ABIOTIC AND BIOTIC FACTORS INFLUENCING EASTERN HEMLOCK (*Tsuga canadensis*) HEALTH AND HEMLOCK WOOLLY ADELGID (*Adelges tsugae*) SUCCESS IN THE SOUTHERN APPALACHIAN MOUNTAINS

by

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(Under the Direction of Kamal J.K. Gandhi)

ABSTRACT

For over 30 years, the exotic hemlock woolly adelgid (*Adelges tsugae* Annand) (Hemiptera: Adelgidae) has caused widespread deterioration of eastern hemlock [*Tsuga canadensis* (L.) Carrière] in the eastern U.S. In the southeastern portion of the range, this infestation is expected to cause the local extinction of *T. canadensis*, a foundation species in the Southern Appalachian Mountains. *Tsuga canadensis* and *Adelges tsugae* were examined at the southernmost part of *T. canadensis*'s contiguous range in the Chattahoochee National Forest of Georgia to better understand the effects of abiotic and biotic factors in this region. *Adelges tsugae* were exposed to high temperatures (20 – 40°C) to assess if summer temperatures observed at the edge of the Southern Appalachians influence *A. tsugae* survivorship. Results from laboratory bioassays found a positive correlation between increasing temperatures and duration of exposure (2 – 8 days), and *A. tsugae* mortality. The effects of high temperatures were cumulative and the ability of *A. tsugae* to survive markedly decreased above 30°C. Field observations supported these results as *A. tsugae* mortality (9 – 63%) increased at warmer sites. A predictive model was created that can be used to estimate percent *A. tsugae* mortality based on temperature recordings from *T. canadensis* sites. We also documented the rate of *T. canadensis* dieback and *A. tsugae* densities over a four-year period, and factors that may influence these rates/densities. The average dieback rate of *T. canadensis* in Georgia (6.3% year^{-1}) was found to be lower than rates reported in other locations in the Southeast (20 – 33% year^{-1}). None of the sampled stand or tree characteristics (e.g. diameter, elevation, years infested, and *A. tsugae* density) were found to significantly affect *T. canadensis* dieback rates. *Adelges tsugae* densities were positively influenced by the length of *T. canadensis* new growth. When comparing release and non-release sites, biological control beetles were not found to significantly affect *T. canadensis* dieback rates or *A. tsugae* densities.

INDEX WORDS: *Adelges tsugae*, biological control, Chattahoochee National Forest, eastern hemlock, hemlock woolly adelgid, supraoptimal temperatures, *Tsuga canadensis*

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DEDICATION

I dedicate this dissertation to my family (and framily) that have given me the support and love necessary to accomplish greatness – to the Mechs, the Brashers, the Goons, the Ashevillians, and the Warnellians.

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DISSERTATION INTRODUCTION AND LITERATURE REVIEW

1.1 Exotic Forest Insects

All around the world, insects and pathogens have been accidently introduced into naïve forest systems. These introductions may cause a multitude of negative ecological effects including an alteration in biodiversity, tree defoliation and/or death, temporary reduction in photosynthesis, variation in light and microclimate conditions, shifts in ecosystem processes, nutrient leaching, and decreases in shelter and/or food resources for birds and mammals (Liebhold et al. 1995, Vitousek et al. 1996, Jenkins et al. 1999, Yamasaki et al. 2000, Pimental et al. 2005, Eschtruth et al. 2006, Lovett et al. 2006, Gandhi and Herms 2010). The main stages for biological invasion are transport and introduction, establishment, spread, and impact (Liebhold 1995, Lockwood et al. 2013). Insects are typically introduced anthropogenically via the movement of people and goods, with the majority of forest pests transported by way of air and maritime cargo on live plant or timber material (Work et al. 2005, Liebhold et al. 2012, Lockwood et al. 2013). Once released into a new habitat, non-native insects must then establish as a self-sustaining population or suffer extinction. In general, establishment requires a number a favorable factors including suitable hosts, available mates (if reproduction occurs sexually), optimal environmental conditions, naïve host(s) with lack of defenses/resistance, absence of interspecific competition, and reduction in natural enemies (Liebhold et al. 1995, Niemelä and Mattson 1996, Lovett et al. 2006, Lockwood et al. 2013). Once established, the population can then increase in size and expand its geographical range.

It has been found that establishment rate of introduced non-native species is relatively low (2 – 11%), but the probability of an established species spreading can be over 50%, resulting in the potential for widespread impacts (Williamson and Fitter 1996, Jeschke and Strayer 2005). Overall, approximately 50,000 non-native species have been introduced in the United States, the majority of which were intentional (food crops, livestock, biological control etc.). However, of the > 4500 established non-native insects in the United States, about 95% were accidental (Pimental et al. 2002). More than 350 exotic insects have become established in U.S. forests with approximately 30% of them considered serious pests. Economic losses from non-native forest insects cost $2.1 billion per year based on the costs of border inspections, eradication programs, control methods, loss of property values, reduced productivity, and habitat restoration (Pimental et al. 2002, Pimental et al. 2005, Aukema et al. 2010).

In an effort to conserve native trees and forests, a number of methods have been used against exotic forest pests including exclusion, quarantine, biological and chemical control, and silvicultural practices (Liebhold et al. 1995, Lockwood et al. 2013). In addition, multiple policies and practices have been created by global governments to prevent further damage to ecosystems by non-native insects. For example, in 1999, the interagency National Invasive Species Council was established in the U.S. As a result, there have been advancements in the prevention (e.g. phytosanitary measures and importation restrictions), detection (e.g. Early Detection Rapid Response and Cooperative Agricultural Pest Survey programs), and control of exotic invasive species. Also, the council has focused attention and funding to projects for the restoration of high-value ecosystems (US Department of the Interior 2008).

1.2 Study Organism: Eastern Hemlock

Eastern hemlock [*Tsuga canadensis* (L.) Carrière], an evergreen tree in the Pinaceae family, is found in eastern North America from Canada down to Georgia and east of Alabama, Kentucky, Indiana, Minnesota, Tennessee and Wisconsin, where it is typically a dominant or codominant species in coniferous or mixed hardwood stands (Godman and Lancaster 1990). The southern limit of *T. canadensis*'s contiguous range is located in the Chattahoochee National Forest of north Georgia's Blue Ridge Mountains. At maturity, *T. canadensis* is one of the largest trees in eastern North America with specimens generally reaching 18 – 21 m tall and having a diameter greater than 100 cm; the largest diameter and height recorded was 213 cm 49 m respectively (Godman and Lancaster 1990). The bark of *T. canadensis* is relatively thin, especially on young trees, making them highly susceptible to even low intensity fires, and their potentially shallow root system makes them vulnerable to drought conditions (Godman and Lancaster 1990, Brown and Smith 2000).

One of the most important characteristics of *T. canadensis* is that it is amongst the most shade tolerant tree species, with seedlings being able to survive and grow in as little as 5% full sunlight (Godman and Lancaster 1990). *Tsuga canadensis* is also a slow growing, late successional tree that can take up to 300 years to reach maturity and specimens have been found to live more than 600 years (Hough and Forbes 1943, Godman and Lancaster 1990). Based on these characteristics, *T. canadensis* can form dense and nearly homogenous stands with unique microenvironments. Typically, the environment within stands is dark and cool with acidic soils and diverse understory vegetation (Godman and Lancaster 1990). In the Southeast, *T. canadensis* is considered a foundation species, typically in riparian and cove habitats where they are found in narrow bands along year-round streams (Ellison et al. 2005, USDA Forest Service 2005).

Historically, *T. canadensis* bark was extensively used for tanning leather, but the creation of synthetic products has eliminated the need for the bark. The wood of *T. canadensis* is not considered commercially valuable due to its brittle and inferior quality, but can be used for pulp production as well as fences, barns, crates, sheds, etc. (Godman and Lancaster 1990, Taylor 1993). Aesthetically, *T. canadensis* is one of the most widely used horticultural species, with over 200 cultivars being used for ornamental purposes including dwarf, weeping, and variegated forms (Godman and Lancaster 1990, Taylor 1993).

Tsuga canadensis is an ecologically valuable species, providing food and shelter to wildlife, and shade/thermal protection to aquatic ecosystems (Godman and Lancaster 1990, Yamasaki et al. 2000, Tingley et al. 2002). In the northeastern U.S., 96 bird and 47 mammal species were found to be *T. canadensis* associates, with a portion of each (8 – 10) considered strongly associated (Yamasaki et al. 2000). For example, *T. canadensis* provides a winter seed source, roosting locations, hunting perches and nesting cavities for birds. In addition, white-tail deer and moose browse on *T. canadensis* and other wildlife use the trees for warmth and shelter from the snow during the winter months (Godman and Lancaster 1990, Yamasaki et al. 2000). In addition, *T. canadensis* can moderate stream temperatures and flows, creating a unique habitat that is utilized by a number of fish and aquatic arthropod species (Ross et al. 2003, Hadley et al. 2008, Rohr et al. 2009).

Overall, there are at least 25 insects that can colonize *T. canadensis*. These include native insects, such as the hemlock borer (*Melanophila fulvoguttata* Harris), hemlock looper (*Lambdina fiscellaria* Guenée), and spruce budworm (*Choristoneura fumiferana* Clemens), as well as non-native insects such as the elongate hemlock scale (*Fiorinia externa* Ferris), hemlock woolly adelgid (*Adelges tsugae* Annand), and gypsy moth (*Lymantria dispar* L.) (Godman and

Lancaster 1990). Of these, *A. tsugae* is the most economically and ecologically damaging pest, the stress from which can then lead to a *M. fulvoguttata* outbreak resulting in a more rapid mortality of *T. canadensis*.

1.3 Study Organism: Hemlock Woolly Adelgid

Adelgids (Hemiptera: Adelgidae) are a group of sap-sucking insects in the Northern Hemisphere that feed exclusively on conifers in the Pinaceae family (Havill and Foottit 2007). It is believed that the diversity of Adelgidae is a result of cospeciation with host trees and their ability to utilize up to five generations based on ecological niches or geographic regions (Steffan 1964, Havill and Foottit 2007). *Adelges tsugae* has been historically associated with all worldwide species of hemlock (*Tsuga* spp.) except those found in eastern North America – *T. canadensis* and Carolina hemlock (*T. caroliniana* Engelm.). The lack of coevolution between these hemlock species and *A. tsugae* is believed to be the main reason why these trees are susceptible to *A. tsugae* attack. In its native ranges, *A. tsugae* does not cause serious damage to the hemlock hosts. This is partially due to *Tsuga* evolving a tolerance of *A. tsugae* and partially due to natural predators evolving alongside populations of *A. tsugae* (Havill et al. 2006).

Adelges tsugae was first documented in North America near Richmond, Virginia in 1951 where it was accidentally introduced from Japan (McClure 1989, Havill et al. 2006). Since then, *A. tsugae* has expanded its range slowly and by 2005 it was found encompassing 25.6% of *T. canadensis*'s and 100% of *T. caroliniana*'s ranges (Morin et al. 2005). Currently, *A. tsugae* can be found in 19 states and, based on the contiguous availability of the host, is approaching the edge of its potential distribution in the south and west (Morin et al. 2009, USDA Forest Service 2013). Low winter temperatures have been found to cause *A. tsugae* mortality indicating that the northern distribution of the pest may be limited in North America (Parker et al. 1998, 1999, McClure and Cheah 2002). *Adelges tsugae* infestation in the state of Georgia was first noticed in 2002 in the northeastern region of the Chattahoochee National Forest and has continuously spread in a southern and westward direction (Fig 1.1) (Georgia Forestry Commission 2012).

Adelges tsugae has a complex lifecycle that includes multiple parthenogenetic generations and a rapid growth rate (Havill and Foottit 2007). The sistens generation, which hatches during the early summer, undergoes aestivation (summer diapause) as first instars and actively feeds through the fall and winter. Eggs laid by the sisten mothers, typically 50-175 eggs per ovisac, will either become the wingless progredien generation or the winged sexuparae generation. The progredien generation does not enter diapause, has a much shorter lifecycle, and typically lays fewer eggs (25-100) (McClure 1989, McClure et al. 2003, Cheah et al. 2004). The sexuparae generation, which is usually produced during overcrowded conditions, requires a spruce (*Picea* spp.) host to produce eggs on, but there are no suitable spruce species present in North America that they are able to continue development on (McClure 1989, McClure 1991, McClure et al. 2003, Havill and Foottit 2007). Because temperature influences the development rates of *A. tsugae*, locations in the southeastern U.S. may see earlier hatches of the generations caused by milder winters (Fig. 1.2) (McClure et al. 2003). While feeding, both the sisten and progredien generations produce a protective, waxy, wool-like flocculence that covers the nymphs, adults and eggs, allowing the minute insects (0.5 – 1.3 mm) to become easier to detect (Havill and Foottit 2007).

Due to their small size, *A. tsugae* crawlers and eggs can passively spread by wind, birds, animals, or humans (McClure 1990). Once hatched, an *A. tsugae* crawler will be mobile for a few days searching for a *T. canadensis* needle base, preferring to colonize the youngest twigs available (McClure 1991, McClure et al. 2003). Once found, *A. tsugae* settle by inserting its stylet bundle intercellularly, terminating inside the xylem ray parenchyma cells where they feed on the tree's stored nutrients (Young et al. 1995, McClure et al. 2003). Feeding by large numbers of *A. tsugae* results in the reduction or halting of new growth production, bud mortality, twig dieback, and defoliation of *T. canadensis*. The reduction in host quality in turn causes *A. tsugae* populations to crash, which allows *T. canadensis* to produce new growth and then *A. tsugae* populations to rebound. These peaks and crashes in *A. tsugae* densities will continue until mortality of the tree eventually occurs, as early as 3-5 or as long as 15 years (McClure 1991, Orwig and Foster 1998, McClure et al. 2003). *Tsuga canadensis* has no known resistance or defense against *A. tsugae*, and no tree or forest stand characteristics have been shown to influence mortality (McClure 1991, McClure et al. 2003, Faulkenberry et al. 2009).

1.4 Hemlock Woolly Adelgid Impacts and Control Methods

Since the 1990's, when *Adelges tsugae* spread into the natural range of *T. canadensis* and began to cause tree mortality, researchers have been looking at the implications of losing a foundation species in forest ecosystems. Because all size and age classes of *T. canadensis* can be attacked by *A. tsugae,* and because seeds do not remain viable in the forest floor beyond one year, successful regeneration of *T. canadensis* is unlikely to occur which would result in a permanently altered forest composition (Marquis 1975, Orwig and Foster 1998, Ellison 2014). Observational and manipulative projects have shown that there are many impacts from *T. canadensis* loss. For example, 1) salamander densities temporarily decreased (Brooks 2001), 2) pulses in stand litterfall (Ellison 2014), 3) understory light availability increased resulting in a shift in vegetation (Eschtruth et al. 2006), 4) a decrease in the uptake of carbon that is not predicted to rebound until after 2030 (Albani et al. 2010), 5) an increase in stand soil and air temperatures that can influence soil moisture levels and decomposition rates (Cobb et al. 2006, Lustenhouwer et al. 2012), 6) an increase in available inorganic nitrogen and nitrification rates (Jenkins et al. 1999), and 7) an increase in transpiration/water use when the stand transitions to a deciduous forest that can result in lower water yields (Daley et al. 2007, Brantley et al. 2014). In summary, the traditionally dark, cool, moist, coniferous *T. canadensis* forests will eventually become brighter, warmer, dryer, deciduous forests.

Although *T. canadensis* is typically concentrated in riparian areas in the Southern Appalachian Mountains, the overall number of trees is relatively lower in Georgia. Along with the fact that *T. canadensis* mortality will be gradual, it indicates that the effects on streams (i.e. temperature, species composition, etc.) will be minimal to immeasurable (USDA Forest Service 2005). In addition, dead *T. canadensis* can benefit birds by providing increased available habitat, and increased rhododendron (*Rhododendron* spp.) and mountain laurel (*Kalmia latifolia* L.) can give suitable cover for wildlife during the winter. It is not feasible to save all *T. canadensis* trees, but various measures are currently being used in North America to control *A. tsugae* populations and/or conserve the genetic variability of *T. canadensis* including chemical and biological control of *A. tsugae*, as well as hybridization, seed collecting and planting of conservation orchards of *Tsuga* spp.

The most widely used chemical treatment is imidacloprid, a neonicotinoid that affects the central nervous system of insects eventually causing mortality. Both soil injections and soil drenches of imidacloprid have been found to be highly effective at reducing *A. tsugae* populations (Webb et al. 2003, Cowles et al. 2006). However, imidacloprid can have negative non-target effects on insects associated with *T. canadensis* and other soil or aquatic organisms (Dilling et al. 2009, Knoepp et al. 2012). In addition, applying chemical control over large forest landscapes is impractical, time consuming, and expensive. The current hope for suppressing *A. tsugae* populations in North American forests lies with natural enemies.

Although native predators such as tooth-necked fungus beetles (Derodontidae), lacewings (Chrysopidae), flower flies (Syrphidae), and midges (Cecidomyiidae) have been found attacking *A. tsugae* in eastern North America, they were not abundant enough to cause a significant reduction in *A. tsugae* densities (McClure 1987, Wallace and Hain 2000, Zilahi-Balogh 2005). Because of this, classical biological control, the introduction of exotic natural enemies from the native range of *A. tsugae*, has become widely researched and utilized (Cheah et al. 2004). This control method, versus chemical control, is feasible in large forested areas, self-perpetuating and a long-term method with minimal non-target effects (Perrings et al. 2000). When successful, biological control is an inexpensive method of reducing pest densities below economic injury levels (Debach and Rosen 1991). The two most abundant predatory beetles released since the late 1990's to combat *A. tsugae* are *Sasajiscymnus tsugae* Sasaji and McClure from Japan and *Laricobius nigrinus* Fender from British Columbia (McClure 1995, Sasaji and McClure 1997, Zilahi-Balogh et al. 2002, Cheah et al. 2004). The life cycles of these predatory beetles overlap and are synchronized with that of *A. tsugae* so that there is the potential for predation on all life stages throughout the entire year – *S. tsugae* feeds during the spring and summer, and *L. nigrinus* feeds during the fall and winter (Sasaji and McClure 1997, Zilahi-Balogh et al. 2002, Cheah et al. 2004).

