

**Eastern Hemlock Needle Physiology as Impacted by Hemlock Woolly Adelgid and  
Treatment with Imidacloprid**

Kelly Marie McDonald

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John R. Seiler, Chair

Scott M. Salom

James R. Rhea

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ABSTRACT

Eastern hemlock (*Tsuga canadensis* [L.] Carr.) is a foundation species that occupies a unique niche in forest ecosystems and which often forms pure stands throughout the eastern United States. Throughout the last half of a century, widespread mortality of *T. canadensis* had been occurring with the introduction of the invasive pest, hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA). HWA now threatens to destroy millions of hectares of hemlock dominated forests and to disrupt its associated ecosystems. In order to determine how HWA impacts hemlock physiology, three sites with various degrees of infestation were chosen and half of the trees at each site were treated with imidacloprid (Merit® 2 F, Bayer, Kansas City, MO) while the rest were left untreated. Needle gas exchange was assessed monthly using a LI-COR 6400 portable open path gas exchange system (LI-COR Inc, Lincoln NE). Chlorophyll fluorescence and bud break were also characterized for all trees at two of the three sites. After one complete growing season, we found a slight increase in photosynthetic rates (4.98 %), increased bud break at Fishburn (562 %) and Mountain Lake (25.6 %) sites, and no change in chlorophyll fluorescence for imidacloprid treated trees. These results suggest that HWA is causing tree mortality largely through a reduction of leaf area and not a reduction in leaf level photosynthetic capacity. By quantifying the physiological response of *T. canadensis* to HWA, better insights can be made into understanding tree decline as it relates to HWA.

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## **Chapter 1: Introduction and Justification**

Forests are a public good that are currently being threatened by many non-native pests, including insects, pathogens, and plants. Although biological invasions have been accelerated by human activity, namely international trade and globalization, they have occurred regardless for millions of years due to extreme climate changes that eliminated forms of geographical isolation. These pests are often a threat to natural forests and constitute a major type of disturbance with severe socioeconomic impacts. The threat to forest ecosystems generated by invasive species is an environmental problem on the same scale as rising atmospheric CO<sub>2</sub> levels and global warming. Invasives can significantly alter forest structure and composition on a global scale, which therefore has many implications for goods and services such as wildlife habitat, nutrient cycling, and timber markets associated with these ecosystems. The impact on nonmarket goods, such as recreation and aesthetics, can also be substantial (Liebhold, MacDonald et al. 1995).

The invasion of one exotic insect, hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA), has already begun to have severe implications on ecosystem productivity and, given the large spatial scale of the invasion, may have a great impact on carbon (C) budgets in North America (Hicke, Allen et al. 2012). Currently, 17 states have been affected by HWA and over 2 million m<sup>2</sup> of U.S. basal area of *Tsuga* spp. are at risk (Hicke, Allen et al. 2012). Since the extent of the invasion is so great, eradication of the insect is virtually impossible (Liebhold, MacDonald et al. 1995). HWA is also highly mobile, as it is distributed by wind and animals, making any attempt to halt the spread along the front of invasion inevitably unsuccessful (Liebhold, MacDonald et al. 1995).

As defined by the U.S. government, an invasive species is “an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human



health” (Register 1999). In order to formulate policies and management strategies to deal with invasive species, attempts to quantify economic losses associated with an invasion have been made (Holmes, Aukema et al. 2009). In the case of hemlock, for which there is generally a low timber demand, the greatest losses incurred are in nonmarket goods (Holmes, Aukema et al. 2009). Private-property losses are also substantial since hemlock is widely used as an ornamental tree. As hemlock mortality and dieback increase, property values are expected to decline as well (Holmes, Aukema et al. 2009). A study in West Milford, NJ found an 8.3 % decrease in property values for every one acre increase in hemlock area (Huggett, Murphy et al. 2008). This is presumed to be a result of the loss of aesthetical value from defoliation of ornamental hemlocks (Huggett, Murphy et al. 2008). Such losses can equate to \$49,383 per hectare of dead and declining hemlocks (Holmes, Aukema et al. 2009). Currently, taxpayers and private landowners are responsible for paying for the losses and costs incurred as a result of HWA invasion (Holmes, Aukema et al. 2009).

In addition to the economic costs associated with HWA infestation, biotic disturbances can also significantly impact forest C dynamics by causing a decrease in Net Primary Productivity (NPP) through defoliation and consequential tree mortality. A hemlock dominated ecosystem may eventually become a C source, with direct implications for climate change, as tree mortality reduces C uptake and dead biomass inputs simultaneously cause an increase in C losses. This will result in a decline of Net Ecosystem Productivity (NEP). Such dynamics between infestation and C cycling have a strong temporal component, as the stage of decline and dieback, or of recovery, will strongly influence what is happening in that ecosystem and whether it is acting as a C source or sink (Hicke, Allen et al. 2012).

In order to create more accurate C budget models to predict the impacts of climate change on C dynamics in forest ecosystems invaded by nonnative pests, more studies are needed to explore how pests such as HWA are themselves impacted by climate change and how pests alter climate-dependent physiological processes. Additionally, these processes interact with other disturbances or stressors, such as drought and fire, and eventually feed back to the climate system; however, these interactions are poorly understood. Development of models may be used to predict whether an ecosystem will serve as a C source or sink, and can thus be used to predict the relationships between pest invasions and climate change.

The major limitations to the development of such models come from lack of current studies that explore the dynamics among pest invasions, physiological processes, climate, and abiotic disturbances like drought. Little is currently known about how pests and pathogens will impact C dynamics as a function of the extent of an outbreak and in relation to other biotic and abiotic disturbances. The effects of pests on physiological properties such as stomatal conductance, transpiration, and specific leaf area have also not been quantified or studied in great detail. Tree mortality as a result of the interplay between tree physiology, pests, and stressors such as drought and heat stress is not well understood. Thus, predicting how HWA will impact hemlock decline and C dynamics remains difficult (Hicke, Allen et al. 2012).

With no natural predators in its nonnative range of the eastern U.S., and its spread limited only by suitable hosts and very low temperatures in the North, HWA poses a critical threat to many forest ecosystems. Understanding changes in eastern hemlock physiological processes, such CO<sub>2</sub> uptake and transpiration, in response to HWA infestation is critical in order to develop methods to slow or prevent further infestation of hemlock-dominated stands, and also in understanding the carbon balance of infested trees. For example, at what point in decline is a

specific tree unable to recover from an infestation? The development of methods to protect and save hemlocks from the effects of HWA is critical in order to prevent major economic losses and to preserve hemlock stands that are considered a public good with great aesthetic value. There are no studies to date that thoroughly characterize such physiological changes and thus the objectives of this study are listed below:

**Study Objectives:**

1. Determine the impact of HWA on hemlock needle gas exchange at sites with varying levels of HWA infestation.
2. Examine initial changes in needle physiology in response to treatment with imidacloprid.
3. Examine the impact of HWA on hemlock chlorophyll fluorescence.

## Chapter 2: Literature Review

### *Background on Hemlock Woolly Adelgid*

With approximately 930,777 ha of eastern hemlock (*Tsuga canadensis* [L.] Carr.) dominated forests in the eastern United States alone, the non-native HWA poses a serious threat to the health and vigor of some of America's most productive ecosystems (Salom, Kok et al. 2008). Originating from southern Japanese hemlock (*Tsuga sieboldii* ([L.] Carr) at low elevations in Osaka, HWA was first observed in the 1950's in Richmond, VA. Spread occurred slowly via long-distance transport into nearby forests, causing the formation of new colonies (Morin, Liebhold et al. 2009). It has since become endemic to boreal and temperate environments in the Northern Hemisphere (Havill 2005; Havill and Footitt 2007). The threat is being realized as HWA is spreading through dispersion by wind and animals at an average rate of approximately 13 km/yr where limited by cold temperatures, and up to 21 km/year where unconstrained (Kimple and Schuster 2002; Evans and Gregoire 2006; Havill and Footitt 2007).

The major limiting factors in its spread are the lack of suitable *Tsuga canadensis* hosts in the South, and temperature in the North (Evans, Aust et al. 2011). Despite its sensitivity to very cold temperatures, some HWA can survive below freezing and the individuals that do survive can continue to support a damaging population (Costa, Trotter et al. 2008). Since its introduction, HWA has spread relatively slowly west and more rapidly north and south (Morin, Liebhold et al. 2009). A period of rapid dispersal occurred in the mid 1980's, and by 2005, HWA infested 25.6 % of the potential area of host habitat, or 25 % of the potential density of host basal area (Morin, Liebhold et al. 2005; Spaulding and Rieske 2010). HWA is currently found from southern Maine to northern Georgia, and in isolated stands in Minnesota (Paradis, Elkinton et al. 2007). Variation in spread rates has been observed, with the fastest spread (km/year) occurring in the northeast

and the slowest spread occurring to the southwest (Morin, Liebhold et al. 2009). These trends can be explained by a positive relationship with hemlock basal area and a negative relationship with minimum January temperatures (Morin, Liebhold et al. 2009).

Although the range of many uninfested hemlocks occur in areas with relatively low minimum winter temperatures, rising temperatures due to global climate change may allow HWA to spread into northern areas once believed to be immune to infestation (Paradis, Elkinton et al. 2007; Morin, Liebhold et al. 2009). Population spread is estimated to cease only when 91 % overwintering mortality occurs. Furthermore, overwintering mortality of this degree is likely only when mean winter temperatures reach  $-5^{\circ}\text{C}$ . Although current temperatures reach this low along the northern front of HWA spread, climate models predict the average winter temperature to increase by  $1.5-2^{\circ}\text{C}$  by 2039, and  $2-3^{\circ}\text{C}$  by 2069. Thus, it is predicted that HWA will eventually spread into these currently uninfested areas to the north as temperatures continue to rise (Paradis, Elkinton et al. 2007).

The lifecycle of HWA is cyclically parthenogenic, with both sexual (holocyclic) and asexual (anholocyclic) generations (Havill and Footitt 2007). The cycle typically lasts two years and has five generations, three of which are spent on the primary or sexual host, and two on the secondary or asexual host. Both of the generations on the secondary host occur annually. While spruce is the sole primary host, the secondary host can be one of several *Tsuga* species, such as *T. canadensis*, making HWA highly host specific (Havill and Footitt 2007). Although HWA is sensitive to extremely low temperatures which can reduce the population size of future generations, it is active during the winter months when it feeds and grows (McClure 1989). This active winter generation is known as sistens while the spring and summer generation is referred to as progrediens, each of which produces crawler offspring (Butlin, Preisser et al. 2007).

Progrediens also produce winged sexuparae which die due to the lack of its necessary spruce host in North America (Cheah, Montgomery et al. 2004).

Trees are attacked regardless of their age or size, but HWA does preferentially settle on new growth (Mayer, Chianese et al. 2002; Narayanaraj, Bolstad et al. 2010). Adelgids settle at the base of needles and feed on hemlock ray parenchyma cells using a stylet that is inserted between epidermal cells before moving both intercellularly and intracellularly to reach the xylem ray cells (Shields, Young et al. 1995). After the first year of infestation, hemlocks enter a state of decline and respond by putting out less new growth. As a result of reduced new growth, HWA populations decline, allowing the tree to rebound the following year and put out more new foliage. Once again, HWA will return and settle on this new growth but in larger numbers and eventually overtake the tree (Mayer, Chianese et al. 2002). Environmental factors, such as drought, also impact infestation as new growth is limited when the tree is water stressed (Mayer, Chianese et al. 2002). HWA nymphs will colonize the youngest branches first, especially of trees that have never been infested. Branch age and HWA survival rates are negatively correlated and previously infested trees have significantly less new foliage, forcing nymphs on these trees to settle on one year old branches and suffer reduced health and survival (Shields, Young et al. 1995). In addition to branch age, branch position must also be considered as lower branches are more likely to be initially impacted by HWA than branches in the upper crown (Fajvan 2007).

### ***Background on Eastern Hemlock***

Considered a foundation species for its critical role in the functioning of an ecosystem, the loss of hemlock threatens to disrupt many ecological processes both within the forest ecosystem and beyond (McClure 1991). It occupies a unique niche in which most other species

could not survive and which most evergreens do not compete for (Sivaramakrishnan and Berlyn 1999). Hemlock is a late-successional, slow growing tree that does best on moist and fertile soils located on stream ravines and north-facing slopes (Eschtruth, Cleavitt et al. 2006; Ward 2008). Soil moisture is a major factor controlling the distribution of hemlock in the South Appalachian Mountains (Narayanaraj, Bolstad et al. 2010). The density of hemlock decreases rapidly moving away from a nearby stream within the first 50 m until the density approaches zero further than 75 m from the stream. In addition to requiring high soil moisture, hemlock also grows best in areas with adequate drainage and shade (Goerlich and Nyland 1999). Its shallow root system is typical for species commonly found on gentle slopes or flat regions (Goerlich and Nyland 1999). Hemlock is also most abundant at lower elevations, typically 650-750 m above sea level, although it can be found from sea level to above an elevation of 730 m. Above 1250 m, eastern hemlock is absent (Narayanaraj, Bolstad et al. 2010).

