

## ABSTRACT

JETTON, ROBERT MILLER. Biological Control, Host Resistance, and Vegetative Propagation: Strategies and Tools for Management of the Invasive Hemlock Woolly Adelgid, *Adelges tsugae* Annand. (Under the direction of Dr. Fred P. Hain.)

Biological control, host resistance, and vegetative propagation were evaluated as strategies and tools for management of the exotic pest hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) in the southeastern U.S. The suitability of the balsam woolly adelgid (BWA), *Adelges piceae* Ratzeburg, as an alternate rearing host for *Sasajiscymnus tsugae* was compared to the primary prey HWA in a series of laboratory-based paired-choice and no choice (single-prey) experiments that tested adult feeding, oviposition, and long-term survival and immature development of the predator. Results indicated that *S. tsugae* will feed equally on eggs of both prey, will accept both prey for oviposition, and that the predator's rate of immature development did not differ between the prey species, although fewer successfully completed egg to adult development on a diet of BWA compared to a diet of HWA. The long-term survival of predator adults was significantly influenced by both test prey type and the availability of a supplemental food source.

The utility of confined releases for colonization of *S. tsugae* was evaluated in three field studies at forest and ornamental sites in western North Carolina. Predator reproduction, survival, and impact on HWA were investigated following the placement of fifteen adults (10♀:5♂) in mesh sleeves cages on adelgid infested hemlock branches for two or four weeks. In all three studies the predator reproduced inside sleeve cages and oviposition generally began within two or three weeks. Some adult predators were recovered during all three studies, indicating that *S. tsugae* can survive for up to one month inside mesh sleeve cages.

Predator exclusion cages indicated significant local reductions in the density of adelgid ovisacs and aestivating nymphs, but these were not always correlated to the presence of the predator. Where *S. tsugae* was liberated from mesh sleeves two weeks after placement in the cages evidence of predator activity disappeared from the study sites within 4 months and establishment could not be documented during the study period.

Differences in initial infestation rate and fecundity of HWA among three species of North American hemlock were evaluated in a climate-controlled greenhouse. Seedlings of adelgid-susceptible eastern, *Tsuga canadensis* (L.) Carrière, and Carolina, *T. caroliniana* Engelmann, hemlocks and putatively adelgid-resistant western hemlock (*T. heterophylla* Sargent) received either low inoculation (50 adelgid egg masses per seedling) or high inoculation (100 adelgid egg masses per seedling) treatments. Results showed very low and statistically similar rates of initial infestation on seedlings of Carolina and western hemlock and significantly higher rates on eastern hemlock seedlings. Adelgids that fed successfully on Carolina hemlock had significantly higher fecundity than those on either eastern or western hemlock, although this varied depending on inoculation treatment.

The effects of auxin (NAA) concentration and cutting length on adventitious root formation in softwood stem cuttings from mature eastern and Carolina hemlocks were studied in a mist house. Overall rooting percentage (41%) and percent mortality (22%) were higher for eastern hemlock compared with Carolina hemlock (10% rooting and 13% mortality). Rooting percentage of each species responded differently to varying auxin concentrations (0, 1, 2, 4, 8 mM NAA), although the relationship between percent mortality and rooting was similar. Mortality among 6 cm stem cuttings was twice that observed for 3-cm cuttings of both species. However, 6 cm cuttings of eastern hemlock that did form

adventitious roots had significantly more roots and longer total root length compared with 3-cm cuttings, a difference that was not significant for Carolina hemlock.

Biological Control, Host Resistance, and Vegetative Propagation: Strategies and Tools for  
Management of the Invasive Hemlock Woolly Adelgid, *Adelges tsugae* Annand

by  
Robert Miller Jetton

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APPROVED BY:

---

Fred P. Hain  
Chair of Advisory Committee

---

Daniel J. Robison  
Co-Chair of Advisory Committee

---

John Frampton

---

David B. Orr

---

John F. Monahan

## **BIOGRAPHY**

Robert Miller Jetton was born in Greenville, South Carolina on January 2, 1974. He is the youngest of the four children of Dr. Robert L. Jetton of Tryon, North Carolina and Ms. Lee E. Churchill of Greenville, SC. He grew up in Greenville and graduated from Christ Church Episcopal School in June 1993.

In September 1993, Robert enrolled in Furman University in Greenville, SC where he pursued and graduated with a Bachelor of Science degree in Biology in June 1997. From 1997 until 1999 Robert continued to live and work in Greenville where he was employed with the textile firm Nutex, Inc. He served as Director of Health, Safety, and Environmental Affairs and interim Plant Manager.

In January of 1999, Robert enrolled as a graduate student at North Carolina State University. In 2002 he received a Master of Science degree in Forestry and Entomology studying under the direction of Dr. Daniel J. Robison and the Hardwood Research Cooperative. He then continued in graduate school at N.C. State to pursue a Doctor of Philosophy degree in Entomology and Forestry studying under the direction of Dr. Fred P. Hain.

Since December of 2005, Robert has been employed full time as a Research Associate with the Camcore Cooperative while finishing his doctoral research and writing this dissertation. Camcore is an international tree conservation and domestication organization housed in the Department of Forestry and Environmental Resources at N.C. State, and Robert serves as the Project Leader for Camcore projects related to germplasm conservation of eastern and Carolina hemlocks.

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## CHAPTER I

### **A Review of the Pertinent Literature on Eastern and Carolina Hemlock and the Biology, Impacts, and Management of the Hemlock Woolly Adelgid**

#### **DISSERTATION INTRODUCTION**

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), so called because of the cottony white ovisacs produced from wax glands on the dorsum of the abdomen, is an exotic pest that has become a serious threat to ornamental and forest hemlock in eastern North America. First reported and described in western North America (British Columbia, California, Oregon) in the 1920's (Annand 1924), its widespread distribution and innocuous habit throughout central Japan and China suggests Asia as its origin (Takahashi 1937; McClure 1995a). Since the first reported sighting in eastern North America (Virginia) in the early 1950's (Gouger 1971), HWA has caused widespread mortality of both eastern (*Tsuga canadensis* (L.) Carrière) and Carolina hemlock (*T. caroliniana* Engelm.) and is now known to occur in 17 eastern states. Until its most recent invasion into the United States the biology of HWA remained largely unstudied (McClure 1996).

HWA feeds from parenchyma or storage cells located in the xylem tissue (Shields et al. 1996). Feeding depletes stored nutrients causing needles to desiccate, premature needle drop, and bud abortion leaving the tree with a gray cast. Heavy infestations can kill trees in as little as four years although some trees have survived for more than ten (McClure et al. 2001). The speed with which this pest can kill and the economic, ecological, and aesthetic value of hemlock in the eastern forest ecosystem highlight the need for a defense against this



invader. While chemical control (foliar sprays and stem and soil injected pesticides) have been successful for control of HWA in ornamental settings (McClure 1992a, 1995a; Steward and Horner 1994), their use in the forest setting is limited by the scattered nature and sensitivity of these ecosystems (McClure et al. 2001). Therefore, biological control by both native natural enemies (Montgomery and Lyon 1996; Wallace and Hain 2000) and exotic coccinellid (Sasaji and McClure 1997; Lu and Montgomery 2001; McClure 2001) and derodontid (Zilahi-Balogh et al. 2002a) beetles has become the focus of control efforts against this pest. One of these exotic beetles, *Sasajiscymnus* (formerly *Pseudoscymnus*) *tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), has shown potential for controlling HWA in both laboratory and field trials, and it is now being mass reared and released into infested hemlock stands in the eastern United States (Cheah and McClure 1998, 2000). However, there is little evidence to suggest that this beetle has become establish in or has dispersed from release sites. There has been little research on the biology of *S. tsugae* other than its life-cycle, potential for controlling HWA, and potential for mass rearing. More intensive research on this coccinellid's behavior and how it is introduced into the hemlock ecosystem may help forest managers to use it more effectively as a biological control agent.

In addition to chemical and biological control is the ability of eastern and Carolina hemlocks to resist attack by HWA. In Asia and western North America, where HWA is an innocuous pest, it is believed that host resistance coupled with native natural enemies prevent this pest from killing the *Tsuga* species that grow in these regions (McClure 1992b; McClure and Cheah 1999). If biological control is to be effective in controlling HWA in eastern North

America, there will need to exist some level host resistance in order for trees to survive long enough for introduced natural enemies to become established. To date there has been little investigation into potential host resistance mechanisms in eastern or Carolina hemlock. *Tsuga* spp. from Asia and western North America may provide a good starting point from which to identify such characteristics.

The research I present in this dissertation addresses issues related to two of these HWA management options, classical biological control and host resistance. Chapter II focuses on an issue critical to the success of *S. tsugae* mass rearing programs, the aestival diapause of the adelgid and resulting rearing delays. I assessed the utility of an alternate prey (the balsam woolly adelgid, *A. piceae* Ratzeburg), active during HWA's diapause, as a surrogate rearing host for the predator. In Chapter III, I present the results of field studies that evaluated the success of low-density confined releases of *S. tsugae* as a means of establishing the predator in the southern Appalachian Mountains. Chapter IV concerns host resistance and measured the initial infestation success and fecundity of HWA on adelgid susceptible eastern and Carolina hemlocks compared to putatively adelgid resistant western hemlocks (*T. heterophylla* Sargent). Finally, in Chapter V, I present the results of rooted cutting trials that assessed the potential for vegetative propagation of eastern and Carolina hemlocks that could be used to multiply resistant genotypes. The reader will also find two appendices, the first that corrects errors in the data analysis of Chapter V discovered after its publication in *HortScience*, and the second that reports on surveys of arthropod diversity associated with old and secondary growth stands of eastern hemlock in the Great Smoky

Mountains National Park. The remainder of this first chapter is a literature review that summarizes what is known of hemlock ecology, and biology and impacts of the HWA, and efforts to manage this pest in forests of the eastern U.S.

## **THE HEMLOCK RESOURCE**

### Description of the Genus

The genus *Tsuga* is the most shade tolerant and drought susceptible in the Pinaceae (Farjon, 1990). Although as many as 18 individual hemlock species are described by various authors (Krussman, 1985; Gelderen and Smith, 1986), only nine are known to naturally occur while the others are now recognized as subspecies and cultivars or have been placed in sister genera (Farjon, 1990). The world-wide distribution of *Tsuga* species is discontinuous with species concentrated in the temperate regions of North America and eastern Asia (Figure 1). Two concentrations are found in North America. One is in the coastal mountains and islands of the Pacific Northwest extending into the northern Rocky and Sierra Nevada Mountains where *T. heterophylla* Sargent and *T. mertensiana* (Bong.) Carr. are found. The second is in eastern North America, extending from Nova Scotia south to Alabama and west into Minnesota, where Eastern (*T. canadensis* (L.) Carrière) and Carolina hemlock (*T. caroliniana* Engelmann) occur, although the latter is restricted to the southeastern U.S. A third concentration of *Tsuga* is in eastern Asia, with species occurring in Japan, *T. sieboldii* Carrière and *T. diversifolia* Masters, China, *T. chinensis* (Franchet) Pritzel in Diels and *T. forrestii* Downie, and the Himalayan Range, *T. dumosa* (D Don) Eichler (Farjon, 1990). Taxonomists place eight hemlock species in the *Tsuga* (formerly *Micropeuce*) section

(Krussman, 1985; Gelderen and Smith, 1986). The ninth species, *T. mertensiana*, is classified alone in the Hesperopeuce section, a separation based largely on morphological differences among the species and is not universally accepted (Farjon, 1990). Although HWA is now found in all three regions worldwide where hemlocks occur, it causes widespread tree mortality only in the eastern United States where it is exotic.

#### Eastern and Carolina Hemlock

Eastern hemlock is a slow-growing, long-lived, shade-tolerant species that takes 250 to 300 years to reach maturity and may live for more than 800 years. It ranges from eastern Canada west to Minnesota and south along the Appalachian mountains to Georgia and Alabama (Figure 2). Small, natural populations exist as far west as southern Michigan and western Ohio, and east of the Appalachians in the mid-Atlantic states. It is restricted to areas with cool, humid climates that can be found at sea level in the northeastern part of its range and at 600 to 1500 meters elevation on the north and east facing slopes, coves, and cool, moist valleys of the southern Appalachians (Godman and Lancaster 1990).

Eastern hemlock is found as a major component of four forest cover types, a common member of 7 types, and a minor species in 18 types. The four types in which it is found as a major component are: the white pine-hemlock [Society of American Foresters (SAF) Type 22], eastern hemlock (SAF Type 23), and hemlock-yellow birch (SAF Type 24) in the northern forest region, and yellow poplar-eastern hemlock (SAF Type 58) in the central forest region. Throughout its southern range Eastern hemlock is considered a minor component of the southern Appalachian mixed-hardwood forest (Godman and Lancaster 1990).

Historically, eastern hemlock was found in 20 percent of the upland forest ecosystems in eastern North America. Following European settlement and the harvesting that followed, it now occurs in less than six percent (McClure et al. 2001). As of 1999 the gross standing volume of eastern hemlock in eastern North America was approximately 300 million cubic meters. Eastern Canada contained 56 million cubic meters or 20 percent of this inventory. The remaining 22 million cubic meters was found in the United States with the eastern hemlock forest type accounting for 1 million hectares or less than one percent of the total timberland area of states within its natural range. Seventy-nine percent of this inventory was located in the New England and mid-Atlantic states with only eight percent found in the southern Appalachian states. Seventy-five percent of eastern hemlock was concentrated in sawtimber-size stands with only four percent in seedling-sapling stands (McWilliams and Schmidt 2000).

The range of eastern hemlock completely overlaps with that of Carolina hemlock (Figure 2), a closely related species that grows primarily on rock outcrops, talus slopes, and dry, rocky ridges of mountainous regions in Virginia, Tennessee, North Carolina, South Carolina, and Georgia (James 1959) commonly occurring alongside rhododendron, *Rhododendron* spp., and mountain laurel, *Kalmia latifolia* L. (Uttal 1969). Isolated populations have also been identified in the upper Piedmont of Virginia and North Carolina (Stevens 1976). While Carolina hemlock can be found growing in stands alongside eastern hemlock, its propensity for colonizing resource deprived sites in the ridge-top environment may have developed as a competitive advantage against other woody species less tolerant of

such harsh conditions (Humphrey 1989; Rentch et al. 2000). This species can be distinguished from eastern hemlock as a typically smaller tree with more compact growth having larger cones and longer needles that surround the twig instead of being in two ranks (James 1959; Uttal 1969).

Early research concluded that Eastern and Carolina hemlock must be closely related genetically (Szafer, 1949) because the two species' geographic ranges overlap (Figure 2) and they occasionally occur sympatrically. However, subsequent biogeographical and morphological studies indicated that Carolina hemlock is more closely related to *Tsuga* species occurring in Asia than to either Eastern hemlock or *Tsuga* species in western North America (Little, 1970; LePage, 2003). These findings are supported by molecular analyses of ribosomal DNA among the members of the genus that identified two clades within *Tsuga*: 1) a Western North American grouping containing *T. heterophylla* and *T. mertensiana* and 2) an Asian grouping containing the five Asian species and Carolina hemlock. Eastern hemlock was supported as a sister group to the Asian clade (LePage, 1997; Vining, 1999). Further support is found in the success of *T. caroliniana* x *T. chinensis* hybridization on arboretum specimens that could not be replicated in *T. canadensis* x *T. chinensis* crosses (Bentz et al., 2002; Pooler et al., 2002).

#### Insects, Disease, and Hemlock Ecosystem Dynamics

There are several pathogens and pests that may damage hemlocks at various stages of development. Seeds are sensitive to molds, particularly *Botrytis* spp., and desiccation during germination. Young seedlings are susceptible to damping-off and root rot fungi of *Pythium*

spp. and *Rhizoctonia* spp. Several rust diseases (*Melampsora farlowii*, *M. abietiscanadensis*, *Pucciniastrum hydrangeae*, and *P. vaccinii*) can cause shoot and needle blights or attack cones causing seed abortion. There are many insects that attack eastern and Carolina hemlock but only a few are of economic concern. Historically, the most important have been the hemlock borer (*Melanophila fulvoguttata* Harris) and the hemlock looper (*Lambdina fiscellaria* Guenée). There are also three species of armored scale insects that can damage needles. The elongate hemlock scale (*Fiorinia externa* Ferris) and the cryptomeria scale (*Aspidiotus cryptomeriae* Kuwana) are exotics introduced from Japan. The hemlock scale (*Abgrallaspis ithacae* Ferris), is native. The spruce budworm (*Choristoneura fumiferana* Clemens) and the gypsy moth (*Lymantria dispar* L.) are minor pests of hemlock that can become major pests in the absence of their preferred hosts (Godman and Lancaster 1990).

One or all of these pests may play a role in the dynamics of the eastern hemlock ecosystem. Paleoecological studies of pollen sediments reveal that eastern hemlock has been a dominant species at some sites in eastern North America for more than 8000 years. These studies have shown that, following initial colonization, hemlock abundance has undergone abrupt decline following major disturbances every 1000 years with gradual recovery over 500-plus years. These disturbances include fire, hemlock decline, and, most recently, European settlement. It is hypothesized that hemlock decline was caused by a combined influence of pests and climate change with the hemlock looper playing a major role (Foster 2000). Hemlock abundance is just now beginning to recover from the most recent disturbance, the logging associated with European settlement, which occurred only 250 years

ago. This only increases the concern over the newest pest of eastern hemlocks, HWA, which is attacking an already weakened hemlock ecosystem.

### Importance of Hemlock Ecosystems

The eastern hemlock ecosystem is valued for its economic, aesthetic, and ecological importance. Historically, hemlock wood has been utilized for framing, sheathing, subflooring, and crating. It was most commonly used during early settlement by the leather industry that took advantage of the high tannin concentration of hemlock bark. Old-growth hemlock was also favored by the timber barons in the early 1900's who cut hemlock in place of the more preferred eastern white pine (*Pinus strobus* L.) which became unavailable or inaccessible due to excessive harvesting (Godman and Lancaster 1990; Quimby 1996). Today, second-growth eastern hemlock have matured, again opening a northeastern market for hemlock pulpwood and sawtimber (Quimby 1996) and a byproduct, bark mulch, pound for pound the most valuable commercial component of this species (Howard et al. 2000). Similar to trends in the early 1900's, markets for hemlock have opened due to changes in spruce (*Picea* sp.) and fir (*Abies* sp.) availability following spruce budworm outbreaks. However, hemlock markets have never been strong and will not become so due to its poor wood properties. Hemlock wood has an uneven texture, considerable ring shake, moderate strength, low resistance to splitting, is harsh and splintery when worked with tools, and has low decay resistance (Godman and Lancaster 1990).

Aesthetically, the eastern hemlock ecosystem is an important component of many state and national recreation areas. Its broad appeal to humans for coolness in summer and



warmth in winter is a main attraction to such areas. With over 274 cultivars available, hemlocks are also highly favored for their ornamental beauty and the wildlife they attract to landscape settings (Swartley 1984; Quimby 1996).

The ecological importance of the eastern hemlock ecosystem is found in the unique habitat created when hemlocks come to dominate a stand. The resulting dark, cool, moist habitat with altered soil characteristics supports only shade tolerant hemlock seedlings and associated grown covers (Quimby 1996). This environment provides cover and forage for a number of terrestrial and aquatic organisms. The impact of HWA infestation on these and other forest and ecosystem processes is covered below in the section on impacts of the hemlock woolly adelgid.

## **BIOLOGY OF THE HEMLOCK WOOLLY ADELGID**

### Distribution of the Hemlock Woolly Adelgid

The first report of HWA in North America was in the Pacific Northwest (British Columbia, California, Oregon) in the 1920's (Annand 1924) where it remains today but is not considered a serious pest. Its widespread distribution and innocuous habit throughout central Japan and China suggest Asia as its origin (Takahashi 1937; McClure 1995a). Recent analyses of HWA mitochondrial DNA from Asian and western and eastern North American specimens suggests that the adelgid is native to both Asia and the Pacific Northwest, and that the source of introduction to the eastern U.S. is Japan (Havill et al. 2006).

The hemlock woolly adelgid was first reported in eastern North America in the early 1950's, found on eastern hemlocks growing in a municipal park in Richmond, Virginia

(Gouger 1971; Souto et al. 1996). Over the next 30 years it slowly spread through the mid-Atlantic states and was considered no more than an annoying ornamental pest easily controlled with insecticides. However, upon reaching the native hemlocks in the Blue Ridge Mountains of Virginia the rate of spread and intensity of damage associated with HWA increased sharply. It is believed that Hurricane Gloria, that hit the eastern seaboard in 1985, hastened its spread north to Connecticut (Souto et al. 1996). The adelgid has now spread into 16 eastern states from Georgia to Maine at a rate of 10-15 miles per year and infests approximately 50% of the eastern hemlock ecosystem (Souto et al. 1996; J.R. Rhea - personal communication; Figure 3).

Twice during the past 20 years severe winter cold has greatly reduced HWA abundance, once in Virginia (1985) and once throughout the entire eastern North American range (1994) (Souto et al. 1996). Initial laboratory studies support this observation, and an apparent lack of cold-hardiness may limit or prevent the spread into northern New England (Gouli et al. 2000; Parker et al. 1998; 1999). The dispersal of HWA is facilitated by wind, birds, mammals, and humans (McClure 1989a, 1990), although human movement of the pest may be more important than initially thought (Koch et al. 2006).

#### Life Cycle of the Hemlock Woolly Adelgid

The hemlock woolly adelgid has a complex polymorphic life cycle that includes three generations per year, each developing through an egg stage, four nymphal instars, and an adult stage (McClure 1989b). Two generations are wingless parthenogenetic forms called the sistens and progrediens, each completing their life cycle on the secondary hemlock host.

The third is the winged sexual generation called the sexuparae that leaves hemlock in search of a primary spruce (*Picea* spp.) host. The sistens reach maturity in February and produce cottony white ovisacs from March to May containing up to 300 eggs each. Approximately 50 percent of these eggs will hatch into progrediens with the remaining becoming sexuparae, although the actual ratio will vary depending on hemlock health and adelgid density (McClure 1987, 1996). The progrediens remain on hemlock and feed from April through late June when they mature and produce cottony ovisacs containing eggs of the sistens generation. The sistens crawlers hatch, settle on hemlock and feed for a few days before entering aestivation for the duration of the summer. In October, they begin feeding again until maturation in February (McClure 1989b). The developmental times reported here represent observations over two years at two sites in Connecticut. The development of any HWA generation can be accelerated or delayed up to a month depending on climate differences between years or states (McClure 1996; Gray and Salom 1996).

The same polymorphic life cycle of HWA has been observed in both its introduced range of eastern North America and its native range in Japan (McClure 1996). However, in the eastern United States there are no suitable spruce hosts for the sexuparae that mature and leave hemlock in June. In studies on 12 spruce species in Connecticut, winged adults colonized and laid a full complement of eggs on all species. However, the nymphs all died within a few days of hatching indicating a lack of primary hosts in eastern North America. Those sexuparae which remained on eastern hemlock also died (McClure 1987). Observations in Japan revealed adelgid galls on *Picea jezoensis hondoensis* (Sieb. and Zucc.)

and *P. polita* (Carriere) suggesting both of these species as primary hosts for HWA (McClure 1996). The lack of primary hosts and the inability of hemlock to support the sexuparae may help to limit the spread and genetic diversity of HWA in eastern North America.

#### Feeding and Host Effects of the Hemlock Woolly Adelgid

HWA crawlers of both the sistens and progrediens generations settle at the base of hemlock needles upon hatching and insert their piercing-sucking mouthparts, or stylet bundle, into the plant tissues (McClure et al. 2001). The stylet bundle is composed of four individual stylets within a sheath. The bundle is inserted into the underside of the needle, proximal to the stem with respect to the leaf abscission layer. Insertion distal to the abscission layer may be too far away from the preferred feeding tissues, or penetration of the abscission zone may be too difficult and/or trigger needle abscission (Young et al. 1995). A salivary sheath that protects the insect from plant chemical defenses is secreted around the bundle (Shields et al. 1996). The stylet bundle moves through the plant tissues following a mixed intracellular and intercellular pathway until it reaches the feeding site, the xylem ray parenchyma cells, where HWA feeds on stored nutrients. This feeding mechanism differs from other adelgids that feed directly on photosynthate from the phloem of their hosts. This suggests that the impact of HWA on eastern and Carolina hemlock may be related to factors other than its direct consumption of food, such as toxic saliva injected while feeding and/or the depletion of stored nutrients, making trees more susceptible to other biotic or abiotic stressors. (Young et al. 1995; Shields et al. 1996).

Feeding by HWA causes needle desiccation, premature needle drop, bud abortion,

and reduced terminal growth leaving infested stands with signs of poor crown condition typified by trees with a gray cast. Heavy infestations can kill trees in as little as four years although some trees have survived for more than ten (Souto et al. 1996; McClure et al. 2001). The impacts vary greatly for individual trees and may be related to predisposing factors of site condition (drought, nutrient deficiencies, old age) and secondary mortality agents (hemlock borer, hemlock looper, elongate hemlock scale, gypsy moth). In 1995, Virginia reported marked increases in hemlock borer activity in stands already infested with HWA. Trees in Pennsylvania have appeared to be growing vigorously for 10 years despite the presence of HWA (Souto et al. 1996). Individuals of species believed to be generally resistant to HWA, such as *T. diversifolia*, *T. sieboldii*, and *T. heterophylla* have become infested when growing in stressed environments (McClure 1992b, McClure and Cheah 1999). The wide variety of factors involved and the interactions that lead to hemlock mortality make it difficult to predict when and where eastern and Carolina hemlock stands will become susceptible to HWA (Souto et al. 1996).

#### Population Dynamics of the Hemlock Woolly Adelgid

Feeding by HWA and the resulting reduction in growth and survival of eastern and Carolina hemlock have a major impact on the performance and population dynamics of this pest (McClure 1991a; McClure et al. 2001). Adelgid population densities are subject to drastic fluctuations from year to year in response to density-dependent changes in the nutritional quality of hemlock, creating a population dynamic characterized by bimodal peaks of abundance (McClure 1991a). During the initial phases of an infestation (1 to 3 years),

HWA populations increase rapidly and attain peak densities when trees are producing abundant new growth. During the subsequent 1 or 2 years, populations decline quickly when hemlocks are producing very little new growth and adelgids are forced to feed on less nutritious older-growth. This negatively affects survival and fecundity and increases the percentage of sexuparae eggs produced by the sistens generation (up to 93%). This generation is unsuccessful in reproducing due to the lack of a suitable spruce host in the eastern United States (McClure 1987). Following this adelgid decline a very small percentage of buds produce new, stunted growth that is quickly colonized by HWA the following year. This represents a second, smaller peak in adelgid abundance. Hemlock health again declines and adelgid populations crash with 100 percent of sistens eggs becoming sexuparae (McClure 1991a). At this point trees will most likely die or they may survive for several more years in a severely weakened condition. The trees that do hold on have little chance for recovery and usually succumb to secondary insects and disease (McClure 1996). The density-dependent production of the unsuccessful sexuparae is an important factor in decline of local HWA populations. However, this pest will continue to thrive in its new environment due to its two successful annual generations, its high fecundity, and the absence of natural enemies (McClure 1991a, 1996).

### **IMPACTS OF THE HEMLOCK WOOLLY ADELGID**

The decline and eventual loss of hemlock from hemlock-dominated stands in the eastern U.S. is expected to significantly alter forest structure, carbon and nitrogen cycling, and hydrological processes (Orwig and Foster 1998; Hadley and Schedlbauer 2002; Stadler

et al. 2005; Cobb et al. 2006; Weckel et al. 2006; Ford and Vose 2007). Following HWA infestation, eastern hemlock demonstrates elevated (20 – 40 % greater than uninfested trees) foliar nitrogen content and increased litterfall. Higher litterfall rates resulted in less total hemlock canopy biomass and increased canopy precipitation throughfall, leading to significantly higher concentrations of nitrogen compounds, dissolved organic compounds, and cations available in the soil (Stadler et al. 2005; Cobb et al. 2006). Because HWA does not discriminate among hemlock age classes, when canopy gaps are created due to hemlock mortality, there is no advanced hemlock regeneration present to capitalize on the increased availability of light and nutrient resources (Orwig and Foster 1998). As a result, forest stands throughout New England previously dominated by hemlock have regenerated to almost pure stands of primarily black birch (*Betula lenta* L.), but also red maple (*Acer rubrum* L.) and white ash (*Fraxinus americana* L.) following HWA related mortality and presalvage hemlock cutting (Orwig and Foster 1998; Brooks 2004). Similar changes in forest structure are likely to occur in hemlock stands throughout the mid-Atlantic and southern Appalachians and are predicted to significantly alter soil moisture availability and stream flow characteristics (Ford and Vose 2007).

The potential impacts of hemlock decline on terrestrial and aquatic organisms are great, but few have been experimentally evaluated. Ruffed grouse (*Bonasa umbellus* L.), wild turkey (*Meleagris gallopavo* L.), snowshoe hare (*Lepus americanus* Erxleben), eastern cottontail rabbit (*Sylvilagus floridanus* Allen), and white-tailed deer (*Odocoileus virginianus* Zimmermann) all utilize hemlock for cover and forage during the winter months (Quimby

1996, Reay, R.S. 2000). In Vermont (U.S.A.), hemlock canopies were found to significantly moderate extreme winter temperatures and snow depths in stands heavily utilized by white-tailed deer (Lishawa et al. 2007). In Connecticut (U.S.A.), 90 species of birds utilize hemlock as a primary source of food, nesting, and roosting sites (Lapin 1994), and three species, the black-throated green warbler (*Dendroica virens* Gmelin), solitary vireo (*Vireo solitarius* Wilson), and northern goshawk (*Accipiter gentilis* L.), are all thought to be hemlock obligates (Benzinger 1994 a,b,c). Tingley et al. (2002) reported that the abundances of black-throated green warblers, blackburnian warblers (*D. fusca* Müller), and Acadian flycatchers (*Empidonax virescens* Vieillot) are sensitive to hemlock removal from forests.

The eastern redback salamander (*Plethodon cinereus*) is the principle terrestrial salamander species found in New England, but it also occurs throughout the Appalachian Mountains as far south as North Carolina. It is strongly associated with hemlock dominated forest stands and ephemeral reductions in the relative abundance of redback salamanders have been noted following presalvage cutting of hemlock to simulate HWA-related mortality (Brooks 2001).

Native brook trout (*Salvelinus fontinalis* Mitchill) and brown trout (*Salmo trutta* L.) are more abundant in forest streams associated with hemlock than other forest types (Ross et al. 2003). It is thought that the thermal and hydrologic regimes provided by hemlocks that shade streams will become unstable following HWA-related hemlock decline resulting in reduced trout abundance (Evans et al. 1996), a particularly unfortunate consequence for the already threatened brook trout. Similar concerns exist for the abundance and diversity of invertebrate taxa in streams draining hemlock dominated forests (Snyder et al. 2002).



## **MANAGEMENT OF THE HEMLOCK WOOLLY ADELGID**

### **Silvicultural Management**

There are a number of cultural tactics that may limit the spread and prevent large infestations of HWA, particularly in ornamental settings. Actions that limit contact between agents of adelgid dispersal and hemlocks will reduce the risk of new infestations, therefore it is suggested that homeowners remove birdfeeders that are placed near hemlocks because birds are known to transport both adelgid eggs and crawlers (Ward et al. 2004). New infestations may also be prevented by careful inspection of plants, logs, firewood, or bark chips before moving them between infested and non-infested areas, especially during the period from March through June when first instar adelgid crawlers are active. Likewise, inspection and cleaning of vehicles and clothing after visiting infested forests, parks, and recreation areas will help limit the spread (Ward et al. 2004). Removing isolated infested trees may further slow the spread of HWA (McClure 1995a).

Activities that enhance tree health are also important in limiting the size of HWA infestations and promoting hemlock survival, as healthy trees can better tolerate HWA infestation. Mulching to maintain soil moisture and irrigating during periods of drought can improve the health of trees growing on poor sites that might otherwise succumb more quickly to adelgid infestation (Ward et al. 2004). Fertilization of hemlocks is not recommended as a means of promoting hemlock health as it has been shown to enhance HWA survival and reproduction on eastern hemlock (McClure 1991b). However, once adelgids have been controlled in an area, fertilization may be effective in promoting hemlock recovery (McClure 1995a).

In areas where landowners are concerned with recovering some economic value from timber size hemlocks that are dead or dying following HWA infestation, salvage cutting is recommended. Ward et al. (2004) have suggested a set of BMPs (Best Management Practices) for conducting hemlock salvage operations but warn these should not be viewed as a technical guide for harvesting. Rather, they are intended as a set of guiding principles to be discussed between the landowner and the professional foresters who will carry out the actual harvest operation.

### Chemical Control

The most effective method of HWA control has been the use of chemical insecticides. Foliar sprays of diazinon, ethion, fluvalinate, malathion, imidacloprid, horticultural oils, and insecticidal soaps have all given excellent control in ornamental and forest settings, with 95%+ adelgid mortality (McClure 1987; McClure et al. 1989; Rhea 1996). However, complete coverage (drenching of all foliage and branches) was essential for successful control. Stem injections of bidrin and oxydemetonmethyl and stem implants of acephate have also given excellent control, with 93-98 % mortality of the spring and 60-88 % mortality of the summer adelgid generations (McClure 1992a). Stem injections of imidacloprid have also provided control, but multiple applications are sometimes required (Doccole et al. 2007) and are not always effective (Cowles et al. 2006). However, stem injections require wounding of the tree which may increase susceptibility to *A. tsugae* and other pests, and may be rendered ineffective on trees that have been infested for some time due to changes in tree conductivity following adelgid attack (McClure 1995a). An

alternative to these stem injection techniques are soil injections of imidacloprid which caused up to 100% mortality of adelgids (Steward and Horner 1994; Cowles et al. 2006) and resulted in significant tree recovery (Webb et al. 2003).

Bidrin, oxydemetonmethyl, acephate, and imidacloprid may be the best choices for control because of their residual activity (up to six months) (McClure 1992a; Rhea 1996; Cowles et al. 2006), but horticultural oils and insecticidal soaps are favored for their efficacy and low toxicity to applicators, beneficial insects, and the environment (McClure 1995a; Rhea 1996). While most recommendations call for insecticide applications in the spring and fall (McClure 1995a), if only one treatment is possible fall applications have proven most effective in controlling HWA (Rhea 1996). The options for chemical control of *A. tsugae* listed above are best suited to infestations on trees of exceptional value in ornamental or recreational settings and are inappropriate in the forest settings because of difficulty associated with achieving complete coverage, high costs, the scattered nature of stands, the sensitivity of the hemlock ecosystem, and proximity of most trees to water (McClure 1987; McClure et al. 2001; Ward et al. 2004; Cowles et al. 2006).

### Biological Control

The impracticality of chemical pesticides for use in the forest setting has shifted the focus of HWA control efforts to biological control. There are no known parasites of the Adelgidae but there are numerous predators. The most viable option for control of *A. tsugae* in forests of eastern North America appears to be a classical biological control approach, the introduction of exotic natural enemies (Montgomery and Lyon 1996).

Before initiating such a program there are three points to consider. First, it is important to assess the population of native natural enemies in eastern North America to minimize displacement and competition between those natives and introduced agents (Montgomery and Lyon 1996). Several such surveys have been completed and the following families and species of native or naturalized predators have been associated with HWA in eastern North America: Cecidomyiidae (midges), Chrysopidae and Hemerobiidae (lacewings), Syrphidae (flower flies), *Harmonia axyridis* Pallas, *Scymnus suturalis* Thunberg, *Laricobius rubidus* LeConte (McClure 1987; Montgomery and Lyon 1996; Wallace and Hain 2000). However, populations exist at such low levels it is unlikely that these established predators alone will have a significant impact on *A. tsugae* population levels. Second, previous attempts in biological control of the Adelgidae have had limited success for the genus *Adelges* (Zilahi-Balogh et al. 2002b). For example, more than 30 species of predators have been imported and released in North America for control of the balsam woolly adelgid (*Adelges piceae* Ratz.), a serious pest of Fraser fir (*Abies fraseri* (Pursh.) Poir.) in the southern Appalachians and subalpine fir (*A. lasiocarpa* (Hook.)Nutt.) in the Pacific Northwest. However, none of these predators has had an impact on populations of *A. piceae*, a pattern likely attributed to a general lack of prior assessment of predator biology, climatic tolerances, and synchrony with the host species (Montgomery and Lyon 1996). Third, due to the high fecundity of HWA, there will need to be an unusually high level of mortality from natural enemies. In this situation a multi-species approach to biological control of *A. tsugae*, including both native and introduced predators, will be

required to maintain populations below damaging levels (Montgomery and Lyon 1996).

In its native range of Asia and its introduced range of western North America, populations of *A. tsugae* are regulated at innocuous levels by a combination of host resistance and natural enemies (McClure 1992b, McClure and Cheah 1999). Beginning in the 1990's field surveys were conducted in Japan, China, and British Columbia to identify potential biological control agents for importation, evaluation, and release in the eastern United States (McClure 1996, Montgomery et al. 2000, Zilahi-Balogh et al. 2002a). In the Yunnan, Sichuan, and Shaanxi provinces of China, eleven families of predators were found in association with HWA on *T. dumosa*, *T. forrestii*, and *T. chinensis*: Cecidomyiidae, Syrphidae, Hemerobiidae, Chrysopidae, Derodontidae, Anthocoridae, Miridae, Inocellidae, Staphylinidae, Labaiduridae, and Coccinellidae (Montgomery et al. 2000). The coccinellids from these regions are the most promising for control of the adelgid and three in the genus *Scymnus* have been imported for evaluation in the United States (Montgomery et al. 2000; Lu and Montgomery 2001). *Scymnus camptodromus* Yu and Liu, *S. sinuanodulus* Yu and Yao, and *S. ningshanensis* Yu and Yao all prefer *A. tsugae* and will feed on multiple life stages, but all are univoltine which might cause difficulty for mass rearing (McClure 2001).

*Laricobius nigrinus* Fender, a derodontid beetle native to western North America, was found in close association with HWA on *T. heterophylla* in British Columbia and has been imported for evaluation in the United States. While it is also univoltine (Zilahi-Balogh et al. 2003b), it does have attributes that may make it a successful biological control agent of *A. tsugae*. *Laricobius nigrinus* prefers to feed on HWA, its feeding stages are well

synchronized with the spring adelgid generation, and it completes development only on *A. tsugae* (Zilahi-Balogh et al. 2002a, 2003a). This predator has been approved for release and field studies have confirmed its effectiveness (Lamb et al. 2005, 2006; Flowers et al. 2006) and establishment (Salom et al. 2007).