Multiple studies have highlighted the potential for *S. tsugae* to successfully reduce *A. tsugae* populations in the field, with lower *A. tsugae* densities observed following releases and when compared to non-release sites (McClure and Cheah 1999, Cheah et al. 2004, Asaro et al. 2005). In addition, *S. tsugae* are established and spreading in forest stands, and *T. canadensis* has exhibited signs of recovery years following releases (Cheah et al. 2004, McDonald et al. 2008, Hakeem et al. 2010, Cheah 2011, Jones et al. 2014). *Laricobius nigrinus* has also been shown to establish in forest stands, promote healthier *T. canadensis*, and reach population levels high enough to create field insectaries (Mausel et al. 2010, McDonald et al. 2011, Jones et al. 2014). With both species, it appears that at least 5-7 years post establishment are required for densities to reach high enough levels where they may successfully reduce *A. tsugae* populations below their injury level (Hakeem et al. 2010, McDonald et al. 2011). Research has continued to improve rearing tactics for natural predators and new species are being considered and released (e.g. *L. osakensis* Montgomery and Shiyake) (Vieria et al. 2011). It has been shown that the probability of establishment increases with an increase in the number of releases, so continued years of releases will most likely result in more biological control beetle populations becoming established and subsequently effective (Mausel et al. 2010).

To conserve the genetic resources of native *Tsuga* spp., seeds from *T. canadensis* and *T. caroliniana* have been collected for over a decade and ex situ orchards have been planted in *A. tsugae*-free locations in Brazil, Chili, and the Ozark Mountains of Arkansas (Jetton et al. 2008). It is hoped that the orchards will provide a resource for the potential restoration of these threatened *Tsuga* spp. In addition, research has successfully cryostored embryogenic cultures from both *T. canadensis* and *T. caroliniana* that can be recovered for future restoration projects (Merkle et al. 2014). Creating *A. tsugae* resistant hemlocks through hybridization is another focus of research and conservation efforts. Unfortunately, there is the hybridization downfall that only *T. caroliniana* can cross breed with the resistant Asian *Tsuga* spp. (Bentz et al. 2002). This is due to the fact that *T. caroliniana* is not a sister species to the other eastern North American species (*T. canadensis*), but was found to be phylogenetically nested in the Asian *Tsuga* spp.

clade (Havill et al. 2008). *Tsuga caroliniana*, typically found on dry ridges and rocky slopes, is not a riparian species that could ecologically replace *T. canadensis*, but *T. caroliniana* hybrids can be used as an ornamental landscape replacement tree (Humphrey 1989, Montgomery et al. 2009). Researchers are also examining *A. tsugae* devastated *T. canadensis* stands for healthy trees that may be putatively resistant (Caswell et al. 2008). Clones from these trees may provide another source for future reforestation projects.

1.5 Dissertation Objectives

The goal of this research is to better understand the biotic and abiotic factors influencing an invasive forest pest and a susceptible host in the southeastern U.S. The results of these studies may facilitate better pest management by recognizing trends and allowing for better interpretations of the future of *T. canadensis* and *A. tsugae* at the southernmost portion of their range. The specific objectives of this study are to:

1) To examine and quantify the thermal ecology of *A. tsugae* in the southernmost limit of *T. canadensis*'s range by determining *A. tsugae* mortality when exposed to above optimal temperatures.

2) To assess the temporal patterns and factors, including biological control, affecting *T. canadensis* health and *A. tsugae* densities in Georgia.

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Figure Legend

Figure 1.1. Yearly spread of *Adelges tsugae* in Georgia, U.S.

Figure 1.2. Lifecycle of *Adelges tsugae* observed in Georgia, U.S. Modified from figure created by Cheah et al. (2004).

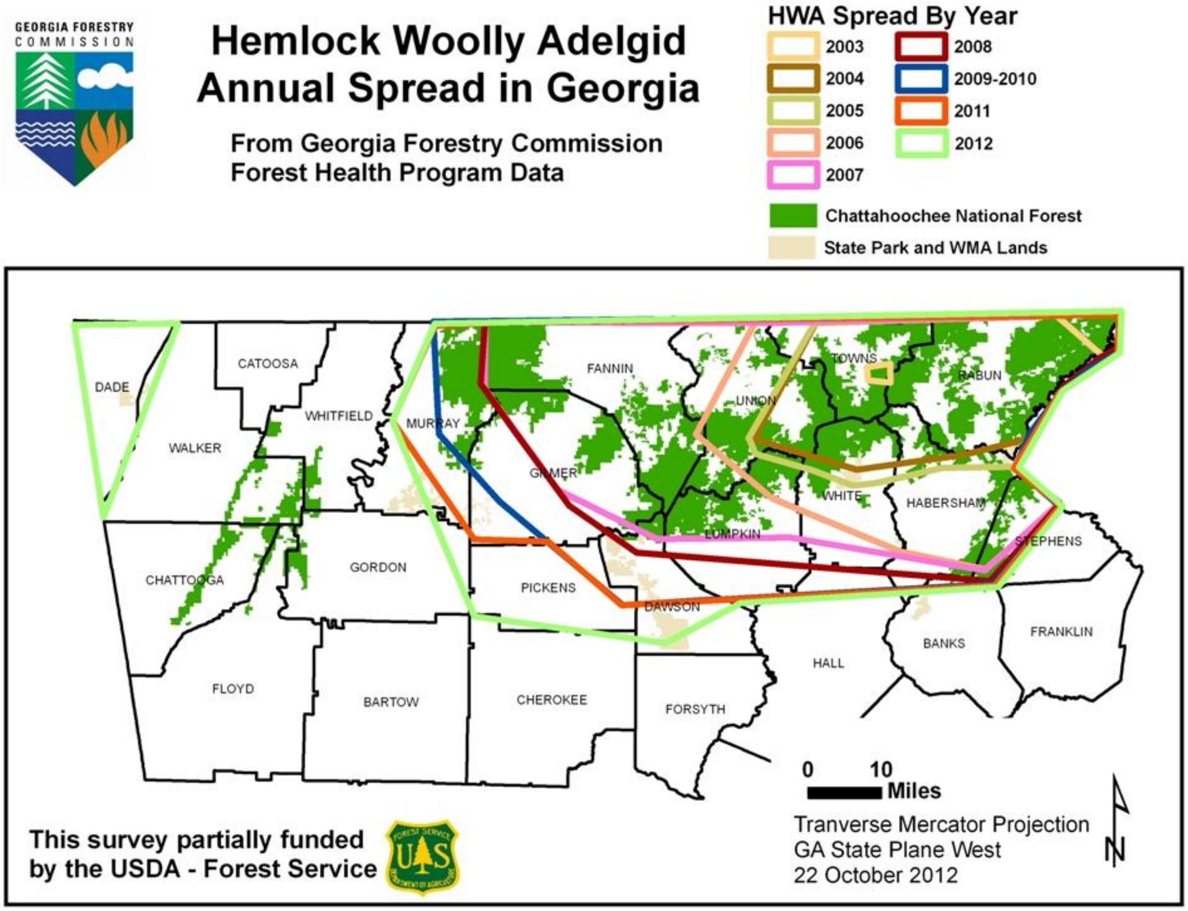
GEORGIA FORESTRY COMMISSION
Hemlock Woolly Adelgid Annual Spread in Georgia
From Georgia Forestry Commission Forest Health Program Data
HWA Spread By Year
2003
2004
2005
2006
2007
2008
2009-2010
2011
2012
Chattahoochee National Forest
State Park and WMA Lands
DADE
CATOOSA
WHITFIELD
MURRAY
FANNIN
UNION
TOWNS
RABUN
WALKER
GILMER
WHITE
HABERSHAM
LUMPKIN
STEPHENS
CHATTOOGA
GORDON
PICKENS
DAWSON
BANKS
FRANKLIN
HALL
FLOYD
BARTOW
CHEROKEE
FORSYTH
0
10
Miles
Tranverse Mercator Projection
GA State Plane West
22 October 2012
N
This survey partially funded by the USDA - Forest Service

Figure 1.1

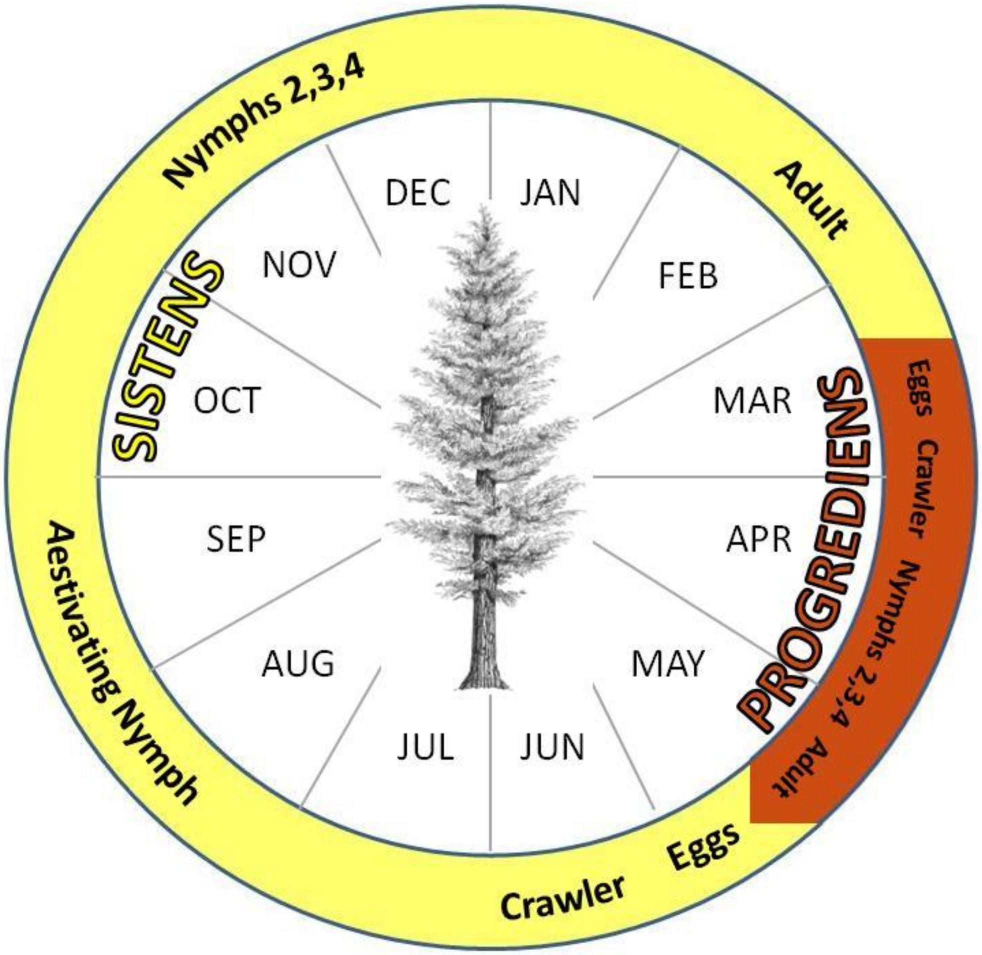
Nymphs 2,3,4
Adult
SISTENS
PROGREDIENS
Eggs
Crawler
Nymphs 2,3,4
Adult
Eggs
Crawler
Aestivating Nymph
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV

Figure 1.2

CHAPTER 2

EFFECT OF SUPRAOPTIMAL TEMPERATURES ON AESTIVATING HEMLOCK WOOLLY ADELGID (*ADELGES TSUGAE*) IN THE SOUTHERN APPALACHIANS[1]

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[1] A M Mech, Tobin P C, Teskey R O, Rhea J, and Gandhi K J K. To be submitted to *Biological Invasions*

Abstract

Higher temperatures predicted under current climate change models are expected to have an overall positive effect on the success of invasive forest pests by increasing biological attributes such as survivability, geographic range, and fecundity. These changes in life history factors may result in negative cascading effects on forested ecosystems. Low temperatures are typically used for predicting forest pest distributions, and consequently, in determining the range where host trees will be protected and maintained. However, heat may also be a limiting factor for invasive insects that undergo summer diapause, such as the exotic hemlock woolly adelgid (*Adelges tsugae* Annand) (Hemiptera: Adelgidae) which is causing widespread mortality of eastern hemlock [*Tsuga canadensis* (L.) Carrière] in eastern North America. We examined the thermal ecology of *A. tsugae* in the southernmost limit of *T. canadensis*' contiguous range by determining *A. tsugae* survivorship under increasing temperature regimens and heat waves. A positive correlation was found between increasing temperatures, duration of exposures, and *A. tsugae* mortality. These results showed evidence for a cumulative effect of temperature. *Adelges tsugae* mortality was minimal (< 20%) when exposed to 20° and 25°C, but markedly increased at temperatures above 30°C (up to 100%). All temperatures resulted in a significant increase in *A. tsugae* mortality over time and similar trends were exhibited in field populations. Field mortality of *A. tsugae* ranged from 8.54% to 62.79% in *T. canadensis* stands in the Chattahoochee National Forest, Georgia and was strongly correlated with temperature regimes. Using the field results, a predictive model was created to calculate the estimated percent mortality of *A. tsugae* based on site heat indices. In response to lower *A. tsugae* densities, *T. canadensis* appears to exhibit signs of possible tolerance rather than the anticipated rapid dieback and mortality. This study shows the potential of climate change to maintain pockets of *T. canadensis* at the southernmost edge of their natural range due to the negative effects of higher temperatures on *A. tsugae*.

Keywords: *Adelges tsugae*, aestivation, supraoptimal temperatures, *Tsuga canadensis*

2.1 Introduction

By the end of the 21st century, it is predicted that global surface temperatures may increase by more than 2°C (IPCC 2013). Further, there may be major alterations in the amount and intensity of precipitation and other precipitation-related disturbance events (e.g. hurricanes and ice storms) that will present challenges for biotic communities and populations. One of the major taxa that are expected to directly and/or indirectly respond to climate changes are herbivorous insects. Although responses to temperatures are species-specific and variable, for most insects, warmer temperatures will be favorable by increasing voltinism (Tobin et al. 2008, Stoeckli et al. 2012), decreasing winter mortality (Ayres and Lombardero 2000), and increasing distributions to higher latitudes and altitudes due to warmer winter climates (Musolin 2007, Cudmore et al. 2010). Climate change may also have negative effects on insects due to asynchronicity with their host (Bale et al. 2002), changes in host plant material chemistry (Ayres and Lombardero 2000), and range retraction due to warmer summer temperatures (Tobin et al. 2014).

One way insects have adapted to extreme weather conditions is to conserve energy by entering a state of hibernation (diapause) where development is typically halted and metabolism is significantly decreased (Tauber et al. 1986, Hahn and Denlinger 2007). This strategy allows insects to survive unfavorable changes in their environment such as extreme temperatures or low food abundance/quality (Masaki 1980). Although aestivation (summer diapause) is not as prevalent as winter diapause, it is a life cycle component of many major insect orders (e.g. Coleoptera, Diptera, Hemiptera, and Lepidoptera) and is found to occur in most parts of the world (Masaki 1980). Temperature plays a large role in the mechanics of diapause by influencing the initiation, length, and termination of dormancy. Although reduced, insect metabolism remains responsive to temperature; as temperatures increase, metabolic rates increase. This may add stress to aestivating insects whose metabolic reserves can be depleted by summer temperatures, thus influencing post-aestivation survival (Tauber and Tauber 1976, Masaki 1980, Hahn and Denlinger 2007). Little work has been done to examine how increasing summer temperatures under climate change regimes will influence aestivating insects, especially non-natives in North America.

The hemlock woolly adelgid (*Adelges tsugae* Annand) (Hemiptera: Adelgidae) is the most important aestivating exotic invasive insect present in eastern North America. *Adelges tsugae* is a small, sessile, sap-sucking insect native to Asia and western North America that is causing widespread mortality of both eastern and Carolina hemlocks [*Tsuga canadensis* (L.) Carrière and *T. caroliniana* Engelmann, respectively] in their native range (McClure et al. 2003, Havill et al. 2006, Havill and Foottit 2007). Since its introduction from Japan into Virginia in the 1950's, *A. tsugae* has spread in all directions and can now be found in 19 states (Fig. 2.1, USDA Forest Service, 2013). Once infested, tree decline and subsequent mortality can occur in as little as 4-5 years (McClure 1991, Orwig and Foster 1998).

Tsuga canadensis, a foundation species in many eastern North American forests, is an ecologically valuable species that plays an important role in regulating community dynamics and ecosystem processes (Ellison et al. 2005, Ellison 2014). The unique characteristics of *T. canadensis*, including shade-tolerant, late-successional, and long-lived, contribute to the eventual establishment of *T. canadensis* as a dominant tree in forest stands (Godman and Lancaster 1990). *Tsuga canadensis* stands are characterized by having a cool, damp microclimate, nutrient-poor acidic soils with diminished understory vegetative cover, low light levels in the understory, and a reasonably stable forest composition. In its southern range, *T. canadensis* is typically limited to cool moist coves and riparian areas with good drainage (Rogers 1978, Godman and Lancaster 1990). *Tsuga canadensis* is considered to be a foundation species, and no co-existing conifer can mimic the characteristics necessary to maintain their unique ecosystem (Ellison et al. 2005).

The impact of *A. tsugae* on hemlock forest ecosystems have been, and will continue to be, high. In forests with *T. canadensis* decline and mortality there are: 1) decreases in *T. canadensis* canopy and a transition from a coniferous to deciduous forest structure (Orwig and Foster 1998, Small et al. 2005, Orwig et al. 2008); 2) increases in available light to the forest floor and alterations in vegetative composition (Eschtruth et al. 2006); 3) decreases in soil moisture and changes in soil nitrogen cycling (Jenkins et al. 1999, Orwig et al. 2008); 4) changes in the carbon and nutrient cycles (Nuckolls et al. 2009); 5) local losses in hemlock-dependent populations of birds, mammals, and arthropods (Yamasaki et al. 2000, Tingley et al. 2002, Rohr et al. 2009); and 6) alterations in stream hydrology, species composition, and water temperature regimes (Snyder et al. 2002, Ross et al. 2003, Roberts et al. 2009, Brantley et al. 2013).

