivity (K_h) adjusted for the total leaf area distal to the stem (Zimmerman 1983, Tyree 1988). Identifying the LSC of a tree can provide information on how the tree responds to stress (Panek 1996). According to the “plant segmentation hypothesis,” xylem pressure is prevented from decreasing to a point where “runaway embolism” occurs by allowing embolism to happen in segments with a low LSC (Tyree 1993, Rood et al. 2000). It has been shown that LSC is much lower in branches than in the trunk of a tree (Ewers and Zimmerman 1983). As a result, branches have a greater potential to experience embolism in times of water-stress.

Conductance is determined by stomatal activity (Jones and Sutherland 1991). If VPD increases too much during the day, the stomata will close to preserve water and prevent cavitation. Net photosynthesis decreases when stomata close because CO_2 is no longer permitted to enter the leaf tissue (Sandford and Jarvis 1986). This process will result in a change in carbon isotopes integrated into the tissues of the plant.

It is believed that carbon discrimination occurs during the carboxylation reaction during photosynthesis, due to the reduced affinity for ^{13}C , versus ^{12}C , to ribulose-1,5-bisphosphate carboxylase. However, when the partial pressure of carbon dioxide is decreased inside the leaf due to the reduction of stomatal conductance, discrimination will decrease and ^{13}C is expected to increase (Farquhar et al. 1982). Several studies strongly support the hypothesis that trees

with greater drought stress will have increased ^{13}C , greater water-use efficiency, decreased stomatal conductance and reduced photosynthesis (Zhang et al. 1994, Zhang and Marshall 1994, Zhang et al. 1997).

Water-use efficiency (WUE) is the ratio of plant yield (dry matter accumulation) to amount of water transpired during the production of dry plant matter (Hsiao and Acevedo 1974). When photosynthesis is high, transpiration is low, or both occur at the same time, WUE will be increased. Since stomatal closure is a limiting factor in both photosynthesis and transpiration during water-stress events, photosynthesis must decrease less than transpiration as a result of stomatal closure in order for WUE to increase (Zhang et al. 1997).

In addition to embolism, xylem structure can prevent movement of water within the tree. An example of altered xylem is rotholz, the abnormal wood found in Fraser fir infested with BWA described by Balch (1964). It is comprised of shorter tracheids, thicker cell walls, and smaller lumens, much like compression wood. The formation of rotholz results in a greater number of annual rings, a greater amount of ray tissue, a fewer number of conducting pit pores and encrusted pit membranes (Mitchell 1967, Timell 1986, Hain 1988, Hollingsworth et al. 1991). Most of these attributes cause decreased water conduction within the tree, resulting in increased susceptibility to environmental stress and external symptoms of water-stress.

THESIS OBJECTIVES

This study was designed to compare water relations in healthy and HWA-infested eastern and Carolina hemlocks. In order to get a complete picture of potential effects on tree water relations, the objectives of this study were to:

- 1) Determine if branch water potential and plant water status are affected by HWA infestation,
- 2) Determine if conductivity within the branches is changed due to infestation,
- 3) Determine if $\delta^{13}\text{C}$ ratios are altered in infested trees,
- 4) Determine if stomatal conductance is changed in infested trees, and
- 5) Determine if wood structure has been altered by HWA infestation.

CHAPTER II

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is a significant ecological pest that was introduced into the eastern United States in the twentieth century. Since its introduction, HWA has caused decimation of eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *Tsuga caroliniana* Engelm., populations, in the southern Appalachian Mountains and along the eastern seaboard (Ford and Vose 2007). Eastern and Carolina hemlock are integral to southern Appalachian forests because of the habitat they provide and their ability to transpire throughout the entire year (Godman and Lancaster 1990, Ford and Vose 2007). In addition, eastern hemlock acts as an important riparian buffer, shielding sensitive waterways from intense sunlight.

Once eastern and Carolina hemlocks are infested with HWA, most do not recover and usually succumb to death in as little as four years in northern states (McClure 1991, Orwig and Foster 2000). The hemlocks are quickly replaced by herbaceous and hardwood species such as oak, birch, and maple that cannot fill the important ecological niche left behind by hemlock mortality (Orwig and Foster 1998, Kizlinski et al. 2002, Ford and Vose 2007). Hemlocks are not expected to regenerate because of damage caused by deer-browsing, sunlight occlusion from deciduous leaf litter, and HWA infestation of the seedlings (Mladenoff and Stearns 1993, Lei et al. 2002).

HWA was discovered to originate in Asia, where it does not cause severe destruction of its hosts due to native host resistance and natural predators (McClure 1996, Havill et al 2006). In the United States, HWA harms the native hemlocks as it feeds from the xylem ray parenchyma cells at the base of needles from current year growth (Young et al. 1995). HWA indiscriminately infests seedlings, saplings, and mature trees. The tree responds by cessation of new growth and dropping of the most current needles (McClure 1987, 1991). After the current year's needles have been expelled from the tree, HWA will move to the next most recent growth but the vigor of the insect is greatly reduced. Eventually, HWA will leave the tree, at which point the tree begins new growth, only to be reinfested (McClure 1991). This pattern will continue until the tree dies. It is not known what causes eastern and Carolina hemlocks to respond in this manner but some believe that an excessive amount of photosynthate from the tree is extracted by infestation (Broeckling and Salom 2003).

Alternatively, the trees may be responding to the feeding of HWA by a reaction similar to that of Fraser fir, *Abies fraseri* (Pursh.) Poir., to balsam woolly adelgid (BWA), *Adelges picea* Ratz. BWA feeds on the bole of Fraser fir, which, in a hypersensitive response, increases the production of heartwood and creates abnormal xylem, termed rotholz, both of which obstruct water movement within the tree (Balch 1952, Puritch 1977). Rotholz is very similar in composition to compression wood, which consists of shorter tracheids, thicker cell walls, and

smaller lumens. Rotholz permeability, and consequently conductivity, is reduced as much as it is in compression wood and heartwood (Puritch 1971, Spicer et al 1998). Heartwood formation is thought to occur when conductivity is diminished and pressure gradients within the tree increase, causing permanent embolism in the sapwood tracheids (Zimmerman 1983). This process may be what is occurring in rotholz. If embolism proliferates enough within the infested tree as a result of a hypersensitive reaction, the tree will die of water-stress (Hollingsworth et al. 1991).

Crown infestations do occur in some firs infested with balsam woolly adelgid, causing a swelling of the terminal and nodal branch tissue (Balch 1964). When a fir produces this swelling, termed gout, in response to the feeding adelgids, it is at the base of buds. Mortality of a tree with BWA crown infestation takes much longer, up to twenty years, or may not occur at all (Newton and Hain 2005). In Fraser fir, where the trunk of the tree is impacted with BWA, the trees die in as little as two years (Hain et al. 1991). Given the similarity of time infested, mortality, and the proportion of the tree infested (throughout the entire needle-bearing fraction, not just the terminal ends of the branch) it can be hypothesized that eastern and Carolina hemlocks infested with HWA are incurring a reaction similar to the one experienced by Fraser fir in response to BWA.

The objective of this study was to ascertain if water relations of eastern and Carolina hemlock infested with HWA are being affected in a manner similar to those in BWA-infested Fraser fir. The hypothesis of this study was that HWA infestation causes the production of abnormal xylem that changes water relations and causes water-stress in eastern and Carolina hemlocks. In order to test this hypothesis branch water potential, branch conductivity, carbon isotope ratios, stomatal conductance, and wood anatomy data were measured and analyzed for differences between healthy and infested hemlocks.

MATERIALS AND METHODS

Study site and experimental design

The field site for this study was the campus of Montreat College in the southern Appalachian mountains (N35° 38' 44.62", W82° 18' 14.35", elevation 797m). The mean annual temperature is 13.3° C and the average annual precipitation is 1218 mm (Black Mountain Digital Media, Black Mountain, NC). Laboratory procedures were completed at North Carolina State University in Raleigh, North Carolina unless otherwise specified.

The field site was chosen because of the close proximity of mature control (chemically protected) and infested trees. The control trees were being treated with soil-injected imidacloprid every two to three years to limit infestation. The field sample size originally consisted of fifteen trees ranging from 30.5 cm to 73.0

cm dbh. Nine trees were eastern hemlock (*Tsuga canadensis* (L.) Carr.) and six were Carolina hemlock (*Tsuga caroliniana* Engelm.). Trees of both species were placed into vigor classes of three trees each. *T. canadensis* had three vigor classes (healthy, medium health, poor health), while *T. caroliniana* had two (healthy, medium health). The vigor classes were determined according to visual estimation of crown needle loss as a result of adelgid infestation. The healthy (control) class had trees with minimal to no needle-loss (*T. canadensis* 1, 2, 3 and *T. caroliniana* 10, 11, 12). The medium health class had trees with twenty percent to sixty percent needle-loss (*T. canadensis* 4, 5, 6 and *T. caroliniana* 13, 14, 15). The poor health class had trees with seventy percent or greater needle-loss (*T. canadensis* 7, 8, 9). There were not any poor health Carolina hemlocks on or near the campus. Due to the dynamics of the natural system, the trees were in constant exposure to the elements and adelgid attack over the two year study period. As a result, most trees continued to decline, changing their original vigor classification. Two of the trees suffered mortality (4 and 7) before the end of this study. Over the course of the study some of the healthy, control trees became partially infested with HWA and elongate hemlock scale (*Fiorinia externa* Ferris), most likely due to the lack of timely and effective chemical treatment.

Water potential

Water potential (Ψ) was determined for each tree using a Scholander pressure chamber (PMS Instrument, Albany, OR; Scholander et al. 1965). Branch samples were cut from the bottom of the tree crown with pole pruners. Measurements were taken from two year's growth of the branch, within two minutes from the time of the initial cut from the tree.