Surveys of 33 forest sites and 34 ornamental sites in Honshu, Japan yielded 4 families of predators (Cecidomyiidae, Syrphidae, Chrysopidae, and Coccinellidae) and one species of oribatid mite associated with HWA on *T. diversifolia* and *T. sieboldii*. The two most promising findings for biological control of *A. tsugae* from these surveys were the oribatid mite, *Diapterobates humeralis* (Hermann), and the coccinellid beetle, *Sasajiscymnus* (formerly *Pseudoscymnus*) *tsugae* Sasaji and McClure, and both have been imported for evaluation in the United States (Cheah and McClure 1996). *Diapterobates humeralis*, the most common natural enemy of HWA in Japan occurring in 42 of the 76 sites surveyed, does not feed directly on adelgid eggs, nymphs, or adults but, rather, feeds on the white woolly filaments that surround the ovisac. In doing so, the adult mites dislodge 95-99 % of adelgid eggs which fall to the ground and desiccate. However, its use as an introduced predator in the eastern United States has been hampered by its low fecundity and difficulty in mass rearing (McClure 1995b).

*Sasajiscymnus tsugae* was widespread, found in 37 of 76 sites surveyed in Honshu, Japan, and killed 86-99 % of all *A. tsugae* encountered (Sasaji and McClure 1997). It belongs to the tribe Scymnini, a group of characteristically small coccinellids that feed preferentially on small homopteran hosts such as aphids, mealybugs, and adelgids (Pang and

Gordon 1986). *Sasajiscymnus tsugae* has several characteristics that make it a good candidate for biological control of HWA in eastern North America. It feeds preferentially on HWA, although it will feed on other adelgid species when encountered (Butin et al. 2004) and anecdotal evidences suggests it can complete immature development on the balsam woolly adelgid (Cheah and McClure 1996). *Sasajiscymnus tsugae* has demonstrated bivoltine (F1 and F2 generations) synchrony with the sistens and progrediens generations of *A. tsugae*, remaining with its prey throughout the year. It is able to survive the critical summer aestivation period of the adelgid, feeding, but not reproducing, on aestivating first-instar nymphs and, perhaps, supplementing its diet with aphid honeydew or extra-floral nectaries of other forest plants. Adults overwinter in the same forest habitat as their prey, moving to the duff layer during harsh winters or remaining on hemlock foliage during mild winters. Finally, *S. tsugae* has a shorter generation time than HWA (*S. tsugae* = five weeks for F1 and F2 generations; *A. tsugae* = 32 weeks for sistens and 10 weeks for progrediens), a factor which may prove critical in the ability of this predator to respond numerically to increasing adelgid populations (Cheah and McClure 1998, 2000; McClure et al. 2000).

Following importation in 1992, *S. tsugae* life history, development, behavior, non-target effects, and mass rearing protocols were evaluated under quarantine laboratory conditions, and it was approved for release by APHIS-PPQ (USDA) in 1996 (Cheah and McClure 1998). Field evaluations of its efficacy as a biological control agent for *A. tsugae* began in 1995 (Connecticut), and since 1999 it has been released in all eastern states reporting adelgid infestations (Cheah and McClure 2000). Long-term efficacy studies

evaluating the release of 2,500 to 10,000 adult beetles in 5-10 acre infested hemlock stands were initiated in 1998 and 1999 in Connecticut, Maryland, Massachusetts, New Jersey, New York, North Carolina, Pennsylvania, Rhode Island, Virginia, and West Virginia (McClure et al. 2000). To date, nearly 2.5 million *S. tsugae* have been released in eastern U.S. hemlock forests (J.R.Rhea – personal communication).

*Sasajiscymnus tsugae* shows remarkable short-term success in some release areas reproducing, dispersing, and reducing HWA densities. Five months following initial field releases in Connecticut this predator had reduced adelgid populations 47-88 % (McClure et al. 2000). However, as *A. tsugae* densities continue to rise, new infestations continue to occur, and hemlocks continue to decline, there is little indication that *S. tsugae* has become successfully established in the eastern United States, other than in a few instances where releases are periodically augmented (Cheah and McClure 2002; Cheah et al. 2005). The following discussion examines several reasons why long-term establishment and effective *A. tsugae* biological control by *S. tsugae* remains elusive.

The list of successfully introduced coccinellid predators is long, but there is still a general lack of understanding about the conditions necessary for the successful establishment of exotic biological control agents. In fact, most successful biocontrol programs have depended largely on trial and error (Hodek and Honěk 1996). However, one condition is understood: the need to maintain the genetic diversity of the imported species. It is recommended that large samples from the insect's central area of distribution be collected for importation. Small samples tend to be ecologically marginal with a high degree of



inbreeding and homozygosity. Mass rearing programs based on such small populations successfully produce large numbers of individuals, but those individuals tend to be adapted to a very narrow range of environmental conditions (Remington 1968; Lucas 1969; Mackauer 1976; Hodek and Honěk 1996). It is likely that *S. tsugae* mass rearing programs in the eastern United States are producing such genetically marginal and ill-adapted populations. Initial shipments of *S. tsugae* adults received from Japan by the Connecticut Agricultural Experiment Station (CAES) were very small, less than 50 individuals (M.S. McClure, personal communication). Mass rearing was initiated, and in 1997 CAES provided 100 adults to the New Jersey Department of Agriculture, Alampi Beneficial Insects Laboratory where mass rearing protocols were optimized (Palmer and Sheppard 2002). Starting with this small population the Alampi lab has mass reared 300,000-plus beetles per year, providing the bulk of beetles that have been release in the eastern United States and small starter populations (100-200 beetles) to all subsequent mass rearing programs. While some effort has been made by the Alampi lab to maintain sub-colonies of *S. tsugae* under differing environmental conditions to promote greater genetic diversity and adaptability, a serious effort has not been possible while faced with the demands of mass rearing (D.J. Palmer, personal communication). The fact is that all *S. tsugae* in mass rearing programs originated from the original shipment of fewer that 50 individuals. Greater gains in ecological quality and establishment success may be possible with periodic infusion of wild-type beetles into mass rearing programs or more rigorous attempts to challenge the culture and increase adaptability in those characteristics deemed most important (Mackauer 1972, Messenger et al. 1976).

The three remaining explanations for the limited success of *S. tsugae* establishment all concern the release protocol being used for this predator. The current method calls for the introduction, in mass, of 5,000-10,000 adult beetles onto 1 or 2 trees at the center of a 5-10 acre infested hemlock stand (Jacobs 2005). The first concern over this protocol is the unusually high density at which beetles are released. It is well documented that under such high density the competitive interactions among coccinellids negatively affect insect survival, developmental rates, fecundity, and ovipositional behavior (Hodek and Honěk 1996). High population densities directly affect the quantity of food available to each individual larval or adult beetle. When food is limited larval development is prolonged and survival decreased as has been documented for several coccinellid predators: *Adalia bipunctata* (L.), *Hippodamia quinquesignata* (Kirby), *H. axyridis*, and *Propylea japonica* Thunburg (Kaddou 1960; Hukusima and Ohwaki 1972; Wratten 1973; Dimetry 1976; Kawauchi 1979). Competition for a limited food supply will also affect coccinellid fecundity and ovipositional behavior, where females will produce fewer eggs when food is lacking and lay fewer eggs in areas with a high density of conspecific larvae and adults in order to avoid intraspecific cannibalism (Hemptinne and Dixon 1991; Hemptinne et al. 1992; Hodek and Honěk 1996). Studies of *S. tsugae* in both the field and laboratory indicate that this predator is cannibalistic and will lay fewer eggs in the presence of conspecifics (Flowers et al. 2005, 2006).

Experience indicates establishment of exotic coccinellid predators is most successful with the introduction of low density populations, avoiding the problems of survival,

developmental rate, fecundity, and ovipositional behavior associated with high-density. For example, the most successful biological control program ever initiated was the 1889 introduction of *Rodolia cardinalis* (Vedalia beetle) for control of *Icerya purchasi* Maskell (cottony cushion scale) in California (Debach 1964; Hodek and Honěk 1996). Establishment of *R. cardinalis* and subsequent control of the scale pest was achieved with the release of 10,555 beetles into 220 citrus orchards (approximately 50 beetles per orchard). Current protocol calls for the release of 25-100 beetles per 10 acre block to achieve establishment and control (Grafton-Cardwell 2003). Low-density release protocols (40-1200 individuals) have also proven successful for the establishment of the coccinellid predators *Serangium parcesetosum* Sicard., *Stethorus picipes* Casey, *Aphidecta oblitterata* Larch, and *Scymnus impexus* (Mulsant) (Dowden 1962; McMurtry et al. 1969; Malausa et al. 1988).

A second concern over *S. tsugae* release protocol is the age of HWA infestations at the time of release. Confined sleeve cage studies have shown *S. tsugae* may not be able to respond numerically to high density adelgid populations and significantly reduces adelgid numbers only in low density adelgid populations (Butin et al. 2003). Typically, introductions are made in stands where trees support peak adelgid populations. Given the cyclic nature of *A. tsugae* population density discussed earlier, beetles are being released onto trees where adelgid populations will decline to near zero the year following introduction (McClure 1991a). Therefore, only one or maybe two *S. tsugae* generations (one adelgid generation) will be completed before there is a scarcity of available food (Cheah and McClure 2000). The resulting competition for food will negatively affect survival, developmental rate,

fecundity, and ovipositional behavior (Kaddou 1960; Hukusima and Ohwaki 1972; Wratten 1973; Dimetry 1976; Kawauchi 1979; Hemptinne and Dixon 1991; Hemptinne et al. 1992; Hodek and Honěk 1996), resulting in slower population growth and a lack of successful control when *A. tsugae* populations rebound in subsequent years. As a general rule of thumb, it generally takes between five and six prey generations for predator populations to reach densities where control will be successful (DeBach 1964). In addition, once HWA populations have reached peak density the bulk of the damage to the tree has already occurred and, if the tree survives, it will exist in a declined state. This pattern is common to most aphid-like pests (Hodek 1967).

The final concern over *S. tsugae* release protocol is that introduced beetles are not allowed time to acclimate to the forest environment. Beetles are moved from the confined and constant (78°C and 16:8 L:D) environment of mass rearing to the field where they are released in mass directly onto hemlock trees. Past experience has demonstrated that the success of coccinellid establishment may be compromised by unfavorable combinations of temperature and photoperiod. Such conditions may decrease survival and promote dispersal away from the food source. For many species a period of acclimation/adaptation may be required if establishment is to be successful (Messenger et al. 1976; Hodek and Honěk 1996).

#### Host Resistance

Research on hemlock host resistance against HWA is in its early stages. Resistance against other adelgid pests has been studied in detail, particularly with respect to three that are considered among the most damaging in North America; the cooley spruce gall adelgid

(*A. cooleyi* Gillette), the eastern spruce gall adelgid (*A. abietis* (L.)), and the balsam woolly adelgid (*A. piceae*).

*Adelges cooleyi* is a serious pest of Norway (*Piceae abies* (L.)Karsten), Colorado blue (*P. pungens* Engelm), Sitka (*P. sitchensis* (Bong.)Carr.), Engelmann (*P. engelmanni* Parry ex Engelm.), and white (*P. glauca* (Moench)Voss) spruce (primary hosts) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco) (secondary host) in ornamental plantings and Christmas tree plantations (Cumming 1959; Drooz 1985). It is a holocyclic (primary and secondary hosts) adelgid feeding from the cortical parenchyma cells of spruce (causing characteristic pineapple galls) and from the phloem sieve cells of Douglas-fir (Carter 1971;Rohfritsch 1990). Variable resistance to *A. cooleyi* has been documented among natural populations of Norway spruce and Douglas-fir, clonal Norway spruce, and half-sib families of Engelmann spruce (Mejnartowicz and Szmidt, 1978; Hertel and Guttenberger 1999; Mattson et al. 1999). Although no resistance mechanisms have been identified and/or correlated with decreased adelgid development and fecundity, resistant trees often have lower levels of free nitrogen, increased concentrations of free amino acids, and a build up of phenolic compounds in foliage, all associated with a drop in host quality following adelgid attack (Parry 1978a,b; 1980; 1982; Hertel and Guttenberger 1999).

*Adelges abietis* is a pest of Norway and white spruce in ornamental plantings and Christmas tree plantations (Plumb 1953; Carter 1971; Drooz 1985). It is an anholocyclic (no secondary host) adelgid feeding from the cortical parenchyma cells of spruce causing characteristic pineapple galls (Rohfritsch 1990). Variable resistance to *A. abietis* has been

documented among natural populations and half-sib and full-sib families of Norway spruce (Thalenhorst 1972; Tija 1973; Tija and Houston 1975; Gaumont and Gaumont 1976; Eidmann and Eriksson 1978; Mattson et al. 1998; Bjorkman 2000). Resistant Norway spruce reacts to *A. abietis* attack by a local necrosis in the cortical tissues at the base of buds, resulting in a plug that encapsulates the adelgids' stylets and prevents penetration into the bud (Thalenhorst 1972). Resistant trees typically have higher concentrations of phenols in the foliage and buds, with one as yet unidentified phenolic compound that is always absent in susceptible trees and consistently present in resistant trees (Tija 1973; Tija and Houston 1975; Bjorkman 2000). Delayed bud burst phenology has also been suggested as a potential mechanism of Norway spruce resistance to *A. abietis* (Gaumont and Gaumont 1976).

*Adelges piceae* is an introduced pest of balsam (*Abies balsamea* (L.) Mill.) and Fraser (*A. fraseri*) fir in eastern North America and subalpine (*A. lasiocarpa*), Pacific silver (*A. amabilis* Dougl.), and grand (*A. grandis* Dougl.) fir in the Pacific Northwest, attacking forest trees as well as those in ornamental plantings and Christmas tree plantations (Drooz 1985; Hain 1988). It is an anholocyclic adelgid infesting only true firs (*Abies* spp.) and feeds from the cortical parenchyma cells (Carter 1971; Rohfritsch 1990). *Adelges piceae* is believed to have originated in Europe, and the native silver fir (*A. alba* Miller) of the region responds to attack with two resistance mechanisms. The first is a typical three-step conifer wound response: 1) wound (feeding site) cleansing by primary resin flow, 2) wound containment by a hypersensitive response where resin is concentrated in cells adjacent to the wound site, 3) wound healing by the formation of an impervious tissue layer that walls-off the wound,

preventing damage to the underlying conductive tissues (xylem and phloem) (Berryman 1972). It is a delay or inhibition of wound healing that allows the hypersensitive response to deposit resin into the conductive tissues of susceptible North American firs, causing the formation of rotholz (red wood) and tree death. The second defensive response of silver fir is the production of thicker outer bark that is corky, less nutritious, and prevents the stylets of *A. piceae* from reaching the preferred feeding site (Kloft 1957).

Host resistance mechanisms have also been investigated in susceptible North American firs. Similar to silver fir, balsam fir responds to *A. piceae* attack by the production of thicker outer bark (Brower 1947; Schooley and Bryant 1978), a response that has also been reported in Fraser fir (Hollingsworth and Hain 1992). Among the many anatomical, structural, and physiological changes in tissues of North American firs caused by *A. piceae* is the production of juvabione and juvabione-like compounds (Hain et al. 1991), substances that mimic the juvenile hormone of insects and can have significant effects on insect growth, development, and reproduction. Infested grand and Pacific silver fir contained two juvabione-like compounds [(+)-todomatuic acid and dehydrotodomatuic acid] near attack sites, while non-infested trees contained none (Puritch and Nijholt 1974). In some cases juvabione levels increased with increasing *A. piceae* infestations on Fraser fir, and such increases may be induced by adelgid attack (Zhang 1994; Fowler et al. 2001). The presence of juvabione in endogenous Fraser fir wood and topical applications directly to *A. piceae* were negatively correlated with adelgid fecundity (Fowler 1999).

Preliminary studies have revealed the possible existence of host resistance

mechanisms against *A. tsugae* in eastern hemlock. Broeckling and Salom (2003) found increased release rates of monoterpenes from previous years foliage following *A. tsugae* infestation and suggested this as evidence for a potential induced defense in eastern hemlock. Similar variations in terpenoid release and profile have been suggested as a potential resistance mechanism of eastern hemlock against exotic scale insects (McClure and Hare 1984).

*Tsuga* species from Asia (*T. dumosa*, *T. forrestii*, *T. chinensis*, *T. diversifolia*, *T. sieboldii*) and western North America (*T. heterophylla* and *T. mertensiana*) are all believed to be either putatively resistant to or susceptible but highly tolerant of *A. tsugae*, and several have been shown to support significantly lower HWA populations in infestation studies (McClure 1992; del Tredici and Kitajima 2004). Studies have also demonstrated significant differences in foliar phosphorus, lignin, aluminum (Pontius et al. 2006), and terpenoids (Lagalante and Montgomery 2003) between these species and adelgid susceptible eastern and Carolina hemlocks. Attempts to capture the resistance of the Asian hemlocks through interspecific hybridization with eastern hemlock have proven unsuccessful, but more than 50 authentic *T. caroliniana* x *T. chinensis* hybrids have been produced (Bentz et al. 2002; Pooler et al. 2002). These hybrids are currently being evaluated for adelgid resistance (Bentz et al. 2007).



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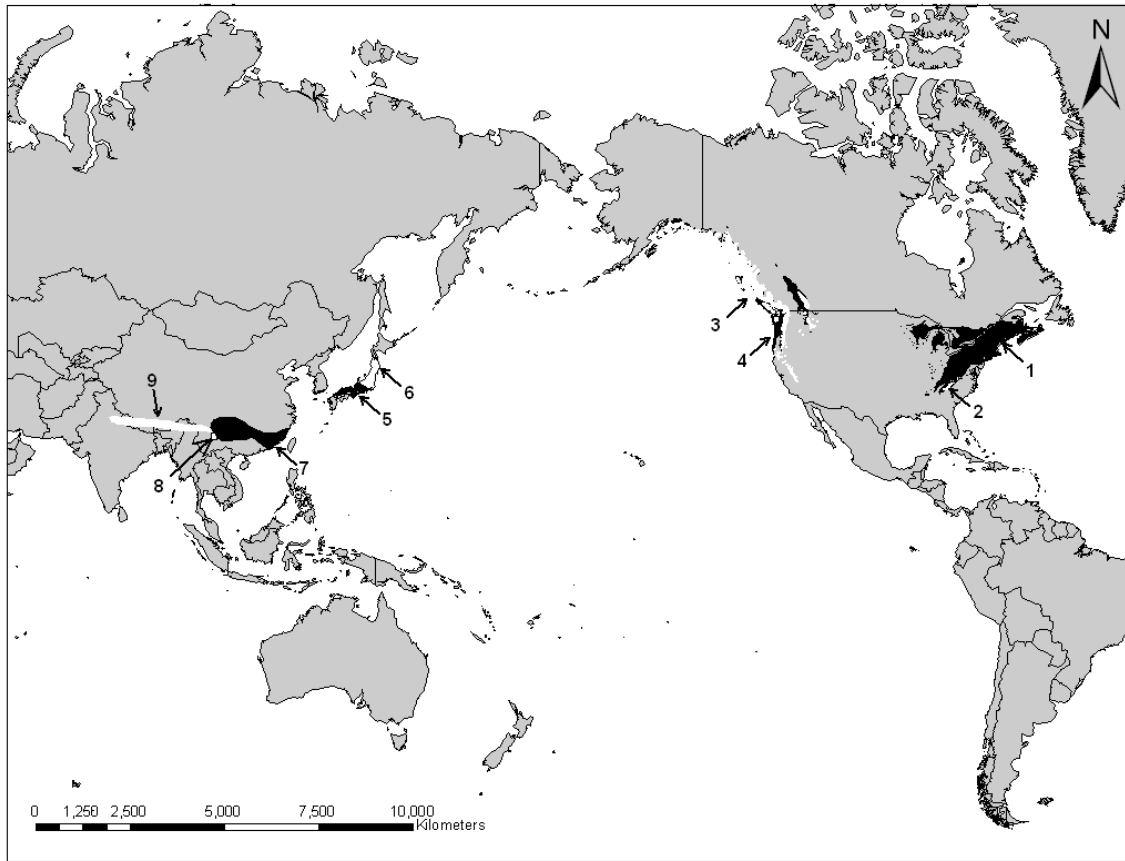


Figure 1. World-wide distribution of *Tsuga* species. 1) *Tsuga canadensis*, 2) *T. caroliniana*, 3) *T. mertensiana*, 4) *T. heterophylla*, 5) *T. sieboldii*, 6) *T. diversifolia*, 7) *T. chinensis*, 8) *T. forrestii*, 9) *T. dumosa*. Map produced by and used here with permission from Camcore, Department of Forestry and Environmental Resources, N.C. State University.



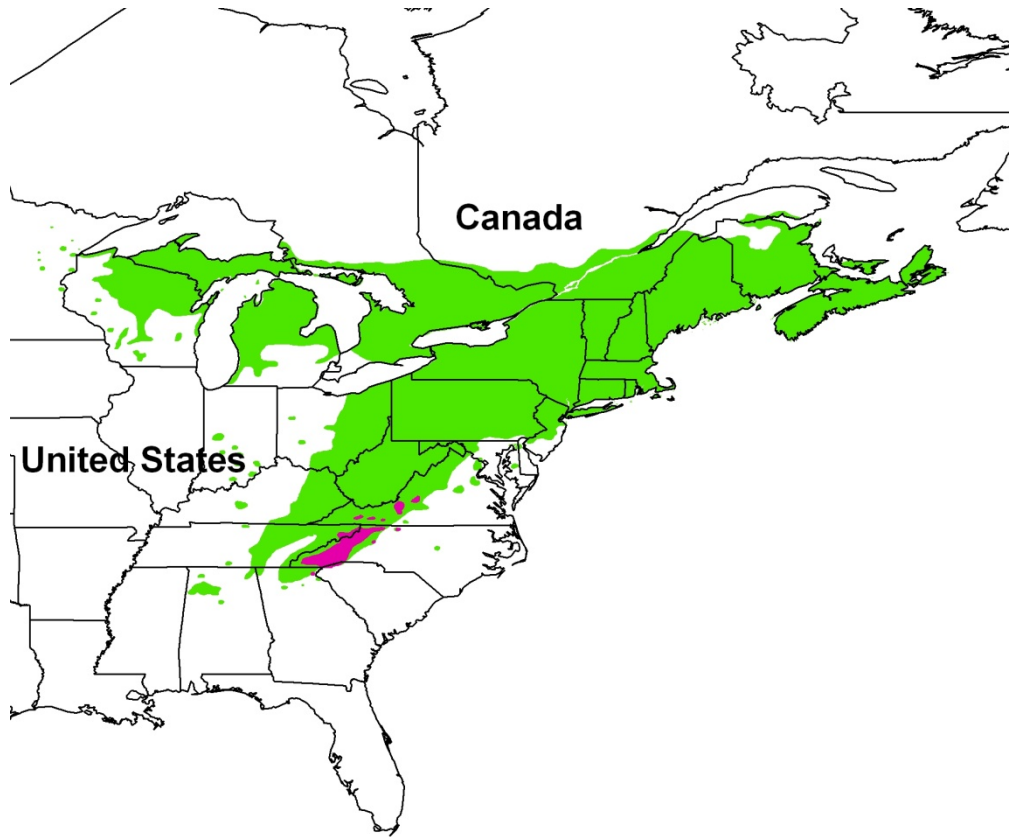


Figure 2. Native range of *T. canadensis* (green) and *T. caroliniana* (red) in eastern North America. Map produced by and used here with permission from Camcore, Department of Forestry and Environmental Resources, N.C. State University.

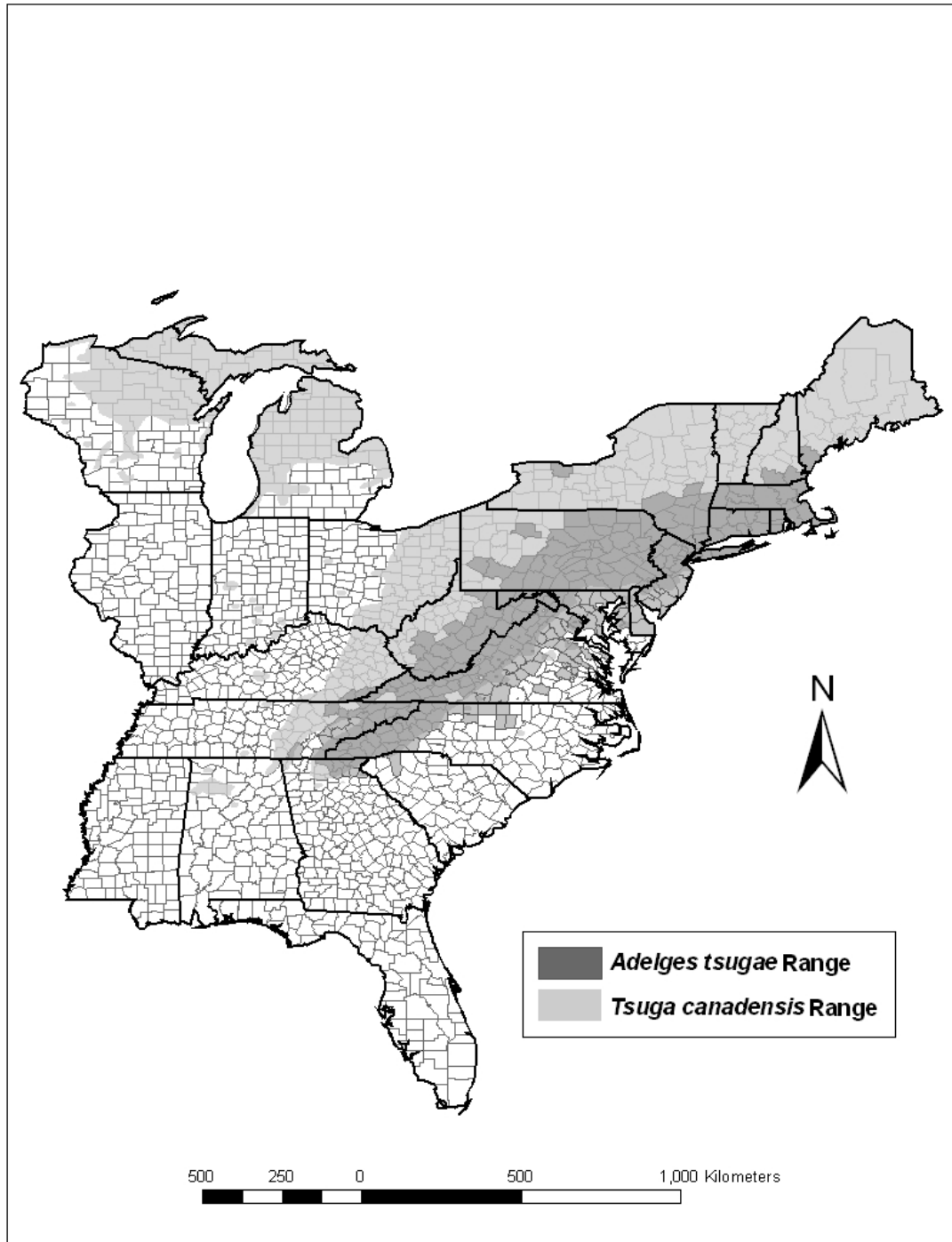


Figure 3. Distribution of the Hemlock Woolly Adelgid (*A. tsugae*) in the eastern U.S. as of December 2006. Map produced by and used here with permission from Camcore, Department of Forestry and Environmental Resources, N.C. State University.

## Chapter II

### **Feeding and Oviposition Preference, Developmental Performance, and Survival of *Sasajiscymnus tsugae* on the Hemlock and Balsam Woolly Adelgids in the Laboratory**

#### **ABSTRACT**

The suitability of *Adelges piceae* Ratzeburg (Hemiptera: Adelgidae) as an alternate mass rearing host for the adelgid predator *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) was studied in the laboratory. This predator is native to Japan and has been introduced to eastern hemlock (*Tsuga canadensis* (L.) Carrière) forests throughout the eastern United States for biological control of *Adelges tsugae* Annand (Hemiptera: Adelgidae), also of Japanese origin. *S. tsugae* feeding, oviposition, immature development, and adult long-term survival were tested in a series of no choice (single-prey) and paired-choice experiments between the primary host prey *A. tsugae* and the alternate host prey *A. piceae*. In paired-choice feeding tests the predator did not discriminate between eggs of the two adelgid species, but did eat more eggs of *A. piceae* to those of *A. tsugae* in the no choice tests. *S. tsugae* accepted both test prey for oviposition and preferred to lay eggs on adelgid infested versus non-infested host plants. Overall oviposition rates were very low (< 1 egg per predator female) in the oviposition preference tests. Predator immature development rates did not differ between the two test prey, but only 60% of *S. tsugae* survived egg to adult development when fed *A. piceae* compared to 86% when fed *A. tsugae*. *S. tsugae* adult long-term survival was significantly influenced by prey type and the availability of a supplemental food source (diluted honey) when offered aestivating *A. tsugae* sistens nymphs or ovipositing aestivosistens *A. piceae* adults but not when offered ovipositing *A. tsugae* sistens adults.

These results suggest that the development of *S. tsugae* laboratory colonies reared on a diet consisting only of *A. piceae* may be possible. However, supplementing *A. tsugae* based mass rearing colonies with the alternate prey during the aestival diapause of the primary prey is unlikely to resolve current rearing delays due to increased predator mortality when fed *A. piceae*.

## INTRODUCTION

*Sasajiscymnus* (formerly *Pseudoscymnus*) *tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) is an adelgid predator native to Japan that has been introduced into the Appalachian forests of the eastern United States for classical biological control of the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), also of Japanese origin (Annand 1924; Sasaji and McClure 1997; Havill et al. 2006). Since its introduction to Richmond, Virginia on imported hemlock nursery stock sometime between 1920 and 1950 (Stoetzel 2002), *A. tsugae* has become a serious threat to natural hemlock ecosystems throughout the eastern U.S., causing widespread mortality of both eastern (*Tsuga canadensis* (L.) Carrière) and Carolina (*T. caroliniana* Engelmann) hemlocks. The adelgid has now spread to at least 16 eastern states from New England south to Georgia, and infests approximately 50% of hemlock ecosystems in the region where it kills trees in as little as four years (McClure et al. 2003).

The balsam woolly adelgid, *Adelges piceae* Ratzeburg, is also an introduced forest pest in North America that attacks true firs (*Abies* spp.). It was introduced from central Europe to the native fir forests of Maine and Nova Scotia sometime around 1900 (Balch

1952). *A. piceae* has since spread and significantly altered the composition of fir forests throughout eastern Canada, New England, the Pacific Northwest, and, most notably, the southern Appalachian Mountains (Dull et al. 1988; Mitchell and Buffam 2001; Jenkins 2003). In this latter region it has eliminated approximately 95% of mature Fraser fir (*Abies fraseri* [Pursh] Poir.) from the high elevation spruce-fir forest type (Dull et al. 1988). It has also become a significant pest management issue for U.S. producers of Christmas trees (Chastagner 1997; Potter et al. 2005).

Both adelgids have a complex polymorphic life cycle and reproduce mostly through parthenogenesis in North America. *Adelges tsugae* has two generations per year on the hemlock host called the sistens (over-wintering generation), that is present from July–March, and the progrediens (spring generation), that is present from March-June (McClure 1989). A third winged sexual generation called the sexuparae also occurs but lacks a suitable host in North America (McClure 1987). *Adelges piceae* has two to four generations per year (Balch 1952). The first is called the hiemosistens (spring generation) and is present from September-June. Subsequent generations are called aestivosistens (summer generation) and are present from June-September. The winged sexual generation of *A. piceae* is called the progrediens but is formed only in populations that occur in the northeastern U.S. and eastern Canada on balsam fir (*Abies balsamea* [L.] Mill.) (Footitt and MacKauer 1983). All generations for both adelgid species develop through four nymphal instars before molting to the adult stage (Balch 1952; McClure 1989).

Logistic, economic, and ecological concerns over the use of chemical pesticides in

forest settings have focused *A. tsugae* management efforts on biological control. Due to a lack of effective native or naturalized adelgid predators in the eastern U.S. (Wallace and Hain 2000), emphasis has been placed on a classical biological control approach (Cheah et al. 2004). There are no known parasitoids of the Adelgidae, but a number of promising predators of *A. tsugae* have been identified and imported into the U.S. for evaluation (Cheah et al. 2004). Several have been approved for release, but to date *S. tsugae* is the most widely distributed predator. As of December 2007, the predator had been introduced to every state reporting an *A. tsugae* infestation with a total of approximately 2.5 million beetles released (J.R. Rhea – personal communication).

*Sasajiscymnus tsugae* was the first predator of *A. tsugae* described and was imported from Japan to the U.S. in 1992 for quarantine and field evaluation (Sasaji and McClure 1997). Following approval by the USDA-APHIS-PPQ, mass rearing was initiated and free releases of *S. tsugae* into adelgid- infested hemlock stands began in 1995 in Connecticut and 1999 throughout New England and the mid-Atlantic states (Cheah et al. 2004). Laboratory and field studies revealed that this predator is well suited for biological control of *A. tsugae*; feeding preferentially on and having a life cycle well synchronized with the adelgid and overwintering in the hemlock habitat (Cheah and McClure 1998, 2000). Under natural conditions, *S. tsugae* has two generations per year that overlap with those of *A. tsugae*, each developing through 4 larval instars, prepupal, and pupal stages before emerging as an adult (Cheah and McClure 2000).

*Sasajiscymnus tsugae* produced in mass rearing facilities are reared on a diet of live

*A. tsugae* collected from naturally occurring infestations. Given the large number of beetles already released into hemlock stands it is clear that these mass rearing programs have been successful. However, the occurrence of aestival diapause during the *A. tsugae* sistens generation places an important constraint on predator production (Palmer and Sheppard 2002). This dormant period occurs from July to October of each year and begins immediately after sistens nymphs eclose from their eggs and begin feeding (McClure 1987), providing the predators with a less nutritious food source that they may need to supplement with feeding from extra-floral nectaries (Cheah and McClure 2000). *Sasajiscymnus tsugae* adult feeding on a diet of aestivating adelgid nymphs in mass rearing colonies in the absence of nectar producing plants has two important negative consequences. Predator survival rates are reduced and female beetles produce very few eggs (Palmer and Sheppard 2002). The result is at least a four-month period during which mass rearing stocks are in decline, no new predators are being produced, and predators are largely unavailable for release. It is known that *A. tsugae* aestival diapause is maternally regulated and temperature dependent (Salom et al. 2001), but it is not clear if these cues can be efficiently and reliably manipulated to prevent the induction of diapause and avoid the *S. tsugae* mass rearing delays associated with the discontinuous supply of suitable prey material.

The use of alternate rearing hosts that are active during *A. tsugae*'s aestival diapause may provide a means to overcome this constraint on *S. tsugae* mass rearing programs. Although the predator shows feeding preferences for most *A. tsugae* life stages, it will feed on eggs, nymphs, and adults of other adelgids in the *Adelges* and *Pineus* genera (Butin et al.

2004), and preliminary evidence suggests that *S. tsugae* not only feeds on but may also complete development on *A. piceae* (Cheah et al 2004). This is significant because the *A. piceae* aestivosistens generation is actively feeding, developing, and reproducing during the same period that the *A. tsugae* sistens generation is in diapause (Balch 1952; McClure 1989). This means it may be possible to augment or substitute *A. piceae* for *A. tsugae* as the main prey item in *S. tsugae* mass rearing facilities during the late summer months to maintain colony survival and beetle production, providing a year round supply of beetles for *A. tsugae* biological control. Additionally, if *S. tsugae* colonies that feed only on *A. piceae* can be developed, the breadth of the predator's biological control utility might be expanded to include both natural stands and plantations of Fraser fir.

The objective of this study was to determine if *A. piceae* is suitable as an alternate rearing host for *S. tsugae* during the aestival diapause of *A. tsugae*. The predator's feeding and ovipositional preference, developmental performance, and adult survival on *A. piceae* versus *A. tsugae* were tested in a series on no choice (single prey) and paired- choice bioassays. The effect of a supplemental food source (diluted honey) alone and in combination with adelgid prey on *S. tsugae* survival was also evaluated.

## **MATERIALS AND METHODS**

### Source of Predators and Test Prey

*Sasajiscymnus tsugae* eggs and adults were obtained from mass rearing colonies at the N.C. State University (NCSU) Insectary reared under protocols developed at the New Jersey Department of Agriculture's Phillip Alampi Beneficial Insect Laboratory (Trenton,



NJ; Palmer and Sheppard 2001). The NCSU colony was developed from small, 100 beetle (50♂:50♀) starter colonies obtained from the Alampi Lab and the North Carolina Department of Agriculture Beneficial Insect Laboratory (Cary, NC). All eggs used were < 24 hours old and adults were reared the same year that experiments were conducted and were > 1 month and < 6 months old.

The test prey were obtained from naturally occurring adelgid populations in Ashe and Avery Counties, North Carolina. The primary host prey, *A. tsugae*, was collected by cutting adelgid infested eastern hemlock branches that were placed in buckets of water and held in a rearing room at the NCSU Insectary (16°C; 50% RH; 12:12 L:D). Branches were collected in March for the Adelgid Preference and Development Tests and Survival Test 2 and were infested with ovipositing sistens (overwintering generation) adults. Branches infested with aestivating sistens nymphs were collected in late July for Survival Test 1.

The secondary host prey, *A. piceae*, was collected by felling infested Fraser fir trees in abandoned Christmas tree plantations. Felled trees were limbed and cut into 1 m bolts. Each bolt was set upright in a bucket of moist sand and the top end sealed with paraffin wax to maintain hydration. Because *A. piceae* overwinters as an early instar nymph, bolts collected in March were held at room temperature to accelerate adelgid development so that ovipositing adults of the hiemosistens (overwintering) generation would be available concurrently with *A. tsugae* for the Adelgid Preference and Development Tests. For Survival Test 1, bolts infested with ovipositing adults of the aestivosistens (summer) generation were collected in August and held in a rearing room at the NCSU Insectary (16°C; 50% RH; 12:12

L:D). The various life stages of predators and test prey used in each experiment are summarized in Table 1.

#### Predator Feeding, Oviposition, and Development

Petri Dish Setup: All tests of *S. tsugae* feeding and oviposition preference and developmental performance among *A. tsugae* and *A. piceae* were conducted using the same basic experimental design. Experimental units were 9 x 2 cm polystyrene petri dishes (Fisherbrand™) with a 1.5 cm diameter ventilation hole covered with a fabric mesh. Each dish was lined with a single layer of filter paper (Whatman No. 1™) that was moistened with a methylparaben solution (0.50 g / 250 ml distilled water) to inhibit fungal growth. A 2 cm piece of dental cotton wick moistened with distilled water was provided as a water source for *S. tsugae* larvae and adults, and the dishes were sealed with Parafilm™. Adelgid prey were presented intact on their host plant and consisted of 5 cm long *A. tsugae* infested eastern hemlock twigs and 2 cm diameter *A. piceae* infested bark rounds of Fraser fir. Bark rounds were extracted from fir bolts using a laboratory 2 cm diameter cork borer. All experiments were conducted in a 1700 Series HOTPACK laboratory incubator at 26°C, 16:8 (L:D), and 70-80 % relative humidity, the same environmental conditions used for the mass rearing of *S. tsugae* in the N.C. State University Insectary.