The parthenogenetic and multivoltine life cycle of *A. tsugae* in North America allows the population to rapidly increase and spread (Havill and Foottit 2007). The sistens generation, present from summer to the following spring, lay eggs that will either become the wingless progredien generation or the winged sexuparae generation. The sexuparae generation requires a spruce (*Picea* spp.) host to produce eggs on, but they are unable to continue development on any of the spruce species present in North America (McClure 1989, McClure et al. 2003). The temporal presence and developmental rates of *A. tsugae* generations are significantly influenced by temperatures (McClure et al. 2003). For example, at more southern latitudes, sistens, the aestivating generation, can hatch as early as mid-spring (*A. Mech, personal observation*).

When sisten eggs hatch, the mobile first instar nymphs (crawlers) will search for and eventually settle at a needle base, preferring to colonize the youngest available twigs (McClure 1991, McClure et al. 2003). Once a needle has been selected, *A. tsugae* will insert their stylet bundle at the abscission layer of the needle until they reach the xylem ray parenchema cells where the host tree transfers and stores nutrients (Zimmermann and Brown 1971, Young et al. 1995). Following settlement, the sisten *A. tsugae* will enter aestivation which will last for 4-6 months. Typically in October, *A. tsugae* will emerge from aestivation, molt, and begin to produce their characteristic wool while continuing to develop and produce the progredien progeny. The progredien generation follows identical trends to the sisten generation, but with the four instars reaching maturity without aestivation (McClure 1989, McClure et al. 2003). Currently, *T. canadensis* and *T. caroliniana* have no known resistance or defense against *A. tsugae*, which will cause mortality regardless of size, age, or location of the tree (McClure 1991, McClure et al. 2003, Faulkenberry et al. 2009).

The *A. tsugae*/*T. canadensis* system is ideal to test the effects of supraoptimal temperatures on an aestivating insect because *A. tsugae* settles on the host tree for the duration of aestivation without their protective wool, receives direct exposure, and is unable to escape temperature extremes. Also, the range of *T. canadensis* encompasses a multitude of climate zones, from zone 3b in Maine to zone 8a in Georgia, with the warmest climates occurring at the southern-most edge of the Southern Appalachian Mountains in northern Georgia (USDA Agricultural Research Service 2012). Our research objectives were to quantify the effects of supraoptimal temperatures on the survival of an aestivating, exotic forest pest and to create a predictive model that can be used to calculate the estimated proportion of mortality from stand heat indices.

2.2 Methods

2.2.1 Laboratory Experiments

Tsuga canadensis twigs were collected around Raper Creek (34°45.024N, -83°34.739W) in the Chattahoochee National Forest, Habersham County, Georgia. Collections were made from a single site to reduce potential effects caused by differences in environmental conditions at multiple locations. A total of 130 twigs were collected from four trees in July 2011 when *A. tsugae* were aestivating, and each twig's offshoots had 2-4 cm of new growth to ensure preferred conditions for *A. tsugae* sistens. Aestivating *A. tsugae* present on the new growth of each twig were evaluated and the needles with those deemed to be alive at the node were marked (13-27 *A. tsugae* twig^{-1}). Vitality was determined by the convexity of the body and the bright white coloration of the dorsal wool; an extra ten twigs were examined to determine the accuracy of this method. Needle nodes where more than two *A. tsugae* settled were not included in the experiment as intraspecific competition could be a confounding factor.

Marked twigs were placed in 55.5 ml vials with wet florist block to prevent desiccation and then randomly assigned both a temperature and a duration treatment using a random number generator. Since *T. canadensis* in the southeastern U.S. is typically found in cool, high elevation areas, three summer temperatures were selected that encompassed typical (20°C), occasional (30°C), and rare (40°C) conditions in *T. canadensis* stands (Godman and Lancaster 1990). In addition, four durations (2, 4, 6, and 8 days) were tested to determine if the length of exposure to the specific temperatures was a factor in *A. tsugae* heat tolerance. Ten twigs per temperature-time treatment were placed in growth chambers (n = 3) (Model GC36, Environmental Growth Chambers, Chagrin Falls, Ohio) with 55% humidity and a daily 14 light and 10 hour dark periods. A decrease of 5°C was implemented in all treatments during the dark phase to mimic cooling temperatures after sunset. A total of 2,164 *A. tsugae* were examined for this portion of the study.

Adelges tsugae were examined immediately following their temperature-time treatment to assess survival. Survivorship was assessed by using up to three steps: 1) *A. tsugae* were gently pulled out from the plant tissue about half the length of their stylet bundle and observed for body movement caused by attempted stylet reinsertion; 2) if no body movement was observed, *A. tsugae* were fully removed from the plant tissue and examined for approximately 15 sec for stylet movement; and 3) if no stylet movement was observed, *A. tsugae* were crushed to see if appropriate viscosity and color of the hemolymph were present (i.e. deep red-purplish). *Adelges tsugae* that were concave, desiccated, and had no movement were considered dead. The proportion of dead *A. tsugae* from the total number marked was calculated for each twig and used in analyses.

An additional experiment was conducted in August 2011 using 25/20°, 30/25°, and 35/30°C light/dark period temperatures for 2, 4, 6, and 8 days to provide a finer-level gradient for *A. tsugae* responses to temperature conditions. A total of 130 *T. canadensis* twigs were collected from three trees at Raper Creek, Georgia, and the same bioassay was performed. A total of 2,051 *A. tsugae* were examined for this part of the study and the method for determining *A. tsugae* vitality was found to be 95.7% and 98.5% accurate for July and August samples respectively.

2.2.2 Field Studies

To test if the effect of temperature observed in the growth chambers were comparable to those found in forest stands, *A. tsugae* were examined from sites in the Chattahoochee National Forest that varied in temperature regimens. Temperature data loggers (HOBO UA-001-64, Onset

Computer Corporation, Bourne, Massachusetts) were placed in 18 *T. canadensis* stands (Appendix A) to record hourly temperatures during 2011 – 2013. A total of nine sites were sampled – three in late July 2012 and six in late November 2012 (Fig. 2.1). At each site, four trees within 30 m of the temperature logger were randomly selected and four twigs per tree were collected. To assess if variations in microclimate caused by aspect may play a role in *A. tsugae* mortality, half of the collected twigs were north-facing and half were south-facing. To ensure that the progredien generation was not included in counts, only the *A. tsugae* present on new growth were examined. Sixteen twigs collected per site were examined within 24 hrs, and the number of live and dead *A. tsugae* were recorded. In July, *A. tsugae* were deemed alive using the methods described in section 2.2.1, while in November, *A. tsugae* were deemed to have survived aestivation if they molted and initiated wool production. To reduce variability based on where *A. tsugae* settled, the mortality proportion was calculated by examining all *A. tsugae* present on the new growth section(s). If a minimum number of *A. tsugae* was not reached by one new growth section, additional sections were examined in their entirety until that number was reached. In July, when *T. canadensis* had less new growth compared to November, sections were analyzed until a minimum of 25 *A. tsugae* were reached ($\bar{x} = 33.4 \pm 0.6$ *A. tsugae* twig^{-1}). In November, sections were examined until a minimum of 45 *A. tsugae* were reached ($\bar{x} = 70.3 \pm 1.2$ *A. tsugae* twig^{-1}). Overall, 8,348 *A. tsugae* were examined from *T. canadensis* stands.

In 2012, an unseasonably warm late winter and early spring resulted in a rapid maturation of both the previous year's sistens and their progredien offspring; progredien hatching was witnessed in early February, 6-8 weeks earlier than normal. Hence, temperature exposure for the sistens was estimated to have begun in mid-April. Temperature data collected from loggers were categorized by cumulative hours spent at each one degree class greater than 15°C. This cutoff temperature was selected based on the assumption that aestivating *A. tsugae* are not sensitive to exposure below this temperature.

2.2.3 Statistical Analyses

2.2.3.1 Laboratory Experiments

All analyses were conducted using R statistical software (R Core Team 2011). A mixed effects logistic regression model was fit to the combined results of the growth chamber studies to examine the relationship between *A. tsugae* mortality at the twig level and the fixed effects of temperature and duration of exposure (Equation 1) (lme4 v0.999375-42 package, Bates et al. 2011). To reduce multicollinearity among the parameters due to the inclusion of an interaction term (temperature x hours), all variables were centered (subtracting the average from each value). The collection tree was included as a random effect to reduce potential variability and to focus on the main parameter effects. Plotted residuals confirmed variance homogeneity of the model variables, and significance of predictor variables were determined at the $\alpha = 0.05$ level.

$$M = \frac{e^{\left(\beta_0 + \beta_1 * \left(T_i^{'}\right) + \beta_2 * \left(H_j^{'}\right) + \beta_3 * \left(T_i * H_j\right)\right)}}{1 + e^{\left(\beta_0 + \beta_1 * \left(T_i^{'}\right) + \beta_2 * \left(H_j^{'}\right) + \beta_3 * \left(T_i * H_j\right)\right)}} \qquad i = 1, 2 \ldots 5; j = 1, 2 \ldots 4. \qquad (1)$$

Where M = predicted *A. tsugae* mortality proportion (0 - 1); T_i'= the centered average temperature (°C; $T_i - 27.9$°C) reached in the growth chamber; H_j'= the centered duration of time (hours; $H_j - 120$ hours) exposed to the i^{th} temperature; and $T_i * H_j$ = the interaction between the i^{th} temperature and the j^{th} duration. Individual linear regression models were tested for each temperature (T_i) to determine if the increased exposure time at particular temperatures had a significant effect on *A. tsugae* mortality. Since all *A. tsugae* were deemed to be alive prior to being placed in growth chambers, all linear regression models had a zero y-intercept (β_0 = 0). Post hoc Chow tests were used to examine potential differences between the duration of a temperature treatment to better elucidate whether the effect of time varies significantly based on temperature (gap v1.1-3 package, Zhao et al. 2011).

To create a model that better represents the effect of temperature and duration on *A. tsugae* mortality, a function of temperature and time was established (F_i; Equation 2) that will be referred to as the heat index. This composite of temperature and length of exposure was defined as the weighted sum of the total hours under different temperature classes greater than 15°C.

$$F_i = \sum W_j \, (H_k) \qquad i = 1, 2 \ldots 9;\ j = 1, 2 \ldots 23;\ k = 1, 2 \ldots n. \qquad (2)$$

Where F_i = the heat index for the i^{th} site/chamber, W_j = the weight for the j^{th} degree class, and H_k = the number of j^{th} degree class's hours logged. The duration coefficient (i.e. slope) from each individual temperature linear regression model (β_1) represented the weight for the average temperature reached in that chamber. An exponential model was then fit to the five β_1 values and the weight estimates (W_j) for each of the 23 degree classes (15° - 38°C) were extrapolated. Weights were multiplied by the number of hours exposed to the corresponding degree class and then the products were summed for an overall chamber heat index (F_i).

2.2.3.2 Field Studies

Due to the lack of normally distributed data, nonparametric analysis (Kruskal-Wallis) was used to test for a significant difference in *A. tsugae* mortality based on twig aspect (north- or south-facing). Since six of the sites were sampled after *A. tsugae* came out of aestivation, natural mortality that occurs due to the transition from 1st to 2nd instar needed to be recognized. An indicator variable for the collection time was established, with November collections receiving a value of one. The percent *A. tsugae* mortality was modeled against this and the site heat index variable (F_i). The resultant linear regression coefficient for the collection variable was treated as the natural mortality for the November collections and was subsequently subtracted from the six site mortality values prior to the final model analysis.

Logistic regression was used to create a final model that could be used to predict aestivating *A. tsugae* mortality based on the heat index experienced at sites in the Chattahoochee National Forest (Equation 3). Adjusted percent mortality (minus natural mortality) was used as the response variable to ensure that predicted estimates were those associated only with temperature.

$$M = \frac{e^{[\beta_0 + \beta_1(F_i)]}}{1 + e^{[\beta_0 + \beta_1(F_i)]}} \qquad i = 1, 2 \ldots n \qquad (3)$$

Where M = predicted *A. tsugae* mortality proportion (0 - 1); and F_i = the heat index for the i^{th} site. The Hosmer-Lemeshow test will be used to examine the goodness of fit for this model. Heat indices were calculated for all sites with temperature loggers (n = 18, Fig. 2.1) and the final model was used to estimate the effect of 2012 summer temperatures on aestivating *A. tsugae* mortality in the Chattahoochee National Forest.

2.3 Results

2.3.1 Laboratory Experiments

Overall, 4,215 *A. tsugae* were tested for an effect of temperature and duration on mortality in growth chambers. Temperature ($\chi^2 = 11.823$, $p < 0.001$), hours of heat exposure ($\chi^2 = 5.547$, $p < 0.001$), and their interaction ($\chi^2 = 2.734$, $p = 0.006$) were all found to have a significantly negative effect on *A. tsugae* survivorship. As temperature and duration increased, *A. tsugae* mortality increased. At higher temperatures there was greater and more rapid *A. tsugae* mortality than at lower temperatures (Fig. 2.2). Post hoc pairwise Chow tests found significantly different effects of duration between each of the temperatures tested, implying that the effect of exposure time on *A. tsugae* mortality varied with the temperature treatment (all *p*-values < 0.01). Slopes from the individual temperature linear regression models (Fig 2.2) were plotted and an exponential model was fit (Fig 2.3). The exponential model exhibits the effect of the increasing temperatures and results in extrapolated weights for each degree class that best represent these effects (Table 2.1). For example, based on these temperature weights, a few hours spent at 35°C would have a larger impact on *A. tsugae* mortality than 100 hours spent at 15°C. Inclusion of the random effect of tree selection accounted for 0.0069 and 0.00002 of the variability for the parameters of temperature and time respectively, indicating that the tree did not play a large role on the effects of these factors.

2.3.2 Field Studies

A total of 8,348 *A. tsugae* were examined from natural *T. canadensis* stands where higher site heat indices were found to significantly increase *A. tsugae* mortality ($p = 0.007$) (Fig. 2.4). Heat indices for sampled sites were calculated by cataloging the number of hours spent at each degree class above 15°C from April to July or mid-October (when the majority of *A. tsugae* came out of aestivation), multiplying those hours by the corresponding temperature weights (Table 2.1), and then summing for an overall heat index value (Equation 2). *Adelges tsugae* mortality from temperature exposure ranged from 8.54% to 62.78% and the heat indices ranged from 1.52 to 4.53 for the nine sampled sites (Table 2.2). Heat indices and elevation were found to be significantly correlated ($p = 0.006$, $R^2 = 0.64$) with heat indices decreasing as elevation increases (Table 2.2).

Logistic regression analysis was conducted to produce the final predictive model of *A. tsugae* mortality as a function of the site heat index (Equation 4):

$$M = \frac{e^{(-3.71+0.92\,(F_i))}}{1+e^{(-3.71+0.92(F_i))}} \qquad i = 1, 2\ldots n \qquad (4)$$

Where M = the predicted *A. tsugae* mortality proportion site^{-1}; and F_i = the heat index for the i^{th} site. The Hosmer-Lemeshow test resulted in a χ^2 of 0.21 that was found to be insignificant (p = 0.65) indicating that the predictive model fits well to the observed data. The relationship between *A. tsugae* mortality and site heat indices was found to be significant (p = 0.007) and can be seen when the two factors are plotted against each other (Fig. 2.4). The model predicted 67% of the sites to within 10% of the observed *A. tsugae* mortality, and the observed-predicted differences ranged from 0.4 to 21% (Table 2.2).

Linear regression analysis used to determine natural *A. tsugae* mortality resulted in a coefficient of 0.1641 for the collection time (July or November) indicator variable. This value, an estimate of natural mortality for *A. tsugae* coming out of aestivation, was subsequently subtracted from November samples' proportion *A. tsugae* mortality prior to all analyses. The spatial aspect of the collected *T. canadensis* twigs were compared using the Kruskal-Wallis test and found that the direction the twigs faced, and potential differences in microclimate, did not have a significant effect on *A. tsugae* mortality (χ^2 = 0.03, df = 1, p = 0.86).

Heat indices for 2012 were calculated for each of the 18 sites where temperature was recorded, and the final model (equation 4) was used to calculate the predicted *A. tsugae* mortality for each of those sites (Table 2.3). Based on this study, the aestivation season of 2012 resulted in a predicted *A. tsugae* temperature-induced mortality ranging from 15.15% to 60.93% in the Chattahoochee National Forest.

2.4 Discussion

This study demonstrates that increasing summer temperatures, including those predicted to occur with climate change, may have negative influences on insect survivorship, particularly to those that enter aestivation. The northern Georgia summer populations of *A. tsugae* appear to be sensitive, under laboratory and field conditions, to exposure to temperatures above 25°C, with substantial mortality occurring above 30°C with < 100 hours of exposure. Results also showed that the effect of heat increases over time at all exposure temperatures used in this study, indicating a cumulative effect of heat stress.

Indirectly, warmer summer temperatures in Georgia may be affecting *A. tsugae* mortality by causing a heat-induced change in host suitability compared to cooler locations in its distribution. The region examined in Georgia is the southernmost portion of *T. canadensis*' contiguous range (Fig. 2.1). It is often assumed that individuals located at the edge of a range are exposed to less favorable conditions making them more sensitive to environmental factors such as temperature extremes. However, researchers have tested *T. canadensis*' growth rates, using tree-ring chronology, at both the northern and southern edges of the range, and found that there were no significant differences when these growth rates were compared with those found at the core region (Kavanagh and Kellman 1986, Hart et al. 2010). This suggests that the temperature extremes found at the edges may not be influencing *T. canadensis* vigor, but it is possible that the extreme temperatures may be altering other factors such as defensive terpenes. In this study, field samples were collected from *T. canadensis* trees within sites that exhibited similar stages of tree health. This reduced the potential that variable host condition/suitability may have indirectly affected *A. tsugae*'s ability to survive.