In June 2007, pre-dawn (Ψ_P ; 430 h to 700 h) and mid-day (Ψ_M ; 1130 h to 1400 h) measurements were conducted on all of the trees. Measurements for a diurnal water potential curve, from 530 h to 1930 h were collected every two hours for two *T. canadensis* (2, 7) and two *T. caroliniana* (10, 15) trees.

In August 2007, pre-dawn (430 h to 600 h) and mid-day (1130 h to 1300 h) measurements were conducted from five different cardinal and intermediate directions on one tree from each vigor class and species combination. The branch health from each tree was classified on a scale from one to five, with one being completely healthy/minimal needle loss, two being 20 to 40 percent defoliated, three being 40 to 60 percent defoliated, four being 60 to 80 percent defoliated, and five being 80 percent to almost entirely defoliated.

In June 2008, water potential measurements every two hours from 430 h to 1930 h were obtained to develop diurnal curves for four *T. canadensis* (1, 2, 8, 9) and four *T. caroliniana* (11, 12, 14, 15) trees.

Carbon isotope composition

Wood tissue samples were collected in August 2007 and January 2008 for carbon isotope analysis. The samples were cut from the bottom of the crown of three *T. canadensis* (2, 4, 7) and two *T. caroliniana* (10, 15) trees. In August 2007, three branches were sampled from each tree. In January 2008, four branches from the medium and poor health trees, and two branches from the healthy trees were collected. Two of the branches from the poor and medium health trees were healthy, while the other two were unhealthy. Both branches from the healthy trees were healthy. Each sample from both collection times was immediately separated into year of growth (current, 2nd year, 3rd year, and 4th year). The second year's growth (i.e., 2006 for the August 2007 samples) was selected for analysis.

The oven-dried samples were separated into twig and leaf tissue, then ground to a fine powder with mortar and pestle, after being frozen with liquid nitrogen. All of the samples were analyzed by an isotope ratio mass spectrometer at the Cornell University Stable Isotope Laboratory (COIL) in Ithaca, New York. $\delta^{13}\text{C}$ was reported based on the PeeDee Belemnite standard (Craig 1957).

Hydraulic conductivity

Hydraulic conductivity measurements were performed on branch samples cut from the bottom crown of the trees in January 2008 and July 2008. Samples

included four branches from the medium and poor health trees, and two branches from healthy trees. Two of the branches from the medium/poor health trees were relatively healthy (10-30 % needle-loss), the other two branches were of poor health (70-90 % needle-loss). Both branches from the healthy trees had minimal needle-loss. The samples from January and July were from each treatment group, including three *T. canadensis* (2, 4/6, 9) and two *T. caroliniana* (11, 15) trees. The *T. canadensis* tree used for the January sample (4) died over the winter, so during the July sampling, it was replaced with another medium health tree (6).

The samples were immediately frozen and transported back to the laboratory at North Carolina State University. Samples were stored in a chest freezer (-20° C) for two months before analysis with a hydraulic conductance flow meter (HPFM) (HCFM-XP, Dynamax Inc., Houston, TX). In order to determine if freezing affected the measurements by the HCFM, a fresh *T. canadensis* sample was cut from the arboretum in the Schenk Memorial Forest, Raleigh, NC, for immediate analysis on the HCFM. It was then frozen for 5 days (-20° C). The sample was allowed to thaw in a refrigerator (2° C) for 24 hours, and then allowed to acclimate to ambient room temperature (20° C) for 4 hours. Measurement with the HCFM was repeated on the sample, resulting in the same conductance. The samples from Montreat College were thawed and analyzed in the same manner.

The analysis procedure began with attaching the whole branch sample, freshly cut, at approximately the level of four years of growth, to the HCFM in a

controlled environment. Filtered, degassed water perfused the branch at a constant pressure until it was assumed that the water had completely infused the branch, after 1000 seconds. This measurement was recorded as the conductivity of the entire branch's stem and leaves, K_b . Conductivity was then recorded for each successive trim of one year's growth until the leafless stem of the fourth year remained. The last conductivity measurement was used to calculate specific conductivity, K_s , which conveys the permeability of the xylem:

$$(K_{\text{stem}} \times L_s)/A_s \quad (1)$$

where, K_{stem} is hydraulic conductivity of the stem without the leaves, L_s is the length of the stem, and A_s is the xylem area.

Leaf area was determined by scanning analysis (Epson Perfection V700 PHOTO, Epson America, Inc., Long Beach, CA). Some needles from each branch were spread on a clear sheet then scanned into a computer that calculated area with ImageJ (National Institutes of Health, Bethesda, MD). The scanned needles and the remaining needles of each sample were weighed separately. The total leaf area was estimated based on the weight:area ratio of the scanned needles. Leaf hydraulic conductance, K_L , was determined by:

$$K_b/A_L \quad (2)$$

where, A_L is the total leaf area.

Leaf specific conductivity, LSC, was determined by:

$$K_h/A_L. \quad (3)$$

where, K_h is hydraulic conductivity.

Stomatal conductance

Stomatal conductance measurements were recorded on five branches from one tree of each treatment (1, 6, 9, 11, 15) between 1100h and 1300h in August 2008. Conductance was determined for the current year's growth by use of a steady state porometer (Model Li-1600, Li-Cor Inc., Lincoln, NE). All measurements were performed in full sunlight saturation. Due to the difficulty of the small needles, the narrow aperture was used on the cuvette and the leaf area in the cuvette was subsequently determined by calipers.

Wood anatomy

The samples from the January 2008 conductivity measurements were reserved for a wood anatomy study. The fourth year stems from the branches of trees 2, 4, 9, 11, and 15 were frozen after the conductivity study until August 2008. After removal from the freezer, they were submerged in water to maintain moisture and then cut into approximately 30 μm cross-sections on a microtome (Model 57951, Spencer Lens Co., Buffalo, NY). The cross-sections were placed back in the water with 0.1% safranin O stain added.

Analysis of the cross-sections was conducted under light microscopy (4x – 40x) with a digital camera. Samples were photographed and analyzed with Image-Pro software (Media Cybernetics, Inc., Bethesda, MD).

Statistical Analysis

SAS version 9.1 was used for all statistical analyses. The mixed model procedure (PROC MIXED) and the general linear model procedure (PROC GLM) were used.

The June pre-dawn and mid-day water potentials and August 2007 measurements were subjected to analysis of variance (ANOVA) using PROC GLM. A fixed effect model including species, vigor class, and their interaction as sources of variation was applied to the data from healthy and medium vigor classes. A reduced fixed effect model with only vigor class was completed separately for each species. In addition, simple linear regressions of branch health onto pre-dawn and mid-day water potential values were performed on the August 2007 data.

The data from the June 2008 water potential measurements were analyzed using PROC MIXED. Species, time and their interaction were employed as sources of variation for data from healthy and medium health vigor classes, with species as a fixed effect. A separate PROC MIXED was executed for vigor classes, time, and their interaction for data from each species.

Both sets of carbon isotope data were analyzed in an ANOVA using PROC MIXED with the fixed effects of vigor, tissue, and their interaction as sources of variation. The analysis from January included branch health as a covariate.

Conductivity data was analyzed in an ANOVA using PROC MIXED. The main effect was branch health.

Stomatal conductance data were analyzed by ANOVA with PROC MIXED. Data from healthy and medium health vigor classes were analyzed with the fixed effects of species, vigor class, and their interaction as sources of variation. A reduced model of vigor class as the sole source of variation was executed for each species separately.

RESULTS

Water potential

Pre-dawn water potential (Ψ_P) from June 2007 was significantly different between species (p-value = 0.05) (Table 1). Ψ_P was significantly lower in medium health trees than in healthy trees for both species (means = -0.61 and -0.36 MPa, respectively; p-value = 0.01), but there was not an interaction between species and vigor class. Species and vigor class main effects were not significantly different for mid-day water potential (Ψ_M), but there was a significant interaction (p-value = 0.05). The healthy trees of eastern hemlock had a more negative Ψ_M than the

medium health trees, while the healthy trees of Carolina hemlock had a less negative Ψ_M than the medium health trees (Table 2).

The August 2007 Ψ_P measurements were significantly lower for medium health trees than healthy trees of both species (p-value = 0.05) (Table 1). Ψ_P for eastern hemlock was significantly different between healthy and medium health trees, but not for Carolina hemlock vigor classes or Ψ_M for both species. In the regression analysis, branch health was a significant predictor for Ψ_P for Carolina hemlock (p-value = 0.005, $r^2 = 0.78$) and eastern hemlock (p-value = 0.0001, $r^2 = 0.84$), but not for Ψ_M (Figures 1a and b).

The healthy trees of both species reached a more negative water potential during the day than the non-healthy trees (Figure 2). In Carolina hemlock, the healthy tree reached its most negative water potential earlier in the day and returned to near pre-dawn levels far before the medium health tree.

In June 2008, the healthy eastern hemlock reached a more negative Ψ than the poor health eastern hemlock during the middle of the day, while Carolina hemlock healthy and medium vigor classes were about the same at that time (Figures 3a and b). Healthy Carolina hemlock was able to recover to a less negative Ψ earlier than the medium health Carolina. One of the poor health eastern hemlocks was unable to recover to near its pre-dawn values by the time measurements stopped in the evening (1900 h). Statistical analysis showed that there was a significant difference among times in both species (p-value = 0.0001)

but not between species (Table 3). There were significant differences in time (p-value = 0.0001), vigor class (p-value = 0.05), and the T x V interaction (p-value = 0.05) for Carolina hemlock and only for time in eastern hemlock (p-value = 0.0001).