Feeding Preference: The acceptance of *A. tsugae* and *A. piceae* eggs for feeding by adult *S. tsugae* was evaluated in no choice (single-prey) and paired-choice experiments. The no choice test consisted of 40 petri dishes split among the two prey treatments ( $n=20$  dishes / adelgid species), each containing 50 eggs intact within woolly masses of its assigned test

prey. In the paired-choice test 50 eggs each of *A. tsugae* and *A. piceae* were placed together in petri dishes (n=20). Prior to each experiment all active, first instar adelgid crawlers were removed from host material and a single *S. tsugae* adult that had been starved for the preceding 12 hours was randomly assigned to each dish. Dishes were completely randomized in the incubator and predators were allowed to feed freely for 72 hours, after which the number of adelgid eggs consumed was recorded. This number was calculated via the following equation:

$$\text{eggs consumed} = 50 - (\text{eggs remaining} + \text{crawlers present})$$

Because host material was cleared of all adelgid crawlers prior to these experiments, any crawlers present afterwards would have hatched from the 50 eggs placed in each dish and could not be considered consumed by *S. tsugae*.

The egg volume of *A. tsugae* and *A. piceae* was also estimated. An ocular micrometer fixed to the eyepiece of a dissecting scope was used to measure the long and short axis of 100 eggs of each adelgid species. Egg volume in cubic micrometers was estimated based on the volume of a prolate spheroid via the following equation:

$$\text{adelgid egg volume} = \frac{4}{3} \pi ab^2$$

where a is the length of the long axis and b is the length of the short axis.

Oviposition Preference: The acceptance of *A. tsugae* and *A. piceae* for oviposition by *S. tsugae* females was evaluated in no choice (single-prey) and paired-choice experiments. The no choice test included 80 petri dishes split among the following 4 treatments: *A. tsugae* infested eastern hemlock, *A. piceae* infested Fraser fir, non-infested eastern hemlock, and

non-infested Fraser fir ( $n=20$  dishes / treatment). Each dish contained a single section of adelgid infested host plant with  $10 (\pm 2)$  woolly egg masses or non-infested host plant in accordance with its assigned treatment. In the paired-choice test,  $10 (\pm 2)$  egg masses of *A. tsugae* and *A. piceae* were paired together in petri dishes ( $n=20$ ). For both experiments, dishes were completely randomized in the incubator, and *S. tsugae* male-female pairs were randomly assigned to petri dishes and allowed to feed, mate, and oviposit over a 72-hour period. After this time, the number of predator eggs laid on each test prey or host plant was counted. Because *S. tsugae* eggs closely resemble the eggs of both adelgid prey species and females tend to lay eggs in concealed locations, all test prey and plant material was held in its assigned petri dish for 10 days following completion of the test at experimental conditions to rear out the predator larvae from eggs in order to verify egg counts. Both the number of *S. tsugae* eggs laid and larvae hatched were recorded, although the following discussion will refer mostly to the counts for number of larvae hatched.

Developmental Performance: The suitability of *A. piceae* as a developmental host for *S. tsugae* from the egg to the adult stage was compared to that of *A. tsugae* in a no choice (single-prey) test. The test included 30 petri dishes split among the two prey treatments ( $n=15$  dishes / adelgid species). A single *S. tsugae* egg, < 24 hours old, was transferred with a fine brush to each dish and placed on the host plant section containing  $10 (\pm 2)$  woolly egg masses of the assigned test prey. Petri dishes were completely randomized in the incubator and examined daily for *S. tsugae* egg hatch or molt to the next life stage and adult emergence. Larval molt was determined by noting the presence of an exuvium, and fresh

prey was added to the dish each day. The pre-pupal stage was determined to be when mature fourth instar larvae became sedentary and had a pronounced woolly covering. For each *S. tsugae* individual the duration of (in days) and survival to (1 = alive; 0 = dead) each life stage was recorded.

### Predator Adult Survival

The long-term survival of *S. tsugae* adults was evaluated in no choice tests among the test prey alone or in combination with a supplemental food source. *Adelges tsugae* was presented on 10 cm infested eastern hemlock twigs and *A. piceae* on 10 x 3 cm sections of infested Fraser fir bark (see Table 1 for insect life stages used in these experiments). The supplemental food source consisted of diluted, store bought honey (50:50 honey:distilled water) presented on 5 x 3 cm pieces of filter paper. In Survival Test 1, the diet treatment combinations were *A. tsugae* plus food supplement, *A. tsugae* alone, *A. piceae* plus food supplement, *A. piceae* alone, food supplement alone, and a control (no test prey or food supplement). In Survival Test 2 the treatments were *A. tsugae* plus food supplement, *A. tsugae* alone, food supplement alone, and the control (*A. piceae* was not available at sufficient densities for inclusion in this test).

All tests were conducted in 20 x 6 x 6 cm polystyrene rearing cages (Consolidated Plastics Co., Inc) with a 2 cm diameter ventilation hole covered with a fabric mesh ( $n = 5$  boxes / diet treatment). Each cage was lined with a double layer of paper towel (Georgia Pacific) moistened with a methylparaben solution (0.50 g / 250 ml distilled water) to inhibit fungal growth, and a 5 cm piece of dental cotton wick moistened with distilled water was

provided as a water source for adult beetles. Plant sections with test prey were placed on the paper towel in the bottom of the cage and the filter paper with the food supplement was attached to the sidewall. Each cage received five randomly assigned adult *S. tsugae*, were sealed with Parafilm™, and all cages were completely randomized in the incubator. The cages were examined daily for 36 days. During each day's observation the number of live adult beetles remaining in each cage was recorded as well as the location of each live beetle. Locations were recorded as resting or feeding on the host plant (hemlock twig or Fraser fir bark section), feeding at the food supplement, drinking at the cotton water wick, or wandering about the test arena (cage). Fresh test prey and/or food supplement was added every other day.

### Statistical Analysis

The no choice (single-prey) tests for feeding preference and developmental performance and egg volume estimates were analyzed using two sample *t* tests to determine the effect of prey type on *S. tsugae* adult feeding rate and the developmental time for each predator life stage. A paired *t* test was performed to determine adult *S. tsugae* prey preference in the paired-choice test for feeding preference. All *t* tests were performed using the Analyst Application in SAS 9.1 (SAS Institute, 2003). A Chi-Square test (PROC FREQ, SAS 9.1) was performed to determine if the frequency of *S. tsugae* survival in each life stage was significantly different between prey types in the developmental performance test.

Logistic regression analyses were performed using the General Model Procedure (PROC GENMOD, SAS 9.1) to determine the probability of *S. tsugae* adult survival on day

36 when fed different diet treatment combinations in the adult survival tests. For Survival Test 1, the main effect of test prey type (*A. tsugae*, *A. piceae*, or none) on survival was tested for diet treatments with (*A. tsugae* + supplement; *A. piceae* + supplement; Supplement alone) or without (*A. tsugae*; *A. piceae*; Control) the food supplement, and the likelihood estimates for these probabilities were calculated via the following formulae:

$$P_S = (e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2}) / (1 + e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2})$$

$$P_{NS} = (e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2}) / (1 + e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2})$$

where  $P_S$  = the probability of *S. tsugae* adult survival with the food supplement present,  $P_{NS}$  = the probability of survival when no food supplement is present,  $\beta_0 \dots \beta_2$  = regression coefficients and  $\beta_0$  is the effect on survival when test prey type = none,  $X_1$  is the effect on survival when test prey = *A. tsugae*, and  $X_2$  is the effect on survival when test prey type = *A. piceae*.

For Survival Test 2, the main effects of prey type (*A. tsugae* or none) and food supplement (present or absent) on *S. tsugae* adult survival were tested, and the likelihood estimates for this probability (P) was calculated via the following formula:

$$P = (e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3}) / (1 + e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3})$$

where  $\beta_0 \dots \beta_3$  = regression coefficients and  $\beta_0$  = is the effect on survival when test prey type = none and food supplement = absent,  $X_1$  = the effect on survival of having *A. tsugae* available,  $X_2$  = the effect on survival of having the food supplement available, and  $X_3$  = the effect on survival of having both *A. tsugae* and the food supplement available.

## RESULTS

### Feeding Preference

In the paired-choice test, the predator demonstrated no feeding preference between the primary host prey *A. tsugae* and the secondary host prey *A. piceae*, consuming the same number of eggs of both prey (Table 3). However, when given no choice (single-prey test), *S. tsugae* ate significantly more *A. piceae* than *A. tsugae* eggs (Table 2), indicating a preference for the former. The volume of *A. piceae* eggs was significantly larger than that of *A. tsugae* eggs ( $t = 12.80$ ,  $df = 198$ ,  $p < 0.0001$ ); 5,205.53 versus 3,855.47  $\mu\text{m}^3$ , respectively.

### Oviposition Preference

In all test arenas, the number of larvae hatching several days after the completion of the oviposition trials exceed the number of eggs as counted by the experimenter. *Sasajiscymnus tsugae* females found both test prey species to be acceptable for oviposition, but host plants alone were not suitable (Table 4). In the no choice (single-prey) oviposition test, the predator demonstrated a clear preference for laying eggs on adelgid infested versus non-infested host plant material. Oviposition occurred only in test arenas containing test prey. In the same experiment, *S. tsugae* did not discriminate between *A. tsugae* infested hemlock or *A. piceae* infested Fraser fir as an oviposition substrate, laying similar numbers of eggs in close proximity to both test prey. In the paired-choice experiment, twice as many *S. tsugae* larvae hatched from *A. piceae* infested Fraser fir as from *A. tsugae* infested hemlock.



### Developmental Performance

There were no significant differences between *A. tsugae* and *A. piceae* in the duration of *S. tsugae* egg ( $t = 0.78$ ,  $df = 28$ ,  $P = 0.4380$ ), instar 1 ( $t = 0.17$ ,  $df = 28$ ,  $P = 0.8597$ ), instar 2 ( $t = 0.49$ ,  $df = 23$ ,  $P = 0.6252$ ), instar 3 ( $t = 1.51$ ,  $df = 22$ ,  $P = 0.1449$ ), instar 4 ( $t = 0.94$ ,  $df = 21$ ,  $P = 0.3545$ ), pre-pupal ( $t = 0.24$ ,  $df = 21$ ,  $P = 0.8106$ ), or pupal ( $t = 0.72$ ,  $df = 20$ ,  $P = 0.4773$ ) life stages. Overall, the total developmental time to the adult stage for *S. tsugae* was slightly shorter on a diet of *A. piceae* compared with *A. tsugae* (Table 5), but again, this difference is not significant ( $t = 0.51$ ,  $df = 20$ ,  $P = 0.6173$ ). The percentage of predators surviving to complete each life stage was lower when *S. tsugae* was fed *A. piceae* compared to *A. tsugae* beginning with Instar 2 (Table 5). This difference in survival was marginally significant at  $\alpha = 0.10$  for the pupal and adult stages ( $X^2 = 2.72$ ,  $df = 1$ ,  $P = 0.09$ ).

### Predator Adult Survival

Long-term Survival: In Survival Test 1, the probability of *S. tsugae* adult survival on day 36 was significantly affected by test prey type (*A. tsugae*, *A. piceae*, or no prey) in diet treatment combinations that included the food supplement ( $X^2 = 7.38$ ,  $df = 2$ ,  $P = 0.0249$ ). The presence of *A. piceae* in combination with the supplement significantly reduced predator survival ( $X^2 = 7.51$ ,  $df = 1$ ,  $P = 0.0061$ ) compared to the *A. tsugae* plus food supplement and supplement alone treatments (Figure 1). The probability of predator survival did not differ between *A. tsugae* plus supplement and supplement alone ( $X^2 = 2.00$ ,  $df = 1$ ;  $P = 0.1573$ ), although *S. tsugae* survival was slightly higher on the former compared to the latter (Figure 1). Among diet treatments that did not include the food supplement (*A. tsugae*, *A. piceae*, or

Control), the probability of predator adult survival was not significantly affected by test prey type ( $X^2 = 0.59$ ,  $df = 2$ ,  $P = 0.7452$ ) and survival in all three treatments was  $\leq 8\%$  (Figure 1).

Survival Test 2 demonstrated that the importance of a supplemental carbohydrate source for the long-term survival of *S. tsugae* adults is much reduced when the predators are provided a diet consisting of actively developing and ovipositing *A. tsugae* sistens adults and progrediens eggs (Figure 2). Although *S. tsugae* survival was best in the supplement alone and *A. tsugae* plus supplement treatments and lowest when predators were offered only *A. tsugae*, these trends were not significant and neither the test prey ( $X^2 = 1.84$ ,  $df = 1$ ;  $P = 0.1753$ ) nor the food supplement ( $X^2$ ,  $df = 1$ ;  $P = 0.4292$ ) affected the probability of *S. tsugae* adult survival on day 36. No predators survived to day 36 in the control treatment (no test prey, no food supplement).

Predator Location: In Survival Test 1, adult *S. tsugae* were found most often feeding or resting on the host plant material in treatments that included aestivating *A. tsugae* sistens nymphs (Figure 3). The percentage of beetles found wandering in the test arena was less when this test prey was combined with the food supplement (Figure 3a) than when it was presented alone (Figure 3b). In treatments that included *A. piceae* aestivosistens adults and hiemosistens eggs, beetles were most often found wandering in the test arena and less often feeding or resting on the host plant material (Figure 4). When the food supplement was presented alone (Figure 5a) and in the control treatment (Figure 5b) the beetles were almost always found wandering. Adult *S. tsugae* were occasionally found feeding on the food supplement in all treatments where it was included, although the percentage of beetles doing

so varied with the type and presence/absence of the host prey (Figures 3a, 4a, and 5a). A similar trend was seen for the percentage of beetles at the water source.

In Survival Test 2, for treatments that included *A. tsugae* sistens adults and progrediens eggs, adult *S. tsugae* were found most often feeding or resting on the host plant material (Figure 6), and the percentage of beetles found wandering in the test arena was slightly less when this test prey was combined with the food supplement (Figure 6a) than when it was presented alone (Figure 6b). In the control and supplement alone treatments beetles were most often seen wandering in the test arena (Figure 7).

## **DISCUSSION**

### **Predator Feeding, Oviposition, and Development**

Prior to its first release in the eastern U.S. for biological control of *A. tsugae*, *S. tsugae*'s potential host range had not been well defined. The predator had been anecdotally reported to feed on other adelgid species (Cheah and McClure 1996), and studies subsequent to its widespread free-release indicated that it will feed on multiple life stages of *A. laricis*, *A. cooleyi*, and *Pineus strobi* (Butin et al. 2004). In this study, tests of preference and performance indicate that *S. tsugae* will accept the alternate host prey *A. piceae* equally to the primary host prey *A. tsugae* for feeding and oviposition, and that the alternate host is as suitable as the primary host to support predator immature development (Tables 2, 3, 4, and 5).

In feeding preference tests, *S. tsugae* did not discriminate between feeding on the eggs of *A. tsugae* or *A. piceae* in paired-choice experiments, consuming equal numbers of

eggs of both adelgids (Table 3). Similarly, this predator readily feeds on the eggs of *A. laricis*, *A. cooleyi*, and *P. strobi* and prefers the eggs of *A. tsugae* only to those of *A. laricis* (Butin et al. 2004). In the no choice (single-prey) test for feeding *S. tsugae* did prefer the eggs of *A. piceae* to those of *A. tsugae* (Table 2), but this result may not indicate a true feeding preference for the alternate host prey. Rather, this may be an artifact of the relative nutritional value among eggs of the two adelgid species. Although *A. piceae* eggs were significantly larger (volume) than those of *A. tsugae*, eggs of the primary host prey species might constitute a more concentrated and nutritious food resource for the predator given that *A. tsugae* feeds primarily on the nutrient rich xylem ray parenchyma of eastern hemlock (Young et al. 1995). *Adelges piceae* feeds mostly on the relatively nutrient poor cortical parenchyma of Fraser fir outer bark (Balch 1952) and its eggs may be of lower nutritional value. This might necessitate that, when forced to feed only on the alternate host prey, *S. tsugae* would need to consume a larger volume of eggs to meet its nutritional needs.

Interestingly, there was a disparity in the number of adelgid eggs consumed by individual *S. tsugae* adults between the no choice (single-prey) and paired-choice feeding experiments. In the no choice test, when offered 50 eggs of either *A. tsugae* or *A. piceae*, each predator consumed, on average, fewer than 40 eggs each during the 72-hour feeding period (Table 2). However, when individual *S. tsugae* were present with 100 eggs (50 *A. tsugae* + 50 *A. piceae*), each predator consumed an average of (38.30 + 38.70) 77 eggs during the same 72-hour period (Table 3). It is not uncommon for predatory coccinellids to adjust their rate of food intake with changes in prey density, and many species have been

noted to reduce consumption as the amount of available prey decreases (Hodek and Honěk 1996). This allows for the conservation of limited food resources while maintaining basic levels of development, reproduction, and survival, and may partly explain why *S. tsugae* consumed less when offered a total prey resource of only 50 adelgid eggs.

Similar to the results for egg feeding, *S. tsugae* accepted both *A. piceae* infested Fraser fir bark and *A. tsugae* infested eastern hemlock twigs as suitable substrates for oviposition, laying eggs on both host plants in close proximity to the adelgid prey (Table 4). However, these oviposition trials indicate only host acceptance and not that the alternate host prey is a suitable nutritional resource to stimulate oogenesis in predator females.

In all cases, the number of newly hatched *S. tsugae* larvae counted in test arenas several days after the trials ended was greater than the number of predator eggs counted. This result is likely due to the fact that *S. tsugae* eggs resemble those of adelgids (although they are slightly larger) and predator females prefer to oviposit singly in concealed locations under bud scales, in empty seed cones, or within adelgid egg masses (Cheah and McClure 1998), conditions that made obtaining accurate egg counts difficult. This same propensity for laying its eggs in concealment may also explain why *S. tsugae* females demonstrated a preference for oviposition on *A. piceae* infested Fraser fir in the paired-choice experiment (Table 4). Fraser fir bark has numerous cracks and crevices and lenticels and is often covered with lichens (Krussman 1985; Beck 1990) that the predator may find suitable for providing unhatched offspring a stable microenvironment and protecting them from parasitoids and predators.

Behavioral analysis of the daily activity patterns of *S. tsugae* indicated that females probe and evaluate only the host plant substrate and not adelgid ovisacs prior to initiating oviposition (Flowers et al. 2007). However, in a related study to the current, *S. tsugae* females were noted to lay no eggs on non-infested hemlock and had much higher rates of oviposition on *A. tsugae* infested eastern hemlock than on *A. tsugae* ovisacs alone (no host plant present) or when ovisacs were placed on acrylic sealed hemlock twigs (R. Jetton – unpublished data). This indicates that the predator females may require cues from both host prey and host plant to stimulate oviposition, and may explain why *S. tsugae* demonstrated a clear preference for laying eggs on host plants only in the presence of adelgid prey and not on non-infested host plants (Table 4).

In this study, the overall oviposition rate of *S. tsugae* females was very low (< 1 egg laid per female; Table 4), and may have been affected by experimental conditions. Oviposition by this predator is very sensitive to variations in prey quality and host plant health (Palmer and Sheppard 2002) and is negatively influenced by intraspecific interaction. In laboratory studies the predator cannibalized conspecific eggs under conditions of low *A. tsugae* density, and under both laboratory and field conditions *S. tsugae* females laid fewer eggs in the presence of conspecifics (Flowers et al. 2005, 2006). As *A. tsugae* density increases cannibalism is reduced and oviposition rates increase (Flowers et al. 2005). In this study, the amount of prey made available to individual beetles in test arenas was obviously much less than what *S. tsugae* would be expected to encounter in nature or even the mass rearing colony, and each petri dish contained an adult male mate for the female beetle. These

conditions may have increased the potential for intraspecific resource competition and egg cannibalism in test arenas and stimulated female *S. tsugae* to lay fewer eggs.

Similar to its feeding preferences, the developmental performance of *S. tsugae* on prey species other than *A. tsugae* had not been thoroughly studied prior to its release. Cheah and McClure (1996) reported that the predator could complete development on a diet of *A. piceae* but provided no supporting data. This study confirms their conclusion, and indicates that not only can *S. tsugae* develop from the egg to adult stage on a diet of the alternate prey, but it does so at the same rate as when provided the primary prey *A. tsugae* (Table 5). These developmental rates on *A. piceae* and *A. tsugae* compare favorably with the previously published rate of 17.9 days for *S. tsugae* egg to adult development on the primary host prey at 25°C (Cheah and McClure 1998).

Although *S. tsugae* successfully completed immature development on the alternate host, the percentage of predators surviving was lower for most life stages (Table 5). Overall, only 60% of beetles survived to the adult stage when fed *A. piceae* compared to 86% when fed *A. tsugae*. However, despite the lower overall survival, the fact that 60% of the predators in the developmental performance test completed immature development on the alternate prey suggests that *A. piceae* is a suitable host for *S. tsugae*, and that this predator may be able to readily switch prey when its primary host becomes scarce. Prey switching has been noted for several species of both aphidophagous and coccidophagous coccinellids where they move between different habitats and feed on alternate prey while maintaining suitable rates of reproduction, development, and survival (Sloggett and Majerus 2000 and references therein).

This trait would be very useful in *S. tsugae*'s native Japan, where *A. tsugae* population density is typically very low or ephemeral due to high natural enemy pressure and the apparent resistance of the hemlock species native to that region (McClure and Cheah 1999; McClure et al. 2003).

When predaceous coccinellids switch prey some period of conditioning to the new host is typically required for predators to gain experience in handling, adopt more vigorous feeding on, and adapt physiologically to better utilize the novel prey and realize maximum fitness (Hodek and Honěk 1996; Sloggett and Majerus 2000). The predator eggs utilized for the developmental performance test were obtained from *S. tsugae* colonies reared on *A. tsugae* where no preconditioning to *A. piceae* occurred. This may explain why a smaller proportion of *S. tsugae* successfully completed immature development on the alternate host. If the predator were reared through multiple generations on a diet consisting only of *A. piceae*, it is possible that it would become conditioned to the alternate prey and its developmental fitness on the *A. piceae* would improve to equal that when reared on *A. tsugae*.

#### Predator Adult Survival

The adult survival tests indicate that *S. tsugae* longevity is heavily influenced by the presence or absence of supplemental food resources (in this case diluted honey), especially when the predators are offered either aestivating *A. tsugae* sistens nymphs or actively developing and ovipositing *A. piceae* aestivosistens adults as the prey resource (Figure 1). The importance of supplemental honey was much less when prey consisted of active *A.*



*tsugae* sistens adults (Figure 2). Predator location data indicate that *S. tsugae* did utilize the supplemental food resource in all treatments where present (Figures 3a, 4a, 5a, 6a, and 7a), and the percentage of beetles found actively feeding on the honey increased in treatments that included the less favorable *A. piceae* or contained no prey (Figures 4a, 5a, and 7a). Elevated levels of predator mortality associated with these tests may be partly explained by increased wandering behavior and the expenditure of energy reserves when supplemental food and prey were absent or less favorable *A. piceae* was present (Figures 3b, 4a&b, 5b, and 7b). However, in the supplement alone treatments predator survival was higher (Figures 1 and 2) and beetles were mostly found wandering (Figures 5a and 7a), indicating that the diluted honey was sufficient to restore energy reserves depleted by wandering beetles and sustain *S. tsugae* survival.

The importance of supplemental food sources to the survival of adult *S. tsugae* being stored during *A. tsugae* aestival diapause in mass rearing facilities or shipped for eventual release has been previously recognized, and rearing protocols call for the regular usage of diluted honey similar to its use in these experiments (Palmer and Sheppard 2001; Conway et al. 2005). While the adult survival tests in this study were conducted in the laboratory and do not reflect the variety of conditions that the predator is expected to experience in the field, they are suggestive that alternative foods of plant origin may be important for *S. tsugae* under field conditions. Even strictly carnivorous coccinellids are widely known to feed on pollen and nectar sources from the flowers or extra-floral nectaries of flowering plants they encounter. Doing so allows coccinellids to survive with reduced mortality when insect prey

are scarce and maintain fat and protein reserves so that oogenesis and oviposition can resume soon after their prey reappears (Hodek and Honěk 1996). Such feeding on nectar or pollen in addition to alternative prey might be expected to be important for *S. tsugae* as well, especially given the low density and ephemeral nature of *A. tsugae* populations in the predators native range (McClure and Cheah 1999; McClure et al. 2003).

The results of the adult survival tests are also cause for concern with the current classical biological control program for *A. tsugae* in the eastern U.S. that utilizes *S. tsugae* as an adelgid predator. Current release protocols call for a one-time release of 2,500 – 5,000 adult beetles in infested hemlock stands in late winter or early spring (Jacobs 2005) under the assumption that the predator will readily begin feeding on *A. tsugae*, become established, increase its population density, and reduce the pest's population numbers below damaging levels. This is sometimes true and *S. tsugae* has had remarkable short-term success in some release areas reproducing, dispersing, and reducing *A. tsugae* densities 47–88 % when the preferred spring sistens and summer progrediens adelgid generations are present (McClure et al. 2000). However, field-cage release studies have demonstrated that *S. tsugae* does not respond numerically to high density *A. tsugae* populations and only appears to have significant impacts on adelgid populations of low density (Butin et al. 2003). Likewise, as adelgid populations continue to grow and spread and hemlocks continue to decline, there has been little evidence presented to suggest that *S. tsugae* has established and significantly reduced *A. tsugae* populations over the long-term in the eastern U.S. despite the large number of beetles already released (Cheah and McClure 2002; Cheah et al. 2005). The only

significant predator recoveries and impacts on *A. tsugae* have been noted in a few areas where predator releases have been periodically augmented (Cheah and McClure 2002).

One scenario to explain this seemingly lack of widespread establishment and long-term impact on *A. tsugae* populations by *S. tsugae* is the characteristically low functional diversity of plants in eastern hemlock dominated forest stands (Farjon 1990; Godman and Lancaster 1990). The soils under these canopies are highly acidic and decomposing hemlock foliage is allelopathic, conditions that prevent understory development beyond hemlock seedlings and ericaceous shrubs such as *Rhododendron* spp. and *Kalmia* spp (Ward and McCormick 1982; Godman and Lancaster 1990). When significant understory development does occur, the species that dominate are typically non-flowering ferns (*Dryopteris* spp.) and club mosses (*Lycopodium* spp.) or flowering perennials in the *Mianthemum*, *Trientalis*, *Oxalis*, *Captis*, and *Carex* genera (Godman and Lancaster 1990). As a whole, these flowering plants together with the ericaceous shrubs flower from May through July (Radford et al. 1968). This means that abundant nectar and pollen producing sources are lacking in hemlock stands from late July through October when *A. tsugae* is in aestival diapause, a prey resource that, in the laboratory, is not sufficient in the absence of supplemental honey to sustain high rates of *S. tsugae* survival (Figure 1). Thus, the predator may experience large-scale die off during this period, at least partially explaining why *S. tsugae* has failed to establish and have lasting impacts on *A. tsugae* in the eastern U.S. Conversely, the presence of highly suitable adelgid life stages (Figure 2) and, where available, abundant flowering plants in the understory during the spring and early summer months have likely contributed

to the remarkable short-term successes that have been noted immediately following initial predator releases in some hemlock stands (McClure et al. 2000).

### Implications for Mass Rearing and Biological Control

The predator preference and performance trials indicate that *S. tsugae* can complete immature development on *A. piceae* and will accept this alternate host prey for feeding and oviposition. This suggests that *A. piceae* is a suitable alternative to *A. tsugae* for the mass rearing of the predator. However, the results of the *S. tsugae* adult survival tests suggest that the production delays and losses incurred in rearing colonies during *A. tsugae*'s aestival diapause are unlikely to be alleviated simply by substituting the alternate prey for the primary prey during this three to four month period. The availability of *A. piceae* ovipositing aestivosistens adults did not improve survival compared to *A. tsugae* aestivating sistens nymphs in the absence of the food supplement and reduced survival when the diluted honey was present (Figure 1).

A better approach may be to develop *S. tsugae* laboratory colonies that are reared exclusively on a diet consisting of *A. piceae*. While this study did reveal significant reductions in predator survival associated with the alternate prey in both the developmental performance and adult survival tests, these losses may be the result of a lack of conditioning for *A. piceae* among *S. tsugae* eggs and adults utilized in these experiments. All predators were obtained from a laboratory colony reared exclusively on *A. tsugae*. This might be overcome if the predator can be reared through multiple generations and conditioned to the alternate prey. Of course, the resulting predators may be less effective biological control

agents of *A. tsugae* than those produced in the traditional rearing programs, but *A. piceae* conditioned *S. tsugae* may be very useful for biological control in Christmas tree plantations and natural stands of Fraser fir where *A. piceae* is a serious pest. Christmas tree production depends on the use of broad spectrum insecticides to control *A. piceae*, a management practice that significantly reduces the density of native natural enemies in plantations. This leads to increased pressure from other Fraser fir pests such as spider mites, rust mites, and the balsam twig aphid that, in turn, lead to the use of additional insecticides and miticides. If *S. tsugae* were able to replace broad spectrum insecticides for *A. piceae* control in these plantations, mite and twig aphid natural enemies might be conserved and overall chemical use would be reduced (Potter et al. 2005).

However, questions remain as to the possibility of utilizing *A. piceae* as an exclusive rearing host for *S. tsugae*. This study tested predator preference and performance on ovipositing adults of *A. piceae*'s hiemosistens generation and adult survival on the aestivosistens generation. Preference and performance on aestivosistens and survival on hiemosistens should also be evaluated. Furthermore, the oviposition data indicates only that *S. tsugae* will lay eggs in close proximity to *A. piceae* and not if the alternate prey is suitable to stimulate oogenesis. Finally, mass rearing protocols for utilizing *A. piceae* need to be developed. However, before time and resources are dedicated to this pursuit, the utility of *S. tsugae* as a biological control agent of *A. piceae* in natural stands and plantations of Fraser fir should be thoroughly evaluated. A large classical biological control program for this pest in natural stands of Fraser fir was previously attempted and largely failed due to poor

synchronization with the prey, inefficient searching ability, and low over-wintering capacity, although several species of natural enemies are established (Zilahi-Balogh et al. 2002).

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Table 1. Summary of predator and test prey life stages used in *S. tsugae* preference, suitability, and survival experiments.

Experiment	Initial Stage <i>S. tsugae</i>	Final Stage <i>S. tsugae</i>	<i>Adelges tsugae</i>	<i>Adelges piceae</i>
Feeding Preference	Adult	Adult	Sistens Adults/Progrediens Eggs	Hiemosistens Adults/Aestivosistens Eggs
Oviposition Preference	Adult	Adult	Sistens Adults/Progrediens Eggs	Hiemosistens Adults/Aestivosistens Eggs
Developmental Performance	Egg	Adult	Sistens Adults/Progrediens Eggs	Hiemosistens Adults/Aestivosistens Eggs
Survival Test 1	Adult	Adult	Aestivating Sistens Nymphs	Aestivosistens Adults/ Hiemosistens Eggs
Survival Test 2	Adult	Adult	Sistens Adults/Progrediens Eggs	n/a

Table 2. Mean ( $\pm$  SE) number of adelgid eggs consumed by adult *S. tsugae* in 72-hour feeding rate no choice (single-prey) tests conducted at 26°C, 16:8 (L:D), and 70-80 % RH.

Host	Mean no. eggs consumed ( $\pm$ SE)	<i>n</i>	<i>t</i> statistic	df	<i>P</i> -value
<i>Adelges tsugae</i>	30.00 $\pm$ 3.39	20			
			2.06	38	0.0462
<i>Adelges piceae</i>	39.30 $\pm$ 2.96	20			

Table 3. Mean ( $\pm$  SE) number of adelgid eggs consumed by adult *S. tsugae* in a 72-hour feeding rate paired-choice test conducted at 26°C, 16:8 (L:D), and 70-80 % RH.

Host	Mean no. eggs consumed ( $\pm$ SE)	<i>n</i>	Difference ( $\pm$ SE)	<i>t</i> statistic	df	<i>P</i> -value
<i>Adelges tsugae</i>	38.30 $\pm$ 2.23					
		20	0.40 $\pm$ 3.32	0.12	19	0.9054
<i>Adelges piceae</i>	38.70 $\pm$ 2.00					

Table 4. Mean ( $\pm$  SE) number of *S. tsugae* eggs laid and larvae hatched after the 72-hour no choice (single-prey) and paired-choice ovipositional preference tests conducted at 26°C, 16:8 (L:D), and 70-80 % RH.

Host	<i>n</i>	Mean no. eggs observed ( $\pm$ SE)	Mean no. larvae hatched ( $\pm$ SE)
<u>No Choice (single-prey) Test</u>			
<i>Adelges tsugae</i>	20	0.20 $\pm$ 0.13	0.60 $\pm$ 0.30
<i>Adelges piceae</i>	20	0.30 $\pm$ 0.15	0.50 $\pm$ 0.40
Uninfested eastern hemlock	20	0	0
Uninfested Fraser fir	20	0	0
<u>Paired-choice Test</u>			
<i>Adelges tsugae</i>	20	0	0.40 $\pm$ 0.40
<i>Adelges piceae</i>		0.40 $\pm$ 0.22	0.80 $\pm$ 0.42

Table 5. Developmental time (days) and percent (%) survival of *S. tsugae* from egg to adult stages on two adelgid hosts at 26°C, 16:8 (L:D), and 70-80 % RH.

Life Stage	<i>Adelges tsugae</i>			<i>Adelges piceae</i>		
	days ( $\pm$ SE)	%	$n^x$	days ( $\pm$ SE)	%	$n^x$
Egg <sup>y</sup>	3.3 $\pm$ 0.3	100	15	3.6 $\pm$ 0.4	100	15
Instar 1 <sup>y</sup>	2.8 $\pm$ 0.2	100	15	2.7 $\pm$ 0.3	100	15
Instar 2 <sup>y</sup>	2.5 $\pm$ 0.5	86	13	2.8 $\pm$ 0.3	80	12
Instar 3 <sup>y</sup>	2.8 $\pm$ 0.3	86	13	2.2 $\pm$ 0.3	73	11
Instar 4 <sup>y</sup>	2.8 $\pm$ 0.5	86	13	2.2 $\pm$ 0.4	66	10
Pre-pupa <sup>y</sup>	1.5 $\pm$ 0.1	86	13	1.4 $\pm$ 0.2	66	10
Pupa <sup>y</sup>	6.5 $\pm$ 0.2	86	13	7.2 $\pm$ 1.1	60	9
Adult <sup>z</sup>	19.1 $\pm$ 0.6	86	13	18.4 $\pm$ 1.2	60	9

<sup>x</sup>Number beginning each life stage.

<sup>y</sup>Duration in days of given lifestage.

<sup>z</sup>Total developmental time to adult stage.

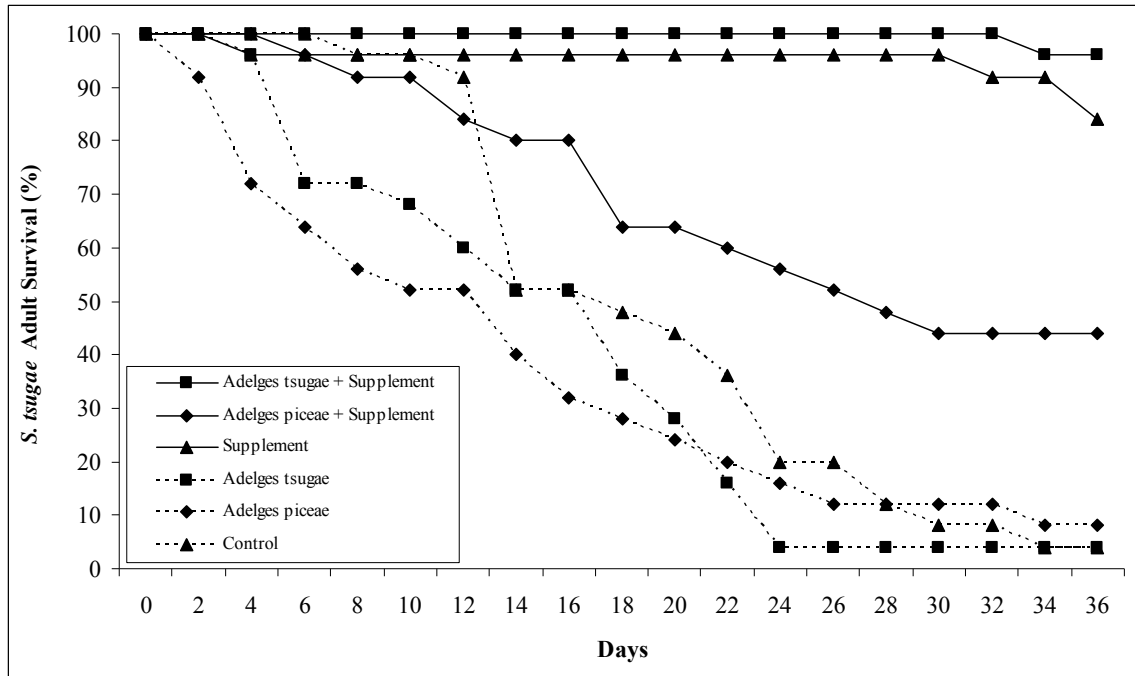


Figure 1. Adult *S. tsugae* percent (%) survival over a 36 day period in long-term survival test 1, conducted between August and September 2005 at 26°C, 16:8 (L:D), and 70-80 % RH.

