

Fitness and Physiology of the Hemlock Woolly Adelgid, *Adelges tsugae*, in Relation to the Health of the Eastern Hemlock, *Tsuga canadensis*

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Sternorrhyncha: Adelgidae) is a small invasive insect that frequently causes hemlock (*Tsugae* spp.) mortality in the eastern United States. Studies have shown that once healthy hemlocks become infested by the adelgid, nutrients are depleted from the tree, leading to both tree decline and a reduction of the adelgid population. Since *A. tsugae* is dependent on hemlock for nutrients, feeding on trees in poor health may affect the insect's ability to obtain necessary nutrients and consequently affect their population and physiological health. A cluster analysis, based on quantitative and qualitative tree health measurements, grouped sample trees into categories of lightly and moderately impacted trees. The *A. tsugae* population health on each tree was determined by measuring insect density, survival from aestivation, and peak fecundity. *A. tsugae* physiological health was determined similarly by measuring insect biomass, total carbon, carbohydrate, total nitrogen, and amino nitrogen. *A. tsugae* from moderately impacted trees exhibited significantly greater fecundity; however, *A. tsugae* from lightly impacted hemlocks contained significantly greater levels of carbohydrates, total nitrogen, and amino nitrogen. All *A. tsugae* physiological parameters increased significantly over time as the insects matured and reproduced regardless of tree health classification. While the results of the physiological analysis generally support our hypothesis that *A. tsugae* on lightly impacted trees are healthier than those on moderately impacted trees, this was not reflected in the population fitness measurements of the insects. Further examination of *A. tsugae* egg health may elucidate this apparent contradiction.

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Chapter 1

Introduction to Hemlock Woolly Adelgid and Eastern Hemlock

1.1 The Hemlock Woolly Adelgid

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Sternorrhyncha: Adelgidae) is a small exotic insect (1.3-1.5 mm long) that has caused hemlock (*Tsuga* spp.) mortality throughout North American eastern forests. *A. tsugae* can decimate both the Carolina hemlock (*Tsuga caroliniana* Engelm.) and eastern hemlock (*T. canadensis* (L.) Carriere) in ornamental and forest settings (McClure 1987). Eastern hemlock is particularly susceptible to this pest; infested hemlocks survive from four to ten years in declining health (McClure 1989, McClure 1991a, Cheah and McClure 2000).

Adelges tsugae are endemic to China, Taiwan, Japan, and western North America (Havill and Footitt 2007). In Japan, *A. tsugae* inhabits *T. diversifolia* Masters and *T. sieboldii* Carriere (McClure 1987). These native *A. tsugae* populations are greatly regulated by host resistance with assistance from natural enemies (McClure and Cheah 1999). The *A. tsugae* from western North America were derived from the Japanese lineage approximately 6 million years ago (mya) (Havill et al. 2006). They appear to be endemic and to have higher genetic variation than *A. tsugae* found in eastern North America (Havill et al. 2006). *A. tsugae* in western North America inhabits *T. heterophylla* (Raf.) Sarg., which it can weaken and occasionally kill in ornamental settings. However, *A. tsugae* has not caused widespread *T. heterophylla* death (Keen 1938, Furniss and Carolin 1977, Havill and Footitt 2007). There are no adelgid species associated with *Tsuga* spp. endemic to eastern North America (Havill and Footitt 2007). The introduced *A. tsugae* present throughout that area are from a population native to southern Japan (Havill et al. 2006).

The lack of co-evolved tree resistance and specialized natural enemy complexes with the *A. tsugae* and the eastern hemlocks system is likely the reason introduced *A. tsugae* have become a serious pest in this region (Havill et al. 2006, Havill and Footitt 2007). In the early 1950s, *A. tsugae* were first reported in Richmond, VA (McClure 1989). Since that time, it has spread throughout about 50% of the native range of eastern and Carolina hemlock (United States 2011). This spread has been facilitated by wind, birds, forest-dwelling mammals, and humans (McClure 1990). Since *A. tsugae* is native to southern Japan (Osaka region), it may be limited by cold temperatures in northern climates (Parker et al. 1998, 1999, Parker et al. 2002).

Hemlock trees cover 7.7 million hectares of forest in the eastern United States (Morin et al. 2011). They are a foundation species in eastern hardwood forests and provide moist, shady habitats for a variety of plants and animals (Daley et al. 2007). Hemlock is also a popular ornamental tree and is frequently used in landscaping (Lagalante et al. 2007). *A. tsugae* infestation of hemlock causes defoliation and an increase of light to the understory, changing the forest ecology (Jenkins 1999). Hemlock mortality also influences nutrient cycling rates in eastern forests by reducing surface water quality and forest productivity (Yorks et al. 2000).

Eastern forests have undergone many changes as hemlocks continue to deteriorate (Stadler 2006). Increased light availability to the understory has led to increases in seedling regeneration in stands affected by *A. tsugae*-infested hemlock (Jenkins 1999). Net nitrogen mineralization, nitrification, and nitrogen turnover increased at sites experiencing hemlock mortality (Jenkins 1999). Such changes in nutrient cycling can lead to nutrient loss, resulting in reduction in site nutrient capital and a decline in forest productivity (Jenkins 1999, Yorks et al. 2000).

Black birch (*Betula lenta* L.) is profiting from hemlock decline and becoming the dominate northeastern hardwood forest species in stands decimated by *A. tsugae* (Stadler et al. 2005). This shift from a coniferous to a deciduous dominant tree species may decrease forest water balance during the peak growing season. A separate study, comparing northern red oak (*Quercus rubra* L.) and hemlock transpiration rates demonstrates a similar effect on forest water (Hadley et al. 2008). In southeastern forests, other broad leaf trees may become the dominate hardwood species; however, they will not be able to fill the ecohydrological niche of hemlock (Ford and Vose 2007). From these reports spreading *A. tsugae* infestations and continuing hemlock mortality can cause significant changes to the forest ecosystems of the eastern United States.

1.1.1 *Adelges tsugae* Lifecycle

In Japan, *A. tsugae* alternates hosts using tigertail spruce, *Picea torano* (K. Koch) Koehne as a primary host and *Tsuga* spp. as the secondary hosts (Havill and Footitt 2007). It appears that *A. tsugae* cannot reproduce on North American *Picea* spp. and has reverted to living solely asexually on its secondary host (Rohfritsch 1990, Havill and Footitt 2007).

The asexual lifecycle of *A. tsugae* is polymorphic and parthenogenic, developing through two generations annually (McClure 1989). The life cycle of *A. tsugae* in the northeast has been documented by McClure (McClure 1987, 1989, McClure 1991a, McClure 1992). In June, the females from the rapidly developing and brief progrediens generation oviposit about twenty-five eggs at the base of hemlock needles (McClure and Cheah 1999). These eggs develop into the sistens generation. The sistens eggs generally hatch in July; the emerging crawlers are mobile and search for a nutrient-rich needle cushion. From August to mid-October or November, the first instar sistens generation undergoes an aestivating period. After breaking aestivation, they begin feeding and developing through four instars during winter. Adults mature and lay between

50-175 eggs in March and April. The eggs hatch as either progrediens (asexual *A. tsugae*) or sexuparae (sexual *A. tsugae*). Sexuparae adults are winged and do not feed on hemlocks but disperse to search for suitable spruce species to complete a holocyclic life cycle (McClure 1989). However the sexuparae cannot survive on the spruce available in eastern North American and will die (McClure 1989).

1.1.2 *Adelges tsugae* Aestivation and Feeding

In order to survive extreme weather conditions, an organism may enter diapause, a neurohormonally mediated state of low metabolic activity. When this state occurs during summer, it is referred to as aestivation (Tauber and Tauber 1987, Salom et al. 2001). In many organisms, this period of inactivation is cued by photoperiod, temperature, and sometimes diet (Beck 1982). Aestivation occurs during the adelgid sistens generation from approximately July to mid-October as the *A. tsugae* sistens crawlers find an acceptable feeding site on the hemlock needle cushion and then enter aestivation (Parker et al. 1998, Eisenback et al. 2010). Aestivation in *A. tsugae* is maternally activated when temperatures average $> 14.5^{\circ}\text{C}$ during early progrediens nymphal instar development (Salom et al. 2001).

A. tsugae is considered a pest to eastern hemlocks particularly because it depletes carbohydrate stores in the tree's xylem ray parenchyma cells (Shields et al. 1996, Schwartzberg and Montgomery 2010). *A. tsugae* have a simple gut that is designed to filter out particulate matter and to prefer nutrient-dense particulate matter over liquid solutions (Cohen et al. 2008). To begin feeding, a crawler settles at the base of a hemlock needle and inserts its lengthy stylet bundle into the needle cushion. The stylet bundle is composed of several probing stylets surrounded by sheaths that probably protect the feeding *A. tsugae* from plant defenses (Young et al. 1995). The stylet is inserted proximal to the abscission zone of the needle and intracellularly through the

plant tissue. The feeding stylet will emerge from the protecting sheaths and continue to follow an intercellular path into tree tissue (Young et al. 1995, Shields et al. 1996).

Elevated levels of either myrcene or germacrene D have been detected during cushion probing and may indicate the presence of nutrient-rich new growth to *A. tsugae* (Lagalante et al. 2007). It was previously thought that the insect's stylet may produce a toxic substance, or that *A. tsugae* feeding may expose the needle cushion to infection (Young et al. 1995, Shields et al. 1996). *A. tsugae* initiates a defensive hypersensitive response in its host; it is possible that certain components of *A. tsugae* saliva may be toxic at high levels (i.e. during *A. tsugae* infestation) (Radville et al. 2011). Recent studies on the presence of trypsin-like enzyme activity in *A. tsugae*, speculate on its extra-oral use to digest structural proteins of the plant (Oten et al. 2012). A complete understanding of *A. tsugae* saliva toxicity and the hemlock's response to feeding is still unclear.

1.1.3 *Adelges tsugae* Mortality Factors

Adelges tsugae populations are susceptible to several mortality factors. Intra- and inter-specific competition, extreme low and high temperatures, native predators, and the health of the host hemlock are natural adelgid population-limiting factors. Native and exotic predators and insecticides are additional measures that have been developed to further decrease adelgid populations in impacted forests and ornamental tree settings (McClure and Cheah 2002). Intra-specific competition leads to a density-dependent feedback mechanism that is discussed later (Section 1.2.2). The elongate hemlock scale (*Fiorinia externa* Ferris) and circular hemlock scale (*Nuculaspis tsugae* Marlatt) (Homoptera: Diaspididae) also feed on hemlock, leading to interspecific competition. In some locations, *F. externa* out-competes *A. tsugae* by feeding on the hemlock and limiting the *A. tsugae* population size (Preisser et al. 2008).

Weather has an impact on the distribution and abundance of invading herbivorous insect populations (McClure 1989). Comparisons of *A. tsugae* overwintering survival between populations in northern and southern locations show that harsh winter conditions lead to low overwintering survival (Skinner et al. 2003, Butin et al. 2005, Butin et al. 2007). As *A. tsugae* spreads throughout New England, the insect may develop cold hardiness (Parker et al. 1999, Butin et al. 2005). Adelgid resistance to cold temperatures is dependent on the time of year as well as the geographical location (Parker et al. 2002). Complete *A. tsugae* mortality generally occurs below -35°C. High mortality reduces the sistens population, and fewer progrediens eggs are laid, allowing the hemlock an opportunity to produce new growth in the spring that will not be infested by *A. tsugae* (Skinner et al. 2003). However, such extreme winter temperature is not a dependable occurrence for *A. tsugae* control, and southern areas do not experience such cold weather conditions (Parker et al. 1999, Gouli et al. 2000).