The most shade tolerant species in the eastern U.S., eastern hemlock is able to successfully grow in the understory or as a sub-dominant in the overstory (Rentch, Fajvan et al. 2008). Hemlocks create a very extreme understory environment that few species can tolerate due to the excessive shade provided by the canopy, relatively low available nitrogen in the litter, and the coarse soils it is found in (Rogers 1980). The dense hemlock canopy also serves to regulate seasonal light levels and daily temperature fluctuations in the understory (Eschtruth, Cleavitt et al. 2006). These conditions often lead to pure hemlock stands, with well defined edges (Eschtruth, Cleavitt et al. 2006; Daley, Phillips et al. 2007). It thus has an important ecological role which includes increased habitat diversity, watershed protection, habitat for animals, stream flow regulation, moderation of water temperature, and thermal cover (Ward 2008). Very few

coniferous species can replace *T. canadensis*, especially in the southern and mid-Atlantic states (Evans, Aust et al. 2011).

### ***Hemlock Physiology and the Effect of HWA***

There is some suggestion that sustained photosynthesis throughout winter months is a critical component to the functioning of hemlock and their surrounding ecosystem. During the autumn months, photosynthetic rates and leaf conductance are influenced by the previous nights' minimum temperature only if that temperature is below freezing. With a minimum temperature below 0 °C, maximum photosynthetic rates are less than  $2 \mu\text{mol m}^{-2}\text{s}^{-1}$ . It is suggested that at -1 °C, the xylem water freezes and thus stomates are unable to open. After weeks of temperatures below freezing, chloroplast structure is altered which results in lower photosynthetic rates until warmer temperatures return. If light levels are high during this time, trees can experience photo-oxidative damage, observable by needle discoloration. Since nighttime minimum temperatures exert the greatest influence over winter photosynthetic rates in hemlock, global warming may significantly increase winter carbon fixation, which already makes up about 10 percent of hemlock's yearly carbon fixation, and provide more carbohydrate stores to be used for growth the following season (Hadley 2000).

Just as carbon fixation varies temporally in hemlock, it is also heterogeneous throughout the canopy. Needles more than 2-years old sustain lower photosynthetic rates than both current season needles and one-year old needles, on average, during the growing season (Catovsky and Bazzazz 2000). Rates in the lower canopy are also lower than those at the top of the canopy due to the high Leaf Area Index (LAI) of hemlock and the resulting reduced light levels in the lower canopy, as well as a greater proportion of old foliage in the lower canopy (Catovsky and Bazzazz



2000). This also explains the lack of well defined light saturation in hemlock. While needles in the lower canopy often have significant increases in photosynthetic rates with increasing photosynthetic active radiation (PAR), those at the top of the canopy have already become saturated due to the prolonged higher light level (Hadley and Schedlbauer 2002). Thus, the upper canopy generally contributes more to total canopy C uptake compared to the lower canopy (Catovsky and Bazzazz 2000).

Although light levels are the primary factor controlling gas exchange, photosynthetic rates are also correlated with both the season and needle age. Throughout the summer season, hemlock photosynthetic rates are the greatest due to a better developed photosynthetic apparatus in that season's needles, resulting in an increase in total canopy carbon uptake. Additionally, the shade tolerance of hemlock allows more CO<sub>2</sub> uptake on cloudy days relative to hardwoods such as red maple (*Acer rubrum* L.). Compared to broad-leaf species; however, photosynthetic rates in hemlock are consistently much lower during the main growing season. Thus, total canopy C uptake in hemlock is less than most hardwoods despite its greater LAI and the ability to fix C during leaf-off months for hardwoods. The potential transition to hardwood-dominated forests will thus have a large impact on ecosystem-level carbon dynamics (Catovsky and Bazzazz 2000).

HWA is a major concern as it can kill a mature hemlock in as little as four years after infestation, and negative health effects are often noticeable within only the first year (Rentch, Fajvan et al. 2008; Siderhurst, Griscom et al. 2010). Once crown transparency exceeds 60 percent, tree mortality rapidly increases and the chance of recovery is slim (Mayer, Chianese et al. 2002). Trees may initially respond to infestation by producing new growth, but as transpiration rates decline and infestation increases, sap flow can be reduced by almost half even during moist soil conditions (Kimple and Schuster 2002; Rentch, Fajvan et al. 2008). After the

first year of infestation, production of new growth is almost completely halted. McClure (1991) found that in a study of 40 trees, only three percent of buds produced new growth after one year of infestation, with all of this new growth occurring on trees with very low adelgid density ( $<4$  adelgids/ $20 \text{ mm}^2$  branch). As overstory trees lose foliage, understory seedlings exposed abruptly to high light levels suffer photodamage to existing foliage that is not adapted to high light levels, even if there is adequate water availability (Mohammed and Parker 1999). Loss of these seedlings further exacerbates the decline of the stand. Additionally, in the years following infestation, hemlock basal area increment (BAI), fine root biomass, and soil  $\text{CO}_2$  efflux all show patterns of decline (Nuckolls, Wurzbarger et al. 2008).

### ***Hemlock Susceptibility to HWA***

Once HWA is introduced into a stand, all trees are equally vulnerable to becoming infested. They do not; however, have an equal likelihood of succumbing to the detrimental effects caused by HWA. Crown conditions are the best indicator to predict hemlock decline after infestation (Rentch, Fajvan et al. 2008). Healthy trees with a full crown are just as likely to become infested as unhealthy trees, but they are less likely to suffer mortality as a result and have a greater chance of recovery. Second, tree crown position also influences tree susceptibility. Codominant trees are likely able to offset some of the negative impacts of HWA infestation during a good growing season as they receive more light and can thus maximize new shoots compared to intermediate trees. Because intermediate trees experience lower light levels on average, even a light infestation can be very damaging. Loss of needles and HWA feeding further depletes already reduced carbohydrate stores in these trees (Rentch, Fajvan et al. 2008). Suppressed trees are smaller overall but have a lower energy demand than intermediates, which may allow them to survive longer after infestation (Rentch, Fajvan et al. 2008).

A third factor contributing to hemlock susceptibility is site conditions. Hemlocks growing outside of their optimal range, such as trees on ridge tops, are more likely to suffer mortality after infestation compared to those in riparian zones (Sivaramakrishnan and Berlyn 1999). Hemlocks on north-northeast facing slopes where conditions are cooler and wetter tend to do better than trees with a southern aspect where increased radiation and warmer temperatures lead to drier soils (Rentch, Fajvan et al. 2008). The location where HWA first appears in a stand tends to suffer the most damage within the first few years and trees infested for a longer time period often have higher mortality rates as well (Mayer, Chianese et al. 2002).

Chemical characteristics of hemlock trees also influence their susceptibility to HWA-induced damage. High nitrogen and potassium content are linked to a greater susceptibility to HWA, while trees rich in calcium and phosphorous, such as western hemlock, demonstrate greater resistance to HWA damage (Pontius, Hallett et al. 2006). Trees with relatively high nitrogen content sustain larger HWA populations and are more susceptible to infestation compared to trees with lower foliar nitrogen concentrations (Pontius, Hallett et al. 2006). The quality of foliage, determined by nitrogen content, is critical to HWA survival since it is unable to move great distances and thus HWA will exploit available resources when possible (Pontius, Hallett et al. 2006). Since hardwoods produce leaf litter containing more nitrogen than conifers, hemlocks within mixed stands may be more vulnerable to infestation (Pontius, Hallett et al. 2006). During the first year after infestation, new growth has higher nitrogen levels and lower C:N ratios than old-growth foliage, possibly a result of tree responses stimulated by HWA saliva injected during feeding (Miller-Pierce, Orwig et al. 2010).

Once an eastern or Carolina hemlock becomes infested, it will rarely recover fully on its own as it has no natural defense to prevent HWA reproduction (Butlin, Preisser et al. 2007;

Narayanaraj, Bolstad et al. 2010). Hemlocks do have an isobornyl-acetate mixture of terpenes which effectively deters some chewing insects, but *T. canadensis* remains susceptible to phloem and xylem feeding insects such as HWA (Miller-Pierce, Orwig et al. 2010). HWA feeding induces a localized hypersensitive response which increases hydrogen peroxide levels and causes cell death. This was observed most pronouncedly in the needles where the insect feeds, but also on new growth which did not have any settled adelgids. The continued decline of hemlock suggests that this hypersensitive response does not provide hemlock with any immunity against HWA, but may actually be costly to the trees and further increase susceptibility to infestation (Radville, Chaves et al. 2011). An increase in release of monoterpenes from old foliage of infested trees has also been observed and is thought to be a potential defense mechanism by attracting natural predators of HWA (Broeckling and Salom 2002).

In the same study on the induced hypersensitive response of hemlock to HWA feeding, it was found that new growth needles of infested trees are longer than old growth needles of infested trees. It is hypothesized that feeding by HWA causes remobilization of nutrients to new growth in an attempt to withstand the infestation, but it may actually be beneficial to future HWA generations that settle on the nutrient-rich new growth (Radville, Chaves et al. 2011).

### ***Ecological Impacts of HWA***

As hemlock populations decline due to HWA, stands will rapidly be transformed into hardwood stands resulting in a cascade of physical and chemical changes to eastern forests (Evans, Aust et al. 2011). In the Appalachian Mts., loss of hemlock will result in a transition to a hardwood dominated overstory as existing hardwoods will have more space available to capture sunlight and expand in size (Evans, Aust et al. 2011). In Central Appalachia, hemlock basal area

is predicted to decline 82 % within 10 years following infestation, and many of eastern hemlock forests will likely be converted to white oak-red oak-hickory stands after 20 years (Spaulding and Rieske 2010). The deep, dense canopy formed by hemlock will thus become short and thin, causing an increase in light on the forest floor that supports deciduous trees, such as black birch, and shrubs (Eschtruth, Cleavitt et al. 2006; Ward 2008). An increase in shrub growth may in turn prevent new hardwoods or conifers from growing (Evans, Aust et al. 2011). A thinning canopy will also create more gaps, which may be exploited by nonnative plants (Spaulding and Rieske 2010).

A problem associated with shrub growth was observed in Connecticut, where hemlock was replaced with thorny exotic shrubs that supported an increase in white footed mice populations and a subsequent increase in the number of blacklegged ticks (Ward 2008). Since heavily infested trees have lower transpiration rates, soil moisture content will increase which further supports shrubs and hardwoods, or can result in an increase in runoff. Ford et al (2007) found that the loss of hemlock will result in a 10 % decline in annual transpiration for the entire stand being studied (Ford, Vose et al. 2007). Adding to the increase in soil moisture is a decrease in interception of precipitation that occurs as needles fall off and leaf area index declines (Ellison, Bank et al. 2005).

The impacts of HWA infestation on forest ecosystems can be characterized by:

- Lower organic soil moisture content
- Higher mineral soil moisture content
- Higher soil temperatures (1-2 °F)
- Higher soil nitrogen availability
- Lower root ectomycorrhizal colonization
- Higher foliar nitrogen content
- Greater bacteria, yeast, and filamentous fungi populations (Jefts and Orwig 2005).

Another related ecosystem impact stems from higher available nitrogen concentration and lower lignin concentration in hardwood litter. This litter is preferred by microbes and as microbial decay increases, nutrient loading may occur in nearby streams and reduce dilution of pollutants downstream (Evans, Aust et al. 2011). Water temperatures will increase as more sunlight reaches the stream, which will further contribute to headwater quality decline and may have severe implications for the survival of brook trout (Siderhurst, Griscom et al. 2010). In areas where hemlock is replaced by black birch (*Betula lenta* L.), stream levels are predicted to decline due to transpiration rates that may be up to 1.6 times greater in *B. lenta* compared to *T. canadensis* (Daley, Phillips et al. 2007). Particularly during the growing season, small streams may completely dry up due to the higher water use of black birch, which is often close to the amount of cumulative precipitation (Daley, Phillips et al. 2007).

In the Delaware Water Gap National Recreation Area (DEWA), a 27 800 ha park incorporating lands of Pennsylvania and New Jersey along the Delaware River, approximately 20% of hemlocks are dead and 60% are in decline (Eschtruth, Cleavitt et al. 2006). Invertebrate populations living in hemlock-drained streams within DEWA show greater diversity than invertebrate communities in hardwood-drained streams despite having fewer rare taxa and a lower total density (Snyder, Young et al. 2005). The hemlock-drained streams have less extreme temperatures which support a more diverse fish and macroinvertebrate community. These streams also had consistently higher base flows, contributing to higher diversity by withstanding stream drying. These effects will be greatest in riparian areas where co-occurring *Rhododendron maximum* is less abundant. *R. maximum* is more prevalent alongside streams and may be able to serve some of the same functions as hemlock, mediating hemlock loss to a degree (Narayanaraj, Bolstad et al. 2010).

As hemlock mortality increases, pre-emptive and salvage logging will also increase despite the typically low demand for and usage of hemlock wood. These management practices; however, have the potential to disturb the ecosystem as an indirect result of HWA. Following harvests, Kizlinkski et al (2002) found that birch seedlings quickly became established while there was a low abundance of hemlock seedlings. The lack of hemlock seedlings is likely a result of poor seed viability, low regenerative capacity, and the lack of seedling generation due to increased incident radiation on the forest floor. Black birch; however, has a high annual seed production and a greater N use ability that allow its establishment following harvests. This suggests that despite the presence of HWA, hemlock harvests will result in conversion to hardwood stands (Kizlinkski, Orwig et al. 2002).