Based on the results of the controlled laboratory experiment, the observed mortality of field *A. tsugae* was most likely directly caused by the exposure to high temperatures. *Adelges tsugae* had not begun feeding and were therefore unlikely to be influenced by host health or defenses. Also, aestivating sistens had not produced their protective wool, leaving them directly exposed to summer temperatures on the branches of the tree. Other studies on aestivating *A. tsugae* have reported that mortality increases substantially (up to 100%) when trees are exposed to direct sunlight (Mayfield and Jetton 2013, Sussky and Elkinton 2015). This suggests that soft-bodied *A. tsugae* may be sensitive to heat-induced desiccation. Further, increasing temperatures causes an increase in insect metabolic rates (Neven 2000) which may have depleted *A. tsugae* reserves.

Based on phylogenetic analyses of bacterial symbionts, *A. tsugae* in eastern North America is believed to have been introduced from Osaka Prefecture, Japan (von Dohlen et al. 2013). The direct effect of supraoptimal temperatures on *A. tsugae* is based on an underlying assumption that temperatures in Georgia are higher than those in the native range of Japan. Historical weather data for Osaka, Japan (34.6939°N, 135.5022°E) and Dahlonega, Georgia (34.7636°N, 84.1156°W) were compared for 2010 – 2014 to see if this assumption holds true. The average maximum daily temperatures were examined for the main aestivating months (July – September) for the five years (n = 15) and compared between the two locations. Surprisingly, Japan was warmer than Georgia for 87% of the summer months, with the remaining two months reaching the same average temperatures (University of Georgia 2011, The Weather Channel 2014). While the level of *A. tsugae* summer mortality in Japan is unknown, this could mean that *A. tsugae* is more sensitive to temperatures in a non-native range or on a non-native host. This is supported by other research that has examined winter cold hardiness of *A. tsugae* and found that field and laboratory populations in the eastern United States suffer greater mortality than native populations in Japan (Parker et al. 1998, 1999, McClure and Cheah 2002).

Summer mortality of *A. tsugae* may also be due to maternal effects on the aestivating generation. For many insects, the temperatures experienced by the parental generation significantly influence the fitness/quality of the diapause stage (Mousseau and Dingle 1991). This is evident in *A. tsugae*'s life history where it has been found that progrediens preconditioned at different temperatures resulted in different aestivation regimens for the sisten generation (Salom et al. 2001). Progrediens reared at lower temperatures (12°C) resulted in sistens which molted without entering aestivation, whereas those reared at the highest temperature tested (22°C) produced sistens that never broke aestivation (Salom et al. 2001). When comparing average maximum daily temperatures during progredien maturation (March – May) for 2010 - 2014, Georgia was warmer than Japan 87% of the times and reached a maximum of 33°C (University of Georgia 2011, The Weather Channel 2014). It is possible that the progredien's exposure to higher spring temperatures in Georgia may be limiting the ability for the sistens generation to respond appropriately to summer temperatures.

Results from this study indicate that future climatic conditions will continue to influence the survivorship of *A. tsugae*. Previously, most predictions regarding exotic insects, including *A. tsugae*, examined only the effects of warming winter temperatures. Based on climatic models, locations that reach low enough winter temperatures to cause *A. tsugae* mortality will be limited to small pockets in the northern region of *T. canadensis*' range by the year 2070 (Dukes et al. 2009). If warmer winter temperatures are solely considered, this implies that *T. canadensis* could experience a severe range retraction and be removed from the Southern Appalachian landscape. However, it is becoming clear that increasing spring and summer temperatures should be taken into consideration and that *T. canadensis* may persist in pockets in the southern region under the current climate change models.

The hypothesis that southern populations of *T. canadensis* will survive is contingent upon at least three factors. Firstly, *T. canadensis* would have to be able to survive low densities of *A. tsugae*. In warmer, low-elevation (< 450 m) *T. canadensis* stands in Georgia, trees are surviving long past the often observed mortality 3-5 years post attack (McClure 1991). Most trees in these areas are in low to moderate decline after being infested with *A. tsugae* for over ten years. Although it is unknown what an appropriate density of *A. tsugae* is for *T. canadensis* to survive, *Tsuga* spp. in western North America and Asia can all survive with low densities of *A. tsugae* (McClure et al. 2003). In Georgia, healthier than expected *T. canadensis* stands were associated with sisten mortality greater than 70%. In addition, warmer spring temperatures can result in an increase in the development rates of the progrediens (Salom et al. 2002) resulting in less carbohydrate reserves being depleted due to a shorter feeding period for that generation. These reductions in nutrient loss and *A. tsugae* densities may be enough for *T. canadensis* to survive and perpetuate at the southern edge of their range.

Secondly, *A. tsugae* would have to not acclimate to the current heat exposure in Georgia as well as the longer heat waves and higher temperatures expected with climate change. In the northeastern portion of its range, *A. tsugae* has been found to be adapting to the cold temperatures, allowing it to expand its range further northward than anticipated (Skinner et al. 2003, Butin et al. 2005). Preliminary results from a latitudinal study appear to show that *A. tsugae* is not behaving similarly to warmer temperatures; *A. tsugae* in Georgia did not experience a decrease in mortality over a three year period, and had an overall higher percent mortality than those from more northern latitudes when exposed to identical temperature treatments (unpublished data).

Lastly, *T. canadensis* would have to survive the effects of climate change regardless of *A. tsugae* presence. Iverson and Prasad (1998) examined habitat requirements for various tree species and determined the effect of a two-fold increase in CO_2 on land-use variables. The model predicted that *T. canadensis* will experience a decrease in overall basal area and number of stems, and a northward range shift of greater than 250 km (Iverson and Prasad 1998). This would move the southernmost part of *T. canadensis*' range from the northern Georgia mountains up to the North Carolina-Virginia border where temperature regimens are much cooler. In addition, *T. canadensis* is only moderately drought tolerant, so the increased drought conditions predicted with climate change may increase stress and/or cause tree mortality (Godman and Lancaster 1990, IPCC 2013).

Results from this project provide evidence of the negative effects of prolonged, above-optimal temperatures on the survivability of an aestivating insect. Combined with climate change predictions, this has implications for the future of *T. canadensis* stands in the southeastern United States. With an increase in temperature of 2°C, *A. tsugae* mortality would increase by an average of 31.22% (± 2.95% SE) resulting in a predicted summer mortality of up to 92%. Warmer temperatures may also be beneficial for the future of *T. canadensis* by assisting biological control by lowering *A. tsugae* densities that need to be combated, and by increasing the length of time for predatory beetles to become established and reach population levels high enough to be effective. Climate change may have a positive influence on the conservation of *T. canadensis*, an important foundation species in the Southern Appalachian Mountains.

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Table 2.1. Estimated weights for individual degree classes used for calculating heat indices for each site/chamber examined. Weights determined via exponential extrapolation of observed linear regression individual temperature coefficients (β_1).

Temperature Class (°C)	**Weight (W_j)**
14 – 15	0.000201
15 – 16	0.000246
16 – 17	0.000301
17 – 18	0.000369
18 – 19	0.000452
19 – 20	0.000553
20 – 21	0.000676
21 – 22	0.000828
22 – 23	0.001013
23 – 24	0.001240
24 – 25	0.001517
25 – 26	0.001857
26 – 27	0.002272
27 – 28	0.002781
28 – 29	0.003403
29 – 30	0.004165
30 – 31	0.005098
31 – 32	0.006239
32 – 33	0.007635
33 – 34	0.009344
34 – 35	0.011436
35 – 36	0.013995
36 – 37	0.017128
37 – 38	0.020962
38 – 39	0.025654

Table 2.2. Site data for all nine locations sampled in 2012 in the Chattahoochee National Forest, Georgia. Predicted morality (M) based on logistic regression model: $M = 1/1 + e^{-(-3.71 + 0.92(F_i))}$.

Site	**Elevation (m)**	**Collection Month**	**Heat Index (F_i)**	**Observed Average *A. tsugae* Mortality**	**± 95% CI**	**Predicted Average *A. tsugae* Mortality**
1	229	November	4.5321	0.6278	0.0709	0.6093
2	444	November	4.0931	0.5907	0.0765	0.5105
3	457	July	2.8686	0.3791	0.0813	0.2536
4	457	November	4.0876	0.5545	0.0511	0.5093
5	524	July	2.2531	0.2598	0.0833	0.1620
6	647	November	2.8969	0.0908	0.0495	0.2585
7	660	November	3.4224	0.1514	0.0765	0.3607
8	929	November	2.1701	0.1661	0.0637	0.1515
9	972	July	1.5238	0.0854	0.0423	0.0902

Table 2.3. Predicted *A. tsugae* mortality based on temperature regimens (F_i) experienced in 2012 in the Chattahoochee National Forest, Georgia.

Elevation (m)	**Heat Index (F_i)**	**Predicted Average Mortality**
229	4.53210	0.60927
444	4.09307	0.51051
457	4.08762	0.50927
521	3.70965	0.42331
523	3.37992	0.35177
523	3.11747	0.29908
535	3.04327	0.28503
545	2.16635	0.15148
560	3.54904	0.38786
647	2.89686	0.25850
660	3.42239	0.36070
717	2.42302	0.18424
720	2.48636	0.19312
807	2.17915	0.15300
818	2.75481	0.23435
839	2.29228	0.16692
929	2.17008	0.15192
972	2.22391	0.15839

Figure Legend

Figure 2.1. Location of sites sampled in 2012 for effects of heat on *A. tsugae* (distribution in red) in the Chattahoochee National Forest, Georgia. Modified from map produced by USDA Forest Service (2013).

Figure 2.2. Linear relationships between *A. tsugae* mortality and duration of exposure (hours) for each growth chamber mean temperature (°C). Regression model: $y = \beta x$, where y is *A. tsugae* mortality (0-1) and x is hours. 17.917°C: $y = 0.00040x$, df = 39, $P = 0.000217$, $R^2 = 0.281$; 22.917°C: $y = 0.00101x$, df = 39, $P = 1.1\text{e-}11$, $R^2 = 0.690$; 27.917°C: $y = 0.002883x$, df = 79, $P = 2\text{e-}16$, $R^2 = 0.646$; 32.917°C: $y = 0.011446x$, df = 19, $P = 6.3\text{e-}12$, $R^2 = 0.917$; 37.917°C: $y = 0.018731x$, df = 9, $P = 2.26\text{e-}11$, $R^2 = 0.994$.

Figure 2.3. Exponential model for estimating temperature weights (β*) based on observed coefficients from five growth chamber linear regression models. Exponential model: $y = Ar^x$, where y = the estimated temperature coefficient (β*), A = initial value, r = rate of increase, and x = temperature; $\beta^* = (1.08\text{e-}5)(1.22)^{\text{Temp}}$.

Figure 2.4. Logistic relationship between *A. tsugae* mortality and heat indices of natural stands. Predictive model based on nine *Tsuga canadensis* stands sampled in 2012 in the Chattahoochee National Forest, Georgia. Logistic model: $M = 1/1 + e^{-(\beta_0 + \beta_1 x)}$, where M = predicted *A. tsugae* mortality proportion (0-1), and x = heat index (F_i). $M = 1/1 + e^{-(-3.71 + 0.92(F_i))}$.

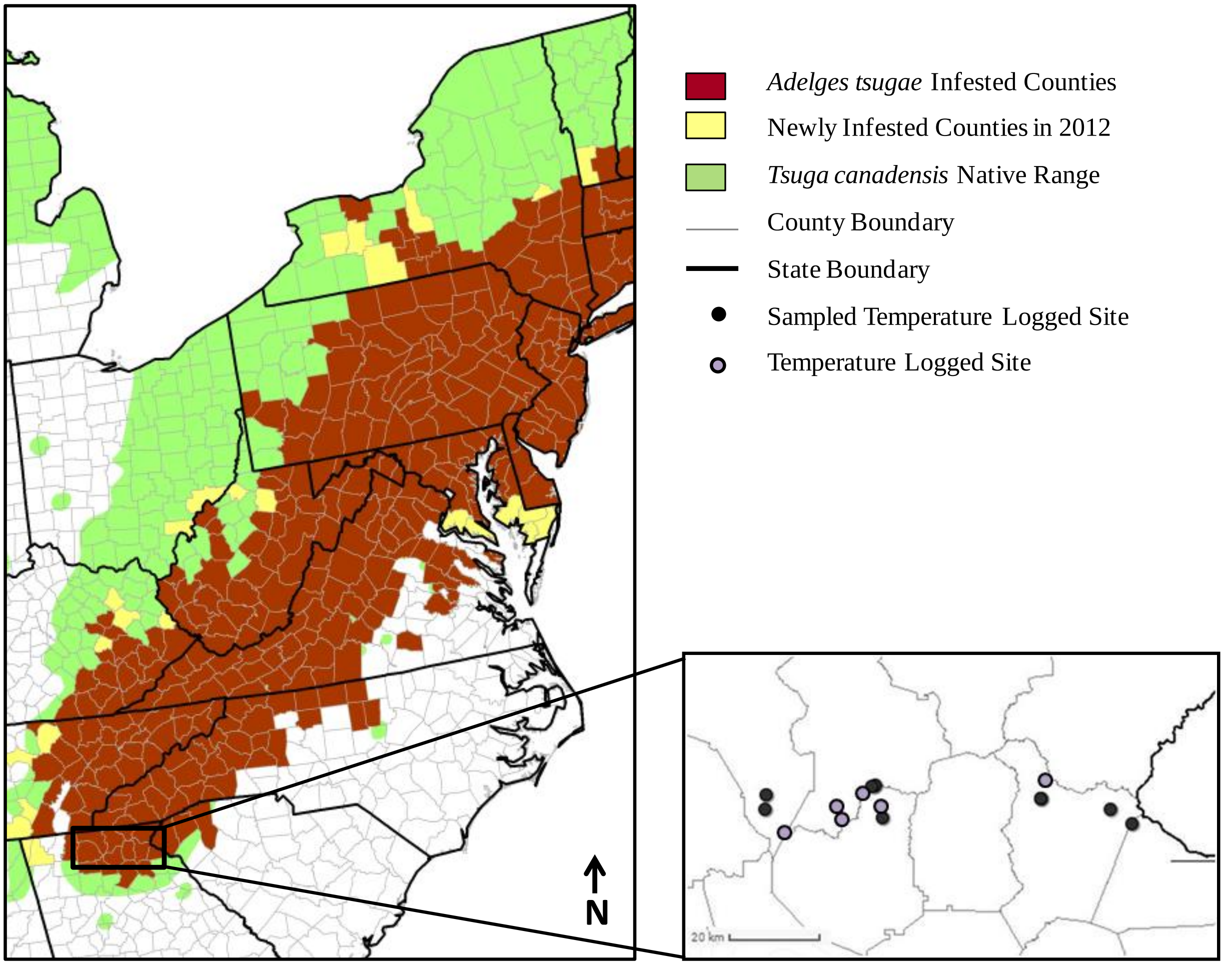

Adelges tsugae Infested Counties
Newly Infested Counties in 2012
Tsuga canadensis Native Range
County Boundary
State Boundary
Sampled Temperature Logged Site
Temperature Logged Site
N
20 km

Figure 2.1

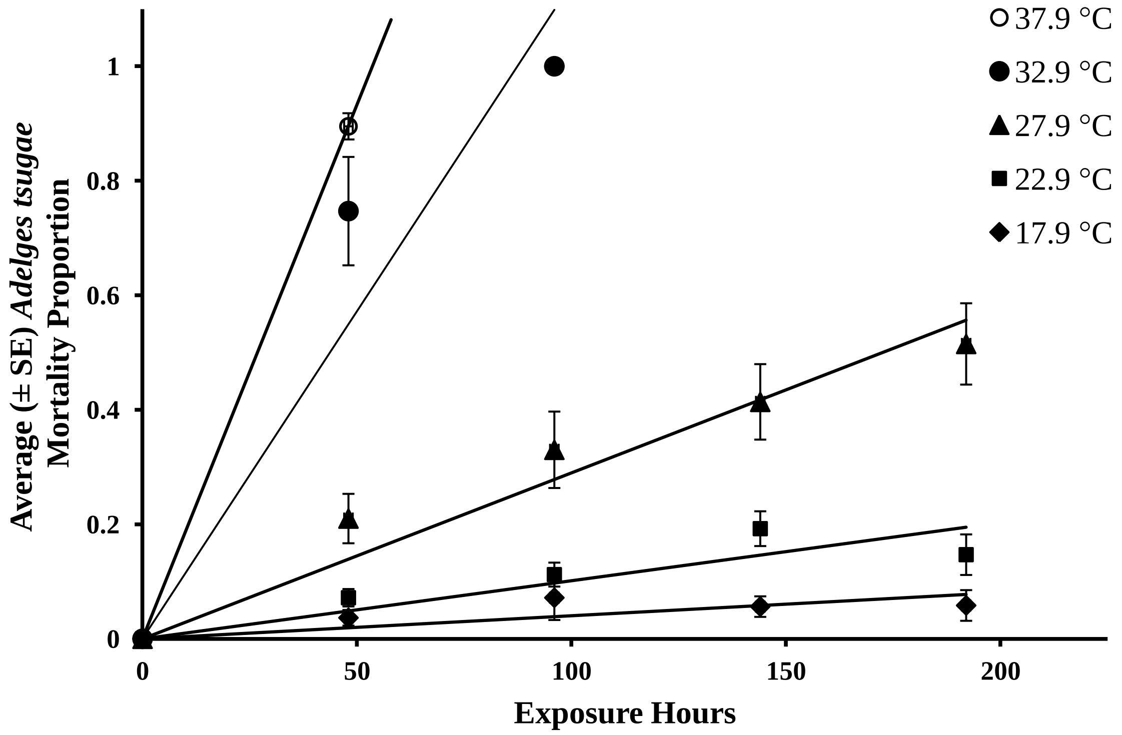
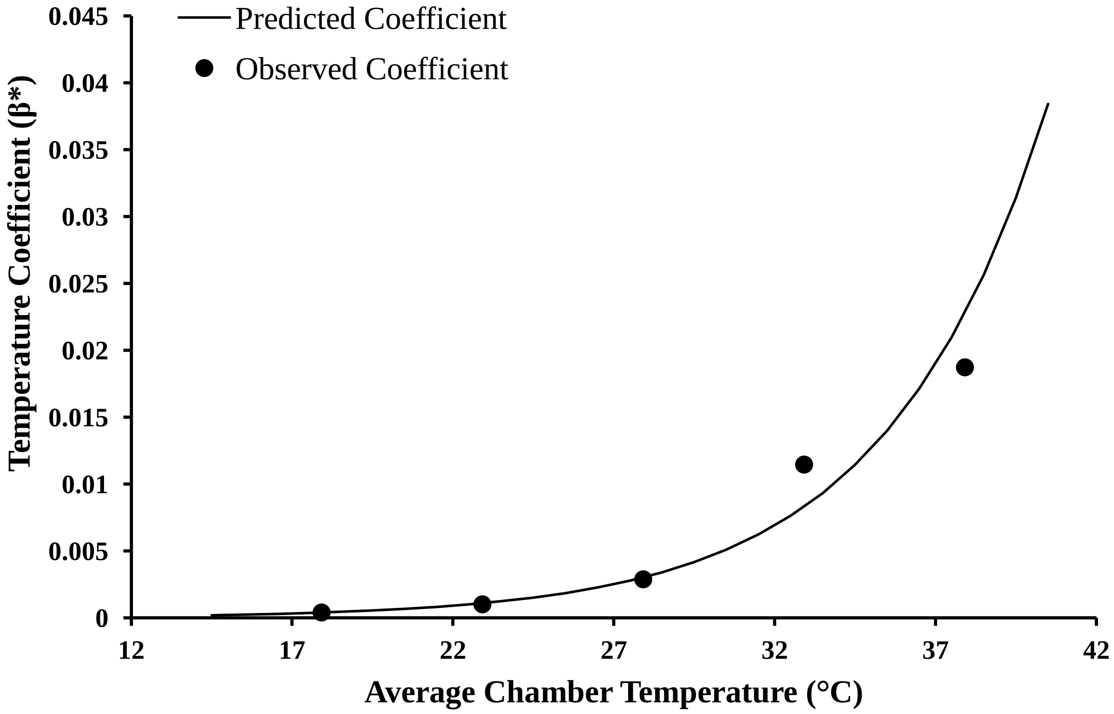