Asian and North American populations of *A. tsugae* contain up to three species of symbiotic bacteria belonging to the *Gammaproteobacteria* that are transmitted maternally through eggs (Dohlen et al. 2013). These bacteria are found in the hemocele and in specialized cells known as mycetocytes or bacteriocytes. *A. tsugae* from infested hemlock seedlings that were treated with the antibiotic rifampicin experienced inhibited development and began dying by the second instar (Shields and Hirth 2005). Continued examination of bacterial endosymbionts in *A. tsugae* may help develop a form of biological control against this insect.

There are several native predators of *A. tsugae* in eastern forests, but the insect has no known hymenopteran parasitoids (Havill and Footitt 2007). Some fly (Diptera: Syrphidae) and lacewing (Neuroptera: Chrysopidae and Hemerobiidae) species will feed on *A. tsugae*. *Laricobius rubidus* LeConte (Coleoptera: Derodontidae), a predator to pine bark adelgid (*Pineus strobi* Hartig) is

commonly found feeding on *A. tsugae* and can complete development solely on this insect (Zilahi-Balogh et al. 2005). While these native predators may successfully attack the adelgid, they fail to control its populations. (McClure and Cheah 2002). Several exotic predatory beetles have been examined as potential biocontrol agents for *A. tsugae*. Those released in large scale efforts include *Sasajiscymnus (Pseudoscymnus) tsugae* Sasaji and McClure and *Laricobius nigrinus* Fender (Cheah 2011, Mausel et al. 2011).

The neonicotinoid imidacloprid was developed in 1985 and is effective against *A. tsugae* (LC₅₀ 300 ppb-20 days). Imidacloprid binds with insect postsynaptic nicotinic acetylcholine receptors (nAChRs) causing permanent activation, resulting in stimulation along the nerve synapse, and leading to neurotoxicity poisoning symptoms and death (Eisenback et al. 2010). Imidacloprid can translocate throughout a treated tree to the *A. tsugae* feeding location at the needles. *A. tsugae*-fed *L. nigrinus* and *S. tsugae* are both susceptible to the insecticide. Both beetles experienced intoxication symptoms; although, *L. nigrinus* mortality was greater on imidacloprid-treated branches than those of *S. tsugae* (Eisenback et al. 2010). Various *A. tsugae* control options have a degree of effectiveness against this pest, although they are generally location- and environment-dependent.

1.2 Plant and Insect Herbivore Interactions

While herbivorous insects cause plant stress, there is no unequivocal measure of stress. It is particularly difficult to relate the stress level in experimental situations to that in naturally stressed plants (Larsson 1989). In some systems, plant stress and reduced growth is deleterious for herbivores, but in other systems, suboptimal growth conditions may favor herbivores (Price 1991). An early hypothesis regarding the relationship between insect herbivores and their host plants was defined as the Plant Stress Hypothesis (PSH). This hypothesis proposed that plants

become more susceptible to herbivores when under stress, due to reduced protein synthesis in the plant, increased amino acids in tissues, and reduced secondary metabolite production for plant defense (White 1974, McClure 1980, Cornelissen et al. 2008). Stressed plants tend to exhibit elevated foliar nitrogen and may become a particularly nutritious food source for nitrogen-limited organisms (White 1974, Rhoades 1979, Cornelissen et al. 2008).

Price (1991) introduced and described the Plant Vigor Hypothesis (PVH) which suggests that herbivore abundance and fitness will be optimal on vigorous plants or plant sections. Cornelissen et al. (2008) divided insect herbivores into feeding guilds (i.e. gall-formers, chewers, sap suckers, etc.) to compare their relationship with their host plant and found negative effects of plant stress on sap-feeding insect performance. However, in the same study, insect preferential colonization of vigorous plants or plant modules did not always result in higher herbivore survivorship. It is clear that both hypotheses are not the strict alternative of each other, but are more likely to be relevant to two ends of a spectrum of plant-herbivore interactions.

Plants provide all the nutrients insect herbivores require, but the absolute amounts and ratios of these nutrients can be variable. Since plant nutrient variation is the result of genotypic differences and environmental conditions, both generalist and specialist insect herbivores experience a heterogeneous nutritional landscape (Behmer 2009). The physiological status of a host plant is particularly important to the success of specialist herbivore insects (McClure 1980). Plant stress should be related to herbivorous insect performance. Stressed plants exhibit loss of vigor, characterized by decreased cell growth, leading to smaller plant structures such as buds, leaves and shoots; however, plant stress may lead to increased cone production, epicormic branching, and the movement of resources to alternative locations in the plant to increase plant survival (Larsson 1989).

1.2.1 Hemlock Response to *A. tsugae* Herbivory

Although the plant response to herbivore damage is generally confined to tissue near the feeding site, phytotoxic herbivore feeding can induce a stress response throughout the plant. This exploitation may be especially harmful for hosts that lack a co-evolutionary history with this herbivore. Many Adelgidae are specialized feeders that have evolved ways to exploit the phytochemistry and metabolic pathways of their native hosts, and can increase local plant nutrient concentrations while decreasing their defenses (Miller-Pierce et al. 2010). While this has not been fully documented in the *A. tsugae* and hemlock system, *A. tsugae* may induce a type of phytotoxic response in hemlock (Miller-Pierce et al. 2010, Radville et al. 2011). The hypersensitivity of the host may initially induce a more favorable nutritional state for the *A. tsugae*, but eventually lead to reduced growth, decline in vigor, and eventual host death (Schwartzberg and Montgomery 2010).

1.2.1.1 Chemical Response

Eastern hemlocks synthesize a complex mixture of terpenoids and have adapted their terpenoid chemistry against defoliators, leaving them susceptible to non-native sucking pests such as *A. tsugae*. Minor terpenoids from the hemlock profile appear to be strongly linked to speciation in *Tsuga* spp. whereas the major terpenoids in eastern and Carolina hemlock are associated with their susceptibility to *A. tsugae* (Lagalante et al. 2007).

Hemlock foliar chemistry is strongly associated with *A. tsugae* and hemlock interactions (Pontius et al. 2002). Terpenoid compounds in plants are often toxic or repellent to insects partly due to acetylcholinesterase inhibitors at the neuromuscular junction (Lagalante et al. 2006). Most of the allelochemicals identified in hemlock xylem fluid are toxic alkaloids that limit feeding to insect species that have specific pathways for detoxifying these compounds (Brodbeck et al. 1990).

Hemlock terpenoids are less variable in mature needle cushions than in young tissue. Since *A. tsugae* enters a nonfeeding diapause during the late spring and summer, it avoids the variable levels of terpenoids in the immature needle cushion and needles. Foliage from infested hemlocks tends to release monoterpenes at a higher rate, often more than double that of foliage from uninfested trees (Broeckling and Salom 2003). Pontius et al. (2002) concluded that hemlock decline may occur in part from a combination of two interrelated factors: 1) hemlock foliar chemistry (an indicator of nutritional quality) influencing *A. tsugae* population densities which ultimately result in tree mortality, and 2) the stress of *A. tsugae* infestation changes hemlock defense physiology, which results in changes to the foliar chemistry.

1.2.1.2 Physical Response

There is a substantial amount of evidence indicating hemlocks become stressed in response to adelgid infestation. Visible physical decline of the tree begins at 30% infestation of shoots (Fidgen et al. 2006). Crown transparency at 60% adelgid infestation marks the beginning of tree mortality (Mayer et al. 2002). Plant growth on infested trees was reduced by two years compared to uninfested plants. Declining trees came under increased water stress and are also more susceptible to other infections (McClure 1991a, Young et al. 1995, Walker-Lane 2009). Adelgid feeding can induce hemlock water stress which will result in increased false ring formation (Gonda-King et al. 2012).

Individual hemlock trees and adjacent stands often respond differently to *A. tsugae* attack (Sivaramakrishnan and Berlyn 1999). In general, *A. tsugae* infestation causes needle desiccation and loss, bud mortality, and a reduction of new growth (Eisenback et al. 2010). Infested trees produce less new foliage and shed relatively more needles than uninfested trees (Stadler et al. 2005). It is possible that *A. tsugae* causes subtle changes in needle development, as nitrogen,

calcium, potassium, and manganese all increased in infested hemlocks. Specifically, the nitrogen content of needles was found to be 20 - 40% higher in infested trees (Stadler et al. 2005).

1.2.1.3 Nutrient Allocation

Insect feeding can alter nutrient allocation within a plant. Herbivore-induced stress increases the free amino acid pools available in the plant and can lead to insect outbreaks (Brodbeck et al. 1990, Miller-Pierce et al. 2010). Hemlock xylem ray parenchyma cells store starch and transport nutrients between the xylem vessels and the phloem (Schwartzberg and Montgomery 2010). Xylem fluid is generally greater than 98% water with the majority of solutes consisting of amino and organic acids (Brodbeck et al. 1990). Also, the feeding mechanism of many adelgids may cause a disadvantageous “sink” dynamic in which the insect removes solutes and nutrients from developing bud tissue faster than it can be replaced (Brodbeck et al. 1990).

Hemlock health and adelgid infestation are affected by tree macromolecule composition. The presence of *A. tsugae* reduces hemlock growth and percent nitrogen during the first year of infestation (Miller-Pierce et al. 2010). However infested branches exhibit higher phosphorus, calcium, nitrogen, and potassium concentrations. Resistant hemlocks contain significantly lower levels of nitrogen and higher concentrations of phosphorous, lignin, and aluminum (Pontius et al. 2006).

1.2.2 Host Stress Effect on the *A. tsugae* Population

Due to its primarily sessile lifestyle, *A. tsugae* health is completely dependent on the health of the host tree. Similarly, hemlock health is affected by the *A. tsugae* population size. This leads to a density-dependent relationship between alternating host and insect health. McClure (1991a) found that less than four individual *A. tsugae* per 20 mm² of branch would inhibit new growth on

branchlet tips. The hemlock will become stressed and will be unable to support *A. tsugae* feeding pressure indefinitely. When the hemlock nutrient pools are depleted, the *A. tsugae* population will decline and as this feeding stress decreases, the tree will recover and produce more new growth. However, this will enable *A. tsugae* to take advantage of a new nutrient sink and the insect population will again increase, and the cycle between tree health and *A. tsugae* population size will continue. About every 4 years, *A. tsugae* cycles through bimodal peaks of abundance indicative of this density-dependent feedback mechanism (McClure 1991a, Eisenback et al. 2010). This cycle can continue for up to ten years, placing the tree in a weakened state and result in mortality (McClure 1991a).

1.3 Insect Nutrition

The interactions between insect life histories and dynamics of the reproductive nutrient/energy budget are complex. Since many insects feed as immatures and as adults, reproductive effort at any given age will involve a combination of larval- and adult-derived nutrition (Boggs 1981).

The change of reproductive reserves over the lifetime of an insect are dependent on the patterns of intake and reproductive output of nutrients, as well as body ageing due to life processes (Boggs 1981).

Insects can control their total nutrient uptake by simultaneously regulating multiple nutrients (Behmer 2009). It is unlikely that insect herbivores would actively regulate the intake of all nutrients, since doing so would demand massive neurological effort and would be costly. Rather, nutrients that are probably most effectively regulated are those that cannot be biosynthesized *de novo* (i.e. sterols, vitamins, and many amino acids). Additionally, carbohydrates are also often considered a limiting nutritional component. However since *A. tsugae* feeds on the carbohydrate

stores of the hemlock, it is possible that carbohydrates are present in excess (Behmer 2009, Schwartzberg and Montgomery 2010).

General insect nutrition has been covered in several reviews by multiple researchers (Lipke and Fraenkel 1956, House 1962, Dadd 1973, Nation 2002). Specifically, three broad components of insect nutrition, total carbohydrates, total nitrogen, and amino nitrogen (proteins, soluble peptides, amino acids, ammonia and various amines) are discussed below (Sections 1.3.1, 1.3.2, and 1.3.3).