### ***Impacts of HWA on Carbon and Nutrient Cycling***

Predicted shifts in species composition have the potential to significantly alter carbon and nitrogen dynamics and nutrient availability in the once hemlock-dominated stands. Hemlock mortality will result in decreased nutrient uptake, thereby increasing the likelihood of nutrient leaching to nearby streams. Reduced evapotranspiration rates will likely lead to higher soil moisture levels, while canopy gaps will allow more sunlight to reach the forest floor, causing an increase in soil temperature. When combined with root decomposition and microbial activity, these conditions can cause an increase in nitrogen mineralization and nitrification rates. Until the stand is regenerated, likely by hardwood species, this inorganic N will be lost from the soil, along with associated cations (e.g  $\text{Ca}^{2+}$ ,  $\text{Mg}^{+2}$ ). The loss of nutrient capital may also result in long-term reduced productivity for the site, depending upon stand composition and the rate of seedling regeneration. The amount of hemlock mortality necessary to impact nutrient cycling,

and for how long those impacts may persist are questions that currently remain unknown (Yorks, Jenkins et al. 1999).

In southern Appalachian forests, the species shift that will likely be observed is towards an increase in great rhododendron (*Rhododendron maximum* L.) or to a mixture of early successional tree species (Nuckolls, Wurzburger et al. 2008). Whereas replacement by rhododendron would likely increase litter C: N ratios and may thus reduce future productivity, replacement by advance regeneration species with easily decomposable litter would likely cause lower litter C: N ratios, thus increasing N availability and potential productivity of the stand (Nuckolls, Wurzburger et al. 2008).

As previously mentioned, hemlock logging has increased throughout many areas impacted by HWA. Harvests will not only alter stand composition, but will also play a role in nutrient cycling and decomposition processes. Logging causes an abrupt change in microclimate, compared to that caused by the relatively slow decline of hemlock due to HWA, which includes an increase in light levels and temperature, and a decrease in soil moisture content. The implications of such changes include an increased inorganic N pool, slower cellulose decomposition, and enhanced net nitrification rates. These conditions are of concern due to the potential for nitrate leaching, although the presence of seedlings which rapidly uptake nitrate may diminish this threat on particular sites. Thus, logging of hemlock may have a more dramatic impact on ecosystem properties compared to the direct impact of HWA (Kizlinkski, Orwig et al. 2002).



## ***Control Techniques***

With HWA having the potential for such a tremendous impact on forest ecosystems, much attention has shifted to biological control of the pest by use of natural enemies. The major problem in controlling HWA has been understanding the complex interactions between the host plant and its ecosystem, pest insect, and potential natural enemies (Salom, Kok et al. 2008). Furthermore, no natural enemies of HWA exist in the eastern U.S. (Salom, Kok et al. 2008). Thus, predators must come from the western U.S. and Asia where hemlock is unaffected by HWA. The derodontid beetle, *Laricobius nigrinus* Fender, is one predator found associated with western hemlock in seed orchards of British Columbia (Zilahi-Balogh, Humble et al. 2003). The primary food source of this beetle is HWA, allowing it to control HWA populations even when the beetle's population size is low. It was first released in 2003 throughout the eastern coast of the U.S., from Massachusetts to Georgia (Mausel, Salom et al. 2010). *L. nigrinus* is active in winter, emerging from aestivation simultaneously with HWA sistens and feeding on those sistens as they develop. It lays eggs in HWA ovisacs so that the larvae can feed on the eggs. Other predatory beetles have been studied and released as well, with most being able to feed down to 2.5 °C ambient, and likely lower when considering the microclimate at the needle surface (Costa, Trotter et al. 2008).

Although it is not likely to stop the spread of HWA at a large scale, chemical control is an alternative and effective method to control HWA populations that can be used to control local spread or preserve landmark trees (Salom, Kok et al. 2008). HWA is highly susceptible to systemic application of imidacloprid at low concentrations; the LC<sub>50</sub> is 105- 411 ppb (95 % CL) (Cowles, Cheah et al. 2005). The response of a tree to treatment is dependent upon its health status beforehand (Webb, Frank et al. 2003). The unhealthiest trees show great improvements but

recover more slowly than trees that are slightly healthier but haven't lost as many needles and are not considered in dieback. The latter are still able to produce new growth after treatment and the unhealthiest trees will resemble these less damaged trees within three years of treatment (Webb, Frank et al. 2003). Once severely damaged, hemlocks ability to transport imidacloprid throughout the tree is compromised, as adequate sap flow is needed to transport the insecticide throughout the tree (Webb, Frank et al. 2003).

There are several methods to apply chemical treatments, with the most common being the Kioritz soil injection (Kioritz® Corporation, Tokyo, Japan). One study found 100 % mortality of HWA using this method near tree trunks, compared to trunk injections which resulted in no significant reductions of the HWA population (Cowles, Cheah et al. 2005). The soil injection also resulted in a long-term moderate imidacloprid concentration in tree sap, suggesting the potential for multiple-year control. Another study found supporting evidence that after only one year of imidacloprid soil injections, HWA populations decreased to less than 10 % of their pre-application levels (Pais and Demko 2005). Although the efficacy of treatment depends on sufficient sap flow to distribute the chemical, which may be diminished in severely impacted trees, it has been shown that even unhealthy trees in a state of dieback can recover after treated with soil injections (Webb, Frank et al. 2003).

In addition to imidacloprid, other chemical treatments have been researched. Thiamethoxam, like imidacloprid, is a thianicotinyl effective in treating HWA that works more rapidly than imidacloprid but is required at a higher dosage and thus higher cost, which may be why it is not widely used (Fidgen, McClellan et al. 2002). Dinotefuran (Safari) is a neonicotinoid that has been shown effective against HWA and can be used as a soil drench (Raupp, Ahern et al. 2008). Since it is more water soluble than imidacloprid, it can be taken up faster by the tree,

although the expected residual time is shorter (Royalty 2008). Dinotefuran has been shown to completely control HWA only 28 days after treatment using potted trees (Frank and Lebude 2011).

Environmentally friendly organic biostimulants or mineral nitrogen have also been shown to help hemlock resist the harmful effects of HWA, especially when trees are also suffering from drought (Sivaramakrishnan and Berlyn 1999). These biostimulants often contain a mixture of humic acids, peptides, polysaccharides, and anti-oxidants, which support the microbial community and thus may enhance plant nutrient availability and ultimately plant growth (Tejada, Benítez et al. 2011). Despite the effectiveness of chemical treatment, it is important to acknowledge that cost of such treatments and, for imidacloprid in particular, the ability to harm non-target aquatic organisms (Cowles, Cheah et al. 2005). Biostimulants are an attractive option as they reduce the need for fertilization and thus minimize the risk of nutrient losses to nearby water bodies, although they would be best used in combination with other chemical treatments that kill HWA (Sivaramakrishnan and Berlyn 1999). Studies are now currently exploring silvicultural treatments, such as thinning of mixed hardwood stands, in order to promote hemlock health and the ability of hemlocks to withstand HWA (Fajvan 2007).

Hemlock stands generally do not regenerate naturally after infestation without chemical or biological treatments, but have the ability to do so if conditions are right (Ellison, Bank et al. 2005). Most new trees will become established in an opening near a seed bearing or in a shaded area near the edge of a stand (Goerlich and Nyland 1999). Canopy cover exceeding 70- 80 % will prevent growth of shade intolerant species while allowing space for new hemlock to grow. Although seedlings have a high susceptibility to sudden increases in temperature and photon flux

density, they do have the ability to acclimate to smaller increases when gaps in the canopy occur, such as through the loss of dominant trees (Mohammed and Parker 1999).

The reestablishment of seedlings depends greatly on soil moisture conditions as well and is thus likely to occur along a stream where there is both adequate moisture and shade (Narayanaraj, Bolstad et al. 2010). Drought induced mortality is likely for a few years once a seedling becomes established (Narayanaraj, Bolstad et al. 2010). Several years of moisture are needed in order to combat the slow growth rates of seedlings and a shallow root system. Although soil moisture is a major factor affecting the distribution and reestablishment of hemlock, it is not the only limiting factor. Hemlocks are not found at high elevations where lower temperatures lead to greater precipitation and thus high soil moisture content. Other factors include past climate regimes, disturbance history, distribution of competing taxa, and human intervention (Narayanaraj, Bolstad et al. 2010).

The recovery of previously infested trees is impacted by a lack of microelements, which are needed in order to reach maximum photosynthetic capacity and subsequently for the production of new growth (Pais and Demko 2005). HWA also causes hemlock to stop vegetative and reproductive budding, resulting in a decrease in cone production. One method that has been developed in order to ensure that hemlock do not go extinct due to HWA is “ex situ conservation” (Jetton, Whittier et al. 2008). This involves collecting seeds from representative hemlock populations and either cold storing them or germinating the seeds and planting them in Latin America or Arkansas where they are protected from HWA. Ex situ conservation was developed to preserve the gene pool of hemlock, as well as to facilitate the breeding of HWA resistant hybrids (Jetton, Whittier et al. 2008).

### ***Using Physiology to Predict Tree Health***

The study of tree physiology allows researchers and land managers to understand how a forest system may respond to disturbances, such as those caused by an insect attack, and to predict subsequent changes in tree growth and productivity. Quantifying photosynthesis, stomatal conductance, and other gas exchange parameters allows better predictions to be made within the framework of models, as any of these physiological parameters alone cannot be used to make accurate predictions. Mathematically expressing such parameters in the form of a model serves as a hypothesis as to how the system is functioning and allows that data to be used in practice by managers and foresters. Physiologically process-based models in particular are more general than empirical models; one model can be used to make predictions in different systems. These models are based on the quantification of the underlying physiology which drives growth responses. Ultimately process-based models depend on empirically collected data. (Landsberg and Sands 2011).

Insect attacks constitute a major disturbance to forest ecosystems that can cause great changes in stand growth and productivity. Knowledge of how tree physiology is altered by a pest attack is needed in order to create and use process-based models that can indicate how the system is changing, i.e. if net productivity is increasing or decreasing. Particularly during large outbreaks, pests have the ability to change photosynthetic rates substantially on host trees and thus alter carbon dynamics within the system (Pinkard, Battaglia et al. 2011). For example, in a study of red pine (*Pinus resinosa* Ait), photosynthesis was stimulated 25-50% on remaining foliage six weeks after artificial defoliation compared to non-defoliated plants (Reich, Walters et al. 1993). Models offer a way to use collected data in order to predict such changes in the future.

Furthermore, these models may provide additional insights into the dynamics and interactions between multiple physiological parameters and environmental conditions.

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## **Specific Hypotheses and Objectives**

**Objective 1:** Determine the impact of HWA on hemlock needle gas exchange at sites with varying levels of HWA infestation.

Ho: Leaf level gas exchange rates (e.g. photosynthetic rate, transpiration rates, needle conductance) rates will not differ between tree size classes

Ho: Leaf level gas exchange rates will not differ between trees of different ages.

Ho: Leaf level gas exchange rates will not differ between study sites.

Ho: Water use efficiency will not differ between tree size classes.

**Objective 2:** Examine initial changes in needle physiology in response to treatment with imidacloprid.

Ho: Leaf gas exchange rates will not differ between trees treated with imidacloprid and those left untreated.

Ho: Adelgid presence will not differ between trees treated with imidacloprid and those left untreated after one year.

Ho: Numbers of flushing shoots will not differ between trees treated with imidacloprid and those left untreated after one year.

Ho: Leaf level gas exchange rates will not be related to shoot vigor as indicated by flushing.

Ho: Specific leaf weight will not differ between trees treated with imidacloprid and those left untreated after one year.

Ho: Water use efficiency will not differ between trees treated with imidacloprid and those left untreated.

***Objective 3:*** Examine the impact of HWA on hemlock chlorophyll fluorescence.

Ho: Chlorophyll fluorescence will not differ between trees treated with imidacloprid and those left untreated after one year.

Ho: Chlorophyll fluorescence will not be related to leaf level gas exchange rates

### **Chapter 3: Physiological Effect of Hemlock Woolly Adelgid on Eastern Hemlock**

#### **Abstract**

Eastern hemlock (*Tsuga canadensis* [L.] Carr.) is a foundation species that occupies a unique niche in forest ecosystems and often forms pure stands throughout the eastern United States. Over the last 30 years, widespread mortality of *T. canadensis* had been occurring with the introduction and spread of the invasive pest hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) and millions of hectares of hemlock dominated forests and the associated ecosystems are threatened. Since HWA feeds at the base of needles and removes stored carbohydrates, it is seems reasonable that the insect would impact leaf level physiology, which could then contribute to the decline of the tree. We investigated hemlock leaf physiology at three sites with various degrees of infestation. At each site, half of the trees were treated with imidacloprid (Merit® 2 F, Bayer, Kansas City, MO) while the rest were left untreated. Following treatment, needle gas exchange was measured monthly using a LI-COR 6400 portable gas exchange system (LI-COR Inc, Lincoln NE). Chlorophyll fluorescence and bud break were also characterized at two of the three sites. After one complete growing season, we found a slight increase in photosynthetic rates (4.98 %), increased bud break at Fishburn (562 %) and Mountain Lake (25.6 %) sites, and no change in chlorophyll fluorescence for treated trees. These results suggest that HWA is causing tree mortality largely through a reduction of leaf area caused by decreased bud break and not a reduction in leaf level photosynthetic capacity. By quantifying the physiological response of *T. canadensis* to HWA, better insights can be made into developing strategies that allow trees to withstand and recover from HWA attack.