○ 37.9 °C
● 32.9 °C
▲ 27.9 °C
■ 22.9 °C
◆ 17.9 °C
Average (± SE) *Adelges tsugae* Mortality Proportion
0
0.2
0.4
0.6
0.8
1
0
50
100
150
200
Exposure Hours

Figure 2.2



Predicted Coefficient
Observed Coefficient
Temperature Coefficient (β*)
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
12
17
22
27
32
37
42
Average Chamber Temperature (°C)

Figure 2.3

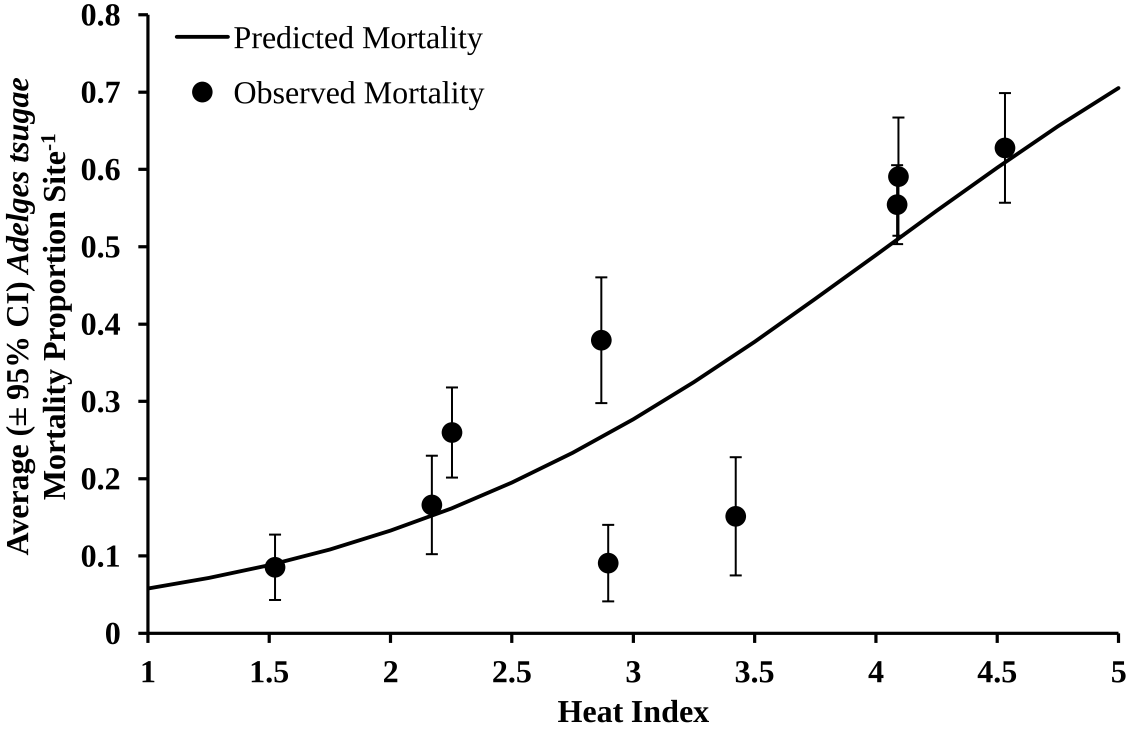

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
1.5
2
2.5
3
3.5
4
4.5
5
Heat Index
Average (± 95% CI) *Adelges tsugae* Mortality Proportion Site^{-1}
Predicted Mortality
Observed Mortality

Figure 2.4

CHAPTER 3

STATUS OF EASTERN HEMLOCK (*TSUGA CANADENSIS*), HEMLOCK WOOLLY ADELGID (*ADELGES TSUGAE*), AND BIOLOGICAL CONTROL IN GEORGIA[1]

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[1] A M Mech, Rhea J, and Gandhi K J K. To be submitted to *Environmental Entomology*

Abstract

The non-native hemlock woolly adelgid (*Adelges tsugae* Annand) (Hemiptera: Adelgidae) is currently causing widespread mortality of a foundation tree species, eastern hemlock [*Tsuga canadensis* (L.) Carrière], in eastern North America. *Adelges tsugae* was first documented in Georgia in 2002 and has spread within the past 12 years across the entirety of the Chattahoochee National Forest of northern Georgia. Tree mortality in the southeastern U.S. was expected to occur in as little as 3 – 5 years post infestation, yet *T. canadensis* trees in multiple locations of Georgia experienced < 25% dieback during this timeframe. Since 2006, over 1.4 million biological control beetles have been released to combat *A. tsugae* in over 50 sites within the Chattahoochee National Forest. Plots were established (n = 33) in 2010 or 2011 and examined over a four-year period to determine the rate of *T. canadensis* dieback/decline and if any stand or tree conditions, including the release of biological control, played a role in *T. canadensis* rates of decline and *A. tsugae* population levels. Results indicate that the dieback of *T. canadensis* trees (n = 165) increased by 6.3% (± 0.4%) per year. If these trends continue, some trees in Georgia may survive for > 15 years post *A. tsugae* infestation. None of the stand or tree characteristics examined (e.g. tree diameter, years infested, elevation, *A. tsugae* density) were found to significantly influence *T. canadensis* dieback rates. However, the length of new growth present on *T. canadensis* had a significantly positive effect on *A. tsugae* densities. There were no significant differences in *T. canadensis* dieback rates or *A. tsugae* densities between sites that did and did not receive biological control. Results suggest that, although established, biological control may require more time and/or more releases before reaching population levels necessary to exert significant pressure on *A. tsugae* in these forest stands.

Keywords: *Adelges tsugae*, biological control, *Laricobius* spp., *Sasajiscymnus tsugae*, *Tsuga canadensis*

3.1 Introduction

Non-native insects that become established in a new habitat have the potential to cause substantial economic losses, threaten biodiversity, and alter ecosystem processes (Vitousek 1996, Pimental et al. 2005, Lovett et al. 2006, Gandhi and Herms 2010). Most of these insects are transported via air and maritime cargo on live plant or timber material, and it is estimated that only 20 – 54% of the exotic insect species are detected at ports of entry (Work et al. 2005, Liebhold et al. 2012). In the United States, over 4,500 exotic insects have become established with nearly 14% affecting forest trees (including urban forest trees) resulting in $2.1 billion in losses/damages per year (Pimental et al. 2005, Aukema et al. 2010). With greater trade and commerce occurring amongst countries, there is the potential that these numbers could increase.

Short-term effects of non-native pests include tree defoliation and/or death, temporary reduction in photosynthesis, changes in microclimate and light conditions, and nutrient leaching (Liebhold et al. 1995, Jenkins et al. 1999, Eschtruth et al. 2006, Lovett et al. 2006, Gandhi and Herms 2010). In addition, exotic forest pests have the potential to produce more long-term changes in ecosystems than some abiotic disturbances, such as fire or severe storms, where regeneration can replenish the forest composition (Lovett et al. 2006). This is mainly due to the fact that, once established, the insect can perpetuate in the ecosystem affecting the target host species, including regeneration, thereby altering species composition, forest productivity, and nutrient cycles (Liebhold et al. 1995, Lovett et al. 2006). Most long-term effects are experienced when a damaging, host-specific pest colonizes a dominant tree species, such as the emerald ash borer (*Agrilus planipennis* Fairmaire) from China on North American ash trees (*Fraxinus* spp.) and the hemlock woolly adelgid (*Adelges tsugae* Annand) from Japan on eastern hemlock [*Tsuga* *canadensis* (L.) Carrière] trees (McClure et al. 2003, Lovett et al. 2006, Herms and McCullough 2014).

Multiple control efforts have been made to target exotic forest pests and conserve natural forest stands. These efforts can range from exclusion, quarantine, biological and chemical control, and silvicultural practices (Liebhold et al. 1995). Once a forest pest has become established, classical biological control, the introduction of exotic natural enemies, becomes a widely used management approach (Pschorn-Walcher 1977). Overall, more than 5,000 natural enemies have been released to combat insect and mite pests, and although establishment (~25%) and pest control (~10 – 15%) rates are not very high, biological control is still deemed a cost-effective plan (Pschorn-Walcher 1977, Debach and Rosen 1991, Simberloff 2014). Although biological control requires a financial investment for exploration, host-testing and rearing, the benefits from successful biological control projects outweigh the initial costs as well as the costs of failed projects (Hoddle 2004, Simberloff 2014). An advantage of biological control is that it is feasible in large forested areas, self-perpetuating, and a long-term method that has minimal non-target effects on other organisms (Perrings et al. 2000).

In the U.S., insects in the Order Hemiptera account for the majority of non-native forest pests (41.5% of species), with most of them (69%) in the suborder Sternorrhyncha (aphids, adelgids, scales, etc.) (Aukema et al. 2010). Of these, *A. tsugae* (Hemiptera: Adelgidae) is one of the most damaging species by causing widespread mortality of a dominant foundation species, *T. canadensis,* and costing $215 million per year (McClure et al. 2003, Ellison et al. 2005, Aukema et al. 2011, Ellison 2014). Like most non-native pests, *A. tsugae* has been successful in eastern North America due to a lack of natural predators, a suitable environment, inadequate host resistance, an open niche, and high reproductive ability. As a result, *A. tsugae* has spread to 19 states since its introduction from Japan in the 1950’s (Fig.1) (Havill et al. 2006, USDA Forest Service 2013).

Tsuga canadensis is a shade-tolerant, slow-growing, long-lived conifer that can take up to 300 years to reach maturity and live to > 800 years (Godman and Lancaster 1990). Due to its late-successional life history, *T. canadensis* can create dense, nearly homogenous stands that have unique microenvironments (Ellison et al. 2005, Orwig et al. 2012). In the Southern Appalachian Mountains, *T. canadensis* is considered a foundation species in riparian and cove habitats where they are typically found in narrow bands on the sides of streams that flow year-round (Ellison et al. 2005, USDA Forest Service 2005). *Tsuga canadensis* forests are known to be dark and cool, have acidic soils with diverse understory vegetation, and have a unique habitat for insects, birds, and wildlife (Godman and Lancaster 1990, Tingley et al. 2002, D’Amato et al. 2009, Rohr et al. 2009). In addition, *T. canadensis* plays a strong role in stream flow, bank stability, aquatic species composition, and water temperature fluxes, making their loss of significant ecological importance (Snyder et al. 2002, Ross et al. 2003, Ellison et al. 2005, Hadley et al. 2008). Unfortunately, *A. tsugae* induced *T. canadensis* decline and mortality can occur in as little as 4-5 years (McClure 1991, Orwig and Foster 1998).

In North America, *A. tsugae* is parthenogenetic and multivoltine, allowing the population to grow rapidly and spread at rates of 8 – 15.6 km per year (Evans and Gregoire 2007, Havill and Foottit 2007). Due to its small size (< 2 mm), *A. tsugae* can be spread via birds, mammals, wind, and humans, making containment of this pest nearly impossible (McClure 1990). *Adelges tsugae* has two wingless generations per year that are responsible for *T. canadensis* dieback – the sistens (summer to following spring) and progrediens (spring to summer) (McClure et al. 2003). Each *A. tsugae* generation has six life stages (egg, four instars, and adult) with the first instar nymphs (crawlers) being the only mobile stage. Once the crawler finds a suitable *T. canadensis* needle node, *A. tsugae* will settle and insert its stylet bundle at the needle base to allow feeding of the nutrients stored in the tree's xylem ray parenchyma cells (Young et al. 1995, McClure et al. 2003). Unlike the progredien generation, the sisten generation will enter aestivation (summer diapause) as soon as they settle and will remain in that state for 4-6 months (McClure 1989, McClure et al. 2003). Following high levels of *A. tsugae* feeding, stressed *T. canadensis* trees will reduce or halt production of new growth, causing the *A. tsugae* population to subsequently crash. As a response to the drop in *A. tsugae* densities, *T. canadensis* will produce some new growth which is typically stunted. This new growth causes a resurgence in *A. tsugae* populations resulting in further dieback and eventual mortality of the tree (McClure 1991, McClure et al. 2003).

In its native range, *A. tsugae* populations are controlled by both a natural resistance of *Tsuga* spp. and the presence of natural enemies (Cheah et al. 2004). Various strategies are currently being used to control *A. tsugae* in North America, including chemical and biological control. Both soil injections and soil drenches of imidacloprid, a systemic insecticide, have been found to be highly effective at reducing *A. tsugae* populations (Webb et al. 2003, Cowles et al. 2006). However, chemical control is not a permanent solution and can have negative non-target effects on insects associated with *T. canadensis* and other soil or aquatic organisms (Dilling et al. 2009, Knoepp et al. 2012).

Beginning in the early 1990's, researchers went to the native ranges of *A. tsugae* in Japan, China, and the Pacific Northwest to search for natural enemies that can be used for biological control. Originally, three main species were selected that were reared and released in eastern North America – *Sasajiscymnus tsugae* Sasaji and McClure from Japan, *Scymnus sinuanodulus*

Yu and Yao from China, and *Laricobius nigrinus* Fender from British Columbia (Sasaji and McClure 1997, Zilahi-Balogh et al. 2002, Cheah et al. 2004). Due to its lack of establishment, *S. sinuanodulus* is no longer being released, but other predators are being attempted, such as *L. osakensis* Montgomery and Shiyake from Japan (Vieria et al. 2011, Jones et al. 2014). In addition, *L. rubidus* LeConte, a native predator of pine bark adelgid (*Adelges strobi* Hartig) in North America has been shown to successfully complete its development on *A. tsugae* and has been recovered from *T. canadensis* samples (Zilahi-Balogh et al. 2005, Jones et al. 2014). With *P. strobus* experiencing dieback in its native range due to a scale insect (*Matsucoccus macrocicatrices* Richards) and pathogen complex (Mech et al. 2013), *L. rubidus* may utilize more *A. tsugae* as populations of *A. strobi* decrease.

The southern limit of *T. canadensis*'s contiguous range is located in the Chattahoochee National Forest (CNF) of northern Georgia's Blue Ridge Mountains (Fig. 3.1). In Georgia, *T. canadensis* is found in the Blue Ridge and Piedmont ecoregions where there is ample precipitation (> 200 cm) with well-drained, sometimes acidic, loamy to clayey soils (Griffith et al. 2001). In the 303,573 ha CNF, *T. canadensis* is on 9,200 ha (~3%) and is typically found between 600 and 900 m elevation. *Tsuga canadensis* is also found in disjunct populations extending into the upper piedmont of Georgia where elevations are as low as 250 m (USDA Forest Service 2005). Most *T. canadensis* in the CNF are associated with white pine (*Pinus strobus* L.) and rhododendron (*Rhododendron maximum* L.) as well as cove hardwoods such as American beech (*Fagus grandifolia* Ehrhart), northern red oak (*Quercus rubra* L.), red maple (*Acer rubrum* L.), and yellow poplar (*Liriodendron tulipifera* L.) (USDA Forest Service 2005).

Adelges tsugae infestation in the state of Georgia was first noticed in the northeastern region of the CNF in 2002 and spread in a southern and westward direction. The USDA Forest

Service took a pro-active approach to the pest's arrival and designated ~150 hemlock conservation areas (HCA) as locations for biological and/or chemical control of *A. tsugae* (USDA Forest Service 2005). Beginning in 2006, the USDA Forest Service, in cooperation with the predatory beetle rearing laboratories at Clemson University, University of Georgia, University of North Georgia, and Young Harris College, began mass releases of the three main biological control beetles in the CNF. As of 2014, over 1.4 million beetles had been released in over 50 HCA's: 1,081,481 *S. tsugae*, 352,082 *L. nigrinus*, and 46,329 *S. sinuanodulus* individuals.

Although research has examined multiple stand variables (elevation, slope, aspect, etc.) on the decline and susceptibility of *T. canadensis* (Orwig et al. 2002, Faulkenberry et al. 2009), little work has been conducted over a longer period of time and with variables at the tree level (size, dieback, etc.). Our research objective was to assess the temporal patterns and factors, including biological control, affecting *T. canadensis* health and *A. tsugae* densities in the CNF of northern Georgia. Observations may lead to a better understanding of the effects of an invasive forest pest on a dominant forest species in the southeastern U.S.