1.3.1 Carbohydrates

While plant phloem contains high concentrations of both amino acids and carbohydrates, carbohydrate levels are low in xylem tissue. However, the xylem ray parenchyma cells serve as the carbohydrate stores of the hemlock (Brodbeck et al. 1990, Wäckers 2000). The analysis of carbohydrate levels in insect hemolymph has formed the basis of numerous physiological and metabolic studies (Mullins 1985, Fell 1990). In general, insect hemolymph has low sugar levels (Wyatt 1957, Mullins 1985). The principal blood sugar of most insects is the non-reducing disaccharide, trehalose (Wyatt 1957, Mullins 1985). Carbohydrates are not only a major component of insect metabolism but are also used in exoskeleton formation and egg production (Arrese and Soulages 2010). The presence of different sugars usually reflects a degree of insect feeding specialization (Wyatt 1957, Mullins 1985, Fell 1990).

1.3.2 Total Nitrogen

In order to estimate any animal's nutritional budget, the amount and composition of foods consumed and the resultant waste eliminated must be quantified. In order to maintain these budgets, animals regulate the uptake of their remaining nutrient requirements. (Studier and

Sevick 1992). A common plant metabolic response to insect herbivory stress may include the breakdown and mobilization of soluble nitrogen away from stressed tissues, the reduction of protein synthesis, and the increase of amino acids in these tissues. Feeding insects will encounter a readily available supply of nitrogenous materials in their food, resulting in an increase in insect abundance (White 1974, Price 1991, Meloni et al. 2012). However, tree-feeding insects may be affected by nitrogen variability in the xylem, phloem, and other plant tissues, as these nutritious sinks are depleted over time. In particular, insect populations dependent on sap-feeding will decline (Brodbeck et al. 1990, Cornelissen et al. 2008). Insect nitrogen content variation may be directly traceable to differences in dietary nitrogen. As a result, dietary nitrogen availability may directly influence insect nitrogen content. Insects consuming nitrogen-poor diets may have low total nitrogen content available for their metabolic processes (Fagan et al. 2002).

There is some experimental evidence that availability of host nitrogen influences *A. tsugae* health. Hemlocks treated with nitrogen fertilizer supported an increased *A. tsugae* population, and nymph and egg survival also doubled (McClure 1991b). During a two-year study of nitrogen fertilization of infested hemlocks, *A. tsugae* reduced tree growth and caused a significant reduction in percent tree nitrogen during the first year. During the second year, *A. tsugae* continued to reduce hemlock growth, but the percent nitrogen remained the same. At this point, nutrients may have moved to plant sinks or may have been undetectable due to a reduced photosynthesis rate caused by the loss of needles. (Miller-Pierce et al. 2010). From these studies, it is likely that nitrogen is a limiting resource for *A. tsugae*, and while initial tree stress may encourage high adelgid densities, continual stress on the tree will cause negative effects to their populations (McClure 1991a, Cornelissen et al. 2008).

1.3.3 Amino Nitrogen

Proteins are an important component of living organisms, and amino nitrogen (proteins, soluble peptides, amino acids, ammonia and various amines) often can be limiting nutrients for some herbivorous insects (Schroeder 1986, McMurry 2008, Haq et al. 2010). Amino acids contained in xylem fluid are a valuable nutrient source for insects. Increased pools of free amino acids during host stress, may induce outbreaks of insects, including *A. tsugae* (Brodbeck et al. 1990, Shields et al. 1996, Schwartzberg and Montgomery 2010). High concentrations of amino acids in host plants was associated with high levels of feeding leafhoppers, *Homalodisca coagulata* (Brodbeck et al. 1990). Nitrogen and protein are essential for egg production in female adult insects (Brodbeck et al. 1990).

1.4 Justification

In the *A. tsugae* and eastern hemlock system, all trees infested by *A. tsugae* are under stress. However, individual trees exhibit vastly different levels of decline and *A. tsugae* populations (McClure 1991). When considering the density-dependent feedback cycle prevalent in *A. tsugae*-impacted hemlock, it is possible that *A. tsugae* populations and physiological health are affected by the nutrients available from their host trees, which in turn, are affected by the depletion of nutrients caused by the adelgid. Initial studies examining the relationship between *A. tsugae* and hemlock have focused primarily on assessing *A. tsugae* population response in terms of their density (Broeckling and Salom 2003; Lagalante et al. 2006). There is little information on *A. tsugae* population fitness and physiological composition with regard to the health status of their host. This information should help us better understand the nature of *A. tsugae* population changes over time in lightly and moderately impacted hemlocks.

1.5 Research Objectives

The overall hypothesis explored in this research is that the health of the trees impacts *A. tsugae* population density, survivorship, fecundity, and physiological composition of the adelgids.

Specific experiments were conducted to evaluate:

1. *A. tsugae* population health based on insect density, survival from aestivation, and fecundity on lightly and moderately impacted hemlock trees.
2. *A. tsugae* physiological health based on insect biomass, total carbon, carbohydrates, total nitrogen, and amino nitrogen from lightly and moderately impacted hemlock trees.

Chapter 2

The Relationship between Eastern Hemlock and Hemlock Woolly Adelgid Populations

Abstract

The hemlock woolly adelgid is a small invasive insect that settles at the base of hemlock needles (*Tsugae* spp.). Studies have shown that when hemlocks become infested by the *A. tsugae* and are depleted of nutrients, a subsequent reduction of the *A. tsugae* population will follow. It has been observed that hemlocks will make a partial health recovery, only to undergo re-colonization by the *A. tsugae* the following season. Since *A. tsugae* is dependent on hemlock for nutrients, feeding on trees in poor health may affect the insect's ability to obtain necessary nutrients and consequently affect their population and reproduction. Thirty-two hemlock trees were selected in both 2011 and 2012. The combined cohort of trees was classified into two tree health categories based on the combined factors of a Hemlock Health Index and mean new shoot length (cm). The *A. tsugae* population fitness levels collected from each tree was the sistens density, survival from aestivation, and fecundity. There was significantly greater *A. tsugae* density and survival from aestivation on current year growth than on previous year growth ($p < 0.05$). Moderately impacted hemlocks had similar *A. tsugae* density and survival but significantly greater fecundity than on lightly impacted hemlocks.

2.1 Introduction

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Sternorrhyncha: Adelgidae) is a small exotic insect (1.3-1.5 mm long) that has caused hemlock (*Tsuga* spp.) mortality throughout eastern North America. This insect can decimate both the Carolina hemlock (*T. caroliniana* Engelmann) and eastern hemlock (*T. canadensis* (L.) Carriere) in ornamental and forest settings (McClure 1987). Eastern hemlock is particularly susceptible to this pest; infested

hemlocks survive from four to more than 10 years in declining health eventually resulting in tree mortality (McClure 1989, Cheah and McClure 2000, Eschtruth et al. 2013)

Individual hemlock trees and adjacent stands often respond differently to *A. tsugae* attack (Sivaramakrishnan and Berlyn 1999). In general, *A. tsugae* infestation causes needle desiccation and loss, bud mortality, and a reduction of new growth. The presence of less than four individual *A. tsugae* per 20 mm² per shoot are enough to inhibit new growth (McClure 1991a, Stadler et al. 2005, Eisenback et al. 2010). Visible physical decline of the tree begins at 30% infestation of shoots while crown transparency at 60% adelgid infestation marks the beginning of tree mortality (Mayer et al. 2002, Fidgen et al. 2006). New shoot growth is reduced on infested trees; additionally, such trees are under increased water stress (McClure 1991a, Young et al. 1995, Walker-Lane 2009).

Many Adelgidae are specialized feeders and have evolved ways to exploit their native hosts by increasing local plant nutrient concentrations while decreasing their defenses (Miller-Pierce et al. 2010). While this has not been documented with the *A. tsugae* and hemlock system, *A. tsugae* may induce a type of phytotoxic response in hemlock (Miller-Pierce et al. 2010, Radville et al. 2011). The hypersensitive response of the host initially induces a more favorable nutritional state for the adelgid, but eventually leads to reduced growth, decline in vigor, and eventual tree death (Schwartzberg and Montgomery 2010).

Due to its primarily sessile lifestyle, *A. tsugae* health is dependent on the health of the host tree. Similarly, hemlock health is affected by the *A. tsugae* population size. This relationship represents a density-dependent feedback system of alternating host and insect health. Healthy hemlocks that become infested by the adelgid are depleted of nutrients, leading to a reduction of

the adelgid population. Hemlocks will make a partial health recovery, only to undergo adelgid re-colonization the following season. While unusual weather may alter this pattern, typically *A. tsugae* populations cycle through these bimodal peaks of abundance on hemlock every four years until tree mortality occurs (McClure 1991a, Eisenback et al. 2010).

In the *A. tsugae* – hemlock system in the eastern United States, all trees are considered stressed. However, individual trees exhibit vastly different levels of decline and levels of *A. tsugae* population (McClure 1991a). Although many studies in this system focus on the effect *A. tsugae* have on the hemlock stands and forest ecosystems, few studies have examined the effect of hemlock health on *A. tsugae* populations (Jenkins 1999, Stadler et al. 2005, Stadler 2006, Radville et al. 2011). Initial studies examining the relationship between *A. tsugae* and hemlock have focused primarily on assessing adelgid health in terms of population density and the number of ovisacs per tree (McClure 1991a, Broeckling and Salom 2003, Stadler et al. 2005, Lagalante et al. 2006). This study seeks to determine the relationship between hemlock health and *A. tsugae* population health using insect density, survival from aestivation, and fecundity of the sistens generation as response variables. We hypothesize that *A. tsugae* populations from lightly impacted hemlocks will be healthier than those populations from moderately impacted hemlocks.

2.2 Materials and Methods

2.2.1 Site Selection and Hemlock Sampling

Adelges tsugae were collected from hemlocks at three locations (Virginia Tech College of Natural Resources and Environment, Fishburn Forest, Montgomery Co., VA; University of Virginia Biological Station at Mt. Lake in Giles Co., VA; and Twin Falls State Park, Wyoming Co., WV) during the 2011 - 2012 field season. Due to the lack of *A. tsugae* populations and

foliage at the Fishburn site, only two sites were selected for the 2012 - 2013 field season (University of Virginia Biological Station at Mt. Lake in Giles Co., VA and Twin Falls State Park, Wyoming Co., WV). Mt. Lake Biological Station is located in the USDA plant hardiness zones 5b and 6a, Fishburn Experimental Forest is in zone 6a, and Twin Falls State Park is in zones 6a and 6b (USDA 2012). We were unable to obtain continuous temperature and rainfall records for 2011 - 2013 for all three sites. However these data were recorded during both years at Mt. Lake using a Campbell CR10 data logger and at Kentland Farm, (a part of the Virginia Agricultural Experimental Station located 14.3 km from the Fishburn site) using a Campbell Scientific CR10X data logger. The general fluctuations in temperature and rainfall between the two years are shown in Figure 2.1. Since the presence of adequate hemlock foliage and sufficient *A. tsugae* populations was critical for tree selection, location was considered to be of secondary importance.

Thirty-two hemlocks exhibiting symptoms of decline were selected for destructive sampling each year. The trees selected for the 2011 - 2012 field season study had a DBH (diameter at breast height) range from 4.5 – 22.1 cm with a mean of 11.8 cm. Those trees selected for the 2012 - 2013 field season study had a DBH range from 4.8 - 21.8 cm with a mean of 10.5 cm. Most of the hemlocks sampled showed typical signs of adelgid infestation, (i.e., needle loss and some bare branches), but also contained healthy branches with new growth and moderate levels of adelgid populations.