## Introduction

Non-native pests are often intractable threats to natural forests and constitute a major type of disturbance with severe socioeconomic impacts. One such exotic pest, hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) has already begun to have severe implications on ecosystem productivity and, given the large spatial scale of the invasion, may have a great impact on carbon (C) budgets in North America (Hicke, Allen et al. 2012). With no natural predators in its non-native range of the eastern U.S., little or no resistance in the trees, and its spread limited only by suitable hosts and very low temperatures in the North, HWA poses a critical threat to many forest ecosystems. Currently, 17 states have been affected by HWA and over 2 million m<sup>2</sup> of U.S. basal area of *Tsuga* spp. are at risk (Hicke, Allen et al. 2012). Since the extent of the invasion is so great, eradication of the insect is virtually impossible (Liebhold, MacDonald et al. 1995). HWA is also highly mobile, as it is distributed by wind and animals, making any attempt to halt the spread along the front of invasion inevitably unsuccessful (Liebhold, MacDonald et al. 1995).

In order to formulate policies and management strategies to deal with invasive species, attempts to quantify economic losses associated with an invasion have been made (Holmes, Aukema et al. 2009). In the case of hemlock, for which there is generally a low timber demand, the greatest losses incurred are in nonmarket goods such as recreation, wildlife habitat, and aesthetics (Holmes, Aukema et al. 2009). In addition to the economic costs associated with HWA infestation, biotic disturbances can also significantly impact forest C dynamics by causing a decrease in Net Primary Productivity (NPP) through defoliation and consequential tree mortality. Such dynamics between infestation and C cycling have a strong temporal component,

as the stage of decline and dieback, or of recovery, will strongly influence what is happening in that ecosystem and whether it is acting as a C source or sink (Hicke, Allen et al. 2012).

Development of C budget models may be used to predict whether an ecosystem will serve as a C source or sink as well as their overall health, and can thus be used to predict the relationships between pest invasions and ecosystem function. The major limitation to the development of such models is a lack of data on the dynamics among pest invasions, tree physiological processes, climate, and abiotic disturbances like drought. The effects of pests on leaf physiological properties such as photosynthesis, stomatal conductance, transpiration, and specific leaf area have not been quantified or studied in great detail. Thus, predicting how HWA will impact hemlock physiological decline and ecosystem C dynamics remains uncertain (Hicke, Allen et al. 2012). Although these physiology parameters, such as photosynthetic and leaf conductance rates, alone cannot predict tree growth or decline, they are one part of understanding what is occurring in the system as a whole and can be used to guide control methods for HWA.

Therefore, the specific objectives of this study were to:

**Study Objectives:**

1. Determine the impact of HWA on hemlock needle gas exchange at sites with varying levels of HWA infestation.
2. Examine initial changes in needle physiology and bud break in response to treatment with imidacloprid.
3. Examine the impact of HWA on hemlock chlorophyll fluorescence.

## Methods

### *Sites*

Three sites at different stages of infestation, ranging from slightly impacted to severely impacted, were selected for study. Site one is located near Christiansburg, VA in Montgomery County on the Virginia Tech College of Natural Resources and Environment Fishburn Forest (elevation 610 meters). It is considered severely impacted with an average crown class score of 2.1 (where 1= dead, 2= <25 % live crown, 3= 25-50 % live crown, 4= 50-75 % live crown, 5= >75 % live crown) at the beginning of the study. Site two, located in Pembroke, VA in Giles County on the University of Virginia, Mountain Lake Biological Station (elevation 1170 meters) is overall the least impacted site with an average crown class of 3.06. Site three is in Twins Falls State Park, WV (elevation 610 meters) in Wyoming County and is less impacted than Fishburn but more so than Mountain Lake with a beginning crown class score of 2.5.

At all three sites a series of plots were established where half of the trees were treated with imidacloprid and the other half were left untreated. A minimum distance of 15 m was used between plots in order to ensure no contamination of chemical in control plots, and all trees in a plot were within approximately 9.3 m<sup>2</sup> of each other. At Fishburn and Mountain Lake, 12 plots (6 treated and 6 control) were installed in a completely randomized design with a split-plot with imidacloprid treatment as the whole plot factor and tree size classes as the split plot factor ([Appendix A](#)). The size classes used were based on the diameter at breast height (DBH) and consisted of small (<5 cm), medium (10-15 cm), and large (20-35 cm) tree within each plot for a total of 12 trees per size class. At Twin Falls, 10 plots were installed (5 treated and 5 control) and no tree size classes were used. The study design was completely randomized.



An analysis of the initial DBH distribution revealed that there were no significant differences between trees selected to be treated and control trees in any of the three size classes at Fishburn and Mountain Lake and no differences between treated and control at Twin Lakes ([Appendix B](#)). At Fishburn, tree size ranged from approximately 3 cm to 25 cm DBH. At Mountain Lake, tree size ranged from about 4 cm to 27 cm DBH. At Twin Falls, trees averaged around 20 cm. The large and medium sized trees from each plot were cored at their base using an increment borer to determine tree age. The mean tree age for treated and control trees across all sites was 50.9 and 48.5 years, respectively.

Plots at Fishburn and Mountain Lake were randomly chosen to be treated with imidacloprid (Merit® 2 F, Bayer, Kansas City, MO) at a rate of 1.2 g (AI)/ 2.5 cm dbh with Kioritz® soil injectors (Kioritz Corporation, Tokyo, Japan). Treatment was done in August 2011 at Fishburn and November 2011 at Mountain Lake. At Twin Falls, trees were treated in October 2011.

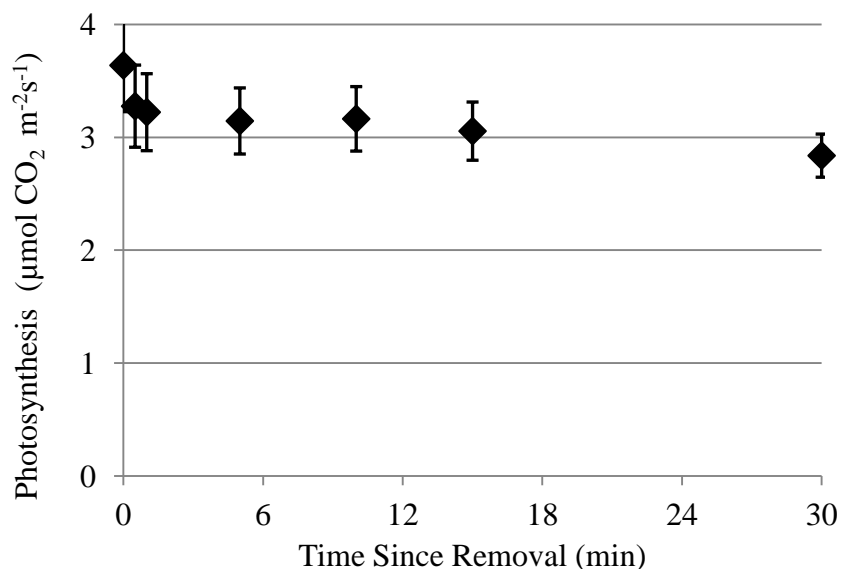
### *Gas Exchange*

Needle gas exchange measurements, including light saturated photosynthesis ( $A_{max}$ ) ( $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) and stomatal conductance ( $g_s$ ) ( $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ), and transpiration ( $T_r$ ) ( $\text{mmol m}^{-2} \text{sec}^{-1}$ ) were collected using a LI-COR 6400 portable open path gas exchange system (LI-COR Inc, Lincoln NE). Chamber conditions were set to a reference  $\text{CO}_2$  of 385 ppm, quantum flux was saturating at  $1200 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , and block temperature was set to near ambient. A flow rate of 200 was used as it was determined to provide a measurable differential between reference and sample  $\text{CO}_2$  concentrations ([Appendix C](#)). Measurements were taken in a 2-3 hr period encompassing solar noon and were recorded after  $A$  and  $g_s$  stabilized, typically within 2 min.

Measurements were taken on prepared attached branch sections of one year old foliage at Fishburn and Mountain Lake. Small segments on randomly selected branches of the lower crown were tagged and a small amount of neighboring foliage on that twig was removed to isolate the segment of foliage so it would fit cleanly into the cuvette. When possible, the newest mature growth on the tree was selected and prepared for measurements. Periodically, when it was obvious that needles were beginning to decline, branch segments were harvested and newer mature branch segments were prepared for the next month's measurements. Visible flushing of shoot segments after they had been prepared and repeatedly measured indicated that handling foliage in this manner did not cause additional stress to the branch (Appendix A).

At Twin Falls, not all trees had low hanging branches on which measurements could be taken; therefore, clipped branch segments were used for all trees at this site. Randomly selected branches were clipped, prepared as described above, and measured immediately. It was determined that clipped branches have stable photosynthetic rates for approximately 15 min and all measurements on clipped branches were taken well within this time frame (Figure 3.1). Soil moisture (measured from 0 to 12 cm soil depth) was recorded at the base of each tree using a soil moisture meter (Hydrosense TM, Campbell Scientific, Australia Pty Ltd.).

Gas exchange measurements were taken at roughly monthly intervals throughout the year at each site. For days in which weather conditions caused poor water data (i.e. damp foliage over estimating transpiration rates), water related data for the entire day was discarded. This was done by determining typical value ranges for each parameter and examining the data set for unreasonable water exchange rates.



**Figure 3.1.** Hemlock photosynthetic rates before branch was detached (0 min) and after branch was removed for a period of 30 min shows no significant decrease in rates after 15 min from detachment. Measurements were taken in approximately 5 min intervals. (n=3).

### *Chlorophyll Fluorescence*

At the Fishburn and Mountain Lake sites, chlorophyll fluorescence was measured monthly from June through October, 2012 for each tree using a Handy PEA fluorometer (Hansatech instruments Ltd., King's Lynn, England). Since there are not low hanging branches on all trees at Twin Falls, fluorescence measurements could not be obtained. Randomly selected needles were dark acclimated for 15 mins using dark adaptor clips. Needles were placed in the clips so as to maximize the amount of needle area in the clip. The most recent mature growth was chosen for measurement. Leaves were then subjected to a 1 second flash of saturating light ( $3000 \mu\text{mol}/\text{m}^2 \text{ s}$ ) using a high intensity LED centered at a wavelength of 650 nm.  $F_o$  (represents emissions by chlorophyll a molecules in PSII) is determined by generating a best fit line between data points 4 -16 recorded from the onset of illumination and extrapolating to time zero.

Maximum fluorescence ( $F_m$ ) is determined at the peak of variable fluorescence ( $F_v=F_m-F_o$ ) over  $F_m$  and this measure is independent of leaf area.

### *Bud Break*

The percent of flushing shoots was recorded in May 2012 and 2013 by randomly selecting five branches in the lower crown, selected from around the tree when possible, and counting the total number of buds and flushing buds. The total number of flushing buds divided by the total number of buds was used as the percent bud break.

### *Needle Age Class*

Gas exchange measurements were collected on current year, one-year-old, and two-year-old needles for ten randomly selected study trees at Mountain Lake in August 2012. Mountain Lake was selected for this study since the trees were in the healthiest condition and most likely to have multiple needle age classes still attached. Needle age was determined by visibly inspecting the branch for needle and twig color, as well as for bud scale scars.

### *Statistical Analysis:*

Day to day treatment differences in gas exchange and fluorescence were analyzed using an analysis of variance separately for each measurement day. At Fishburn and Mountain Lake, a two-way ANOVA was used to test the effect of chemical treatment, tree size class, and the interaction of treatment and size class. The Expected Mean Squares (EMS) method was used to analyze the split-plot. At Twin Falls, a one-way ANOVA was used to test the effect of chemical treatment only. To test the effect of chemical treatment and needle age cohort on tree physiology, a two-way ANOVA using EMS was performed. These were done using JMP Pro 10 software

(SAS Institute Inc., Cary NC). Data were tested at the 0.10, 0.05, and 0.01 significance levels. Differences detected at a 0.10 or lower significance level were deemed significant. Weather data were collected from the National Ocean and Atmospheric Administration (NOAA) website, as well as from the Mountain Lake Biological Station Meteorological Data website. Direct statistical comparisons will not be made between study locations although quantitative comparisons will be made.

Models for gas exchange were also developed using environmental variables collected from nearby meteorological stations and a stepwise regression procedure. When a significant model was developed, treatment effects (imidacloprid, tree class, etc.) on parameter estimates (slopes and intercepts) were examined using an analysis of covariance. This was done to determine whether any of the treatments (e.g. chemical treatment) modified how needles were responding to the environment.

