ASSESSMENT OF *SASAJISCYMNUS TSUGAE, SCYMNUS SINUANODULUS,* AND *LARICOBIUS NIGRINUS*: PREDATOR BEETLES RELEASED TO CONTROL THE HEMLOCK WOOLLY ADELGID IN GEORGIA

by

CERA ELIZABETH JONES

(Under the Direction of James L. Hanula)

ABSTRACT

Eastern hemlock, *Tsuga canadensis,* and Carolina hemlock, *Tsuga caroliniana,* are being threatened by the invasive hemlock woolly adelgid, *Adelges tsugae. Adelges tsugae*, is currently found throughout most of the range of *T. canadensis* and the entire range of *T. caroliniana* and arrived in Georgia in 2003. In an attempt to preserve the hemlocks, biological control predatory beetles *Sasajiscymnus tsugae*, *Laricobius nigrinus*, and *Scymnus sinuanodulus* are being mass reared and released. The objectives considered in this research are to:

1. Determine establishment of these biological control agents in the North Georgia mountains.
2. To determine aestivation survival and time of emergence of *L. nigrinus*.

INDEX WORDS: *Adelges tsugae*, *Sasajiscymnus tsugae*, *Laricobius nigrinus*, *Scymnus sinuanodulus, Tsuga canadensis, Tsuga caroliniana*, biological control, emergence, recovery, establishment, aestivation

ASSESSING THE ESTABLISHMENT OF *SASAJISCYMNUS TSUGAE, SCYMNUS SINUANODULUS,* AND *LARICOBIUS NIGRINUS*: PREDATOR BEETLES RELEASED TO CONTROL THE HEMLOCK WOOLLY ADELGID IN GEORGIA

by

CERA ELIZABETH JONES

B.S., Baldwin-Wallace College, 2006

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

ASSESSING THE ESTABLISHMENT OF *SASAJISCYMNUS TSUGAE*, *SCYMNUS SINUANODULUS,* AND *LARICOBIUS NIGRINUS*: PREDATOR BEETLES RELEASED TO CONTROL THE HEMLOCK WOOLLY ADELGID IN GEORGIA

by

CERA ELIZABETH JONES

Major Professor: James L. Hanula

Committee: S. Kristine Braman

Kamal Gandhi

Electronic Version Approved

Maureen Grasso
Dean of Graduate School
The University of Georgia
December 2013

ACKNOWLEDGEMENTS

I would like to especially thank my advisor Dr. James Hanula for his assistance and encouragement, and for always making time to meet with me over the years. This research could not have been done without the rest of my advisory committee Drs. S. Kristine Braman, and Kamal Gandhi for whose guidance and support I greatly appreciate. In addition, there are multiple people who helped contribute in many ways including Angela Mech, for her advice and friendship, James Wentworth and James Sullivan for collecting foliage, setting up cages, and releasing beetles, Scott Horn, Yanzhuo Zhang, and Mike Cody for field assistance that made my emergence study possible, Nathan Havill for DNA identification of the beetles and providing comments and input on the recovery study and LayLa Burgess, Dr. Paul Arnold, and Mark Dalusky who providing valuable insight on rearing these predators. Lastly, I would like to thank my parents and my husband whose encouragement and support motivated me to pursue and finish my master's degree. Funding for this research was provided by the University of North Georgia, an assistantship through the University of Georgia, and the USDA Forest Service, Southern Research Station work unit SRS 4552, Insects, Diseases and Invasive Plants.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1:

LITERATURE REVIEW

Throughout the world there are nine extant hemlock species that occur in three regions (Havill et al., 2008, Jetton et al., 2009). In North America *Tsugae heterophylla* (Raf.) Sargent and *T. mertensiana* (Bong.) Carrière are found on the Pacific Northwest and *T. canadensis* (L.) Carrière and *T. caroliniana* Engelmann on the East Coast. In Asia there are three species found in China, *T. chinensis* (Franch.) E. Pritz, *T. dumosa* (D. Don) Eichler, and *T. forrestii* Downie, and two species in Japan *T. diversifolia* (Maxim.) Mast. and *T. sieboldii* Carrière. Results from Havill et al. (2008) indicate that two island species found on Taiwan and Ullung Island (Korea) should be given their own species status. It is the two east coast species that are of interest for this paper. *Adelges tsugae* can be found naturally occurring on all hemlock species throughout the world except for the two species found on the east coast of North America (Montgomery et al., 2009), which now occurs due to an introduction from *A. tsugae* infested ornamental *T. sieboldii* determined by DNA analysis (Havill et al., 2006) as well as identification of proteobacterial endosymbionts within *A. tsugae* (von Dohlen et al., 2013).

Hemlock Woolly Adelgid

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an aphid-like insect that is associated with *Picea* and *Tsuga* species. Its common name is

attributed to the waxy secretion that encases the adelgid throughout most of its life and houses the eggs as well (McClure, 1987). While it is currently described as a single species, *A. tsugae* contains several divergent lineages endemic to different regions in Asia and western North America (Havill et al., 2006, 2007, 2009). The primary host of *A. tsugae* is spruce (*Picea*) with hemlocks (*Tsuga*) species being their secondary host. Havill and Foottit (2007) have shown that *A. tsugae* and hemlocks in western North America and Asia have long evolved together.

Hemlock species in their native range show resistance to *A. tsugae* (Havill et al., 2011), however, the level of tree resistance or tolerance to *A. tsugae* varies among the host species. Both eastern and Carolina hemlock show no resistance to *A. tsugae* (McClure, 1987) while *T. chinensis* exhibits a high resistance (Hoover et al., 2009). Artificial infestation of *Tsuga* spp. allowed for the highest densities to survive on *T. canadensis*, *T. caroliniana*, and *T. sieboldii*, intermediate densities surviving on hybrids of *T. chinensis* crossed with either *T. caroliniana* or *T. sieboldii*, and the lowest densities on *T. chinensis* (Montgomery et al., 2009). *Tsuga canadensis* was not able to hybridize with any other species tested (Bentz et al., 2002) which is most likely due to this species evolutionarily diverging a long time ago from the Asian clade, while *T. caroliniana* is nested within the clade (Havill et al., 2008). Joseph et al. (2011a) compared the two western hemlock species with *T. canadensis* and *T. chinensis* and found that *T. mertensiana* had significantly more progredien ovisacs or sisten eggs compared to *T. canadensis*, with *T. heterophylla* between the two and *T. chinensis* significantly lower than the rest. In addition, *T. mertensiana* had significantly higher progredien and sisten

ovisacs that could be explained by a high foliar nitrogen percent since there was also an increase in number of ovisacs on *T. canadensis* and *T. heterophylla* when given a short term fertilizer (Joseph et al., 2011a). These results suggest that the western species might not be resistant to the eastern North America *A. tsugae* and individual adelgid populations should be considered to determine level of resistance in their native range (Joseph et al., 2011a).

The first east coast sighting of *A. tsugae* was in 1951 in Richmond, VA (McClure, 1989). *Adelges tsugae* can currently be found in 18 eastern U.S. states, from Maine to Georgia and west into Tennessee and Kentucky, and infests at least 50% of hemlock habitat (Jetton et al., 2009). There is no relationship between hemlock stand size or age of the trees and the spread of *A. tsugae* seems to be influenced by distance of the hemlock stand to other infested stands (Faulkenberry et al., 2009, Orwig and Foster, 1998). On the East Coast, the lifecycle of *A. tsugae* has allowed the infestation to spread rapidly, with the help of wind, migratory birds, and mammals (McClure, 1990) as well as lack of resistance seen in the host species (Montgomery et al., 2009) and biological control (Montgomery and Lyon, 1996, Wallace and Hain, 2000). Unconstrained *A. tsugae* spread can occur at 20.8 km/yr., however, it is more likely that *A. tsugae* will spread at an average rate of 12.5 km/yr. over its entire range, spreading faster in the South, 15.6 km/yr., and slower in the North at 8.13 km/yr. (Evans and Gregoire, 2007).

Like all adelgids, *A. tsugae* display multiple generation with complex life cycles with many different morphological forms (Havill et al., 2007). In eastern North America

and Japan, *A. tsugae* has three generations with the two spring generations, that includes a winged sexual form, developing simultaneously (McClure et al., 2001). A sexual generation is not found in western North America and therefore only has two generations a year (Havill et al., 2011). The winged sexuparae generation flies off to reproduce on its primary host, spruce (*Picea* spp.) and in Japan galls are formed on the tigertail spruce, *Picea torano* (k. Koch) Koehne (Foottit et al., 2009, Montgomery et al., 2009). In eastern North America *A. tsugae* eggs deposited by the winged sexuparae generation, on either native or exotic spruce trees, were not suitable for development of the nymphs and this stage does not survive (McClure, 1987). The other spring generation is the parthenogenetic progrediens that develop between March and June. The progredien eggs hatch into the mobile crawler sisten (winter) generation between June and mid-July which then ideally settle at the base of new growth (Lagalante et al., 2006). Instead of feeding at this time, the settled sisten 1st instars aestivate over the summer, becoming active again once temperatures cool, October-Early November, maturing over winter and giving rise to the progredien/winged sexuparae generation the next spring (McClure, 1987). Aestivation is maternally determined, but can be manipulated by artificially changing temperature and light regimes (Salom et al., 2001). Fecundity varies among generations with the sisten generation producing more offspring (50) than the progredien generation (20) and winged sexuparae (25) (McClure, 1987).

Adelges tsugae settle at the base of hemlock needles on the underside of branches and insert their piercing-sucking mouth parts (stylet bundles) into the leaf

cushion (Lagalante et al., 2006) and remain sessile as they feed on the xylem ray parenchyma cells, extracting stored nutrients (Young et al., 1995). Heavily infested trees exhibit reduced bud production, lack of new growth, and tip dieback (McClure, 1991). Hemlock mortality can occur in 4-6 years (Mayer et al., 2002, McClure, 1991) and faster in the southern range due to other factors such as drought stress (Ward et al., 2004) and mild winters that are positively related to *A. tsugae* survival (McClure and Cheah, 2002, Trotter and Shields, 2009). Smaller hemlock trees exhibit higher mortality rates (Orwig and Foster, 1998). In the North, *A. tsugae* infestations are not widespread and infested trees have survived over 10 years without succumbing (Paradis et al., 2008).