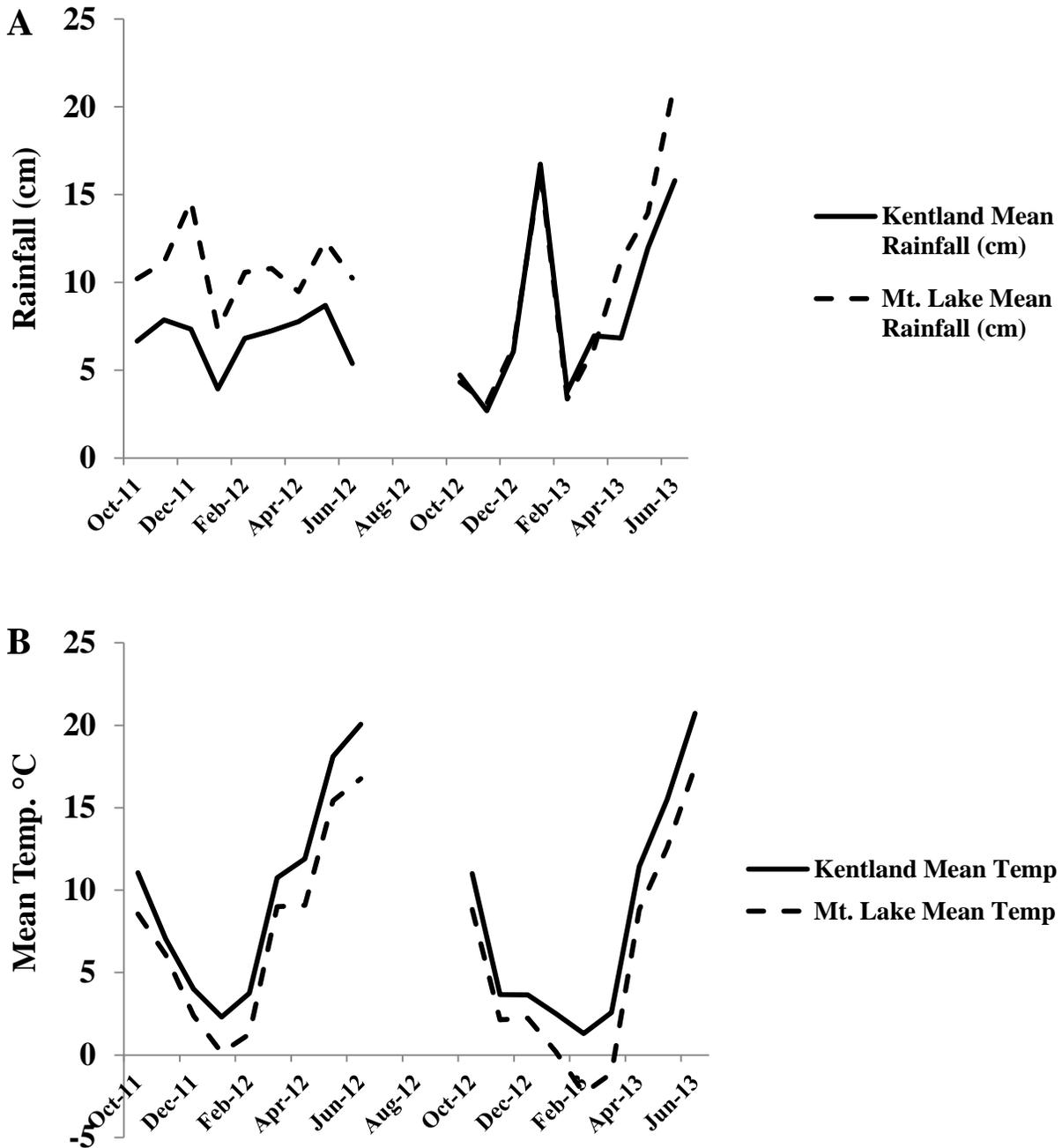


Figure 2.1 Comparison of (A) mean rainfall (cm) during the 2011-2012 and 2012-2013 *A. tsugae* sampling period and (B) mean temperature (°C) during the 2011-2012 and 2012-2013 *A. tsugae* sampling period. Mt. Lake data obtained from the University of Virginia Mt. Lake Biological Station meteorological data collection. Kentland Farm data obtained from the Virginia Agricultural Experiment Station.

Individual tree health was qualitatively evaluated using five parameters: percent foliage density, live crown ratio, live branches, live tips, and new foliage. All evaluations were made by the author to maintain consistency. The entire tree was examined visually when evaluating each health parameter for each tree. All of the parameters were rated on a scale of 0 – 100% in 5% increments such that a high value would indicate a healthy condition while a low value would indicate an unhealthy condition. Percent foliage density was an estimate of the remaining foliage on the tree. This is the inverse of foliage transparency as defined by Schomaker et al. (2007) which is the amount of skylight visible through microholes in the live portion of the crown. Foliage density was used instead of foliage transparency so that a high value would indicate a healthy condition whereas a high value using foliage transparency would indicate poor health. Recently defoliated branches were included in the measurement. Dead branches and trunk were not included in the estimate. Percent live crown ratio was determined by estimating the ratio of the length of the tree that supported live foliage relative to the total tree length (Schomaker et al. 2007). Percent live branches were determined by estimating the number of branches supporting live needles relative to the number of dead branches. Percent live branch tips were determined by estimating the hemlock branch tips for live foliage relative to the branch tips that did not support needles. Percent new foliage was determined by estimating the amount of new growth present on the branch tips relative to the live and dead branch tips. New growth that developed during the current year was distinguished from old needle growth by having a more yellow-green color and more feathery and flexible appearance. When branches were within reach from the ground the terminal 1 m of two branches from each ordinal direction were examined and the number of branch tips with live needles and no needles (dead tip) and percent new growth was determined. If branches were not within reach, percent live tips and new growth was estimated

by standing at the edge of the crown or dripline and looking up at the perimeter of the live crown. Samples taken for HWA analysis which were cut from 3 m (low-canopy) to 5 m (mid-canopy) above the ground with a pole pruner were also examined to determine live tip and new growth values. The mean of these five parameters (percent foliage density, live crown ratio, live branches, live tips, and new foliage) was used to calculate a Hemlock Health Index (0-100) similar to the Crown Condition Rating Guide used for Forest Health Monitoring assessments of forest stands by the USDA Forest Service (Millers et al. 1992, McAvoy 2011). A high index indicates a high health and a low index indicates poor health (Table 2.1).

To evaluate hemlock health quantitatively, new shoot growth was measured (cm). New growth was differentiated from the previous year growth by twig and needle color and intact bud scales. Monthly cuttings (Dec. 2011 – Apr. 2012 and Oct. 2012 – Apr. 2012) were obtained from each tree at low- and mid-canopy heights for *A. tsugae* analysis. These sample cuttings were placed in labeled self-sealing plastic bags and transported from the field in a cooler containing several cold blue ice packs. They were stored at ~4°C in a cold room until hemlock measurements and *A. tsugae* counts and collections could be performed. All hemlock health parameters were taken once per field season – in December 2011 and October 2012.

2.2.2 *Adelges tsugae* Density, Survival from Aestivation, and Fecundity

Adelges tsugae density was determined by counting the total adelgids/cm of hemlock growth at the beginning of each field season (December 2011 and October 2012). All settled adelgids on 50 cm of both low- and mid-canopy (25 cm of previous and 25 cm of new shoot growth) were counted. The *A. tsugae* that survived aestivation were determined by observing the production of wax from the settled insect, and their survival was calculated in the same manner as density. The

raw data for *A. tsugae* density and survival was divided by 25 to determine the number of adelgids/cm of hemlock growth inclusive of all growth and canopy types.

Adelgids were sampled bi-monthly (Feb. 21; Mar. 12, 13; Mar. 29, 31; Apr. 14, 16; Apr. 28, 30 2012 and Feb. 14, 15; Mar. 4, 5; Mar. 20, 21; Apr. 2; Apr. 17; May 6 2013) during the sistens oviposition period. Multiple sampling dates per period reflect our inability to sample from all sites in one day. To count the eggs per ovisac, an individual ovisac was removed from hemlock new growth (when possible). Seventy percent ethanol was dripped onto the ovisac and the surrounding wax was teased away using probes under a dissection microscope (Nikon SMZ800). Adelgid fecundity for each tree was calculated by counting the eggs and chorions (the newly hatched crawlers) from 10 ovisacs per canopy type on new growth (total of 20 ovisacs per tree) (McClure 1991b). The mean eggs per ovisac were calculated for each tree from each sample period. The sample period during which the maximum number of eggs was present was used to determine peak fecundity.

2.2.3 Statistical Analysis

A cluster analysis was carried out using Ward's method to group sample trees into two hemlock health categories based on hemlock new shoot growth and the Hemlock Health Index (Hair et al. 2010). Site data were pooled from both years to improve the distribution for tree health categories. The analysis resulted in clusters that categorized the hemlock trees as lightly or moderately impacted by *A. tsugae*.

During both years, several trees did not contain *A. tsugae* at the levels needed for sampling. As a result, these trees were eliminated from the study, and the sample size was reduced from 32 to 28 trees during 2011 - 2012 season and from 32 to 30 trees during 2012 - 2013 season. All data

were normalized by square-root transformation, and an analysis of variance (ANOVA) was used to compare hemlock shoot length, *A. tsugae* density, and survival from aestivation between hemlock shoot age (previous and new growth) and also between low- and mid-canopy. Since egg counts were only made on new growth, peak fecundity was compared between canopy levels with an ANOVA test.

A multivariate analysis of variance (MANOVA) was used to compare the relationship between the hemlock health categories and the response variables for *A. tsugae* population health: density, survival from aestivation, and peak fecundity of the sistens generation. All statistical analyses were performed using JMP Pro 10.0.0 (SAS 2012)

2.3 Results

2.3.1 Hemlock Health

The various hemlock measurements for the Hemlock Health Index and new shoot growth showed that most of the selected trees came from a relatively narrow range of the hemlock health (Table 2.1). This Index, combined with new shoot growth measurements, was used to categorize sample trees. The hemlock categories from the analysis were defined as ‘lightly impacted’ (average percent tree health: mean = 80.4 ± 1.3 and shoot length: mean = 5.3 ± 0.3 cm) and ‘moderately impacted’ (average percent tree health: mean = 68.4 ± 1.4 and shoot length: mean = 2.2 ± 0.1 cm) with *A. tsugae* (Table 2.1 and Figure 2.2).

Previous year’s hemlock shoot growth averaged 3.3 ± 0.1 [SE] cm, and new shoot growth averaged 3.7 ± 0.2 cm (Table 2.2A). Low-canopy shoot lengths averaged 3.5 ± 0.2 cm, while mid-canopy shoot lengths averaged 3.4 ± 0.2 cm. Shoot lengths from low- and mid-canopy on either previous or new growth were not significantly different (Table 2.2B).

Table 2.1 Comparison of the mean \pm standard error (SE) of health measurements for lightly and moderately *A. tsugae*-impacted hemlock trees combined from the 2011-2012 and 2012-2013 sampling periods¹.