Carbon isotopic composition

The carbon isotope ratio ($\delta^{13}\text{C}$) from August 2007 was significantly different for tissue in Carolina (p-value = 0.05) and eastern hemlock (p-value = 0.005) (Table 5). $\delta^{13}\text{C}$ for the Carolina hemlock healthy tree was significantly more negative than the ratio for the medium health tree (means = -28.48 and -27.51 normalized $\delta^{13}\text{C}$ vs. VPDB, respectively; p-value = 0.005) (Table 5). All vigor classes for eastern hemlock varied significantly (p-value = 0.0001). Eastern hemlock healthy tree $\delta^{13}\text{C}$ was significantly more negative than the ratio for medium and poor health trees (means = -29.42, -26.93, and -27.90 normalized $\delta^{13}\text{C}$ vs. VPDB, respectively). There was not a significant vigor class-by-tissue interaction for either species.

For January 2008, there were significant differences between branch health, vigor class, and tissue (all p-values = 0.005) for Carolina hemlock (Table 4). However, the medium health tree had a more negative $\delta^{13}\text{C}$ (mean = -27.47 normalized $\delta^{13}\text{C}$ vs. VPDB) than the healthy tree (mean = -26.25 normalized $\delta^{13}\text{C}$ vs. VPDB). Differences for the eastern hemlock $\delta^{13}\text{C}$ were significant for vigor

class and tissue (both p-values = 0.05), but not branch health. The healthy tree mean (-29.10 normalized $\delta^{13}\text{C}$ vs. VPDB) was significantly more negative than the medium health tree mean (-26.61 normalized $\delta^{13}\text{C}$ vs. VPDB), but not the poor health mean (-27.67 normalized $\delta^{13}\text{C}$ vs. VPDB).

Hydraulic conductivity

There was not a significant difference for specific conductivity (K_s), leaf hydraulic conductance (K_l), or leaf specific conductivity (LSC) between poor health branches and healthy branches (Table 6). There was significantly less leaf area (0.0005 vs. 0.0020 m^2 ; p-value = 0.05) and leaf-to-sapwood area (0.0163 vs. 0.0652 $\text{m}^2:\text{cm}^2$; p-value = 0.05) of poor health branches compared to healthy branches (Figure 4).

Stomatal conductance

Differences in stomatal conductance (g_s) were significant for species (p-value = 0.0001) and vigor classes (p-value = 0.01) (Table 1). Healthy tree g_s for both species (mean = 211.65 $\text{mmol m}^{-2} \text{s}^{-1}$) was significantly higher than g_s for medium health trees (mean = 118.99 $\text{mmol m}^{-2} \text{s}^{-1}$) (Table 7). Carolina hemlock g_s was lower than eastern hemlock (means = 96.09 and 234.21 $\text{mmol m}^{-2} \text{s}^{-1}$, respectively). There was not a species x vigor class interaction.

When analyzed separately by species, conductance for the Carolina hemlock healthy tree was significantly greater than the medium health tree (means = 125.91 and 66.28 mmol m⁻² s⁻¹, respectively; p-value = 0.005). There was a significant difference between medium and healthy vigor classes in eastern hemlock (means = 171.69 and 297.39 mmol m⁻² s⁻¹, respectively; p-value = 0.05).

Wood anatomy

Microscope analyses of samples taken from healthy branches of all of the hemlock vigor classes showed normal hemlock characteristics, such as distinct rings with abrupt changes in earlywood to latewood structure, very rare longitudinal parenchyma in the margin of the latewood, and straight tracheid development in the longitudinal cut (Figures 5a and 6a). The unhealthy branches had a large number of false rings, especially in the outermost ring, where it appears that the trees repeatedly slowed down growth enough during the growing season to form some latewood and marginal parenchyma cells (Figure 5). Longitudinal analysis of the unhealthy branches showed that there was more longitudinal parenchyma and a distortion of the grain with large abnormal, unidentified cells (Figure 5).

DISCUSSION

Infestation of the eastern and Carolina hemlocks by HWA caused significant changes in water relations of the trees. Water potential (Ψ), carbon isotope ratios ($\delta^{13}\text{C}$), and stomatal conductance (g_s) were all affected and may provide insight into the mechanisms of decline.

Healthy trees of both species start the day at a less negative Ψ_p than the medium health trees, especially when the measurements accounted for branch health. In addition, the healthy trees reached a less negative Ψ earlier in the evening than the poor and medium health trees. In general, as conductivity decreases in a tree, water potential is expected to become more negative when the tree attempts to continue to draw water from the roots. In a study of Fraser fir infested with BWA, Ψ of the bole for the infested Fraser fir was more negative than in non-infested trees, but the differences were not significant (Arthur and Hain 1986). Statistically significant results for this hemlock study followed these trends during pre-dawn measurements, showing that conductivity was reduced in HWA-infested trees.

The water status of a tree is partially influenced by its ability to uptake water from the roots. Pre-dawn water potential measurements are assumed to be an indicator of available water in the soil (Ritchie and Hinckley 1975, Barataud et al. 1995). In healthy trees, pre-dawn water potential measurements are expected to be equal on the same site because available soil water is the same. However, the

decreased pre-dawn water potential of infested trees from this study indicates that infestation must cause resistance in the roots because they were growing on the same site as the healthy trees. It has been shown that if roots are compromised at the soil-root interface, soil-root resistance will increase and the ability of the tree to uptake water from the soil will be diminished (Barataud et al. 1995, Sellin 1996). If faced with a drought event, infested trees will be further compromised because of this added resistance and healthy vegetation will be more competitively fit for available water uptake.

While the midday Ψ measurements from 2007 did not confirm this, most of the diurnal curves indicate that the healthy trees reach a position of greater transpiration during the midday. This was supported by the $\delta^{13}\text{C}$ and the g_s data.

When stomata close due to drought stress, the partial pressure of carbon dioxide will decrease inside the leaf. Factors that were previously selective towards the lighter isotope of ^{12}C will be forced to assimilate ^{13}C into the plant tissue (Farquhar et al. 1982, Zhang & Marshall 1994). It has been shown that $\delta^{13}\text{C}$ will increase with decreasing stomatal conductance and increasing drought-stress (Zhang et al. 1994, Zhang et al. 1997). The increased $\delta^{13}\text{C}$ data of poor and medium health hemlocks showed that the trees were undergoing drought-like conditions. Water-use efficiency (WUE) may have increased, but this could not be confirmed because photosynthesis data was not obtained. As further evidence of water-stress, the medium health tree of each species had significantly lower g_s

than the healthy tree of that species which indicates that the stomata were remaining closed during the time of day that the measurements were taken.

In June 2007, Ψ , $\delta^{13}\text{C}$, and g_s measurements implicate that the water relations of medium health trees were poorer than in poor health trees. The $\delta^{13}\text{C}$ data show that the medium health trees have an even greater water-stress response than poor health trees, while g_s shows that the needles are transpiring at a lower level. Both indicate that medium health trees are suffering from a greater drought-like reaction to infestation. The mechanisms for this are not known, but it may be that during the course of infestation, trees initiate a defense response, followed by some recovery. This result is supported by the fact that hemlocks normally recommence needle growth after the adelgid population initially declines (McClure 1987).

In general, the medium health trees that were studied had a large population of adelgids, while the poor health trees were free of adelgids because of the severe defoliation that had previously occurred. Many of the poor health trees appeared to have commenced some new growth but, presumably, they will once again become infested with adelgids in the 2009 growing season.

Conductivity measurements were not significantly different. Water relations within the tree can be connected to each other according to Ohm's law analogue (van den Honert 1948):

$$\Delta P / \Delta x = E / LSC \quad (4)$$

where $\Delta P/\Delta x$ is the xylem pressure gradient, E is the transpiration (conductance (g_s)/vapor pressure deficit (D)), and LSC is the leaf-specific conductivity (Tyree and Sperry 1989). LSC is expected to be positively correlated with stomatal conductance and water potential and negatively correlated with $\delta^{13}C$. This could not be substantiated in this study perhaps because the reduced K_s of poor health trees was offset by a significantly lower $A_L:A_s$ ratio, resulting in non-significant differences of LSC. In addition, it is possible that this study may have resulted in non-significance of conductivity because of large variation within a small sample size.

Hollingsworth et al. (1991) found that there was a significant difference in trunk conductivity between BWA-infested and non-infested Fraser fir. Results may be different for hemlock because of the hydraulic architecture of the infested branches, as opposed to the infested trunk of Fraser fir. It has been shown that branches have a much lower LSC than the larger trunk of a tree because of the smaller tracheids (Ewers and Zimmerman 1984b). Ewers and Zimmerman (1984a) confirmed this finding in balsam fir (*Abies balsamea* L. Mill.), a close but geographically separated relative of Fraser fir (Frank 1990).

In order for the branches to effectively compete for resources, resistance within the trunk must be low. When water reaches the branches it meets greater resistance and increased pressure gradients due to smaller tracheids within the xylem, as a result branches are more susceptible to embolism. Tyree and Sperry

(1988) concluded that if embolism can occur to the point where many of the branches with low LSC are killed and leaf area is significantly reduced, the branches that are still surviving will have a greater LSC. An increased LSC for all surviving branches of a medium or poor health hemlock would explain why the differences between the healthy and poor branches on that tree were not significant.

Wood anatomy showed that there were obvious differences in healthy branches and poor branches but the significance of those differences were not established in this study. Balch et al. (1964) found that BWA induces hormonal changes in the xylem of native infested firs by injecting saliva into the tree for feeding. The saliva stimulates the tree to exhibit the characteristics of rotholz as growth ring portions contiguous to feeding increase in size and the tracheids begin to resemble compression wood. The cells in contact with the adelgid stylet enlarge and increase cell wall thickness, and the cells adjacent to feeding multiply. Perhaps in a similar hormonal reaction to the saliva of HWA, infested eastern and Carolina hemlock produced more latewood in the form of false rings, more longitudinal parenchyma and unidentified large, abnormal cells, as seen in the anatomy of these study trees. Further work that can relate growth rings with years of infestation may be able to provide more answers to how trees react to infestation. It is also necessary to establish what cells are being produced in the abnormalities of the xylem.