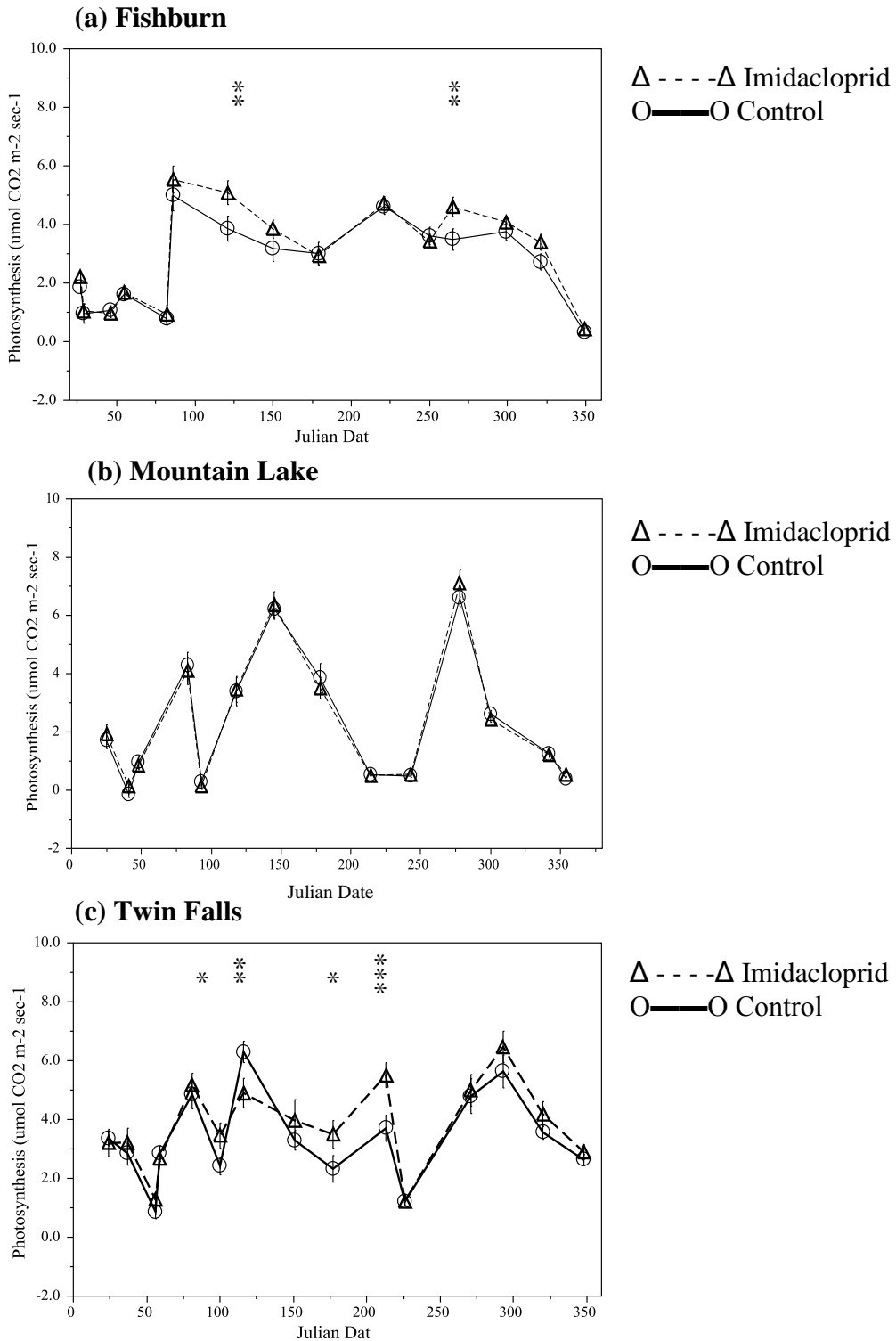
## **Results**

### *Photosynthesis Patterns and Means*

All photosynthetic rates are expressed in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$ . At Fishburn, the mean ( $\pm$ SE) photosynthetic rate for the growing season (May-Oct) was  $2.95 \pm 0.09$  when the average temperature on measurement days was  $25.56 \pm 0.16$  °C. During the winter months (Nov-Apr) the average photosynthetic rate was  $2.05 \pm 0.10$  and the average measurement day temperature was  $18.13 \pm 0.31$  °C. At Mountain Lake, the mean rates were  $3.16 \pm 0.19$  for the growing season, average measurement day temperature  $20.34 \pm 0.15$  °C, and  $1.49 \pm 0.11$  for the winter months, average measurement day temperature  $15.21 \pm 0.29$  °C. At Twin Falls, it was  $3.88 \pm 0.20$  for the growing season, average measurement day temperature  $24.06 \pm 0.35$  °C and  $3.56 \pm 0.12$  for the winter months, average measurement day temperature  $18.71 \pm 0.43$  °C. A one-way ANOVA

among sites indicates the overall rates were significantly highest at Twin Falls, followed by Fishburn and lowest at Mountain Lake ( $p < 0.0001$ ).

The split-plot analysis examining the effects of treatment, tree class, and their interaction at Fishburn revealed three days when significant differences occurred. On Julian date (JD) 121, treated trees had significantly higher photosynthetic rates compared to control trees ( $p = 0.0322$ ) (Figure 3.2a). On JD 265, treated trees had higher photosynthetic rates than control trees ( $p = 0.0440$ ) and the largest tree class had higher rates than medium and small size classes ( $p = 0.0457$ ) (Figure 3.2a). On JD 315, the interaction between treatment and size class was significant ( $p = 0.0310$ ), with treated trees in size class 3 having the highest rates and control trees in size class 1 having the lowest rates. There were no significant treatment or tree class differences detected at Mountain Lake (Figure 3.2b). At Twin Falls, there were four dates when photosynthesis differed significantly between chemical treatments (Figure 3.2c). On JD 116, treated trees had lower photosynthetic rates compared to control trees ( $p = 0.0360$ ) while on JD 100, 177, and 213 treated trees had higher photosynthetic rates ( $p = 0.0648$ ,  $p = 0.0895$ , and  $p = 0.0087$ , respectively).



**Figure 3.2 a-c.** Eastern hemlock photosynthesis rates comparison between imidacloprid treated and untreated trees at Fishburn (a), Mountain Lake (b), and Twin Falls (c) from 2012 to 2013. Points show mean  $\pm$  SE. Single asterisk denotes difference at  $\alpha = 0.10$ , double asterisk denotes a significant difference at  $\alpha = 0.05$ , and a triple asterisk denotes a difference at  $\alpha = 0.01$ .

### *Photosynthesis model comparisons*

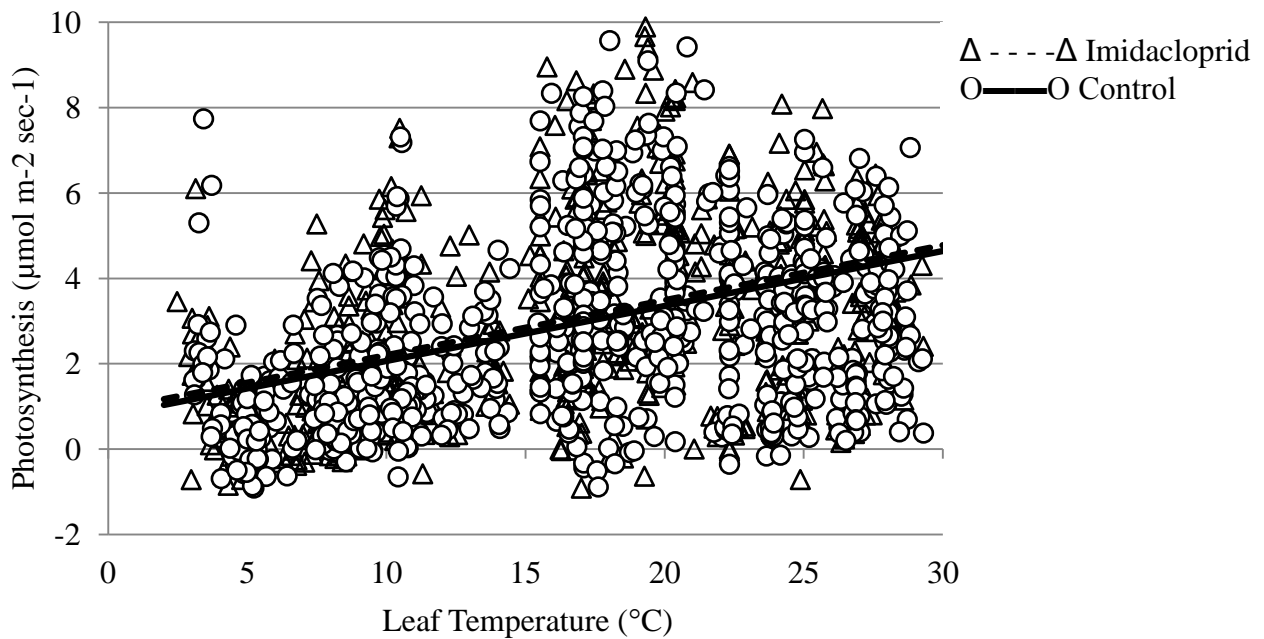
Using available environmental variables and data from all three sites, a simple model for photosynthesis was developed. Model selection was done with a stepwise regression procedure for initial screening of variables followed by examination of best  $R^2$  values. A simple model with three parameters containing leaf temperature, relative humidity and soil moisture explained 26.5 % of the variability in photosynthesis (Table 3.1). Using this simple model we then tested using analysis of covariance if chemical treatment, site, tree class, and presence of adelgid on the twig had any influence on the slope and intercept estimate.

There were significant differences ( $p = 0.0213$ ) between the intercepts of treated (-1.22) and control (-1.35) trees, suggesting that treated trees had on average higher photosynthetic rates compared to control trees (Figure 3.3). In fact, when all photosynthetic rates for treated and untreated trees are averaged, treated trees had rates 6.4% higher (2.82 versus 2.65). This difference was significant ( $p=0.0213$ ) when tested using a one-way ANOVA with leaf temperature, relative humidity and soil moisture used as covariates. In order to make sure this increase was not due to the presence of adelgid respiration on the branch segment measured, the presence of adelgid was tested using analysis of covariance and no significant differences in the intercepts were found ( $p > 0.10$ ). Tree class and site had no significant difference on model parameters. We also tested to see if there were any interactions between treatment and the environmental variables. None of the interactions were significant ( $p > 0.10$ ).



**Table 3.1.** Photosynthesis parameter estimates and p-values for the treatment models, with a total of three significant parameters. Only the intercept was found to differ significantly (<.0001) between treated and control trees.

PARAMETER	ESTIMATE		P-VALUE
Intercept	Imidacloprid -1.22	Control -1.35	< 0.0001
Soil Moisture Content (%)	0.039		< 0.0001
Relative Humidity of Leaf (%)	0.039		< 0.0001
Temperature of Leaf (°C)	0.129		< 0.0001
R <sup>2</sup> = 0.265			



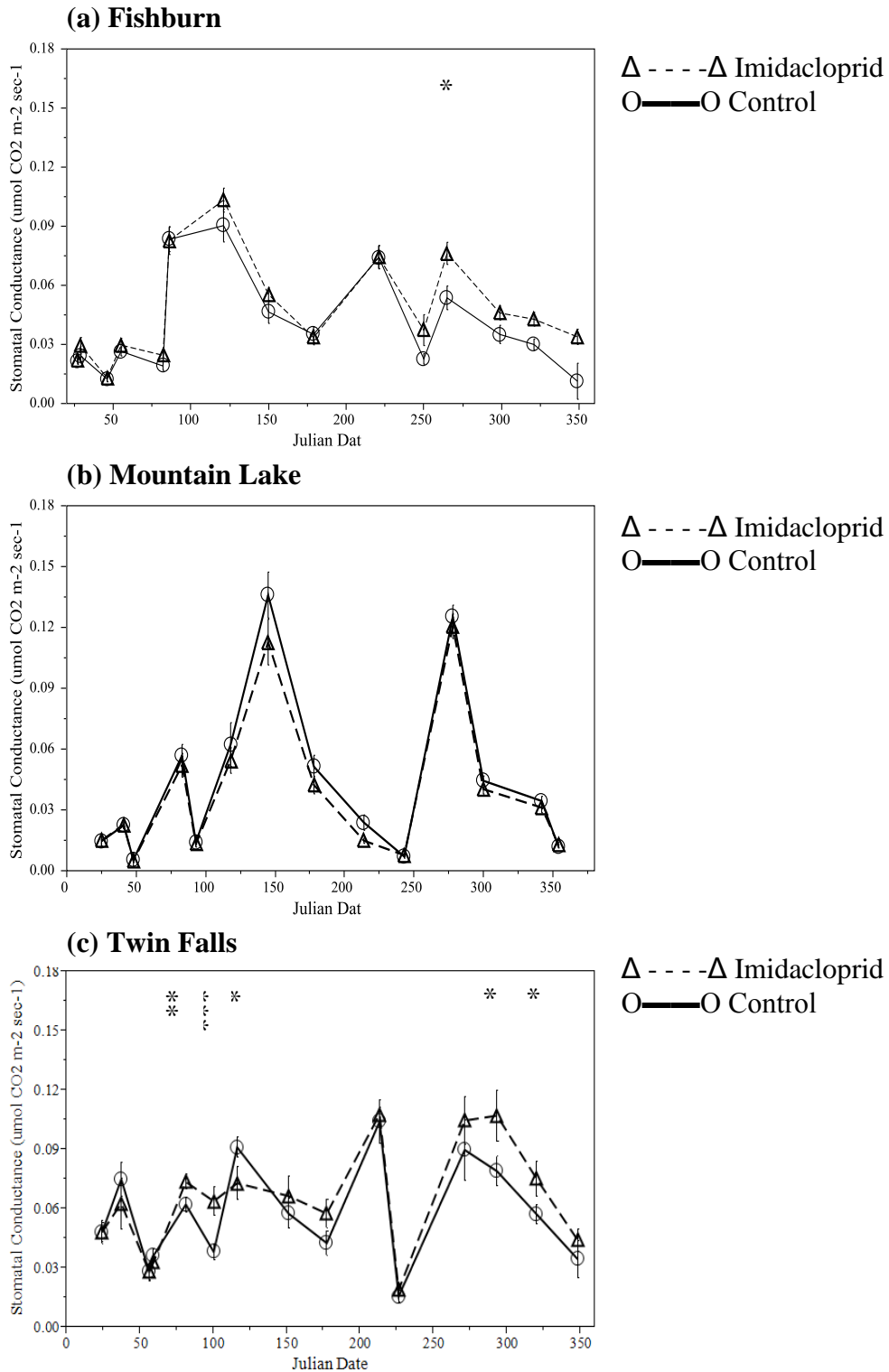
**Figure 3.3.** Eastern hemlock photosynthetic rates influenced by leaf temperature and the modeled best fit lines for treated and control trees. All other variables in the model were held at their seasonal average for calculating the fitted lines.