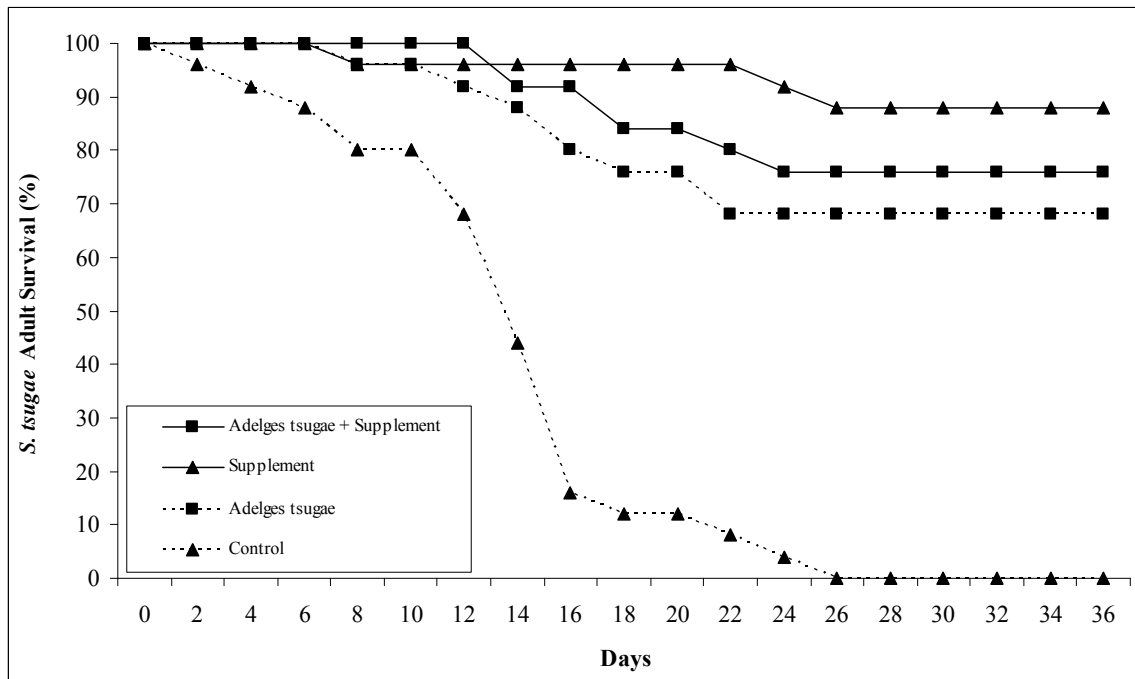


Figure 2. Adult *S. tsugae* percent (%) survival over a 36 day period in long-term survival test 2, conducted between April and May 2006 at 26°C, 16:8 (L:D), and 70-80 % RH.



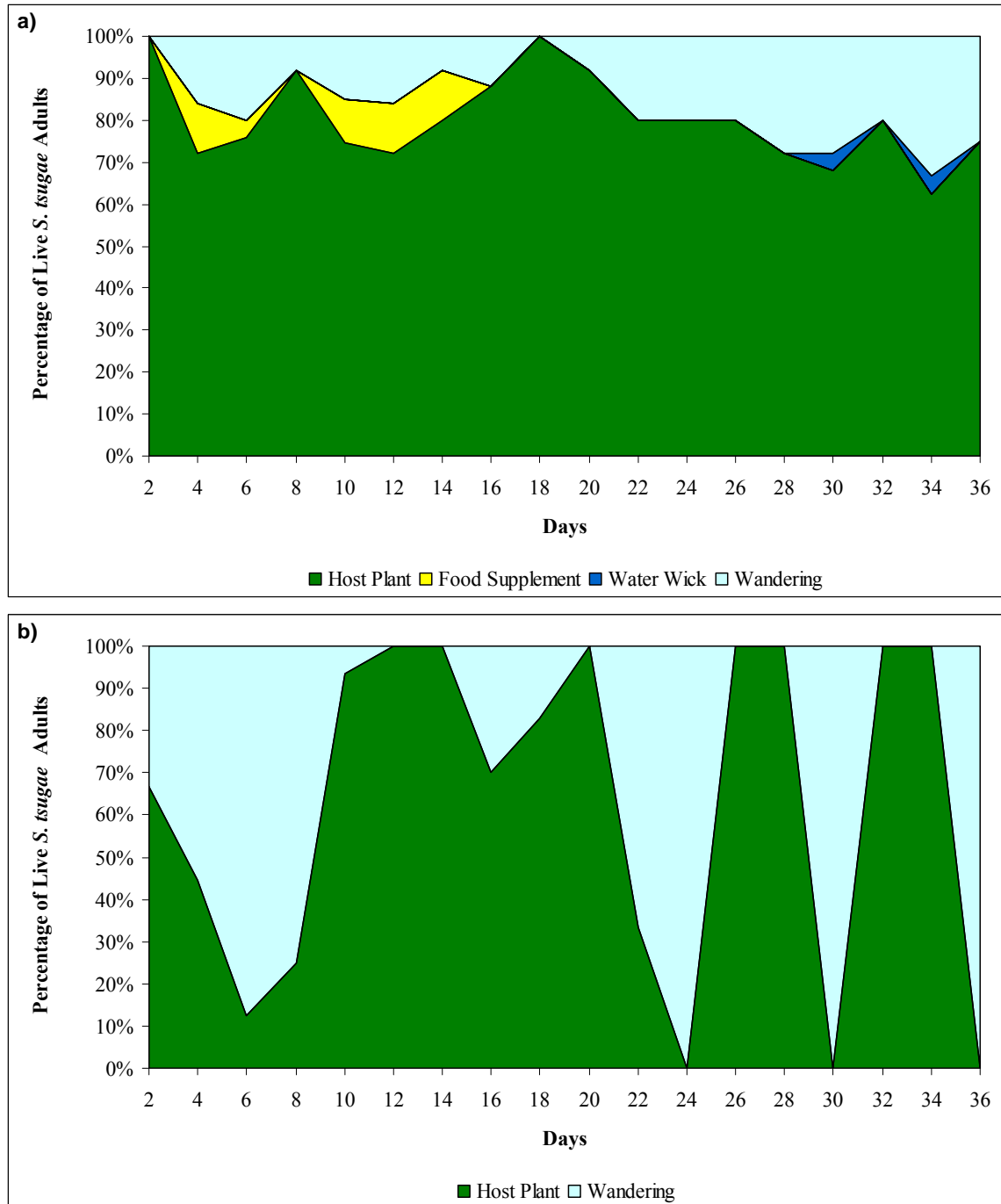


Figure 3. Percentage (%) of live *S. tsugae* adults found at specified locations within test arenas in the *Adelges tsugae* + Supplement (a) and *Adelges tsugae* (b) treatments during 36 day long-term survival test 1.

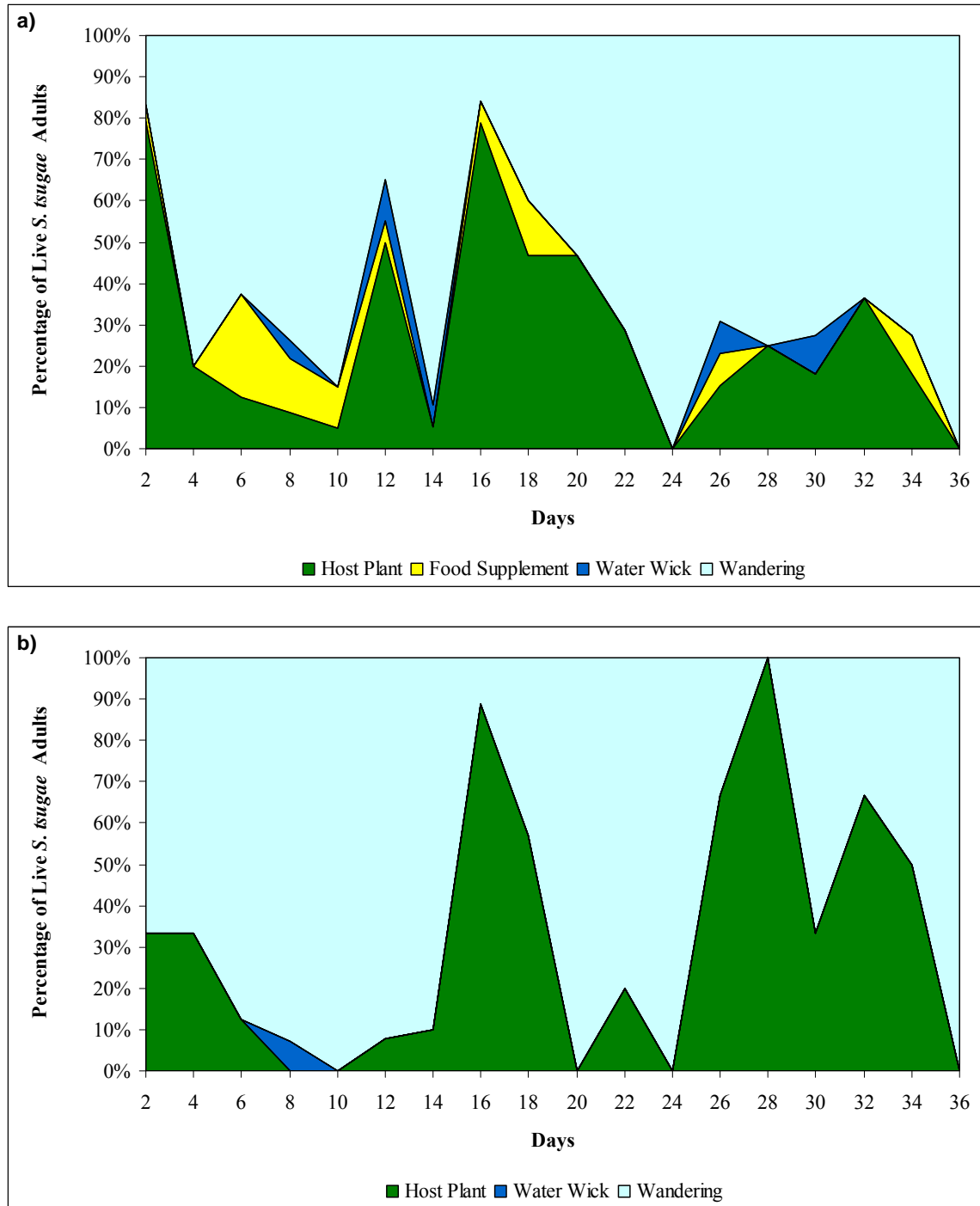


Figure 4. Percentage (%) of live *S. tsugae* adults found at specified locations within test arenas in the *Adelges piceae* + Supplement (a) and *Adelges piceae* (b) treatments during 36 day long-term survival test 1.

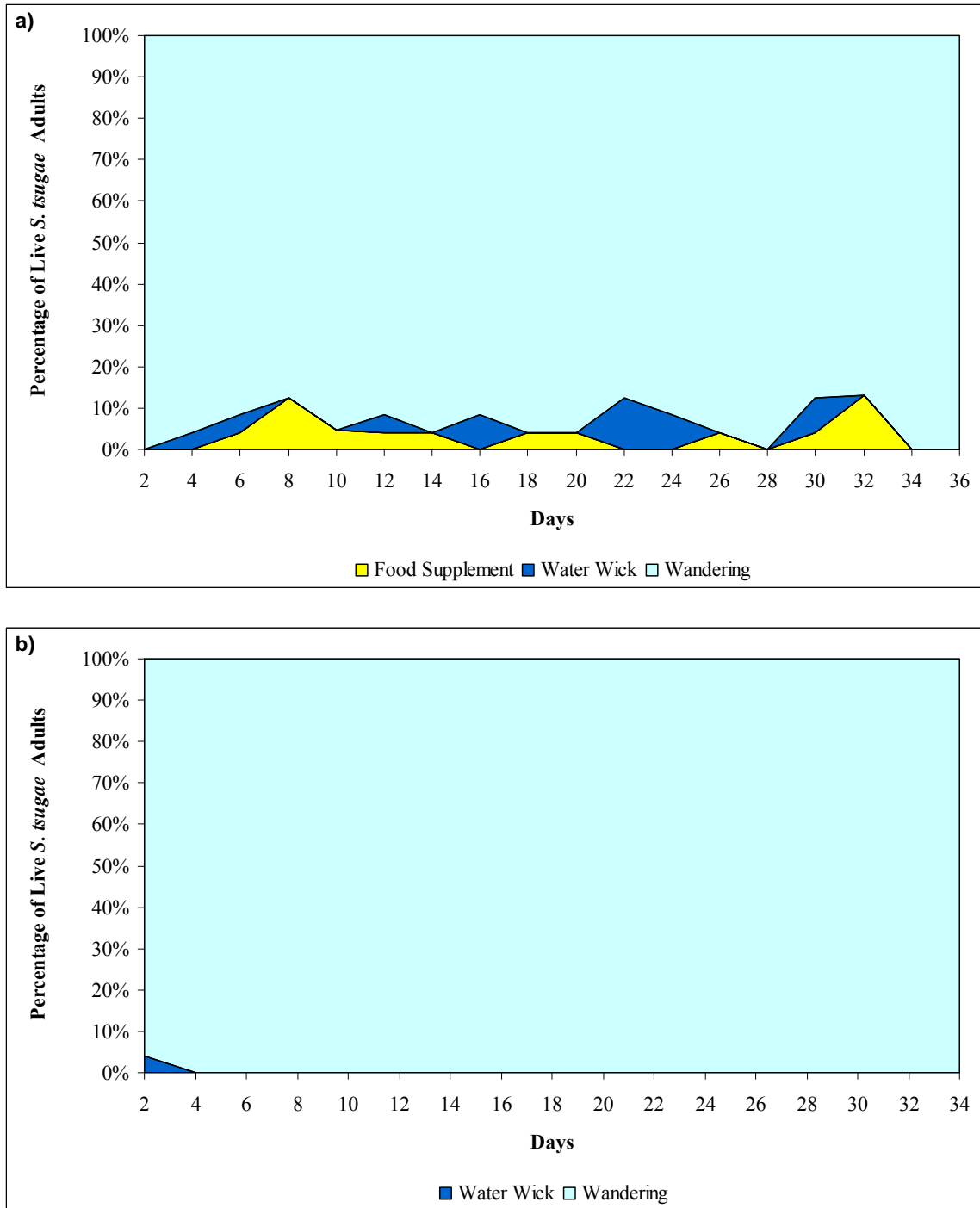


Figure 5. Percentage (%) of live *S. tsugae* adults found at specified locations within test arenas in the Supplement (a) and Control (b) treatments during 36 day long-term survival test 1.

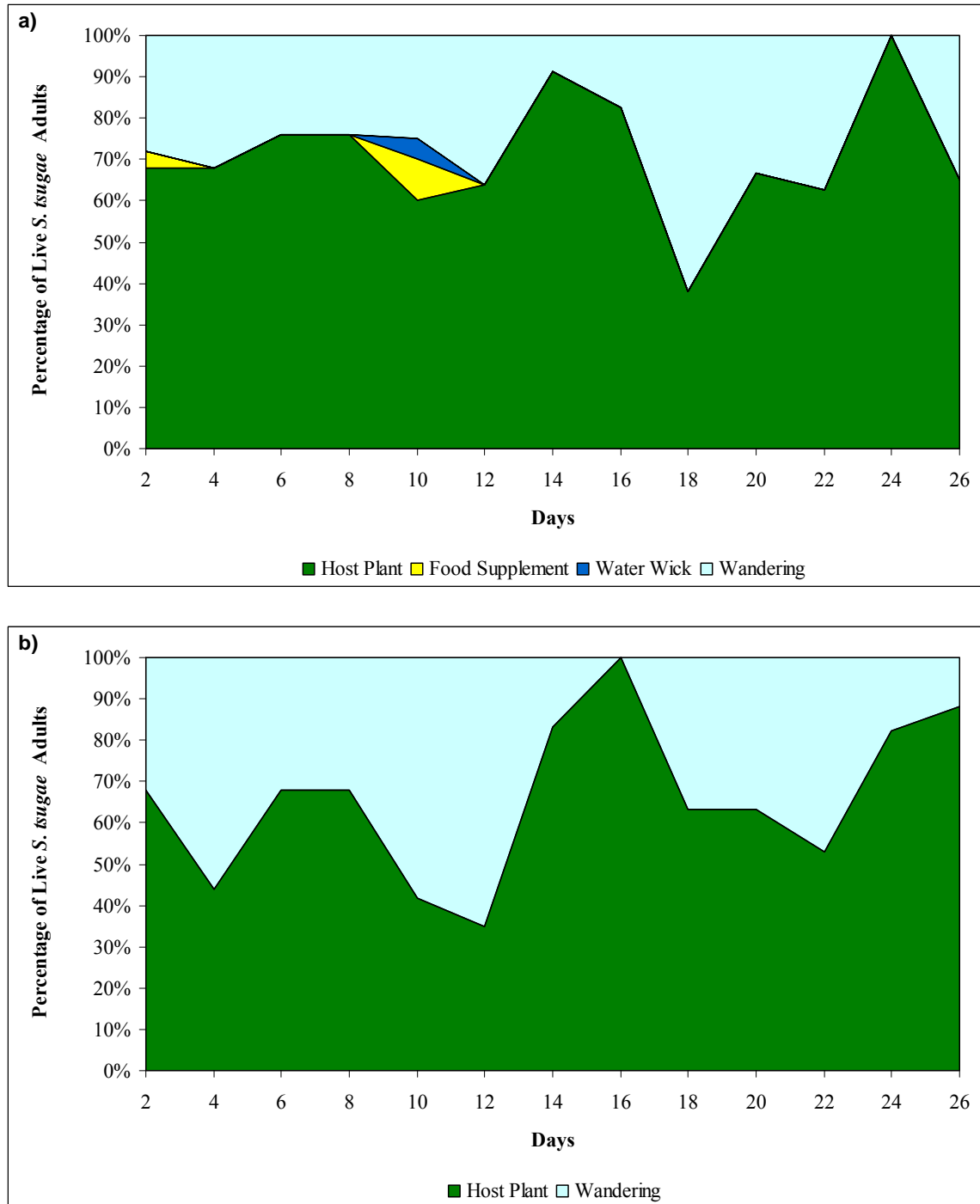


Figure 6. Percentage (%) of live *S. tsugae* adults found at specified locations within test arenas in the *Adelges tsugae* + Supplement (a) and *Adelges tsugae* (b) treatments during 36 day long-term survival test 2.

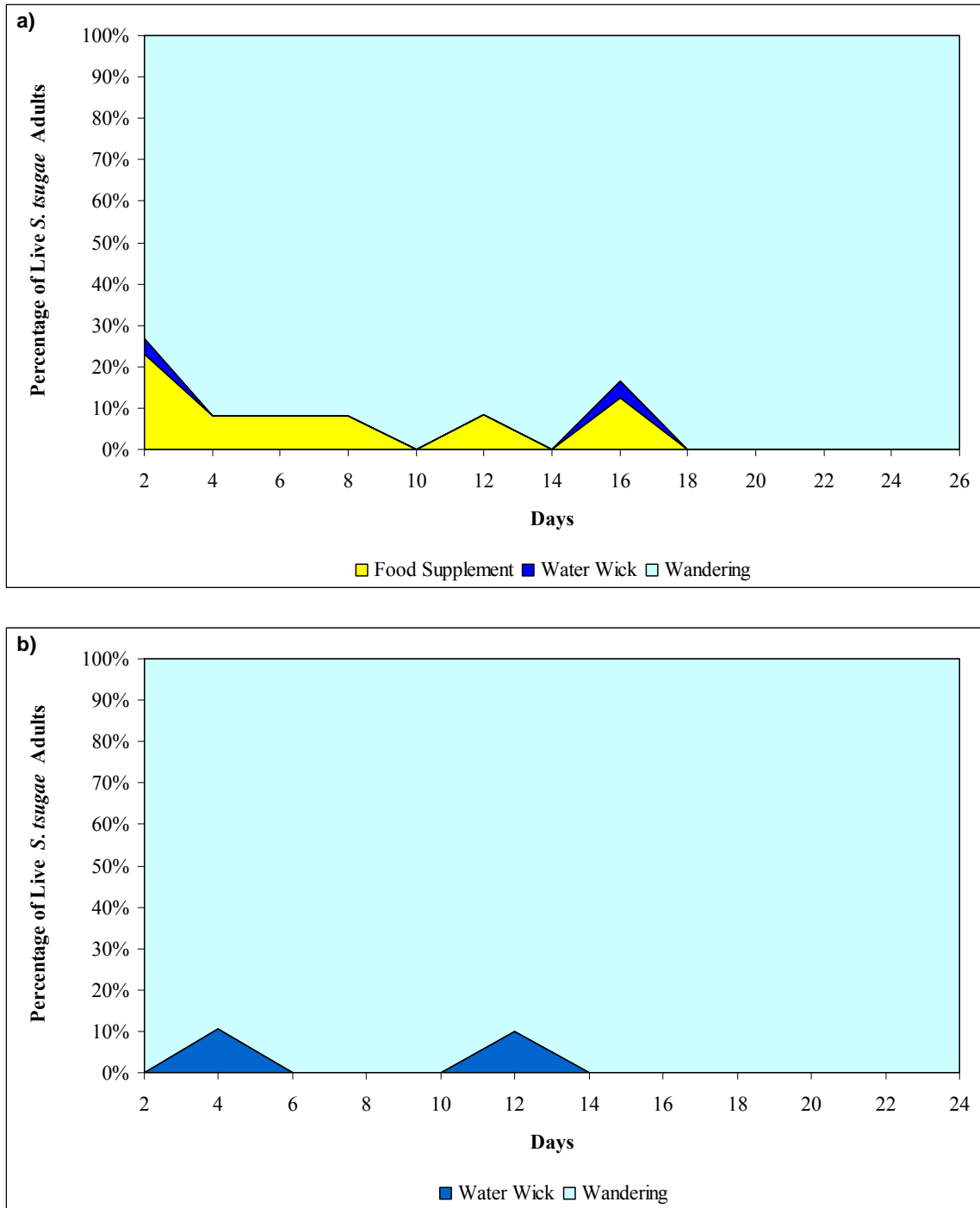


Figure 7. Percentage (%) of live *S. tsugae* adults found at specified locations within test arenas in the Supplement (a) and Control (b) treatments during 36 day long-term survival test 2.

### Chapter III

#### Low Density Field Release Studies of *Sasajiscymnus tsugae* for Biological Control of the Hemlock Woolly Adelgid in Western North Carolina

##### ABSTRACT

The utility of confined releases for colonization of *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), a classical biological control agent of the hemlock woolly adelgid (HWA) *Adelges tsugae* Annand (Hemiptera: Adelgidae) was evaluated in three field studies over three years at forest and ornamental sites in western North Carolina (USA). Predator reproduction, survival, and impact on HWA were investigated following the placement of fifteen adults (10♀:5♂) in mesh sleeve cages on adelgid infested hemlock (*Tsuga*) branches for two or four weeks. In all three studies the predator reproduced inside sleeve cages and oviposition generally began within two or three weeks. Some adult predators were recovered during all three studies, indicating that *S. tsugae* can survive for up to one month inside mesh sleeve cages. In one study the number of adults recovered was negatively correlated with the density of predator progeny. Predator exclusion cages were used to evaluate the impact of predation by *S. tsugae* on HWA density by comparison to branches on which the predator was initially confined for two weeks (Field Study 2) or to predator inclusion cages that were left on adelgid infested branches for the duration of the study (Field Study 3). Significant local reductions in the density of adelgid ovisacs and aestivating nymphs were found but were not always correlated to the presence of the predator. Where *S. tsugae* was liberated from mesh sleeves two weeks after placement in the cages (Field Studies 1 and 2) all evidence of predator activity disappeared from the study

sites within 4 months and establishment could not be documented during the study period.

## INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an invasive forest pest in eastern North America that causes significant tree decline and mortality in populations of eastern (*Tsuga canadensis* (L.) Carrière) and Carolina (*T. caroliniana* Engelmann) hemlock. The insect is native to the hemlock forests of Asia and western North America where it is considered an innocuous pest and rarely causes tree damage (McClure et al. 2001). The adelgid was first described from specimens collected on western hemlock (*T. heterophylla* Sargent) in California and Oregon (Annand 1924), but the source of introduction to the eastern US was most likely adelgids on *T. sieboldii* Carrière from southern Japan (Havill et al. 2006). The current infestation may be the result of a single introduction of the adelgid on imported nursery stock that was planted in the Richmond, Virginia area in the early 1950s (Souto et al. 1996). So far, HWA's spread and damage in eastern North America has been confined to the US where it infests 50% of hemlock ecosystems and can be found in 17 states from Maine south to Georgia (Cheah et al. 2004).

The adelgid feeds at the base of hemlock needles where it inserts its feeding stylet into the xylem and extracts stored nutrients from ray parenchyma cells (Young et al. 1995). Tree damage associated with HWA infestations is typically described as needle desiccation and defoliation, bud abortion, and reduced terminal growth (McClure et al. 2001). It is not known if these symptoms are a direct result of adelgid feeding or are indirect consequences

of hemlock defensive responses similar those seen in Fraser fir (*Abies fraseri* [Pursh] Poir.) following infestation by the balsam woolly adelgid (*Adelges piceae* Ratz.) (reviewed by Newton and Hain 2005). Under favorable climatic and site conditions some hemlocks can survive HWA feeding for more than ten years in a declined condition (Souto et al. 1996), but severe infestations associated with drought and secondary attacking insects can kill trees in as few as four years (McClure et al. 2001).

The innocuous infestations of HWA on hemlocks found in the insect's native range is attributed to the presence of natural enemies and host resistance mechanisms, factors that are absent in the eastern US (Wallace and Hain 2000; McClure et al. 2001). Chemical insecticides, particularly stem and soil injections of imidacloprid, are the most effective management option for reducing HWA impacts on hemlock in the insects introduced range (Cowles et al. 2006). However, the high cost associated with these treatments and the environmental sensitivity of hemlock ecosystems makes their widespread use in forested settings impractical. They are best suited for use on high value or specimen trees in recreational and ornamental settings (Ward et al. 2004).

Classical biological control, the importation, culture, and release of natural enemies from HWA's native range, is an alternative to insecticidal management that has received much attention (Cheah et al. 2004). Because there are no known parasitoids of the Adelgidae much emphasis has been placed on the identification of adelgid predators, particularly those in the order Coleoptera. Explorations are ongoing (Montgomery et al. 2007), but four natural enemies have been evaluated in quarantine and experimentally or operationally released into



HWA infested forests of the eastern US. These are *Laricobius nigrinus* Fender (Derodontidae) from western North America, *Scymnus ninghanensis* Yu and Yao and *S. sinuanodulus* Yu and Yao (Coccinellidae) from China, and *Sasajiscymnus* (formerly *Pseudoscymnus*) *tsugae* Sasaji and McClure (Coccinellidae) from Japan (Cheah et al. 2004).

*Sasajiscymnus tsugae* was the first predator of HWA to be identified and imported to the US for quarantine evaluation (Sasaji and McClure 1997; Cheah and McClure 1998, 2000). Since 1995, nearly 2.5 million beetles have been released in adelgid infested forests (J.R. Rhea, personal communication). It is considered a good candidate for successful biological control of HWA because it feeds preferentially on HWA, although, it will feed and complete development on other adelgid species (Butin et al. 2004; Jetton, Chapter II this volume), is able to survive the summer aestivation of the adelgid, although, mortality is high if supplemental food sources are lacking (Jetton Chapter II, this volume), overwinters in hemlock forests remaining in close association with its prey (McClure et al. 2000), and has demonstrated bi-voltine synchrony to the complex polymorphic life cycle of the adelgid (Cheah and McClure 2000).

The predatory Coccinellidae are the most commonly utilized predators in classical biological control programs despite the historically low rate of success for colonization of these species. Since 1900, approximately 180 different species have been intentionally introduced to North America to combat plant pests. Only 18 of these have been documented as established and even fewer have successfully controlled their target pest (Obrycki and Kring 1998). The success rate in forestry applications is even lower for coccinellids

specifically, and for predators in general is half that for parasitoids (Dahlsten and Mills 1999). The classical biological control effort for the balsam woolly adelgid (BWA) in the Fraser fir forests of the southern Appalachian Mountains is a good example. In total, 31 species of predators, 16 of which were coccinellids, were introduced to combat this pest. A few are now established but have no impact on BWA population levels (reviewed by Zilahi-Balogh et al. 2002).

A primary issue related to the difficulty classical biological control programs face in achieving successful coccinellid establishment is that at the time of release the adult insects typically disperse very quickly from the release area, often into inappropriate environments where the target prey is not present (Hodek and Honek 1996). This is due to a number of factors including wind or temperature conditions at the time of predator liberation (Etzel and Legner 1999), or density-dependent movement of the insects to avoid intra-specific competition for prey resources and cannibalism (Obrycki and Kring 1998). The dispersal of the adults after release reduces the likelihood that males and females will be able to locate one another at times appropriate for mating and oviposition and that predator density will increase rapidly in the targeted control area (Hodek and Honek 1996).

An alternative to free releases that may aid the establishment of coccinellids and other predators with strong dispersal tendencies is to initially cage the insects on host plants in the desired release area (DeBach and Bartlett 1964; Van Driesche 1993). Confined releases restrict dispersal while allowing one or more reproductive cycles in an environment of prey abundance and protecting the agents from competition or depredation by other natural

enemies. Confined releases may also help to better acclimate laboratory reared biological control agents to field conditions. Larvae and adults that emerge in the cages may be better adapted to natural conditions and less likely to disperse long distances when the release cages are removed (Van Driesche 1993). For the predaceous Coccinellidae specifically, due to the potential for intra-specific competition and cannibalism, it is probably important to restrict the number of insects placed in each release cage. The establishment of the vedalia beetle *Rodolia cardinalis* (Mulsant), an important component of the biological control complex for the cottony cushion scale *Icerya purchasi* Maskell in the orange groves of California, was routinely achieved with as few as 10 adult insects per release cage (DeBach and Bartlett 1964).

It has been suggested that the potential for intra-specific competition and cannibalism that can negatively influence *S. tsugae* feeding, mating, and oviposition behaviors may necessitate low density releases of this predator (Flowers et al. 2005, 2006). Throughout many areas of the eastern US where *S. tsugae* has been released it is considered established (Blumenthal 2002; Cheah et al. 2004; Blumenthal and Werner 2005), including dozens of sites in western North Carolina where predator releases have occurred since 2001, the first year that widespread adelgid infestations were noted in the state. However, repeated attempts to recover the predator in and around most of these release sites have met with sporadic success, and, as HWA infestations continue to grow and spread and hemlocks continue to decline and die, there is little evidence to suggest that *S. tsugae* is having any impact even if it is established. The only sites in the eastern US where post-release recovery

has been consistent and trees appear to be recovering are located in Connecticut where predators have been released in low densities; 2000 to 4000 beetles per stand in groups of 30 distributed throughout the site (McClure et al. 2000; Cheah and McClure 2002; Cheah et al. 2005). Conversely, the release protocol in North Carolina, and other states in the southern Appalachian region, is to conduct single-point releases where between 2000 and 10,000 beetles are released as a group on one or two trees near the center of a HWA infested hemlock stand (USDA FS 2005a,b,c). While efficient and less costly in terms of time and resources, this method does not account for density-dependent dispersal common to many coccinellids that may be related to avoidance of intra-specific competition and cannibalism.

It is hypothesized that the high density conditions and lack of an appropriate acclimation period imposed by current single-point release methods stimulate *S. tsugae* adults to disperse from HWA infested hemlock stands prior to mating and oviposition, and may, in part, explain the lack of predator establishment in western North Carolina. The three field studies presented here evaluate a low density release protocol, where a small number of adult beetles are placed in sleeve cages distributed throughout an HWA infested hemlock stand or the crown of a single infested tree and allowed to acclimate, mate, and oviposit in their new environment prior to liberation from the cages. The objectives were to test the effects of cages on the survival, reproduction, and subsequent establishment of *S. tsugae* in the release areas. The potential for the predator to have a significant local impact on HWA population density at the point of introduction was also studied.

## MATERIALS AND METHODS

### Predator Rearing

*Sasajiscymnus tsugae* adults used in the field release studies were obtained from mass rearing colonies maintained at NC State University (NCSU). Beetles were reared using standard protocols and environmental conditions (26°C; 16:8 L:D; 70-80 % RH) developed for the predator at the New Jersey Department of Agriculture Phillip Alampi Beneficial Insect Laboratory (Trenton, NJ; Palmer and Sheppard 2001). The NCSU colony was developed from two small, 100 beetle (50♂:50♀) starter colonies obtained from the Alampi Lab and the North Carolina Department of Agriculture Beneficial Insect Laboratory (Cary, NC). All predators were reared the same year that experiments were conducted and had eclosed at least 2 months prior to field release. In all three field studies, *S. tsugae* adults were released in groups of 15 that consisted of 10 female and 5 male beetles. This is the same density and sex ratio used by mass rearing facilities to obtain maximum reproductive output (Palmer and Sheppard 2001).

### Study Sites

Field release studies were conducted over 3 consecutive springs (2003-2005) in the mountains of Western North Carolina. Field Study 1 (2003) was conducted in the Back Creek area of the Pisgah National Forest in Burke, County at N35°49.898', W81°51.833', and 412 m elevation. The eastern hemlock stand chosen for the study was approximately three hectares in sized and occupied a riparian area bordering Reedy's Fork Creek. The trees on which predators were released ranged in size from 8–15 m in height and 20–50 cm DBH

and were all newly infested by HWA (within the previous 2 years). Associated forest cover was dominated by eastern white pine (*Pinus strobus* L.), yellow poplar (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.), *Paulownia tomentosa* (Thunb.) Sieb. and Zucc. ex Steud., *Quercus* spp., and *Carya* spp with an understory dominated by *Rhododendron maximum* L. This stand had not been treated (either chemical or biological controls) for HWA prior to this study.

Field Study 2 (2004) was conducted in an ornamental planting of hemlock near the town of Laurel Springs in Ashe County located at N36°24.323', W81°17.952', and 916 m elevation. Predators were released on a single open grown eastern hemlock that measured 25 m in height and 75 cm DBH at the beginning of the study. Surrounding vegetation consisted of ornamental eastern and Carolina hemlocks (4 trees total), several species of ornamental shrubs, and a fescue (*Festuca sp.*) lawn. All hemlocks at the site were newly infested with HWA, had received no adelgid control treatment, and, having been transplanted as seedlings from nearby forests, were local in origin.

Field Study 3 (2005) was conducted in a nearly pure stand of mature eastern hemlocks with an understory dominated by hemlock regeneration in the seedling, sapling, and pole size classes. The study site is located approximately 1 mile east of the Tennessee border near the Beech Mountain community in Avery County at N36°13.409', W81°56.517', and 949 m elevation. Predators were released on understory hemlocks that were newly infested by HWA, measuring approximately 15 m in height and 20 cm DBH, and had received no previous adelgid control treatments.

### Field Study 1: Predator Release in the Forest Environment

Fifteen trees heavily infested with HWA and lacking visible signs of branch dieback were selected for this experiment. Care was taken to choose trees with a large proportion of live crown assessable from the ground to facilitate predator release and adelgid sampling. Two lower crown branches, one in the sun and one in the shade, supporting high adelgid densities were identified on each tree (30 branches total). On 5 May 2003, 120cm x 60cm mesh sleeve cages sewn closed on three sides were pulled over the terminal end of each branch. Fifteen (10♀: 5♂) adult *S. tsugae* were placed in each cage and the open ends were closed around the branch using plastic zip-ties fastened tight around foam weather stripping. A total of 450 *S. tsugae* were released. Mesh sleeve cages remained on the trees for two weeks to facilitate predator conditioning to the release environment and reproduction on release branches. After this two week period the release cages were removed to facilitate predator dispersal throughout the stand.

Pre and post-release sampling to track changes in the density of adelgid life stages, new growth production by the trees, and *S. tsugae* abundance consisted of: 10cm twig sample collected from each release branch (release branch samples) immediately prior to *S. tsugae* release and 2, 4, 6, and 8 weeks after release, 10cm twig samples collected at high and low heights and north and south orientations on each tree (crown samples, four per tree) collected prior to and 1, 2, 4, 10, 11, 12, 15, 23, and 24 months after release, and two beat sheet samples per tree collected concurrently with crown samples. Beat sheet sampling utilized a 1 m square section of bed sheet held 12 inches below a branch that was beaten 3 times with a

Wiffle® ball bat. These samples were collected from lower crown branches on the north and south side of each tree.

All twig samples were collected using hand or pole pruners and were evaluated in the laboratory using a dissecting microscope. Beat sheet samples were evaluated in the field and the number of *S. tsugae* larvae and adults captured was recorded. The number of predator eggs, larvae, larval exuviae, pupae, and adults observed was recorded for each release branch and crown sample, and the bi-weekly or monthly counts were totaled by predator life stage. The number of adelgid ovisacs, the number of aestivating adelgid nymphs, and the amount (cm) of succulent green new growth was also recorded for each crown sample. The seasonal density of the adelgid life stages and seasonal change in the production of new growth by hemlocks were summarized by each crown height and orientation combination.

#### Field Study 2: Predator Release in the Ornamental Environment

A single ornamental Eastern hemlock with fourteen first order branches that supported high adelgid densities, lacked visible signs of dieback, and were distributed throughout the lower half of the crown was selected for predator release. The terminal portion of four second order branches on each larger branch (56 second order branch terminals total) were then randomly assigned to one of four sleeve cage treatments: predator release cage, predator exclusion cage, open cage, and no cage (open branch). All cages were 120cm x 60cm mesh sleeves. The release and exclusion cage treatments consisted of mesh sleeves that were sewn closed on three sides. One side of each exclusion cage had a window with Velcro closure to facilitate post-release sampling. Mesh sleeves in the open cage



treatment were closed along each side but were left open at the terminal end. In all treatments, the basal end of each sleeve cage was attached to the branch with a plastic zip-tie fastened tightly around foam weather stripping. Branch terminals in the no cage treatment did not received mesh sleeves and were marked with flagging tape for identification. Fifteen (10♀: 5♂) *S. tsugae* adults were placed in each release cage on 3 May 2004. A total of 210 beetles were released. The predators remained caged on release branches for two weeks to facilitate *S. tsugae* conditioning to the release environment and reproduction on release branches. After this two week period the release cages were removed to facilitate predator dispersal throughout the tree.

One pre-release sample was conducted immediately prior to placement of cages and predators to document initial HWA density and amount of new growth. Five post-release samples were made 1, 2, 4, 10, and 12 months after predators were introduced to estimate changes in HWA density and new growth production among the cage treatments and to evaluate predator reproduction, dispersal, and establishment. Monitoring involved sampling of one 10 cm twig section from each caged or un-caged treatment branch during the pre-release and post release sample periods (56 twig sections per sample date). All samples were collected from a ladder using hand pruners and were evaluated in the laboratory using a dissecting microscope. Variables recorded for each twig sample included the number of adelgid ovisacs, the number of aestivating adelgid nymphs, the amount (cm) of succulent green new growth, and the number of *S. tsugae* eggs, larvae, larval exuviae, pupae, and adults.

*S. tsugae* dispersal throughout the crown of the tree was also evaluated at the pre- and post- release sample periods. This was done using pole pruners to destructively sample 10 cm twig sections from branches (excluding those in sleeve cage treatments) at high, medium, and low heights in the crown on the north, south, east, and west sides of the tree (12 twig sections per sample date). These crown samples were also evaluated in the laboratory using a dissecting microscope and the number of *S. tsugae* eggs, larvae, larval exuviae, pupae, and adults per twig was recorded.