CONCLUSIONS

Eastern and Carolina hemlocks infested with HWA are experiencing drought-like water stress symptoms. However, this study did not determine if there is a hormonal reaction of eastern and Carolina hemlock to a salivary compound injected by feeding HWA or if the trees are dying as a direct response to the water-stress caused by infestation. Arthur and Hain (1986) speculated that while it wasn't obvious that the infested Fraser fir were dying as a direct consequence of infestation, they were more susceptible to drought-stress. When hemlock is infested with HWA, the water relations may be reduced in a manner that results in tree mortality when the tree is faced with any significant environmental water-stress event.

If this true, it can be suspected that Carolina hemlock may have more resistance to HWA than eastern hemlock because of its ability to withstand water-stress in its normal habitat on slopes with rocky, shallow soils (Harrar and Harrar 1962). There were a few significant differences in reactions of the different species to infestation in stomatal conductance and water potential. Further analyses may indicate that one species may be more tolerant of infestation than the other. A better understanding of this phenomenon could also be useful for selecting tolerant trees within each species.

Results of this study were complicated since the trees were constantly degrading in health. Further analysis that can allow for a truly healthy control

group may allow for more clearly interpretable data. A study of whole-tree data could also provide further answers as to what is occurring in eastern and Carolina hemlock infested with HWA.

LITERATURE CITED

- Anagnostakis, S.L. 1987. Chestnut blight: the classical problem of an introduced pathogen. *Mycologia* 79: 23-37.
- Annand, P.N. 1924. A new species of Adelges (Hemiptera, Phylloxeridae). *Pan-Pacific Entomologist* 1: 79-82.
- Arthur, F.H. and F.P. Hain. 1986. Water potential of Fraser fir infested with balsam woolly adelgid (Homoptera: Adelgidae). *Environ. Entomol.* 15: 911-913.
- Balch R.E, J. Clark, and J.M. Bonca. 1964. Hormonal action in production of tumours and compression wood by an aphid. *Nature* 202: 721-722.
- Barataud, F., C. Moyne, N. Bréda, and A. Granier. 1995. Soil water dynamics in an oak stand. *Plant and Soil* 172: 29-43.
- Broeckling, C.D. and S.M. Salom. 2003. Volatile emissions of eastern hemlock, *Tsuga canadensis*, and the influence of hemlock woolly adelgid. *Phytochemistry* 62: 175-180.
- Cheah, C., and M.S. McClure. 2000. Seasonal synchrony of life cycles between the exotic predator, *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae) and its prey, the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). *Agricultural and Forest Entomology* 2: 241-251.
- Cheah, C., M.E. Montgomery, S. Salom, B.L. Parker, S. Costa, and M. Skinner. 2004. Biological control of hemlock woolly adelgid. For. Health Technol. Enterprise Team, U.S. Dept. Agric.-Forest Service. FHTET-2004-04.
- Cowles, R.S., M.E. Montgomery, and Cheah, C. 2006. Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *Forest Entomology* 99: 1258-1267.
- Craig, H. 1957. Isotopic standards for carbon and oxygen and correlation factors for mass spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta*, 12:133-149

- Dixon, H.H. and J. Joly. 1894. On the ascent of sap. *Philosophical Transactions of the Royal Society of London*. B 186: 563-576.
- Ellison, A.M., et al. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 9: 479-486.
- Evans, R.A., E. Johnson, J. Shreiner, A. Ambler, J. Battles, N. Cleavitt, T. Fahey, J. Sciascia, and E. Pehek. 1996. Potential impacts of hemlock woolly adelgid (*Adelges tsugae*) on eastern hemlock (*Tsuga canadensis*) ecosystems. In: Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.), *Proceedings of the First Hemlock Woolly Adelgid Review*, Charlottesville, Virginia, 12 October 1995. Forest Health Technology Enterprise Team 96-10. USDA Forest Service, Morgantown, WV.
- Ewers, F.W. and M.H. Zimmerman. 1984a. The hydraulic architecture of balsam fir (*Abies balsamea*). *Physiologia Plantarum* 60: 453-458.
- Ewers, F.W. and M.H. Zimmermann. 1984b. The hydraulic architecture of eastern hemlock (*Tsuga canadensis*). *Canadian Journal of Botany* 62: 940-946.
- Farquhar, G.D., O'Leary, M.H., and Berry, J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9: 121-37.
- Ford, C.R. and J.M. Vose. 2007. *Tsuga canadensis* (L.) Carr. mortality will impact hydrological processes in southern Appalachian forest ecosystems. *Ecological Applications* 17: 1156-1167.
- Frank, R.M. 1990. *Abies fraseri* (Pursh) Poir. In: Burns R.M. and B.H. Honkala, eds. *Silvics of North America*. USDA Forest Service, Washington, D.C., USA.
- Godman, R.M. and K. Lancaster. 1990. *Tsuga canadensis* (L.) Carr. In: Burns R.M. and B.H. Honkala, eds. *Silvics of North America*. USDA Forest Service, Washington, D.C., USA.
- Hain, F.P. 1988. The balsam woolly adelgid in North America. In *Dynamics of Forest Insect Populations: Patterns, Causes, Implications*. Ed. A.A. Berryman. Plenum Press, New York. 603p.

- Harrar, E.S. and J.G. Harrar. 1962. Guide to southern trees, 2nd edition. Dover Publishers Inc. New York. 709p.
- Havill, N.P., M.E. Montgomery, G. Yu, S. Shiyake, and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Ann. Entomol. Soc. Am.* 99: 195-203.
- Hollingsworth, R.G., U. Blum, and F.P. Hain. 1991. The effect of adelgid-altered wood on sapwood conductance of Fraser fir Christmas trees. *IAWA Bulletin n.s., Vol. 12 (3)*: 253-239.
- Hubbard, R.M., M.G. Ryan, V. Stiller, and J.S. Sperry. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell, and Environment* 24: 113-121.
- Humphrey, D.L. 1989. Life history traits of *Tsuga caroliniana* Engelm. (Carolina hemlock) and its role in community dynamics. *Castanea* 54: 172-190.
- Jenkins, J.C., J.D. Aber, and C.D. Canham. 1999. Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. *Canadian Journal of Forestry Research*. 29: 630-645.
- Jones, H.G. and R.A. Sutherland. 1991. Stomatal control of xylem embolism. *Plant, Cell, and Environment* 14: 607-612.
- Kaufmann, M.R. 1976. Stomatal response of Engelmann spruce to humidity, light, and water stress. *Plant Physiology* 57: 898-901.
- Kizlinski, M.L., D.A. Orwig, R.C. Cobb, and D.R. Foster. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. *Journal of Biogeography* 29: 1489-1503.
- Lei. T.T., S.W. Semones, J.F. Walker, B.D. Clinton, and E.T. Nilsen. 2002. Effects of *Rhododendron maximum* thickets on tree seed dispersal, seedling morphology, and survivorship. *International Journal of Plant Sciences*. 163: 991-1000.
- McClure, M.S. 1987. Biology and control of hemlock woolly adelgid. *Bulletin of The Connecticut Agricultural Experimental Station* 851.

- McClure, M.S. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). *Annals of the Entomological Society of America* 82: 50-54.
- McClure, M.S. 1990. Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology* 19: 36-43.
- McClure, M.S. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environmental Entomology* 20: 258-264.
- McClure, M.S. 1996. Biology of *Adelges tsugae* and its potential for spread in the northeastern United States. In: Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.), *Proceedings of the First Hemlock Woolly Adelgid Review*, Charlottesville, Virginia, 12 October 1995. Forest Health Technology Enterprise Team 96-10. USDA Forest Service, Morganton, WV.
- Mitchell, R.G. 1967. Abnormal ray tissue in three true firs infested by the balsam woolly aphid. *Forest Science* 13: 327-332.
- Mladenoff, D.J. and F. Stearns. 1993. Eastern hemlock regeneration and deer browsing in the northern Great Lakes region: a re-examination and model simulation. *Conservation Biology* 7: 889-900.
- Orwig, D.A. and D.R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society* 125: 60-73.
- Orwig, D.A. and D.R. Foster. 2000. Stand, landscape, and ecosystem analysis of hemlock woolly adelgid outbreaks in southern New England: an overview. In: McManus, K.A., K.S. Shields, and D.R. Souto (eds.), *Proceedings: Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America*. USDA General Technical Report 267. Newtown Square, PA.
- Orwig, D.A. 2002. Stand dynamics associated with chronic hemlock woolly adelgid infestations in southern New England. In: Reardon, R.C., B.P. Onken, and J. Lashomb (eds.), *Symposium on the Hemlock Woolly*

Adelgid in eastern North America. New Jersey Agricultural Experiment Publication, New Brunswick, NJ.