Densities of *A. tsugae* have been recorded as high as 25 per cm of hemlock twig (McClure, 1991, Paradis, 2011) and have personally been observed with up to three settled nymphs per needle node and potentially higher densities than recorded. Higher densities also cause the proportion of nymphs that develop into sexuparae to increase (McClure, 1991). There is a negative correlation between densities of *A. tsugae* and percent new growth and could be the cause for the increase in monoterpenes released, possibly as a defense mechanism of hemlocks (Broeckling and Salom, 2003). It is suggested that earlier hatched progredien crawlers have more success at establishing on their host (Butin et al., 2007) and could be attributed to chemical changes in the hemlocks (Lagalante et al., 2006, Lagalante and Montgomery, 2003). Considering the volatile terpenoid composition of *T. canadensis*, three particular compounds may influence *A. tsugae* settling and survival. In 2003 Lagalante and Montgomery found that

elevated levels of germacrene D could be found in higher concentrations in trees resistant to *A. tsugae* mortality (*T. mertensiana* and *T. sieboldii*) while higher concentrations of isobornyl acetate was found in susceptible species (*T. canadensis and T. caroliniana*). In 2006, Lagalante et al. examine the specific feeding location of *A. tsugae*, the leaf cushion, and found three dominant terpenoids that varied throughout the season. Myrcene was the dominant component found during the summer months within the new growth, but germacrene D was increased as well in the leaf cushion and both terpenoids were equivalent to previous year's growth by November (Lagalante et al., 2006). In addition, concentrations of isobornyl acetate are higher in the older growth. These findings could indicate that germacrene D and myrcene are feeding deterrents, and explain why the progrediens generation feeding on new growth has poor survival compared to those that feed at older leaf cushions, and that isobornyl acetate is an attractant. Though the sistens generation settles on new growth they do not actively feed until the fall which coincides with the deterrents decreasing in concentration. Along with chemical composition, physical characteristics of the leaf cushion, like the thickness of the cuticle at the insertion point, may play a role in where *A. tsugae* settles while other characteristics, like trichomes, seem to not have an influence (Oten et al., 2012).

Biological Control

Biological control is the use of natural enemies (predators, parasites, pathogens) to limit pest populations to a lower density than what would exist without that agent (Montgomery, 2011). With no specialized predators found on the East Coast

(Montgomery and Lyon, 1996, Wallace and Hain, 2000) and no known parasitoids of adelgids (Elkinton et al., 2011), biological control agents were identified in the native range of *A. tsugae*. Biological control agents include *Sasajiscymnus tsugae*, *Laricobius spp.*, and *Scymnus spp.* primarily, but several Hemipteran species in the family Anthocoridae, including *Tetraphleps galchanoides* Ghauri (Li et al., 2011) and a *Leucopis spp*. (Diptera: Chamaemyiidae) (Grubin et al., 2011) have also been evaluated. In addition fungi associated with *A. tsugae* have been identified (Gouli et al., 1997, Reid et al., 2010), and one, *Lecanicillium muscarium,* was tested as a control agent resulting in decreased adelgid densities (Costa, 2010, Gouli et al., 1997).

Predatory Beetles

Sasajiscymnus tsugae Sasaji and McClure (formally *Pseudoscymnus tsugae*) (Coleoptera: Coccinellidae), a small black beetle (1.5 -2.5 mm) native to Japan, is primarily found associated with hemlock but can also be found on grasses and shrubs (Sasaji and McClure, 1997). *Sasajiscymnus tsugae* life stages will feed on all life stages of *A. tsugae* (Cheah and McClure, 1998) and while they prefer *A. tsugae*, other adelgids will be consumed (Butin et al., 2004). *Sasajiscymnus tsugae* is multivoltine with two generations a year, adults are active from March to November where on average 280 eggs are laid over 14 weeks that are concealed in hemlock bud scales, male cones, or underneath the woolly ovisac of *A. tsugae* (Cheah and McClure, 1998, 2000). Egg production of *S. tsugae* is not increased with higher *A. tsugae* density which could be a result of its multivoltine lifecycle (Butin et al., 2003). In order to complete development,

larvae consume roughly 500 *A. tsugae* eggs or 50 to 100 *A. tsugae* nymphs (Cheah, 2011). *Sasajiscymnus tsugae* has the longest history of releases in the Eastern U.S., with beetles first released in Connecticut in 1995, followed by multiple state releases there after (Cheah, 2011). Recovery of *S. tsugae* has been recorded in Connecticut (Cheah et al., 2005), North Carolina (McDonald et al., 2008), Tennessee (Hakeem et al., 2010, 2011), and Georgia (Chapter 2).

Species in the genus *Laricobius* Rosenhauer (Coleoptera: Derodontidae) are small predatory beetles (2-3 mm) that specialize on adelgids (Lawrence and Hlavac, 1979). *Laricobius nigrinus* (Fender) is a pure black beetle native to the Pacific Northwest that specializes on *A. tsugae*, which is essential for larval development, and has a univoltine life cycle (Zilahi-Balogh et al., 2002, 2006). *Laricobius nigrinus* is well synched with the *A. tsugae* life cycle and also aestivates, as pupae that eventually develop to adults in the soil during the summer. Adults emerge in late September and remain active through May. Eggs of *L. nigrinus* are laid between late January to May, and larvae occur from March to early June (Mausel et al., 2011a, Zilahi-Balogh et al., 2003a). Over a 13 week period mean fecundity was 101 eggs and larval development and egg consumption was temperature dependent, with a quicker developmental time (47 day) and a higher consumption rate of 252 *A. tsugae* eggs at 18°C compared to 12°C (89 days and 226 eggs), but development could not be completed at 21°C (Zilahi-Balogh et al., 2003b). Though morphologically identifiable as *L. nigrinus*, an inland Idaho population has developed more cold tolerance than the coastal Washington population and are potentially more suitable for biocontrol in the northern states (Mausel

et al., 2011b). Releases have occurred in hemlock stands along the eastern United States since 2003 (Lamb et al., 2006). Recovery of *L. nigrinus* has been documented in Tennessee (Hakeem et al., 2011, Mausel et al., 2010), Pennsylvania, Maryland, Virginia, North Carolina (Mausel et al., 2010), and Georgia (Chapter 2).

Laricobius rubidus LeConte is a small (2-3 mm) red and black beetle, native to eastern North America with its primary host being pine bark adelgid *Pineus strobi* Hartig, (Clark and Brown, 1960), but it is also found associated with *A. tsugae* (Montgomery and Lyon, 1996, Wallace and Hain, 2000). *Laricobius rubidus* can complete development on *A. tsugae* just as well as *P. strobi*, but they prefer to oviposit in the later species ovisacs (Zilahi-Balogh et al., 2005). Adults were found on *A. tsugae* infested hemlock from October through May. After mating with *L. nigrinus* was observed (Mausel et al., 2008), it was confirmed that *L rubidus* and *L. nigrinus* are sister species in the North American clade (Montgomery et al., 2011b) and hybridization can occur (Havill et al., 2012).

Laricobius osakensis Montgomery and Shiyake is a brownish (females bicolored brownish and tan) predatory beetle of *A. tsugae* from Japan, the same origin as *A. tsugae* introduced to eastern North America, and can be distinguished from other *Laricobius* by their lack of ocelli (Montgomery et al., 2011b). Just like *L. nigrinus*, this predator is also specific to *A. tsugae* (Vieira et al., 2011), however *L. osakensis* laid at least as many, if not more, eggs and the larvae fed more voraciously on *A. tsugae* compared to *L. nigrinus* (Story et al., 2012, Vieira et al., 2012). *Laricobius osakensis* is also in a separate Asian clade, from *L. nigrinus* and *L. rubidus* (Montgomery et al.,

2011b). In addition, with Japan being more closely related in regards to summer temperature and rainfall experienced in the southern hemlock range on the east coast, (Vieira et al., 2013) and *L. osakensis* emerging later than *L. nigrinus*, this predator might be more suited for the southern range where its life cycle would be better synched with *A. tsugae* breaking diapause (Lamb et al., 2008). Another species *Laricobius kangdingensis* Zilahi-Balogh and Jelinek from China (Gatton et al., 2009), could only complete development between 12-15°C and due to small numbers found in China, a colony could not be maintained (Montgomery et al., 2011a). With all *Laricobius* larvae being morphologically similar, the mitochondrial COI gene can be used to distinguish species, as well as the coastal and inland *L. nigrinus* species (Davis et al., 2011, Mausel et al., 2011b).

One of the largest genera in the family Coccinellidae are the Scymnus, a group of small (1-3 mm) oblong-oval shaped beetle that are uniform in appearance (Wilson, 1927). Three small (2 mm), univoltine beetle species native to China showed the most promise as well as a preference for *A. tsugae*, but other adelgids could be consumed (Montgomery and Keena, 2011). *Scymnus sinuanodulus* (Yu and Yao) (Coleoptera: Coccinellidae) is red-brown in color with one black spot on each elytron. *Scymnus sinuanodulus* average annual fecundity is 130 eggs and larvae will feed on all life stages of *A. tsugae* but they cannot survive on *A. tsugae* nymphs alone (Lu and Montgomery, 2001, Lu et al., 2002). *S. sinuanodulus* was not recovered in Tennessee following release in whole tree cages (Hakeem et al., 2011) or field releases in North Carolina (McDonald et al., 2008) and Georgia (Chapter 2). *Scymnus camptodromus* Yu

and Liu has a unique life cycle in that the eggs appear to enter into an unusual egg diapause that would allow them to hatch when *A. tsugae* eggs are being laid (Keena et al., 2012). The third Chinese species is *S. ningshanensis*, which lays eggs in the spring after adults have overwintered and will increase egg production with higher densities of *A. tsugae* (Butin et al., 2003). *Scymnus sinuanodulus* and *S. ningshanensis* have not been able to be recovered after release, possibly due to low numbers, and *S. camptodromus* still shows potential now that colonies can be maintained in the lab. In addition, a new species introduced to the east coast is *Scymnus coniferarum*, found on *A. tsugae* infested hemlocks in the Pacific Northwest (Darr et al., 2012, Wager-Page, 2012).