3.2 Methods

3.2.1 Study Sites

Study sites were located in the CNF in Fannin, Habersham, Lumpkin, Stephens, and Union counties of northern Georgia. Two types of forest sites were selected for this study: 1) biological control release sites (HCA's) and 2) non-biological control release sites. HCA's were selected if they had > 15,000 predatory beetles released of three species (*S. tsugae*, *S. sinuanodulus*, and *L. nigrinus*) for more than two consecutive years (including 2009). Preference was also given to HCAs where predatory beetles had previously been recovered (*C. Jones,* *personal communication*). Six HCAs were selected that met these criteria – the sites ranged in elevation from 229 – 972 m and across 76 km of the Chattahoochee National Forest (Fig. 3.1, Table 3.1). Within each study site, three 18 m radius plots were established. Locations of plots within the site were based on GPS coordinates of release locations provided by the predatory beetle rearing laboratories (Clemson University, University of Georgia, University of North Georgia, and Young Harris College). Due to the longitudinal variation, sites became infested with *A. tsugae* at different times (2004 – 2007), and therefore had been receiving biological control for different lengths of time (2 – 4 years) at the onset of this study. Estimated year of *A. tsugae* infestation was based on the annual spread map made by the Georgia Forestry Commission (2012).

Corresponding control (non-release) sites were located for each selected HCA. To reduce confounding factors, such as years and levels of *A. tsugae* infestation, and to remain in the same geographic area, *T. canadensis* control stands were approximately 1.5 – 8 km away from the predatory beetle release sites. Similar to the biological control sites, three 18 m radius plots were established within each site, but specific locations of plots were selected randomly. For Panther Creek, HCA 29, there was no *T. canadensis* stand in the vicinity to qualify as a control site, but was nonetheless kept as a release site.

All selected plots (Appendix A) had one of four Society of American Foresters (SAF) forest cover types: 1) Hemlock-Hardwood (SAF 59; 30.3% of plots), 2) Hemlock (SAF 23; 27.3%), 3) Hemlock-White Pine (SAF 22; 21.2%), and 4) Hemlock-White Pine-Hardwood (SAF 20; 21.2%). Within each plot, an average of 39.6% of the woody stems were *T. canadensis*. Other tree species commonly found within these plots include *Acer rubrum*, hickory (*Carya* spp.), sourwood [*Oxydendrum arboretum* (L.) de Candolle], *P. strobus*, *Liriodendron tulipifera*, and white oak (*Quercus alba* L.) (Table 3.2).

Data were collected for four consecutive years (2010 – 2013 or 2011 – 2014) to assess patterns in host tree and pest dynamics. All trees within each plot were identified to the genus or species level and the diameter at breast height (DBH) was measured to calculate the basal area of *T. canadensis* and *P. strobus*. In addition, the elevation, slope, and aspect were recorded for each plot, and temperature data loggers (HOBO UA-001-64, Onset Computer Corporation, Bourne, Massachusetts) were placed in 18 of the 33 plots. Aspect values were transformed for statistical analyses using Beers et al. (1966):

$$\text{Transformed aspect} = \text{cosine}\ (45 - \text{azimuth}) + 1.$$

This yields a value 0 – 2 where 0 correlates to an azimuth of 225°, representing southwestern slopes with more direct sunlight and xeric conditions, and 2 correlates to an azimuth of 45°, representing a northeastern direction with less sunlight and more mesic conditions.

3.2.2 *Tsuga canadensis* Health

Within each plot, five *T. canadensis* trees taller than 2 m were randomly selected for crown assessments (percent dieback) and collections (n = 165 trees). To reduce sampling bias, a single surveyor estimated the crown dieback, to the nearest 5%, for each sample tree for all four years. In addition, whether or not the tree directly received predatory beetles (if in a HCA) and the DBH were recorded.

3.2.3 *Adelges tsugae* Biology

To examine *A. tsugae* trends over time, four 10 cm twigs were collected in July from each of the 165 sampled *T. canadensis* per year (n = 660 twigs per year). Two twigs from the tree, randomly clipped at a level of 1.0 – 5.5 m, were collected from the north or east facing direction and the other two were south or west facing to reduce potential spatial variability. The length of each twig offshoot was measured and the composite twig length was determined. Examining only the underside of the *T. canadensis* twig, the number of needles present and absent were counted and the resultant proportion present was multiplied by the composite length to determine the overall length of the twig that *A. tsugae* utilized. This value provided a more accurate and comparable estimate of *A. tsugae* density since some twigs had more dieback than others and would therefore have less *A. tsugae*.

On each twig, the number of live *A. tsugae* nymphs were counted for a density calculation (total number *A. tsugae* divided by utilized length of twig) and then averaged with other twigs from the same tree to establish the overall tree *A. tsugae* density for each year. In addition, other factors were noted that may potentially influence *A. tsugae* dynamics, such as percent new growth present on the twigs, number of bare tips (indicating a shoot blight), and presence of the circular hemlock scale (*Nuculaspis tsugae* Marlatt).

3.2.4 Statistical Analyses

All analyses were conducted using R statistical software (R Core Team 2011). Linear mixed effects models were used to determine if any measured fixed factors had a significant effect on the response variables (lme4 v0.999375-42 package, Bates et al. 2011). For data collected at the tree-level, the analyses included the random intercept of plot nested in site and the random intercept of site was included for all analyses conducted at the plot level. These random effects are included to take into account the variation due to the plot and/or the site. Pearson correlation values were examined for potential correlations between the fixed effects, and residual plots for all analyses were examined for deviations from homoscedasticity and normality (R Core Team 2011).

3.2.4.1 *Tsuga canadensis* Health

The resultant coefficient (β) from modeling the individual tree's percent dieback by year (simple linear regression, R Core Team 2011) was treated as the response variable for *T. canadensis* health analyses at the tree-level. The five *T. canadensis* rates of decline were then averaged for analyses at the plot level. At the tree level (n = 165 trees), covariates included DBH (cm), average peak *A. tsugae* density (cm^{-1}), estimated number of years infested (5 -10 years), presence of circular hemlock scale (0,1), and if the tree directly received biological control (0,1). Analyses were initially separated for HCA's and control sites to examine the biological control factor, and then combined if found to be insignificant. At the plot level (n = 33 plots), the average tree rate of decline was modeled against multiple plot-level stand conditions including elevation, slope, aspect, heat index (section 2.2.3.1), years of *A. tsugae* infestation, basal area of *T. canadensis* (m^2/ha), and basal area of *P. strobus* (m^2/ha).

Tsuga canadensis health was compared between release and non-release plots (excluding HCA 29) to determine if healthier trees were correlated with the release of predatory beetles. Both the average rate of *T. canadensis* decline and the final sampling year's *T. canadensis* percent dieback were modeled to determine if they were a function of predatory beetle presence as well as any other factors found to be significantly correlated to tree health from the previous analyses. If the predatory beetle indicator variable was found to be significant, further analyses examined which factors within the treatment (i.e. number of beetles per hectare, years of releases, beetle species, and/or life stage released) are correlated to *T. canadensis* health in the 18 plots that received biological control.

3.2.4.2 *Adelges tsugae* Biology

To assess whether any tree characteristics influence *A. tsugae* densities, the peak average density per tree (n = 165 trees) was modeled against tree variables that were measured from the peak year. The peak *A. tsugae* density was used to sync up fluctuating population levels that varied between sites and years. Fixed effects in the model included tree DBH (cm), average length of new growth present per twig (cm), average proportion of bare tips (twig^{-1}), presence of the circular hemlock scale (0,1), estimated number of years the tree had been infested, and tree dieback (%). Stand dynamics were also analyzed to determine if they have a significant effect on *A. tsugae* densities at the plot level (n = 33). The fixed effects of plot elevation, aspect, slope, years of infestation, *T. canadensis* basal area (m^2/ha), and *P. strobus* basal area (m^2/ha) were included in the linear mixed effects model with the average peak *A. tsugae* density as the response variable.

To determine if biological control beetles had an effect on *A. tsugae* populations, the plot average peak *A. tsugae* density for the paired release and control plots (n = 30) were modeled against the presence/absence of predatory beetles and any other fixed effects found to be significantly correlated to *A. tsugae* densities. If the predatory beetle indicator variable was found to be significant, further analyses examined which factors within the treatment were correlated to *A. tsugae* densities in the 18 plots that received biological control.

3.3 Results

3.3.1 *Tsuga canadensis* Health

The average rate of decline for the 165 *T. canadensis* trees sampled in the Chattahoochee National Forest was 6.3% ± 0.4% dieback per year over the four years examined (Table 3.1), indicating that if trends continue at the same rate, trees would suffer mortality at an average of

15.87 years. Rates ranged from -0.12 to 0.18 with 20 trees (12.12%) experiencing a negative rate of decline (i.e. trees had an increase in new growth rather than needle loss over the four year period). When analyzing tree factors to determine if anything influences the rate of tree dieback, no factors were found to be significant including whether or not the tree directly received biological control beetles (*p*-values ranged from 0.081 to 0.629) (Table 3.3). Due to a strong correlation with elevation (r = 0.87), the heat index was removed from analyses. At the plot-level, no stand characteristics had a significant effect on *T. canadensis* dieback (*p*-values ranged from 0.082 to 0.916) (Table 3.4). With no significant tree or stand covariates, biological control was analyzed as a single factor in the analysis and found to have no effect on the rate of decline of *T. canadensis* in the Chattahoochee National Forest ($p = 0.865$) (Table 3.3, Fig. 3.2). In addition, biological control did not have a significant effect when examining the final year's percent dieback of *T. canadensis* ($p = 0.121$, Fig. 3.3).

3.3.2 *Adelges tsugae* Biology

Adelges tsugae followed expected trends of increasing and decreasing densities over the four-year study period. The average peak density was 5.2 ± 0.17 *A. tsugae* cm^{-1} and the average low density was 0.6 ± 0.06 *A. tsugae* cm^{-1} with an overall range of 0 – 13.7 *A. tsugae* cm^{-1} (Table 3.1). At the tree and plot level, the length of *T. canadensis* new growth was the only factor significantly correlated to *A. tsugae* density ($p < 0.001$ and $p = 0.013$ respectively) (Table 3.5 – 6). This relationship was evident over the four years in all sites except Panther Creek (HCA29) (Fig. 3.4). No stand characteristics were significantly correlated with *A. tsugae* densities (*p*-values ranged from 0.155 to 0.881) (Table 3.6). Although initial comparisons between release and control sites indicated that biological control releases could have influenced densities (Fig.

3.5A), the analyses found that the trend appears to be due to the differences in *T. canadensis* new growth between the sites (*p* = 0.013) (Table 3.7, Fig. 3.5B).

3.4 Discussion

During the four-year study, the majority of *T. canadensis* trees in both HCA's and non-release sites in the CNF were declining, however none of them died. Following the final measurement year (2013 or 2014), the average percent dieback for *T. canadensis* trees in the CNF was 58% (± 2%) after being infested with *A. tsugae* for an average of 8.36 (± 0.24) years (Table 3.1). There was variation in the year to year tree-level percent dieback that could be attributed to the production/halting of new growth as a response to *A. tsugae* population outbreaks and collapses (McClure 1991), but dieback nonetheless increased over the four years (Fig. 3.2). No stand or tree characteristics significantly influenced the rate at which *A. tsugae* is causing *T. canadensis* decline (Table 3.3 – 4). This was expected since the host has no known defenses or resistance, the non-native pest has no known native parasitoids or predators in the eastern U.S., and the environment in the Southern Appalachians is suitable for *A. tsugae* to reach high densities.

Increasing elevation marginally increased the rate of *T. canadensis* dieback (*p* = 0.08, Table 3.4) implying that trees at lower elevations may have longer life expectancies. This is supported when comparing HCAs and their corresponding control plots – with all five pairs, the lower elevation plots had lower average *T. canadensis* percent diebacks. When we focused on the 20 trees that recovered slightly from their initial measurement (i.e. had 5 – 15% decrease in dieback over the four year period), 95% of them were found at elevations below 550 m. In addition, 47% of all the trees examined below 460 m had negative dieback rates (recovery). Elevation was found to have a significant negative correlation to heat indices (r = 0.87), with higher elevations experiencing cooler temperatures. Previous research has found that higher heat indices are associated with increasing *A. tsugae* mortality (section 2.3), implying that these lower elevation/warmer sites may have recovering *T. canadensis* trees as a result of lower *A. tsugae* densities. If dieback rates are separated for trees at low elevations (< 460 m), the trees at higher elevations are experiencing dieback at an average rate almost six times faster than those at these lower elevations. Regardless of elevation, the region examined in the CNF did not experience *T. canadensis* mortality in the predicted time of 3-5 years post *A. tsugae* infestation (McClure 1991) and may not experience the anticipated 90% mortality by 2015 (USDA Forest Service 2005). Instead, mortality of *T. canadensis* in the CNF may closely resemble trends seen in the northeastern U.S. where stands still have a small portion of trees alive 15-20 years post infestation (Small et al. 2005). Of the 165 *T. canadensis* trees examined, only 45 (27%) had dieback greater than 75% at the end of this study (7 – 10 years post infestation). Of those, 85% were under- or mid-story trees. These smaller trees may have exhibited greater dieback than the larger trees due to less extensive root systems or fewer carbohydrate reserves.

In a similar study conducted in New England, Orwig et al. (2002) found that the main characteristic influencing the rate of *T. canadensis* decline was the duration of *A. tsugae* infestation. The number of years infested was not found to be a significant factor in Georgia (p = 0.63, Table 3.3) and sites infested with *A. tsugae* for 10 years had less dieback and slower rates of decline than those infested for 7 – 9 years. However, this counterintuitive trend is most likely due to the fact that the sites infested for 10 years (HCA 29 and 145) are also the lower elevation sites (< 460 m) implying that the effect of elevation/temperature may be stronger than the effect of infestation time.

As expected, increases in the length of *T. canadensis* new growth significantly increased *A. tsugae* densities (*p* < 0.001, Fig. 3.4). Since *T. canadensis* twigs were examined in July, *A. tsugae* density values were based on the number of aestivating sistens - the generation that preferentially settles on new growth (McClure 1991). In 2010, all sizes and conditions of *T. canadensis* in the CNF were exhibiting signs of a tip blight, later identified as *Sirococcus tsugae* Castlbury, Farr, and Stanosz (Stanosz et al. 2011). Samples from multiple sites in this study were positive for *S. tsugae* (*S. Fraedrich, personal communication*), but the potential pest-pathogen interaction that may occur with *A. tsugae* has not been well studied. The proportion of bare tips present on the *T. canadensis* samples marginally decreased the number of settled *A. tsugae* (*p* = 0.06, Table 3.5) indicating that an interaction may be occurring.

Results from this study imply that the release of biological control beetles is not reducing *A. tsugae* densities (*p* = 0.12) enough to slow the rate of *T. canadensis* decline (*p* = 0.87), or increase *T. canadensis* tree health (*p* = 0.12). However, multiple factors may need to be taken into consideration. Both *Sasajiscymnus tsugae* and *L. nigrinus* have been recovered from locations sampled 0.4 – 1.6 km away from release locations, including HCA's used in this study, indicating that the control sites used in this study may not have been true controls (McDonald et al. 2011, Jones et al. 2014). In addition, *L. rubidus*, a native species whose effects on *A. tsugae* populations is unknown, was recovered from *T. canadensis* sampled in both HCA's and control sites, potentially confounding the results regarding biological control (Jones et al. 2014).

According to biological control theory, the main goal for these releases is to reduce the pest population to a low, stable equilibrium, where there are no severe economic losses/damages (Caltagirone 1981, Murdoch et al. 1985). In the *T. canadensis*/*A. tsugae* system, effective control should eventually result in *A. tsugae* densities that remain at a stable level, avoiding the peaks and crashes, and trees that consistently produce new growth. Simulation models predict that an equilibrium density for overwintering sistens is approximately 2.1 cm^{-1}, with densities below this value if egg predation by biological control beetles is effective (~0.03 – 1.8 *A. tsugae* cm^{-1} from 80 – 20% predation respectively) (Elkinton et al. 2011). Of the six HCA's examined in this study, one site, HCA 29 (Panther Creek) is showing signs of *A. tsugae* densities at or approaching equilibrium. Over the four years studied, average densities at this site started at 3.19 *A. tsugae* cm^{-1} and gradually decreased to 1.93 *A. tsugae* cm^{-1} regardless of the length of *T. canadensis* new growth available (Fig. 3.4). This decrease could be a result of biological control releases, which was more than twice the number in other HCAs (n = 117,464 individuals since 2007) and/or warmer temperatures at this lower elevation site (229 – 445 m). Unfortunately, the effects of these two factors are difficult to separate at the present time. Further, Panther Creek is a relatively isolated area of *T. canadensis* which may have led to lower emigration of the biological control beetles allowing for greater pest control.

Biological control beetles, if established, have the potential to control *A. tsugae*, but pest suppression will not occur quickly. Many other factors negatively affect *T. canadensis* health, such as drought, hemlock borer (*Melanophila fulvoguttata* Harris), and elongate hemlock scale (*Fiorinia externa* Ferris). Hence, tree mortality may occur before biological control becomes effective. There is no known standard time in which biological control of *A. tsugae* will become successful and multiple factors may play a role such as the number released, the time of year releases occur, the life stage released, the density of *A. tsugae* in the stand, temperature, etc. (Cheah et al. 2004). In other studies, visible improvements in *T. canadensis* health have been observed as early as 4 – 7 years post release of biological control (Cheah et al. 2004, McDonald et al. 2011). The stands examined in the CNF have been receiving biological control releases for

6 – 8 years implying that the recovery seen (more new growth than dieback) may be a result of biological control efficacy.