- Orwig, D.A. and M.L. Kizlinski. 2002. Vegetation response following hemlock woolly adelgid infestation, hemlock decline, and hemlock salvage logging. In: Reardon, R.C., B.P. Onken, and J. Lashomb (eds.), Symposium on the Hemlock Woolly Adelgid in eastern North America. New Jersey Agricultural Experiment Publication, New Brunswick, NJ.
- Orwig, D.A., D.R. Foster, and D.L. Mausel. 2002. Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. *Journal of Biogeography* 29: 1475-1487.
- Panek, J.A. 1996. Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. *Tree Physiology* 16: 747-755.
- Puritch, G.S. 1971. Water permeability of the wood of Grand fir (*Abies grandis* (Doug.) Lindl.) in relation to infestation by the balsam woolly aphid, *Adelges piceae* (Ratz.) *J. Exp. Bot.* 22:936-945.
- Puritch, G.S. 1977. Distribution and phenolic composition of sapwood and heartwood in *Abies grandis* and the effects of the balsam woolly aphid. *Canadian Journal of Forest Research* 7:54-62.
- Quimby, J.W. 1996. Value and importance of hemlock ecosystems in the eastern United States. In: Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.), Proceedings of the First Hemlock Woolly Adelgid Review, Charlottesville, Virginia, 12 October 1995. Forest Health Technology Enterprise Team 96-10. USDA Forest Service, Morganton, WV.
- Rentch, J.S., H.S. Adams, R.B. Coxe, and S.L. Stephenson. 2000. An ecological study of a Carolina hemlock (*Tsuga canadensis*) community in southwestern Virginia. *Castanea* 65: 1-8.
- Ritchie, G.A. and T.M. Hinckley. 1975. The pressure chamber as an instrument for ecological research. *Advances in Ecological Research* 9: 165-254.
- Royle, D.D. and R.G. Lathrop. 2000. The effects of site factors on the rate of hemlock decline: a case study in New Jersey. In: McManus, K.A., K.S. Shields, and D.R. Souto (eds.), Proceedings: Symposium on Sustainable

Management of Hemlock Ecosystems in Eastern North America. USDA General Technical Report 267. Newtown Square, PA.

- Rood, S.B., S. Patiño, K. Coombs, and M.T. Tyree. 2000 Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees* 14: 248-257.
- Sandford, A.P. and P.G. Jarvis. 1986. Stomatal responses to humidity in selected conifers. *Tree Physiology* 2: 89-103.
- Sasaji, H. and M.S. McClure. 1997. Description and distribution of *Pseudoscymnus tsugea* sp. nov. (Coleoptera: Coccinellidae), an important predator hemlock woolly adelgid in Japan. *Annals of the Entomological Society of America* 90: 563-568.
- Scholander, P.F., H.T. Hammel, E.D. Bradstreet & E.A. Hemmingsen. 1965. Sap pressure in vascular plants. *Science* 148:339-346.
- Sellin, A. 1996. Base water potential of *Picea abies* as a characteristic of the soil water status. *Plant and Soil* 184: 273-280.
- Skinner, M., B.L. Parker, S. Gouli, and T. Ashikaga. 2003. Regional responses of hemlock woolly adelgid (Homoptera: Adelgidae) to low temperatures. *Environmental Entomology* 32: 523-528.
- Smith, G.F. and N.S. Nicholas. 1998. Patterns of overstory composition in the fir and fir-spruce forests of the Great Smoky Mountains after balsam woolly adelgid infestation. *The American Midland Naturalist* 139: 340-352.
- Souto, D., T. Luther, and B. Chianese. 1996. Past and current status of HWA in eastern and Carolina hemlock stands. In: Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.), *Proceedings of the First Hemlock Woolly Adelgid Review*, Charlottesville, Virginia, 12 October 1995. Forest Health Technology Enterprise Team 96-10. USDA Forest Service, Morganton, WV.
- Spicer, R. and B.L. Gartner. 1998. Hydraulic properties of Douglas-fir (*Pseudotsuga menziesii*) branches and branch halves with reference to compression wood. *Tree Physiology* 18:777-784

- Stadler, B., T. Muller, and D. Orwig. 2006. The ecology of energy and nutrient fluxes in hemlock forests invaded by hemlock woolly adelgid. *Ecology* 87:1792-1804.
- Timell, T.E. 1986. *Compression wood in gymnosperms*. Vol. 3. Springer Verlag, Berlin.
- Tyree, M.T. 1988. A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. *Tree Physiology* 4: 195-217.
- Tyree, M.T. and J.S. Sperry. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology* 88: 574-580.
- Tyree, M.T. and J.S. Sperry. 1989. Vulnerability of xylem to cavitation and embolism. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 40: 19-38.
- Tyree, M.T., H. Cochard, P. Cruiziat, B. Sinclair, and T. Ameglio. 1993. Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. *Plant, Cell and Environment* 16: 879-882.
- van den Honert, T.H. 1948. Water transport as a catenary process. *Discussions of the Faraday Society* 3: 146-153.
- Wallace, M.S. and F.P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. *Environ. Entomol.* 29: 638-644.
- Young, R.F., K.S. Shields, and G.P. Berlyn. 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America* 88: 827-835.
- Zhang, J.W. and Marshall, J.D. (1994) Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Canadian Journal of Forest Research* 24(1): 92-99.

- Zhang, J., Fins, L. and Marshall, J.D. (1994) Stable carbon isotope discrimination, photosynthetic gas exchange, and growth differences among western larch families. *Tree Physiology* 14: 531-539.
- Zhang, J.W., Feng, Z., Cregg, B.M., and Schumann, C.M. (1997) Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. *Tree Physiology* 17: 461-466.
- Zimmermann, M.H. 1983. Xylem structure and the ascent of sap. Springer-Verlag. Berlin. 143p.

TABLES AND FIGURES

Table 1. Results of analysis of variance for effects of species, vigor, and species-by-vigor interaction for healthy and medium vigors only, and vigor for analysis of species separately. P-value significance shown for pre-dawn and mid-day water potentials (Ψ) from June and August 2007, and leaf conductance (g_s).

| Effect | Ψ - June 2007 | | Ψ - August 2007 | | g_s |
|--------------------------------|--------------------|---------|----------------------|---------|---------|
| | Pre-dawn | Mid-day | Pre-dawn | Mid-day | |
| ---Healthy and medium vigor--- | | | | | |
| Species (S) | < 0.05 | ns | ns | ns | < 0.001 |
| Vigor (V) | < 0.01 | ns | < 0.05 | ns | < 0.01 |
| S x V | ns ¹ | < 0.05 | ns | ns | ns |
| ---Carolina hemlock--- | | | | | |
| Vigor (V) | < 0.01 | ns | ns | ns | < 0.001 |
| ---Eastern hemlock--- | | | | | |
| Vigor (V) | ns | ns | 0.05 | ns | <0.05 |

¹ ns = not significant at p-value = 0.05

Table 2. June 2007 pre-dawn and mid-day water potential (Ψ) vigor class means

| Condition | Carolina | Eastern |
|----------------|------------------|------------------|
| ---Pre-Dawn--- | | |
| Healthy | -0.40 ± 0.04 | -0.33 ± 0.07 |
| Medium | -0.75 ± 0.08 | -0.48 ± 0.18 |
| Poor | na | -0.43 ± 0.12 |
| ---Mid-Day--- | | |
| Healthy | -1.44 ± 0.11 | -1.65 ± 0.22 |
| Medium | -1.78 ± 0.34 | -1.17 ± 0.02 |
| Poor | na | -1.23 ± 0.31 |

Table 3. Significance results (P-value) for analysis of variance of the June 2008 branch water potential (Ψ) of species, time, and species-by-time interaction for trees of healthy and medium vigor classes only, and effects of time, vigor, and time-by-vigor interaction for species separately.

| Effect | P < F |
|--------------------------------|-----------------|
| ---Healthy and medium vigor--- | |
| Species (S) | ns ¹ |
| Time (T) | < 0.0001 |
| S x T | ns |
| ---Carolina hemlock--- | |
| Time (T) | < 0.0001 |
| Vigor (V) | < 0.05 |
| T x V | < 0.01 |
| ---Eastern hemlock--- | |
| Time (T) | < 0.0001 |
| Vigor (V) | ns |
| T x V | ns |

¹ ns = not significant at p-value = 0.05

Table 4. Significance results (p-value) for effects of vigor, tissue, and vigor-by-tissue interactions for separate species in August 2007 and January 2008 carbon isotope ratios ($\delta^{13}\text{C}$), with added effect of branch health for January 2008.

| Effect | Carbon isotope | |
|------------------------|------------------|--------------|
| | August 2007 | January 2008 |
| ---Carolina hemlock--- | | |
| Branch health | --- ¹ | < 0.001 |
| Vigor (V) | < 0.01 | < 0.01 |
| Tissue (T) | < 0.05 | < 0.01 |
| V*T | ns | ns |
| ---Eastern hemlock--- | | |
| Branch health | --- ¹ | ns |
| Vigor (V) | < 0.0001 | < 0.01 |
| Tissue (T) | < 0.001 | < 0.05 |
| V*T | ns | ns |

¹ not recorded for this sampling period

Table 5. Means (\pm S.E.) of carbon isotope ratio ($\delta^{13}\text{C}$) results from August 2007 and January 2008. Units are normalized $\delta^{13}\text{C}$ vs. VPDB.

| Condition | August 2007 | | January 2008 | |
|---------------------|-------------------|-------------------|-------------------|-------------------|
| | Carolina | Eastern | Carolina | Eastern |
| ---Branch health--- | | | | |
| Healthy | -28.48 ± 0.18 | -29.42 ± 0.23 | -26.25 ± 0.18 | -29.10 ± 0.53 |
| Medium | -27.51 ± 0.18 | -26.93 ± 0.26 | -27.47 ± 0.13 | -26.61 ± 0.30 |
| Poor | na | -27.9 ± 0.23 | na | -27.67 ± 0.30 |
| ---Tissue type--- | | | | |
| Twig | -27.66 ± 0.18 | -27.46 ± 0.19 | -26.34 ± 0.14 | -27.34 ± 0.28 |
| Leaf | -28.32 ± 0.18 | -28.71 ± 0.20 | -27.38 ± 0.15 | -28.23 ± 0.28 |

na = not applicable

Table 6. Means (\pm S.D.) of stem radii, leaf area (A_L), leaf area-to-sapwood area ratio ($A_L:A_S$), specific conductivity (K_s), leaf conductance (K_L), and leaf specific conductivity (LSC) for healthy and poor health branches from both species combined, Carolina hemlock, and eastern hemlock separately. Means in the same column followed by an * are significantly different at the < 0.05 level.