The use of multiple biological control agents allows for introduction of a year round predator complex with the fall-spring active *Laricobius spp*. and the spring-fall active coccinellid beetles. Research indicates that *L. nigrinus* and *S. tsugae* (Flowers et al., 2006, Flowers et al., 2007, Hakeem et al., 2011) and Laricobius *spp*. (Story et al., 2012, Vieira et al., 2012) do not display any negative interactions and a multiple species complex is recommended in the fight against *A. tsugae*.

Chemical Control

Another method being employed to control *A. tsugae* is insecticide treatment, while not a long term solution, it does bide some time until biological control agents can establish (Dilling et al., 2010). The most commonly applied insecticide is imidacloprid, a systemic neonicotinoid that acts on the nervous system of insects (Churchel et al., 2008). The use of imidacloprid as a soil application eliminates unwanted side effects,

such as pesticide drift and dermal exposure, along with wounds caused by implants or stem injections (Steward and Horner, 1994). Stem injections also were found to distribute the insecticide less uniformly throughout the tree compared to soil applications (Dilling et al., 2010). Another product Safari (dinotefuran) acts more rapidly than imidacloprid, but only persists up to two years (Joseph et al., 2011b) Application of imidacloprid began translocation in the tree within 3 months and continued for 12 months, with concentrations peaking between 9 -12 months after treatment (Dilling et al., 2010) and could provide control for up to six years (Doccola et al., 2012). However, it is the secondary metabolite olefin that is found at concentrations much higher than the effective dose (12-15ppb), while concentrations of imidacloprid are below the effective dose, and is most likely the cause for this long term suppression of *A. tsugae* (Benton et al., 2012). It has even been suggested that an application dose of 0.25g a.i. per 2.5 cm in trees up to 30 cm dbh will provide 85% reduction in *A. tsugae* population after three years, a dose that is much less than the recommended 0.75 – 1.5g a.i. per 2.5 cm estimated to be necessary in trees above 60 cm dbh (Cowles, 2009). The use of a lower dosage would allow for more trees to be treated with an area and maximum yearly application rates (Cowles, 2009).

The use of systemics have allowed hemlocks to recover dramatically after showing such signs of decline as tip dieback caused by *A. tsugae* both in a landscape (Webb et al., 2003) and nursery setting (Frank and Lebude, 2011). While this insecticide has remarkable effects in suppressing *A. tsugae*, there are concerns with non-target species being negatively influenced. Low levels of application, 0.25g a.i. per 2.5 cm combined with fertilization, known to enhance *A. tsugae* survival (McClure, 1992) and predator egg consumption (Joseph et al., 2011a), would allow tree health to improve as *A. tsugae* levels are reduced, but not eliminated which would provide prey for the beetles as they establish (Joseph et al., 2011c).

Results studying the effects of imidacloprid on species richness and abundance of arthropods have found that species abundance was significantly lower in soil injected trees than control, showing a reduction in fungivore and transient phytophaga insects, and while there was no significant difference overall in species richness, this was significantly lower with the transient phytophaga species when considering the separate guilds (Dilling et al., 2009). Falcone and DeWald (2010) found similar results that while there was no significant difference overall in species richness, composition, and abundance, non-target herbivorous such as Hemiptera and Lepidoptera were greatly reduced. Research on this insecticide entering streams has shown that only trace amounts were detected with no observed effect on aquatic macroinvertebrates (Churchel et al., 2008).

Literature Cited

Benton, E. P., R. J. Webster, R. Cowles, C. I. Coots, A. Lagalante and J. F. Grant. 2012. Persistence of imidacloprid and its metabolites in eastern hemlock in the great smoky mountains, p. 45-46. *In* L. Rieske-Kinney and S. Enebak [eds.]. Proceedings: 54th Southern Forest Insect Work Conference/2012 Southwide Forest Disease Workshop.

Bentz, S. E., L. G. H. Riedel, M. R. Pooler and A. M. townsend. 2002. Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (*Tsuga*) species. J. Arboric. 28: 200-205.

Broeckling, C. D. and S. M. Salom. 2003. Volatile emissions of eastern hemlock, *Tsuga canadensis*, and the influence of hemlock woolly adelgid. Phytochemistry. 62: 175-180.

Butin, E., J. Elkinton, N. P. Havill and M. E. Montgomery. 2003. Comparison of numerical response and predation effects of two coccinellid species on hemlock woolly adelgid (Homoptera: Adelgidae). J. Econ. Entomol. 96: 763-767.

Butin, E., E. Preisser and J. Elkinton. 2007. Factors affecting settlement rate of the hemlock woolly adelgid, *Adelges tsugae*, on eastern hemlock, *Tsuga canadensis*. Agric. For. Entomol. 9: 215-219.

Butin, E. E., N. P. Havill, J. S. Elkinton and M. E. Montgomery. 2004. Feeding preference of three lady beetle predators of the hemlock woolly adelgid (Homoptera: Adelgidae). J. Econ. Entomol. 97: 1635-1641.

Cheah, C. and M. S. McClure. 1998. Life history and development of *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae), a new predator of the hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 27: 1531-1536.

Cheah, C. A. S.-J. 2011. *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae*, a ladybeetle from Japan. 42-53 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid USDA Forest Service publication FHTET: 2011-04

Cheah, C. A. S.-J., M. A. Mayer, D. Palmer, T. Scudder and R. Chianese. 2005. Assessments of biological control of hemlock woolly adelgid with *Sasajiscymnus tsugae* in Connecticut and New Jersey, pp. 116-130. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Third Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2005-01.

Cheah, C. A. S. J. and M. S. McClure. 2000. Seasonal synchrony of life cycles between the exotic predator, *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae) and its prey, the hemlock woolly adelgid *Adelges tsugae* (Homoptera:Adelgidae). Agric. For. Entomol. 2: 241-251.

Churchel, M. A., J. Hanula, C. W. Berisford and J. M. Vose. 2008. Environmental fate of imidacloprid, pp. 99-100. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Fourth Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2008-01.

Clark, R. C. and N. R. Brown. 1960. Studies of predators of the Balsam woolly aphid, *Adelges piceae* (Ratz.) (Homoptera: Adelgidae). VII. *Laricobius rubidus* Lec. (Coleoptera: Derodontidae), a predator of *Pineus strobi* (Htg.) (Homoptera: Adelgidae). Can. Ent. 92: 237-240.

Costa, S. D. 2010. Hemlock woolly adelgid suppression with aerial application of Mycotal in a microfactory formulation, can it work?, pp. 36-42. *In* B. Onken and B. Reardon [eds.]. Proceedings of the Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA For. Serv. FHTET-2010-07.

Cowles, R. S. 2009. Optimizing dosage and preventing leaching of imidacloprid for management of hemlock woolly adelgid in forests. For. Ecol. Manage. 257: 1026-1033.

Darr, M. N., T. J. McAvoy, R. C. McDonald, M. E. Montgomery, M. J. Dalusky, S. M. Salom and L. T. Kok. 2012. Rearing, host feeding and biology of *Scymnus coniferarum* (Coleoptera: Coccinellidae) a predator of Adelgidae from western North America, p. 45. *In* L. Rieske-Kinney and S. Enebak [eds.]. Proceedings: 54th Southern Forest Insect Work Conference/2012 Southwide Forest Disease Workshop.

Davis, G. A., N. P. Havill, Z. N. Adelman, A. Caccone, L. T. kok and S. M. Salom. 2011. DNA barcodes and molecular diagnostics to distinguish an introduced and native *Laricobius* (Coleoptera: Derodontidae) species in eastern North America. Biol. Control. 58: 53-59.

Dilling, C., P. Lambdin, J. Grant and R. Rhea. 2009. Community response of insects associated with eastern hemlock to imidacloprid and horticultural oil treatments. Environ. Entomol. 38: 53-66.

Dilling, C., P. Lambdin, J. Grant and R. Rhea. 2010. Spatial and temporal distribution of imidacloprid in eastern hemlock in the southern appalachians. J. Econ. Entomol. 103: 368-373.

Doccola, J. J., W. Hascher, J. J. Aiken and P. M. Wild. 2012. Treatment strategies using imidacloprid in hemlock woolly adelgid (*Adelges tsugae* Annand) infested eastern hemlock (*Tsuga canadensis* Carrière) trees. Arboric. & Urban For. . 38: 41-49.

Elkinton, J. S., R. T. Trotter and A. F. Paradis. 2011. Simulations of population dynamis of hemlock woolly adelgid and potential impact of biological control agents. 15-24 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid USDA Forest Service publication FHTET: 2011-04

Evans, A. M. and T. G. Gregoire. 2007. A geographically variable model of hemlock woolly adelgid spread. Biol. Invasions. 9: 369-382.

Falcone, J. F. and L. E. DeWald. 2010. Comparisons of arthropod and avian assemblages in insecticide-treated and untreated eastern hemlock (*Tsuga canadensis* [L.] Carr) stands in Great Smoky Mountains National Park, USA. For. Ecol. Manag. 260: 856-863.

Faulkenberry, M., R. Hedden and J. Culin. 2009. Hemlock susceptibility to hemlock woolly adelgid attack in the Chattooga River watershed. Southeastern Naturalist. 8: 129-140.

Flowers, R. W., S. M. Salom and L. T. Kok. 2006. Competitive interactions among two specialist predators and a generalist predator of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae) in south-western Virginia. Agric. For. Entomol. 8: 253-262.

Flowers, R. W., S. M. Salom, L. T. Kok and D. E. Mullins. 2007. Behavior and daily activity patterns of specialist and generalist predators of the hemlock woolly adelgid, *Adelges tsugae*. J. Insect Sci. 7: 1-20.

Foottit, R. G., H. E. Maw, N. P. Havill, R. G. Ahern and M. E. Montgomery. 2009. DNA barcodes to identify species and explore diversity in the Adelgidae (Insecta: Hemiptera: Aphidoidea). Mol. Ecol. Res. 9 188-195.

Frank, S. D. and A. Lebude. 2011. Season-long insecticide efficacy for hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), management in nurseries. Fl. Entomol. 94: 290-295.

