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*University of Tennessee - Knoxville*

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To the Graduate Council:

I am submitting herewith a thesis written by Isaac K. Deal entitled "Life History of Hemlock Woolly Adelgid, *Adelges tsugae* Annand, on Eastern Hemlock, *Tsuga canadensis* (L.) Carriere, in the Southern Appalachians and Assessment of Egg Releases of *Sasajiscymnus tsugae* (Sasaji and McClure) for Its Management." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Paris L. Lambdin, David S. Buckley, James R. Rhea

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
To the Graduate Council:

I am submitting herewith a thesis written by Isaac Kent Deal entitled “Life history of hemlock woolly adelgid, Adelges tsugae Annand, on eastern hemlock, Tsuga canadensis (L.) Carriere, in the southern Appalachians and assessment of egg releases of Sasajiscymnus tsugae (Sasaji and McClure) for its management.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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David S. Buckley

James R. Rhea

Accepted for the Council:

Linda Painter
Interim Dean of Graduate Studies

(Original signatures are on file with official student records)
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A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Isaac K. Deal
May 2007
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Abstract

Studies were conducted in 2005 and 2006 at Baxter Orchard, Great Smoky Mountains National Park, Tennessee to optimize survival of Sasajiscymnus tsugae (St) (Sasaji and McClure) released as eggs on eastern hemlock, Tsuga canadensis (L.) Carriere, for biological control of Adelges tsugae (HWA) Annand. Studies investigated annual abundance and seasonality of HWA lifestages, weekly field and laboratory survival of St, and survival of St egg cohorts of various ages and densities. HWA was determined to be bivoltine on eastern hemlock in Tennessee with an aestivation period between July and October. Lifestages preferred as food by St were present from February through June with peak abundance ca. late March. Weekly survival of St placed in the field and laboratory as eggs was investigated. Less than 10% of St eggs placed in the field between 8 February to 22 March survived because of freezing field temperatures. Survival rose to 30-40% on 30 March and persisted at that level until 7 June, when survival decreased below 10% due to scarcity of food. Studies were conducted to investigate the effect of St egg density on survival of St. Four densities of St eggs (50, 100, 150, and 200 eggs/container) were placed in the field and laboratory. In the laboratory, survival was higher for lower densities of St eggs, implying cannibalism increases with density. Three egg age classes (0-2, 3-5, and 6-8 days old) were used to test the effect of St egg age on survival. St in field cages showed poor survival, likely due to freezing temperatures. However, the oldest (6-8 day) age class showed significantly greater survival in the field than the younger age classes. The two oldest age classes showed significantly greater survival than the youngest (0-2 day) age class in the
laboratory. Results of multiple studies over two years support the release of $S_t$ eggs to enhance biological control efforts against HWA. Studies showed $S_t$ established as eggs in the field could successfully develop into adults. Survival of $S_t$ from egg releases is highest when $S_t$ eggs are released between late March and May, in low densities, and in older age classes.
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Chapter I. Introduction

i. Importance of Invasive Species

Increases in human commerce, migration, and transport, along with faster modes of transportation, have facilitated the spread of species outside of their native ranges (Lounibos 2002, Mack et al. 2000). The spread of species may be incidental, such as the spread of marine organisms via ballast water in ships, or intentional, such as the spread of exotic plants in the nursery trade (Mack et al. 2000, Simberloff 2003). While many of these displaced species may be unsuited to their new environments, some successfully establish, thrive, and spread. The number of species that are spread to environments where they are not indigenous and the number of such displaced species that establish in their new environments are virtually impossible to quantify. However, it is believed that the vast majority of displaced species perish rather than establish in their new environment. A number of synonyms, such as alien, adventive, exotic, neophytes, and introduced, are used, often interchangeably, when referring to displaced species that successfully establish in environments they are not native to and compete with, or otherwise impact, species native to those environments (Mack et al. 2000). The term “invasives” will be used in this thesis to refer to such species.

Quantifying the environmental and economic costs associated with invasives is difficult. Cost estimates, both worldwide and national in scope, are complicated by the fact that many species are yet undescribed. Merely ca. 1.5 million of the ca. 10 million species worldwide have been described and only ca. 375,000 of the ca. 750,000 species in the United States (U.S.) have been described (Pimentel et al. 2000, 2001). However, it is clear that invasives pose a threat to the survival of native species by preying on and
parasitizing native species, vectoring pathogens that affect native species, altering native habitat, competing with native species for resources, and hybridizing with native species (Pimentel et al. 2001, Simberloff 2003). Few regions on earth have not been affected by invasives (Mack et al. 2000). In the U.S., invasives are second only to habitat degradation/loss as a threat to species listed under the Endangered Species Act and in the Natural Heritage system databases (Groat 2001, Simberloff 2003). The U.S. hosts ca. 50,000 invasive species (Pimentel et al. 2000).

Numerous examples of losses due to invasives in the U.S. are present in the literature including: $51.4 billion annual losses in agriculture due to invasive weeds and plant pathogens, $7 billion annual losses of forest products due to exotic insects, $1 billion annual damage to structures due to the Formosan termite, *Coptotermes formosanus* (Shiraki), and $1 billion annual damages from the red imported fire ant, *Solenopsis invicta* Buren (Pimentel et al. 2000, 2001). Invasives are recognized both nationally and internationally as having negative economic and ecological impacts. As a result, legal regulation, research, and management of invasives are being pursued more aggressively (Leung et al. 2005, Simberloff 2003). Charles Groat (2001), director of the U.S. Geological Survey (1998-2005), articulated the threats posed by invasive species:

The spread of invasive plants, animals and pathogens is considered one of the most serious ecological problems facing our nation in the 21st century, second only to habitat destruction...

…Many invaders cause huge losses in agriculture, livestock, fisheries, and other resource production systems. Some significantly alter ecosystems, resulting in costly damages due to increases in fire, flooding, and erosion...

…Invasive species are a growing threat to the Department of the Interior's stewardship of the Nation's natural resources. They are currently estimated to infest more than 30 percent of the acreage of the National Park System in the lower 48 states. By various estimates, these species contribute to the decline of 35 to 46 percent of U.S. endangered and threatened species.
In response to the growing economic and ecological threats caused by invasive species, President Clinton issued an executive order in 1999 which formed a national Invasive Species Council. The council was given the prerogative of providing national leadership for the management of invasive species. Duties of the Invasive Species Council include: organizing effective, cost-efficient cooperation between federal agencies with regard to invasive species, encouraging planning and action regarding invasive species at many organizational levels both nationally and internationally, facilitating the development of a coordinated network among federal agencies to document, evaluate, and monitor impacts of invasive species, facilitating the establishment of an information-sharing system that facilitates access to and exchange of information regarding invasive species, and preparing and issuing a national Invasive Species Management Plan which details and recommends performance-oriented objectives and specific measures of success for Federal Agency efforts concerning invasive species (USFG 1999).

Many important invasive species are insects. Important invasive insects include crop pests such as the diamondback moth, *Plutella xylostella* (L.), European corn borer, *Ostrinia nubilalis* (Hubner), and Hessian fly, *Mayetiola destructor* (Say), forest pests such as the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), balsam woolly adelgid, *Adelges piceae* (Ratzeburg), and gypsy moth, *Lymantria dispar* (L.), and pests of medical importance such as the Asian tiger mosquito, *Aedes albopictus* Skuse, Africanized honey bee, *Apis mellifera scutellata* Lepeletier, and red imported fire ant (Bugwood 2005). Invasive insects impact agriculture, forestry, and urban areas, causing innumerable losses each year in degraded food, health, industry, and shelter to billions of people worldwide (Lounibos 2002, Pimentel et al. 2000, 2001). This thesis addresses the
hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, an invasive insect of particular concern for hemlocks in eastern North America.

**ii. Hemlock**

**Distribution of Hemlock**


The Carolina hemlock is found along the Appalachians from Virginia and West Virginia, through the Carolinas and Tennessee and into Georgia (Godman and Lancaster 2003, Stupka 1989). The eastern hemlock ranges from Michigan, east and north to Nova Scotia and south following the Appalachians to Georgia (Fig. 1) (Godman and Lancaster 2003). Hemlock is dominant or codominant in four forest cover types (eastern hemlock, hemlock-yellow birch, white pine-hemlock, and yellow poplar-eastern hemlock) and is a component or associate in many other forest types in the eastern U.S. and Canada (Brisbin 1970, Eyre 1980). The ranges of eastern and Carolina hemlock together span ca. 1.3 million hectares (Rhea 1995).
Figure 1. Native range of eastern and Carolina hemlock (adapted from USGS 2006)

**Hemlock in Great Smoky Mountains National Park**

Great Smoky Mountains National Park (GRSM) was established in 1935 and encompasses 212,460 hectares of the Blue Ridge Mountains in western North Carolina and eastern Tennessee. GRSM is the largest area in the eastern U.S. managed as wilderness. Also, it is more visited than any other national park in the U.S.; with ca. 10 million visitors annually. Although 80% of the land in GRSM has been logged, 294 hectares of old growth hemlock, some exceeding 400 years old, have been reported in the park (Godman and Lancaster 2003, J. F. Grant, personal comm., Johnson et al. 1999, 2005a; Little 1997).

Eastern hemlock is the only native hemlock species found in the GRSM. Eastern hemlock commonly occurs in forests below 1,520 m and prefers shaded habitats along streamcourses, cool ravines, north slopes in the lower elevations, and ridges above 1,070 m (Stupka 1989). Johnson et al. (1999) reported that eastern hemlock was widely distributed in the Park with elevations ranging from 460 to 1,740 m.

Eastern hemlock prefers moist soils with good drainage and is the most shade-tolerant (able to tolerate as little as 5% normal sunlight) of the eastern conifers. Pure
stands of eastern hemlock form such a dense canopy that an understory usually can not
develop. However, eastern hemlocks can persist in heavily shaded understories for as
long as 200 years with a diameter at breast height (dbh) of only several cm in mixed
stands. Eastern hemlock is slow-growing and may take 250-300 years to fully mature but
may live 800 or more years, reaching a diameter of 193 cm, and height of 53 m (Brisbin

GRSM hosts many of the largest and, presumably, oldest specimens of eastern
hemlock in the world, many measuring more than 4.5 m in circumference. The largest
hemlock ever recorded, measuring 6 m in circumference, is located ca. 1.6 km west of
Brushy Mountain in GRSM, along Surry Fork at an altitude of 1,051.6 m. Another large
specimen located along the Indian Camp Creek Trail to Maddron Bald in GRSM
measures over 5.2 m in circumference (Stupka 1989).