Forest systems have been found to have the lowest potential for biological control successes (vs. agricultural or orchard settings) due to their large, complex, and heterogeneous landscape (Kimberling 2004). However, there are biological control management plans that have worked in forest settings: the winter moth (*Operophtera brumata* L.) was maintained at low population levels by *Cyzenis albicans* Fallén and *Agrypon flaveolatum* Gravenhorst in North America (Embree 1966), the Eurasian pine adelgid (*Pineus pini* Macquart) was controlled by *Leucopis obscura* Haliday and *L. nigraluna* McAlpine in Hawaii (Culliney et al. 1988, Lai 1988), the larch casebearer (*Coleophora laricella* Hübner) was successfully maintained by *Agathis pumila* Ratz. and *Chrysocharis laricinellae* Ratz. in the Northwest (Kimberling 2004), and *Compsilura concinnata* Meigen has lowered populations and retracted the range of the browntail moth (*Euproctis chrysorrhoea* L.) in New England (Elkinton et al. 2006). In addition, it has been found that over 60% of successful biological control scenarios, regardless of the system, involve the management of insects in the suborder Sternorrhyncha (aphids, adelgids, scales, etc.) due to their immobile, external feeding, indicating that the control of *A. tsugae* may have a better chance than other forest pests (Kimberling 2004).

The benefits of controlling *A. tsugae* in eastern North America are undoubtedly great, and based on this study, there is the potential that *T. canadensis* stands in Georgia may survive due to higher temperatures and/or biological control releases. Regardless, the majority of the CNF will likely experience a massive loss of *T. canadensis* basal area and a shift in forest composition within the next decade. However, the temporal information given herein can be important for future management efforts by predicting decline rates and prioritizing locations for biological control releases.

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Table 3.1. Characteristics of *T. canadensis* stands sampled in the Chattahoochee National Forest, Georgia (n = 165 trees or 33 plots).

Forest Attribute	**Mean ± SE**	**Range**
Plot Elevation (m)	658.03 ± 32.76	228.5 – 972.1
Plot Slope (%)	18.55 ± 2.14	0 – 39.0
T. canadensis Basal Area (m^2ha^{-1})	0.78 ± 0.10	0.09 – 1.78
P. strobus Basal Area (m^2ha^{-1})	0.79 ± 0.15	0 – 2.67
T. canadensis DBH (cm; all trees)	12.70 ± 0.40	1.4 – 92.7
T. canadensis DBH (cm; sampled trees)	15.71 ± 1.02	1.7 – 61.7
T. canadensis Dieback Rate ($year^{-1}$)	0.063 ± 0.004	-0.12 – 0.18
Final year *T. canadensis* Dieback (%)	0.58 ± 0.02	0.10 – 0.90
Years infested with *A. tsugae*	8.36 ± 0.24	7 – 10
Peak *A. tsugae* Density (cm^{-1})	5.21 ± 0.17	1.56 – 13.68
Maximum *T. canadensis* New Growth (cm)	9.39 ± 0.35	2.36 – 20.59
Number of Biological Control Beetles (HCA^{-1})	51,136 ± 13,722	26,590 – 117,464
- *Sasajiscymnus tsugae* (HCA^{-1})	34,698 ± 7,279	15,806 – 65,963
- *Laricobius nigrinus* (HCA^{-1})	14,574 ± 7,044	4,194 – 49,278
- *Scymnus sinuanodulus* (HCA^{-1})	1,864 ± 443	408 – 3,123

Table 3.2. Species composition of woody stems found in at least five (15%) *T. canadensis* plots sampled in the Chattahoochee National Forest, Georgia.

Species	**Occurrence (%)**
Tsuga canadensis	100
Acer rubrum L.	81.48
Carya spp.	66.67
Oxydendrum arboretum (L.) de Candolle	66.67
Pinus strobus	66.67
Liriodendron tulipifera L.	62.96
Quercus alba L.	62.96
Kalmia latifolia L.	55.56
Ilex opaca Aiton	48.15
Betula lenta L.	40.74
Nyssa sylvatica Marshall	37.04
Cornus florida L.	33.33
Hamamelis virginiana L.	29.63
Quercus rubra	29.63

Table 3.3. Result summary for linear mixed effects model examining multiple tree-level characteristics on the response variable of *T. canadensis* dieback rate: coefficient estimates β, standard errors SE (β), associated *t*-score, and significance level *p* for all independent variables in the analysis.

Independent Variable	**Coef. β**	**SE(β)**	***t***	***p***
Intercept	0.0762	0.0370	2.0598	0.0394*
DBH	0.0005	0.0003	1.7440	0.0812
Peak *A. tsugae* Density	-0.0013	0.0016	-0.8259	0.4088
Circular Hemlock Scale	0.0064	0.0080	0.8088	0.4187
Years Infested	-0.0024	0.0049	-0.4827	0.6293
Biological Control Tree$^{\Psi}$	0.0046	0.0121	0.3766	0.7065

*Significant at the $\alpha = 0.05$ level.

$^{\Psi}$ Results from analysis conducted using only release plot data.

Table 3.4. Result summary for linear mixed effects model examining multiple plot-level stand characteristics on the response variable of average *T. canadensis* dieback rate: coefficient estimates β, standard errors SE (β), associated t-score, and significance level p for all independent variables in the analysis.

Independent Variable	**Coef. β**	**SE(β)**	t	p
Intercept	0.0736	0.0488	1.5095	0.1312
T. canadensis Basal Area	-0.0134	0.0147	-0.9150	0.3602
P. strobus Basal Area	-0.0142	0.0105	-1.3522	0.1763
Peak *A. tsugae* Density	-0.0062	0.0059	-1.0549	0.2915
Elevation	0.00008	0.00004	1.7378	0.0823
Slope	-0.00008	0.0007	-0.1050	0.9164
Aspect (Transformed)	-0.0066	0.0110	-0.6015	0.5475
Biological Control[£]	-0.0013	0.0491	-1.5490	0.8652

*Significant at the $\alpha = 0.05$ level.

[£] Results from analysis using biological control as the only independent variable

Table 3.5. Result summary for linear mixed effects model examining multiple tree-level characteristics on the response variable of *A. tsugae* density: coefficient estimates β, standard errors SE (β), associated t-score, and significance level p for all independent variables in the analysis.

Independent Variable	**Coef. β**	**SE(β)**	t	p
Intercept	5.0322	1.3109	3.8386	0.0001*
DBH	0.0131	0.0123	1.0688	0.2852
T. canadensis New Growth	0.1653	0.0334	4.9436	< 0.001*
Proportion Bare Tips	-4.8222	2.5837	-1.8664	0.0620
Years Infested	-0.1881	0.1699	-1.1070	0.2683
Circular Hemlock Scale	0.0613	0.3591	0.1708	0.8644
T. canadensis Dieback	0.5510	0.9887	0.5574	0.5773

*Significant at the $\alpha = 0.05$ level.

Table 3.6. Result summary for linear mixed effects model examining multiple plot-level stand characteristics on the response variable of *A. tsugae* density: coefficient estimates β, standard errors SE (β), associated t-score, and significance level p for all independent variables in the analysis.

Independent Variable	**Coef. β**	**SE(β)**	**t**	**p**
Intercept	4.4587	2.5427	1.7535	0.0795
Elevation	0.0009	0.0015	0.5757	0.5648
Slope	-0.0350	0.0279	-1.2564	0.2090
Aspect (Transformed)	-0.1392	0.3867	-0.3599	0.7189
Years infested	-0.0497	0.3315	-0.1500	0.8807
T. canadensis Basal Area	-0.2789	0.5084	-0.5486	0.5833
P. strobus Basal Area	-0.5350	0.3766	-1.4204	0.1555
T. canadensis New Growth	0.2485	0.1000	2.4862	0.0129*

*Significant at the $\alpha = 0.05$ level.

Table 3.7. Result summary for linear mixed effects model examining biological control at the plot-level on the response variable of *A. tsugae* density: coefficient estimates *β*, standard errors SE (*β*), associated *t*-score, and significance level *p* for all independent variables in the analysis.

Independent Variable	**Coef. *β***	**SE(*β*)**	***t***	***p***
Intercept	3.4423	0.6237	5.5191	< 0.001*
Biological Control	0.7209	0.4568	1.5782	0.1145
T. canadensis New Growth	0.2055	0.0828	2.4827	0.0130*

*Significant at the α = 0.05 level.

Figure Legend

Figure 3.1. Location of plots sampled from 2010 to 2014 on *A. tsugae* in the Chattahoochee National Forest, Georgia. Numbers represent Hemlock Conservation Area (HCA) numbers. Modified from map produced by USDA Forest Service (2013).

Figure 3.2. Average percent *T. canadensis* dieback compared between sites that did (Release) and did not (Control) receive biological control beetles in the Chattahoochee National Forest, Georgia. Lines represent site trends over a four-year period. Most sites were observed from 2010 – 2013 with HCA 29 observed from 2011 – 2014. Overall, the presence of biological control did not have a significant effect on the rate of *T. canadensis* dieback ($p = 0.865$).

Figure 3.3. Comparison of *T. canadensis* dieback between sites that received biological control beetles (Release) and those that did not (Control) for each HCA studied in the Chattahoochee National Forest, Georgia. The presence of biological control was not a significant predictor of tree dieback ($p = 0.121$).

Figure 3.4. Yearly trends between the average sisten *A. tsugae* density and the average length of new growth present on *T. canadensis* trees within each site (HCA and control plots combined) studied in the Chattahoochee National Forest, Georgia. Most sites were observed from 2010 – 2013 with HCA 29 (Panther Creek) observed from 2011 – 2014. Overall, *T. canadensis* new growth length was a significant positive predictor of *A. tsugae* densities ($p < 0.001$).

Figure 3.5. Comparisons of *A. tsugae* densities (A) and *T. canadensis* new growth (B) between sites that received biological control beetles (Release) and those that did not (Control) for each HCA studied in the Chattahoochee National Forest, Georgia. The presence of biological control did not significantly influence *A. tsugae* densities ($p = 0.115$), but the length of *T. canadensis* new growth was found to be significant ($p = 0.013$).

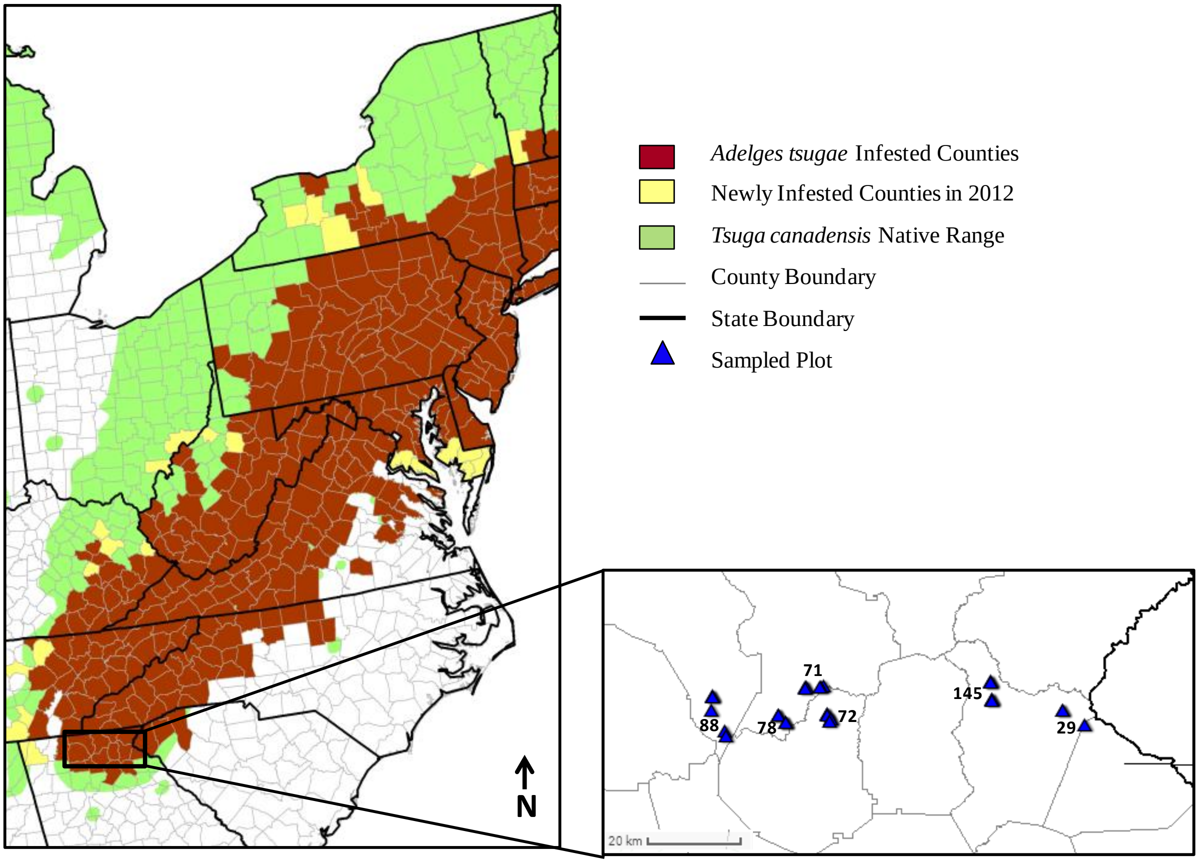

Adelges tsugae Infested Counties
Newly Infested Counties in 2012
Tsuga canadensis Native Range
County Boundary
State Boundary
Sampled Plot
N
71
145
88
78
72
29
20 km

Figure 3.1

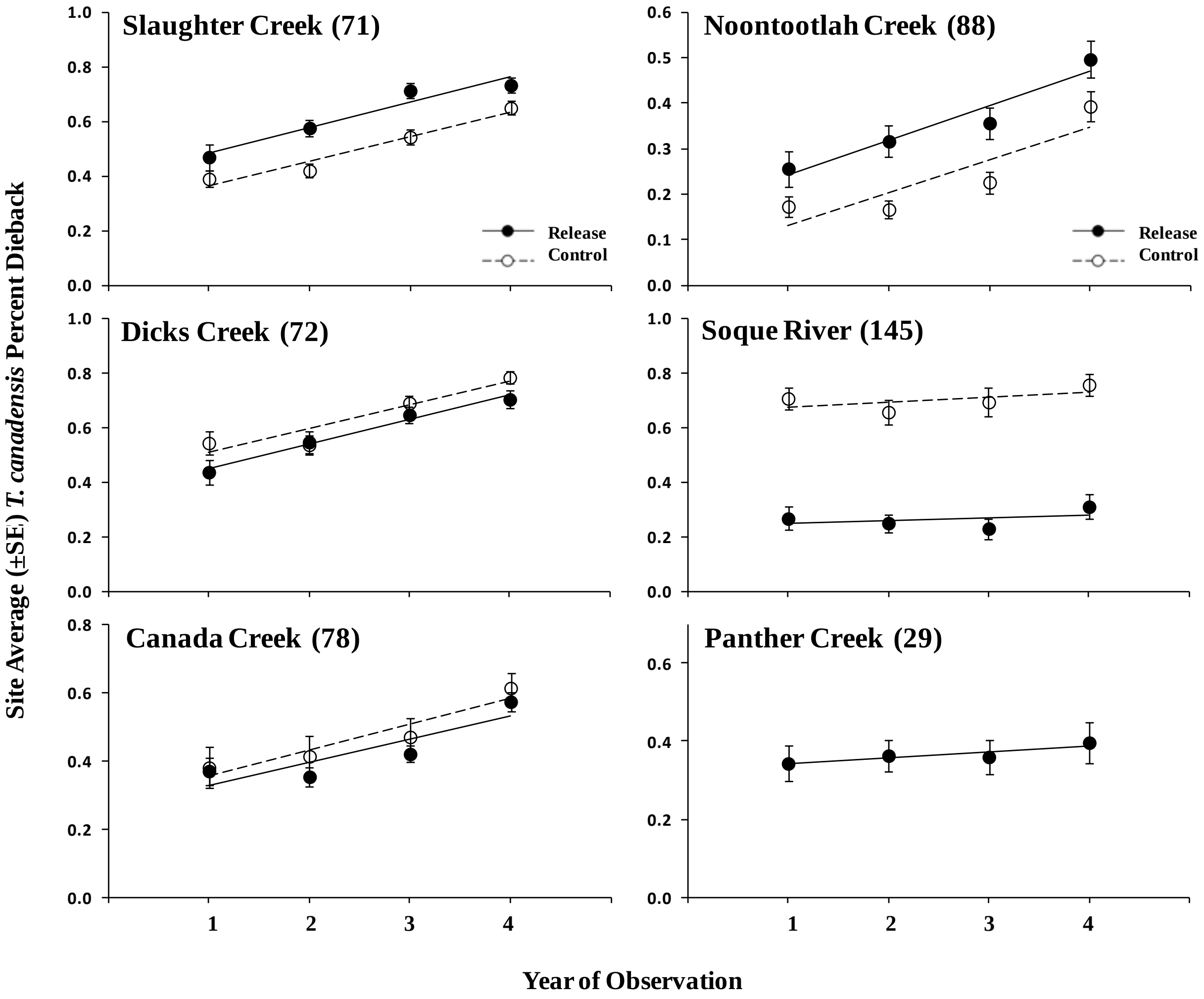
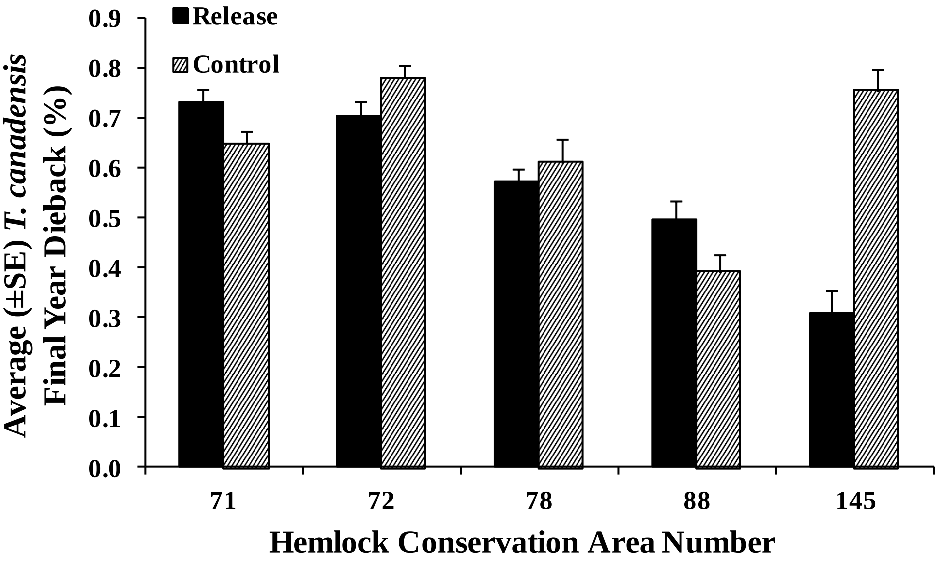