Gatton, H. A., G. M. G. Zilahi-Balogh, S. M. Salom and L. T. Kok. 2009. Biology and development of *Laricobius kangdingensis* Zilahi-Balogh and Jelínek, a newly discovered predator of *Adelges tsugae*. BioControl. 54: 219-228.

Gouli, S., B. L. Parker and M. Skinner. 1997. Isolation of fungi associated with hemlock woolly adelgid (Homoptera: Adelgidae). J. Invert. Pathol. 70: 76-77.

Grubin, S. M., D. W. Ross and K. F. Wallin. 2011. Prey suitability and phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) associated with hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. Environ. Entomol. 40: 1410-1416.

Hakeem, A., J. F. Grant, P. L. Lambdin, D. Buckley, F. A. Hale, J. R. Rhea, G. J. Wiggins and G. Taylor. 2010. Recovery of *Sasajiscymnus tsugae*, released against hemlock woolly adelgid, *Adelges tsugae*, in the southern Appalachians. Biocontrol Sci. Tech. 20: 1069-1074.

Hakeem, A., J. F. Grant, G. J. Wiggins, P. L. Lambdin and J. R. Rhea. 2011. Establishment and coexistence of two predators, *Laricobius nigrinus* and *Sasajiscymnus tsugae*, introduced against hemlock woolly adelgid on eastern hemlock. Biocontrol Sci. Tech. 21: 687-691.

Havill, N. P., C. S. Campbell, T. F. Vining, B. LePage, R. J. Bayer and M. J. Donoghue. 2008. Phylogeny and biogeography of *Tsuga* (Pinaceae) inferred from nuclear ribosomal ITS and chloroplast DNA sequence data. Syst. Botany. 33: 478-789.

Havill, N. P., G. Davis, D. L. Mausel, J. Klein, R. McDonald, C. Jones, M. Fischer, S. Salom and A. Caccone. 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biol. Control. 63: 359-369.

Havill, N. P. and R. G. Foottit. 2007. Biology and evolution of Adelgidae. Ann. Rev. Entomol. 52: 325-349.

Havill, N. P., R. G. Foottit and C. D. v. Dohlen. 2007. Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. Mol. Phylogenet. Evol. 44: 357-370.

Havill, N. P., M. Montgomery, S. Shiyake, A. Lamb, M. A. Keena and A. Caccone. 2009. Hemlock woolly adelgid population genetics, p.75. *In* K. A. McManus and K. W. Gottschalk [eds.]. Proceedings 20th U. S. Department of Agriculture Inteagency Research Forum on Invasive Species. USDA, For. Serv., Northern Research Station.

Havill, N. P., M. E. Montgomery and M. Keena. 2011. Hemlock woolly adelgid and its hemlock hosts: A global perspective. 3-14 *In* B. Onken and R. C. Reardon [eds.]. USDA Forest Service publication FHTET: 2011-04

Havill, N. P., M. E. Montgomery, G. Yu, S. Shiyake and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann. Entomol. Soc. Am. 99: 195-203.

Hoover, B. K., R. M. Bates, J. C. Sellmer and G. A. Hoover. 2009. Challenging Chinese hemlock (*Tsuga chinensis*) with hemlock woolly adelgid (*Adelges tsugae*) ovisacs. Arboric. & Urban For. 35: 1-4.

Jetton, R., B. Dvorak, K. Potter, A. Whittier and R. Rhea. 2009. Genetics and conservation of hemlock species threatened by the hemlock woolly adelgid, *In* [eds.]. Proceedings of the 30th Southern Tree Improvement Conference.

Joseph, S. V., S. K. Braman and J. L. Hanula. 2011a. Effects of fertilization of four hemlock species on Adelges tsugae (Hemiptera: Adelgidae) growth and feeding preference of predators. J. Econ. Entomol. 104: 288.

Joseph, S. V., S. K. Braman, J. Quick and J. L. Hanula. 2011b. The range and response of neonicotinoids on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). J. Environ. Hort. 29: 197-204.

Joseph, S. V., J. L. Hanula, S. K. Braman and F. J. Byrne. 2011c. Effects of fertilizer and low rates of imidacloprid on *Adelges tsugae* (Hemiptera: Adelgidae). J. Econ. Entomol. 104: 868-878.

Keena, M. A., R. T. Trotter, C. Cheah and M. E. Montgomery. 2012. Effects of temperature and photoperiod on the aestivo-hibernal egg diapause of *Scymnus camptodromus* (Coleoptera: Coccinellidae). Environ. Entomol. 41: 1662-1671.

Lagalante, A. F., N. Lewis, M. E. Montgomery and K. S. Shields. 2006. Temporal and spatial variation of terpenoids in eastern hemlock (*Tsuga canadensis*) in relation to feeding by *Adelges tsugae*. J. Chem. Ecol. 32: 2389-2403.

Lagalante, A. F. and M. E. Montgomery. 2003. Analysis of terpenoids from hemlock (*Tsuga*) species by solid-phase microextraction/gas chromatography/ion-trap mass spectrometry. J. Ag. and Food Chemistry. 51: 2115-2120.

Lamb, A., S. Shiyake, M. Montgomery and L. Kok. 2008. Evaluation of the Japanese *Laricobius* sp. n. and other natural enemies of hemlock woolly adelgid in Japan, pp. 29-36. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Fourth Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2008-01.

Lamb, A. B., S. M. Salom, L. T. Kok and D. L. Mausel. 2006. Confined field release of *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of the hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in Virginia. Can. J. For. Res. 36: 369-375.

Lawrence, J. F. and T. F. Hlavac. 1979. Review of the Derodontidae (Coleoptera: Polyphaga) with new species from North America and Chile. Coleopt. Bulletin. 33: 369-414.

Li, L., Y. Guo-Yue, T. J. McAvoy, R. C. Reardon, W. Yun and S. M. Salom. 2011. The biological characteristics, habitat and food selection of *Tetraphleps galchanoides* (Hemiptera: Anthocoridae). Acta Entomologica Sinica. 54: 800-808.

Lu, W. and M. E. Montgomery. 2001. Oviposition, development, and feeding of *Scymnus* (Neopullus) *sinuanodulus* (Coleoptera: Coccinellidae): a predator of *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 94: 64-70.

Lu, W., P. Souphanya and M. E. Montgomery. 2002. Descriptions of immature stages of *Scymnus* (Neopullus) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) with notes on life history. Coleopt. Bulletin. 56: 127-141.

Mausel, D. L., G. A. Davis, A. S. Lamb, G. M. G. Zilahi-Balogh, L. T. Kok and S. M. Salom. 2011a. *Laricobius nigrinus* Fender (Coleoptera: Derodontidae). 77-89 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid USDA Forest Service publication FHTET: 2011-04

Mausel, D. L., R. G. V. Driesche and J. S. Elkinton. 2011b. Comparative cold tolerance and climate matching of coastal and inland *Laricobius nigrinus*

(Coleoptera: Derodontidae), a biological control agent of hemlock woolly adelgid. Biol. Control. 58: 96-102.

Mausel, D. L., S. M. Salom, L. T. Kok and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39: 440-448.

Mausel, D. L., S. M. Salom, L. T. Kok and J. G. Fidgen. 2008. Propagation, synchrony, and impact of introduced and native *Laricobius spp.* (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. Environ. Entomol. 37: 1498-1507.

Mayer, M., R. Chianese, T. Scudder, J. White, K. Vongpaseuth and R. Ward. 2002. Thirteen years of monitoring the hemlock woolly adelgid in New Jersey forests, pp. 50-60. *In* B. Onken, R. C. Reardon and J. Lashcomb [eds.]. Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium New Brunswick, NJ: Rutgers University.

McClure, M. S. 1987. Biology and control of hemlock woolly adelgid. Bulletin - Connecticut Agricultural Experiment Station, New Haven. 851: 9 p.

McClure, M. S. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 82: 50-54.

McClure, M. S. 1990. Role of winds, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 19: 36-43.

McClure, M. S. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environ. Entomol. 20: 258-264.

McClure, M. S. 1992. Effects of implanted and injected pesticides and fertilizers on the survival of *Adelges tsugae* (Homoptera: Adelgidae) and on the growth of *Tsuga canadensis*. J. Econ. Entomol. 85: 468-472.

McClure, M. S. and C. Cheah. 2002. Important mortality factors in the life cycle of hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) in the Northeastern United States. In: Onken, B; Reardon, R; Lashomb, J./ eds. Proceedings, Hemlock woolly adelgid in the Eastern United States symposium; 2002 February 5 - 7; East Brunswick, NJ. New Brunswick, NJ: Rutgers University; 13-22.

McClure, M. S., S. Salom and K. S. Shields. 2001. Hemlock woolly adelgid. Forest Health Technology Enterprise Team. U.S. Forest Service Publication FHTET-2001-03. Morgantown, WV. 14 pp.

McDonald, R., D. Mausel, S. Salom, L. Kok, M. Montgomery, G. Luker, S. Steury, G. Spears, S. Skeate, J. Graham and B. Hamstead. 2008. Recovery of hemlock woolly adelgid predators in the high country of northwestern North Carolina, 2004-2008, pp. 53-60. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Fourth Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2008-01.

Montgomery, M. E. 2011. Understanding fegeral regulations as guidelines for classical biologcial control programs. 25-40 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid USDA Forest Service publication FHTET: 2011-04

Montgomery, M. E., S. E. Benta and R. T. Olsen. 2009. Evaluation of hemlock (*Tsuga*) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. J. Econ. Entomol. 102: 1247-1254.

Montgomery, M. E. and M. A. Keena. 2011. *Scymnus* (Neopullus) lady beetles from China. 53-76 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid.

Montgomery, M. E. and S. M. Lyon. 1996. Natural enemies of adelgids in North America: Their prospect for biological control a *Adelges tsugae* (Homoptera: Adelgidae), pp. 89-102. *In* S. M. Salom, T. C. Tigner, and R. C. Reardon [eds.]. Proceedings, First Hemlock Woolly Adelgid Review. U.S.D.A. For. Serv. FHTET 96 - 10.