Tree Health Measurement	Mean of Measurements ²	
	Lightly Impacted Trees	Moderately Impacted Trees
% Live Crown Ratio ³	83.2 \pm 1.7	76.3 \pm 1.5
% Live Branches	79.8 \pm 1.8	68.5 \pm 1.8
% Tips Alive	82.8 \pm 1.6	70.3 \pm 1.6
% New Foliage	77.6 \pm 1.6	62.1 \pm 1.8
% Crown Density	78.7 \pm 1.5	64.7 \pm 1.9
Health Index⁴	80.4 \pm 1.3	68.4 \pm 1.4
New Shoot Growth (cm)	5.3 \pm 0.3	2.2 \pm 0.1

¹Total trees sampled (N = 57)

²A Ward Cluster analysis (JMP 10.0.0 SAS Institute) of the Hemlock Health Index and the new shoot growth (cm) on each tree, categorized sampled trees over both years into two groups

³The Percent Live Crown Ratio is the approximate ratio of live crown from ground to the top of the tree

⁴The Hemlock Health Index is the mean \pm SE of all Tree Health Measurements for each tree



Lightly Impacted



Moderately Impacted

Figure 2.2 Representative specimens of lightly and moderately impacted hemlock trees. The lightly impacted tree is from Twin Falls State Park, WV during the 2012-2013 sampling season. The moderately impacted tree is from Twin Falls State Park, WV during the 2011-2012 sampling season.

2.3.2 *Adelges tsugae* Density, Survival from Aestivation, and Fecundity

Adelges tsugae density, survival from aestivation, and peak fecundity did not differ significantly within canopy position (Table 2.2B). *A. tsugae* density and survival from aestivation was significantly greater on new growth than previous growth (Table 2.2A).

2.3.3 Relationship between Hemlock Health and *A. tsugae* Population Health

A MANOVA test indicated a significant difference between hemlock health categories with respect to the three *A. tsugae* population response variables, density, survival from aestivation, and peak fecundity (Pillai's Trace, $V = 0.5792$; $df = 4,46$; $P = 0.0118$). Individual analyses by ANOVA determined that mean *A. tsugae* density and survival from aestivation were not significantly different between lightly and moderately impacted hemlocks. However, mean *A. tsugae* fecundity was significantly greater on moderately impacted than on lightly impacted trees. (Figure 2.3)

Table 2.2 Comparison of mean \pm standard error (SE) hemlock shoot length, *A. tsugae* density, survival from aestivation, and peak fecundity between (A) hemlock shoot age and (B) canopy type during two years. *Adelges tsugae* eggs were not counted for comparison between previous and new hemlock shoot growth¹.

A. Hemlock Shoot Age

Test	Mean \pm SE		ANOVA ⁵		
	Previous Growth	New Growth	Exact F	Degrees of Freedom (df)	Prob > F
Hemlock Shoot Length (cm)	3.3 \pm 0.1	3.7 \pm 0.2	1.41	1, 231	$P = 0.09$
Adelgid Density ²	5.1 \pm 0.4	15.7 \pm 0.6	189.46	1, 218	$P < 0.05$
Adelgid Aestivation Survival ³	1.2 \pm 0.1	6.5 \pm 0.4	229.22	1, 231	$P < 0.05$

B. Hemlock Canopy Type

Test	Mean \pm SE		ANOVA ⁵		
	Previous Growth	New Growth	Exact F	Degrees of Freedom (df)	Prob > F
Hemlock Shoot Length (cm)	3.5 \pm 0.2	3.4 \pm 0.2	0.32	1, 231	$P = 0.6791$
Adelgid Density ²	10.9 \pm 0.8	9.9 \pm 0.7	0.06	1, 218	$P = 0.8050$
Adelgid Aestivation Survival ³	3.9 \pm 0.4	3.8 \pm 0.4	0.19	1, 231	$P = 0.6623$
Adelgid Peak Fecundity ⁴	76.7 \pm 5.0	77.0 \pm 4.8	0.05	1, 115	$P = 0.8274$

¹Total trees sampled (N = 57)

²Adelgid density is the total mean \pm SE sistens adelgids per cm shoot growth

³Adelgid survival from aestivation is the mean \pm SE sistens adelgids that broke aestivation per cm shoot length

⁴Peak adelgid fecundity is the highest mean \pm SE of eggs and chorions (newly hatched crawlers) per 10 ovisacs

⁵ANOVA performed in JMP Pro 10.0.0 (SAS Institute 2013)

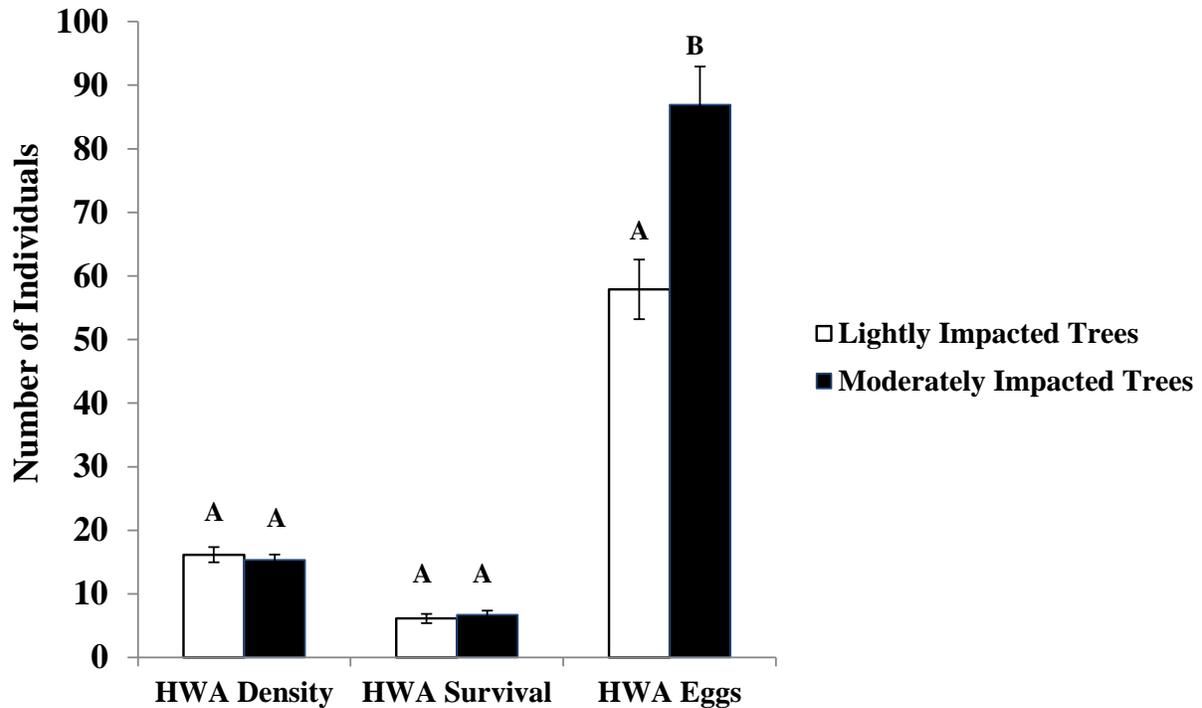


Figure 2.3 Comparison of hemlock health categories with respect to *A. tsugae* population response variables. Total trees sampled (N = 57). Letters indicate significant differences using MANOVA. Error bars indicate standard error as calculated by JMP 10.0.0 (SAS Institute). HWA density: $F = 0.2284$, $df = 1, 56$, $P > 0.05$; HWA survival from aestivation: $F = 0.4218$, $df = 1, 56$, $P > 0.05$; HWA fecundity: $F = 13.6227$, $df = 1, 57$, $P < 0.05$

2.4 Discussion

The tree health cluster analysis grouped all sampled hemlocks into two categories using a cluster analysis that combined both quantitative and qualitative hemlock measurements. These categories were defined as lightly and moderately impacted by *A. tsugae*. After classification, it was noted that the tree health cluster analysis primarily grouped trees from the 2011 - 2012 season into the moderately impacted category and trees from 2012 - 2013 into the lightly impacted category. However, the hemlocks were categorized based on new shoot growth and the Hemlock Health Index and not by sample year.

The hemlock trees examined throughout our study sites ranged from those with plentiful foliage and large *A. tsugae* populations, to those with meager foliage and large *A. tsugae* populations. In between these two extremes were many trees that exhibited moderate foliage and scattered pockets of *A. tsugae* populations throughout the tree canopy (personal observation). Our tree health classification categorized the trees, not as broadly as ‘healthy’ and ‘declining’, but as lightly and moderately impacted by *A. tsugae*. This difference in tree classification may better reflect the re-colonization of trees by *A. tsugae*, but due to the limited tree variability may not allow for a full assessment of hemlock health impact on *A. tsugae*.

Although there are guidelines for assessing tree health, hemlock health determination is still fairly subjective. To decrease subjectivity, a single individual estimated the Hemlock Health Index measurements for both years to eliminate inter-observer variability. Additionally the Hemlock Health Index used was based on the Crown Condition Rating Guide (Millers et al. 1992). This guide divides tree health into three classes (good, average, and poor) based on the live crown ratio, crown diameter, crown density, and crown dieback, but it is not specific to hemlocks and is primarily used for evaluating dominant trees. This index, encompasses live crown ratio, live branches, tips alive, new foliage and crown density parameters (Table 2.1) (McAvoy 2011). Our classifications of lightly and moderately impacted trees include both qualitative (the hemlock health index) and quantitative (new shoot length) hemlock tree measurements. Lightly impacted trees are analogous to those in the Crown Condition Rating Guide Class 1 (Good), whereas moderately impacted trees are analogous to those in Class 2 (Average). We were unable to sample from trees that would fall into a ‘heavily impacted’ (Class 3 – Poor) ranking since we needed ample foliage for the periodic destructive sampling in this study.

The narrow range of tree health among all the sampled trees was a result of two factors. Heavily impacted trees are lightly colonized by *A. tsugae* and sampling insects from such trees was not sustainable over the two-year study. On the other end of the tree health spectrum, we observed very few completely healthy trees with heavy infestation (such as a first time infestation) at our sampling locations. Therefore, we were limited to sampling trees that were classified as lightly or moderately impacted by *A. tsugae*. Based on their physical appearance and reduced new growth, moderately impacted trees typically appeared to be in poorer health than lightly impacted trees.

Our results show sistens adults laid more eggs on moderately impacted trees than on lightly impacted trees, but their density and survival from aestivation did not differ significantly on trees between these health categories (Figure 2.3). The measures of *A. tsugae* density and survival both represent the ability of *A. tsugae* sistens to successfully find suitable feeding sites and to break aestivation, respectively. *A. tsugae* fecundity directly reflects the accessibility of hemlock nutrients to the growing sistens. It may be that *A. tsugae* have access to increased levels of nutrients and were able to lay more eggs on moderately impacted trees as the host responds to stress and mobilizes nutrients away from the area. *A. tsugae* nymphs prefer to colonize the youngest available branches on hemlock. If little new growth is available, the nymphs are forced to colonize older growth (Table 2.2A) (McClure 1991a). Infestations of the adelgid are vertically uniform on forest trees (Table 2.2B) (McClure 1990, Parker et al. 1998).

Perhaps the plant vigor hypothesis as described in White (1974) can offer an explanation for *A. tsugae* adults laying more eggs on moderately impacted trees. Plant stress exists along a continuum that varies in response to the stressors and the overall health of the plant (Price 1991). Typically plants respond to herbivore attack by mobilizing soluble nitrogen away from stressed tissues to nutrient sinks or secondary metabolite synthesis, allowing herbivores feeding on such

tissues a ready supply of nitrogen in their food (White 1984). However at times of very high stress levels, the tree may become a non-resource to insects, either because it no longer provides the insect with adequate food sources or because it is dead (Larsson 1989, Schwartzberg and Montgomery 2010, Radville et al. 2011). While all hemlocks colonized by *A. tsugae* are stressed, individual trees exhibit vastly different levels of decline and contain varying levels of *A. tsugae* populations (McClure 1991a, Parker et al. 1998). *A. tsugae* health is dependent on the health of the host tree to obtain enough nutrients for growth and reproduction, and hemlock health is affected by the *A. tsugae* population size. (McClure 1991a, Eisenback et al. 2010).