*Stomatal Conductance Patterns and Means*

All leaf conductance rates are expressed in μmol CO<sub>2</sub> m<sup>-2</sup> sec<sup>-1</sup>. At Fishburn, the mean leaf conductance rate (± SE) for the growing season was 0.03 ± 0.001 and 0.03 ± 0.002 for

winter months.. At Mountain Lake, the mean leaf conductance rates for the growing season and winter months were  $0.05 \pm 0.003$  and  $0.02 \pm 0.001$ , respectively. At Twin Falls, it was  $0.06 \pm 0.004$  and  $0.05 \pm 0.002$ , respectively.

At Fishburn, leaf conductance differed significantly by tree class on JD 46, with the largest tree size class having the highest rates and smallest tree size class having the lowest rates ( $p = 0.0315$ ) (Figure 3.4a). Treatment differences were detected on JD 265 ( $p = 0.0430$ ) with treated trees having higher rates than control trees. The interaction between treatment and size class was significant on JD 82 with treated trees in size class 3 having the highest rates ( $p = 0.0611$ ). At Mountain Lake, the interaction of treatment and tree class was significant on JD 83, with control trees of size class 2 having the highest rates and treated trees in the same size class having the lowest rates ( $p = 0.100$ ). At Twin Falls, on JD 81, 100, 293, and 320, higher leaf conductance rates were recorded for treated trees ( $p = 0.0455$ ,  $p = 0.0075$ ,  $p = 0.0747$ ,  $p = 0.0900$ , respectively), while JD 116 higher rates were recorded on the control tree ( $p = 0.0803$ ) (Figure 3.4c).



**Figure 3.4 a-c.** Eastern hemlock leaf conductance rates comparison between imidacloprid treated and untreated trees at Fishburn (a), Mountain Lake (b), and Twin Falls (c) from 2012 to 2013. Points show mean  $\pm$  SE. Single asterisk denotes a difference at  $\alpha = 0.10$ , double asterisk denotes a significant difference at  $\alpha = 0.05$ , and a triple asterisk denotes a difference at  $\alpha = 0.01$ .

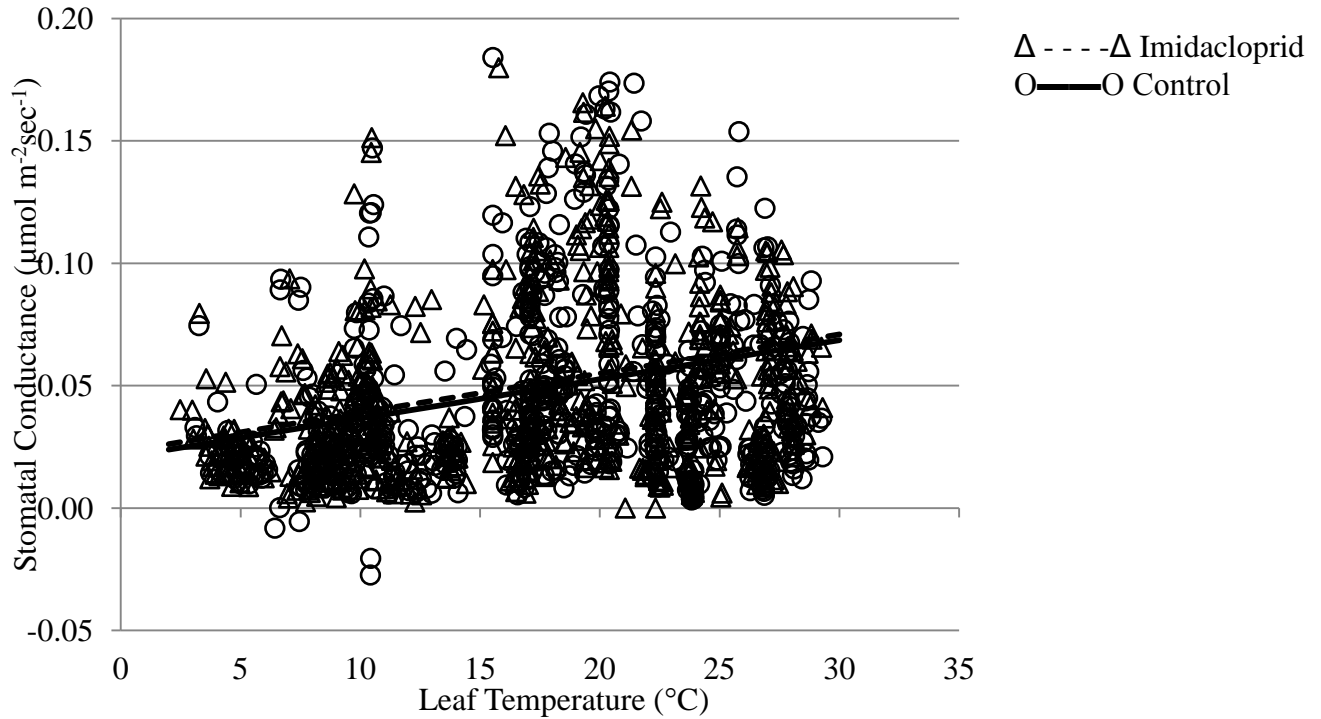
### *Leaf conductance model comparisons*

The same procedure used to develop a model for photosynthesis was used to develop a model for leaf conductance. The same parameters: leaf temperature, relative humidity, and soil moisture content, were found to be significant and explain the greatest amount of variation in the data (Table 3.2). Using this simple model, we then tested using analysis of covariance if chemical treatment, site, tree class, and presence of adelgid on the twig had any influence on the slope and intercept estimate.

There were significant differences ( $p = 0.0287$ ) between the intercepts of treated (-0.0270) and control (-0.0293) trees, suggesting that throughout the measurement period, treated trees had higher leaf conductance rate compared to control trees (Figure 3.5). Average needle conductance during the study period was 7.1 percent higher in treated trees (0.0482 versus 0.0450). As with photosynthesis this change in leaf conductance was not due to the presence of adelgid on the branch segment; the presence of adelgid was tested and no significant differences in the intercepts were found. Tree class and site had no significant difference on model parameters. We also tested to see if there were any interactions between treatment and the environmental parameters. None of the interactions were significant.

**Table 3.2.** Leaf conductance parameter estimates and p-values for the treatment models, with a total of three significant parameters. Only the intercept was found to differ significantly between treated and control trees.

PARAMETER	ESTIMATE		P-VALUE
Intercept	Imidacloprid -0.0270	Control -0.0293	< 0.0001
Temperature of Leaf (°C)	0.0016		< 0.0001
Relative Humidity of Leaf (%)	0.0008		< 0.0001
Soil Moisture Content (%)	0.0011		< 0.0001
$R^2 = 0.230$			



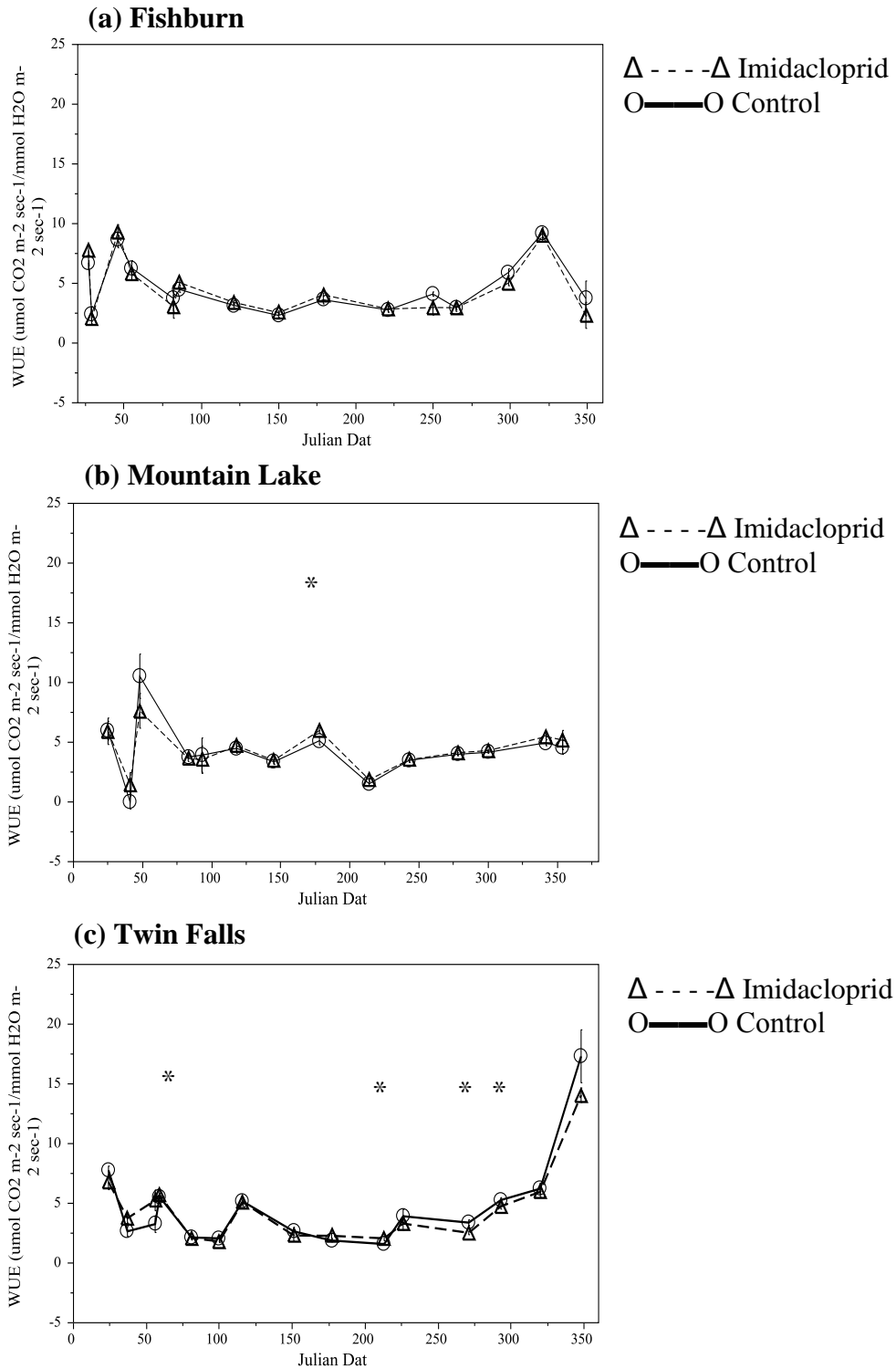
**Figure 3.5.** Eastern hemlock leaf conductance as influenced by leaf temperature and the modeled best fit lines for treated and control trees. All other variables in the model were held at their seasonal average for calculating the fitted lines.

#### *Water Use Efficiency patterns and means*

All water use efficiency is expressed in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ sec}^{-1}$ . At Fishburn, the mean water use efficiency (WUE  $\pm$  SE) for the growing season was  $4.45 \pm 0.17$  and  $6.05 \pm 0.24$  for winter months. Note all WUE data are expressed in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ sec}^{-1}$ ). At Mountain Lake, the mean WUE for the growing season and winter months were  $3.74 \pm 0.10$  and  $4.46 \pm 0.22$ , respectively. At Twin Falls, it was  $3.09 \pm 0.14$  and  $5.83 \pm 0.29$ , respectively. Overall means for the entire year did not differ between treatments at any of the three sites.

At Fishburn, the interaction between treatment and tree class on WUE was significant on two days. These were JD 250 and 299 ( $p = 0.0438$ , and  $p < 0.0001$ , respectively). On both days,

control trees in the largest size class had the greatest WUE. At Mountain Lake, WUE varied significantly by tree class on JD 48 and 145 ( $p = 0.0805$  and  $p = 0.0762$ , respectively). On JD 48, the medium tree size class had the greatest WUE while the largest tree size class had the greatest on JD 145. There was also a significant interaction effect on JD 48 at Mountain Lake ( $p = 0.0882$ ) with treated trees in the medium size class having the greatest WUE. On JD 178, there was a significant treatment effect ( $p = 0.0872$ ), with treated trees having greater WUE than control trees (Figure 3.6b). At Twin Falls, JD 56 and 213 treated trees had greater WUE than control trees ( $p = 0.0916$  and  $p = 0.0309$ , respectively) (Figure 3.6c). Twin Falls also had two days, JD 271 and 293, where control trees had greater WUE than treated trees ( $p = 0.0107$  and  $p = 0.0991$ , respectively).