Montgomery, M. E., T. J. McAvoy and S. M. Salem. 2011a. Other species considered. 116-122 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. USDA Forest Service publication FHTET: 2011-04

Montgomery, M. E., S. Shiyake, N. P. Havill and R. A. B. Leschen. 2011b. A new species of *Laricobius* (Coleoptera: Derodontidae) from Japan with phylogeny and a key for native and introduced congeners in North America. Ann. Entomol. Soc. Am. 104: 389-401.

Orwig, D. A. and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Bulletin of the Torrey Botanical Club. 125: 60-73.

Oten, K. L. F., G. R. Bauchan, J. Frampton and F. P. Hain. 2012. Biophysical characteristics of the stem and petiole surface of six hemlock (*Tsuga*) species and a hybrid: implications for resistance to *Adelges tsugae*. Botany. 90: 1170-1178.

Paradis, A., J. Elkinton, K. Hayhoe and J. Buonaccorsi. 2008. Role of winter temperature and climate change on the survival and future range expansion of the hemlock woolly adelgid (*Adelges tsugae*) in eastern North America. Mitig. Adapt. Strat. Glob. Change. 13: 541-554.

Paradis, A. F. 2011. Population dynamics of hemlock woolly adelgid (Hemiptera: Adelgidae) University of Massachusetts, Amherst. *Electronic Doctoral Dissertations for UMass Amherst*. Paper AAI3482653. http://scholarworks.umass.edu/dissertations/AAI3482653

Reid, W. R., B. L. Parker, S. Y. Gouli, M. Skinner, V. V. Gouli and H. B. Teillon. 2010. Fungi associated with the hemlock woolly adelgid, *Adelges tsugae*, and assessment of entomopathogenic isolates for management. J. Insect Sci. 10: 1-18.

Salom, S. M., A. A. Sharov, W. T. Mays and J. W. N. Jr. 2001. Evaluation of aestival diapause in hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 30: 877-882.

Sasaji, H. and M. S. McClure. 1997. Description and distribution of *Pseudoscymnus tsugae* sp. nov. (Coleoptera: Coccinellidae), an important predator of hemlock woolly adelgid in Japan. Ann. Entomol. Soc. Am. 90: 563-568.

Steward, B. V. and T. A. Horner. 1994. Control of hemlock woolly adelgid using soil injections of systemic insecticides. J. Arboric. 20: 287-288.

Story, H. M., L. C. Vieira, S. M. Salom and L. T. Kok. 2012. Assessing performance and competition among three *Laricobius* (Coleoptera: Derodontidae) species, predators of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). Environ. Entomol. 41: 896-904.

Trotter, R. T. and K. S. Shields. 2009. Variation in winter survival of the invasive hemlock woolly adelgid (Hemiptera: Adelgidae) across the eastern United States. Environ. Entomol. 38: 577-587.

Vieira, L. C., A. B. Lamb, S. Shiyake, S. M. Salom and L. T. Kok. 2013. Seasonal abundance and synchrony between *Laricobius osakensis* (Coleoptera: Derodontidae) and its prey, *Adelges tsugae* (Hemiptera: Adelgidae), in Japan. Ann. Entomol. Soc. Am. 106: 249-257.

Vieira, L. C., T. J. McAvoy, J. Chantos, A. B. Lamb, S. M. Salom and L. T. Kok. 2011. Host range of *Laricobius osakensis* (Coleoptera: Derodontidae), a new biological control agent of hemlock woolly adelgid (Hemiptera: Adelgidae). Environ. Entomol. 40: 324-332.

Vieira, L. C., S. M. Salom and L. T. Kok. 2012. Functional and numerical response of *Laricobius spp*. predators (Coleoptera: Derodontidae) on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). Biol. Control. 61: 47-54.

von Dohlen, C. D., U. Spaulding, K. Shields, N. P. Havill, C. Rosa and K. Hoover. 2013. Diversity of proteobacterial endosymbionts in hemlock woolly adelgid (*Adelges tsugae*) (Hemiptera: Adelgidae) from its native and introduced range. Environ. Microbiol. 15: 2043-2062.

Wager-Page, S. 2012. Release of the Predatory Beetle *Scymnus coniferarum* (Coleoptera: Coccinellidae), for Biological Control of the Hemlock Woolly Adelgid (*Adelges tsugae*) in the Eastern United States. http://www.aphis.usda.gov/plant_health/ea/downloads/HWA-biocontrol-EA-FONSI.pdf.

Wallace, M. S. and F. P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. Environ. Entomol. 29: 638-644.

Ward, J. S., M. E. Montgomery, C. A. S.-J. Cheah and R. S. Cowles. 2004. Eastern hemlock forest: guidelines to minimize the impacts of hemlock woolly adelgid. U.S. Department of Agriculture. Forest Service, Washington, DC.

Webb, R. E., J. R. Frank and M. J. Raupp. 2003. Eastern hemlock recovery from hemlock woolly adelgid damage following imidacloprid therapy. J. Arboric. 29: 298-302.

Wilson, J. W. 1927. The male genital tube of some of the species of the genus *Scymnus* (Coleoptera, fam. Coccinellidae). Psyche. 34: 167-171.

Young, R. F., K. S. Sheilds and G. P. Berlyn. 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. Ann. Entomol. Soc. Am. 88: 827-835.

Zilahi-Balogh, G. M. G., C. D. Broeckling, L. T. Kok and S. M. Salom. 2005. Comparison between a native and exotic adelgid as hosts for *Laricobius rubidus* (Coleoptera: Derodontidae). Biocontrol Sci. Tech. 15: 165-171.

Zilahi-Balogh, G. M. G., L. M. Humble, L. T. Kok and S. M. Salom. 2006. Morphology of *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of the hemlock woolly adelgid. Can. Ent. 138: 595-601.

Zilahi-Balogh, G. M. G., L. M. Humble, A. B. Lamb, S. M. Salom and L. T. Kok. 2003a. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). Can. Entomol. 135: 103-115.

Zilahi-Balogh, G. M. G., L. T. Kok and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Biol. Control. 24: 192-198.

Zilahi-Balogh, G. M. G., S. M. Salom and L. T. Kok. 2003b. Temperature-dependent development of the specialist predator *Laricobius nigrinus* (Coleoptera: Derodontidae). Environ. Entomol. 32: 1322-1328.

CHAPTER 2

HEMLOCK WOOLLY ADELGID PREDATOR BEETLE RELEASES AND RECOVERY EFFORTS IN THE NORTH GEORGIA MOUNTAINS

Jones, C. E., Havill, N. P., Hanula, J. L., and Braman, S. K. To be submitted to the *Journal of Entomological Science*.

Abstract

Eastern hemlock, *Tsuga canadensis,* and Carolina hemlock, *Tsuga caroliniana,* provide unique habitat that is threatened by the invasive hemlock woolly adelgid, *Adelges tsugae,* which arrived in Georgia in 2003. In an attempt to conserve a portion of the mature hemlocks in north Georgia, the USDA Forest Service created over 100 Hemlock Conservation Areas throughout the Chattahoochee National Forest and designated them to receive chemical and/or biological control. *Sasajiscymnus tsugae*, *Laricobius nigrinus*, and *Scymnus sinunodulus* are predatory beetles reared in the laboratory and released in these areas. To determine establishment of these predators, infested hemlock trees were sampled during spring 2010 – 2012 at some of these release sites. Additionally, non-release sites, 0.4 to 1.6 km from release areas, were sampled in 2012 to evaluate predator spread from release trees. 592 *Sasajiscymnus tsugae*, 232 *L. nigrinus*, 262 native *L. rubidus,* and 58 *Laricobius* hybrids were recovered at multiple sites over the years. *Sasajiscymnus tsugae* was found at three sites, three years after release and at two other sites, two years after release. *Laricobius nigrinus* was found at one site, three years after release and at two sites, two years after release. *Scymnus sinunodulus* was never recovered. Our results demonstrate that *S. tsugae* and *L. nigrinus* are established in North Georgia, and that the native *L. rubidus* is commonly associated with *A. tsugae*, and is hybridizing with *L. nigrinus*; however, the population sizes, efficacy, and survival rates of all these predators are still unknown.

Keywords: *Adelges tsugae*, *Laricobius nigrinus*, *Laricobius rubidus*, *Sasajiscymnus tsugae*, biological control

Introduction

Eastern (Canadian) hemlock, *Tsuga canadensis* (L.) Carrière, is one of the most shade tolerant and long-lived tree species in eastern North America (Ward et al., 2004), where it can be found ranging from Georgia into Canada. It also occurs in Michigan and Wisconsin along with isolated pockets in Alabama and Indiana. The dense canopy and location of these trees provides a unique habitat for many plant and animal species (Ward et al., 2004). In contrast, Carolina hemlocks (*Tsuga caroliniana* Engelmann) are limited to a small area of western North Carolina and patches in the surrounding states. Both species are threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), and the loss of this unique habitat they provide would alter forest community and ecosystem dynamics (Ellison et al., 2005).

Hemlock woolly adelgid is an invasive, aphid-like insect that, while currently described as a single species, contains several divergent lineages endemic to different regions in Asia and western North America (Havill et al., 2006). *Adelges tsugae* feeds on the xylem ray parenchyma cells of hemlock trees (Young et al., 1995). Hemlock species in their native ranges are resistant or tolerant to *A. tsugae* and native predators prevent it from damaging the trees (Montgomery et al., 2009). The parthenogenetic *A. tsugae* has two generations a year, with a longer sistens generation (summer –early spring generation) that aestivates as 1st instar nymphs, becoming active in late fall where they produce cottony ovisacs that give rise to the short progrediens generation (spring generation) (McClure, 1989).

The first east coast record of *Adelges tsugae* was in 1951 in Richmond, VA (McClure, 1989), most likely brought over on ornamental hemlocks from Japan (Havill et al., 2006). Both eastern and Carolina hemlock show no resistance to *A. tsugae* and no specialized predators are found on the East Coast (Montgomery and Lyon, 1996, Wallace and Hain, 2000). This lack of population control, along with the adelgids bivoltine asexual lifecycle and mobile crawler stage has allowed them to spread rapidly with the help of wind, and migratory birds, and mammals (McClure, 1990). As of 2013, *A. tsugae* can be found as far north as Maine, south to Georgia, and west into Tennessee and Kentucky. Hemlock mortality can occur in four to six years (Mayer et al., 2002, McClure, 1991) and faster in the southern range due to other factors such as drought stress (Ward et al., 2004) and mild winters that are positively related to *A. tsugae* survival (Trotter and Shields, 2009).