Initial studies examining the relationship between *A. tsugae* and hemlock have focused primarily on assessing adelgid health in terms of population density and fecundity (Broeckling and Salom 2003, Lagalante et al. 2006). *A. tsugae* density calculations are standard (number *A. tsugae*/cm hemlock shoot), and McClure (1991a) measured *A. tsugae* fecundity by counting the number of eggs and chorions from ovisacs with dead adults (indicating the termination of oviposition). In addition, he obtained fecundity measurements once, potentially missing the peak of *A. tsugae* fecundity on certain trees (McClure 1991a). In order to obtain a more accurate estimate of fecundity, we determined the mean eggs present in a set number of ovisacs bi-monthly. We also expanded our assessment of the insect's health to include survival from aestivation, which described *A. tsugae*'s fitness during aestivation.

The occurrence of increased fecundity on moderately impacted trees is of interest in light of McClure's (1991a) findings that *A. tsugae* exhibits a higher rate of reproduction on healthy rather than on declining trees. McClure measured tree health using only the percent viable buds and affected crown as a parameters. We used assessments that encompass a wider degree of tree health parameters, which should provide a more accurate assessment of hemlock health.

Considerable health variability among hemlocks will influence *A. tsugae* populations (McClure 1991b, Parker et al. 1998). We determined that lightly and moderately impacted hemlocks have similar *A. tsugae* densities and survival from aestivation. *A. tsugae* feeding on moderately impacted hemlocks exhibit higher fecundity than those insects from lightly impacted trees. Increased mobilization of hemlock nutrients in response to *A. tsugae*-related stress may provide insects feeding on such trees with more nutrients for oogenesis and oviposition than may be available to insects feeding on lightly impacted hemlocks. Future studies should sample tree tissue for presence of nutrient mobilization at different levels of hemlock decline to better understand this relationship.

Chapter 3

The Relationship between Eastern Hemlock and Hemlock Woolly Adelgid Physiology

Abstract

The hemlock woolly adelgid (*Adelges tsugae*) is a small invasive insect that settles at the base of hemlock needles (*Tsugae* spp.). While infested eastern hemlock (*T. canadensis* Carriere) can die within as little as four years, many hemlocks survive for ten years or more in poor health.

Healthy trees infested by *A. tsugae* are depleted of nutrients, leading to a reduction of the adelgid population. After initial colonization by *A. tsugae*, hemlocks often make a partial health recovery, only to undergo re-colonization of the insect the following season. Since the *A. tsugae* is dependent on hemlock for nutrients, feeding on trees in poor health may affect the insect's ability to obtain necessary nutrients. We examined the physiological state of *A. tsugae* collected from lightly and moderately impacted hemlocks during the sistens generation by measuring the biomass, total carbon, carbohydrate, total nitrogen, and amino nitrogen. The physiological components of *A. tsugae* on lightly impacted hemlocks was either the same or significantly greater than that of insects on moderately impacted trees. These results support our hypothesis that *A. tsugae* physiological measurements could be used to estimate the relative HWA health, and that those insects feeding on lightly impacted trees are physiologically healthier than those on moderately impacted trees. These results may be explained by the plant stress hypothesis as well as the early stages of the density-dependent feedback interaction that exists between *A. tsugae* and their host.

3.1 Introduction

Plants provide all the nutrients insect herbivores require, but the nutrient amounts may vary based on genotypic differences and environmental conditions (Behmer 2009). Stressed plants

generally slow down cell growth, which in turn, leads to investment in smaller plant structures such as buds, leaves and shoots, all of which will eventually impact insect performance (McClure 1980, Larsson 1989). In plants, a common metabolic response to stress (such as weather, herbivory, and infections) is the breakdown and mobilization of nutrients, to other areas of the plant (White 1984, Price 1991). Since these nutrients are necessary for many plant-feeding insects, a ready supply of them moving away from the stressed location will increase insect fitness and abundance (White 1984, Cornelissen et al. 2008, Meloni et al. 2012). Over time, as this nutritious flow is depleted, sap-feeding insect populations will decline (Cornelissen et al. 2008).

The hemlock woolly adelgid, *Adelges tsugae*, Annand (Hemiptera: Adelgidae) is a small invasive insect (1.3-1.5 mm long) that has caused eastern and Carolina hemlock (*Tsuga canadensis* Carriere and *T. caroliniana* Engelm, respectively) mortality throughout eastern North America in ornamental/urban and forest settings (Carter 1971, McClure 1987, 1989, McClure 1991a, Cheah and McClure 2000). Eastern hemlock is particularly susceptible to this pest, as infested hemlocks survive from four to ten years in declining health. *Adelges* spp. colonizes spruce as primary hosts and other conifers as secondary hosts. Various *Tsuga* spp. are the secondary hosts of *A. tsugae* (Havill and Footitt 2007). In its native range, *A. tsugae* alternates sexual and asexual generations between its primary and secondary hosts. In eastern North America, *A. tsugae* cannot reproduce on neotemperate spruce species and has reverted to living solely asexually on its secondary host (Carter 1971, Rohfritsch 1990, Havill and Footitt 2007). The asexual lifecycle of *A. tsugae* is polymorphic and parthenogenic, developing through two generations annually (McClure 1989).

In the hemlock-*A. tsugae* system, trees treated with nitrogen fertilizer support an increased *A. tsugae* population and nymph and egg survival (McClure 1991b). Based on this study, it is evident that nitrogen is a limited resource for *A. tsugae*, and while initial tree stress may encourage high adelgid densities, continual stress on the tree will cause negative effects to the population (McClure 1991b, Cornelissen et al. 2008). It follows that other physiological factors such as biomass, carbon, and carbohydrates would have a similar relationship in the *A. tsugae*-hemlock system. However, little is known about the effect hemlock health may have on the physiological processes of *A. tsugae* feeding on hemlocks.

We hypothesize that a series of *A. tsugae* physiological health parameters can be used to estimate *A. tsugae* health, and that *A. tsugae* physiological health is influenced by the health of its hemlock host. Specifically, *A. tsugae* collected from lightly impacted trees are healthier than those from moderately impacted trees. This study employs the use of a Hemlock Health Index and hemlock shoot measurements to categorize tree health, followed by an analysis of *A. tsugae* biomass, total carbon, carbohydrates, total nitrogen, and amino nitrogen compared with host tree health throughout the sistens generation.

3.2 Materials and Methods

3.2.1 Hemlock Health

The sample hemlocks were the same trees, and the same tree health evaluation was used as described in Section 2.2.1.

3.2.2 *Adelges tsugae* Sample Preparation

When possible, three samples of approximately 30 *A. tsugae* were taken per tree from new foliage from both lightly and moderately impacted hemlocks. The majority of the wax covering

was removed from the insects by manipulation with probes under a dissecting microscope (Nikon SMZ800), and insects were micro-aspirated with a vacuum pump system into 0.6 mL microcentrifuge tubes (Fisherbrand). Half these insect samples were obtained from low-canopy foliage and the other half from mid-canopy foliage. These samples were designated for elemental, amino nitrogen, and total carbohydrate analyses. Initial wet sample weights were obtained using a microbalance (Orion Cahn C-36, Thermo Scientific). The samples destined for elemental analysis were placed in new microcentrifuge tubes and frozen at -16°C until analysis. The samples for the amino nitrogen and carbohydrate assays were homogenized in 30 µL 70% EtOH in a 0.6 mL pre-weighed microcentrifuge tube. The homogenates were sonicated for 2-3 minutes (Solid State/Ultrasonic FS-28, Fisher Scientific), heated to approximately 80 °C in a water bath (Isotemp water bath, Fisher Scientific), and centrifuged for 5 min. between 1050-1300 rpm with a 12-place rotor (Micro-Centrifuge Model 59A, Fisher Scientific). The samples were stored at -16°C until the assays were run.

3.2.3 Elemental Analysis

Each sample was reweighed prior to drying at 78-83°C overnight in the presence of absorbent silica beads. The dried adelgid samples were re-weighed to obtain the dry weight (mg) and stored in a vacuum chamber with absorbent silica beads. A series of 0.25, 1.0, and 3.0 mg BSA (Sigma-Aldrich, St. Louis, MD) standards and 0.25 and 2.0 mg uric acid standards (Sigma Chemical Company, St. Louis, MD) were prepared for each group of 30 *A. tsugae* samples. Standards and dried *A. tsugae* samples were placed in aluminum or silver capsules (Elementar, Germany). Standards and samples underwent elemental analysis which involved complete combustion of samples, the trapping of carbon and nitrogen in columns, and final analyzing by a thermal

conductivity detector. Percent carbon and nitrogen content were determined with this method using the vario EL Cube (Elementar, Germany).

3.2.4 Amino Nitrogen Assay

The ninhydrin assay was used to determine total amino nitrogen contained in the *A. tsugae* tissue homogenates (ninhydrin positive materials include soluble proteins, peptides, amino acids, ammonia, and various amines) (McMurry 2008). The method was miniaturized from Starcher (2001). The ninhydrin reagent and L-leucine standard were obtained from MP Biomedicals, LLC, Solon, OH and Nutritional Biochemicals Corporation, Cleveland, OH, respectively. The ninhydrin reagent was prepared a day prior to running the assay according to Starcher (2001) at 19.5 mg/mL. A 2 mg/mL (DI H₂O) leucine standard from which a series of dilutions of 1000 µg/mL, 100 ng/µL, 50 ng/µL, and 10 ng/µL were made.

The adelgid samples for this assay were reconstituted in 70% EtOH, vortexed briefly, and centrifuged for 5 min at either 1050-1300 rpm or 3000 rpm (Micro-Centrifuge Model 59A, Fisher Scientific or Sorvall fresco, Thermo Scientific centrifuge with a Ch. 522801 PP 31/05 rotor, respectively) for a well-formed pellet. Assays from the 2011-2012 field season were run on 96-well polypropylene microplates (Thermo Fisher Scientific) and covered with adhesive PCR film (Thermo Scientific) to reduce evaporation. Assays from the 2012-2013 field season were run on 0.2 mL 96-polypropylene PCR plates (TempPlate, USA Scientific) and covered with well caps to reduce sample evaporation.

Each plate contained a control, at least three leucine standards, and (when possible) triplicate subsamples of *A. tsugae* extracts. Each reaction consisted of 100 µL ninhydrin reagent and 10 µL DI H₂O as a control, 10 µL of each standard, or 5 µL sample *A. tsugae* homogenate plus 5 µL DI

H₂O (total volume of 110 μ L for each well). All plates were heated in a 100 °C digital dry bath (GeneMate, BioExpress) for 20 min. Plates with PCR film were spun in a mini plate spinner (Labnet) for approximately 1 min while plates with caps were shaken and tapped to mix in condensation that may have accumulated on the caps. Aliquots of 80 μ L of reaction were read at 570 nm (M2 SpectraMax, Molecular Devices). The sample optical densities were corrected for control optical densities.

3.2.5 Total Carbohydrate Assay

The total carbohydrate assay was miniaturized from that described by Van Handel and Day (1988). The anthrone reagent and D (+)-glucose standard were both obtained from Acros Organics (Morris Plains, New Jersey). The anthrone reagent was prepared according to Van Handel and Day (1988) at 1.4 mg/mL. A 2000 μ g/mL (DI H₂O) leucine stock was prepared, and a series of dilutions of 1000 μ g/mL, 100 ng/ μ L, 50 ng/ μ L, and 10 ng/ μ L were made.