**Economic Importance of Eastern Hemlock**

The wood of eastern hemlock is not of great economic value. Hemlock wood is
course-grained, of uneven texture, and prone to splintering. Lumber made from hemlock
is susceptible to rot (Brisbin 1970). However, eastern hemlock wood lacks odor and taste
and has been used to make food containers (Howard et al. 2000). Eastern hemlock wood
pulp is dark and difficult to bleach, making it undesirable for use in high-quality paper,
instead it is primarily used for newsprint and wrapping paper (Brisbin 1970).

The principle use of hemlock lumber is for light framing, sheathing, roofing, and
subflooring. Eastern hemlock wood also is used in boxes, crates, pallets, signs, and other
low-value wood products (Brisbin 1970). An informal survey of eastern hemlock
roundwood purchasers indicated the wood was used for pulpwood, dimension door lumber including studs, boards, construction timber, post and beam house frames, bridges, plywood core veneers, landscape timbers (despite eastern hemlock timber’s susceptibility to decay), and landscaping mulch. The author suggested that the latter was likely the most valuable commercial use of eastern hemlock today. New Hampshire and Vermont each annually produced 10 to 20 million board feet (bf) of eastern hemlock over the last four decades. Maine annually produced 40 to 65 million bf of hemlock sawtimber between the mid-1970s and mid-1980s. Since then, annual eastern hemlock sawtimber production in Maine has risen to between 80 and 110 million bf (Howard 2000). Although eastern hemlock lumber is not of great value, losses due to HWA may have a substantial effect on the wood products industry of the northeastern U.S. (Brisbin 1970, Rhea 1995).

Eastern hemlock is one of the most desired ornamental conifers (Brisbin 1970). Eastern hemlock has, by far, the most known, or previously grown, varieties of any hemlock species (Welch and Haddow 1993). Qualities that contribute to eastern hemlock’s appeal as an ornamental include: conical shape, drooping fan-shaped branches, propensity to provide densely shaded areas, longevity, and large size potential (Holmes et al. 2006). Approximately $34 million of eastern hemlock growing stock is maintained in the nursery industry in North Carolina and Tennessee. Spread and public awareness of HWA has manifested as reduced sales in hemlock nursery stock in these states (Rhea 1995). A property value study in Sparta, New Jersey, found empirical evidence that decline in eastern hemlock due to HWA is associated with the value of residential private property. Reductions in value of private property occurred not only on property
containing eastern hemlocks in decline but also adjacent property; indicating that the reductions in property value were due to aesthetics (Holmes et al. 2006).

**Ecological Importance of Eastern Hemlock**

Eastern hemlock is an integral component of many old growth communities (Buck et al. 2005). The precise consequences of loss of eastern hemlock due to HWA are not well understood (Snyder et al. 2002). However, wide consensus amongst the literature suggests that important changes in abiotic factors, such as light penetration, and ecological processes, such as nutrient cycling and decomposition, will occur in ecosystems where eastern hemlock is common (Kizlinski et al. 2002, Orwig and Foster 1998, Snyder et al. 2002, Stadler et al. 2005). Loss of eastern hemlock trees due to HWA causes gaps in the forest canopy which facilitate the invasion of invasive plant species, such as tree of heaven, *Ailanthus altissima* (Miller) Swingle, Japanese barberry, *Berberis thunbergii* DC, and Japanese stilt-grass, *Microstegium vimineum* (Trin.) A. Camus (Evans et al. 1996). Black birch, *Betula lenta* L., a native tree, also aggressively fills gaps left by dead and dying hemlocks in the northeastern U.S. (Orwig and Foster 1998, Stadler et al. 2005).

Many animals are directly, indirectly, or partially reliant on eastern hemlock trees for habitat (Jordan and Sharp 1967, Quimby 1996, Snyder et al. 2002, Tingley et al. 2002). Eastern hemlock is important to several bird species including the black-throated green warbler, *Dendroica virens* (Gmelin), and Acadian flycatcher, *Empidonax virescens* (Vieillot) (Tingley et al. 2002). It also is associated with a diverse array of insects (ca. 280 species in ca. 80 families) (Buck et al. 2005). Eastern hemlock’s dense foliage
produces a distinct microclimate, which along with its slow growth, shade tolerance, and extensive range make it the most important shelter species for game among the coniferous trees in the eastern U.S. (Buck et al. 2005, Jordan and Sharp 1967). It provides cover for many species including ruffled grouse, *Bonasa umbellus* (L.), turkey, *Meleagris* spp., snowshoe hare, *Lepus americanus* Erxleben, and rabbit, *Oryctolagus* spp. (Jordan and Sharp 1967, Quimby 1996). White-tailed deer, *Odocoileus virginianus* Zimmerman, and porcupines, *Erethizon dorsatum* (L.), are dependent on eastern hemlock for winter shelter. Eastern hemlocks shade streams, mediate stream temperatures, increase diversity of stream invertebrates, and facilitate the survival of some trout species (Snyder et al. 2002).

iii. Hemlock Woolly Adelgid

*Origin and Spread of Hemlock Woolly Adelgid*

HWA is a minute (ca. 1 mm long) hemipteran pest of hemlock trees (McClure 1989). HWA was first reported in North America in the western U.S. ca. 1924 where it had a minor impact on western hemlock and mountain hemlock (Annand 1924, Havill et al. 2006, McClure and Cheah 1999, Stoetzel 2002). Recent analysis of HWA mitochondrial DNA indicates HWA in eastern North America is of a different lineage than HWA in western North America, and matches the lineage of HWA in Honshu, Japan. Spread and establishment of HWA from western North America to eastern North America has not been documented. The variety of HWA in western North America was either introduced from a yet unknown location or is endemic (Havill et al. 2006).
The HWA poses a dire threat to the existence of hemlock trees in eastern North America. In Honshu, Japan, HWA is a ubiquitous, yet innocuous, herbivore of the northern Japanese hemlock, and the southern Japanese hemlock. HWA densities are believed to be low in Japan because of a combination of host plant resistance and an effective guild of natural enemies of HWA (McClure and Cheah 1999).

HWA was first collected in the eastern U.S. in 1951 in Richmond, Virginia, and spread to Pennsylvania by 1969 and Maryland by 1973 (Stoetzel 2002). Since then, it has spread north to Massachusetts, south to North Carolina, and west to West Virginia and Tennessee (Fig. 2) (Broeckling and Salom 2003b).

**Previous Life History Studies on Hemlock Woolly Adelgid**

The life history of HWA has previously been studied in the northeastern U.S. HWA is parthenogenic on eastern hemlock, with two non-migratory generations: sistens, and progrediens, and one migratory generation: sexupara. Sistens are present from August through March, progrediens are present from February through August, and sexupara are present February through July. Eggs produced by sistens develop into progrediens. Eggs produced by sistens appear homologous but each egg develops into a crawler of one of two different generations: progrediens or sexupara (McClure 1989).

All generations of HWA that occur on eastern hemlock progress through four nymphal instars before reaching adulthood. Eggs hatch into a mobile first instar (crawler) which walks along the host plant until finding a suitable site to insert its stylet. Once the adelgid inserts its stylet into the eastern hemlock, it becomes sessile (hereafter referred to
Figure 2. Distribution of hemlock woolly adelgid, *Adelges tsugae* Annand, in the eastern United States in 2005 (USDA 2005)

as settled) for the remainder of its life, in the sisten and progredien generations, or until reaching adulthood in the sexupara generation (McClure 1989).

Sexupara adults are winged and mobile (McClure 1989). All other known adelgid species that produce winged adults complete part of their life cycle on spruce (*Picea* spp.). Likewise, winged adult HWA sexupara are believed to migrate to, and complete a lifecycle on, spruce. Field and laboratory experiments demonstrated that sexupara lay eggs in June, which hatch into a sexual generation in July, known as sexuales on spruce (McClure 1989, 1992). However, prolonged survival and development of sexuales on
spruce have not been demonstrated (McClure 1989). *Picea jezoensis hondoensis* (Sieb. and Zucc.) and *P. polita* (Carriere) are suspected hosts for sexupara and sexuales in Japan (McClure and Cheah 1999). Fortunately, sexuales do not survive more than a few days on tested native and introduced spruce species common in the eastern U.S. However, little is known about the sexuale generation of HWA (McClure 1989, 1992).

HWA exhibits an unusual tolerance to prolonged low temperatures (Cheah and McClure 2000). However, seasonal development of HWA in Japan varies greatly and is dependent on elevation and the phenology of the hemlock species (McClure and Cheah 1999). Given the differences due to elevation in Japan, along with the fact that differences in latitude approximate differences in elevation, development of HWA in the southeastern U.S. would be expected to be different than in the northeastern U.S. where previous life history studies on HWA have been conducted.

**Mechanisms of Hemlock Woolly Adelgid Spread**

HWA crawlers are ambulatory and are capable of dispersing short distances by walking, which may explain the patchiness of HWA infestations observed on hemlock. Tree to tree dispersal is facilitated by wind, birds, deer, and likely any other mechanical vector. HWA eggs and crawlers have been captured on sticky traps over 1,300 m from an infested eastern hemlock stand, likely blown there by the wind. HWA suddenly appeared in Connecticut in 1986. The distribution of HWA infestations in Connecticut along the coast, major waterways, and relatively high altitudes suggests that HWA was spread to Connecticut by Hurricane Gloria in 1985 from Long Island, New York, a distance of about 25 km. Other anecdotal evidence suggests birds as a major agent of HWA
dispersal. Several residential neighborhoods that stocked bird feeders year round in Connecticut and Rhode Island harbored isolated infestations of HWA before HWA became widespread in those states. Birds have been found capable and likely to carry HWA (primarily crawlers) on their bodies in areas where HWA-infested hemlocks are present. Browsing deer can spread HWA to hemlock seedlings. Human activities, such as salvage cutting of eastern hemlock and shipment of infested eastern hemlock plant material, also are suspected to spread HWA (McClure 1990).

iv. Outlook and Management

Overview

No eastern or Carolina hemlock has recovered, without the aid of insecticides, after being infested with HWA. Furthermore, no recovery at the stand level has been documented (Snyder et al. 2002). Feeding by HWA causes death of the hemlock within four to six years (McClure 1991, Cheah and McClure 1998). No known limiting factors exist for the expansion of HWA in the eastern U.S. If left unchecked, HWA may spread throughout the entire range of eastern and Carolina hemlock (Orwig and Foster 1998).