Hemlock occupies only about 4% of approximately 685,000 acres in the Blue Ridge and Mountain-Piedmont transition zone in Georgia with two-thirds of hemlocks being a minority species in hardwood and white pine (*Pinus strobus* L.) mixed composition stands (Meyer, 2005). *Adelges tsugae* was first discovered in Georgia in 2003 in Rabun County. Since then it has spread west and now, in 2013, the infestation covers the entire range of hemlock in Georgia (Georgia Forestry Commission, 2012).

In response to *A. tsugae* arrival in north Georgia, the U.S. Forest Service created 144 Hemlock Conservation Areas (HCAs) within the Chattahoochee National Forest to be treated either with biological controls and/or insecticides to try to save a portion of the mature hemlock trees in this region (Meyer, 2005, Save Georgia's Hemlocks, 2013).

The HCAs range between 3 – 447 hectares, typically containing multiple beetle release sites that are generally separate from the insecticide (imidacloprid soil drench or injection) treatments (see Joseph et al., 2011). Within each release site, multiple trees will have beetle releases, though not every release tree is always individually marked. The three biological control predators released in Georgia and other eastern states include: *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae), and *Scymnus sinuanodulus* (Yu and Yao) (Coleoptera: Coccinellidae).

Sasajiscymnus tsugae, a small black beetle native to Japan, has the longest history of releases in the eastern U.S. Beetles were first released in Connecticut in 1997 (Cheah, 2011) and has since been released repeatedly throughout the introduced range of *A. tsugae*. *Sasajiscymnus tsugae* was first released in Georgia in 2004 (Asaro et al., 2005). They are active from March to November and have two generations per year with eggs of *S. tsugae* found in the spring starting when daytime temperatures average 15ºC (Cheah, 2011). Both larvae and adults feed on all stages of *A. tsugae* (Cheah and McClure, 1998). In Georgia, adult *S. tsugae* are typically released from March to June.

Laricobius nigrinus is a small black beetle native to the Pacific Northwest. Adults are active during late September to May, with eggs being found between late January to May and larvae from March to early June (Mausel et al., 2011, Zilahi-Balogh et al., 2003a). *Laricobius nigrinus* spends the summer underground as pupae. They have one generation per year and need *A. tsugae* eggs to mature into adults which can then feed

on all life stages (Zilahi-Balogh et al., 2002). In Georgia, the typical release method for *L. nigrinus* involves allowing females to oviposit on adelgid infested branches in the lab, and these twigs with beetle eggs are placed in the field. Some adult releases of lab reared beetles have also occurred. Relatively few adults are released, however, due to high pupal mortality in the lab and asynchronous emergence times following summer aestivation (Salom et al., 2012). Currently adult releases are more likely to include wild caught adults from either North Carolina or Washington. Adults are released from fall to late spring.

Scymnus sinuanodulus is a small beetle from China that is red-brown in color with one black spot on each elytron. Adult activity begins in spring and they have one generation per year. Larvae will feed on all life stages of *A. tsugae* but they survive significantly better on *A. tsugae* eggs (Lu and Montgomery, 2001, Lu et al., 2002). In Georgia, the typical release methods for *S. sinuanodulus* were either as eggs laid on twigs or release of adults at the end of the rearing season. However, rearing efforts have halted for this predator since data suggests *S. sinuanodulus* was not able to survive in Tennessee following release in whole tree cages (Hakeem et al., 2011) or field releases in North Carolina (McDonald et al., 2008).

Beetle releases started in eastern Georgia in 2004 (Asaro et al., 2005) where *A. tsugae* was initially discovered and the releases then moved west following the spread of the infestation. By the end of the 2011 release season, roughly 1,300,000 predator beetles have been released in the North Georgia mountains with *S. tsugae* totaling 998,531 (871,869 adults, 126,662 eggs), *L. nigrinus* totaling 263,018 (24,591 adults,

238,427 eggs), and *S. sinuanodulus* totaling 47,461 (3,333 adults, 44,128 eggs) (appendix 2.1). Most areas receive predator releases over multiple-years if the hemlocks remain healthy enough to support the predatory beetles. As hemlock trees succumb to *A. tsugae* infestation, visible decline can be seen as lack of new growth, followed by tip die-back and thinning of the crown. This decline in tree health also leads to declining *A. tsugae* populations to the point where no new ovisacs can be found as there are no suitable feeding sites. However, if the hemlocks start to produce new growth (often the year after *A. tsugae* is no longer observed on the tree) additional predatory beetle releases have been made.

Although beetle releases began in Georgia as early as 2004 (Asaro et al., 2005, Mausel et al., 2010) the sites being monitored for beetle establishment had predators released from 2007-2012. This has given the predators up to six years to establish and grow in these areas. In addition, *Laricobius rubidus* (LeConte), a native predator of pine bark adelgid, *Pineus strobi* (Hartig), is also found on *A. tsugae* infested hemlocks where it can complete development (Mausel et al., 2008, Zilahi-Balogh et al., 2005) and interbred with *L. nigrinus* (Havill et al., 2012).

With effective biological control agents characterized in part as those that easily establish (Clausen, 1951), our objectives were to determine which of the three predator beetle species released in Georgia are established in the forest and if they are spreading from the points of release.

Methods and Materials

Selection of Release Sites and Trees. GPS coordinates, provided by the four laboratories doing beetle releases in North Georgia, were used to locate release areas and trees on which beetles were released and were further identified by metal tags and/or flagging when possible. If *A. tsugae* could be found on the release tree, then it was selected for sampling. However, if no infestation was found on the tree, then surrounding trees were examined until *A. tsugae* was located. Therefore, sampling was sometimes conducted on other nearby trees.

In 2010, 13 sites in 11 Hemlock Conservation Areas (HCAs) were selected for sampling based on having prior beetle releases and no signs of severe hemlock decline (Fig. 2.1, Table 2.1). In 2011, 14 sites in 11 HCAs were sampled. Four of the original sites from 2010 were excluded due to road closure, a tornado, or because additional beetles were released in 2011 which would have confounded sampling results. Five additional sites were selected (Fig. 2.1, Table 2.1). In 2012, 17 sites in 14 HCAs were sampled including seven sites that were sampled the previous two years, five sites that were sampled the prior year, two sites that were sampled in 2010 but not 2011, and three new sites where beetles had been released two to three years previously with no subsequent releases (Fig. 1, Table 1).

Determination of Establishment. To determine establishment of the predator beetles, branch clippings were collected using the sampling methods of Mausel et al. (2010) which are an effective method for collecting larvae. Since our goal was to sample all predators at the same time, this method was used to sample for eggs and larvae

when average daytime temperatures were approximately 15°C as *L. nigrinus* (Zilahi-Balogh et al., 2003b), *S. tsugae* (Cheah, 2011), and *S. sinuanodulus* (Lu and Montgomery, 2001) oviposit at this temperature.

In 2010, 10 trees at each site were sampled using a pole pruner (6.1m long) to remove two or three, 1m long adelgid infested limbs per tree (Table 2.1). Each limb was placed on a white tray while being processed to catch larvae or adults that were dislodged. From those limbs, 15 adelgid infested twigs (ca. 15-20cm long) were clipped from each tree and placed immediately into 7.6L zip lock bags. The remaining branches were beaten over a sheet of white cloth to dislodge any additional larvae or adults. If beetles were found, they were placed into Petri dishes, returned to the lab, and identified. A total of 150 twigs were collected per site. These twigs were placed into floral foam blocks, and kept in separate BugDorm Insect Tents (60 x 60 x 60cm, model BD2120, MegaView Science Co., Ltd, Taichung, Taiwan). Branches in the rearing tents were elevated by placing them on hardware cloth (1.27cm mesh) so that *L. nigrinus* larvae, which drop from the branches to pupate, could be collected more easily (Fig. 2.2). Foam blocks were wrapped in plastic wrap except the top which had a fine screen cover. This allowed for easy weekly watering through the screen top but prevented larvae from being lost in the block (Fig. 2.3). Twigs were held at 20°C, 14L:10D, and 65%RH. Supplemental adelgid infested hemlock twigs collected from non-release areas were added each week to provide additional food for the predators during 2010. However, since in 2011 *L. rubidus* was found in the area where infested hemlocks were harvested for the lab colony, supplemental foliage was not added the following years.

During this time emerging larvae or adult beetles were collected and placed in 95% EtOH and stored in a freezer until voucher specimens were prepared and/or DNA analysis was conducted. Vouchers were deposited at Yale University's Peabody Museum of Natural History. For *Laricobius* samples, the identity was determined using the methods described in Havill et al. (2012). Briefly, individual genotypes were scored using six microsatellite markers, and were compared to known reference samples using the software Structure 2.3.2 (Pritchard et al., 2000) and NewHybrids 1.1 (Anderson and Thompson, 2002) to determine whether a beetles was *L. nigrinus, L. rubidus,* or a hybrid. After nine weeks the tents were emptied and each branch carefully examined for any remaining beetles.

In 2011, four sites (3 HCAs) were sampled using the 2010 methods described above. The remaining 10 sites were sampled using pole pruners or hand shears to collect branches from nearby trees due to declines in *A. tsugae* populations. If 150 adelgid infested twigs were not found, the number of twigs collected and trees sampled were recorded (Table 2.1). Beetles were reared from the twigs using the rearing tents as in 2010 or if less than 30 twigs were available, in smaller 64.4 liter clear plastic container with locking lid (68 x 45 x 30 cm) that were modified by cutting 38 x 23 cm holes in the longer sides and lid and hot gluing no-see-um netting (Mosquito Curtains, Inc., Atlanta, GA) to cover the hole (Fig. 2.4). In 2012, the modified sampling techniques of 2011 were used again due to lack of adelgid infested branches to sample (Table 2.1).