| Condition | Radius (mm) | A_L (m^2) | $A_L:A_S$ ($m^2:cm^2$) | K_s (kg/m/MPa/s) | K_L (mmol/m ₂ /s/MPa) | LSC (kg/m/MPa/s) |
|------------------------|-----------------|----------------------|-----------------------------|-----------------------|---------------------------------------|---------------------|
| ---Both Species--- | | | | | | |
| Healthy | 1.07 \pm 0.27 | 0.0020 \pm 0.0010* | 0.0652 \pm 0.0112* | 0.70 \pm 0.085 | 134.16 \pm 50.08 | 0.0045 \pm 0.0009 |
| Poor | 1.02 \pm 0.18 | 0.0005 \pm 0.0002* | 0.0163 \pm 0.0030* | 0.52 \pm 0.11 | 215.77 \pm 48.35 | 0.0082 \pm 0.0014 |
| ---Eastern Hemlock--- | | | | | | |
| Healthy | 1.10 \pm 0.06 | 0.0019 \pm 0.0002* | 0.0558 \pm 0.0084* | 0.78 \pm 0.13 | 196.06 \pm 67.39 | 0.0056 \pm 0.0013 |
| Poor | 1.08 \pm 0.08 | 0.0005 \pm 0.0003* | 0.0122 \pm 0.0103* | 0.42 \pm 0.15 | 241.39 \pm 82.50 | 0.0078 \pm 0.0016 |
| ---Carolina Hemlock--- | | | | | | |
| Healthy | 1.03 \pm 0.10 | 0.0023 \pm 0.0030* | 0.0726 \pm 0.0108* | 0.47 \pm 0.13 | 92.78 \pm 24.72 | 0.0033 \pm 0.0012 |
| Poor | 0.85 \pm 0.17 | 0.0005 \pm 0.0004* | 0.0217 \pm 0.0176* | 0.60 \pm 0.21 | 106.83 \pm 34.94 | 0.0074 \pm 0.0017 |

Table 7. Means (\pm S.E.) of stomatal conductance (g_s) for species combined and separate, by tree condition. Units in $\text{mmol m}^{-2} \text{s}^{-1}$.

| Condition | Both species | Carolina | Eastern |
|-----------|--------------------|-------------------|--------------------|
| Healthy | 211.65 \pm 18.12 | 125.91 \pm 7.29 | 297.39 \pm 30.15 |
| Medium | 118.99 \pm 18.12 | 66.28 \pm 7.29 | 171.69 \pm 30.15 |
| Poor | na | na | 196.4 \pm 30.15 |

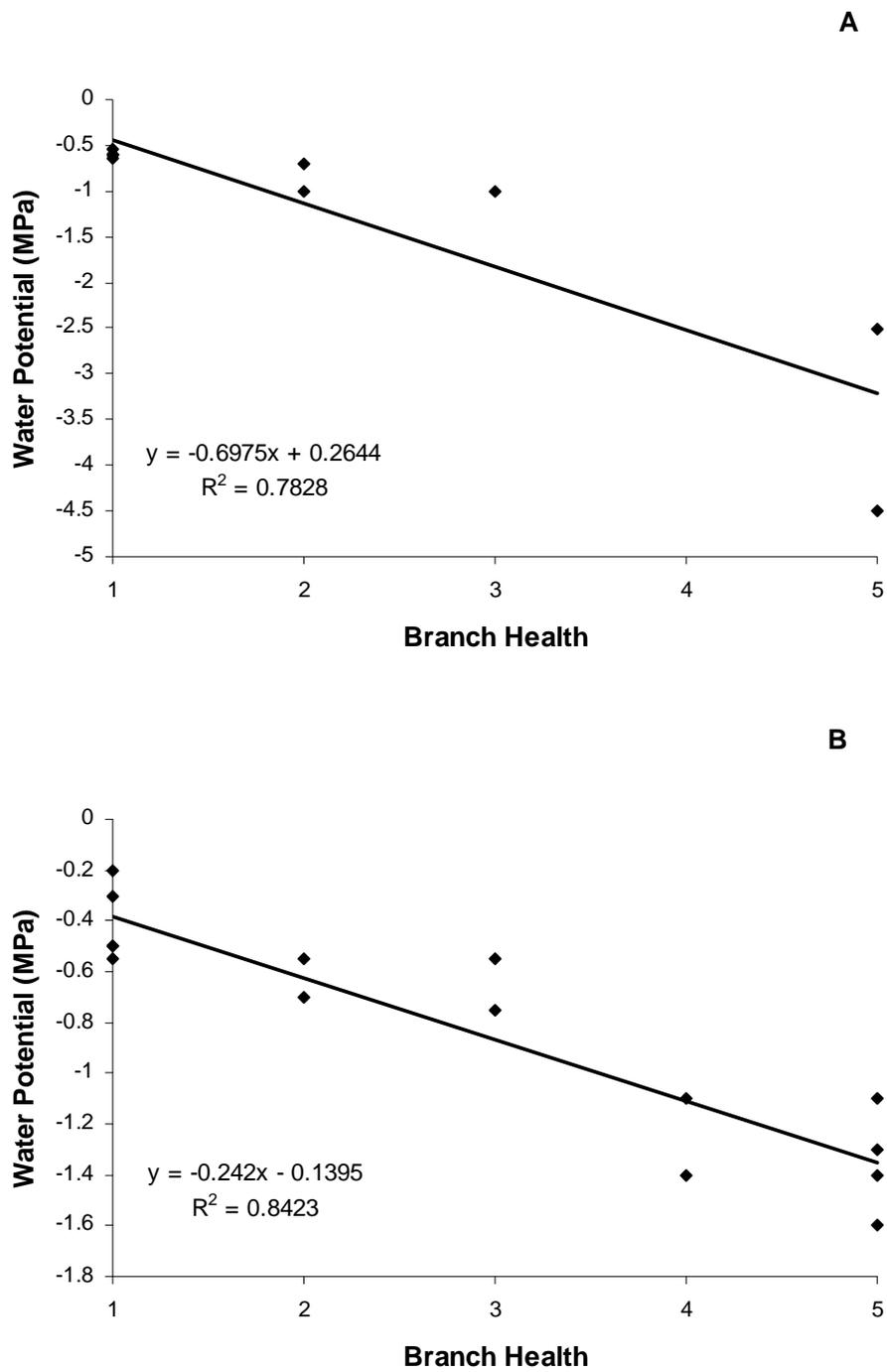


Figure 1. August 2007 Simple linear regression equation, and r^2 of Carolina (A) and eastern (B) hemlock pre-dawn water potentials (Ψ_p), by branch health (1 = healthy branch, 5 = most unhealthy branch)

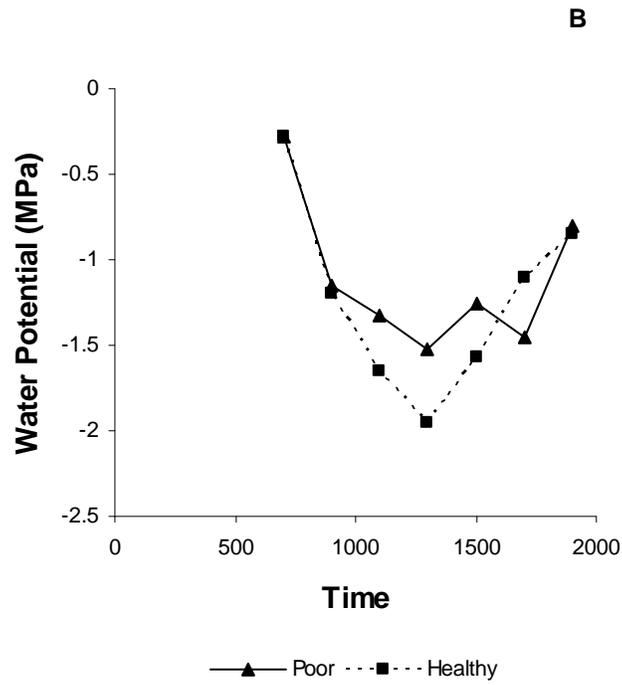
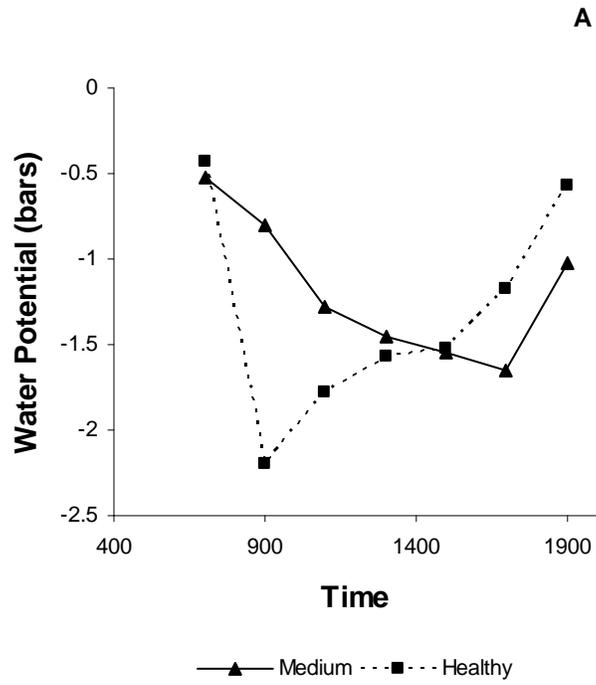


Figure 2. June 2007 water potential (Ψ) diurnal curves of a healthy and medium health Carolina (A) hemlock, and a healthy and poor health eastern (B) hemlock.