Adelges tsugae samples were reconstituted from the freezer in the same manner as was done for the amino nitrogen assay (Section 3.2.4). One hundred μ L of a 1:1 chloroform:methanol solution and 75 μ L DI H₂O were used to separate the carbohydrates from the *A. tsugae* homogenate. Each 96-well polypropylene PCR plate (Thermo Fisher Scientific) included a control, at least three glucose standards, and (when possible) triplicate subsamples of *A. tsugae* carbohydrate extracts. The reactions consisted of 175 μ L anthrone reagent and 25 μ L DI H₂O as a control, 25 μ L of each glucose standard, and 10 μ L sample *A. tsugae* carbohydrate homogenate plus 15 μ L DI H₂O (total volume of 200 μ L for each well). Caps were sealed over each well to reduce evaporation, and the plates were nested on a 100 °C dry heat bath for 30 min, cooled for 1-2 min. and were flipped and shaken to mix in condensation which may have accumulated on the caps.

Aliquots of 150 μ L for each reaction were read at 625 nm (M2 SpectraMax, Molecular Devices). Sample optical densities were corrected for control optical densities.

3.2.6 Statistical Analysis

Since *A. tsugae* sampling did not occur during October and November in 2011, our sample size was further reduced during those two months, prohibiting statistical analysis of these data. This resulted in using only the data obtained for the months of December – April for both years. A cluster analysis was carried out using Ward's method to group sample trees into two hemlock health categories based on hemlock new shoot growth and the Hemlock Health Index (Hair et al. 2010). Site data were pooled from both years to improve the distribution for tree health categories (Section 2.2.3).

Separate analyses were carried out using a linear mixed model ANOVA to compare differences between *A. tsugae* on lightly and moderately impacted hemlocks over time with respect to biomass, total carbon, carbohydrate, total nitrogen, and amino nitrogen (Chaves 2010). Tree health category was the fixed effect factor, and the monthly sampling periods were the random effects. Normality for the response variables could not be achieved by transformation, and the appropriateness of using the data was judged by the lack of departure of the distribution from normality based on skewness (-0.8 – 0.8) and kurtosis (-3.0 – 3.0). All statistical tests were run on JMP Pro 10.0.0 (SAS 2012).

3.3 Results

3.3.1 Hemlock Health

The hemlock trees and health assessment from Chapter 2, Section 2.3.1 was the same.

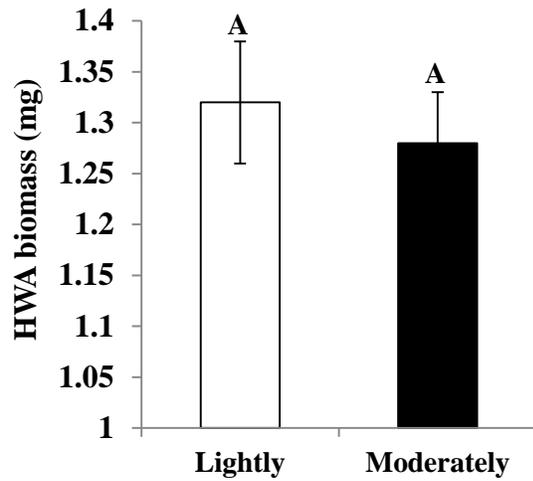
3.3.2 Overall *A. tsugae* Physiological Responses

Biomass and total carbon of *A. tsugae* did not have significantly different means between lightly or moderately impacted trees. ($F = 1.12$; $df = 1, 278$; $P = 0.29$ and $F = 0.96$; $df = 1, 284$; $P = 0.33$, respectively) (Figure 3.1.A and 3.1.B). However, *A. tsugae* from lightly and moderately impacted trees contained significantly higher levels of carbohydrates, total nitrogen, and amino nitrogen (Figure 3.1.C-E).

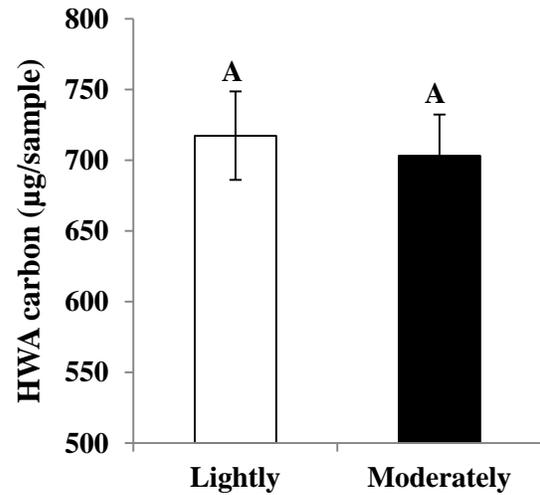
3.3.3 Overall *A. tsugae* Physiological Response Comparisons by Tree and Time

There were significant differences for all *A. tsugae* physiological parameters measured over time (Figure 3.2.A-E). Peak biomass and total carbon occurred in *A. tsugae* from lightly impacted hemlocks in April (mean = 2.17 ± 0.16 mg/*A. tsugae* sample and 1159.3 ± 91.6 μ g/*A. tsugae* sample, respectively). *A. tsugae* reached maximum carbohydrate levels on lightly impacted trees in March (mean = 58.4 ± 6.9 μ g/*A. tsugae* sample). Both total nitrogen and amino nitrogen in *A. tsugae* from lightly impacted trees in April (mean = 108.5 ± 8.2 μ g/*A. tsugae* sample and 42.6 ± 2.9 μ g/*A. tsugae* sample, respectively).

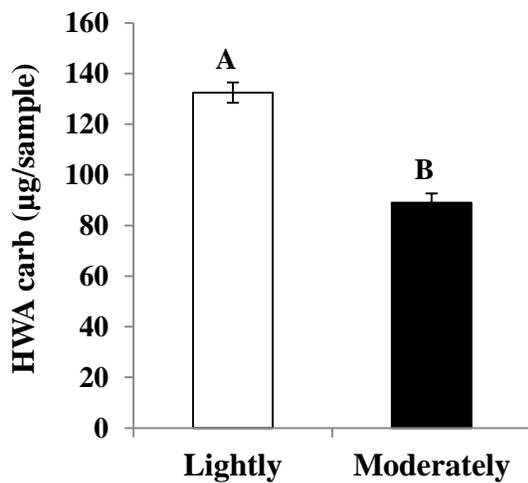
3.1.A HWA Biomass



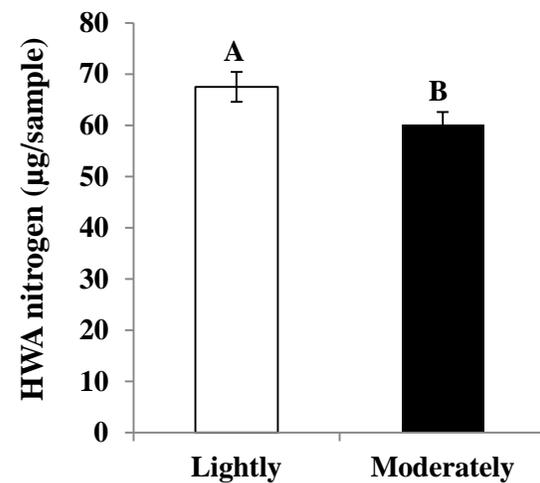
3.1.B HWA Carbon



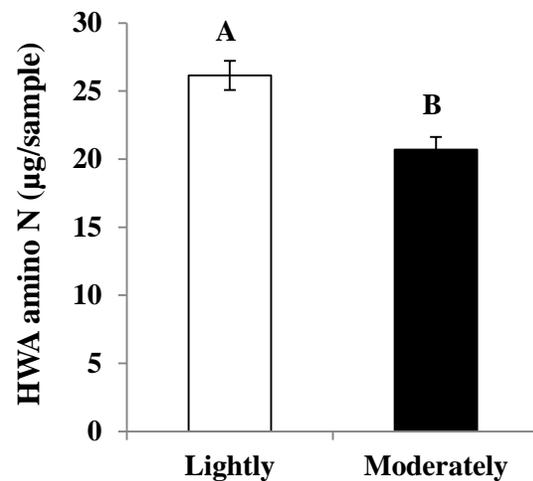
3.1.C HWA Carbohydrate



3.1.D HWA Nitrogen



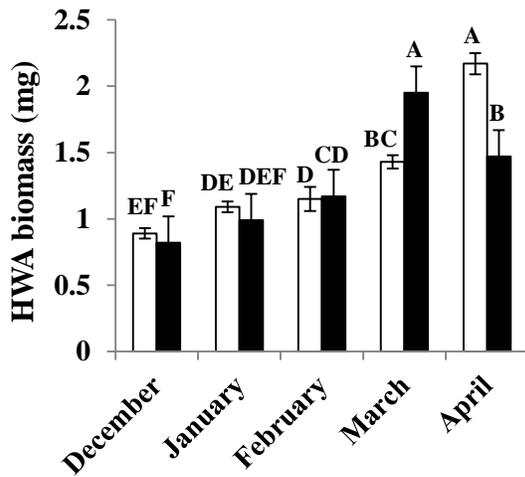
3.1.E HWA Amino N



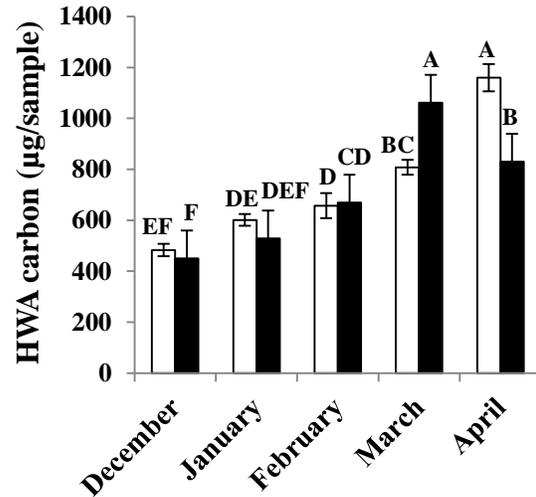
□ Lightly Impacted ■ Moderately Impacted

Figure 3.1 Comparison of mean \pm SE *A. tsugae* (A) biomass ($F = 1.12$; $df = 1, 278$; $P = 0.29$), (B) total carbon ($F = 0.96$; $df = 1, 284$; $P = 0.33$), (C) carbohydrate ($F = 201.97$; $df = 1, 291$; $P < 0.05$), (D) total nitrogen ($F = 8.62$; $df = 1, 284$; $P < 0.05$), and (E) amino nitrogen ($F = 33.05$; $df = 1, 285$; $P < 0.05$) between *A. tsugae* from lightly and moderately impacted hemlocks. Sample size was approx. 30 insects. The data are pooled from December to April for trees sampled during 2011-2012 and 2012-2013. Total sampled trees ($N = 57$). Letters indicate significant difference based on a linear mixed model using JMP Pro 10.0.0 (SAS Institute 2012).