Management Options

Invasive insect pests are managed in similar ways as native insect pests. The most-used method of insect control is the application of chemical insecticides. However, the overuse of this management tactic can lead to the buildup of chemical resistance among pest species, pest resurgence, and secondary pest outbreaks. Furthermore, the
application of chemical pesticides is not cost effective or practical in some situations. Therefore, many pest management plans are adopting a holistic approach to pest control. This approach is termed integrated pest management (IPM) and incorporates alternative control methods (biological, cultural, genetic, legal, physical, and mechanical) with traditional chemical control to manage pest populations.

While the use of chemical insecticides and insecticidal oils are effective in suppressing HWA infestations on individual trees in managed settings, these methods are too costly in terms of dollars and manpower to provide control in large scale natural areas. Because of the cost of chemical control and the ecological sensitivity of hemlock habitat, biological control is seen as the most effective control method at this time (Broeckling and Salom 2003a, Francis 2004).

v. Biological Control

Biological control seeks to reduce pest populations by introducing and establishing natural enemies, generally predators and parasitoids, of the pest into the environment. If successful, biological control usually reduces pest populations indefinitely with the biological control agents reproducing and spreading independently of human support. However, this ability to reproduce, increase in number, and spread independently also presents unique risks from the use of biological control agents (Johnson et al. 2005b).
Native and Established Exotic Predators

Several predatory insects that occur on eastern hemlocks have been documented to consume HWA. Examples from the families Cecidomyiidae, Coccinellidae, Chrysopidae, Hemerobiidae, and Syrphidae have been observed. Of these, the Asian multicolored lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), and brown (Neuroptera: Hemerobiidae) and green lacewings (Neuroptera: Chrysopidae) are likely the most important in terms of abundance. However, none of these established predators have been effective in controlling infestations of HWA (Wallace and Hain 2000).

Biological Control Using Non-native Predators

Several non-native beetle species have been found to feed on HWA. It is believed that a complex of these predators will be necessary to provide maximum control of HWA (J. R. Rhea, personal comm.). *Sasajiscymnus tsugae* (St) (Sasaji and McClure), a Japanese lady beetle, is the favored biological control agent for suppression of HWA (Cheah and McClure 2000). Other beetle species being reared, evaluated, and considered for biological control include: *Laricobius nigrinus* Fender (a derodontid beetle native to western North America) and several Chinese lady beetles in the *Scymnus* genus (personal observation, J. F. Grant, J. R. Rhea, personal comm.).

*Sasajiscymnus tsugae*

In 1992, St was observed feeding on HWA in Honshu, Japan (Sasaji and McClure 1997). This small (ca. 1.6 mm long) lady beetle occurred in more than 30% of forest and
ornamental sites sampled where average adelgid mortality of over 90% was observed (Sasaji and McClure 1997, Cheah and McClure 2000). The potential for *St* as a biological control organism was recognized. The U.S. Department of Agriculture Animal Plant Health Inspection Service Plant Protection and Quarantine (APHIS) evaluated the beetle for nontarget effects, issuing a permit for its release in 1996 (Cheah and McClure 1998). *St* was originally described in 1997 as *Pseudoscymnus tsugae* (Sasaji and McClure 1997) but the name of the genus ‘*Pseudoscymnus*’ was found to have previously been described as a shark genus (Vandenburg 2004). The coccinellid genus ‘*Pseudoscymnus*’ was subsequently renamed ‘*Sasajiscymnus*.’

Life history studies on *St* have mostly occurred in the northeastern U.S. *St* is bivoltine with the first cohort of eggs laid between May and July and the second cohort of eggs laid between August and October. Lifetime fecundity of females is estimated between 64 and 513 eggs (Cheah and McClure 2000). Females generally lay single eggs in cryptic locations, such as curled bud scales, empty male cones, and under the woolly masses produced by HWA (Cheah and McClure 1998). Development from egg to adult in the field ranges from 30 to 60 days (Cheah and McClure 2000). *St* has four larval instars, which is common among aphidophagous coccinellids (Cheah and McClure 1998, Koch 2003). The fourth instar larva experiences the longest developmental time, followed by the first instar larva, a typical occurrence in coccinellids not limited by prey or environmental conditions (Cheah and McClure 1998). *St* are opportunistically cannibalistic on eggs, larvae, pupae, and adults (personal observation). The average lifespan of *St* exceeds 120 days in the laboratory. However, *St* adults overwinter in the field, which likely prolongs their lifespan. Time of oviposition, developmental time of *St*
lifestages, and the lifespan of _St_ is highly dependent on temperature (Cheah and McClure 2000).

The rearing and release of _St_ has long been the focus of biological control efforts against HWA. Mass releases of _St_ between 1995 and 1999 in Connecticut alone totaled 100,000 (Cheah and McClure 2000). Four mass rearing facilities operated by Clemson University, Ecoscientific Solutions, the North Carolina Department of Agriculture, and the University of Tennessee currently supply _St_ used in biological control programs. However, the rearing process for _St_ is costly, time-consuming, and labor-intensive making it impossible for rearing facilities to meet the demand. Releasing _St_ as eggs, rather than adult beetles, has been suggested as a way to reduce labor and costs associated with rearing and to increase production. The germinal idea of _St_ egg releases was developed by Daniel Palmer of the New Jersey Department of Agriculture (J. F. Grant, personal comm.). Although releasing _St_ as eggs is enticing from a rearing perspective, the ability of _St_ released as eggs to develop to adults in the field and factors associated with survival of _St_ released as eggs in the field have not been investigated.

**vi. Objectives of Research**

The main goal of this research was to optimize survival of _St_ placed in the field as eggs in GRSM in Tennessee. Four main objectives were followed to this end: 1) determine the abundance and seasonality of HWA in Tennessee, 2) assess the effect of time of year on survival of _St_ placed in the field, 3) assess the effect of _St_ egg density on survival of _St_ placed in the field, and 4) assess the effect of _St_ egg age on survival of _St_
placed in the field as eggs. Additional studies were conducted to determine the effect of sleeve cages on abundance of HWA and the effect of temperature on survival of St eggs. These latter two studies were conducted to clarify and enhance the results of main objective studies.
Chapter II. Life History of the Hemlock Woolly Adelgid in the Great Smoky Mountains National Park

i. Introduction

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an invasive insect from Honshu, Japan, that attacks eastern hemlock, *Tsuga canadensis* (L.) Carriere, and Carolina hemlock, *Tsuga caroliniana* Engelmann. HWA has recently invaded the Great Smoky Mountains National Park (GRSM), an international biosphere reserve, where infestations of the pest on eastern hemlock are disrupting native ecosystems and degrading scenic beauty and have generated much concern from both the public and private sectors (Anon. 2006).

Most of what is known about the lifecycle of HWA is based on a life history study conducted in Connecticut by McClure (1989). Three parthenogenic generations of HWA occur on hemlock, two of which (the sistens and progrediens) are non-migratory and reproduce on hemlock. The sexupara are a parthenogenic generation which co-develop with progrediens on hemlock from heterogeneous egg masses oviposited by the sistens. Sexupara produce wings upon reaching adulthood and are believed to migrate to, and lay eggs on, spruce (*Picea* spp.) which develop into a sexual generation: the sexuales. Life history of the sexuales is unknown at this time (McClure 1989).

The HWA on eastern hemlock progress through four nymphal instars before reaching adulthood. Eggs hatch into a mobile first instar (crawler) which searches the host plant until finding a suitable site to insert its stylet. Once a HWA crawler inserts its stylet, it becomes sessile for the remainder of its life, in the sisten and progredien generations, or until reaching adulthood in the sexupara generation (McClure 1989).
Differences in latitude between Connecticut and Tennessee translate into differences in climate; Connecticut generally experiences colder winters than Tennessee. Differences in climate between Tennessee and Connecticut likely affect the seasonality of HWA. Differences in latitude between two points result in differences in climate comparable to differences due to elevation. Thus, findings that seasonal development of HWA in Japan is, at least partially, dependent on elevation (McClure and Cheah 1999) lends support to the hypothesis that seasonality of HWA is different in Tennessee than in Connecticut.

Studies were developed to elucidate the life history of HWA in Tennessee as well as the effect that enclosure in sleeve cages (used in biological control studies) has on HWA. The objectives of these studies were to determine: 1) the presence, abundance, and seasonality of various lifestages of HWA in the field throughout the year, and 2) the effect that sleeve cages has on the presence and abundance of these lifestages.

ii. Materials and Methods

Site

This study was conducted in GRSM at Baxter Orchard (35°45’75”N 83°16’60”W) (elevation ca. 610 m) in Cosby in Sevier Co., TENNESSEE (Fig. 3). The Baxter Orchard site consists of farmland that has regenerated into a mixed-hardwood forest. GRSM purchased the land at the Baxter Orchard site in 1955 (Kent Cave, personal comm.). The area contains a substantial number of eastern hemlocks of various sizes and ages with HWA infestations (first documented in late 2004). None of the eastern
Figure 3. Location of Baxter Orchard site in the Great Smoky Mountains National Park hemlocks at the site had been previously treated for HWA (Tom Remaley, personal comm.).