Data were analyzed by analysis of variance (ANOVA) using the general linear model procedure (PROC GLM) in Statistical Analysis System (SAS) Version 9.1 to determine if the pre-release density of adelgid ovisacs and aestivating adelgid nymphs and the length (cm) of new growth differed among the first (branch) and second (cage treatment) order branches selected for the study, and if, after its release, *S. tsugae* significantly affected these same variables among the sleeve cage treatments. Pre-release analysis tested the main effects of branch and cage treatment. Post-release analysis tested the main effects of branch, sample date, cage treatment, and all two-way interactions during the period of peak post-release abundance for the number of adelgid ovisacs (10 and 12 months after predator release) and the number of aestivating adelgid nymphs and length (cm) of new growth (1, 2, and 4 months after predator release).

### Field Study 3: Predator Survival, Reproduction, and Impact in Sleeve Cages

Three trees heavily infested with HWA and lacking visible signs of branch dieback were selected for this experiment. Ten branches supporting high adelgid densities were

chosen from each tree and randomly assigned to one of three sleeve cage treatments: predator inclusion cage, predator exclusion cage, and no cage (open branch). Eight branches were assigned to the inclusion cage treatment while the remaining two were assigned to either the exclusion cage or no cage treatment. Cage size, construction, and attachment to the tree were the same as in Field Study 2. On 2 May 2005, one 10 cm twig sample was cut from each branch using hand pruners to determine the pre-release density of HWA ovisacs, and then fifteen (10♀: 5♂) *S. tsugae* adults were placed in each inclusion cage. Beginning one week after release and continuing once per week for 4 weeks, 2 inclusion branches (with cages intact) were removed from each tree, and one 10 cm twig sample was cut from each branch in the exclusion and no cage treatments (6 release cages and 6 twig samples total per week). These were returned to the laboratory to determine predator survival, reproduction, and impact. Inclusion cage branches were surveyed for the total number of surviving *S. tsugae* adults per cage, predator reproduction by counting the number of progeny (*S. tsugae* eggs, larvae, and pupae) on twenty 10 cm samples per branch, and predator impact on HWA density by counting the number of adelgid ovisacs on one 10 cm sample per branch. For comparison to the release cage treatment, adelgid density in the open and no cage treatments was estimated by counting the number of adelgid ovisacs on the 10 cm twig samples collected from these cage treatments each week. Data were analyzed by ANOVA using PROC GLM in SAS Version 9.1 to determine if the pre-release density of adelgid ovisacs differed among the trees and branches selected for the study, and if *S. tsugae* significantly affected the post release density of adelgid ovisacs among the sleeve cage treatments. Pre-

release analysis tested the main effects of tree, cage treatment, and their interaction, and post-release analysis tested the main effects of tree, sample date, cage treatment, and all two-way interactions.

## RESULTS

### Field Study 1: Predator Release in the Forest Environment

Pre-release sampling indicated that *S. tsugae* was not present at the study site prior to this experiment (Tables 1 and 2). Two weeks after predators were introduced, twig samples collected from the release branches at the time mesh sleeve cages were removed contained eggs, larvae, larval exuviae, and adults of *S. tsugae* (Table 1). Overall, more eggs and adults were found on release branches exposed to the sun than those in the shade. No predator life stages were recovered during subsequent release branch samplings that occurred four, six, and eight weeks after *S. tsugae* was introduced to the stand. Among crown samples that were collected at 9 sampling periods after *S. tsugae* release, the only recovery of predator life stages was on the one month samples (Table 2). No *S. tsugae* larvae or adults were recovered from beat sheet sampling that occurred concurrently with crown sampling (Table 2). Overall, through the duration of the study the number of hemlock woolly adelgid ovisacs increased, the number of aestivating hemlock woolly adelgid nymphs decreased, and the amount of new growth production by eastern hemlock remained relatively unchanged (Figure 1).

An unfortunate design flaw in this field study is the lack of experimental controls, therefore, the results will not be discussed with respect to any potentially significant or

meaningful impacts that the predator may have had on the adelgid population at this site. A more thorough and well designed study would have included additional trees, either paired on site with those trees included in the study or in a similar hemlock stand nearby, on which *S. tsugae* was not released for comparison to those where introductions were attempted.

#### Field Study 2: Predator Release in the Oranamental Environment

All predator recovery data for this study are presented in Table 3. Pre-release sampling indicated that *S. tsugae* was not present on the ornamental hemlock used in this study prior to the experimental introduction of the predator. One month after predator release, twig sampling in the predator release cage treatment documented the presence of *S. tsugae* larvae, larval exuviae, and adults. Although this one month sample occurred two weeks after release cage removal, no evidence of predator dispersal to branches in the other treatments was found at this time. Two months after release, *S. tsugae* had dispersed from the release cage treatment as predator larvae, larval exuviae, and pupae were found in the release, open, and no cage treatments. The only evidence of predator activity that remained on treatment branches four months after release was larval exuviae found on twig samples in the release and no cage treatments. No predator life stages were recovered from cage treatment samples collected 10 and 12 months after release. At no point during the study was *S. tsugae* recovered on samples collected from the exclusion cage treatment. Crown sampling conducted during the five post-release sample periods to detect dispersal of the predator beyond the 14 treatment branches found no evidence of *S. tsugae*.

Prior to *S. tsugae* release, the density of hemlock woolly adelgid ovisacs ( $F = 0.60$ ,  $P$

= 0.8296,  $df = 12,36$  for branch;  $F = 0.21$ ,  $P = 0.8910$ ,  $df = 3,36$  for cage treatments), aestivating hemlock woolly adelgid nymphs ( $F = 1.64$ ,  $P = 0.1244$ ,  $df = 12,36$  for branch;  $F = 0.25$ ,  $P = 0.8583$ ,  $df = 3,36$  for cage treatments), and length of succulent green hemlock growth ( $F = 0.94$ ,  $P = 0.5195$ ,  $df = 12,36$  for branch;  $F = 0.26$ ,  $P = 0.8532$ ,  $df = 3,36$  for cage treatments) did not differ significantly among the 14 first order branches or cage treatments. After release, the overall density of adelgid ovisacs, aestivating adelgid nymphs, and the length of new growth production appeared to be decreasing throughout the study (Figure 2).

The peak density of adelgid ovisacs occurred at 10 and 12 months after predator release and was significantly affected by the main effects of branch ( $F = 4.34$ ,  $P = 0.0004$ ,  $df = 12,33$ ), sample date ( $F = 36.78$ ,  $P < 0.0001$ ,  $df = 1,33$ ), and cage treatment ( $F = 5.78$ ,  $P = 0.0027$ ,  $df = 3,33$ ) and by the interaction between branch and sample date ( $F = 2.30$ ,  $P = 0.0316$ ,  $df = 11,33$ ). During these two sample periods the density of adelgid ovisacs was lowest in the release cage treatment, highest in the exclusion cages, and intermediate in the open and no cage treatments (Figure 2a). Similarly, the lowest density of aestivating adelgid nymphs was found in the release cage treatment during their period of peak density, which occurred between 1 and 4 months after predator release (Figure 2b). At this time, the density of aestivating nymphs was significantly affected by the main effects of branch ( $F = 5.45$ ,  $P < 0.0001$ ,  $df = 12,72$ ), sample date ( $F = 9.27$ ,  $P = 0.0003$ ,  $df = 2,72$ ), and cage treatment ( $F = 2.92$ ,  $P = 0.0399$ ,  $df = 3,72$ ) and by the interaction between branch and cage treatment ( $F = 2.75$ ,  $P < 0.0001$ ,  $df = 36,72$ ).

The longest lengths of succulent, green new growth produced by the treatment

branches during this study were found between 1 and 4 months after release (Figure 2c).

During this period the length of new growth was significantly affected by the main effects of branch ( $F = 7.97$ ,  $P < 0.0001$ ,  $df = 12,72$ ) and sample date ( $F = 7.55$ ,  $P = 0.0011$ ,  $df = 2,72$ ) and the interaction of cage treatment with both branch ( $F = 2.48$ ,  $P = 0.0005$ ,  $df = 36,72$ ) and sample date ( $F = 4.28$ ,  $P = 0.0010$ ,  $df = 6,72$ ). One month after release the length of new growth was shortest in the release cage treatment, highest in the exclusion and no cage treatments, and intermediate in the open cages. The length of new growth was longest in the release cage treatment four months after release, although there was little variation among the cage treatments at this time.

### Field Study 3: Predator Survival, Reproduction, and Impact in Sleeve Cages

*Sasajiscymnus tsugae* adults survived for 4 weeks inside the inclusion cages, although the number of live beetles recovered began to decrease sharply at week 3 (Figure 3a). This corresponds with the first signs of predator reproduction inside the sleeve cages, and as the number of *S. tsugae* progeny present increased the number of adults decreased. Dead adult predators were never found inside the inclusion cages indicating that missing beetles may have escaped from the sleeves.

The impact of the adult *S. tsugae* and their progeny on adelgid populations inside the inclusion cages is illustrated in Figure 3b. Prior to predator release the density of hemlock woolly adelgid ovisacs did not differ significantly among the trees ( $F = 1.36$ ,  $P = 0.3802$ ,  $df = 2,3$ ) or treatment branches ( $F = 0.86$ ,  $P = 0.5071$ ,  $df = 2,3$ ). After release, the density of adelgid ovisacs was significantly affected by cage treatment ( $F = 47.51$ ,  $P < 0.0001$ ,  $df =$

2,24) and the interaction between tree and cage treatment ( $F = 4.02$ ,  $P = 0.0124$ ,  $df = 4,24$ ). Following an initial decrease inside inclusion cages exposed to the predator, the density of ovisacs remained low and relatively constant compared to the other treatments. In the exclusion cages that were not exposed to *S. tsugae* the density of adelgid ovisacs increased throughout the study, while ovisac density in the no cage treatment fluctuated but was the same at the beginning and end of the experiment.

## DISCUSSION

### *S. tsugae* Reproduction Inside Release Cages

The primary objective of the three field studies presented here was to determine if *S. tsugae* will mate and oviposit in field cages when the predator is initially confined to HWA infested branches using mesh sleeves. There are concerns that the placement of predators inside cages on host plants may be detrimental to the fitness of some species of natural enemies and prevent establishment (Van Driesche 1993). The cages may become too hot, too wet, or prey may become too scarce and the biological control agents may die. Other natural enemies simply will not mate and oviposit inside the cages. Previous studies of *S. tsugae* reproduction inside field cages suggested that the latter may be true for this beetle. The predator failed to reproduce inside mesh sleeves containing 2 (1 female and 1 male) or 3 (2 females and 1 male) adult beetles placed on infested eastern hemlocks in Massachusetts (Butin et al. 2003) and Georgia (Asaro et al 2005), respectively. However, *S. tsugae* mating and oviposition might not have occurred in these studies because the small number of adult beetles, in relatively large sleeve cages, may not have been able to easily locate and



recognize one another for mating. Chemoreception in this species as it relates to host and mate finding has been determined to be predominantly tactile (Broeckling and Salom 2003). Additionally, the long periods for which adults were confined to the cages (6 weeks in Georgia and 8 weeks in Massachusetts) may have allowed for egg and larval cannibalism.

In contrast, the set of field studies presented here suggests that, when confined to HWA infested hemlock branches for up to 4 weeks using mesh sleeve cages, a population of 15 (10 female and 5 male) *S. tsugae* adults will mate and oviposit inside the cages. In all three experiments, during the first month after adults were introduced to sleeve cages progeny were detected on branches on which adults had been caged (Tables 1 and 3; Figure 3a). These studies also indicate that *S. tsugae* adults are able to survive inside mesh sleeve cages for the same length of time. Adults that were experimentally released were recovered from release branches in Field Study 1 two weeks after release (Table 1), a few were recovered from the release cage treatment in Field Study 2 one month after release (Table 3), and in Field Study 3 40% of released adults were still alive inside sleeve cages after four weeks (Figure 3a).

The pattern of adult recovery from the latter study is of particular interest. One hundred percent of the adults released at the beginning of the study were recovered from predator inclusion cages examined at one and two weeks after predator introduction at a time when no predator progeny were present (Figure 3a). It was not until the third and fourth weeks that as the number of progeny detected in the inclusion cages began to increase the rate of adult insect recovery from the cages began to decrease. Because no dead adults were

found inside the cages or on the release branches at weeks 3 and 4, it can be reasonably assumed that the predator adults that were not recovered had escaped. This is interesting because it suggests that *S. tsugae* females may adjust their feeding, oviposition, and dispersal behaviors in the presence of conspecific eggs and larvae. For other coccinellid species it has been noted that females will cease laying eggs and initiate activities associated with dispersal as the number of conspecific eggs and larvae encountered (Hemptinne et al. 1992; Hemptinne and Dixon 1997) or contact with conspecific fecal cues (Agarwala et al. 2003) increases. It is thought that this behavior may have evolved as a mechanism to maximize offspring fitness through reduced intra-specific predation and competition for prey resources (Hodek and Honek 1996). Unfortunately, the genders of the adult beetles recovered from release cages in this study were not determined so it is not known if the missing beetles may have been mostly females that are most likely to disperse in response to conspecifics. Nor was the study designed to directly test the effect of conspecifics on the dispersal tendencies of *S. tsugae* females. However, laboratory and field studies have shown that adults will consume eggs of their own species, and that net egg production of individual females is lower in the presence of conspecifics (Grant et al. 2005; Flowers et al. 2005, 2006).

The amount of time from release to the first indications of oviposition and larval activity did differ between the field studies, suggesting that the micro-environmental effect of mesh sleeve cages on *S. tsugae* reproduction will not be the same in all sites. In Field Study 3, all evidence of mating, oviposition, and immature development was delayed until the third week after release (Figure 3a) while in Field Study 1 these activities were well underway on

release branches two weeks after predator release (Table 1). In Field Study 2, the first samples were not collected from the release cage treatment until 1 month after release, so it is not possible to determine at what point during this 4 week period *S. tsugae* mating and oviposition began. The earlier indications of oviposition and larval development in Field Study 1 may also be the result of females being mated prior to release while those in Field Study 3 may not have mated until after release. Additionally, the quality of the adelgid prey available on hemlock branches in Field Study 3 may have been of sub-optimal quality compared to that in Field Study 1, causing *S. tsugae* females to have fewer resources for oogenesis or to spend more time searching for suitable oviposition sites.

Overall, the number of *S. tsugae* progeny detected in these three field release studies was quite low compared to the reproductive potential that has been documented for similar numbers of adults in the laboratory (Cheah and McClure 1998), mass rearing facilities (Palmer and Sheppard 2002, Conway et al. 2005), and in some field studies (Cheah and McClure 2002). The level of predator reproduction might have been slightly improved if in all three studies an effort had been made to place mesh release cages on branches that receive long periods of sunlight during the day. In Field Study 1, the number of *S. tsugae* eggs detected was 30% higher and the number of adults recovered was 50% greater on release branches exposed to the sun compared to those in the shade. This finding is supported by behavioral studies of the daily and seasonal activity patterns of *S. tsugae* (Flowers et al. 2007). Although oviposition was hard to document due to this species' preference for laying its eggs in concealed locations, *S. tsugae* demonstrated significantly higher levels of activity

at warmer temperatures and during daylight periods for behaviors associated with extensive and intensive searching and feeding.

### *S. tsugae* Dispersal and Establishment

*Sasajiscymnus tsugae* had been operationally released for biological control of HWA at sites close to those where Field Studies 1 and 2 were conducted. On the Pisgah National Forest, one month prior to the start of Field Study 1, the predator was released in high densities in a similar adelgid infested stand of eastern hemlock that was located approximately 5 kilometers from the study site. The Field Study 2 ornamental release site in Ashe County was located approximately 10 km from a hemlock site on the Blue Ridge Parkway where another high density release of the predator had occurred the previous year. It was knowledge of these previous *S. tsugae* releases that necessitated the pre-release branch, crown, and beat sheet sampling for the predator at the study sites. These samplings found no evidence that the predator had dispersed from the operational release sites to the experimental sites used in these studies (Tables 1 - 3).

The post-release dispersal capability of *S. tsugae* has not been well documented. The available evidence for long-range dispersal indicates that the predator does not migrate quickly from points of introduction. Field studies in Connecticut showed that predators failed to disperse from release points along transects to hemlock plots established at distances of 100 and 200 m within the first months following release, although, a few beetles were found at distances of up to 2 km from the release point (Cheah and McClure 2002). It has been suggested that in the short-term, within tree dispersal by *S. tsugae* is mostly into the

upper portions of the crown (Cheah and McClure 2002; Cheah et al. 2005). This was not the case in Field Studies 1 and 2, as predators were not recovered in post-release samples from high in the canopies of the release areas (Tables 2 and 3). In fact, there was very little detectable movement of the predator beyond the release branches after sleeve cages were removed. The only significant dispersal occurred in the Field Study 2 where predators readily moved from second order branches in the release cage treatment to nearby branches in the open and no cage treatments (Table 3).

A secondary objective of these two field studies was to determine if the use of mesh sleeve cages to initially confine *S. tsugae* adults to HWA infested hemlock branches and secure reproduction in the targeted release areas would lead to long-term establishment of the predator in the forest and ornamental environments. While reproduction did occur and adults did survive inside sleeve cages, there is no evidence to suggest that at the end of each study *S. tsugae* had established in either environment. All indications of predator activity had completely disappeared from the study sites within the first few months after the release of beetles in early May. The same result was reported by Casagrande et al. (2002) following May releases of *S. tsugae* adults on eastern hemlocks at an ornamental site in Rhode Island, and by Grant et al. (2005) after April releases of *S. tsugae* eggs on eastern hemlocks in the Great Smoky Mountains National Park. These authors suggested that April and May releases may be too late in the season for *S. tsugae*, and that the late season disappearance of the predator from release sites might be due to mortality or dispersal away from hemlock to locate better prey resources. This is a plausible explanation as from July through October

nutritious adelgid eggs that are critical to *S. tsugae* survival and reproduction are not available. The only HWA life stages available to the predator during this period are aestivating nymphs of the sistens generation, a prey resource that in the laboratory does not support high *S. tsugae* survival except when paired with suitable supplemental food resources (Jetton Chapter II, this volume).

A second, related explanation for the lack of predator establishment in Field Studies 1 and 2 has to do with the health of the eastern hemlocks on which *S. tsugae* was released. Interactions between host plant quality and the health of target prey can significantly affect the success with which biological control agents can colonize an area (Etzel and Legner 1999). Reduced new growth production is one of the primary symptoms of HWA infestation and tree decline in hemlocks (McClure et al. 2001) and, based on declining amounts of succulent green growth produced each season, it appears that the trees in the experiments were declining in health throughout each study (Figures 1c and 2c). Consequently, as hemlocks decline and produce less new growth the size of adelgid infestations and the health of the insects themselves also decline (McClure 1991). This may have created a situation where, after release, the quality of the adelgid prey resource available to *S. tsugae* in each environment was in a constant state of decline, decreasing the predator's fitness and likelihood that it would successfully establish.

It is also possible that *S. tsugae* was present at both field sites throughout the duration of each study, but that more than one or two years time is necessary for the predator to build to population levels detectable with the sampling strategies used. A lack of efficient predator

detection methods is one of the most critical problems currently facing HWA biological control programs (Cheah et al. 2005). Destructive twig sampling and beat sheeting are not very effective for detecting the predator in the forest, nor is it clear what density of beetles needs to be present for detection to occur. Furthermore, laboratory and field studies have demonstrated that this coccinellid does not have a strong numerical response to HWA density (Butin et al. 2003), and in both field studies presented here the overall number of adult insects released was relatively small. As a general rule it takes between six and ten prey generations, three to five years in the case of HWA, for a classical biological control agent to become established at detectable densities and begin to reduce target pest populations (DeBach and Bartlett 1964). For predatory insects this may take even longer as most have intrinsically low rates of increase compared to parasitoids (Etzel and Legner 1999). However, because HWA can fully infest the healthiest hemlocks and kill them within 4 or 5 years, even if *S. tsugae* is present for several years after release it may never increase its population to a level where it can control the adelgid before trees have declined beyond the point of recovery.

#### *S. tsugae* Impact on the Hemlock Woolly Adelgid

In many of the hemlock forests of the eastern U.S. where free releases of *S. tsugae* have occurred the predator is considered established (Cheah et al. 2004; Blumenthal and Werner 2005), although recoveries have been very sporadic (Cheah et al. 2005). As mentioned earlier, this could be due to a lack of appropriate sampling techniques or the beetles being present in low densities and difficult to detect. At times, hemlock health has

improved and HWA populations have declined in some stands where *S. tsugae* has been introduced when compared to control areas where releases have not occurred (Cheah et al. 2005). But, the lack of predator recovery makes it difficult to attribute these observations to predation by *S. tsugae*. Further complicating the situation is that severe winter cold can significantly reduce adelgid abundance and rainy spring and summer seasons can result in improved tree health independent of attempts to control the adelgid (Cheah and McClure 2002).

While free release studies may be a good way to evaluate the ability of an introduced natural enemy to successfully colonize a new environment, they are likely not appropriate to determine if a predator or parasitoid has the potential to control a target pest's population. In the case of HWA in particular, by the time a predator such as *S. tsugae* has become established and begun to have a detectable impact on the pest, hemlocks will likely have declined beyond the point where they will support healthy adelgid populations and where predators will be retained in the stand. A better method to evaluate potential for biological control is to use predator exclusion or inclusion techniques (Luck et al. 1988). Several studies have used this experimental approach to evaluate the effectiveness of natural enemies to control HWA (McClure et al. 2000; Wallace and Hain 2000; Butin et al. 2003; Asaro et al. 2005; Grant et al. 2005; Lamb et al. 2005, 2006), and several have indicated that *S. tsugae* can have a significant local (within cages or on release branches) impact on HWA density (McClure et al. 2000; Asaro et al. 2005; Grant et al. 2005). McClure et al. (2000) noted that the use of sleeve cages may have artificially improved adelgid survival in their study and



positively biased their results towards the biocontrol potential of *S. tsugae*. Wallace and Hain (2000) did not find a significant effect of sleeve cages on HWA survival or population density, therefore field cages of the same material, design, and construction were utilized in the field studies presented here.

Field Studies 2 and 3 further demonstrate the potential of *S. tsugae* to have a significant local impact on HWA populations at the point of introduction. In Field Study 2, where the predator was initially confined to the release cage treatment for two weeks before the cages were removed to facilitate the dispersal of the adults and their progeny, the density of adelgid ovisacs and aestivating nymphs was lowest on branches of the release cage treatment during the period of peak post-release abundance for each adelgid life stage (Figure 2a,b). Because there was still evidence of predator activity on release branches 1, 2, and 4 months after release (Table 3), the lower density of aestivating nymphs can be at least partially attributed to predation by *S. tsugae*. Lower adelgid ovisac density on the release branches is not directly attributable to predation by *S. tsugae* because no evidence of predator activity remained in the study 10 and 12 months after release. Although this could be a lingering effect of predator feeding in the first months after release, similar responses of ovisac density in the open and no cage treatments 12 months after release suggest the presence of an unidentified mortality agent affecting those adelgids not protected by the exclusion cages. However, drawing any meaningful conclusions about the biological control potential of *S. tsugae* from this study is tenuous. Although the data analysis indicated a significant effect of the cage treatment, these results are confounded by complicated

interactions among main effects and the fact that the densities of adelgid ovisacs and nymphs were more strongly influenced by the particular first order branch on which they occurred and the date on which field samples were collected.

In Field Study 3, where predators remained in sleeve cages for the duration of the study, the density of adelgid ovisacs was lower during all sample weeks on hemlock branches in the predator inclusion treatment compared to branches caged without predators or those with no cages (Figure 3b). The largest decrease in ovisac density in the inclusion cages occurred during the first week after release, and thereafter remained fairly constant even during the third and fourth weeks when both *S. tsugae* adults and their progeny were present in the inclusion cages (Figure 3a). Adelgid ovisac density on hemlock branches that were not caged or were caged without predators did not change during the first week after release, then increased and fluctuated similarly for the duration of the study. This indicates that the mesh sleeve cages did not affected the survival of HWA on caged branches and the significant reduction in ovisac density in the inclusion treatment was the result of predation by *S. tsugae*.

## CONCLUSIONS

The field studies reported here have demonstrated that *S. tsugae* will mate, oviposit, and survive for a period of up to one month at forest and ornamental sites in western North Carolina when confined to HWA infested hemlock branches at the density and sex ratio used for reproduction of the predator in mass rearing facilities. These findings are significant because they suggest that confined field releases may be a suitable alternative to high density

single-point releases for operational introduction of the predator for biological control of the adelgid in hemlock forests of North Carolina and, perhaps, throughout the eastern United States. However, similar to most free releases that have occurred, after *S. tsugae* was released from confinement longer-term establishment was not documented.

At this time, it remains unclear whether or not classical biological control will remain a viable component of integrated management approaches for reducing the impact of HWA in eastern North America. Several species of predators have been released in hemlock forests in the eastern US, but as the adelgid continues to expand its range and trees continue to decline there is little evidence that any are having a meaningful impact. Admittedly, for most of these HWA biological control agents the numbers that have been released are very small compared to the millions for *S. tsugae*, and it is very unlikely that any one is going to have much impact on its own. Each is likely to have its greatest effect at a particular time of year on a particular adelgid life stage that is present at a certain density. Given *S. tsugae*'s lack of a strong numerical response to adelgid density and a typically localized predatory impact, it may be best suited to low density adelgid populations early in the development of an infestation. This may account for its importance as an adelgid predator in Japan where characteristically low adelgid density is strongly influenced by host tree resistance and a multitude of natural enemies.

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Table 1. Total bi-weekly counts of *S. tsugae* life stages on 10 cm twig samples collected from release branches following low density release in a forest environment.

Weeks After Predator Release	Cage Location	Eggs	Larvae	Larval Exuviae	Pupae	Adults
Pre-Release	Sun	0	0	0	0	0
	Shade	0	0	0	0	0
2 Weeks	Sun	97	6	1	0	32
	Shade	73	5	0	0	15
4, 6, & 8 Weeks	Sun	0	0	0	0	0
	Shade	0	0	0	0	0

Table 2. Total monthly counts of *S. tsugae* life stages from 10 cm twig crown samples and beat sheet samples collected from release trees over a two year period following low density release in a forest environment.

Months After Predator Release	Eggs	Larvae	Larval Exuviae	Pupae	Adults
<u>Crown Samples</u>					
Pre-Release	0	0	0	0	0
1 Month	0	1	1	0	0
2, 4, 10, 11, 12, 15, 23, & 24 Months	0	0	0	0	0
<u>Beat Sheeting</u>					
Pre-Release, 1, 2, 4, 10, 11, 12, 15, 23, & 24 Months	n/a <sup>1</sup>	0	n/a	n/a	0

<sup>1</sup>n/a, not applicable, beat sheet sampling was for detection of larvae and adults only.

Table 3. Total monthly counts of *S. tsugae* life stages on 10 cm twig samples collected from cage treatments and throughout the crown over a one year period following low density release in an ornamental environment.

Months After Predator Release	Cage Treatment	Eggs	Larvae	Larval Exuviae	Pupae	Adults
<u>Cage Samples</u>						
Pre-Release		0	0	0	0	0
1 Month	Release	0	26	7	0	4
	Exclusion	0	0	0	0	0
	Open	0	0	0	0	0
	No Cage	0	0	0	0	0
2 Months	Release	0	3	16	3	0
	Exclusion	0	0	0	0	0
	Open	0	1	2	1	0
	No Cage	0	2	4	1	0
4 Months	Release	0	0	4	0	0
	Exclusion	0	0	0	0	0
	Open	0	0	0	0	0
	No Cage	0	0	4	0	0
10 & 12 Months	Release	0	0	0	0	0
	Exclusion	0	0	0	0	0
	Open	0	0	0	0	0
	No Cage	0	0	0	0	0
<u>Crown Samples</u>						
Pre-Release, 1, 2, 4, 10, & 12 Months		0	0	0	0	0

## FIGURE CAPTIONS

Figure 1. The mean seasonal density of (a) ovipositing hemlock woolly adelgid adults and (b) aestivating adelgid sistens nymphs and (c) seasonal change in the production of new, succulent green growth by eastern hemlock following the low density release of *S. tsugae* in a forest environment.

Figure 2. The mean seasonal density of (a) ovipositing hemlock woolly adelgid adults and (b) aestivating adelgid sistens nymphs and (c) seasonal change in the production of new, succulent green growth by eastern hemlock following the low density release of *S. tsugae* in an ornamental environment.

Figure 3. (a) Percentage of *S. tsugae* adults recovered and mean number of *S. tsugae* progeny (eggs, larvae, pupae) found in predator inclusion cages at each sample period. (b) Density of ovipositing hemlock woolly adelgid adults in each cage treatment at each sample period.

Figure 1.

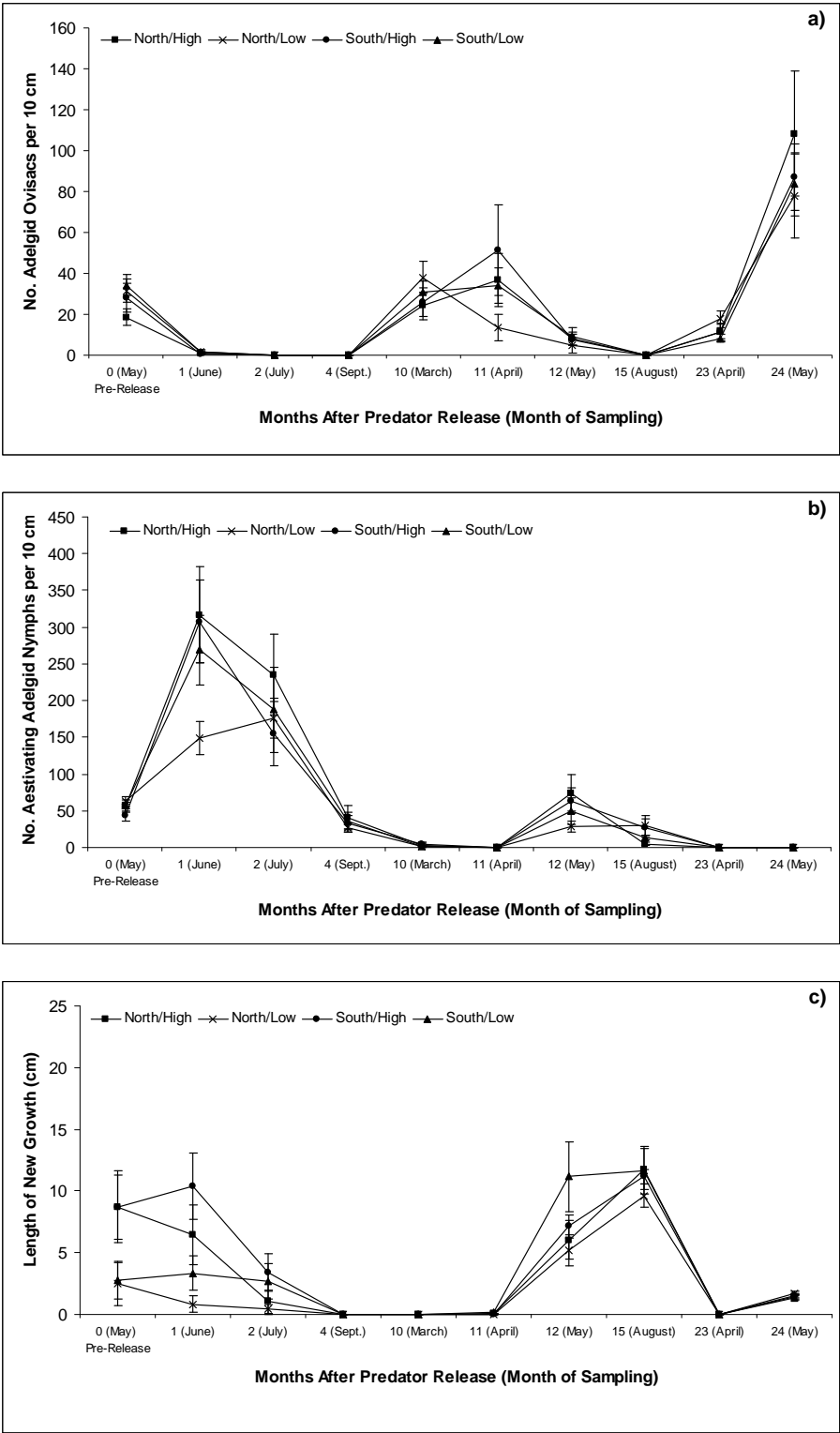


Figure 2.

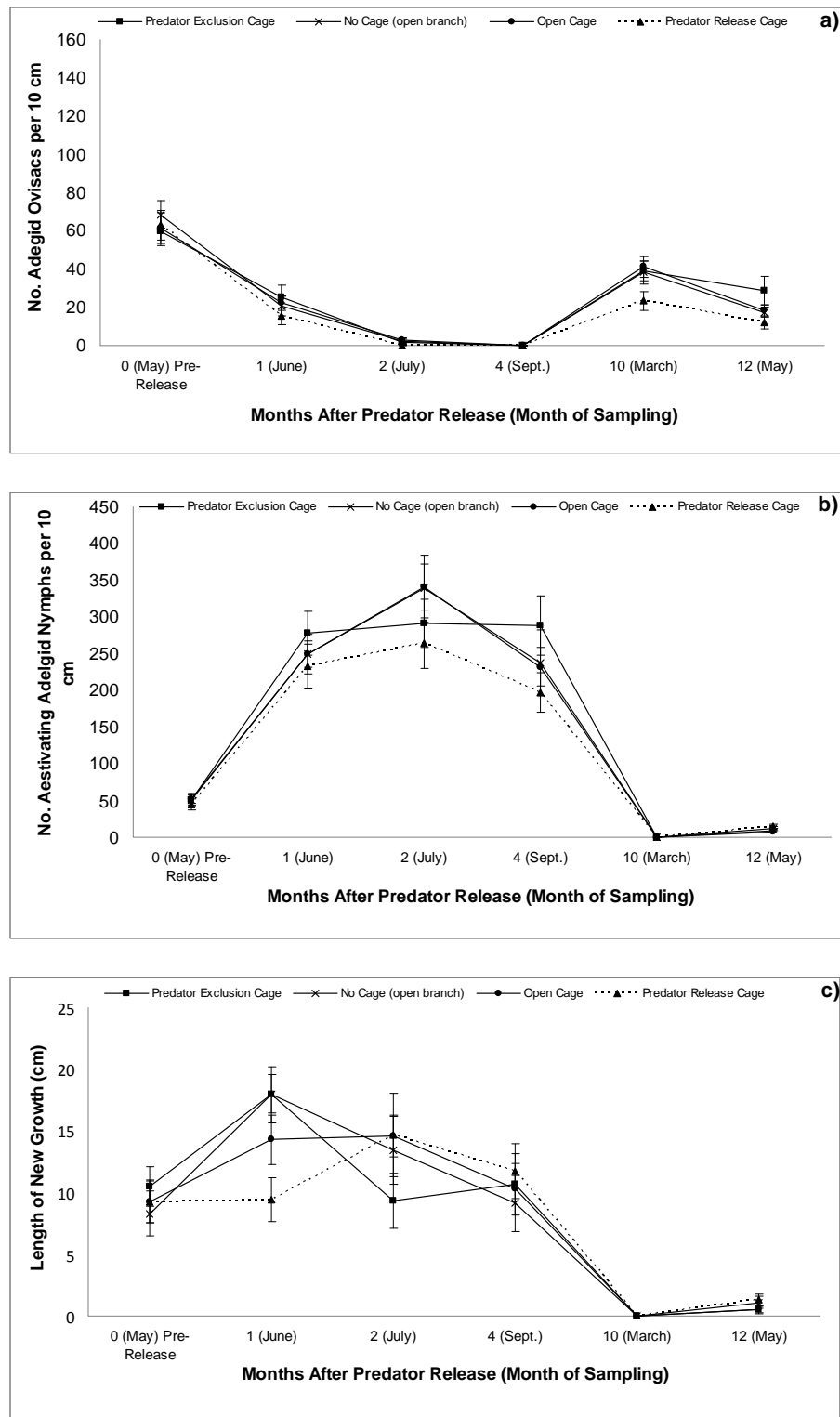
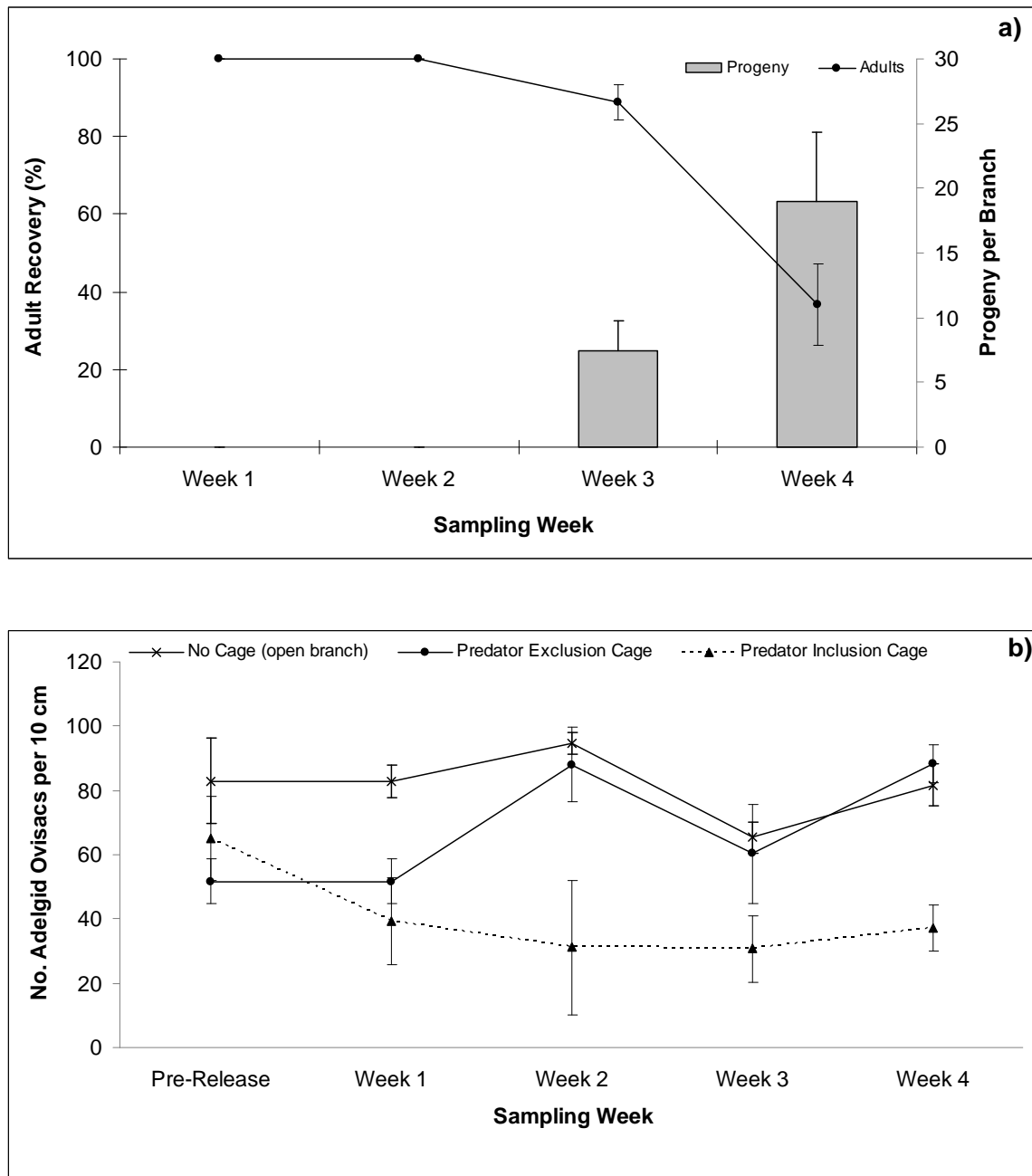


Figure 3.