Determination of Spread from Release Areas. To evaluate spread, we sampled 0.4 to 1.6 kilometers from 13 release sites in 10 HCAs and one site between release

sites in two HCAs (Table 2.2). Sites were selected by entering all release site coordinates into Arc MAP and creating radii of 0.4, 0.8, and 1.6 kilometers. Locations with no overlap with other sites and with sufficient hemlock (i.e. along rivers) were selected for sampling. If roads allowed for easy access in a different direction, these areas were also examined for hemlocks as well as *A. tsugae*. Sampling of the non-release sites were conducted in the same manner as release sites using pole-pruners to sample 150 twigs, when possible, from 10 trees.

Statistical Analyses. Recovery efforts in 2012 had the potential of sampling at least three years after releases which would indicate establishment. Therefore this year's data were analyzed using Fisher's exact test to determine whether recovery of *S. tsugae*, *L. nigrinus*, or *Laricobius* hybrids were independent of the most recent year of corresponding predator releases. *Laricobius* hybrid recovery was compared on the basis of the last year of *L. nigrinus* releases.

Results

Recovery Efforts. *Sasajiscymnus tsugae* was recovered from six sites (153 beetles) in 2010, seven sites (100 beetles) in 2011, and nine sites (312 beetles) in 2012, respectively (Table 2.3). *Laricobius nigrinus* was recovered from four sites (25 beetles) in 2010, three sites (58 beetles) in 2011, and six sites (146 beetles) in 2012 (Table 2.4). *Laricobius nigrinus* x *rubidus* hybrids were found at two sites (20 beetles) in 2010, three sites (15 beetles) in 2011, and four sites (19 beetles) in 2012 (Table 2.4). In addition to recovery of introduced predators, native *L. rubidus* were recovered from four

sites (21 beetles) in 2010, three sites (15 beetles) in 2011, and four sites (19 beetles) in 2012 (Table 2.5). *Scymnus sinuanodulus* was never recovered during the study. Using the Fisher's exact test, 2012 recovery data were compared in regards to year of their last release (2009, 2010, or 2011). There were no significant differences in *S. tsugae* (P = 1.0000), *L. nigrinus* (P = 0.7580), or hybrids (P = 0.5036) recovered based on year they were last released. While there was no significant difference, more sites were positive for *L. nigrinus* and hybrid recovery when sampled one year after release (3 of 9 sites) than two years after release (1 of 4 sites for *L. nigrinus*, 2 of 4 sites for hybrids) and three years post release (0 of 4 sites). *Sasajiscymnus tsugae* was evenly split on whether a site was positive for recovery one year after release (4 of 8 sites), two years after release (2 of 3 sites), and three years after release (3 of 6 sites).

Determination of Establishment. Beetles were considered to be established if they could be found at least three generations after their last release. Due to continual releases at the sampling sites by multiple laboratories, only four sites did not have beetles released in the three years prior to 2012. A single site, HCA# 78 Canada Creek 1, had both *S. tsugae* and *L. nigrinus* established together (Tables 2.3 and 2.4). *Sasajiscymnus tsugae* was also recovered three years after release at HCAs # 63 Wolf Pen Gap and # 71 Slaughter Creek so this beetle can be considered established at these sites as well. *Sasajiscymnus tsugae* was recovered two years after release at HCA sites # 73 Dockery Lake and # 88 Noontootla (Table 2.3). Since *S. tsugae* has two generations per year the recovered beetles should belong to the fourth generation produced in the field. In addition, *L. nigrinus* was recovered two years after release at

HCA sites # 73 Dockery Lake and # 88 Noontootla and *Laricobius* hybrids were recovered from HCA# 71 Slaughter Creek four years after *L. nigrinus* were released at that site (Table 2.4).

Determination of Spread from Release Areas. Of the 13 non-release sites sampled within 0.4 to 1.6 km of release sites, 27 *S. tsugae* were recovered from three sites, three *L. nigrinus* from three sites, two *Laricobius* hybrids from two sites, and 56 native *L. rubidus* from 10 sites (Table 2.2).

Discussion

Sasajiscymnus tsugae and *L. nigrinus* were consistently recovered at three HCAs every year and *S. tsugae* was consistently found at a fourth as well. It is uncertain as to what makes these sites suitable locations, but it could be due to higher elevations (above 700m) and that the tree health and *A. tsugae* populations were rebounding in those areas. It is clear that both *S. tsugae* and *L. nigrinus* can establish in North Georgia, however their population sizes, efficacy, and survival rates are still unknown. Since *Scymnus sinuanodulus* was never recovered it appears that either this predator is not suitable as a biological control agent in this region, or not enough beetles were released for them to establish.

Documented recovery of *S. tsugae* is minimal (Cheah et al., 2005), and to our knowledge, only one other study has kept voucher specimens of recovered beetles (Hakeem et al., 2010). With a total of 565 *S. tsugae* recovered at roughly 50% of the

sites for three years, these results document not only the largest recovery of *S. tsugae* recorded, but also at a high success rate. Hakeem et al. (2010) were able to recover beetles at 21% of their sites using beat sheets, while Cheah et al. (2005) recovered beetles at 65% of the sites one year after release.

The use of beat sheets limit sampling efforts to the lower canopy and the presence of predators can be underestimated when *A. tsugae* is present throughout the trees. Research has shown that the predators can be found high in the canopy for *S. tsugae* (4 - 20 m, Cheah et al., 2005, Cheah and McClure, 2002) and *L. nigrinus* (above 15 m, Davis et al., 2012). However, *L. nigrinus* were recovered below 7 m when *A. tsugae* densities were highest there compared to the rest of the tree strata (Davis et al., 2012).

Collections at non-release sites showed that the native *L. rubidus* is a common predator associated with *A. tsugae*, but its effect on populations is unknown. Because it is so common, its presence raises concerns of bringing *L. rubidus* into the rearing lab on infested foliage intended to feed beetle colonies which could result in competition and hybridization making the colony no longer “pure” *L. nigrinus*.

With hundreds of thousands of predatory beetles released throughout North Georgia, continued recovery efforts would greatly improve our knowledge of establishment and dispersal. Additional methods can be employed, such as using a sweep net that can reach high into the canopy (Shiyake et al., 2008) or tree climbing

(Davis et al., 2012) as well as increased frequency of sampling to determine when life stages are found in Georgia.

Acknowledgements

We thank Paul Arnold, LayLa Burgess, and Mark Dalusky for providing predatory beetle release data, DeAdra Newman for help with genetic analysis, and Kamal Gandhi and Angela Mech for helpful discussion and insight throughout this project. Research was supported through the University of North Georgia and the USDA Forest Service, Southern Research Station work unit SRS 4552, Insects, Diseases and Invasive Plants.

Literature Cited

Anderson, E. C. and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics. 160: 1217-1229.

Asaro, C., C. W. Berisford, M. E. Montgomery, J. R. Rhea and J. L. Hanula. 2005. Biological control of the hemloc woolly adelgid in the southern appalachians, pp. 248-251. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Third Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2005-01.

Cheah, C. and M. S. McClure. 1998. Life history and development of *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae), a new predator of the hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 27: 1531-1536.

Cheah, C. A. S.-J. 2011. *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae*, a ladybeetle from Japan. 42-53 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid USDA Forest Service publication FHTET: 2011-04

Cheah, C. A. S.-J., M. A. Mayer, D. Palmer, T. Scudder and R. Chianese. 2005. Assessments of biological control of hemlock woolly adelgid with *Sasajiscymnus tsugae* in Connecticut and New Jersey, pp. 116-130. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Third Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2005-01.

Cheah, C. A. S.-J. and M. S. McClure. 2002. *Pseudoscymnus tsugae* in Connecticut forests: The first five years, pp. 150-165. *In* B. Onken, R. C. Reardon and J. Lashcomb [eds.]. Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. New Brunswick, NJ: Rutgers University.

Clausen, C. P. 1951. The time factor in biological control. J. Econ. Entomol. 44: 1-9.

Davis, G. A., S. M. Salom, C. C. Brewster, B. P. Onken and L. T. Kok. 2012. Spatiotemporal distribution of the hemlock woolly adelgid predator *Laricobius nigrinus* after release in eastern hemlock forests. Agric. For. Entomol. 14: 408-418.

Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, J. Mohan, D. A. Orwig, N. L. Rodenhouse, W. V. Sobczak, K. A. Stinson, J. K. Stone, C. M. Swan, J. Thompson, B. v. Holle and J. R. Webster. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems Front. Ecol. Environ. 3: 479-486.

Georgia Forestry Commission. 2012. Hemlock woolly adelgid annual spread map in Georgia. http://www.gfc.state.ga.us/forest-management/forest-health/hemlock-woolly-adelgid/HWASpreadinGA.jpg

Hakeem, A., J. F. Grant, P. L. Lambdin, D. Buckley, F. A. Hale, J. R. Rhea, G. J. Wiggins and G. Taylor. 2010. Recovery of *Sasajiscymnus tsugae*, released against hemlock woolly adelgid, *Adelges tsugae*, in the southern Appalachians. Biocontrol Sci. Tech. 20: 1069-1074.

Hakeem, A., J. F. Grant, G. J. Wiggins, P. L. Lambdin and J. R. Rhea. 2011. Establishment and coexistence of two predators, *Laricobius nigrinus* and

Sasajiscymnus tsugae, introduced against hemlock woolly adelgid on eastern hemlock. Biocontrol Sci. Tech. 21: 687-691.

Havill, N. P., G. Davis, D. L. Mausel, J. Klein, R. McDonald, C. Jones, M. Fischer, S. Salom and A. Caccone. 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biol. Control. 63: 359-369.

Havill, N. P., M. E. Montgomery, G. Yu, S. Shiyake and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann. Entomol. Soc. Am. 99: 195-203.

Joseph, S. V., J. L. Hanula and S. K. Braman. 2011. Distribution and abundance of *Adelges tsugae* (Hemiptera: Adelgidae) within hemlock trees. J. Econ. Entomol. 104: 1918-1927.

Lu, W. and M. E. Montgomery. 2001. Oviposition, development, and feeding of *Scymnus* (Neopullus) *sinuanodulus* (Coleoptera: Coccinellidae): a predator of *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 94: 64-70.