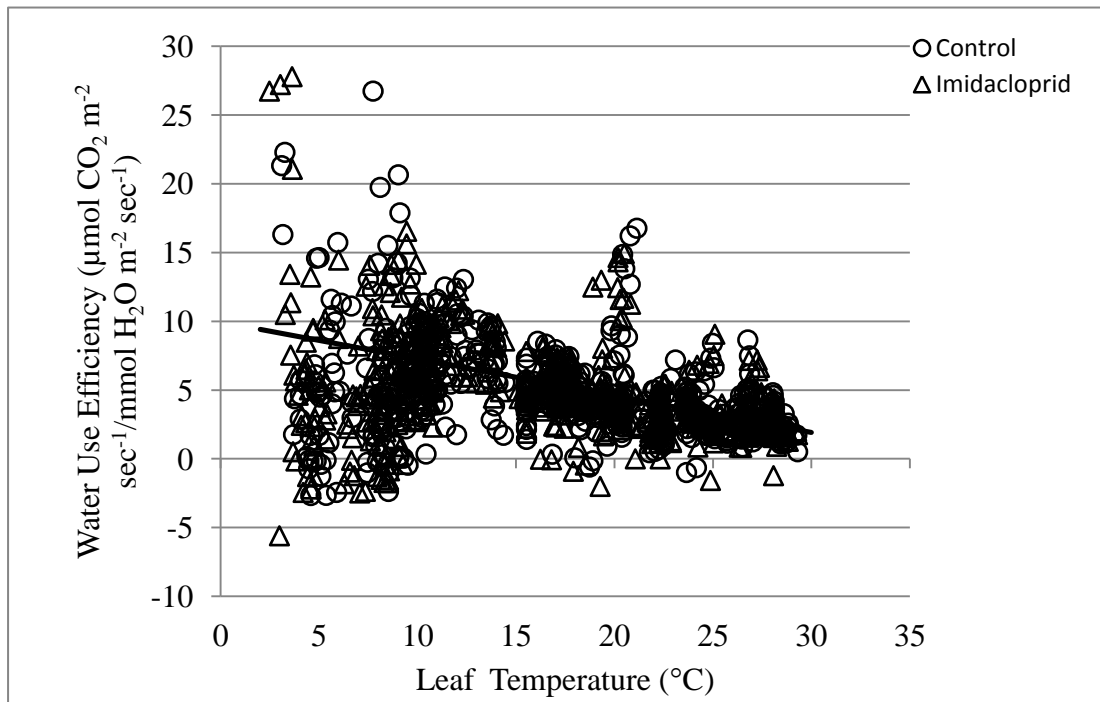
**Figure 3.6 a-c.** Eastern hemlock WUE comparison between imidacloprid treated and untreated trees at Fishburn (a), Mountain Lake (b), and Twin Falls (c) from 2012 to 2013. Points show mean  $\pm$ SE. Single asterisk denotes a difference at  $\alpha = 0.10$  and a double asterisk denotes a difference at  $\alpha = 0.05$ , and a triple asterisk denotes a difference at  $\alpha = 0.01$ .

*Water use efficiency model comparisons*

Since there was no significant treatment effect across all measurement days, only a full model incorporating both treatments was created to explain WUE (Figure 3.7). The parameters included in this model for WUE were temperature of the leaf, relative humidity, soil moisture content, and monthly precipitation to date (Table 3.3).

**Table 3.3.** Water use efficiency parameter estimates for the treatment models, with a total of four significant parameters.

PARAMETER	ESTIMATE	P-VALUE
Intercept	8.894	< 0.0001
Relative Humidity (%)	0.0308	0.0075
Temperature of Leaf (°C)	-0.2680	< 0.0001
Soil Moisture Content (%)	-0.0334	0.0221
Monthly Precip to Date (in)	0.2339	< 0.0001
R <sup>2</sup> = 0.271		



**Figure 3.7.** Eastern hemlock water use efficiency as influenced by leaf temperature with the modeled best fit line. All other variables in the model were held at their seasonal average for calculating the fitted line.



### *Chlorophyll Fluorescence*

Generally there were few treatment effects on chlorophyll fluorescence (Table 3.4). On JD 299 at Fishburn, treated trees had a significantly higher Fv/Fm than control trees ( $p = 0.0057$ ). On JD 243 at Mountain Lake, treated trees had a significantly lower Fv/Fm than control trees ( $p = 0.0019$ ). Both sites showed an overall decrease in Fv/Fm with an increase in JD likely due to decreasing temperatures.

**Table 3.4.** Eastern hemlock chlorophyll fluorescence (Fv/Fm) as affected by imidacloprid treatment at Fishburn and Mountain Lake. Different letters indicate significant differences between treatments within a site ( $p < 0.01$ ).

Julian Date (Fishburn/Mt Lake)	Site			
	Fishburn		Mountain Lake	
	Imidacloprid	Control	Imidacloprid	Control
179/178	0.841±0.004 A	0.841±0.004 A	0.858±0.007 A	0.854±0.007 A
205/214	0.851±0.001 A	0.851±0.001 A	0.853±0.002 A	0.858±0.002 A
250/243	0.846±0.001 A	0.844±0.001 A	0.845±0.003 A	0.859±0.003 B
265/278	0.840±0.002 A	0.841±0.002 A	0.841±0.002 A	0.841±0.002 A
299/300	0.837±0.003 A	0.823±0.003 B	0.796±0.008 A	0.793±0.008 A

### *Bud Break*

Chemical treatment had a significant effect on bud break (Table 3.5). After one year of treatment (2012), Fishburn treated trees had a significantly greater percent bud break relative to control trees (13.37 and 5.78%, respectively), although overall bud break was very low. At Mountain Lake in 2012, bud break was greater than at Fishburn but nearly identical between treated and control trees (46.63 and 46.88%, respectively). After two years of treatment (2013), bud break increased for all trees at Fishburn, but increased to over 88% for treated trees. At

Mountain Lake, bud break of treated trees increased to 58.9% and was significantly greater than control trees which dropped to 24.5% (Table 3.5).

**Table 3.5.** Eastern hemlock percent bud break as affected by imidacloprid treatment at Fishburn and Mountain Lake. Means ( $\pm$  SE) within a year and site followed by different letters differ significantly ( $p < 0.05$ ).

Year	Site			
	Fishburn		Mountain Lake	
	Imidacloprid	Control	Imidacloprid	Control
2012	13.4 $\pm$ 0.032 % A	5.78 $\pm$ 0.011 % B	46.9 $\pm$ 4.60% A	46.6 $\pm$ 4.20 % A
2013	88.7 $\pm$ 2.64 % A	22.5 $\pm$ 2.99 % B	58.9 $\pm$ 5.97 % A	22.7 $\pm$ 4.80 % B

### *Specific Leaf Area*

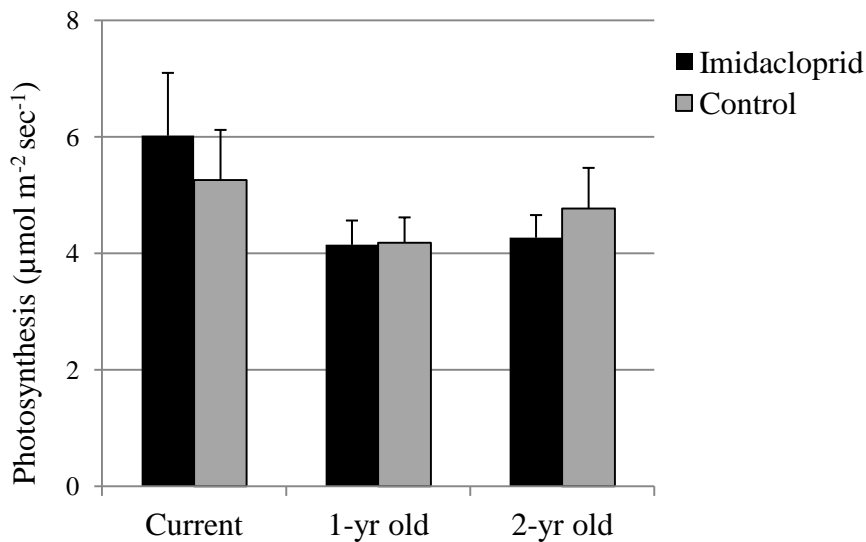
Across all the sites, there were no clear patterns in specific leaf area as a result of chemical treatments (Table 3.6). Some sites showed increases in response to imidacloprid treatment while others showed decreases.

**Table 3.6.** Eastern hemlock specific leaf area as affected by imidacloprid treatment at three sites. Within a site, significant differences are denoted by different letters ( $p < 0.05$ ).

	Site					
	Fishburn		Mountain Lake		Twin Falls	
	Imidacloprid	Control	Imidacloprid	Control	Imidacloprid	Control
SLA (cm/g)	66.9 $\pm$ 0.79 A	71.6 $\pm$ 0.80 B	64.3 $\pm$ 0.89 A	61.7 $\pm$ 0.88 B	64.2 $\pm$ 1.3 A	64.0 $\pm$ 1.3 A

## Needle Age Cohorts

Differences in photosynthetic rates due to the interaction between needle age and chemical treatment were slightly significant ( $p=0.0942$ ), mainly due to the effect of needle age ( $p=0.0566$ ). Current year needles tended to have the highest rates, followed by two-year-old needles and one-year-old needles had the lowest rates. (Figure 3.8).



**Figure 3.8.** Eastern hemlock photosynthetic rates by needle age cohort as affected by imidacloprid treatment at Mountain Lake.

## Discussion

In the year following treatment of eastern hemlock trees, there is some evidence that HWA decreases leaf level photosynthetic rates. However, the change was not dramatic. Photosynthetic rates in imidacloprid treated trees were slightly higher than in control trees. Rates in treated trees tended to be higher at Fishburn and Twin Falls, and although there were few significant differences (seven dates), treated trees were higher on six of them. Further, regression analysis indicated that across all sites, treated trees had photosynthetic rates higher than control trees (Figure 3.2 a-c) and overall rates of treated trees were 6.4 % higher. Fishburn trees had the

worst crown conditions (average score of 2.1 in 2012 and 2.3 in 2013) and had the lowest rates suggesting severely impacted trees may have lower leaf level photosynthetic rates. Mountain Lake had the healthiest crown class (average score of 3.1 in 2012 and 2.9 in 2013) and had photosynthetic rates midway between Twin Falls and Fishburn. These slightly lower rates, on average, at Mountain Lake are likely due to the fact that it is at a higher elevation than the other two sites and thus it is often colder at this site than the others (Figure 3.2b). In fact average temperature when photosynthetic rates were measured at Mountain Lake averaged  $15.2 \pm 0.29$  °C while it averaged  $18.2 \pm 0.42$  °C and  $17.6 \pm 0.28$  °C at Twin Falls and Fishburn, respectively.

Given the only slight change in leaf level photosynthetic rates, other factors must play a role in decline of hemlock in response to HWA. Bud break data suggest that leaf area development may play a large role. Bud break increased significantly at both sites for trees treated with imidacloprid. Webb et al. (2003) also found that even trees in severe decline had the capacity to uptake imidacloprid from the soil and deliver it the foliage in order to kill HWA (Webb, Frank et al. 2003). In that same study, they also found little to no new growth on control trees while treated trees experience significant biomass gains. As trees transported the chemical systemically, adelgid populations decreased and the tree could recover by putting on new growth.

The observed increases in bud break for both treated and control trees at Fishburn may indicate that this stand is in a period of recovery from adelgid infestation. In contrast, the observed decrease in bud break of control trees at Mountain Lake may indicate that this stand is in a period of decline from infestation. These results also suggest that mortality and tree decline caused by HWA are likely due to a decline in leaf area, rather than a reduction in needle physiological capacity. Stadler et al. (2005) found little to no new growth on heavily infested

trees as well as greater needle shed on infested trees during a three month study. Heavily infested trees shed 48 % more needle biomass ( $\text{g/m}^2$ ) compared to moderately infested trees. Heavily infested trees also had shoots that reached a maximum length of only 2 cm compared to uninfested or lightly infested trees with shoots that reached a maximum length of 5 cm. It was found that heavily infested trees had 24,140 one-year-old shoots compared to 139,680 for uninfested trees. While the photosynthetic ability per unit area may not be greatly affected, the loss of needle area causes a reduction in whole tree carbon assimilation that eventually leads to mortality.