## CHAPTER IV

### **Initial Infestation Rate and Fecundity of the Hemlock Woolly Adelgid (Hemiptera: Adelgidae) on Three Species of North American Hemlock (*Tsuga* spp.)**

(A version of this Chapter is In Press with the *Journal of Entomological Science* at this time)

#### **ABSTRACT**

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an exotic pest that is causing widespread mortality among hemlocks (*Tsuga* spp.) in the eastern United States. *Tsuga* species that occur in Asia and the Pacific Northwest are putatively resistant to adelgid infestation while those in the eastern U.S. are considered generally susceptible. The objectives of this study were to determine if a method for artificially inoculating hemlock seedlings with the adelgid is successful at creating infestations of variable density, and if there are differences in initial adelgid infestation rate and fecundity among three species of North American hemlock: eastern hemlock (*T. canadensis* (L.) Carrière) and Carolina hemlock (*T. caroliniana* Engelmann), which occur in the east, and western hemlock (*T. heterophylla* Sargent), which occurs in the west. Seedlings received one of two inoculation treatments in a climate-controlled greenhouse; either low inoculation (50 adelgid egg masses per seedling) or high inoculation (100 adelgid egg masses per seedling). Seedlings of all three species were successfully infested with hemlock woolly adelgid. However, populations of variable density did not develop as a result of initial inoculation density. There was variation among hemlock species with respect to woolly adelgid infestation rate and fecundity. The number of live adelgids feeding on eastern hemlock seedlings was significantly higher than on Carolina and western hemlocks. Adelgids that fed



successfully on Carolina hemlock had significantly higher fecundity than conspecifics on either eastern or western hemlock, although this varied depending on inoculation treatment.

## INTRODUCTION

The hemlocks (*Tsuga* spp.) are slow-growing, long-lived trees and are among the most shade tolerant and drought susceptible in the Pinaceae. The genus consists of nine species, all restricted to regions with maritime to subcontinental climates where rainfall is plentiful throughout the growing season. *Tsuga* worldwide distribution is discontinuous with hemlock species occurring in three main regions (Farjon 1990). Two concentrations are found in North America. One is in the coastal mountains and islands of the Pacific Northwest extending into the northern Rocky and Sierra Nevada Mountains where western hemlock, *T. heterophylla* Sargent, and mountain hemlock, *T. mertensiana* Carrière, are found. The second is in the East, extending from Nova Scotia south to Alabama and west into Minnesota, where eastern hemlock, *T. canadensis* (L.) Carrière, and Carolina hemlock, *T. caroliniana* Engelmann, occur, although Carolina hemlock is restricted to a relatively small number of populations found in the southern Appalachian Mountains. The third concentration of *Tsuga* is in eastern Asia, with two species occurring in Japan, *T. sieboldii* Carrière and *T. diversifolia* Masters, and three present in China and the Himalayan Range, *T. chinensis* (Franchet) Pritzel in Diels, *T. dumosa* (D Don) Eichler, and *T. forrestii* Downie.

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an exotic pest in eastern North America, where it has caused widespread mortality among hemlocks. It was first described in the Pacific Northwest on specimens

collected from western hemlock (Annand 1924). HWA was first observed in the eastern U.S. (Richmond, Virginia) in 1951 (Stoetzel 2002) and was likely introduced to the area on exotic ornamental hemlocks as early as 1920. The adelgid was originally considered no more than an annoying ornamental pest in the East and was easily controlled with insecticides. However, the severity of damage and rate of dispersal associated with HWA increased dramatically in the early 1980s when it reached the southern Appalachian Mountains (Souto et al. 1996). The adelgid now occurs in 16 eastern states from New England to Georgia and infests approximately 50% of the eastern hemlock ecosystem, where it can kill trees in as little as four years (McClure et al. 2003; Cheah et al. 2004).

The hemlock woolly adelgid has a complex polymorphic life cycle that includes three generations per year, each developing through an egg stage, four nymphal instars, and an adult stage (McClure 1989). Two generations are wingless parthenogenetic forms called the sistens (overwintering generation) and progrediens (spring generation), each completing their life cycle on the hemlock host. The third is the winged sexual generation called the sexupara that disperses but lacks a suitable host in North America (McClure 1987). The sistens reach maturity in February and, from March to May, produce white, woolly masses into which they lay eggs that will develop into nymphs of the sexupara and progrediens generations. Progrediens first instar nymphs, called crawlers, remain on hemlock, settle at the base of needles, and feed from April through late June when they mature and produce woolly ovisacs containing eggs of the sistens generation. The sistens crawlers hatch, settle on hemlock and feed for a few days before entering aestivation for the duration of the summer (Salom et al.

2001). In October, they begin feeding again until maturation in February (McClure 1989).

Due to logistical, economic, and ecological concerns over the use of chemical pesticides in forest settings, HWA management efforts remain focused on biological control. Due to a lack of effective native or naturalized adelgid predators in the eastern U.S. (Wallace and Hain 2000), emphasis has been placed on a classical biological control approach (Cheah et al. 2004). To date, a number of promising predators of the hemlock woolly adelgid have been identified and imported into the U.S. for evaluation and several have been approved for release. Additionally, pathogenic fungi have also been identified that show promise for controlling HWA (Costa et al. 2005). *Sasajiscymnus tsugae* Sasaji & McClure (Coleoptera: Coccinellidae), the first adelgid predator described and imported from Japan for evaluation (Sasaji and McClure 1997; Cheah and McClure 1998; Cheah and McClure 2000), is currently being mass-produced and has been released over the entire eastern North American range of HWA. Research on another predator from British Columbia, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), is in the early stages of experimental release (Lamb et al. 2005; Flowers et al. 2006). Four *Scymnus* spp. have been imported into the U.S. from China for evaluation (Cheah et al. 2004), and one, *S. sinuanodulus* (Coleoptera: Coccinellidae), has shown promise for HWA biological control.

It is suggested that a complex of natural enemies, such as those described above, needs to be established if classical biological control is to be successful in regulating adelgid populations below damaging levels in eastern North America (Cheah et al. 2004). In general, it takes between 6 and 10 prey generations (3 to 6 years in the case of HWA) for populations

of introduced natural enemies to become established and offer effective control of pest populations (DeBach 1964.). Because HWA can significantly reduce tree vigor or even kill trees in as little as four years (McClure et al. 2003), hemlocks in predator release areas will likely be in a severe state of decline or will die before natural enemies become established.

One option is to target predator releases in hemlock populations with low adelgid susceptibility. Trees in these areas would be expected to remain healthy and support low-density adelgid populations for an extended period of time and increase the likelihood of successful predator establishment. It is generally accepted that such biological control-host resistance interactions account for the innocuous habit of HWA on hemlock species growing in western North America and Asia (McClure et al. 2003). It is unknown if there are populations of eastern or Carolina hemlock that harbor resistance to HWA, although, there have been reports of trees surviving for ten or more years despite adelgid infestation (Souto et al. 1996; Orwig 2002). If this phenomenon is attributable to genetically controlled resistance traits, better site conditions, or some combination of the two remains unclear. However, the existence of these trees, prospects for their propagation (Jetton et al. 2005), and current gene conservation efforts (Camcore 2005; Tighe et al. 2005) are providing material for better understanding these interactions.

The first step to understanding the role of genetic by environmental interaction in determining hemlock susceptibility to HWA is to evaluate interactions between the adelgid and various hemlock genotypes under homogenous site conditions. This can be accomplished through field studies in the context of common garden or provenance-progeny

trials or in the greenhouse. Because HWA shows no preference among hemlock age classes (Orwig and Foster 1998), greenhouse studies with seedlings seem appropriate. Successful greenhouse studies require artificial inoculation techniques that are reliable at initiating adelgid infestations and can effectively and consistently rank the relative susceptibilities of hemlock species, families, hybrids, or clones to HWA. This paper reports on the development of a bioassay screening protocol for the greenhouse designed to achieve these goals. The objectives of the study were to 1) test a method for artificially infesting hemlocks with hemlock woolly adelgid populations of variable density and 2) use this method to detect differences in initial infestation rate and adelgid fecundity among three North American hemlock species.

## **MATERIALS AND METHODS**

### **Hemlock Seedlings and Hemlock Woolly Adelgids**

Four-year-old eastern hemlock and two-year-old Carolina and western hemlock seedlings were used in this study. Eastern hemlocks were purchased as bulk 2-0 seedlings from Strathmeyer Forests, Inc. (Dover, PA) in October 2004. Upon receipt, the seedlings were planted in 8-liter Treepots™ (Stuewe & Sons, Inc., Corvallis, OR) with a commercial soil media (Fafard® Brand 3-B Bark Mix, Conrad Fafard, Inc, Agawam, MA) consisting of 45% sphagnum peat moss, 25% composted pine bark, 15% perlite, and 15% vermiculite. Carolina and western hemlocks were grown from bulk seedlots as part of a germination trial conducted by Camcore (International Tree Conservation & Domestication Program), Department of Forestry and Environmental Resources, N.C. State University (Raleigh, NC),

in February 2004 (see Tighe et al. 2005 for details on stratification and germination treatments). Carolina hemlock seed was provided by Camcore and western hemlock seed was obtained from Western Forest Products, Inc. (Saanichton, British Columbia). Following germination, seedlings of both species were sown in Ray Leach SC-10 Cone-tainers™ and grown for one year before transplant into 8-liter Treepots™ using the same commercial soil media described above. Seedlings of all three species were maintained in the N.C. State University Method Road Greenhouse Complex for two growing seasons.

One month prior to the current study, the seedlings were moved into an air conditioned greenhouse where they remained for the duration of the infestation experiment. A cooling system maintained the daily air temperature between 23 and 30 °C, the night temperature between 18 and 23 °C, and an average relative humidity of 55%. The doors were sealed with masking tape and the exhaust vent of the air-conditioning unit was fitted with a High Efficiency Particulate Air (HEPA) filter to prevent the escape of hemlock woolly adelgid eggs and crawlers.

Hemlock woolly adelgid egg masses were collected from naturally occurring adelgid populations in Ashe County, North Carolina. Hemlock branches infested with ovipositing adults of the sistens (overwintering) generation were cut from 10 trees in March 2006 and transported to Raleigh, NC, where they were placed in water and stored in a rearing room at the N.C. State University Insectary (18°C; 50% RH; 12:12 L:D). Adelgid egg masses present on these branches contained eggs of the progrediens (spring) generation.

### Inoculation Treatments and Experimental Design

On 28 March 2006, hemlock twigs with adelgid woolly masses containing progrediens eggs were cut from the field collected branches and attached using paper-clips to the underside of hemlock seedling branch tips. The twigs remained attached to seedlings for two weeks, allowing time for eggs to hatch and progrediens crawlers to settle on the hemlocks and begin feeding. Seedlings received one of two inoculation treatments: either low inoculation (50 egg masses) or high inoculation (100 egg masses). At this time, a subset of 100 woolly egg masses was destructively sampled to determine the number of progrediens eggs contained within egg masses on the field collected material. On average, each woolly mass contained 13 eggs, meaning seedlings in the low and high inoculation treatments received approximately 650 and 1300 adelgid eggs, respectively.

The experiment was a split-plot design with two replications. Each replication was divided into two inoculation treatments (whole plots), low and high. Each whole plot contained a total of 12 randomly distributed hemlock seedlings (4 Carolina hemlock, 4 eastern hemlock, and 4 western hemlock), each receiving the assigned inoculation treatment. A total of 16 seedlings per hemlock species were included in the experiment. The infestation experiment lasted 10 weeks (28 March 2006 to 6 June 2006) and encompassed the developmental cycle of the progrediens generation from the egg to ovipositing adult. After this period, the following data was recorded: 1) seedling height (cm), 2) infestation rate determined by counting the number of white, woolly masses containing live progrediens adults per centimeter of seedling height, and 3) adelgid fecundity estimated by counting the number of sistens eggs in 10 woolly masses per seedling.

### Statistical Analysis

Analysis of variance was performed using the General Linear Model procedure (PROC GLM) of SAS version 9.1 (SAS Institute 2003). The main effects of hemlock species, inoculation treatment, and the species by inoculation treatment interaction were tested for infestation rate. The main effects of hemlock species, inoculation treatment, species by inoculation treatment interaction, and the covariate seedling height were tested for adelgid fecundity. The data were analyzed as non-transformed and square root transformed for both infestation variables. Results from the analysis of the transformed and non-transformed data were similar, therefore all results presented are based on non-transformed data. All pairwise mean comparisons were analyzed by the Tukey-Kramer Multiple Comparison Procedure. All means are reported as least squares means.

### **RESULTS**

Adelgid infestation rate was significantly affected by the main effect of hemlock species but not inoculation treatment or the interaction (Table 1). The total number of live progrediens adults per centimeter of seedling height on eastern hemlock was significantly and nearly four fold higher than observed on Carolina hemlock or western hemlock while infestation rates on the latter two species were statistically similar (Figure. 1a). Although not significant, there were slightly more live HWA adults per centimeter height on seedlings receiving the high compared to low inoculation treatment (Figure 2a). Likewise, infestation rates on eastern hemlocks receiving high inoculation were greater than those in the low inoculation treatment. This trend was the reverse for Carolina and western hemlocks (Figure 3a).



Adelgid fecundity was significantly affected by the main effects of hemlock species and inoculation treatment but not by the covariate seedling height (Table 1). Among hemlock species, HWA oviposition rates were highest on Carolina hemlock followed by eastern hemlock and then western hemlock (Figure 1b). Between the inoculation treatments, adelgid oviposition trends were opposite of that of infestation rate with more eggs laid following low compared to high inoculation (Figure 2b).

There was a significant interaction detected between hemlock species and inoculation treatment for adelgid fecundity (Table 1). Among adelgids reared on Carolina hemlock, the number of sistens eggs per egg mass was significantly higher under the low inoculation level compared with the high inoculation level. This trend was opposite but non-significant for adelgids reared on eastern and western hemlocks (Figure 3b).

## **DISCUSSION**

The bioassay method used in this study was successful for artificially inoculating hemlock seedlings with the hemlock woolly adelgid. Previous studies have reported infestation success with similar techniques (McClure 1992; Salom et al. 2001; Pontius et al. 2006), although one reported a one-year time lag between HWA inoculation and heavy infestation rates on eastern hemlock seedlings (Salom et al. 2002). The inoculation treatments, however, failed to produce HWA populations of variable density on hemlock seedlings. This was despite the fact that, on average, 1300 adelgid eggs were placed on high inoculation seedlings and only 650 on low inoculation seedlings. Similar results were reported by Butin et al. (2007) where increasing the number of HWA infested branches

attached to seedlings did not increase infestation rates on artificially inoculated hemlocks. Such results may be explained by an over-crowding effect of adelgid crawlers on seedlings receiving higher inoculation treatments. Although detailed studies on HWA behavior are lacking, it is plausible to conclude that first instar adelgid nymphs may disperse to avoid over-utilization of the host resource during the initial phases of colonization.

The bioassay method also detected differences in adelgid infestation rate and fecundity among the three hemlock species tested. Variation in infestation rate among the species may be the result of either differential susceptibility to HWA or differential seedling age. If differential susceptibility explains the observed infestation patterns, the difference in HWA infestation rate between eastern and western hemlock is not surprising. Western hemlock is a putatively adelgid resistant *Tsuga* species and has been demonstrated to support significantly lower HWA populations than eastern hemlock on seedlings planted in Connecticut (McClure 1992). Likewise, the two species have been shown to differ in chemical characteristics that may be linked to adelgid susceptibility (Lagalante and Montgomery 2003; Pontius et al. 2006). However, hemlock species is confounded by seedling age in this study. The eastern hemlock seedlings were 4-years-old while both the western and Carolina hemlocks were 2-years-old. So, it cannot be ignored that differential seedling age may also explain some or all of the adelgid infestation pattern observed on eastern hemlock compared to the other two species.

Mausel (2005) reported much higher HWA infestations on western hemlocks compared to eastern hemlocks on trees growing in an arboretum in Seattle, WA and

cautioned against considering western hemlock as adelgid resistant. However, recent molecular evidence from mitochondrial DNA analysis of HWA populations around the world has indicated that the adelgids present in the eastern United States originated in Japan while those in the Pacific Northwest likely originated in China or may even be native (Havill et al. 2006). The diverse origins of adelgids in North America suggest differing evolutionary history between HWA populations in the eastern and western U.S., and may explain the differential response of western hemlock to adelgid infestation in these two studies. Therefore, the potential for resistance of western hemlock to HWA in the eastern United States cannot be ruled out, and the species may be a valuable source of genes for reduced adelgid susceptibility in hybrid breeding programs.

The observed response of adelgid infestation to Carolina hemlock in the current study was not expected. Carolina hemlock is generally believed to be as highly susceptible to HWA as eastern hemlock, a conclusion supported by McClure (1992) who reported similar infestation levels on the two species. We report very low adelgid infestation rates (less than one adelgid per centimeter of seedling height) on 2-year-old Carolina hemlock seedlings that are statistically similar to those on putatively HWA resistant western hemlocks of the same age. This suggests that Carolina hemlock may harbor some level of resistance or tolerance to adelgid attack, at the very least during the early stages of infestation. Interestingly, research has demonstrated that there is a high degree of variability in HWA infestation rates among seedlings from different Carolina hemlock seed sources, where in similar greenhouse bioassays initial adelgid infestation rates ranged from very low among seedlings from some

populations to very high in others (Camcore 2006). Additionally, phylogenetic analysis of *Tsuga* has indicated that Carolina hemlock is most closely related to the putatively adelgid resistant Asia hemlock species (Vining 1999). This all may help to explain why, based on the authors' own field experiences and anecdotal evidence gathered from foresters and resource managers in the southern Appalachian Mountains, adelgid infestations on Carolina hemlock grow more gradually and trees decline more slowly when compared with eastern hemlock.

The high fecundity of hemlock woolly adelgids on Carolina hemlock seedlings in the low inoculation treatment is difficult to explain. One might expect this result if the density of adelgids on seedlings was higher at the high inoculation level and lower at the low level. A higher density of insects might indicate fewer resources (nutrients) are available per individual for egg production. This was not the case in this study. Although not significantly different, the average infestation rate on Carolina hemlocks in the low inoculation treatment was 1.07 adelgids per centimeter of seedling height compared with 0.7 per centimeter at the high inoculation level.

Perhaps in the low inoculation treatment, adelgid crawlers are able to locate foliar sites that are suitable for stylet (piercing-sucking mouthpart) penetration more easily and with minimal competition or interference from their cohorts, thus conserving energy for egg production. In the high inoculation treatment, competition from cohorts would be increased and crawlers might expend more energy locating suitable feedings sites, depleting resources that might otherwise be conserved for egg production as adults.

The ovipositional rate of hemlock woolly adelgids on greenhouse seedlings in this study was much lower than similar values reported in the literature, particularly when compared to studies where seedlings were planted in the field (McClure 1992; Del Tredici and Kitajima 2004; Mausel 2005). This indicates that data collection might have been conducted prior to peak adelgid adult development and egg production, and raises the question of whether adelgid fecundity was actually higher on Carolina hemlock or if HWA is able to develop more quickly on this species. If the latter is true, it suggests that Carolina hemlock, while maybe less susceptible (i.e. supports significantly lower adelgid infestation rates) to initial adelgid infestation compared to eastern hemlock, might also be very suitable for adelgid development and reproduction among adelgids that successfully attack. This may explain, in part, why adelgid infestations appear to grow more gradually on Carolina hemlock and trees decline more slowly following infestation.

## **CONCLUSIONS**

The bioassay protocol used in this study resulted in successful artificial infestation of hemlock seedlings with HWA in the greenhouse, and detected differences in adelgid infestation rate and fecundity among three North American hemlock species. Any conclusions based on this data concerning the HWA susceptibility of eastern hemlock relative to results for western or Carolina hemlock are tenuous due to the confounding effect of seedling age. Similar comparisons between the latter species, however, are not confounded by age and suggest that Carolina hemlock may harbor some tolerance for adelgid attack during the early stages of infestation. This finding is significant because it suggests

that efforts to breed for HWA resistance in this rare species need not focus only on hybridization with putatively adelgid resistant species (Bentz et al. 2002), but that opportunities might exist to capture some level of adelgid resistance through traditional selection and breeding within the pure species. Additionally, this and other studies (Camcore 2006) indicate that populations of Carolina hemlock with increased tolerance to HWA might exist, increasing the probability of saving this rare hemlock species from the onslaught of the adelgid through targeted biological control efforts.

Additional research is needed to further refine the screening protocol described in this study so that the nature and stability of HWA resistance in hemlock species can be better defined. Experiments should attempt to determine how adelgid infestation rate is affected by seedling age, how infestations on younger material compares to that on mature trees, and if infestation studies on intact plants are comparable to those on stem cuttings. Related studies could focus on the effects of HWA natal hosts, adelgid generation, and mesh enclosures on adelgid settlement rate and success. Studies should also evaluate multiple inoculation densities so that adelgid competition for feeding sites and resulting fecundity can be better understood.

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Table 1. Analysis of variance for the total number of live progrediens adults per centimeter of seedling height and number of sistens eggs per progrediens egg mass among three species of hemlock following artificial inoculation with the hemlock woolly adelgid. *F*-values with indicated level of significance are given for both measures of adelgid infestation.

Source of Variation	Degrees of Freedom <sup>1</sup>	Infestation Rate	Adelgid Fecundity
Hemlock Species	2	24.28***	9.40**
Inoculation Treatment	1	0.83 <sup>NS</sup>	5.73*
Species*Treatment	2	1.99 <sup>NS</sup>	12.00***
Seedling Height	1	n/a	0.18 <sup>NS</sup>

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, 0.001, respectively.

<sup>1</sup>Error degrees of freedom for Infestation Rate = 42 and for Adelgid Fecundity = 17.

<sup>n/a</sup> Not used as a covariate in this analysis.

## FIGURE CAPTIONS

Figure 1. Effect of hemlock species on hemlock woolly adelgid a) infestation rate and b) fecundity. Columns with different letters are significantly different at  $\alpha = 0.05$ , Tukey-Kramer Multiple Comparison Procedure.

Figure 2. Effect of inoculation treatment on hemlock woolly adelgid a) infestation rate and b) fecundity.

Figure 3. Effect of hemlock species and inoculation treatment on hemlock woolly adelgid a) infestation rate and b) fecundity. Columns with different letters are significantly different at  $\alpha = 0.05$ , Tukey-Kramer Multiple Comparison Procedure.

Figure 1.

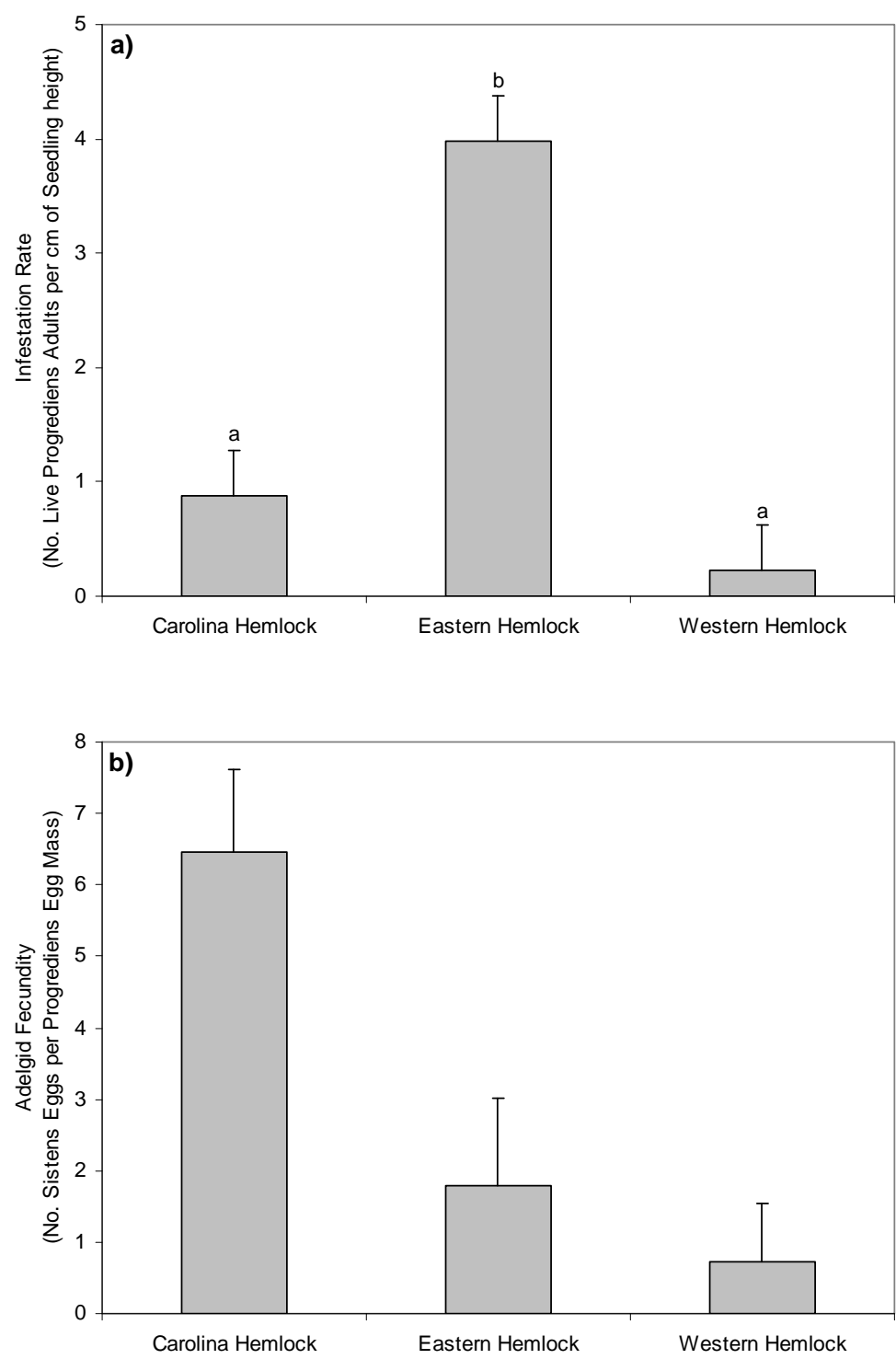


Figure 2.

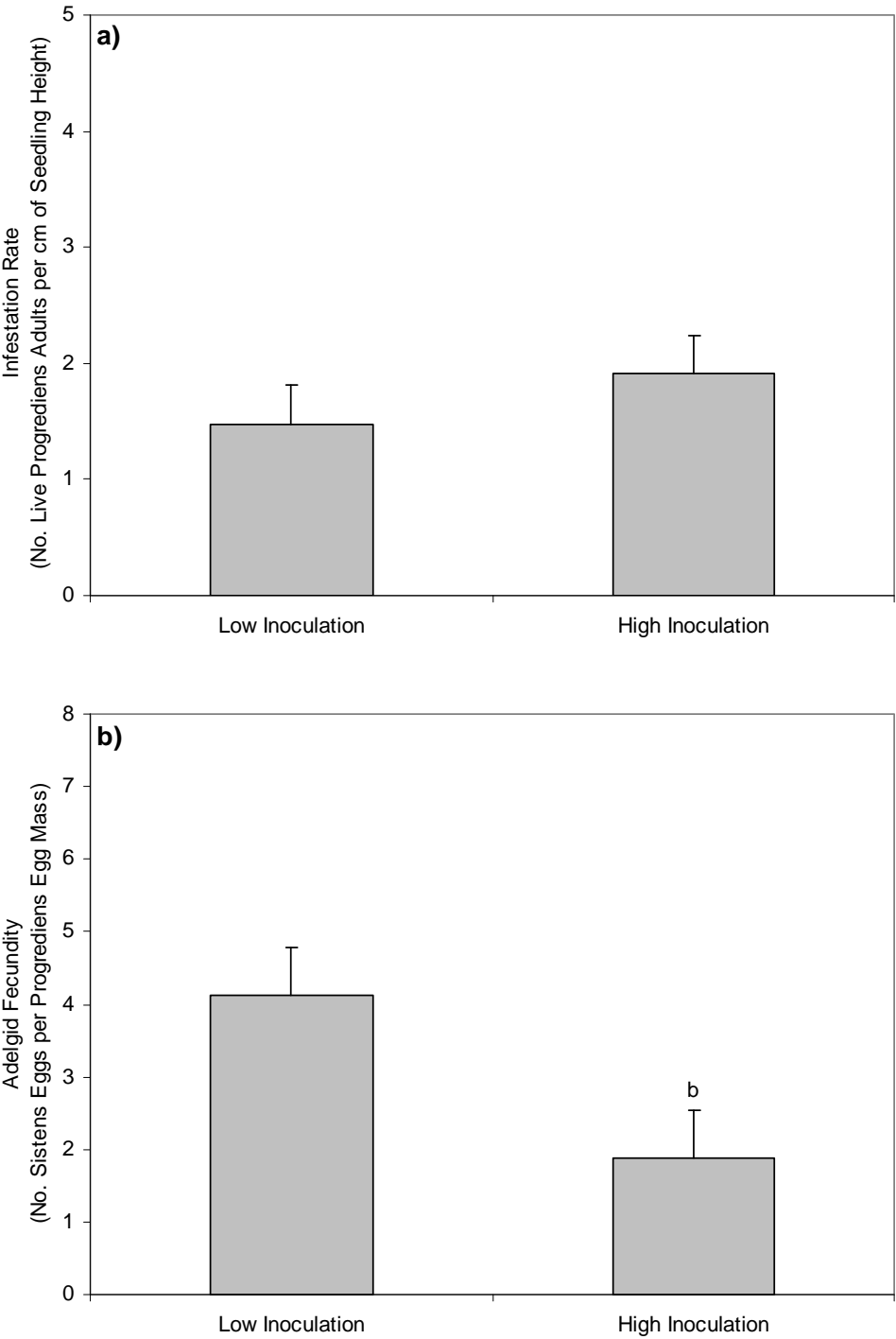
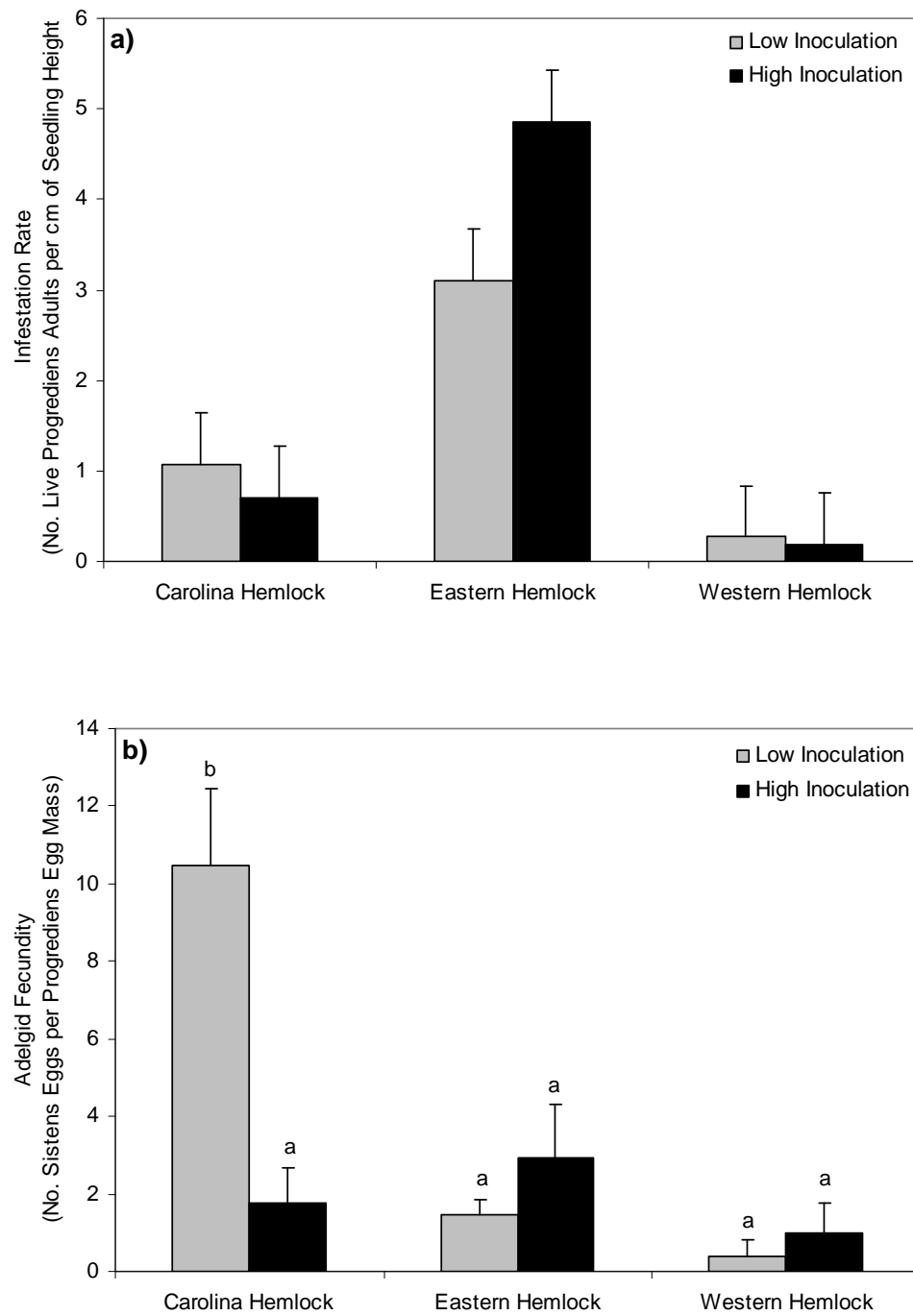


Figure 3.



## CHAPTER V

### **Vegetative Propagation of Mature Eastern (*Tsuga canadensis*) and Carolina (*T. caroliniana*) Hemlocks by Rooted Softwood Cuttings**

(This Chapter was published in *HortScience* 40: 1469-1473 prior to this dissertation)

#### **ABSTRACT**

This study tested the effects of cutting length and auxin (NAA) concentration on adventitious root formation in softwood stem cuttings from mature eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *T. caroliniana* Engelm. Overall rooting percentage (41%) and percent mortality (22%) were higher for eastern hemlock compared with Carolina hemlock (10% rooting and 13% mortality). Rooting percentage of each species responded differently to varying auxin concentrations (0, 1, 2, 4, 8 mM NAA). Maximum rooting (56%) for eastern hemlock occurred at 0 mM NAA; then decreased with increasing auxin concentration. Carolina hemlock rooting percentage increased from the control to a maximum (16%) at 1 mM NAA; then decreased with increasing auxin concentration. For both species, the lowest mortality occurred at the same auxin concentration as maximum rooting. The highest rates of mortality coincided with the same concentrations as the lowest rooting percentages. At all auxin concentrations, eastern hemlock had a higher number of roots and greater total root length relative to Carolina hemlock. Mortality among 6-cm stem cuttings was twice that observed for 3-cm cuttings of both species. However, 6-cm cuttings of eastern hemlock that did form adventitious roots had more roots and longer total root length compared with 3-cm cuttings.



## INTRODUCTION

Recent interest in the vegetative propagation of hemlock (*Tsuga* spp.) by rooted cuttings arises primarily from the threat posed by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera:Adelgidae), on two species: eastern hemlock, *Tsuga canadensis* (L.) Carr, and Carolina hemlock, *T. caroliniana* Engelm. The adelgid is an exotic pest with the potential to cause the extinction of both species. First reported and described by Annand (1924) in the Pacific Northwest from specimens collected on western hemlock, *T. heterophylla* (Raf.) Sarg., this pest is believed to have originated in Asia (Takahashi 1937; McClure 1987). Introduced into the eastern United States (Virginia) on imported nursery stock in the early 1950s, HWA had spread into natural stands by the 1980s. The adelgid has since caused widespread mortality of both eastern and Carolina hemlock throughout 16 eastern states, killing trees in as little as four years (McClure et al. 2001).

The development of techniques for the vegetative propagation of eastern and Carolina hemlock, as well as other *Tsuga* spp., will be of great utility to current and future effort in HWA control and research. Current biological control programs depend on field collection of HWA infested hemlock branches, often from remote sites, for the mass rearing of adelgid predators and this has been recognized as a major limitation to production (Palmer and Sheppard 2002, Cheah et al. 2004). The ability to root cuttings from these species will allow for relatively small field collections that can be vegetatively multiplied and infested with HWA to provide an ample, easily assessable, and local source of host material for predator rearing. Given the low seed viability for both species (< 25%), vegetative propagation via

rooted cuttings could also be used in addition to seed collection for current gene conservation efforts for eastern and Carolina hemlock (Godman and Lancaster 1991; Romero et al. 2000; Staniforth 2001). This technology will also benefit those who propagate, breed, and sell the 80-plus eastern and Carolina hemlock ornamental cultivars (Ouden and Boom 1982; Swartley 1984).

Future efforts to understand hemlock host resistance against HWA will benefit from this technology as well. Although HWA can kill hemlocks in as little as four years, there are some trees that have survived infestation for nearly 20 years. At this time it is unclear if this is due to site conditions or moderate levels of host resistance (Souto et al. 1996; McClure et al. 2001). The ability to root stem cuttings from these surviving hemlocks will be an important tool for determining the basis of this prolonged survival, providing an increased number of trees and genotypes for study that will grow and mature more rapidly than propagules from seed. When hemlock host resistance is understood, the availability of rooted cutting technology will accelerate the breeding and deployment of resistant trees to areas devastated by HWA. While grafting would offer similar opportunities and benefits for the vegetative propagation of hemlocks, rooted cuttings are a more cost effective and less time consuming technique.