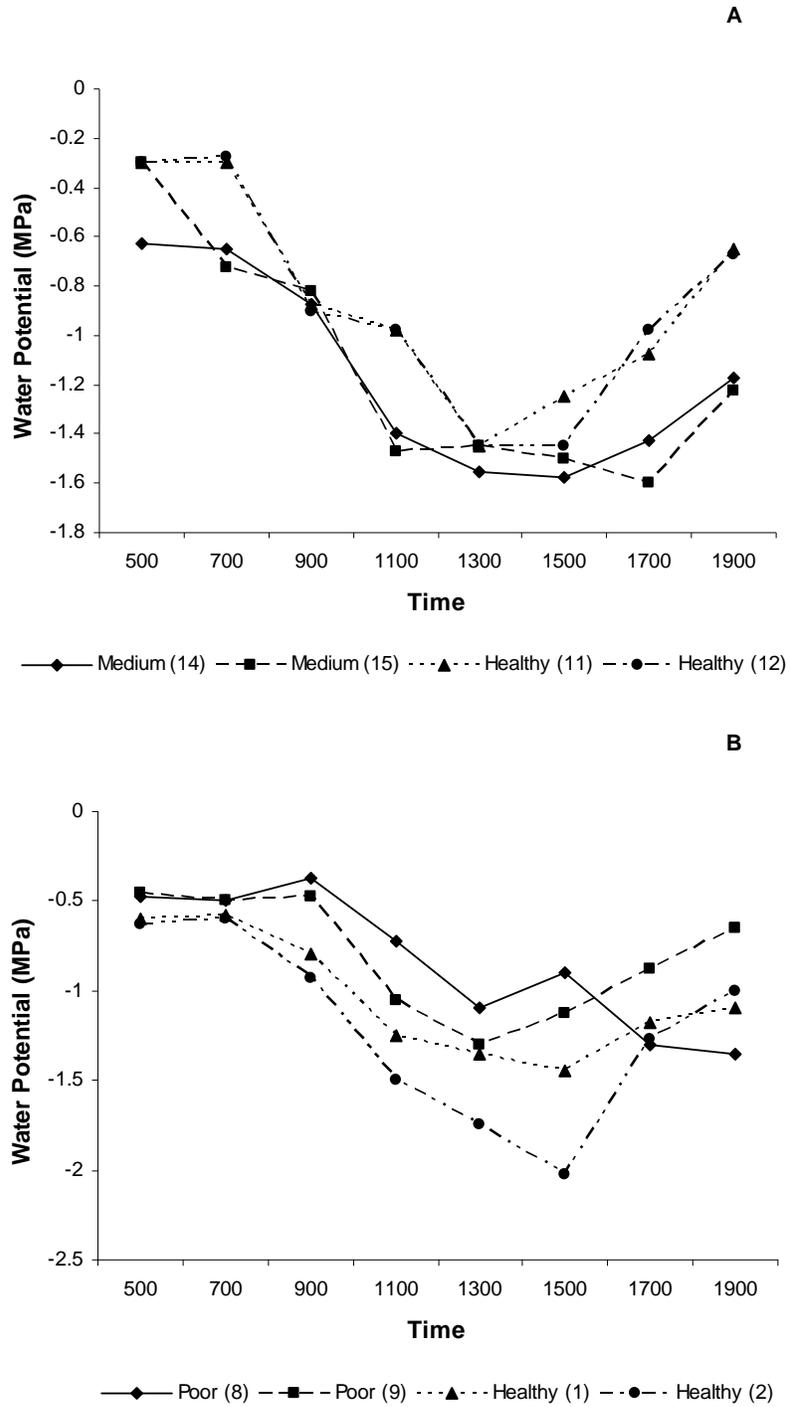


Figure 3. June 2008 water potential (Ψ) diurnal curves of Carolina (A) and eastern (B) hemlock. Carolina hemlock has two medium health and two healthy trees represented, while eastern hemlock has two poor health and two healthy trees measured.



Figure 4. Leaf area-to-sapwood area of healthy and poor health branches from both species and one tree of each vigor class.

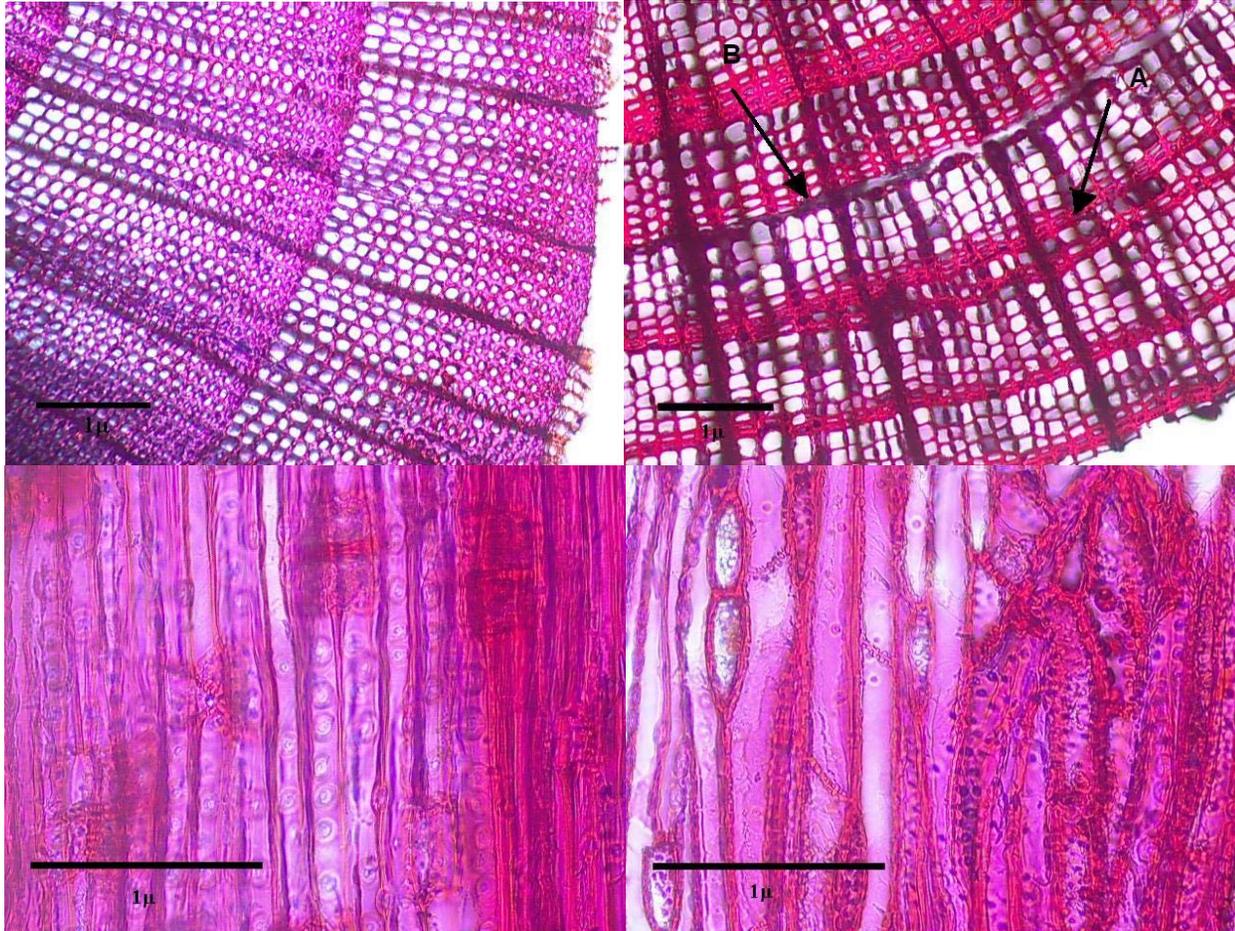


Figure 5. Wood anatomy photographs; Eastern hemlock; clockwise from the top left: healthy hemlock cross-section, poor health hemlock cross-section (arrow A displays false-ring structure, arrow B displays increased longitudinal parenchyma), poor health hemlock tangential section, healthy hemlock tangential section. Scale indicates 1µm.

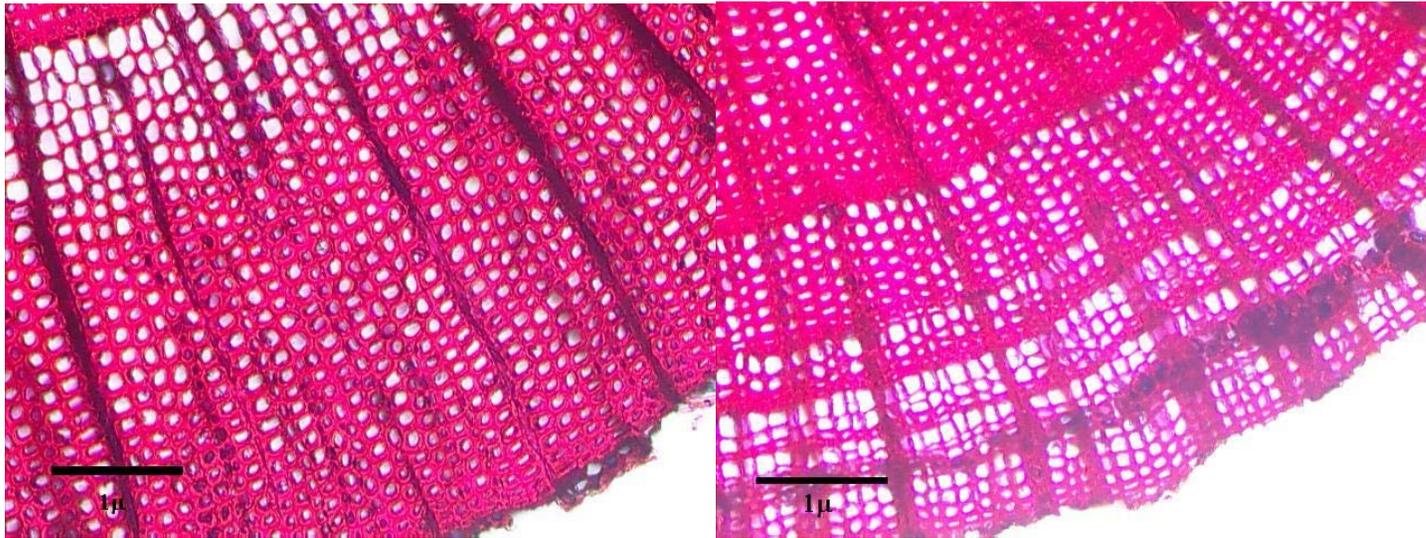


Figure 6. Wood anatomy photographs; Carolina hemlock; from left to right: healthy hemlock cross-section, poor health hemlock cross-section. Scale indicates 1 μ m.