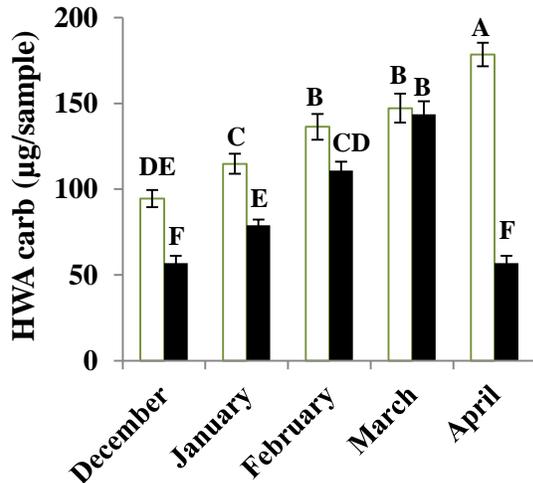
3.2.A HWA Biomass



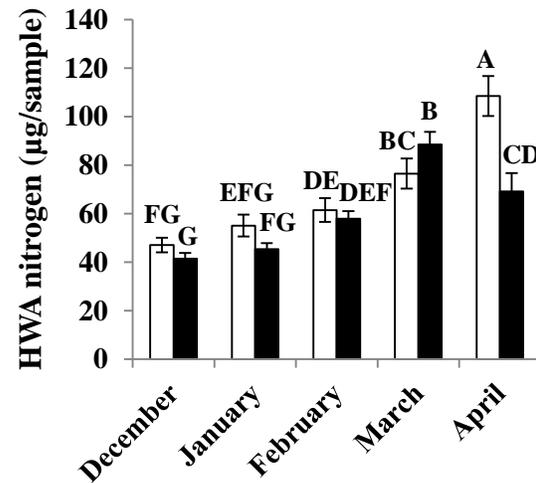
3.2.B HWA Carbon



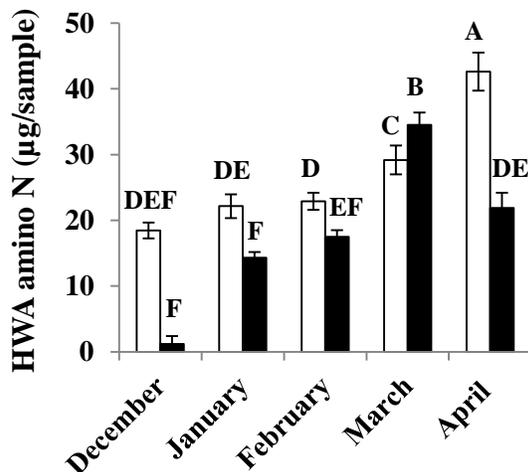
3.2.C HWA Carbohydrate



3.2.D HWA Nitrogen



3.2.E HWA Amino N



□ Lightly Impacted ■ Moderately Impacted

Figure 3.2 Comparison between lightly and moderately impacted hemlocks (from 2011 - 2013) for mean \pm SE *A. tsugae* (A) biomass (mg/HWA sample) ($P < 0.05$), (B) total carbon ($\mu\text{g}/\text{HWA}$ sample) ($P < 0.05$), (C) carbohydrate ($\mu\text{g}/\text{HWA}$ sample) ($P < 0.05$), (D) total nitrogen ($\mu\text{g}/\text{HWA}$ sample) ($P < 0.05$), and (E) amino nitrogen ($\mu\text{g}/\text{HWA}$ sample) ($P < 0.05$) during the sistens generation. Sample size was approx. 30 insects. Total sampled trees ($N = 57$). Letters indicate a significant difference based on a linear mixed model using JMP Pro 10.0.0 (SAS Institute 2012).

3.4 Discussion

The same hemlock health classification from Chapter 2 was used to categorize sample trees as lightly and moderately impacted. For this objective we compared *A. tsugae* physiological health between these two tree health categories. Our data show that *A. tsugae* collected from lightly impacted trees typically had greater levels of nutritional components, with regard to carbohydrates, nitrogen, and amino nitrogen, than those insects from moderately impacted trees. In insect-plant interactions, insects can be influenced by plant phenology, nutritional value, the density and location of the host plants, the mechanical resistance of the leaves, and the environmental community (Fagan et al. 2002, Meloni et al. 2012). Although insects are affected by the nutritional content of their host plants, they can also, to a certain extent, regulate the nutrients they ingest (White 1984, Raubenheimer and Simpson 2003, Behmer 2009). Nutrient regulation is particularly important for sessile, specialist insects, such as *A. tsugae*, as they are limited to obtaining a balance of nutrients for optimum fitness from a narrow tissue range (Slansky 1982, Young et al. 1995, McClure and Cheah 2002, Raubenheimer and Simpson 2003). Insects allocate a portion of nutrients absorbed from the gut for assimilation, growth, and metabolic processes (Slansky 1990). While most studies on insect nutritional ecology focus on insects that actively search for food, this study examines *A. tsugae*, a sessile insect that is limited to the fluctuations in the availability of hemlock resources on which they are feeding. While *A. tsugae* appear to regulate the absorption and utilization of available nutrients, it may ultimately be affected by its host's health (Behmer 2009).

Our results show that *A. tsugae* biomass and carbon measurements were not significantly different between lightly to moderately impacted trees (Figure 3.1.A-B). Since herbivorous insects control the homeostasis of their body elemental composition, their physiological variation

may be related to body size (Studier and Sevick 1992, Fagan et al. 2002). Insect biomass and carbon reflect nutrient utilization destined for assimilation and growth. *A. tsugae*'s restriction to the site location, particular tree, and even the portion of the tree where the stylet bundle is initially inserted may indicate that *A. tsugae* exhibits a limited level of control over nutrient accumulation (McClure and Cheah 2002). Since biomass is predominately composed of carbon-based materials, these measurements may represent the insect's total utilization of available resources to maintain adequate size and carbon for their life processes.

Carbohydrate measurements in *A. tsugae* collected from lightly impacted trees were significantly greater from those in insects from moderately impacted trees (Figure 3.1.C). The xylem ray parenchyma cells are the carbohydrate storage sites of hemlocks, and insect herbivores tightly regulate carbohydrates as a major energy source and is major exoskeleton component and for egg production (Slansky 1982, Mullins 1985, Brodbeck et al. 1990, Hanife 2006, Behmer 2009, Arrese and Soulages 2010). The significantly lower levels of carbohydrates that we found in insects collected from moderately impacted trees may indicate a notable lack of available nutrients in these trees, compared to lightly impacted trees.

Our results show that insects from lightly impacted trees had greater levels of both total and amino nitrogen than insects collected from moderately impacted trees. Nitrogen is an essential nutrient for insect herbivores, and amino nitrogen (destined for protein production and other metabolically important nitrogen-containing materials) is particularly important as a source of nitrogen destined for assimilation, growth, and reproduction (Slansky and Rodriguez 1987, Brodbeck et al. 1990, Slansky 1990). Further nitrogen fluctuations within the plant xylem, phloem, and other tissues may affect nitrogen content in herbivorous insects (Slansky and Rodriguez 1987, Brodbeck et al. 1990, Fagan et al. 2002). Laboratory studies have shown the

importance of amino nitrogen to insect success, with high concentrations of amino acids in host plants exhibiting high levels of feeding by the leafhopper, *Homalodisca coagulata* (Brodbeck et al., 1990). As *A. tsugae* matures and enters oogenesis, the insect's nitrogen content is expected to increase (particularly amino nitrogen) for egg production (Figure 3.2.D-E) (Boggs 1981, Brodbeck et al. 1990).

Adelges tsugae physiological parameters (measured by insect weight and the levels of different nutrients) increased over time as would be expected as the insect matures (Figure 3.1.A-E). An insect's nutritional requirements will change as it matures (House 1962, Hanife 2006). Changes in body composition over time are not only associated with changes in food quality, but also a component of the maturation process (Scriber and Slansky Jr 1981). Poor food availability or composition for the adult female may result in a reduced rate or quality of egg production. She may not waste reproductive effort producing offspring with a low probability of survival since the energetic cost of embryonic development and maintenance may be considerable (Slansky 1982). The differences between insect physiology parameters from insects collected from lightly and moderately impacted trees were most distinctive during *A. tsugae* oogenesis and oviposition of the progrediens generation (February-April) (Figure 3.2.A-E). *A. tsugae* appears to alter their assimilation (ingestion and storage) of nutrients in anticipation for egg production.

This study involved a preliminary assessment of *A. tsugae* physiological parameters in relation to the health of their host hemlock, and of *A. tsugae* physiological parameters throughout sistens maturation. The greater levels of carbohydrate, nitrogen, and amino nitrogen content in *A. tsugae* on lightly impacted trees may be caused by the initial tree stress from *A. tsugae* feeding and the resulting plant nutrient mobilization (White 1984, McClure 1991a, McClure 1991b).

As would be expected, *A. tsugae* biomass and physiological composition increased as the insect matured in preparation for oogenesis and oviposition of the progrediens generation.

Further analysis of *A. tsugae* physiology should be conducted, particularly the lipid composition, representing fat accumulation, which we were not able to explore due to time constraints.

Continuing examination of progrediens egg hatch and development would provide information about *A. tsugae* maternal investment in the next generation. An examination of a wider range of tree health and a physiological assessment of the *A. tsugae* feeding sites on hemlock is needed to more fully understand the relationship between hemlock health and *A. tsugae* health. The apparent ability to reach adulthood and reproduce by *A. tsugae* on both lightly and moderately impacted hemlocks may indicate that *A. tsugae* exerts a degree of control over nutrient assimilation. However, the decline of hemlock host health will ultimately cause *A. tsugae* decline.

Chapter 4 Summary

Hemlock trees are a foundation species in eastern hardwood forests. *A. tsugae* has caused eastern hemlock and Carolina hemlock mortality throughout eastern North American forests. Eastern hemlock is particularly susceptible to this pest; infested hemlocks survive from four to ten years in declining health. Abnormally cold winters and various predatory beetles used for biological control may cause *A. tsugae* mortality, but are not a reliable control or are currently not dispersed at a large scale, respectively. Since *A. tsugae* is primarily sessile, the insect's health is closely related to the health of its hemlock host. We hypothesize that *A. tsugae* will exhibit greater population levels and better physiological health on lightly impacted trees than insects feeding on moderately impacted trees.

Chapter 2 assessed hemlock health using a combination of a Hemlock Health Index and new shoot growth. This method categorized all sampled trees as either lightly or moderately impacted by *A. tsugae*. We examined the effect of hemlock health on *A. tsugae* population density, survival from aestivation, and peak fecundity. *Sistens* adults laid more eggs on moderately impacted trees than on lightly impacted trees. However, *A. tsugae* density and survival from aestivation were not significantly different between hemlock health categories. *Sistens* adults from moderately impacted trees may have access to increased nutrient resources for increased egg production or may be laying many poor-quality eggs.

Chapter 3 compared *A. tsugae* biomass, total carbon, carbohydrate, total nitrogen, and amino nitrogen of insects collected from lightly and moderately impacted hemlocks. *A. tsugae* from lightly impacted trees had greater levels of carbohydrates, total nitrogen, and amino nitrogen

than did insects on moderately impacted trees. Lightly impacted trees, those in better health and probably longer shoot growth, may have greater levels of nutrients, creating a nutritious food source for the feeding insects. However, moderately impacted trees were not as healthy, and may not be able to provide *A. tsugae* with maximum nutrients.

During this study, we performed the first analysis of *A. tsugae* physiological parameters spanning the sistens generation maturation period. As would be expected, these insects assimilate nutrients in preparation of the oogenesis and oviposition of the next *A. tsugae* generation. Furthermore, our results indicate that *A. tsugae* feeding on lightly impacted trees may have better access to carbohydrates, nitrogen, and amino nitrogen than *A. tsugae* feeding on moderately impacted trees.

Future work. Although *A. tsugae* collected from lightly impacted trees appear to contain higher levels of nutrients, the fecundity study in Chapter 2 indicated that those insects on moderately impacted trees laid more eggs. It may be that *A. tsugae* on lightly impacted trees had access to more resources or may be producing fewer high-quality eggs than insects on moderately impacted trees. Due to the nature of this study and the small individual insect size, it was not possible to perform physiological assays on the same insects for which eggs were counted. This necessary variation between insect samples may account for the apparent contradictory nature of the results between Chapters 2 and 3.

Further assessments of the impact of *A. tsugae* on hemlock and the subsequent influence of hemlock on *A. tsugae* are needed to understand at the population and physiological level. A controlled setting (such as a hemlock farm) may allow for better distinctions between tree health and degree of *A. tsugae* infestation. Additional assays could examine *A. tsugae* total protein and

lipid content, and further studies could examine the macronutrient composition of hemlock needles and shoots.

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