Currently, there is a body of information concerning the propagation of semi-dormant and dormant hardwood stem cuttings from eastern, western, and Carolina hemlocks (Doran 1952; Flint and Jesinger 1971; Fordham 1971; Mitsch 1975; Swartley 1984; del Tredici 1985; Waxman 1985; Packee 1991; Wigmore and Woods 2000). However, there is

relatively little known about the cultural techniques, specifically auxin concentrations, necessary for the successful rooting of softwood cuttings from these species. The objective of the current study was to test the effects of cutting length and NAA (1-naphthalenacetic acid) concentration on adventitious root formation in softwood stem cuttings of eastern and Carolina hemlock. While IBA (indole-3butyric acid) or IBA plus NAA are more commonly used to root conifer stem cuttings, NAA alone is effective in promoting adventitious root formation in a number of species, including eastern hemlock, loblolly pine, *Pinus taeda* L., eastern white pine, *Pinus strobus* L., and Fraser fir, *Abies fraseri* (Pursh) Poir (Doran 1952; Diaz-Sala et al. 1996; Goldfarb et al. 1998a,b; Rosier 2003).

## **MATERIALS AND METHODS**

### Plant Material

Eastern hemlock stem cuttings were collected from trees growing along fifteen miles (mile posts 261-275) of the Blue Ridge Parkway in Ashe County, North Carolina. Carolina hemlock stem cuttings were collected from trees growing beside the South Toe River at the Carolina Hemlocks Recreation Area (Pisgah National Forest) in Yancey County, North Carolina. Softwood cuttings were harvested from primary branch tips in the lower crown on 24 and 25 June 2002. Six cuttings were taken from each of approximately 120 trees per species. Following collection, each cutting was immediately wrapped in a moistened paper towel and placed on ice. Cuttings were transported to Raleigh, NC on 26 June 2002 and stored at 4°C until used in the rooting trial.

### Rooting Trial Treatments and Experimental Design

The rooting trial tested the effects of the two cutting lengths (3 and 6 cm) and five concentrations of NAA (0, 1, 2, 4, 8 mM). Beginning with a stock solution of 8 mM NAA, each auxin concentration was prepared by serial dilution into a 50% isopropyl alcohol-deionized water solution. The control (0 mM NAA) consisted of the 50% isopropyl alcohol-deionized water solution. The prepared auxin solutions were placed into opaque bottles and stored at 4 °C until used the same day. On 28 June 2002, the stem cuttings were re-cut from the base to either 3 or 6 cm, and auxin was applied by a 3-second dip of the basal 1 cm. Cuttings were allowed to air dry for 15 minutes before being inserted to a depth of 2 cm into a rooting medium consisting of 3 horticultural perlite: 2 peat. Cuttings were rooted individually in 164-mL Ray Leach SC-10 Super Cells (Stuewe & Sons, Inc., Corvallis, OR).

The experiment was a randomized complete block design with eight blocks, each containing 20 treatment combinations with eight cuttings per treatment for a total 1280 cuttings. The entire study was surrounded by a row of border cuttings. The rooting trial lasted 24 weeks (28 June 2002 to 28 Dec. 2002) after which rooting percentage and percent mortality among all cuttings as well as the number of primary roots and total length of primary roots (cm) among rooted cuttings were recorded.

### Rooting Environment

The rooting trial was conducted in a clear polyethylene-covered greenhouse located at the N.C. State University Horticultural Field Laboratory, Raleigh. Cuttings were rooted under natural photoperiod and irradiance, except for the summer period (28 June 2002

through August 2002) when irradiance was reduced 60% by placing shade cloth on the greenhouse. Heating and cooling systems maintained the daily air temperature between 23 ° and 26 °C and the night temperature between 20 ° and 23 °C for the duration of the rooting trial. Cuttings were maintained under intermittent mist at a frequency varied inversely to the relative humidity within the greenhouse using a Grower Junior™ (McConkey Co., Mt. Puyallup, Wash.) overhead boom irrigation system. Mist delivery rate was held constant at 61 ml\*m<sup>-2</sup> from 28 June 2002 through 31 August 2002 and 53 ml\*m<sup>-2</sup> from 1 September 2002 through 28 December 2002.

#### Statistical Analysis

Analysis of variance was performed using the General Linear Model procedure (PROC GLM) of SAS version 8.1 (SAS Institute, 1999). The main effects of block, species, auxin concentration, cutting length, and all two- and three-way interactions other than those including block were tested for all rooting traits assessed (Table 1). Also tested were the main effects of block, auxin concentration, cutting length, and the interaction between auxin concentration and cutting length on rooting traits assessed for each species individually (Table 2). The variables rooting percentage and percent mortality were transformed using the arcsine of the square root to increase homogeneity among treatment variances. Results from the analysis of the transformed and non-transformed data were similar, therefore all results presented are based on non-transformed data. All means are reported as least squares means.

## RESULTS AND DISCUSSION

### Effect of Hemlock Species on Adventitious Root Formation

Rooting percentage, percent mortality, number of primary roots, and total length of primary roots were all significantly affected by the main effect of species (Table 1). Overall, eastern hemlock softwood stem cuttings rooted at a higher rate (41%) than those of Carolina hemlock (10%). While this result might indicate some inherent difference in rooting capacity between these two species, our study was not designed to investigate this possibility.

Additional explanations for differential rooting between eastern and Carolina hemlock include variation in stock plant age and site type. Both species occur on nutrient poor soils, but eastern hemlock sites are typified by very moist, well-drained soils and Carolina hemlock sites by very dry, rocky soils (Godman and Lancaster 1990, Rentch et al. 2000). This difference in soil moisture availability could have affected the water status of stem cuttings at the time of collection and may at least partially account for the observed differences in stem cutting rooting rates.

Overall mortality among softwood stem cuttings was 22% for eastern hemlock and 13% for Carolina hemlock. Thirty-seven percent of eastern hemlock stem cuttings and 77% of Carolina hemlock stem cuttings that did not root were alive at the end of the six month trial. This indicates that Carolina hemlock softwood stem cuttings might require more time or altered cultural conditions to root at rates equivalent to eastern hemlock.

The number of primary roots and total primary root length among eastern hemlock stem cuttings were 2.6 roots per cutting and 8.6 cm, respectively, while these variables were

non-estimable for Carolina hemlock. The non-estimable means in the analysis were due to the extremely low overall rooting percentage among Carolina hemlock stem cuttings, particularly in the 8 mM NAA treatments (3%).

#### Effect of Cutting Length on Adventitious Root Formation

Percent mortality, number of primary roots, and total length of primary roots were all significantly affected by the main effect of cutting length (Table 1). Significant interactions between species and cutting length were detected for number of primary roots and total length of primary roots (Table 1). There was no effect of cutting length on rooting percentage for either species (Tables 1 & 2; Figure 2a). Mortality among larger cuttings of both eastern and Carolina hemlock was twice that observed among smaller cuttings, with overall mortality of 24% among 6-cm cuttings compared with 11% among 3-cm cuttings (Figure 2b). Increased mortality among 6-cm cuttings may be attributable to a greater exposed length (4-cm) above the soil compared with 3-cm cuttings (1-cm). Cuttings with greater exposed length have more above-soil leaf area that may be subjected to higher rates of transpirational stress. Such stress may have caused mortality directly through desiccation or indirectly through the inhibition of adventitious root formation.

Significant interactions between species and cutting length for the mean number of primary roots and mean length of primary roots are likely due to the extremely low rooting percentage for 3-cm Carolina hemlock stem cuttings. Therefore, the following discussion of cutting length and its effects on root number and length will focus on results for eastern hemlock only.

The number of primary roots and total length of primary roots among eastern hemlock softwood stem cuttings were significantly affected by cutting length (Table 2), with fewer (2.0) and shorter (5.1 cm) roots formed on 3-cm cuttings compared with 6-cm cuttings (3.5 and 12.3 cm; Figures 2c & 2d). Similar responses have been reported for stem cuttings of both sweetgum, *Liquidambar styraciflua* L., and Fraser fir where longer cuttings or cuttings of greater diameter produced more roots than shorter or thinner cuttings (Miller et al. 1982; Rieckermann et al. 1999). While greater above ground leaf surface area may lead to greater transpirational stress and subsequent mortality, it may also benefit cuttings that successfully root providing a larger pool of photosynthetic resources for root formation. Likewise, cuttings of larger size and greater length may also have greater levels of stored carbohydrates for root growth (Rieckermann et al. 1999).

#### Effect of Auxin Concentration on Adventitious Root Formation

Rooting percentage, percent mortality, and number of primary roots were all significantly affected by the main effect of auxin concentration with a significant interaction between species and auxin concentration detected for rooting percentage (Table 1). The application of exogenous auxin is often an effective and, sometimes, necessary treatment for promoting adventitious root formation in conifer stem cuttings (Hinesley and Blazich 1981). This beneficial effect of auxin treatment was detected for softwood stem cuttings of Carolina hemlock. Rooting was significantly affected by auxin concentration (Table 2) increasing from 8% for the control to a maximum of 16% at 1 mM NAA then subsequently decreasing with increasing auxin concentration to 4% at 8 mM NAA (Figure 1a). It appears, however,



that exogenous auxin treatments lack any benefit for rooting in eastern hemlock softwood stem cuttings. Rooting was significantly affected by auxin concentration (Table 2) with a maximum of 56% occurring at 0 mM NAA followed by a decrease with increasing auxin concentration to a low of 19% at 8 mM NAA (Figure 1a).

Overall, mortality decreased from 13% at 0 mM NAA to 11% at 2 mM NAA and then increased with increasing auxin concentration to a high of 32% at 8 mM NAA. Likewise, the number of roots per cutting increased with increasing auxin concentration from 1.5 at 0 mM NAA to 2.5 at 4 mM NAA. The number of primary roots at 8 mM NAA was non-estimable for both the overall analysis and Carolina hemlock. The mortality and root number responses of the individual species to auxin concentration are shown in figures 1b and 1c.

The total primary root length for eastern hemlock softwood stem cuttings was significantly affected by auxin concentration (Table 2). The longest average root length of 10.8 cm occurred at 2 mM NAA decreasing with decreasing and increasing auxin concentration to 7.2 cm and 7.0 cm at 0 mM NAA and 8 mM NAA, respectively (Figure 1d). The effect of NAA concentration on root length of Carolina hemlock softwood stem cuttings (Figure 1d) was not significant ( $P = 0.66$ , Table 2). The number of primary roots at 8 mM NAA was non-estimable among Carolina hemlock owing to the overall low rooting percentage.

## CONCLUSIONS

To date, there has been no direct experimental test of the effect of cutting type (softwood versus hardwood) on rooting in eastern and Carolina hemlock stem cuttings. Overall, softwood stem cuttings of eastern and Carolina hemlock rooted at rates much lower than those reported for dormant and semi-dormant hardwood cuttings from these species. Doran (1952) reported mean rooting rates of 65% and 71% for hardwood stem cuttings, treated with comparable concentrations of auxin (0.5 - 1.0 mM IBA alone or NAA alone), of eastern and Carolina hemlock, respectively. However, direct comparisons between the current study and Doran's are tenuous.

The results of this trial are encouraging and indicate that softwood stem cuttings from eastern hemlock can be rooted at reasonably high rates without exogenous auxin treatments. Additionally, it is clear that cutting size is an important mediating factor for successful rooting in this species, with larger cuttings producing more and larger roots. Rooting rates for Carolina hemlock were significantly lower than those of eastern hemlock, but maximum rooting occurred at 1mM NAA, indicating that very little auxin is required to root softwood cuttings from this species. The high survival rate among Carolina hemlock cuttings that did not root suggests that additional time in the rooting environment or slightly altered cultural conditions may improve rooting rates.

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Table 1. Analysis of variance for traits assessed in a rooting trial of eastern and Carolina hemlock softwood stem cuttings collected from mature trees. *F*-values with indicated level of significance are given for each trait.

Source of variation	DF <sup>x</sup>	Rooting (%)	Mortality (%)	Number of primary roots	Total length of primary roots (cm)
Block	7	0.99 <sup>NS</sup>	2.26*	2.45*	1.54 <sup>NS</sup>
Species	1	161.01***	9.33**	16.59***	20.90***
AC <sup>y</sup>	4	12.33***	8.36***	6.07***	2.05 <sup>NS</sup>
Species x AC	4	5.73***	0.48 <sup>NS</sup>	1.68 <sup>NS</sup>	1.06 <sup>NS</sup>
CL <sup>z</sup>	1	1.14 <sup>NS</sup>	21.11***	21.53***	32.28***
Species x CL	1	2.21 <sup>NS</sup>	0.83 <sup>NS</sup>	4.81*	23.50***
AC x CL	4	0.6 <sup>NS</sup>	1.81 <sup>NS</sup>	2.09 <sup>NS</sup>	1.35 <sup>NS</sup>
Species x AC x CL	4	1.17 <sup>NS</sup>	0.96 <sup>NS</sup>	0.03 <sup>NS</sup>	1.34 <sup>NS</sup>

<sup>NS</sup>, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>x</sup>Error degrees of freedom for rooting and mortality = 133; for number of roots and root length = 82.

<sup>y</sup>Auxin concentration.

<sup>z</sup>Cutting length.

Table 2. Analysis of variance for traits assessed individually for species in a rooting trial of eastern and Carolina hemlock softwood stem cuttings collected from mature trees. *F*-values with indicated level of significance are given for each trait.

Source of variation	DF <sup>x</sup>	Rooting (%)	Mortality (%)	Number of primary roots	Total length of primary roots
<u>Eastern hemlock</u>					
Block	7	0.81 <sup>NS</sup>	1.49 <sup>NS</sup>	2.08 <sup>NS</sup>	1.21 <sup>NS</sup>
AC <sup>y</sup>	4	10.40***	3.58*	3.28*	4.09**
CL <sup>z</sup>	1	2.06 <sup>NS</sup>	13.09***	41.91***	95.24***
AC x CL	4	0.60 <sup>NS</sup>	0.94 <sup>NS</sup>	1.78 <sup>NS</sup>	3.35*
<u>Carolina hemlock</u>					
Block	7	1.19 <sup>NS</sup>	3.07**	1.16 <sup>NS</sup>	0.50 <sup>NS</sup>
AC	4	3.66**	6.22***	6.12**	0.60 <sup>NS</sup>
CL	1	0.20 <sup>NS</sup>	8.98**	1.66 <sup>NS</sup>	0.63 <sup>NS</sup>
AC x CL	4	1.88 <sup>NS</sup>	2.23 <sup>NS</sup>	0.77 <sup>NS</sup>	0.11 <sup>NS</sup>

<sup>NS</sup>, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>x</sup>Error degrees of freedom for rooting and mortality = 63; for number of roots = 56 (eastern) and 19 (Carolina); for root length 56 (eastern) and 19 (Carolina).

<sup>y</sup>Auxin concentration.

<sup>z</sup>Cutting length.

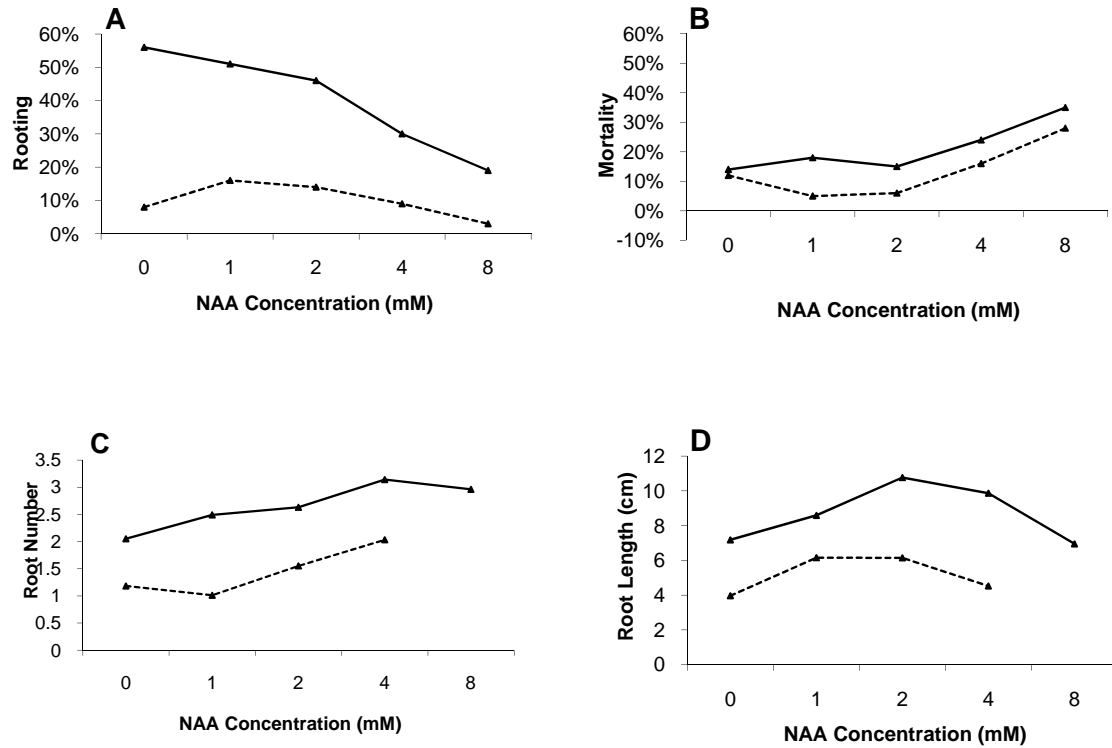


Figure 1. Effect of NAA concentration on (A) rooting percentage, (B) percent mortality, (C) number of primary roots, and (D) total length of primary roots of eastern (—) and Carolina (----) hemlock softwood stem cuttings. The number of primary roots and total root length of Carolina hemlock cuttings at 8 mM NAA were non estimable.



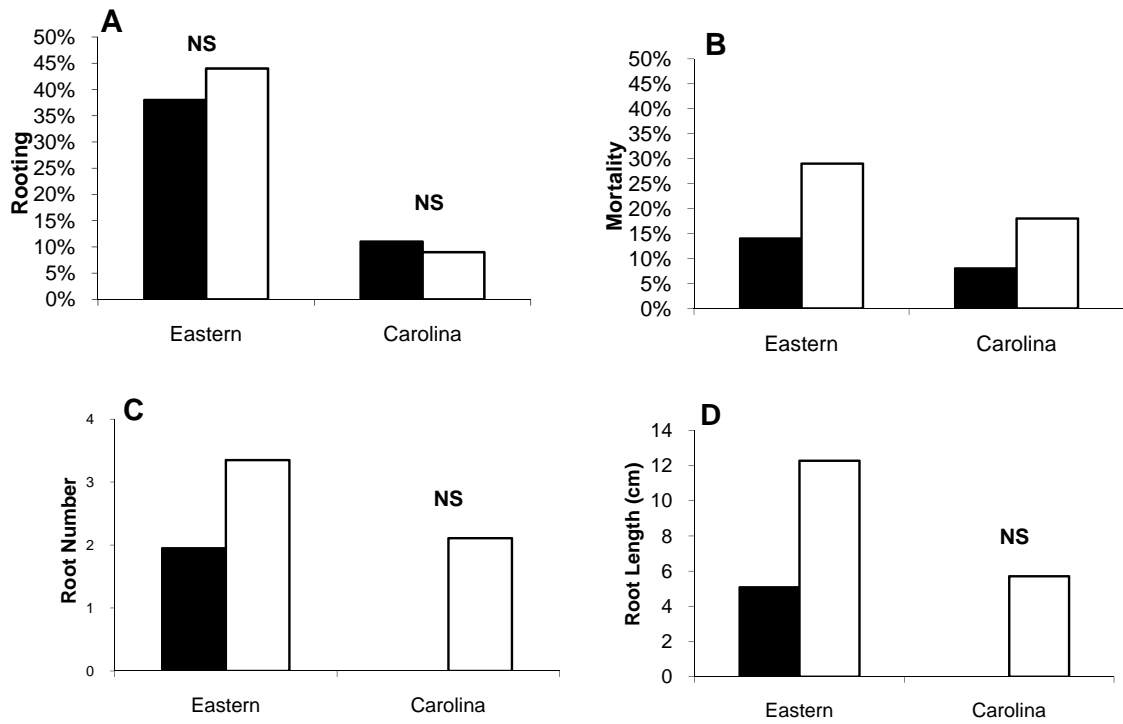


Figure 2. Effect cutting length on (A) rooting percentage, (B) percent mortality, (C) number of primary roots, and (D) total length of primary roots of eastern and Carolina hemlock softwood stem cuttings. The number of primary roots and total root length of 3 cm Carolina hemlock cuttings were non estimable. Cutting length: 3 cm (■), 6 cm (□).

## **Appendix A**

### **Addendum to Chapter V: Revised Data Analysis**

#### **INTRODUCTION**

Chapter V was published prior to the completion of this dissertation and appears here as it did in *HortScience* 40: 1469-1473. While reviewing this chapter prior to my thesis defense, the members of my graduate committee questioned the occurrence of non-estimable LS Means for the number and length of primary roots among Carolina hemlock softwood stem cuttings in the 8 mM NAA and 3 cm treatments (Figures 1 and 2, Chapter V). Specifically, it was questioned if the inclusion of the three-way interaction between species, auxin concentration, and cutting length in the ANOVA model may have limited the ability of SAS to estimate these means. This was the case, and, because this interaction did not significantly affect any of the rooting variables assessed (Table 1, Chapter V), the model was run again excluding the three-way interaction term. Doing so did not substantially alter the results of the study and provided the LS means which were non-estimable in the more complex ANOVA model.

While reviewing the analysis to correct the non-estimable means, a second more serious error was discovered. The variables for rooting percentage and mortality were recorded as binary (0,1) data. The result, because most hemlock stem cuttings in the study did not root and many died, was an unbalanced data set. To adjust for this and attempt to normalize the data, SAS was coded to calculate block means for each species-auxin concentration-cutting length treatment combination, and these means were then analyzed to

determine what main effects and interactions significantly affected rooting percentage and mortality among the hemlock stem cuttings. However, SAS was also inadvertently coded to calculate similar block means for the number and length of primary roots among hemlock stem cuttings that did produce adventitious roots. The effect was to reduce the number of observations available for analysis of these variables by more than half and may have significantly altered their statistical interpretation. In the following discussion I present the results of new analyses for the number and length of primary roots that include LS means previously reported as non-estimable and are based on the whole data set rather than the block means. I also compare the new analysis to those presented in Chapter V.

### **RESULTS OF THE REVISED ANALYSIS**

Two revised ANOVAs were conducted, both similar to those presented in Chapter V. The first tested the main effects of block, species, auxin concentration, cutting length, and all two-way interactions other than those including block on the number and length of primary roots among hemlock stem cuttings. The second tested the main effects of block, auxin concentration, cutting length, and the interaction between auxin concentration and cutting length on these same two rooting variables for each hemlock species individually. The results of these revised analyses are presented in Tables 1 and 2 of this addendum. Quantitative differences in the values of  $F$  statistics and levels of significance do exist between these revised analyses and those presented in Tables 1 and 2 of Chapter V. However, most of these are not qualitative differences and do not alter the interpretation of the data. In other words, most factors that significantly affected the number and length of

primary roots in the original analysis remained significant in the revised analysis, and those that were not significant remained non-significant. Qualitative differences that were found are highlighted in bold type in Tables 1 and 2 of this addendum. Additionally, revised LS means for the number and length of primary roots are presented in Figures 1 and 2 of this addendum and amend Figure 1 (C and D) and Figure 2 (C and D) in Chapter V.

Among the few qualitative differences that were found between the original and corrected analyses, the most important concerns the main effect of auxin concentration on the number of primary roots on rooted Carolina hemlock stem cuttings (Table 2, this addendum). In the original analysis this effect was found to be significant (Table 2, Chapter V), with the number of roots generally increasing with increasing concentrations of NAA (Figure 1, Chapter V). In the revised analysis, the effect of auxin was found to be non-significant for the number of roots on Carolina hemlock stem cuttings (Table 2, this addendum), with very little change in the number of roots with changes in NAA concentration (Figure 1, this addendum).

The Editor-in-Chief of *HortScience* was contacted about the error in the original analysis as published in the journal. He agreed that the qualitative differences between the two analyses were rather minor, but he did suggest that it would be best for a correction to be submitted for publication. I will pursue this avenue to correct my published research.

Table 1. Analysis of variance for number of roots and length of roots in a rooting trial of eastern and Carolina hemlock softwood stem cuttings collected from mature trees. Results reflect corrected data analysis performed subsequent to publication of the data in *HortScience* 40: 1469-1473 as presented in Chapter V of this dissertation. *F*-values with indicated level of significance are given for each trait. Statistics in bold differ qualitatively from the original analysis in Table 1, Chapter V.

Source of variation	DF <sup>x</sup>	Number of primary roots	Total length of primary roots (cm)
Block	7	<b>1.90</b> <sup>NS</sup>	<b>2.41</b> <sup>*</sup>
Species	1	341.91***	7.46**
AC <sup>y</sup>	4	3.85**	1.36 <sup>NS</sup>
CL <sup>z</sup>	1	74.98***	22.45***
Species x AC	4	1.66 <sup>NS</sup>	0.88 <sup>NS</sup>
Species x CL	1	70.66***	27.80***
AC x CL	4	1.93 <sup>NS</sup>	<b>4.35</b> <sup>**</sup>

<sup>NS</sup>, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>x</sup>Error degrees of freedom for Number of Primary Roots = 1051

and for Length of Primary Roots = 291.

<sup>y</sup>Auxin concentration

<sup>z</sup>Cutting length

Table 2. Analysis of variance for number of roots and length of roots, assessed individually for species in a rooting trial of eastern and Carolina hemlock softwood stem cuttings collected from mature trees. Results reflect corrected data analysis performed subsequent to publication of the data in *HortScience* 40: 1469-1473 as presented in Chapter V of this dissertation. *F*-values with indicated level of significance are given for each trait. Statistics in bold type differ qualitatively from the original analysis in Table 2, Chapter V.

Source of variation	DF <sup>x</sup>	Number of primary roots	Total length of primary roots (cm)
<u>Eastern hemlock</u>			
Block	7	1.86 <sup>NS</sup>	2.01 <sup>NS</sup>
AC <sup>y</sup>	4	2.44*	5.50***
CL <sup>z</sup>	1	71.46***	141.40***
AC x CL	4	<b>2.46</b> *	4.77**
<u>Carolina hemlock</u>			
Block	7	0.89 <sup>NS</sup>	0.75 <sup>NS</sup>
AC	4	<b>2.06</b> <sup>NS</sup>	0.82 <sup>NS</sup>
CL	1	0.39 <sup>NS</sup>	0.78 <sup>NS</sup>
AC x CL	4	1.66 <sup>NS</sup>	0.26 <sup>NS</sup>

<sup>NS</sup>, \*, \*\*, \*\*\*Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>x</sup> Error degrees of freedom for Eastern hemlock: Number of Primary Roots = 490

Length of Primary Roots = 243; Carolina hemlock: Number of Primary Roots = 550

Length of Primary Roots = 38.

<sup>y</sup>Auxin concentration

<sup>z</sup>Cutting length

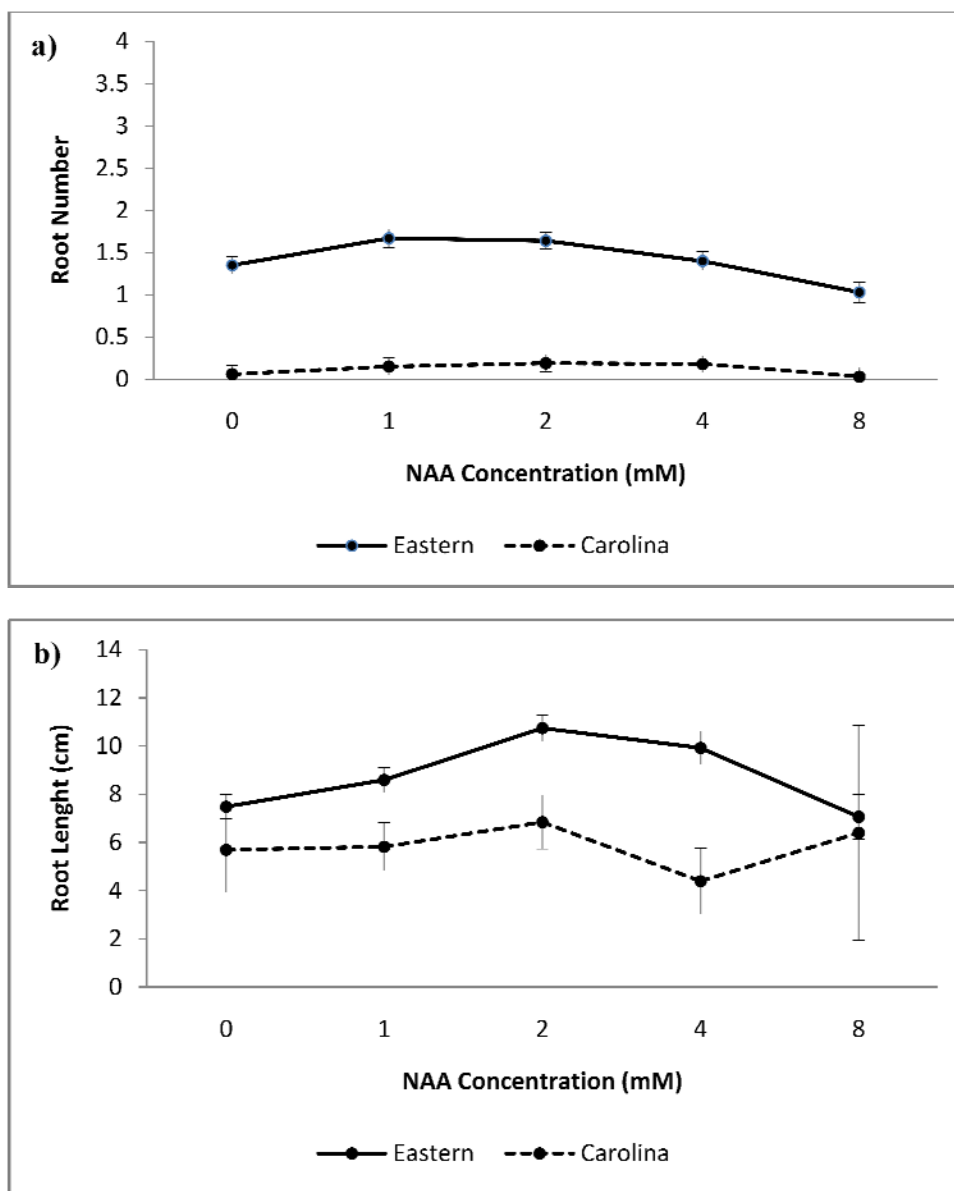


Figure 1. Effect of NAA concentration on (a) number of primary roots and (b) total length of primary roots of eastern and Carolina hemlock softwood stem cuttings. This figure amends Figure 1 C and D in Chapter V and presents LS Means for Carolina hemlock at 8 mM NAA.

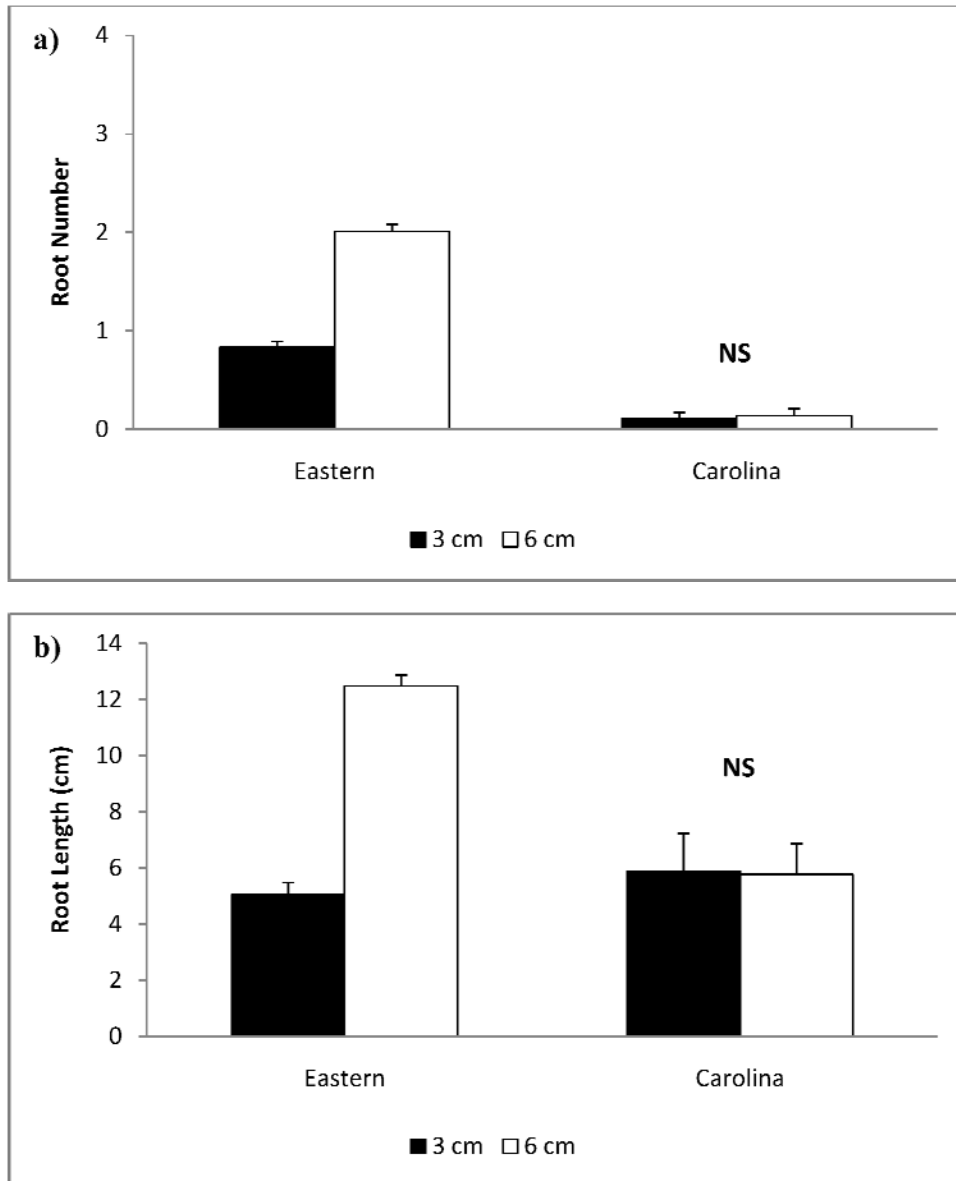


Figure 2. Effect of cutting length on (a) number of primary roots and (b) total length of primary roots of eastern and Carolina hemlock softwood stem cuttings. This figure amends Figure 2 C and D in Chapter V and presents LS Means for 3 cm stem cuttings of Carolina hemlock.



## Appendix B

### Arthropod Diversity Associated with Old and Secondary Growth Eastern Hemlock in the Great Smoky Mountains National Park

#### ABSTRACT

Prior to the introduction of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, into the Great Smoky Mountains National Park (GSMNP), the diversity and seasonal abundance of arthropods (insects and spiders) associated with two old growth and two secondary growth stands of eastern hemlock, *Tsuga canadensis* (L.) Carrière, were assessed. In total, 8,071 insects and 191 spiders were sampled, and both were found to be more abundant in secondary growth than old growth hemlock stands. The insect samples were dominated by the orders Diptera and Lepidoptera that accounted for over 75% of all specimens while spider collections were dominated by three species (*Pirata montanus*, *Wadotes tennesseensis*, and *W. hybridus*) that accounted for over 50% of all specimens. Shannon-Weiner diversity and evenness values for insect families and spider species did not differ greatly by hemlock stand type. The seasonal abundance of insect families in light traps was found to be highest in the summer while the abundance of insect families in pitfall traps varied little throughout the study.

## INTRODUCTION

Eastern hemlock, *Tsuga canadensis* (L.) Carrière, is a slow-growing and long-lived conifer whose native range extends throughout the eastern United States and Canada. It is found at elevations ranging from near sea-level to more than 1500 meters and occupies multiple forest types from the Maritimes west to Minnesota and south along the Appalachian Mountain range from Maine to Georgia (Farjon 1990; Godman and Lancaster 1990). Although it was routinely harvested in the early 1900s, slow growth and mediocre wood quality have limited its economic usefulness. However, eastern hemlock is widely recognized as an ecologically important and aesthetically pleasing species. The dense shade and acidic soil conditions under hemlock dominated forest canopies create a habitat where only a handful of herbaceous and woody plant species are adapted for survival (Quimby 1996). These areas are also ideal roosting and nesting sites for a number of avian species and provide an important source of forage and cover for wild turkey and deer (Quimby 1996; Ross et al. 2004; Lishawa et al. 2007). Where it occurs as a riparian species, eastern hemlock plays a vital role in soil stabilization and moderates water quality and temperature at conditions optimal to the fitness of native brook trout, aquatic insects, and amphibians (Brooks 2001; Snyder et al. 2002; Ross et al. 2003). Hemlocks are also an important component of many recreation and scenic areas throughout the Appalachian region (Quimby 1996), and with numerous cultivars available have been of great value to the nursery industry (Swartley 1984).

There are a number of factors that threaten the stability and long-term survival of

hemlock ecosystems in the eastern U.S. including exotic insects, periodic severe droughts, wildfires, suburban development, and climate change. The most important of these is the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, an exotic insect pest that in recent years has caused widespread mortality of eastern hemlock throughout the mid-Atlantic and southern Appalachian regions (McClure et al. 2001). The adelgid was first described from specimens collected on western hemlock (*T. heterophylla* Sargent) in California and Oregon (Annand 1924) where it is now thought to be native (Havill et al. 2006). It was most likely introduced to the eastern U.S. sometime between 1920 and 1950 on *T. sieboldii* nursery stock that was imported from Japan and planted in Richmond, Virginia (Souto et al. 1996; Stoetzel 2002). Since that time, HWA has spread to 17 eastern states from Maine south to Georgia and currently infests approximately 50% of eastern hemlock ecosystems where it can kill trees in as little as four years (McClure et al. 2001).