APPENDIX

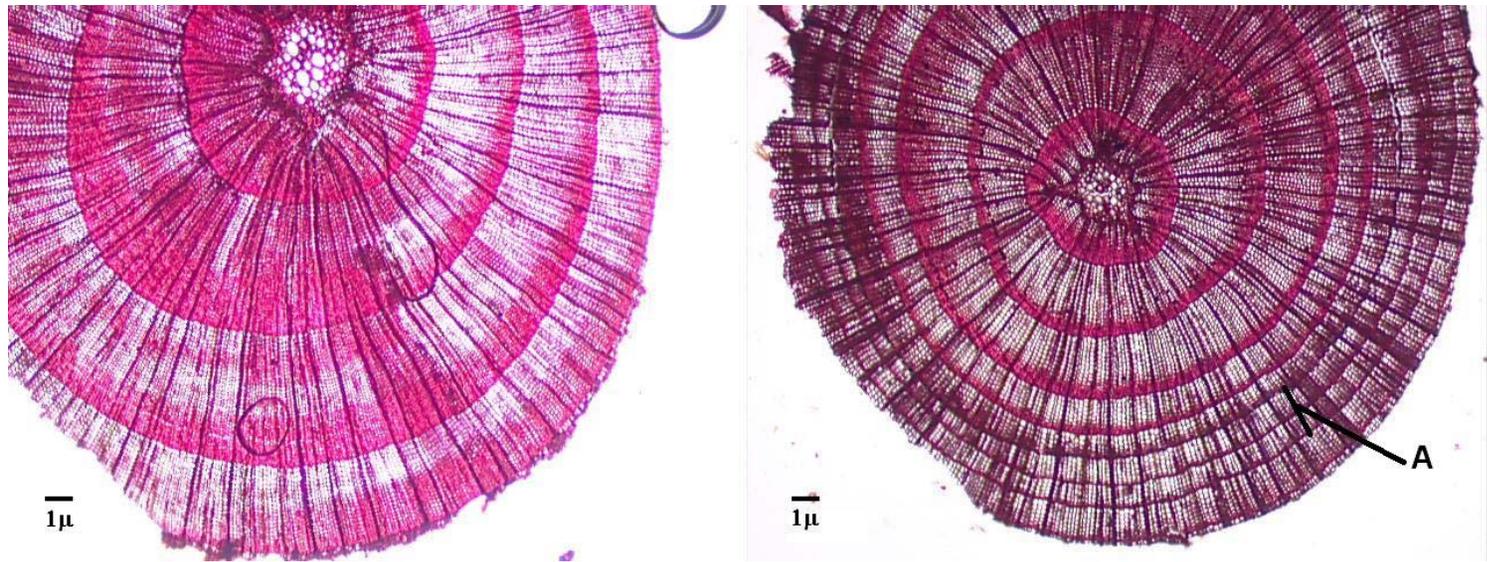


Figure 1. Low-magnification wood anatomy (4x); Eastern hemlock; from left to right: healthy hemlock cross-section, poor health hemlock cross-section (A indicates false-ring structures). Scale indicates 1μm.

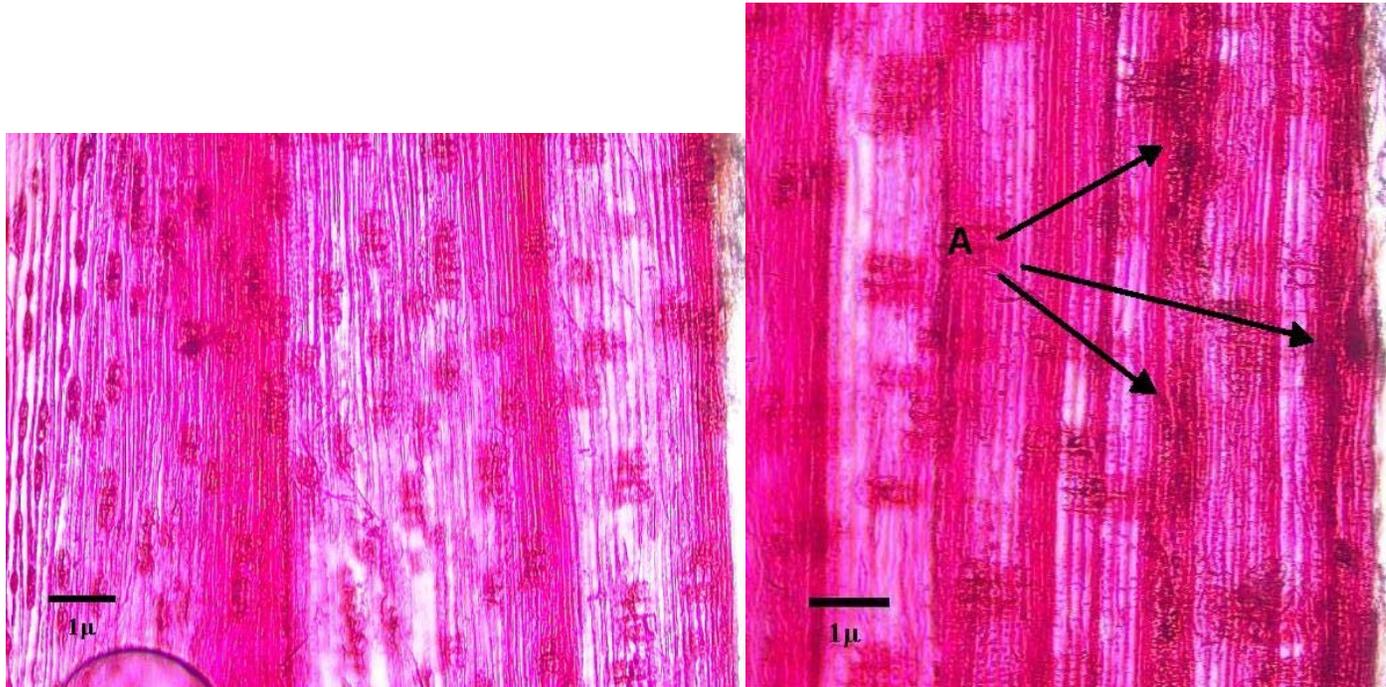


Figure 2. Low-magnification wood anatomy (10x); Eastern hemlock; from left to right: healthy hemlock tangential section, poor health hemlock tangential section (arrows indicate abnormal wood). Scale indicates 1 μm.

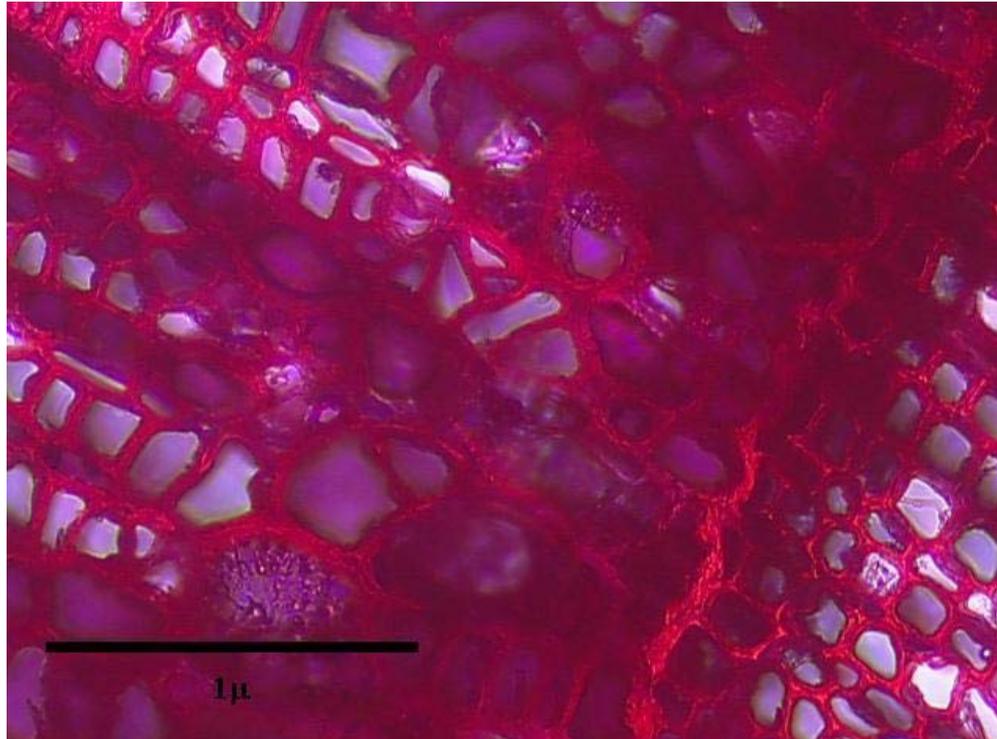


Figure 3. High-magnification wood anatomy of poor health eastern hemlock cross-section (40x). Scale indicates 1 μ m.