Rhododendron and/or laurel dominates the understory vegetation and forms thickets in some areas (personal observation, Tom Remaley, personal comm.). In areas with thin canopy cover, greenbriar, *Smilax* spp., and poison ivy, *Toxicodendron radicans* (L.), dominate the understory vegetation (personal observation).

**Seasonality and Lifecycle of the Hemlock Woolly Adelgid**

Five branches (each ca. 0.6 m long) of *T. canadensis* infested with HWA were cut from selected trees (n=2) at the Cosby site once every two weeks from 8 August 2005 to 13 December 2005 and from 4 January 2006 to 2 August 2006. The branches were placed in separate plastic garbage bags, transported to the laboratory, and processed for HWA. For detailed description of processing and determination of HWA lifestages and generations see the “Processing Branches of *T. canadensis* Infested with HWA” section below.

**Effect of Enclosure in Sleeve Cages on Hemlock Woolly Adelgid**

On 27 February, 12 terminal branches (ca. 0.6 m long) of *T. canadensis* infested with HWA at the Cosby site were chosen for study and on 30 March and 27 April 2005, 24 terminal branches (ca. 0.6 m long) of *T. canadensis* infested with HWA at the Cosby site were chosen for study. These branches were located on the lower 3 m of each tree where branches were accessible within arm’s reach on the ground or on a step ladder. Sleeve cages (12 in February and 24 each month in March and April) (31 cm x 68 cm) made of no-see-um plastic netting with a zipper spanning the length of the cage, sewn closed on one end and with a draw-string closure on the other, were used to encase the
selected branches (Fig. 4). Sleeve cages were secured to the branches and closed by cinching, wrapping, and tying the draw-string cord to closely appress the no-see-um mesh to the branch and prevent the movement of small insects into or out of the sleeve cage. Two to four plastic zip ties were tied below the cord to further increase security of the sleeve cages. Each sleeve cage was marked with a flag.

For each of the monthly field studies, three sleeve cages were removed from the field each week (four weeks in February and seven weeks in March and April). The remaining three sleeve cages in March and April were removed from the field after 13 weeks. All sleeve cages were removed from their respective trees by cutting the enclosed branch to which each was fastened ca. 3 cm above the cage. The sleeve cages, and the

![Figure 4. Typical sleeve cage used in field studies attached to an infested hemlock](image-url)
branches that they enclosed, were taken to the laboratory, opened, and processed for HWA.

Concurrently, uncaged branches (ca. 0.6 m long) of *T. canadensis* infested with HWA were removed from the field on the same schedule and in the same manner as caged branches. Uncaged branches were cut from the tree, placed in separate plastic garbage bags, taken to the laboratory, and processed for HWA. The uncaged branches served as a control for the caged branches to determine differences in the abundance of HWA lifestages due to microclimate effects resulting from sleeve cages.

Data were pooled from three studies conducted in 2005. Mean number of lifestages of HWA on caged and uncaged branches were calculated along with total number of HWA. Percent difference was calculated for total weekly densities of HWA on caged and uncaged branches.

**Processing Branches of *T. canadensis* Infested with HWA**

In the laboratory, ten apical vegetative shoots (branchlets) were randomly selected from each branch. The newest growth and next oldest growth were then measured for each branchlet. Any branchlets that had less than 1 cm of newest or next oldest growth were discarded and new branchlets were measured in their place until ten branchlets of suitable length were acquired. Up to 2 cm of the basal-most newest growth and up to 2 cm of the apical-most next oldest growth (making a total of 2-4 cm of contiguous hemlock foliage) were then viewed. All live HWA were classified, counted, and recorded according to lifestage: egg mass, crawler, settled first instar, second/third/fourth instar, sessile adult, and winged adult.
First-instar settled nymphs were distinguished by their small size, “football-like” shape, lack of dorsal wool, and, often, the presence of a white circumferential ring where the body of the adelgid contacted the host plant. Second-, third-, and fourth-instar nymphs were distinguished by their plump appearance, dorsal woolly secretions, secretions of honeydew, and, oftentimes, by their previous cast skins that became enmeshed in their wool and honeydew. Sessile adults were distinguished by their relatively large size and the presence of one or more closely associated eggs near the anal region. Winged adults were distinguished by their relatively large size, mobility, and the presence of wings.

When possible, HWA also were classified according to generation. Generations were distinguished temporally, with the overwintering generation (hatching in early summer) classified as sistens and the following generation (hatching in late winter) classified as progrediens (McClure 1989). Sexupara hatched and developed along with progrediens and were not classified separately from progrediens until they matured into adults and developed wings. Both progrediens and sexupara extract nutrients from hemlock to grow and develop so there is biological reason to lump the immature stages of the generations for the purpose of this study.

Data Analysis

Multivariate analysis of variance (ANOVA) \((p \leq 0.10)\) was conducted in SPSS 14.0 on HWA lifestage abundance data from caged and uncaged branches pooled from the three time studies in 2005.
iii. Results and Discussion

Seasonality and Lifecycle of the Hemlock Woolly Adelgid

Year-round representations of HWA lifestage abundance (Fig. 5, Table 1) and seasonality (Fig. 6) in the field were developed from biweekly samples collected in 2005 and 2006. All HWA lifestages except settled first instars and winged adults showed two peaks, indicating that HWA is bivoltine on hemlock (Figs. 5 and 6). These data agree with the previous life history study conducted by McClure (1989) in Connecticut. Settled first instars were indistinguishable amongst generations and were most prevalent from March to November. However, they were present year-round, suggesting that the development of various individuals within a generation is staggered. First instars that settled on the trunk, branches, or even branchlets that had no new growth on the trees

Figure 5. Abundance of lifestages of hemlock woolly adelgid, *Adelges tsugae* Annand, on eastern hemlock at Baxter Orchard, Great Smoky Mountains National Park, Tennessee, 2005 and 2006 combined data
Figure 6. Lifecycle of hemlock woolly adelgid, *Adelges tsugae* Annand, on eastern hemlock at Baxter Orchard, Great Smoky Mountains National Park, Tennessee, 2005 and 2006 combined data (Prog/Sexup = Progredien/Sexupara)

Table 1. Monthly lifestages of hemlock woolly adelgid, *Adelges tsugae* Annand, on infested eastern hemlock at Baxter Orchard, Great Smoky Mountains National Park, Tennessee, 2005 and 2006 combined data.  

<table>
<thead>
<tr>
<th>Month</th>
<th>Egg</th>
<th>Crawler</th>
<th>Instar 1</th>
<th>Instar 2-4</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>2.67 ± 1.00</td>
<td>8.34 ± 1.65</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>February</td>
<td>10.92 ± 3.90</td>
<td>0.00 ± 0.00</td>
<td>1.44 ± 0.43</td>
<td>4.60 ± 1.54</td>
<td>2.61 ± 0.78</td>
</tr>
<tr>
<td>March</td>
<td>74.26 ± 29.61</td>
<td>3.35 ± 2.87</td>
<td>6.87 ± 2.93</td>
<td>0.35 ± 0.22</td>
<td>5.66 ± 2.23</td>
</tr>
<tr>
<td>April</td>
<td>77.70 ± 50.59</td>
<td>1.47 ± 0.67</td>
<td>24.68 ± 6.63</td>
<td>0.76 ± 0.36</td>
<td>2.36 ± 1.59</td>
</tr>
<tr>
<td>May</td>
<td>19.32 ± 11.01</td>
<td>0.28 ± 0.21</td>
<td>21.88 ± 1.87</td>
<td>12.97 ± 4.27</td>
<td>2.57 ± 1.36</td>
</tr>
<tr>
<td>June</td>
<td>13.51 ± 6.24</td>
<td>0.55 ± 0.28</td>
<td>30.03 ± 7.47</td>
<td>1.92 ± 0.76</td>
<td>2.73 ± 1.14</td>
</tr>
<tr>
<td>July</td>
<td>0.30 ± 0.23</td>
<td>0.00 ± 0.00</td>
<td>24.07 ± 5.70</td>
<td>0.33 ± 0.39</td>
<td>0.14 ± 0.11</td>
</tr>
<tr>
<td>August</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>26.17 ± 10.13</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>September</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>28.29 ± 9.52</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>October</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>31.78 ± 14.97</td>
<td>0.67 ± 0.55</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>November</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>4.60 ± 2.02</td>
<td>28.11 ± 7.75</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>December</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>4.09 ± 1.98</td>
<td>11.96 ± 2.91</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

2Mean no./cm ± SE
generally did not mature past first instar and died. HWA preferred, and had highest
survival on, the newest growth produced by the tree.

Sistens began ovipositing progredien/sexupara eggs during the first week of
February and the eggs began hatching the first week of March (Fig. 6). Upon hatching,
progrediens/sexupara quickly developed to adulthood. Sisten eggs were oviposited by
progredien adults starting ca. 26 April. Winged adult sexupara were ephemeral and
observed in May with peak abundance recorded on the week of 24 May both years. Sisten
eggs hatched into crawlers which settled and began aestivation. Sistens ceased aestivation
during the week of 16 October 2005, and resumed development.