The Great Smoky Mountains National Park (GSMNP) straddles a portion of the border between North Carolina and Tennessee and encompasses over 200,000 hectares of forested habitat in the southern Appalachian Mountains. The park boasts the largest biodiversity of flora and fauna found in any one place in the eastern U.S., including one of the largest pockets of both old and secondary growth eastern hemlock forest found within the species' native range. In total, hemlock grows on over 35,000 hectares in multiple forest types with more than 2000 hectares occurring as pure hemlock (Johnson et al. 2005). The first infestations of HWA at GSMNP were found near Fontana Lake in 2002. Ten years prior to this (1992) park resource managers initiated an effort to map the hemlock forests at

GSMNP and document the associated flora and fauna in advance of the adelgid (Johnson et al. 2000). Included in this effort was a project to survey and identify the relatively unknown community of arthropods that occupy the eastern hemlock ecosystems within the park. The goal was to develop fundamental knowledge of the season abundance, natural variations, and locations of the species present to help guide decisions about the use of insecticides and biological controls once HWA arrived in the park. We report on a portion of this effort and present the results of light and pitfall trap field surveys to identify arthropods associated with old and secondary growth eastern hemlock stands within GSMNP.

## **MATERIALS AND METHODS**

### Survey Plots

Arthropod sampling plots were established in hemlock dominated forest stands at four locations within GSMNP, two in old growth stands (Inadu Knob and Cataloochee) and two in secondary growth stands (Elkmont and Cosby Creek). Inadu Knob, Cataloochee, and Cosby Creek are all located in the eastern portion of the park and Elkmont is in the central portion. Within each of the four stands, 16 0.1-acre circular plot-monitoring stations were established at representative sites, each separated by a distance of 20 meters. The study had a total of 64 monitoring stations, 32 in old growth and 32 in secondary growth. The design allowed for sampling of all 64 stations within a one week period. Arthropod surveys were conducted 5-8 June 1995, 19-21 July 1995, 3-8 September 1995, and 22-26 April 1996.

### Survey Techniques

Light trap sampling consisted of placing one battery-powered UV light trap in one, centrally located monitoring station in each of the four hemlock stands on successive nights during each sampling week. For approximately one hour after sunset, insects attracted to the light trap were captured with aerial nets or by hand from a white sheet hung near the light. Thereafter for the remainder of the night, insects attracted to the light were capture in a soapy water filled reservoir attached below the trap. Pitfall trap sampling consisted of placing 5 traps in an X-pattern in the center of each of the 64 monitoring stations. Insects were captured using soapy water, and traps were left in place for one 24-hour period during each sampling week.

### Specimen Processing

Insect specimens captured at UV light traps by hand or aerial nets were pinned and labeled with the site, date, and method of collection. Those caught in the soapy water reservoirs were placed in similarly labeled vials of alcohol. For specimens captured in pitfall traps, the larger hard-bodied insects were air dried and then pinned and labeled as above. The smaller soft-bodied insects and spiders were placed in labeled vials of alcohol. All insect specimens were identified to the family level by Dr. Felton L. Hastings with the help of technicians and graduate students in the NCSU Forest Entomology program and taxonomic specialists in the NCSU Department of Entomology. The spider specimens, all of which were capture in pitfall traps, were sent to Dr. Frederick A. Coyle, now Professor Emeritus of Biology at Western Carolina University (Cullowhee, NC), for identification to the species level.

### Data Summary and Analysis

A database for all arthropod specimen data was created using Microsoft Excel® spreadsheets. For insects, the data entered consisted of order, family, sampling site, sampling date, sampling method, and number of specimens (Table 1). The data for spiders included family, genus, species, author, sampling site, sampling date, and number of specimens (Table 2). The insect database was sorted to determine the relative abundance of insect orders over all sampling sites, dates, and methods (Figure 1), and the total number of insect families captured at each sampling site on each sampling date in light (Figure 2) and pitfall (Figure 3) traps. The relative diversity ( $H'$ ) and evenness ( $E$ ) of arthropods was estimated by the Shannon-Weiner Index using the Ecological Measures computer program (Kotila 1986). Insect diversity and evenness values were determined for each sampling site and method individually and for all sites and methods together (Table 3). Spider diversity and evenness were estimated for each site individually and all sites together (Table 4).

### **RESULTS AND DISCUSSION**

Overall, we collected 8,071 insect specimens from 142 families and 11 orders from hemlock stands in GSMNP during this survey (Table 1). The number of insects sampled from secondary growth stands (4,931 specimens from 123 families and 10 orders) was greater than from old growth stands (3,140 specimens from 85 families and 9 orders). Similarly, the number of insects sampled from light traps (6,766 specimens from 106 families and 10 orders) was greater than from pitfall traps (1,305 specimens from 68 families and 7 orders). The orders Diptera and Lepidoptera dominated the survey, accounting for over 75% of all specimens collected (Figure 1).

Several studies have evaluated the insects associated with eastern hemlock with a focus on predators (Montgomery and Lyons 1996; Wallace and Hain 2000), guild structure (Dilling et al. 2007), and species abundance and diversity (Buck et al. 2005). Studies that focused on predators were conducted to evaluate the biological control potential of native and naturalized insects for HWA management in the eastern U.S. The most common natural enemies found feeding on the adelgid in these surveys were from the families Cecidomyiidae, Chrysopidae, Coccinellidae, Hemerobiidae, and Syrphidae, but none were present at high enough densities to offer effective control of HWA (Montgomery and Lyons 1996; Wallace and Hain 2000). All but one of these families, Hemerobiidae, were captured in our study, and for all but Cecidomyiidae fewer than 5 specimens were sampled (Table 1). Interestingly, although native natural enemies are now recognized as ineffective for adelgid control, Dilling et al. (2007) found predators to be the third most common insect guild associated with eastern hemlock in the southern Appalachian Mountains.

Buck et al. (2005) also surveyed the insect associates of eastern hemlock at GSMNP, and similar to our study, they evaluated the seasonal abundance and diversity of insects associated with old growth and secondary growth stands. Overall, they collected fewer insect specimens (2,832) and families (101) and one less insect order using a combination of Malaise, pitfall, and direct sampling. Eight orders were common to both studies, with Psocoptera and Mecoptera unique to Buck et al. and Ephemeroptera, Plecoptera, and Trichoptera unique to our study (Table 1). Both studies found less insect diversity associated with pitfall traps than with other means of sampling. Buck et al. reported fewer specimens

and significantly less species diversity captured in pitfall compared to Malaise traps and direct sampling. Similarly, in our survey the Shannon-Weiner index for the diversity of insect families captured in pitfall traps was lower than that for light traps (Table 3).

Our study did differ from Buck et al. (2005) in the seasonal abundance and numbers of insect specimens associated with old and secondary growth stands of eastern hemlock. They found the seasonal abundance of insect species to be highest in the early spring and fall months. Our survey found the seasonal abundance of insect families associated with light traps to be highest during July and lowest in the fall and spring (Figure 2). The seasonal abundance of insect families associated with pitfall traps varied little by site throughout the study (Figure 3). Additionally, we captured greater numbers of insect specimens from secondary growth stands while Buck et al. sampled higher abundance of specimens in old growth. However, neither our study (Table 3) nor Buck et al. found the diversity and evenness of insects as estimated by the Shannon-Weiner index to differ greatly by hemlock stand type.

The values of the Shannon-Weiner diversity indices calculated for insects associated with eastern hemlock at GSMNP were much lower (0.882 – 1.306) in our study compared to Buck et al. (3.62 – 4.51). However, trying to reach a meaningful conclusion about this difference is difficult for two reasons. First, Buck et al. accomplished the bulk of their insect sampling with Malaise traps while we did the same with light traps, and comparing subsets of specimens from different trapping systems is tenuous due to sampling biases created from insect behavior, activity levels, and size among other factors. Second, diversity indices in



our study are based on specimen identifications to the family level while Buck et al. identified their specimens to the species level, and one would expect that breaking each family down to individual species would increase diversity.

We collected 191 spider specimens in pitfall traps located in hemlock stands at GSMNP (Table 2). Similar to our results for insect sampling, more spiders were captured in secondary growth (149) compared to old growth (42). Twenty-seven individual species were identified with 2 specimens classified only to the family level and 12 to the level of genus (Table 2). The most abundant species were *Pirata montanus*, *Wadotes tennesseensis*, and *W. hybridus* that accounted for over 50% off all spiders sampled. Shannon-Weiner diversity and evenness values for the spiders did not differ greatly among old growth and secondary growth hemlock sites (Table 4).

Spider communities associated specifically with eastern hemlock in the U.S. have not been well studied, but the identities, habitat distribution, and life histories of many of those found in GSMNP have been documented (Aiken and Coyle 2000; Davis and Coyle 2001; Stiles and Coyle 2001). In total, 461 species have been identified in the park as part of the All Taxa Biodiversity Inventory (ATBI), and the 27 spider species identified in our survey are included in the GSMNP Spider Checklist maintained by DiscoverLife.org (<http://www.discoverlife.org/nh/cl/GSMNP/arachnid/araneae/>).

## CONCLUSIONS

Our survey of the arthropod associates of eastern hemlock in GSMNP yielded a total of 8,262 specimens (8,071 insects and 191 spiders) from a combination of light and pitfall

traps. All specimens have been pinned or place in vials of alcohol, labeled and identified to family (insects) or species (spiders). More arthropods were captured in secondary growth hemlock stands compared to old growth, although analyses of community structure indicated that the diversity of insect families and spider species in our samples did not differ greatly by stand type. In 1998, the GSMNP began the All Taxa Biodiversity Inventory (ATBI) to identify, map, and catalog all flora and fauna that occurs within the park boundaries with an emphasis placed on endangered ecosystems. Together, our survey and those by Buck et al. (2005) and Dilling et al. (2007), have provided a robust description of the arthropod fauna found in eastern hemlock ecosystems in the park that are currently threatened by HWA. This data can be used to improve management decisions for the adelgid at GSMNP through the evaluation of potential non-target effects of biological controls and insecticides currently used to combat this invasive insect pest.

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Table 1. Insect families associated with old and secondary growth eastern hemlock in the Great Smoky Mountains National Park.

<b>Order</b>	<b>Family</b>	<b>Site<sup>a</sup></b>	<b>Date<sup>b</sup></b>	<b>Method<sup>c</sup></b>	<b><i>n</i></b>
Blattaria	Cryptocercidae	IK	2,3	PF	2
Coleoptera	Agyrtidae	Elk	1	PF	2
	Alleculidae	Cat,Elk	1, 2	LT	4
	Anobiidae	Cos	2	LT	1
	Anthribidae	IK	4	PF	2
	Byrrhidae	IK	2	PF	1
	Cantharidae	Cat,Cos,Elk,IK	1,2,4	LT	34
	Carabidae	Cat,Cos,Elk,IK	1,2,3,4	LT, PF	433
	Cerambycidae	Cat,Cos,Elk	1,2	LT	6
	Cerylonidae	Cos	3	LT	1
	Chrysomelidae	Cat,Cos,Elk,IK	1,2,3,4	LT, PF	26
	Cicindelidae	Elk	4	PF	1
	Cleridae	Elk,IK	1,2	LT	2
	Coccinellidae	Cos,Elk,IK	2,3,4	LT	4
	Curculionidae	Cat,Cos,Elk	2,3,4	LT,PF	12
	Dermestidae	Elk	3	PF	1
	Elateridae	Cat,Cos,Elk,IK	1,2	LT,PF	33
	Erotylidae	Cat,Cos,Elk	1,2,3	LT,PF	4
	Histeridae	Elk	2	PF	1
	Lampyridae	Cat,Cos,Elk,IK	1,2	LT,PF	32
	Lucanidae	Cat	2	LT	1
	Lycidae	Cat,IK	1,4	LT,PF	4
	Melandryidae	Cat,Cos	1,2	LT	5
	Mycetophagidae	Cos	2	LT	1
	Phalacridae	Cat	2	LT	1
	Pselaphidae	Elk	2	LT	3
	Pyrochroidae	Cos,Elk,IK	1,2	LT	7
	Rhysodidae	IK	2	PF	1
	Scarabaeidae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	25
	Silphidae	Cat,Cos,Elk,IK	1,2,4	LT,PF	43
	Staphylinidae	Cat,Cos,Elk,IK	1,2	LT,PF	8
Diptera	Anisopodidae	Cat,Cos,IK	2,4	LT,PF	18
	Anthomyiidae	Cat,Cos,Elk,IK	1,3,4	LT,PF	10
	Bombyliidae	IK	4	PF	1
	Calliphoridae	Cat,Elk,IK	2,3	LT,PF	9
	Camillidae	IK	4	LT	1
	Cecidomyiidae	Cat,Cos,Elk	2,3,4	LT	68

Table 1 Continued.

Order	Family	Site <sup>a</sup>	Date <sup>b</sup>	Method <sup>c</sup>	<i>n</i>
	Ceratopogonidae	Cat,Cos,Elk,IK	1,2,3,4	LT	147
	Chironomidae	Cat,Cos,Elk,IK	2,3,4	LT	2167
	Conopidae	Elk	4	PF	1
	Culicidae	Cat,Cos,Elk	1,2,3	LT	9
	Dixidae	Cat	2,4	LT	18
	Dolichopodidae	Cat,Cos,Elk	1,2	LT	11
	Drosophilidae	Cat,Cos,Elk	1,2	LT	27
	Empididae	Cat,Cos,Elk,IK	1,2,3,4	LT	305
	Ephydriidae	Elk	1	LT	29
	Heleomyzidae	Cos,Elk	1,2	LT	7
	Muscidae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	43
	Mycetophilidae	Cat,Cos,Elk,IK	1,2,3,4	LT	197
	Oestridae	Cos,Elk	1,2	LT,PF	3
	Phoridae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	137
	Pipunculidae	Cat,Elk	1,2,3	LT,PF	3
	Psychodidae	Cat,Cos	2,3,4	LT	20
	Rhagionidae	Cos,Elk,IK	1,2,3,4	LT,PF	16
	Sarcophagidae	Cos,Elk	2,4	PF	2
	Scathophagidae	Cos,Elk	2,3	LT,PF	4
	Sciaridae	Cat,Cos,Elk,IK	1,2,3,4	LT	195
	Sciomyzidae	Elk,IK	3	LT,PF	3
	Sepsidae	Elk	4	PF	1
	Simuliidae	Cos,Elk	1,2,3,4	LT	11
	Syrphidae	Cat	2	LT	1
	Tabanidae	Elk	2	LT	3
	Tachinidae	Cat,Cos,IK	2,4	LT,PF	6
	Tanyderidae	Elk	4	PF	3
	Therevidae	Elk	1,2	LT,PF	2
	Tipulidae	Cat,Cos,Elk,IK	1,2,3,4	LT	220
	Trichoceridae	IK	4	PF	1
	Xylophagidae	Elk	4	PF	1
Ephemeroptera	Heptageniidae	Cos,Elk	2,4	LT	2
	Leptophlebiidae	Cos	4	LT	2
Hemiptera	Aphididae	Elk,IK	1,2	LT	2
	Cercopidae	Elk	2	LT	7
	Cicadellidae	Cos,Elk	1,2,3	LT	3
	Cixiidae	IK	1,4	LT,PF	2
	Derbidae	Elk	1	LT	1
	Issidae	Elk	2	LT	1

Table 1 Continued.

Order	Family	Site <sup>a</sup>	Date <sup>b</sup>	Method <sup>c</sup>	<i>n</i>
	Lygaeidae	Elk	4	PF	2
	Miridae	Cos,Elk	1,2	LT,PF	4
	Pentatomidae	IK	2	LT	1
	Piesmatidae	Cos	4	PF	1
	Reduviidae	Cos,Elk	3,4	PF	2
Hymenoptera	Andrenidae	IK	4	PF	11
	Anthophoridae	Elk	4	PF	6
	Apidae	Elk	4	PF	1
	Bethylidae	Elk	3	PF	1
	Braconidae	Elk,IK	3	LT,PF	16
	Bradyobaenidae	Elk	3	PF	1
	Chalcididae	Elk	3	PF	5
	Colletidae	Cat,Cos,Elk	1,2,3,4	PF	5
	Cynipidae	Elk	1	LT	2
	Eucoilidae	Elk	3	PF	5
	Figitidae	Elk	3	PF	1
	Formicidae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	409
	Gasteruptiidae	Elk	3	LT	2
	Halictidae	Cos	1	PF	1
	Ichneumonidae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	22
	Megachilidae	Elk	4	PF	3
	Melittidae	Cat,Elk	3,4	PF	11
	Pompilidae	Elk	3	PF	1
	Scelionidae	Cos	3	PF	1
	Sphecidae	Elk	3,4	PF	9
	Tiphiidae	Elk,IK	1,3,4	PF	5
	Trigonalidae	Elk	2,4	PF	2
Lepidoptera	Apatelodidae	Cat	2	LT	1
	Arctiidae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	102
	Blastobasidae	Cos	2	LT	11
	Drepanidae	Cat,Cos,Elk,IK	1,2,3,4	LT	41
	Geometridae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	1190
	Hepialidae	Cos,Elk	1	LT	2
	Hesperiidae	Elk	3	PF	1
	Hyblaeidae	Cos	2	LT	11
	Lasiocampidae	Cat,Cos,Elk,IK	1,2,3,4	LT	20
	Limacodidae	Cat,Cos,Elk,IK	1,2,3	LT	23
	Lymantriidae	Cat,Cos,Elk,IK	1,2,3,4	LT	32



Table 1 Continued.

Order	Family	Site <sup>a</sup>	Date <sup>b</sup>	Method <sup>c</sup>	<i>n</i>
	Noctuidae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	496
	Notodontidae	Cat,Cos,Elk,IK	1,2,3,4	LT	115
	Oecophoridae	Cat	2	LT	4
	Papilionidae	Cat,Cos,Elk	3,4	LT,PF	3
	Pyralidae	Cat,Cos,Elk,IK	1,2,3	LT,PF	271
	Saturniidae	Cat,Cos,Elk	1,2	LT	14
	Sphingidae	Cat,Cos,Elk,IK	1,2,3,4	LT	28
	Thyatiridae	Cat,Cos,Elk	1,2,4	LT	104
	Tineidae	Cat,Elk	3	LT	2
	Tortricidae	Cat,Cos,Elk,IK	1,2,3,4	LT	32
	Zygaenidae	Cos	2	LT	1
Neuroptera	Corydalidae	Elk	1	LT	7
	Chrysopidae	Cos	2	LT	1
Orthoptera	Gryllacrididae	Cat,Cos,Elk,IK	1,2,3,4	LT,PF	323
	Gryllidae	Elk	2	LT	1
	Tettigoniidae	Cat,Cos,Elk,IK	2,3	LT	8
Plecoptera	Capniidae	Elk	1	LT	2
	Leuctridae	IK	3	LT	1
	Perlidae	Cat,Cos,Elk	1,2	LT	36
	Perlodidae	Cat,Elk	1,3	LT	2
Trichoptera	Hydropsychidae	Cat,Cos,Elk,IK	1,2,3	LT	36
	Leptoceridae	Elk	1	LT	3
	Limnephilidae	Cat,Cos,Elk,IK	1,2,3	LT	91
	Philopotamidae	Cat,Elk	1,2,3	LT	23
	Phryganeidae	Cat,Cos,Elk	1,2,3	LT	5
	Polycentropodidae	Cat,Cos,Elk	1,2,3	LT	43
	Psychomyiidae	Elk	1,2	LT	27
	Rhyacophilidae	Elk	1,3	LT	7

<sup>a</sup>Field Sites: Cat = Cataloochee (old growth); Cos = Cosby Creek (secondary growth); Elk = Elkmont (secondary growth); IK = Inadu Knob (old growth).

<sup>b</sup>Sampling Dates: 1 = June 5-8 1995; 2 = July 19-21 1995; 3 = September 3-8 1995; 4 = April 22-26 1996.

<sup>c</sup>Sampling Methods: LT = Light Trap; PF = Pitfall Trap.

Table 2. Spiders associated with old and secondary growth eastern hemlock in the Great Smoky Mountains National Park. All specimens were collected in pitfall traps.

Family	Genus	Species	Author	Site <sup>a</sup>	Date <sup>b</sup>	<i>n</i>
Amaurobiidae	<i>Callioplus</i>	<i>pantoplus</i>	(Bishop & Crosby)	Cos	1,3	2
	<i>Coras</i>	<i>aerialis</i>	Muma	Cat	4	2
	<i>Wadotes</i>	<i>calcaratus</i>	(Keyserling)	Cos,Elk	1,3,4	11
	<i>Wadotes</i>	<i>dixiensis</i>	Chamberlin	Cat,Elk	4	2
	<i>Wadotes</i>	<i>hybridus</i>	(Emerton)	Cos,Elk	3,4	19
	<i>Wadotes</i>	<i>tennesseensis</i>	Gertsch	Cat,Elk,IK	2,3	25
Antodiaetidae	<i>Antrodiaetus</i>	<i>unicolor</i>	(Hentz)	Cos	2,3	8
Araneidae	<i>Neoscona</i>	<i>arabesca</i>	(Walckenaer)	Cat	2	1
Corinnidae	<i>Scotinella</i>	<i>redempta</i>	(Gertsch)	Cat,Cos,Elk	1,3	3
Cybaeidae	<i>Cybaeus</i>	<i>patritus</i>	Bishop & Crosby	Cos,Elk	2,3	7
Dictynidae	<i>Cicurina</i>	<i>breviaria</i>	Bishop & Crosby	IK	4	1
	<i>Cicurina</i>	<i>arcuata</i>	(Keyserling)	Cos,Elk	1,2,4	3
Gnaphosidae	<i>Drassyllus</i>	<i>fallens</i>	Chamberlin	Elk	1	1
Leptonetidae	<i>Leptoneta</i>	sp.		Cos	2	1
	<i>Leptoneta</i>	<i>gertschi</i>	(Barrows)	Cos	2	2
Linyphiidae	<i>Bathyphantes</i>	<i>bishopi</i>	Ivie	Cat,Cos,IK	1,2,3	6
	<i>Eperigone</i>	<i>maculata</i>	(Banks)	Elk	3	1
	<i>Lepthyphantes</i>	<i>zebra</i>	(Emerton)	Elk	4	1
	<i>Meioneta</i>	sp.		Elk	4	7
	<i>Pocadicnemis</i>	<i>americana</i>	Millidge	IK	4	1
	<i>Walckenaeria</i>	<i>minuta</i>	(Emerton)	Elk	4	2

Table 2 Continued.

<b>Family</b>	<b>Genus</b>	<b>Species</b>	<b>Author</b>	<b>Site<sup>a</sup></b>	<b>Date<sup>b</sup></b>	<b><i>n</i></b>
	<i>Walckenaeria</i>	<i>brevicornis</i>	(Emerton)	Elk	4	9
	<i>Walckenaeria</i>	<i>pallida</i>	(Emerton)	Elk	4	2
Linyphiidae	?	?		IK	4	2
Lycosidae	<i>Pirata</i>	<i>montanus</i>	Emerton	Cat,Cos,Elk	1,2,3	59
Nesticidae	<i>Nesticus</i>	<i>tennesseensis</i>	(Petrunkévitch)	Cat,Cos	1,2	2
Pisauridae	<i>Dolomedes</i>	<i>tenebrosus</i>	(Hentz)	Cat	3	1
Salticidae	<i>Habrocestum</i>	<i>parvulum</i>	(Banks)	Elk,IK	1,4	3
	<i>Habrocestum</i>	<i>pulex</i>	(Hentz)	Elk	1	1
Tengellidae	<i>Liocranoides</i>	sp.		Cat,Cos,Elk	1,3,4	4
Tetragnathidae	<i>Leucauge</i>	<i>venusta</i>	(Walckenaer)	Cos,IK	1,2	2

<sup>a</sup>Field Sites: Cat = Cataloochee (old growth); Cos = Cosby Creek (secondary growth); Elk = Elkmont (secondary growth); IK = Inadu Knob (old growth).

<sup>b</sup>Sampling Dates: 1 = June 5-8 1995; 2 = July 19-21 1995; 3 = September 3-8 1995; 4 = April 22-26 1996.

Table 3. Shannon-Weiner diversity ( $H'$ ) and evenness ( $E$ ) values for insect families associated with old and secondary growth eastern hemlock and light and pitfall traps in the Great Smoky Mountains National Park.

	Shannon's $H'$	Shannon's $E$
Cataloochee – Old Growth	1.192	0.655
Inadu Knob – Old Growth	1.048	0.597
Cosby Creek – Secondary Growth	1.070	0.569
Elkmont – Secondary Growth	1.443	0.709
Light Traps	1.192	0.589
Pitfall Traps	0.882	0.481
All Sites/Sampling Methods	1.306	0.607

Table 4. Shannon-Weiner diversity ( $H'$ ) and evenness ( $E$ ) values for spider species associated with old and secondary growth eastern hemlock in the Great Smoky Mountains National Park.

	Shannon's $H'$	Shannon's $E$
Cataloochee – Old Growth	0.826	0.866
Inadu Knob – Old Growth	0.490	0.630
Cosby Creek – Secondary Growth	0.908	0.815
Elkmont – Secondary Growth	0.828	0.687
All Sites	1.058	0.739

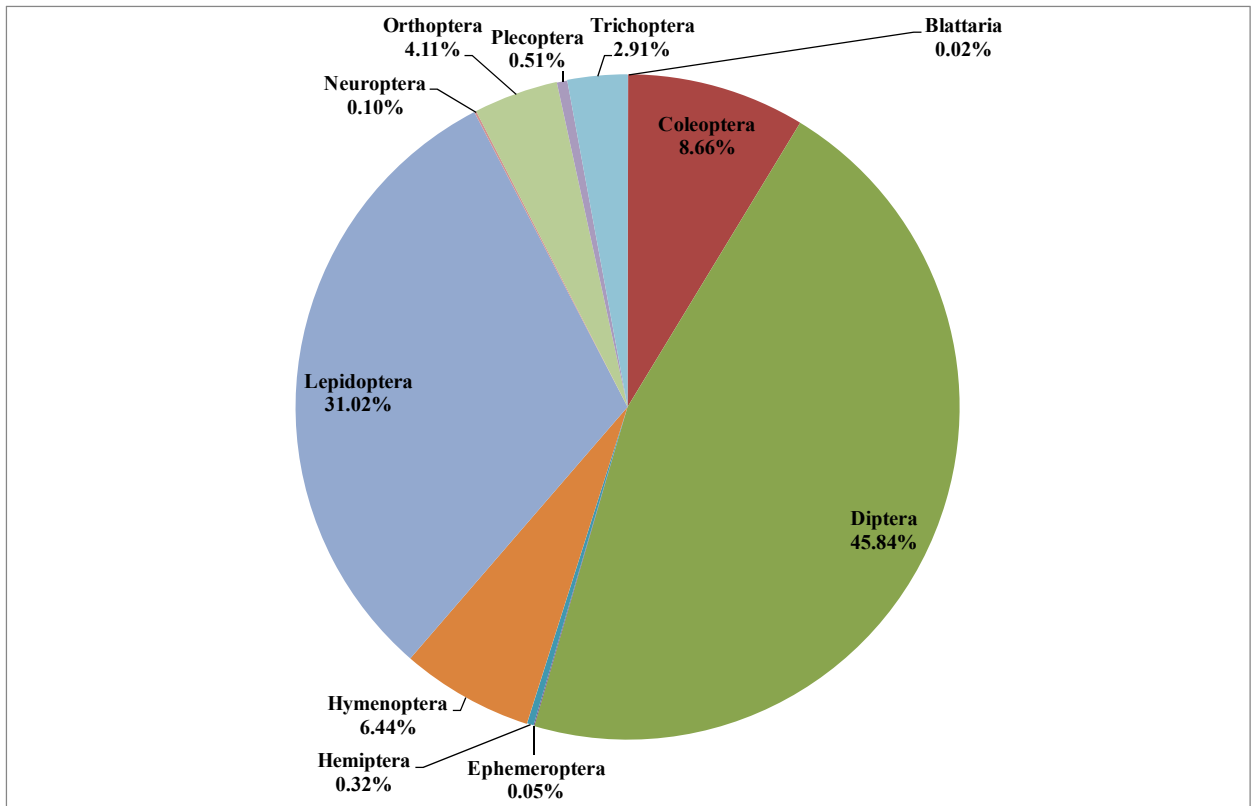


Figure 1. The relative abundance of insect orders captured in light and pitfall traps associated with eastern hemlock stands in the Great Smoky Mountains National Park.

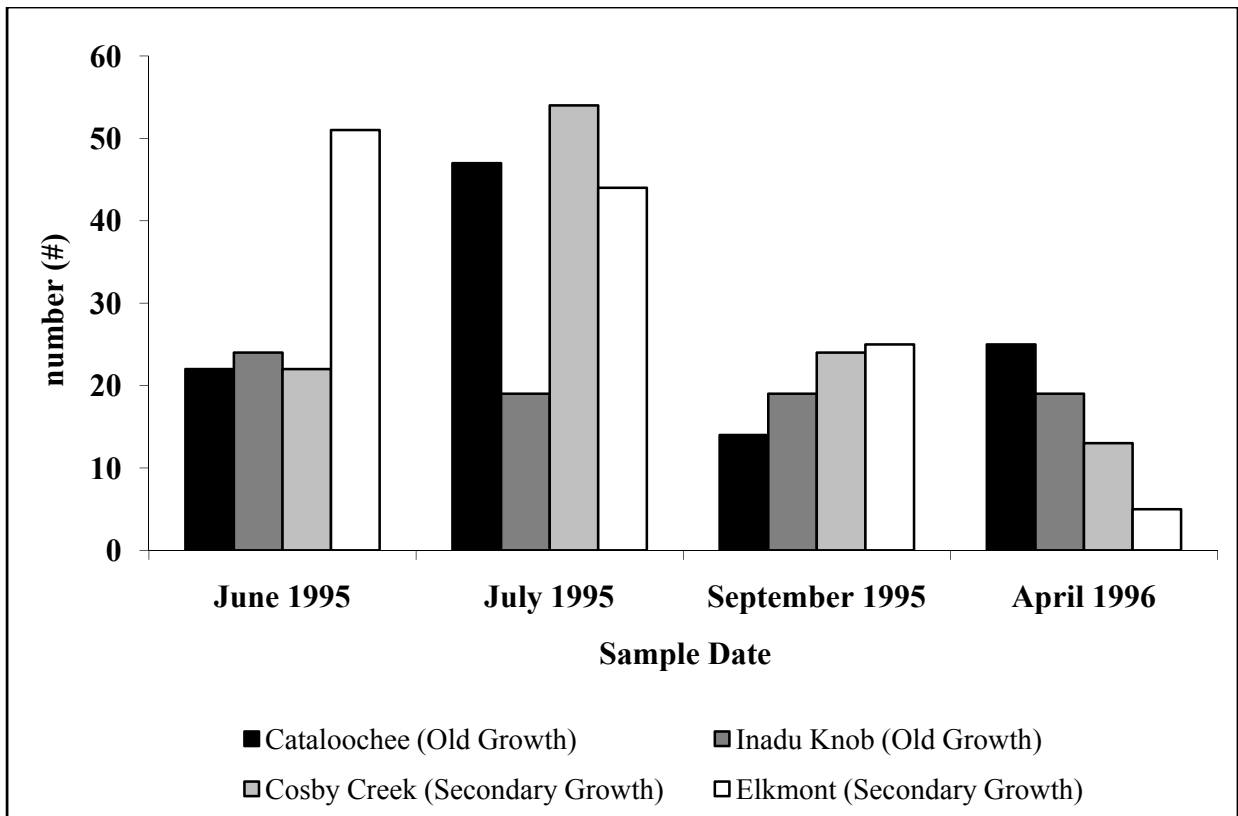


Figure 2. Seasonal abundance of insect families captured in light traps associated with old and secondary growth stands of eastern hemlock in the Great Smoky Mountains National Park.

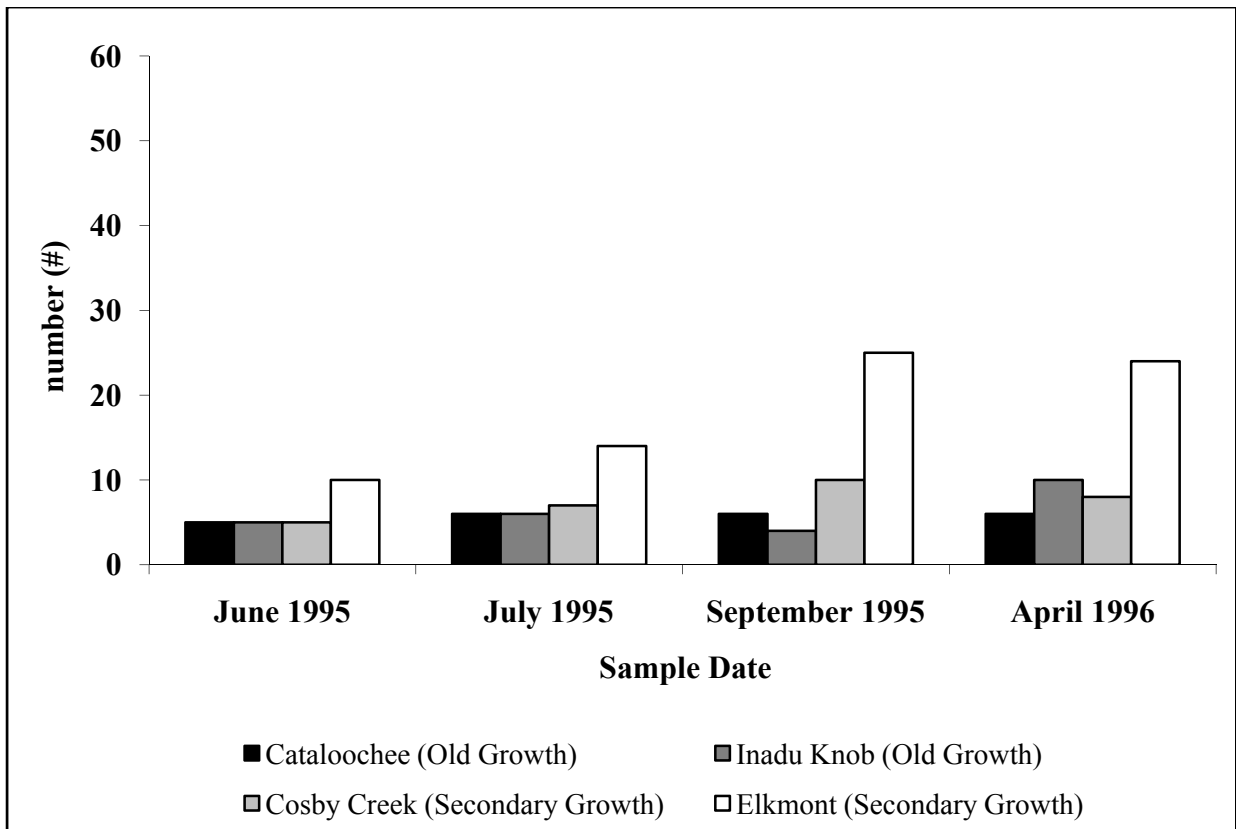


Figure 3. Seasonal abundance of insect families captured in pitfall traps associated with old and secondary growth stands of eastern hemlock in the Great Smoky Mountains National Park.

## DISSERTATION CONCLUSIONS

The hemlock (HWA, *Adelges tsugae*) and balsam (BWA, *A. piceae*) woolly adelgids continue to pose serious threats to forest ecosystems in the eastern United States. The research I presented in the preceding chapters offers several new insights that may be of importance to current and future management strategies involving biological control and host resistance for these invasive pests. Among studies related to adelgid biological control, data in Chapter II indicates that *Sasajiscymnus tsugae* will feed, oviposit, and complete immature development on a diet consisting of BWA, and does so at rates similar to its feeding on the primary prey HWA. This suggests that BWA may be suitable as an alternative rearing host for the predator and might be utilized to alleviate mass rearing delays associated with the aestival diapause of HWA. It also means that there may be a role for *S. tsugae* in biological control of BWA in plantations and, perhaps, natural stands of Fraser fir (*Abies fraseri*). However, additional research is needed to determine if one or both of these are viable strategies for adelgid management.

The data presented in Chapters II and III offer clues to how field releases and the utilization of *S. tsugae* for biological control of HWA might be improved. The predator was found to oviposit and survive for up to one month when introduced to adelgid infested eastern hemlocks (*Tsuga canadensis*) using a low-density, multiple-point, confined release technique. This is important because this release method ensures some level of predator reproduction in targeted release areas prior to adult dispersal, and may offer an improvement over currently utilized high-density, single-point, free releases. However, similar to this



commonly used release protocol, low-density, confined releases did not result in longer-term establishment of *S. tsugae* in the study areas. This may have been due to insufficient detection methods for the predator or, perhaps, a lack of supplemental food resources. The laboratory studies indicated that an additional carbohydrate resource was an important factor for the long-term survival of *S. tsugae* adults. This suggests that predator survival and establishment in the wild might also be improved by targeting predator releases in hemlock stands with abundant flowering plants or by periodically provisioning supplemental food resources, strategies that should be evaluated by future research.

The study I present that relates to hemlock host resistance is preliminary in nature, but its findings revealed a new avenue for research on a subject that until now found its only support in anecdotal field observations. For several years foresters, resource managers, and university researchers working in the southern Appalachian Mountains have noted that trees and stands of Carolina hemlock (*T. caroliniana*) that are infested with HWA do not appear to decline as quickly or suffer mortality at the same levels as those of eastern hemlock. With its close genetic relationship to putatively adelgid resistant Asian hemlock species it was hypothesized that Carolina hemlock may harbor some level of tolerance for HWA infestation that is missing in eastern hemlock. The results of the greenhouse infestation trial reported in Chapter IV indicate that this might be a possibility. Following artificial inoculation of seedlings with the adelgid, initial rates of infestation on Carolina hemlock were very low and statistically similar to those on putatively resistant western hemlock (*T. heterophylla*). Infestation levels on eastern hemlock were nearly four-fold higher. Although more research

is needed to verify this result and determine how tolerance to initial adelgid attack varies across the geographic range of Carolina hemlock, this species might serve as an important source of resistance genes for breeding of HWA resistant stock for restoration efforts. Furthermore, Carolina hemlock stands that tolerate adelgid attack for longer periods of time and remain healthy despite infestation might also be ideal locations for the establishment of field insectaries for HWA biological control agents.

The final study I present in Chapter V assessed the rooting ability of softwood stem cuttings from mature wild specimens of both eastern and Carolina hemlock. Although rooting percentages in eastern hemlock were higher than Carolina hemlock, for both species fewer than 50% of cuttings rooted successfully. Results might have been better for semi-dormant or dormant hardwood cuttings which should be evaluated. Despite the low rooting success the data do show that vegetative propagation via rooted cuttings is a viable low-cost alternative to grafting for cloning mature hemlock specimens of interest. This includes trees that have survived HWA infestation long-term and may harbor adelgid resistance or those that are important to gene conservation programs but have failed to produce seed.