Site Average (±SE) *T. canadensis* Percent Dieback
Year of Observation
Slaughter Creek (71)
Noontootlah Creek (88)
Dicks Creek (72)
Soque River (145)
Canada Creek (78)
Panther Creek (29)
Release
Control
1.0
0.8
0.6
0.4
0.2
0.0
0.5
0.3
0.1
1
2
3
4

Figure 3.2



■ Release
▨ Control
Average (±SE) *T. canadensis* Final Year Dieback (%)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
71
72
78
88
145
Hemlock Conservation Area Number

Figure 3.3

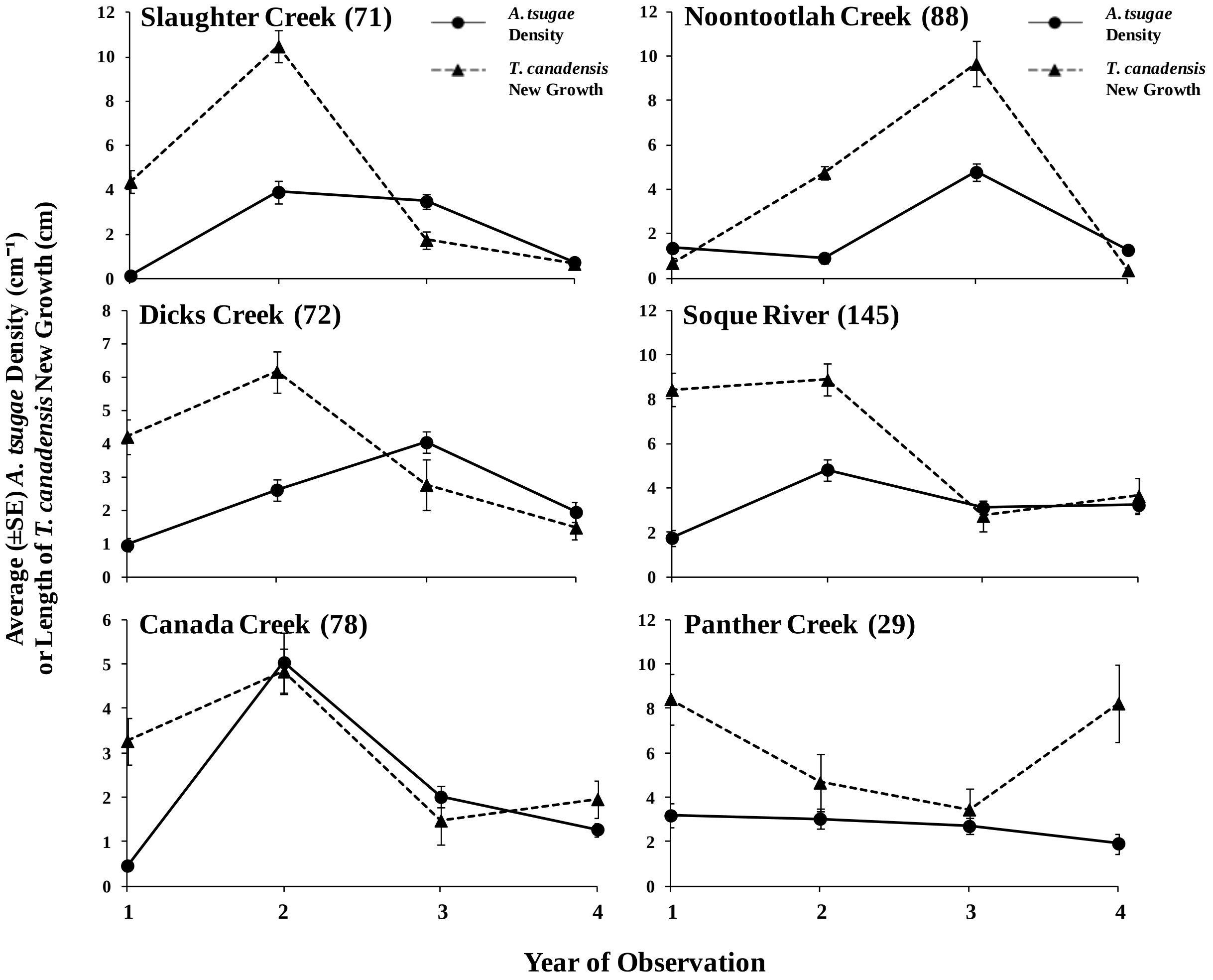
Slaughter Creek (71)
Noontootlah Creek (88)
Dicks Creek (72)
Soque River (145)
Canada Creek (78)
Panther Creek (29)
A. tsugae Density
T. canadensis New Growth
Average (±SE) A. tsugae Density (cm⁻¹) or Length of T. canadensis New Growth (cm)
Year of Observation
0
2
4
6
8
10
12
1
2
3
4

Figure 3.4

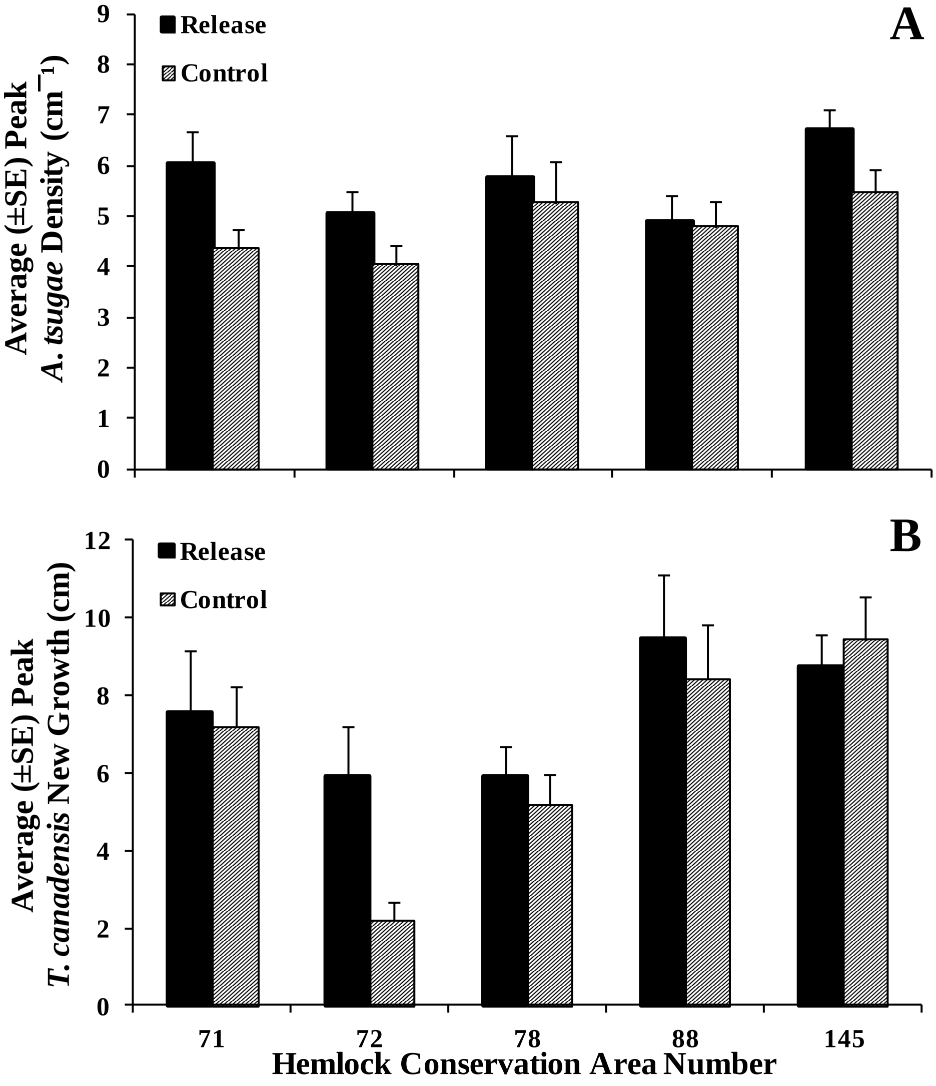

A
■ Release
▨ Control
Average (±SE) Peak
A. tsugae Density (cm⁻¹)
0
1
2
3
4
5
6
7
8
9
B
■ Release
▨ Control
Average (±SE) Peak
T. canadensis New Growth (cm)
0
2
4
6
8
10
12
71
72
78
88
145
Hemlock Conservation Area Number

Figure 3.5

CHAPTER 4

DISSERTATION CONCLUSIONS

4.1 Dissertation Conclusions

Infestation of *Adelges tsugae* in eastern North America is resulting in the widespread mortality of a dominant foundation species, *Tsuga canadensis*. For over 30 years, researchers have been examining this biological invasion with multiple advancements in both the top-down and bottom-up effects of the system. The goal of this dissertation was to examine the abiotic and biotic factors that may potentially influence *A. tsugae* and *T. canadensis* dynamics, and to add to established literature by focusing on the lesser researched region of the southeastern U.S. By having a better understanding of what characteristics play a role in the rate of *T. canadensis* dieback and/or *A. tsugae* densities, land managers can make better decisions on a temporal scale. For example, where the rate of *T. canadensis* dieback is slower, management can focus on long-term possibilities, such as the use of biological control. Where trees are dying at a faster rate, the use of pesticides may be more applicable.

In the southern region of *T. canadensis*'s range, *A. tsugae* was predicted to have a rapid negative effect on *T. canadensis* health due to the lack of cold winters, a limiting factor of *A. tsugae* in the northeastern region (Parker et al. 1998, 1999). However, trees at low elevation stands in the Chattahoochee National Forest, Georgia (< 550 m) were found to be surviving beyond the anticipated mortality of 3-5 years post infestation (McClure 1991) and had less dieback (< 25%) than locations with longer exposure to *A. tsugae*. In chapter two, I examined whether or not the higher summer temperatures at these locations may be a limiting factor in *A.*

tsugae success. Results from both manipulative and observational experiments indicate that increasing temperatures, especially above 30°C, significantly increase *A. tsugae* mortality. The length of exposure to tested temperatures (20 - 40°C) also had a significant negative effect on *A. tsugae* survivorship, implying that the effect of temperature is most likely cumulative. An algorithm was created that converted hourly field temperature readings, based on increasing weights given for each degree class above 15°C, into an overall site heat index for the duration of the summer (sisten) *A. tsugae* generation. These site heat indices were strongly correlated to the corresponding *A. tsugae* mortality (%), and the resultant model can be used to predict *A. tsugae* mortality based on summer temperatures. Following the summer of 2012, observed field *A. tsugae* mortality ranged from 9 to 63%, with the variability most likely due to the differences in elevation, and subsequently temperature regimens, found at the edge of the Southern Appalachian Mountains.

These results highlight the importance of evaluating summer temperatures in research, especially for studies that examine effects of climate change on aestivating insects. Based on the predictive model from this study, increases in temperatures associated with climate change (+2°C) may have a positive influence on *T. canadensis* conservation by reducing *A. tsugae* populations by up to 92% in regions of Georgia. Other predictions regarding *T. canadensis* and climate change have estimated that *A. tsugae* will eliminate *T. canadensis* from the Southeast due to warmer winters (Dukes et al. 2009).

Comparisons of temperatures between Georgia and Japan over a five-year period found that the majority of the maximum temperatures were higher in Japan during the summer months and higher in Georgia during the spring months. Physiologically, temperatures experienced by the parental (progredien) generation have been shown to significantly influence the fitness of the aestivating generation (Mousseau and Dingle 1991, Salom et al. 2001). There is the possibility that the mortality of the summer *A. tsugae* generation in Georgia is a response to the progredien generation being exposed to higher spring temperatures. Future research should evaluate the effects of high temperatures on *both* generations in order to create a more accurate predictive model of *A. tsugae* mortality as a response to temperatures, and to better understand the relationship between temperature and an aestivating forest pest.

A number of studies have examined tree and site characteristics on *T. canadensis* mortality rates and *A. tsugae* densities, but the majority of them have been located in the northeastern U.S., and did not examine both response variables simultaneously or did not include the factor of biological control (Orwig et al. 2002, McClure et al. 2003, Small et al. 2005, Eschtruth 2013). In chapter three I investigated the influence of tree factors, stand dynamics, and biological control on the rate of *T. canadensis* decline and corresponding *A. tsugae* populations over a four-year period in the Chattahoochee National Forest, Georgia. Results found that *T. canadensis* trees are experiencing dieback at an increase of 6.3% (± 0.4%) per year indicating that they may survive for over 15 years with *A. tsugae* infestation. The dieback rate of *T. canadensis* in the Southeast had never been quantified and was merely assumed to occur quickly. All trees observed in this study have survived 7 – 10 years of *A. tsugae* infestation with an average of 58% (± 2%) dieback at the culmination. Results indicate that *T. canadensis* mortality in the Chattahoochee National Forest may resemble trends observed in the northeastern U.S. where a portion of trees survive 15 – 20 years post infestation (Small et al. 2005). These slower rates of decline may result in more subtle forest responses compared to stands that experiences rapid mortality.

No tree or stand characteristics were found to significantly affect the rate of *T. canadensis* dieback. However, elevation was found to have a marginally negative effect on *T. canadensis* health (p = 0.08) with higher elevations experiencing more rapid rates of dieback. In addition, 12% of the *T. canadensis* trees examined over the four-year period showed signs of recovery (i.e. negative dieback rate), 95% of which were found at lower elevations (< 550 m). These slower rates may be the result of higher temperatures at the lower elevation sites reducing *A. tsugae* populations to levels low enough for *T. canadensis* to survive longer with the infestation. However, there are a number of factors that were not examined in this study that may influence the decline rates of *T. canadensis* such as drought history, soil characteristics, proximity to water, etc. Future work should evaluate the role of these factors in dieback rates to better elucidate what conditions are optimal for *T. canadensis* survivorship.

The length of *T. canadensis* new growth was the only factor found to have a significant effect on *A. tsugae* densities. This was anticipated since *A. tsugae* prefer to settle on new growth (McClure 1991). *Adelges tsugae* appeared to be somewhat negatively affected by the proportion of bare tips present on the twigs (p = 0.06). Less *A. tsugae* settled on offshoots with needles present if there were one or more offshoot tips missing needles, an indication of a potential tip blight such as *Sirococcus tsugae* (Stanosz et al. 2011). Research should look into this possible pest-pathogen interaction to determine its effects on both *A. tsugae* populations and *T. canadensis* health.

No significant top-down regulatory effects of biological control beetles on *A. tsugae* densities (p = 0.12) or *T. canadensis* dieback rates (p = 0.87) were found in this study. However, there were a number of potentially confounding factors affecting the confidence of these results. For example, predatory beetles were recovered from sites deemed to be non-release sites and these beetles are known to travel distances greater than those between the release and non-release sites used (McDonald et al. 2011, Jones et al. 2014). Also, the effects of a recovered native predator, *Laricobius rubidus*, on *A. tsugae* are currently unknown. Lastly, the site showing potential signs of effective biological control (i.e. decreasing *A. tsugae* densities and *T. canadensis* recovery following 10 years of infestation) was not included in statistical analyses regarding biological control due to the lack of a corresponding control site.

Interestingly, the trend in *A. tsugae* densities with biological control releases was positive (i.e. adding biological control increased *A. tsugae* densities compared to control sites). At first this seems surprising and counterintuitive, but when examining the length of *T. canadensis* new growth, it becomes evident that *A. tsugae* populations are higher at release sites because these sites had more new growth. If more new growth is interpreted as a sign of healthier trees, then it could be said that sites with biological control beetles are associated with healthier *T. canadensis*. More research is needed before biological control efficacy is determined including attempting to tease apart the effects of high temperatures and biological control at low elevation sites, examining the influence of high temperatures on predatory beetle success, fecundity, and efficacy, and an in-depth analysis of more biological control release sites to determine if there are any within treatment trends that may prevent *T. canadensis* mortality.

4.2 Literature Cited

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Appendix A

Latitude, longitude and elevation of Chattahoochee National Forest plots used in studies. HCA numbers followed by "CP" denote non-release (control) plots and "*" are plots where temperature loggers were placed.

HCA Plot Number	Latitude	Longitude	Elevation (m)
71P1*	34.74127	-83.95538	972.1
71P2	34.74020	-83.95998	936.8
71P3*	34.73933	-83.96188	928.5
71CP1	34.73857	-83.99348	901.5
71CP2*	34.73868	-83.99465	903.8
71CP3	34.73780	-83.99512	900.4
72P1*	34.68093	-83.93885	522.5
72P2*	34.67667	-83.94070	522.6
72P3	34.67968	-83.93722	524.9
72CP1	34.68992	-83.94770	559.9
72CP2*	34.68928	-83.94780	544.7
72CP3*	34.68767	-83.94753	535.2
88P1*	34.69682	-84.20752	646.9
88P2	34.65812	-84.17593	842.2
88P3*	34.64998	-84.17458	838.8
88CP1	34.72205	-84.20397	669.3
88CP2	34.72220	-84.20410	663.7
88CP3*	34.72243	-84.20385	660.4
78P1*	34.68655	-84.05770	717.2
78P2	34.68687	-84.05767	718.1
78P3*	34.68767	-84.05762	720.4
78CP1*	34.67327	-84.04007	827.8
78CP2	34.67387	-84.04122	818.1
78CP3*	34.67458	-84.04118	806.9
145P1	34.71668	-83.57650	458.0
145P2*	34.71590	-83.57632	457.1
145P3	34.71557	-83.57630	457.0
145CP1*	34.75042	-83.57915	520.6
145CP2	34.74890	-83.57777	519.3
145CP3	34.74843	-83.57762	502.7
29P1*	34.69628	-83.41758	443.9
29P2	34.69620	-83.41672	445.1
29P3*	34.66930	-83.36792	228.5