Time of sisten and progredien oviposition differ from McClure (1989), likely due
to warmer climate in Tennessee than in Connecticut (Table 2). Sistens oviposited and
progrediens/sexupara hatched one month earlier and progrediens began oviposition two
months earlier in this study than in McClure (1989). Generations also were more
persistent and staggered in this study than in McClure (1989). Interestingly, the months
that sistens began and ended aestivation were the same in this study as in McClure
(1989). Aestivation of HWA is linked with seasonal sap flow in hemlock; when sap flow
slows during the summer, HWA aestivates. Alternatively, when sap flow resumes, HWA
terminates aestivation. This link between sap flow and aestivation may explain why the
results in this study are similar to those found in Connecticut, despite the differences in
latitude and climate (J. R. Rhea, personal comm.).
Table 2. Comparison of results of current study on life history of hemlock woolly adelgid, *Adelges tsugae* Annand, in Tennessee with a previous study conducted in Connecticut (McClure 1989)

<table>
<thead>
<tr>
<th>Month</th>
<th>Current Study (Tennessee)</th>
<th>McClure 1989 (Connecticut)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generations Present</td>
<td>Event</td>
</tr>
<tr>
<td>January</td>
<td>Sist</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>Sist</td>
<td>Sist Begin Oviposition</td>
</tr>
<tr>
<td>March</td>
<td>Prog/Sexu/Sist</td>
<td>Prog/Sexu Hatch</td>
</tr>
<tr>
<td>April</td>
<td>Prog/Sexu/Sist</td>
<td>Prog Begin Oviposition</td>
</tr>
<tr>
<td>May</td>
<td>Prog/Sexu</td>
<td>Peak of Sexu Adults</td>
</tr>
<tr>
<td>June</td>
<td>Prog/Sist</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>Prog/Sist</td>
<td>Sist Begin Aestivation</td>
</tr>
<tr>
<td>August</td>
<td>Sist</td>
<td></td>
</tr>
<tr>
<td>September</td>
<td>Sist</td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>Sist</td>
<td>Sist End Aestivation</td>
</tr>
<tr>
<td>November</td>
<td>Sist</td>
<td></td>
</tr>
<tr>
<td>December</td>
<td>Sist</td>
<td></td>
</tr>
</tbody>
</table>

1All lifestages of all generations occurring on hemlock, excluding eggs. Generation names abbreviated: Prog = progresiens, Sexu = sexupara, Sist = sistens.

*Sasajiscymnus tsugae* (*St*) (Sasaji and McClure), the principle biological control organism used against HWA, prefers to feed on the egg and crawler lifestages. Eggs and crawlers of HWA were most abundant in March and April and were present until mid-July. Based on these findings, egg releases of *St*, detailed in Chapter III of this thesis, are likely to be most successful during March and April since *St* generally take ca. 4-5 weeks to mature in the field depending on temperature (personal observation). The developmental time for *St* found in this study (see Chapter III) roughly agrees with the developmental time reported by Cheah and McClure (2000) who estimated developmental time from egg to adult in the field between 30 and 60 days. The difference between the developmental time estimate for *St* from egg to adult found in this study and Cheah and McClure (2000) likely is due to the warmer climate in our area.
Effect of Enclosure in Sleeve Cages on Hemlock Woolly Adelgid

The effect of sleeve cages on abundance of HWA lifestages was investigated by comparing counts of HWA lifestages on caged and uncaged branches in 2005 (Table 3). The time of first and/or last appearance for all lifestages of HWA was nearly identical on caged and uncaged branches. The abundance of each HWA lifestage observed, however, varied amongst caged and uncaged branches.

Fewer total HWA were found on caged branches than on uncaged branches for 15 of the 20 weeks in the three studies (Table 3). A large positive outlier on branches removed on 25 July was likely the result of natural variation in HWA crawler location and density in the field along with low total numbers of HWA during that time of the year (HWA aestivates as minute first instars during the summer). Numbers of HWA lifestages and total number of HWA varied by study during three weeks of overlap from 4 June to 18 June 2005. Therefore, differences in the number of HWA on caged and uncaged branches were likely confounded by the natural variation of HWA in the field.

The large positive outlier on branches removed on 25 July caused the average total number of HWA, across all lifestages, found on caged branches to be only 8.78% lower than the total number of HWA found on uncaged branches. However, the average number of each lifestage was consistently higher in uncaged branches than in caged branches when all dates were totaled (Table 3). By combining total HWA found on caged and uncaged branches on all dates, a much more substantial percent difference (-25.06) is observed. Although HWA did survive and develop within sleeve cages, these data suggest that sleeve cages may contribute negatively to HWA survival and development.
Table 3. Weekly mean no.\(^1\) hemlock woolly adelgid, *Adelges tsugae* Annand, lifestages per cm of caged and uncaged infested hemlock branches sampled March-June 2005 at Baxter Orchard, Great Smoky Mountains National Park, Tennessee

<table>
<thead>
<tr>
<th>Study(^2)</th>
<th>Date</th>
<th>Caged</th>
<th>Uncaged</th>
<th>Caged</th>
<th>Uncaged</th>
<th>Caged</th>
<th>Uncaged</th>
<th>Caged</th>
<th>Uncaged</th>
<th>Caged</th>
<th>Uncaged</th>
<th>% Diff(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 Mar</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.49</td>
<td>1.23</td>
<td>0.49</td>
<td>1.23</td>
<td>-60.14</td>
</tr>
<tr>
<td>1</td>
<td>10 Mar</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.78</td>
<td>0.54</td>
<td>0.78</td>
<td>0.54</td>
<td>44.12</td>
</tr>
<tr>
<td>1</td>
<td>18 Mar</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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\(^1\)Means calculated for three caged and three uncaged branches (0.6 m long) removed weekly after establishment of study (n = 10 counts/branch).

\(^2\)Data compiled from three studies in 2005 established 24 February, 30 March, and 27 April, respectively.

\(^3\)Percent difference of HWA density on caged branches from HWA density on uncaged branches: % Diff = (Total no. HWA/cm on caged branches – Total no. HWA/cm on uncaged branches) ÷ (Total no. HWA/cm on uncaged branches).
Significant differences between caged and uncaged branches were found for crawlers ($p \leq 0.10$, $df = 1$, 1281, $F = 8.36$), settled first instars ($p \leq 0.10$, $df = 1$, 1281, $F = 5.35$), and adults ($p \leq 0.10$, $df = 1$, 1281, $F = 20.90$); but not for second through fourth instars ($p > 0.10$, $df = 1$, 1281, $F = 0.38$). However, the model accounted for little of the variation in the data ($R^2 = 0.09$). Therefore, differences in abundance of HWA lifestages on caged branches and uncaged branches are obscured by variables not accounted for by the model. Since $St$ tend to disperse upon release, are minute in size and inconspicuous in coloration, and can not be lured to traps by an attractive pheromone (Broeckling and Salom 2003a), sleeve cages remain the most accurate and reliable tool, by far, for monitoring $St$ survival and development in the field.

iv. Summary

Most lifestages of HWA occur from March through June. HWA is bivoltine in Tennessee but lifestages mature sooner and are more staggered, with greater overlap than reported in Connecticut (McClure 1989). Sistens began oviposition and progrediens/sexupara hatched one month earlier in this study than in Connecticut. Progrediens began ovipositing two months earlier in this study than in Connecticut. Differences in developmental times in this study and in McClure (1989) are most likely due to climatic differences in Tennessee and Connecticut. HWA eggs and crawlers are most abundant in Tennessee in March and April, indicating that these months would be the optimum time of year to release $St$ for biological control of HWA, given adequate
temperature for $St$ survival and development (see Chapter III). Aestivation of HWA occurs between June and October, which is in agreement with McClure (1989).

Sleeve cages have a negative effect on HWA abundance, likely due to microclimate affects. The extent to which sleeve cages reduce the abundance of HWA is uncertain because of variation in the data. However, fewer total HWA were found on caged branches than on uncaged branches for 15 of the 20 weeks in the three studies. The average number of each lifestage also was consistently higher in uncaged branches than in caged branches when all dates were totaled. Therefore, survival of biological control agents that feed on HWA may be lower when tested within sleeve cages than outside of sleeve cages due to reduced availability of food on branches enclosed by sleeve cages. Future testing of biological control agents especially susceptible to cannibalism or starvation, or with particular efficacy or reliance on specific lifestages of HWA, should address the effect that sleeve cages may have on the abundance of HWA lifestages.
Chapter III. Evaluation of Egg Releases to Establish Sasajiscymnus tsugae on Eastern Hemlock

i. Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand (HWA), poses a serious threat to the existence of hemlock trees in eastern North America. HWA was first reported in the U.S. in 1924 on the west coast, where it had a minor impact on western hemlock, *Tsuga heterophylla* Sargent, and mountain hemlock, *Tsuga mertensiana* Carriere (Annand 1924, McClure and Cheah 1999, Stoetzel 2002). HWA was first collected in the eastern U.S. in 1951 in Richmond, Virginia, and spread to Pennsylvania by 1969 and Maryland by 1973 (Stoetzel 2002). Since then, it has spread north to Massachusetts, south to North Carolina, and west to West Virginia and Tennessee (Fig. 2) (Broeckling and Salom 2003b).

HWA feeds on fluids that it extracts via a long stylet from the ray parenchyma cells of hemlock trees, resulting in needle loss and the death of eastern, *Tsuga canadensis* (L.) Carriere hemlock trees within six years (McClure 1991, Young et al. 1995, Cheah and McClure 1998). Recovery of eastern or Carolina, *Tsuga caroliniana* Engelmann, from HWA infestation without human intervention has not been documented. HWA may spread throughout the entire range of eastern and Carolina hemlock as no limiting factors for expansion of HWA are known in the eastern U.S. (Orwig and Foster 1998).

Chemical control of HWA with insecticides and horticultural oils is an effective method for reducing HWA infestations on individual trees in managed settings. However, chemical control is not viable in a forest environment where accessibility and sensitivity of habitat are issues. The most promising method of HWA reduction in forest settings is
biological control (Broeckling and Salom 2003a; Francis 2004, J. R. Rhea, personal comm.).

Members of the insect families Cecidomyiidae, Coccinellidae, Chrysopidae, Hemerobiidae, and Syrphidae are known to feed on HWA. In terms of abundance, the most important predators of HWA established in the native environment of eastern hemlock are the Asian multicolored lady beetle, *Harmonia axyridis* (Pallas), and brown and green lacewings. However, none of these predators have been effective in controlling infestations of HWA (Wallace and Hain 2000). Thus, efforts have been made to identify potential predators in the native habitat of HWA. These natural enemies have been evaluated and several have been approved for release in the U.S.

One of these introduced predator species, *Sasajiscymnus tsugae* (Sasaji and McClure) (*S*_t), has been the focus of biological control efforts against HWA in the eastern U.S. since the 1990s. In the northeastern U.S., *S*_t is bivoltine with the first cohort of eggs laid between May and July and the second cohort of eggs laid between August and October (Cheah and McClure 2000). *S*_t releases between 1995 and 1999 in Connecticut alone totaled 100,000 (Cheah and McClure 2000).