Like many past studies, our findings support that conifer carbon assimilation is reduced during the winter months compared to the growing season. In a study of a mixed coniferous stand, including Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco), silver fir (*Abies alba* Mill.), Scots pine (*Pinus sylvestris* L.), and Norway spruce (*Picea abies* L.), Aubinet et al. (2002) found the stands to be a carbon source during the winter months rather than a carbon sink. Years with milder winters allowed a small amount of carbon assimilation, although this carbon gain was insignificant to total annual carbon sequestration. In a study of red spruce (*Picea rubens* Moench), Schaberg et al. (1995) also found reduced photosynthetic rates during the winter months where carbon gain was also associated with temperature. We tended to measure photosynthesis in the winter on more mild days and in fact found positive rates at all sites during winter months. The warmer the day, the higher photosynthetic rates tended to be. At Fishburn, the highest measured rates during the winter months occurred on JD 86, when photosynthesis averaged  $5.26 \pm 0.35$ . On this day, the maximum temperature reached nearly 18 °C. The lowest measured rates during the winter months at Fishburn occurred on JD 349, when photosynthesis averaged  $0.39 \pm 0.11$ . On this day, the maximum temperature only reached 12 °C. The maximum

and minimum temperatures the day before a measurement day are also likely to influence measured rates. The low temperature on JD 85 at Fishburn was only 46 °C, while on JD 348 it was 22 °C.

Some past studies have observed no significant decreases in photosynthetic capacity of conifers during winter months. In a study done by Burkle and Logan (2003), winter photosynthesis was observed in eastern hemlock in both shaded and exposed microsites. Shaded sites actually had higher rates on mild winter days compared to summer rates, although this difference was not statistically significant. It is important to note; however, that photosynthesis was not directly measured in this study, it was estimated from the incident light intensity and PSII efficiency. Estimating photosynthesis in this way causes an over-estimation of the carbon assimilation rate as some of the incident light is reflected by the leaf.

Similar to the results of the Burkle and Logan (2003) study, we found sustained photosynthetic activity during the winter season. Although we purposely biased our study by selecting only warm winter days to measure photosynthesis, this allowed us to see what the maximum winter potential is for carbon gain in eastern hemlock. Future studies may aim to take measurements on cold winter days in order to determine at what temperature photosynthesis reaches zero. Sustained winter photosynthesis coincides with the fact that eastern hemlock is extremely shade tolerant and also with the fact that HWA is active during the winter months. HWA feeding during the winter suggests that its host cells are still active, likely in maintenance respiration.

Chlorophyll fluorescence measurements indicate that HWA is not causing a significant reduction in photosynthetic capacity. The quantum efficiency of Photosystem II was at or near

optimal across most measurement days for both treatments, indicating that photosynthetic performance was not being hindered. Only one day at each site showed a significant difference between treatments and which treatment was higher was inconsistent. The decline in  $F_v/F_m$  observed on the last measurement day at Mountain Lake can be explained by cooler temperatures since the site is at a higher elevation. On that day, the low temperature of approximately 11 °C was reached by 6:00 AM.

Specific Leaf Area shows conflicting results across sites and while there were some significant days at each site, there are no clear trends across all measurement days (Table 3.6). The significantly higher SLA for control trees at Fishburn may indicate that during recovery, trees put out needles with greater area relative to unit weight in order to capture maximal light to drive photosynthesis. Reich et al. (1998) found that, across species and biomes, higher SLA correlated with higher leaf  $N_{\text{mass}}$  within a species and also with a higher WUE. We found similar results, with trees at Fishburn having the highest SLA as well as the greatest WUE among all sites. The higher SLA of control trees may therefore indicate that those needles have higher  $N_{\text{mass}}$  compared to needles of treated trees. This may be a mechanism by which declining trees allocate resources to rebuild their crown, which subsequently allows greater light capture and photosynthate production during infestation. An alternative explanation may be that needles of control trees lack starch from adelgid feeding and thus weigh less than needles of treated trees. Whether the observed relationship between non-treatment and SLA is a cause or effect of infestation remains unknown and is a topic for further investigation.

The results of this extensive leaf level physiological study suggest that HWA is causing tree mortality through a reduction in leaf area as a consequence of HWA feeding. Some evidence of altered leaf level physiological capability of eastern hemlock was observed over the course of

an entire growing season but in comparison to changing leaf area as a result of decreased bud break it seems rather minor. The significant regression models incorporating several environmental parameters suggest that hemlock physiology tracks environmental parameters closely and suggests that hemlock growth may be predictable using process-based models (Zarter, Demmig-Adams et al. 2006).

Several process-based models are already in existence and have been tested for different species across habitats. One such model currently being used in Europe is BALANCE, which uses environmental parameters to predict individual tree growth and development (Rotzer, Leuchner et al. 2012). Another commonly used model is the Physiological Principles in Predicting Growth, or 3PG model. 3PG calculates gross primary production of a stand from environmental parameters including climate and soil data (Landsberg and Waring 1997). Nole et al. (2009) concluded that the 3PG model could accurately calculate stand gross primary productivity across a region by comparing 3PG predictions to actual eddy covariance measurements. One of the most well known models, FOREST-BGC (BioGeochemical Cycles), also allows regional estimations of nutrient cycling within an ecosystem using primarily remote sensing data (Running and Coughlan 1988; Running and Gower 1991). These are only a few of the many process-based models available for ecological studies. Our current study may allow customization of current models to more accurately predict carbon assimilation in hemlock stands.



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## **Chapter 4: Synthesis**

Our results support the idea that decreased leaf area, not decreased physiological ability, is leading to the widespread mortality of eastern hemlock as a result of HWA. As trees initially become infested, their leaf area decreases and thus their overall carbon assimilation. Eventually, the tree loses enough leaf area to a point that it is no longer a sufficient food source for the adelgid and infestation may decline. This decrease in adelgid levels allows the subsequent recovery of the tree. As the tree attempts to rebuild its crown by putting out new growth, it often becomes reinfested with adelgid as the tree becomes a preferable food source once again. This cyclic infestation continues until the tree eventually succumbs to the adelgid.

These results therefore support the silvicultural practice of thinning of infested stands in order to allow the remaining impacted trees to rebuild their crown at a faster rate as more resources become available. Since healthier hemlocks have a greater ability to withstand HWA infestation, reallocating fixed resources within a stand by thinning gives remaining hemlocks a better chance at survival. It is important to note, however, that this reallocation of resources may alter foliar nutrient contents and actually make hemlock needles more palatable to HWA, increasing infestation levels on a tree. Future studies may examine this dynamic and whether the benefits of thinning outweigh the potential costs. Other factors to consider in how well a particular tree will respond to crown release are prior stocking density and crown position, with suppressed crowns experiencing the greatest benefit from thinning. Salvage harvesting can also be used before infestation, along with chemical treatment of particularly valuable trees. (Fajvan 2007).

Future studies may look at long term consequences of treatment with imidacloprid by studying hemlock physiology over the course of multiple growing seasons. This would allow a

more detailed investigation of tree physiology through infestation to eventual recovery.

Investigation into hemlock water potential and foliar nutrient content as a result of adelgid infestation could also be examined in order to determine if these variables are altered as a result of infestation.

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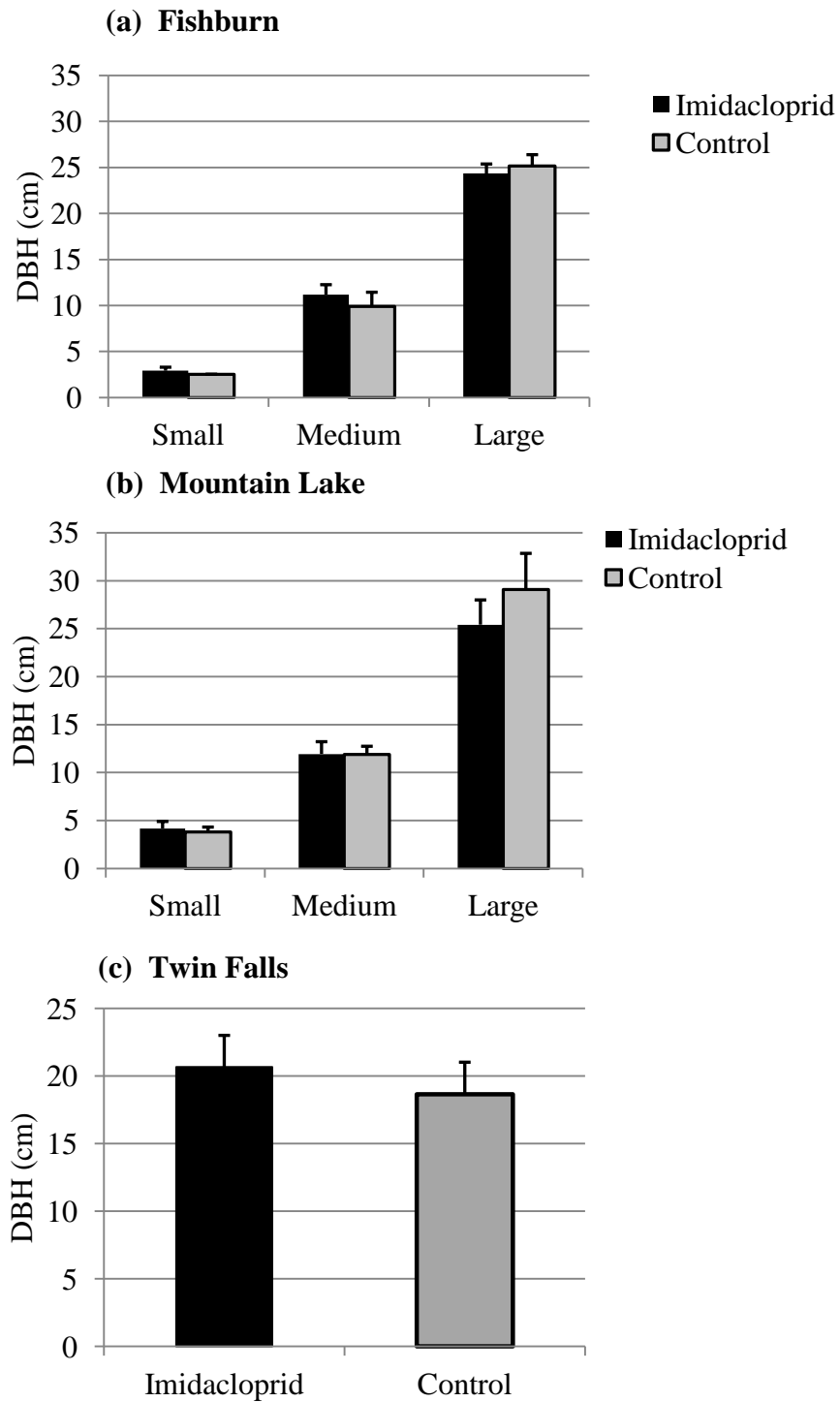
## **Appendix A: Methods**

All plots were on similar topographic positions and soils. The soil series at site one is mapped as the Berks-Clymer complex and the Berks-Wiekert series (Soil Survey Staff 2012). Site two consists of the Lily-Bailegap complex on 2-15% and 15-35% slopes (Soil Survey Staff 2012). The third site is the Berks-Pineville series on 35-70% slopes and the Pineville-Buchanan series on 3-15% slopes (Soil Survey Staff 2012). We were able to determine that preparing branch segments as we did by removing surrounding foliage did not cause any additional harm or stress to the plant. Several months after preparing a branch segment for measurement, it was found that the branch segment had flushed (**Figure A.1**).



**Figure A.1.** Visible flushing of an eastern hemlock branch segment after it had been prepared for measurement.

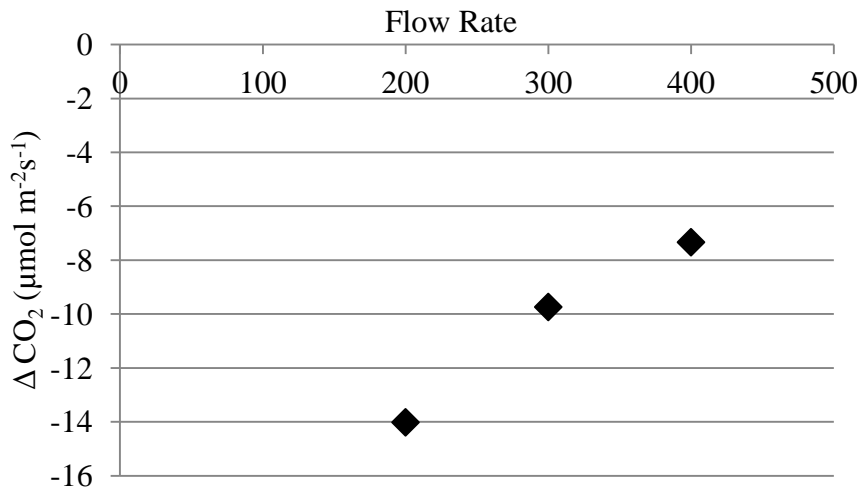
**Appendix B: Eastern hemlock diameter at breast height distribution at three sites**



**Figure B.1.** Average eastern hemlock tree size distributions at the three study site locations (a) Fishburn, (b) Mountain Lake, (c) Twin Falls. No tree size classes were investigated at Twin Falls.

### Appendix C: Effect of flow rate on the differential between the reference and sample gas stream using hemlock needles in the LiCor 6400

Chamber flow rate was determined to give the maximum differential between reference and sample CO<sub>2</sub> at a rate of 200 (Figure C.1). Flow rate was thus set to 200 for the duration of the study in order to achieve this maximal differential. This was important since on many days, carbon assimilation rates were extremely low. Having a higher differential allows the machine to better detect these low photosynthetic rates against background variability.



**Figure C.1.** Differential in sample and reference CO<sub>2</sub> concentrations as a function of the flow rate.