Since 1995, more than 2 million *S*_t adults have been released in the U.S. *S*_t are currently mass reared in several facilities in the eastern U.S.: Clemson University (SC), Ecoscientific Solutions (PA), North Carolina Department of Agriculture, and University of Tennessee. The rearing process is costly, time-consuming, and labor-intensive. Unfortunately, such facilities can not meet the demand for the beetles. Releasing *S*_t as eggs, rather than adult beetles, has been suggested as a way to reduce labor and costs associated with rearing and to increase production of the beetles to better meet demand.
The germinal idea for egg releases of *St* was articulated by Dan Palmer at the New Jersey Department of Agriculture (J. F. Grant, personal comm.).

Studies were conducted in 2005 and 2006 to test the efficacy of releasing *St* as eggs. Studies sought to determine the optimum time of year to release *St* eggs, the effect of temperature on survival of *St* eggs, optimum number of *St* eggs to release per site, and optimum age of *St* eggs to release.

**ii. Materials and Methods**

**Site**

Studies were conducted in GRSM at Baxter Orchard (35°45'75"N 83°16'60"W) (elevation ca. 610 m) in Cosby in Sevier Co., TENNESSEE (Fig. 3). The Baxter Orchard site consists of abandoned farmland that has regenerated into a mixed-hardwood forest. GRSM purchased the land at the Baxter Orchard site in 1955 (Kent Cave, personal comm.). The area contains a substantial number of eastern hemlocks of various sizes and ages with HWA infestations (first documented late 2004). None of the eastern hemlocks at the site had been previously treated for HWA (Tom Remaley, personal comm.). Information on soil type and other site characteristics is described in Chapter II.

**General Methodology for All Studies**

**Obtaining Eggs of *St***

Gauzes, on which *St* eggs had been oviposited (i.e., “egg gauzes”), were obtained from rearing facilities at the University of Tennessee in Knoxville, Tennessee, Clemson
University in Clemson, South Carolina, and the North Carolina Department of Agriculture in Cary, North Carolina. Infested eastern hemlock foliage was obtained from the rearing facility at the University of Tennessee at Knoxville, when available, for use in “gauze twig” construction (see below). Otherwise, infested eastern hemlock foliage was collected from the field.

**Use of Sleeve Cages in the Field and Jars in the Laboratory**

No accurate and reliable sampling method is available to conduct these experiments in a natural, completely open setting. Therefore, sleeve cages were used to contain *St* in the field so accurate counts could be made. Sleeve cages (31 cm x 68 cm) were made of no-see-um plastic netting with a zipper spanning the length of the cage, sewn closed on one end and with a draw-string closure on the other.

Egg gauzes were placed between twigs of eastern hemlock infested with HWA, which were then secured together with plastic tie wraps. The sets of gauzes between twigs, or “gauze twigs,” were attached to branches of infested eastern hemlock trees with tie wraps. Sleeve cages were then placed over the infested branches with attached gauze twigs.

Sleeve cages were secured to the branches and closed by cinching, wrapping, and tying the draw-string cord to closely appress the no-see-um mesh to the branch and prevent the movement of small insects into or out of the sleeve cage (Fig. 4). Two to four plastic zip ties were tied below the cord to further increase security of the sleeve cages. Each sleeve cage was marked with a flag.

Concurrent with field work using sleeve cages, *St* were established and maintained in glass jars in the laboratory (ca. 25°C, 45% relative humidity) as a control.
Each jar enclosed a 3.8 liter volume with a “mouth” 8.5-10.5 cm in diameter, and had a lid with a no-see-um mesh circular central region 6.5-8.0 cm in diameter. Each jar contained a cylindrical container (ca. 5.0 cm wide, ca. 4.5 cm tall) made of glass or plastic with one open end. The cylindrical container was packed with Oasis® floral foam (0020) which was then supersaturated with distilled water. The open end of the container was then covered in Parafilm® (PM-999).

Two infested twigs of eastern hemlock were inserted through the parafilm into the floral foam and egg gauzes were “sandwiched” between the twigs, to simulate a gauze twig. Additional infested foliage was added from the corresponding study tree in the field as needed.

**Processing for St**

Interiors of containers, and everything within them, were scanned for the presence of St by eye or under magnification, as appropriate in the laboratory. All St were counted and classified as living or dead.

**Determining the Optimum Time of Year for St Egg Releases**

**2005**

Gauzes were placed in sleeve cages (n=21) which were attached to branches of infested eastern hemlock trees on 27 February, 30 March, and 27 April. Corresponding jars (n=21) were established in the laboratory within one day of establishment of sleeve cages in the field.

Each week for seven weeks, three sleeve cages with St were removed from the field. All sleeve cages were removed from their respective trees by cutting the branch to
which each was fastened. The sleeve cages, and the branches that they enclosed, were taken to the laboratory and processed for *St* along with their corresponding jars.

**2006**

A season-long study was conducted in 2006 to complement the three monthly studies conducted in 2005. *St* eggs laid on gauze were obtained weekly from the Lindsay Young Beneficial Insects Laboratory at the University of Tennessee in Knoxville, Tennessee, and Cherry Farm Insectary at Clemson University in Clemson, South Carolina. Each week, gauzes were combined to form six groups, each consisting of 50 eggs. Three groups of *St* eggs were used to establish three sleeve cages in the field and three groups of *St* eggs were used to establish three jars in the laboratory.

Efforts were made to provide numbers of HWA proportional to the number of *St* egg placed in each container (sleeve cage or jar). For instance, a sleeve cage with 200 *St* eggs would encompass branches harboring ca. four times the number of HWA that a sleeve cage with 50 *St* eggs would encompass.

Three weeks after establishment of each weekly set of sleeve cages and jars, the sleeve cages were removed from their respective trees by cutting the branch to which each was fastened. The sleeve cages, and their corresponding jars, were processed for *St*.

**Determining the Effect of Temperature on Survival of *St* Eggs**

On 14 and 16 June 2006, petri dishes (n=50 and 25, respectively) containing egg gauzes (n=25 eggs per dish) were sealed using Parafilm® (PM-999) around the circumference of each dish. Petri dishes were then placed in five incubators (n=10 and 5 petri dishes/incubator on 14 and 16 June, respectively) at the following temperatures:
-1.1, 4.4, 10.0, 15.6, and 21.1 °C. Each set of petri dishes at each temperature was processed for *S* *t* every three to five days, for at least two weeks, until no living or dead hatched *S* *t* were recovered from two consecutive processing dates. Then, a final count was taken 7-31 days later to ensure no *S* *t* were overlooked.

**Determining the Optimum Number of *S* *t* Eggs for Release in the Field**

Three studies were conducted on 15 March, 29 March, and 5 June 2006 to investigate the effect of crowding, competition, and cannibalism among *S* *t* established as eggs. Gauzes, on which *S* *t* eggs had been oviposited, were obtained from the Lindsay Young Beneficial Insects Laboratory at the University of Tennessee and North Carolina Department of Agriculture and combined to form groups with the following numbers of eggs: 50, 100, 150, and 200. Sleeve cages were established in the field and jars were established in the laboratory with each density of *S* *t* eggs. The number of replications per density varied based on availability of *S* *t* eggs on each date and were 4, 5, and 3, respectively.

Three weeks after establishment, sleeve cages were removed from their respective trees by cutting the branch to which each was fastened and taken to the laboratory. The sleeve cages, and their corresponding jars, were processed for *S* *t*.

**Determining the Optimum Age of *S* *t* Eggs for Release in the Field**

A study was conducted to investigate the effect of *S* *t* egg age class on survival of *S* *t*. Gauzes, on which *S* *t* eggs had been oviposited, were obtained from the Lindsay Young Beneficial Insects Laboratory at the University of Tennessee in the following age
classes: 0-2, 3-5, and 6-8 days old. Due to logistical considerations, it was not feasible to obtain all three St egg age classes and establish them in the field on the same day. Therefore, the 3-5 and 6-8 day old age classes were established in the field on 22 March and the 0-2 day old age class was established in the field on 23 March. Sleeve cages were established in the field and jars were established in the laboratory with each age class of St eggs in five replications.

Three weeks after establishment, sleeve cages were removed from their respective trees by cutting the branch to which each was fastened and taken to the laboratory. The sleeve cages, and their corresponding jars, were processed for St.

**Data Analysis**

Analysis of variance (ANOVA) and Least Significant Difference (LSD) procedures were run in SPSS 14.0 on field and laboratory St survival data (p ≤ 0.10) from sleeve cages and jars from the time study in 2005 and the age and density studies and on St recovery data (p ≤ 0.05) from the temperature study. Likewise, ANOVA and LSD procedures (p ≤ 0.05) were run in SAS 9.1 on field and laboratory St survival data (p ≤ 0.05) from sleeve cages and jars from the time study in 2006. Statistical differences among means were distinguished by letters.
iii. Results and Discussion

Determining the Optimum Time of Year for St Egg Releases

2005

Virtually no survival (< 1%) was observed for St eggs placed in the field in February (Fig. 7), nor were significant differences among weeks observed (p > 0.10, df = 4, 10, F = 0.55), most likely due to cold temperatures. Neither were significant differences found among weekly survival of St eggs placed in the field in March (p > 0.10, df = 6, 14, F = 2.16) (Fig. 8). Significant differences were found among weekly survival of St eggs placed in the field in April (p ≤ 0.10, df = 7, 16, F = 5.95) (Fig. 9). Survival of St placed in the field as eggs in April was highest in weeks 3 and 4 followed by weeks 2 and 5. Maximum field survival of St was observed on the fourth week of each time study in 2005 and was 0.5% in February, ca. 40% in March, and ca. 60% in April.

Figure 7. Weekly percent survival (± SE) of Sasajiscymnus tsugae (Sasaji and McClure) reared from eggs established in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and in the laboratory on 24 February 2005.
**Figure 8.** Weekly percent survival (± SE) of *Sasajiscymnus tsugae* (Sasaji and McClure) reared from eggs established in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and in the laboratory on 30 March 2005

**Figure 9.** Weekly percent survival (± SE) of *Sasajiscymnus tsugae* (Sasaji and McClure) reared from eggs established in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and in the laboratory on 27 April 2005
Weekly field survival of \( S_t \) in both March and April (Figs. 8 and 9) showed a trend where survival increased during the first 4 weeks after establishment and decreased afterwards. Lower field temperatures caused \( S_t \) to develop more slowly in the field than in the laboratory. Therefore, the lifestages of \( S_t \) from field cages were smaller and less noticeable than those in laboratory jars. Field cages also encompassed more volume and often contained more foliage than laboratory jars, resulting in more surface area to search in sleeve cages than in jars. Therefore, more \( S_t \) from the field may have been overlooked in counts made during the earlier weeks, resulting in the appearance of an increase in \( S_t \) survival before the fourth week of the studies in March and April.

The decline in \( S_t \) survival in the field after 4 weeks (Figs. 8 and 9) is likely due to cannibalism of \( S_t \) as their food source (HWA) became relatively scarcer within the cages. Laboratory jars showed a decline in survival of \( S_t \) over time as well (Figs. 7, 8, and 9). Decline of survival of \( S_t \) in jars was likely due to the combination of cannibalism and the accumulation of mold growth, especially in the study established 27 April 2005 (Fig. 9). Mold growth was a seemingly unavoidable side effect of the containment of cut hemlock along with floral foam supersaturated with distilled water in jars in an incubator in the laboratory (i.e., a humid, mostly-enclosed environment) for seven weeks.

Survival of \( S_t \) placed in the field in March (peak survival ca. 40%) (Fig. 8) was less than those placed in the field in April (peak survival ca. 60%) (Fig. 9). This difference in survival may be attributable to warmer temperatures in April than in March.

Laboratory survival of \( S_t \) declined in a seemingly exponential fashion in the studies established in February and April (Figs. 7 and 9). Laboratory survival of \( S_t \) from the study established in March (Fig. 8) showed a decline over time, but the rate of decline
is not as severe or clear-cut as in the other studies, which may be partially due to the fact that laboratory jars showed less mold growth in the March study than in the April study. However, the reason for the difference of mold growth in the two studies is unknown.

2006

Survival of St eggs placed in the field prior to 30 March was less than 10%, after which field survival varied, ranging between ca. 25 and ca. 40% survival until the week of 7 June when survival again fell below 10% (Fig. 10). Low field survival of St prior to 30 March was likely due to cold temperatures (Fig. 11), while low survival of St after 1 June was likely due to a scarcity of preferred lifestages of HWA (eggs and crawlers) as a food source (Fig. 6).

**Figure 10.** Weekly percent survival of *Sasajiscymnus tsugae* (Sasaji and McClure) reared from eggs three weeks after establishment in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and in the laboratory in 2006 (LSD was run for field and laboratory data separately; non-identical letters indicate significant difference (p ≤ 0.05))
Figure 11. Comparison of weekly percent survival of *Sasajiscymnus tsugae* (Sasaji and McClure) reared from eggs three weeks after establishment to minimum daily temperature (Temp = temperature) in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and in the laboratory in 2006 (Percent survival data occupy light gray region only; temperature data occupy both light and dark gray regions).

The minimum daily temperature is compared with weekly percent survival of *St* reared from eggs placed in the field (Fig. 11). *St* cohorts were removed from the field three weeks after their placement in the field and processed. Thus, the minimum daily temperatures each cohort of eggs was subjected to may be determined by examining temperature data for three weeks after the date that a given cohort was placed in the field. For example, eggs placed in the field on 23 February were removed on 15 March and subjected to the temperature during that period (Fig. 11). No cohorts of *St* eggs were established in the field after 20 June 2006; however, *St* eggs remained in the field until the week of 10 July 2006.

Freezing temperatures within one week of establishment of a cohort of *St* eggs resulted in substantial mortality of *St* (Fig. 11). The first time that field temperatures did
not decrease below freezing within one week of establishment of a cohort of *St* eggs corresponds with the first noteworthy peak of *St* survival (9 March) in 2006. Only one day after 30 March showed a minimum temperature below freezing. This date corresponds with a slight decline in *St* survival on 5 April but the decline in *St* survival is not significantly different (*p > 0.05*) from 30 March (Fig. 10). These results imply survival of *St* placed in the field as eggs is adversely affected by cold temperatures.

**Determining the Effect of Temperature on Survival of *St* Eggs**

Temperature influenced the survival of *St* eggs placed in petri dishes and held in incubators. There was a decrease in live and total (live and dead) recovery of *St* at each successively lower temperature gradient below 15.6 °C (Fig. 12), with little or no live

![Figure 12. *Sasajiscymnus tsugae* (Sasaji and McClure) recovered from petri dishes subjected to five temperatures (Combined data from two studies initiated on 14 and 16 June 2006, respectively)](image-url)
recovery at temperatures at or below 4.4 °C. The numbers of live (p ≤ 0.05, df = 4, 214, F = 14.35) and total (p ≤ 0.05, df = 4, 214, F = 17.39) St recovered were significantly different among temperatures.

The greatest percentage of live St recovered (ca. 30%) and the highest total recovery (ca. 75%) of St was observed at 15.6 °C. At the highest temperature (21.1 °C), significantly fewer (ca. 20%) live St were recovered; this lower survival may be a result of cannibalism due to faster rates of development and metabolism of St. Losses of St due to cannibalism were not discernable unless identifiable body parts remained. However, total recovery of St (live and dead St combined) was not significantly different between 15.6 °C and 21.1°C. Live and total recoveries at 10.0 °C, 15.6 °C, and 21.1 °C were significantly greater than at -1.1 °C and 4.4 °C. Results from these studies support findings in the time studies (Figs. 10 and 11), in that St released as eggs show poor survival at temperatures below or near freezing.

**Determining the Optimum Number of St Eggs for Release in the Field**

Survival of St eggs placed in the field on 15 March 2006 was nearly zero after three weeks (Fig 13), likely because of cold temperatures. These data agree with findings in previous studies (Figs. 7, 10, and 11). In the March 2006 study, no significant differences (p > 0.10, df = 3, 12, F = 0.20) among densities in the field were observed. In the laboratory, survival was significantly higher (p ≤ 0.10, df = 3, 12, F = 2.87) in the lowest density of St eggs (50) than in the two highest densities of St eggs (150 and 200).

Survival of St in the field showed was not significantly different (p > 0.10, df = 3, 16, F = 1.23) among egg densities, although a faint trend of increasing survival among
Figure 13. Influence of density on survival (± SE) of eggs of *Sasajiscymnus tsugae* (Sasaji and McClure) established in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and laboratory on 15 March 2006

Figure 14. Influence of density on survival (± SE) of eggs of *Sasajiscymnus tsugae* (Sasaji and McClure) established in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and laboratory on 29 March 2006
successively larger densities of St eggs on 29 March 2006 was evident (Fig. 14). This trend conflicts with laboratory results of all three density studies (Figs. 13, 14, and 15). Percent survival of St in the laboratory showed a stronger opposite trend of decreasing survival among successively larger densities of St eggs, although these densities were also nonsignificant (p > 0.10, df = 3, 16, F = 2.36).

Both field and laboratory survival of St eggs established on 5 June 2006 (Fig. 15) showed an overall decrease in survival as density increased. However, no significant differences among densities in the field (p > 0.10, df = 3, 8, F = 1.97) and laboratory (p > 0.10, df = 3, 8, F = 1.16) were observed. Hemlock material with adequate HWA lifestages preferred as food by St (eggs and crawlers) was difficult to distinguish in the field on 5 June because the sisten generation of HWA was nearing its period of summer.
aestivation (Fig. 6 and Table 2). Therefore, some sleeve cages may not have received the type of, or amount of, food intended.

Larger numbers of \( S_t \) established together would be expected to show a lower percent survival than smaller numbers of \( S_t \) established together because of higher rates of cannibalism among larger numbers of \( S_t \) resulting from competition among \( S_t \) for food and space. Trends in laboratory data from all density studies support this assertion (Figs. 13, 14, 15). However, only the laboratory data from the density study established on 15 March statistically validates this hypothesis (Fig. 13). There were no consistent trends or significant differences in field data.

Differences in percent survival of \( S_t \) among densities, especially in the field, may have been confounded by a side-effect of attempting to provide proportionate amounts of HWA among densities of \( S_t \); and that is, disproportionately higher amounts of foliage in higher density classes. Unfortunately, the distribution of HWA on infested trees was often so patchy that even adjacent branches varied greatly in density of infestation. Sleeve cages had to be placed around living, intact branches, which made selecting proportionate amounts of foliage among densities impossible in many cases. Ensuring proportionate amounts of HWA were present among densities was considered more important than ensuring proportionate amounts of foliage were present among densities so many of the sleeve cages that contained higher densities of \( S_t \) eggs encompassed a disproportionately larger amount of foliage than sleeve cages that contained lower densities of \( S_t \) eggs.

Disproportionately large amounts of foliage in higher densities translate into a disproportionately higher amount of surface area available to \( S_t \) in higher densities than
lower densities. Cannibalism among $St$ seems to be opportunistic and dependent on the availability of food (personal observation). Since $St$ in higher densities had more available space than $St$ in lower densities, opportunities for cannibalism may have been greater among $St$ in lower densities. Therefore, $St$ in lower densities may have suffered higher attrition due to cannibalism than $St$ in higher densities in field cages.

As in the field, HWA was provided proportionate to the number of $St$ eggs placed in the container in the laboratory. However, greater control of the amount of foliage was possible in the laboratory than in the field. Sprigs of hemlock foliage were selectively pruned from infested branches of eastern hemlock. Generally, jars containing larger densities of $St$ received foliage more densely infested with HWA than jars containing smaller densities of $St$. In this way, larger densities of $St$ received an amount of HWA appropriate for their densities without receiving as much of a disproportionately high amount of foliage.

**Determining the Optimum Age of $St$ Eggs for Release in the Field**

Survival of $St$ eggs placed in the field on 22 and 23 March 2006 was low (Fig. 16), and is in agreement with other studies in this thesis (Figs. 7, 10, 11, and 13). However, a trend of increasing survival with increasing egg age in the field was evident; survival in the 6-8 day age class was significantly greater than all other age classes ($p \leq 0.10$, df = 3, 14, $F = 26.92$) (Fig. 16). These results indicate $St$ released as older eggs are more likely to survive cold temperatures than $St$ released as younger eggs, likely because older eggs hatch sooner into larvae which are mobile and can seek favorable microclimates on hemlock branches during cold weather.
Figure 16. Survival (± SE) of eggs of Sasajiscymnus tsugae (Sasaji and McClure) of various ages established in the field at Baxter Orchard, Great Smoky Mountains National Park, Tennessee and laboratory on 22 and 23 March 2006 (Compiled data from two studies; age study data occupy the light grey region, time study data occupy the dark grey region).

In the laboratory survival of St in the two oldest age classes (3-5 and 6-8 days old) was significantly greater (p ≤ 0.10, df = 3, 14, F = 3.00) than in the youngest age class (0-2 days old). In a concurrent study, survival of 0-8 day old (i.e., “unaged”) St eggs was ca. 50% in the laboratory on 23 March in the 2006 time study (Fig. 16). Percent survival of the unaged eggs from the time study most closely agrees with the youngest (0-2 day old) age class from the age study.

Percent survival across St egg ages was significantly different (p ≤ 0.10, df = 3, 28, F = 5.54) in the older age classes (3-5 and 6-8 days old) than in the youngest age class (0-2 days old) and unaged eggs from the time study (0-8 days old) when field and laboratory data were pooled (Table 4). Thus, age of St eggs upon release affects St
survival. *St* larvae that hatch and become active may cannibalize any *St* eggs that they contact. Groups of *St* eggs of similar ages are expected to hatch and develop around the same time. Thus, releasing *St* eggs of similar ages is expected to minimize losses among *St* due to cannibalism.

iv. Summary

Optimum survival of *St* established as eggs in the field occurs from the last week of March through the end of May (Figs. 8, 9, and 10). Cold temperatures adversely affected survival of *St* (Figs. 11 and 12) and are a limiting factor for utilization of *St* eggs as a biological control against HWA during the early months of the year.

Trends in laboratory data from 2006 indicate that the number of *St* released together as eggs (i.e., density) is inversely proportional to percent survival of *St* (Figs. 13, 14, and 15). Differences in percent survival among age classes in the laboratory are sizable, with a 15-20% difference in percent survival between the smallest and largest density in each of the three studies. However, differences are validated statistically only

Table 4. Percent survival of eggs of *Sasajiscymnus tsugae* (Sasaji and McClure) of various age classes

<table>
<thead>
<tr>
<th>Egg Age Classification</th>
<th>Percent Survival (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>24.60 ± 10.44 a</td>
</tr>
<tr>
<td>3-5</td>
<td>34.64 ± 14.40 b</td>
</tr>
<tr>
<td>6-8</td>
<td>37.05 ± 10.79 b</td>
</tr>
<tr>
<td>0-8</td>
<td>25.11 ± 11.96 a</td>
</tr>
</tbody>
</table>

1. Compiled field and laboratory data from two studies initiated 22 and 23 March 2006
2. Age of eggs in days upon initiation of study
3. Values followed by the same letter are not significantly different (p > 0.10, df = 3, 28, F = 5.54)
in the density study on 15 March (Fig. 13). No significant difference in percent survival among densities was observed in field data, and trends in field data are unclear (Figs. 13, 14, and 15).

Field and laboratory results indicate $St$ from older eggs exhibit higher percent survival than $St$ from younger eggs (Fig. 16). Percent survival of $St$ eggs from the oldest egg age class (6-8) was significantly greater than survival of $St$ eggs from the two younger egg age classes (0-2 and 3-5) in the field when temperatures were cold enough to inhibit $St$ survival (Fig. 11).

Releases of $St$ eggs in the field should occur from late March through May (Figs. 8, 9, and 10), in low densities (Figs. 13, 14, and 15), and with groups of older $St$ eggs (Fig. 16) in order to achieve optimal survival. $St$ eggs 6-8 days old, released in low densities in late March-May will produce the maximum number of larval and adult $St$ to provide the maximum reduction of populations of HWA and possible permanent colonization of the infested hemlock by $St$. 
Chapter IV. Conclusions

Much concern is expressed over the loss of eastern hemlock trees in eastern North America due to infestations by hemlock woolly adelgid, *Adelges tsugae* Annand (HWA). HWA is likely to spread throughout the entire range of native hemlock species in eastern North America if left unchecked (Orwig and Foster 1998). No effective limiting factors have been found to prevent the spread of HWA, as native predators are ineffective in controlling populations of HWA, and native hemlocks have not shown natural resistance to HWA (Orwig and Foster 1998, Snyder et al 2002, Wallace and Hain 2000). Biological control through the use of natural enemies of HWA from Japan and western North America is currently considered the best approach for preserving hemlock in the forests of eastern North America.

Several beetle species including *Laricobius nigrinus* Fender, *Sasajiscymnus tsugae* (*St*) (Sasaji and McClure), and members of the *Scymnus* genus are currently being reared for biological control of HWA (personal observation, J. F. Grant, J. R. Rhea, personal comm.). It is believed that a complex of these predators will be necessary to achieve maximum suppression of HWA. This research project focused on *St*, which has received the most rearing effort to this date (J. F. Grant, J. R. Rhea, personal comm.).

Rearing facilities can not meet demand for *St* because of the great monetary, time, and labor requirements necessary to rear *St* adults. Releasing *St* as eggs, rather than adult beetles, has been suggested as a way to maximize production of rearing facilities. The initial idea for *St* egg releases was proposed by Daniel Palmer at the New Jersey Department of Agriculture. However, without basic information about HWA abundance
and seasonality or knowledge of mortality factors associated with \(St\) egg releases, \(St\) egg releases could not be justified.

During this two-year research project, the life history of HWA in the southern Appalachians (Tennessee) was documented, and the optimum time of year, number, and age of \(St\) eggs to release for biological control of HWA were determined. Furthermore, the effects of sleeve cages on HWA and temperature on survival of \(St\) eggs were evaluated. The results from these studies will be used to determine if egg releases of \(St\) are a viable option for biological control, and if so to optimize the use of \(St\) eggs as a biological control of HWA in the southern Appalachians.

The HWA in Tennessee was determined to have a bivoltine lifecycle on eastern hemlock due to the presence of two peaks in abundance for almost all HWA lifestages in the parthenogenic generations (sistens, progrediens, and sexupara) recorded throughout the year. Sistens began oviposition and progrediens/sexupara hatched one month earlier and progrediens began ovipositing two months earlier in Tennessee than in Connecticut, where a previous HWA life history study has been conducted (McClure 1989) (Table 2). Sistens and progrediens also persisted longer in Tennessee than in Connecticut. Differences in developmental times in Tennessee and in Connecticut are most likely due to climatic differences in Tennessee and Connecticut. Aestivation of HWA occurs between June and October in Tennessee, which is in agreement with results documented in Connecticut.

The period of peak diversity of HWA lifestages (i.e., development of HWA) was March through June. HWA eggs and crawlers are most abundant in Tennessee in March and April, indicating that these months would be the optimum time of year to release \(St\)
for biological control of HWA. However, cold temperatures were determined to adversely affect survival of $St$ and were a limiting factor for utilization of $St$ eggs as a biological control against HWA in the early months of the year (January-March). Results from 2005 and 2006 indicate that optimum survival of $St$ established as eggs in the field occurs from the last week of March through the end of May.

Sleeve cages had a negative effect on HWA abundance (Table 3). However, the extent to which sleeve cages reduce the abundance of HWA is uncertain because of variation in the data. Survival of biological control agents that feed on HWA may be lower when tested within sleeve cages than outside of sleeve cages due to reduced availability of food. However, sleeve cages are the most useful tools available to accurately monitor survival and development of $St$ in the field.

Results of the density studies were somewhat unclear. Statistical differences of survival of $St$ among densities were only found in laboratory data of the density study begun on 15 March. However, trends in the laboratory data from all three density studies indicate that the number of $St$ released together as eggs (i.e., density) is inversely proportional to percent survival of $St$. Field data do not show clear trends in percent survival of $St$ among densities.

Field and laboratory results indicate $St$ established from older eggs exhibit higher survival than $St$ established as younger eggs. $St$ eggs from the oldest age class (6-8 days old) had significantly greater survival than all other age classes of $St$ eggs in the field when temperatures were cold enough to inhibit $St$ survival.

All studies supported the release of $St$ eggs as a supportive tactic to enhance biological control efforts against HWA. Studies showed $St$ placed as eggs in the field
could successfully develop into adults in the field. Egg releases are a viable alternative method for the establishment of *St*.

Results of this research project will help rearing facilities meet the demand for *St* and land managers and release personnel make informed decisions when using *St* eggs to enhance biological control efforts against HWA. Results found during this project have been used to produce the first protocol for *St* egg releases to see widespread use. Optimal survival of *St* eggs in the field should be achieved from releases made late March through May, in low densities, and with groups of older *St* eggs. *St* eggs 6-8 days old, released in low densities in late March-May will produce the maximum number of larvae and adult *St* to provide the maximum reduction of populations of HWA and permanent colonization of infested trees by *St*. *St* egg releases are a promising and important tactic to enhance the number of *St* available to reduce HWA populations in the imperiled hemlock forests of the eastern U.S.
Literature Cited
Literature Cited


VITA

Isaac Kent Deal was born in 1982 in Hickory, North Carolina and raised in the city of Claremont, North Carolina. Isaac participated in a Research Experience for Undergraduates (REU) program in 2003 at Kansas State University, Kansas. During the program, he studied the life history of, and conducted a mark-recapture study on, the Regal Fritillary, *Speyeria idalia* Drury, at the Konza Prairie Long-Term Ecological Research (LTER) Station in the Flint Hills of Kansas. Isaac earned a Bachelor of Science degree in biology with a minor in chemistry from Lenoir-Rhyne College in 2004. He is a member of the Entomological Society of America, Tennessee Entomological Society, and Gamma Sigma Delta Agricultural Honor Society.