Lu, W., P. Souphanya and M. E. Montgomery. 2002. Descriptions of immature stages of *Scymnus* (Neopullus) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) with notes on life history. Coleopt. Bulletin. 56: 127-141.

Mausel, D. L., G. A. Davis, A. S. Lamb, G. M. G. Zilahi-Balogh, L. T. Kok and S. M. Salom. 2011. *Laricobius nigrinus* Fender (Coleoptera: Derodontidae). 77-89 *In* B. Onken and R. C. Reardon [eds.]. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid USDA Forest Service publication FHTET: 2011-04

Mausel, D. L., S. M. Salom, L. T. Kok and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39: 440-448.

Mausel, D. L., S. M. Salom, L. T. Kok and J. G. Fidgen. 2008. Propagation, synchrony, and impact of introduced and native *Laricobius spp.* (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. Environ. Entomol. 37: 1498-1507.

Mayer, M., R. Chianese, T. Scudder, J. White, K. Vongpaseuth and R. Ward. 2002. Thirteen years of monitoring the hemlock woolly adelgid in New Jersey forests, pp. 50-60. *In* B. Onken, R. C. Reardon and J. Lashcomb [eds.]. Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium New Brunswick, NJ: Rutgers University.

McClure, M. S. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 82: 50-54.

McClure, M. S. 1990. Role of winds, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 19: 36-43.

McClure, M. S. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environ. Entomol. 20: 258-264.

McDonald, R., D. Mausel, S. Salom, L. Kok, M. Montgomery, G. Luker, S. Steury, G. Spears, S. Skeate, J. Graham and B. Hamstead. 2008. Recovery of hemlock woolly adelgid predators in the high country of northwestern North Carolina, 2004-2008, pp.

53-60. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Fourth Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2008-01.

Meyer, C. L. 2005. Conservation of eastern hemlock by suppression of hemlock woolly adelgid infestations. Chattahoochee-Oconee National Forest, 1755 Cleveland Hwy. Gainesville, Georgia 30501. (770) 297-3000.

Montgomery, M. E., S. E. Benta and R. T. Olsen. 2009. Evaluation of hemlock (*Tsuga*) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. J. Econ. Entomol. 102: 1247-1254.

Montgomery, M. E. and S. M. Lyon. 1996. Natural enemies of adelgids in North America: Their prospect for biological control a *Adelges tsugae* (Homoptera: Adelgidae), pp. 89-102. *In* S. M. Salom, T. C. Tigner, and R. C. Reardon [eds.]. Proceedings, First Hemlock Woolly Adelgid Review. U.S.D.A. For. Serv. FHTET 96 - 10.

Pritchard, J. K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Salom, S. M., L. T. Kok, A. B. Lamb and C. Jubb. 2012. Laboratory rearing of *Laricobius nigrinus* (Coleoptera: Derodontidae): a predator of the hemlock woolly adelgid (Hemiptera: Adelgidae). Psyche. 2012: 9 p

Save Georgia's Hemlocks. 2013. Hemlock Conservation Areas. http://www.savegeorgiashemlocks.org/Hemlock_Conservation_Areas.htm

Shiyake, S., Y. Miyatake, M. Montgomery and A. Lamb. 2008. Hemlock woolly adelgid phenology and predacious beetle community on Japanese hemlocks, pp. 261-266.

In B. Onken and R. Reardon [eds.]. Proceedings of the Fourth Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2008-01.

Trotter, R. T. and K. S. Shields. 2009. Variation in winter survival of the invasive hemlock woolly adelgid (Hemiptera: Adelgidae) across the eastern United States. Environ. Entomol. 38: 577-587.

Wallace, M. S. and F. P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. Environ. Entomol. 29: 638-644.

Ward, J. S., M. E. Montgomery, C. A. S.-J. Cheah and R. S. Cowles. 2004. Eastern hemlock forest: guidelines to minimize the impacts of hemlock woolly adelgid. U.S. Department of Agriculture. Forest Service, Washington, DC.

Young, R. F., K. S. Sheilds and G. P. Berlyn. 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. Ann. Entomol. Soc. Am. 88: 827-835.

Zilahi-Balogh, G. M. G., C. D. Broeckling, L. T. Kok and S. M. Salom. 2005. Comparison between a native and exotic adelgid as hosts for *Laricobius rubidus* (Coleoptera: Derodontidae). Biocontrol Sci. Tech. 15: 165-171.

Zilahi-Balogh, G. M. G., L. M. Humble, A. B. Lamb, S. M. Salom and L. T. Kok. 2003a. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). Can. Entomol. 135: 103-115.

Zilahi-Balogh, G. M. G., L. T. Kok and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of

the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Biol. Control. 24: 192-198.

Zilahi-Balogh, G. M. G., S. M. Salom and L. T. Kok. 2003b. Temperature-dependent development of the specialist predator *Laricobius nigrinus* (Coleoptera: Derodontidae). Environ. Entomol. 32: 1322-1328.

Table 2.1: Total number of twigs collected and trees sampled for recovery at each sampling site from 2010 to 2012. In 2010, all sites had 10 trees sampled for a total of 150 twigs/site so only the date sampled is listed.

					2010	2011			2012		
HCA #	Site	Altitude (m)	Latitude	Longitude	Date Sampled	Date Sampled	# twigs collected	# trees sampled	Date Sampled	# twigs collected	# trees sampled
29	Lower Panther A	225	34.66906	-83.36742					3/27/2012	150	10
29	Lower Panther B	261	34.67403	-83.37290	4/21/2010				3/27/2012	150	10
29	Upper Panther	449	34.69642	-83.41708	4/21/2010				3/27/2012	150	10
145	Soque River	457	34.71582	-83.57611	4/12/2010	4/23/2011	23	8	3/22/2012	150	10
115	Yahoola Creek	495	34.61288	-83.98640	4/7/2010	4/26/2011	18	6	4/4/2012	150	10
90	Jones Creek A	513	34.60015	-84.13520		4/13/2011	150	10	4/3/2012	6	1

68	Boggs										
	Creek	517	34.68780	-83.88995	4/7/2010	Tornado: area closed					
90	Jones										
	Creek B	522	34.60261	-84.15091	4/14/2010	4/13/2011	150	10	4/3/2012	16	4
72	Waters										
	Creek A	526	34.67639	-83.94282	4/15/2010	4/19/2011	150	10			
72	Waters										
	Creek B	542	34.67708	-83.93812	4/15/2010	4/19/2011	20	4			
91	Cochran										
	Creek	544	34.56968	-84.21544	4/14/2010	Access road closed					
78	Canada										
	Creek 1	718	34.68727	-84.05750					4/11/2012	150	10
74	Coopers										
	Creek	724	34.74991	-84.03668		4/22/2011	14	6	4/19/2012	150	10
73	Dockery										
	Lake	731	34.67544	-83.97596	4/9/2010	4/22/2011	46	4	4/5/2012	150	10
77	Canada										
	Creek 2	739	34.68952	-84.04485	4/23/2010	4/22/2011	24	3	4/11/2012	150	10
140	Mart Helton	775	34.74240	-84.02956		4/22/2011	18	6	4/19/2012	150	10
88	Noontootla	841	34.64995	-84.17449					4/11/2012	150	10
95	Jacks River	852	34.86093	-84.51434	4/22/2010	4/26/2011	19	10	4/20/2012	2	1

84	Blackwell Creek	856	34.64338	-84.04360	4/23/2010	4/11/2011	150	10	4/5/2012	150	10
71	Slaughter Creek	974	34.74132	-83.95564		4/22/2011	19	6	3/22/2012	150	10
63	Wolf Pen Gap	1024	34.76447	-83.95298		4/22/2011	48	18	4/13/2012	150	10

Table 2.2: Recovery of predatory beetles from non-release sites. Locations of sites are indicated by distance (km) from their relative release site.

HCA #	Site	Distance (km) from Release	Date Sampled	# twigs collected	# trees sampled	Beetles Recovered			
						St	Ln	LnxLr	Lr
29	Lower Panther B	0.4	3/27/2012	150	10	0	0	0	0
145	Soque River A	0.4	3/22/2012	150	10	0	0	**1**	**16**
145	Soque River B	1.6	3/22/2012	150	10	0	0	0	**1**
90	Jones Creek B	0.4 - 1.2	4/3/2012	150	10	0	0	0	**8**
78	Canada Creek 1	0.4	4/11/2012	150	10	**25**	**0**	**0**	**0**

74	Coopers								
	Creek	0.4	4/19/2012	150	10	0	0	0	**8**
140	Mart Helton								
73	Dockery								
	Lake	0.4	4/5/2012	45	9	0	0	0	0
77	Canada								
	Creek 2	0.4	4/11/2012	18	3	0	0	0	**3**
88	Noontootla	0.4 - 1.6	4/11/2012	150	10	0	0	0	**1**
84	Blackwell								
	Creek	0.4 - 0.8	4/5/2012	150	10	0	0	0	**4**
71	Slaughter								
	Creek A	0.4	3/22/2012	45	3	**1**	**0**	**0**	**4**
71	Slaughter								
	Creek B	0.4	3/22/2012	150	10	0	**1**	0	**10**
63	Wolf Pen								
	Gap	0.4	4/13/2012	150	10	**1**	**2**	**1**	**4**
					total	**27**	**3**	**2**	**56**

Table 2.3: Recovery of *Sasajiscymnus tsugae* from predatory beetle release sites sampled during 2010-2012. Year of release indicates the year *S. tsugae* was released in the area with releases during the sampling year occurring after the area was sampled. Years (Yrs.) post release indicates how long it has been since the last *S. tsugae* release in the area prior to sampling with (-) for no prior releases.

HCA		No. of *S. tsugae* Recovered			Year of *S. tsugae* Release	Yrs. Post Release		
#	Site	2010	2011	2012		2010	2011	2012
29	Lower Panther A			0	2008, 2009, 2010, 2011			1
29	Lower Panther B	**1**		**1**	2010, 2011	-		1
29	Upper Panther	**2**		**1**	2009, 2010	1		1
145	Soque River	0	0	**3**	2009, 2010, 2011	1	1	1
115	Yahoola Creek	0	0	0	2008, 2009, 2010, 2011	1	1	1
90	Jones Creek A	0	**1**	0	2009, 2010		1	2

68	Boggs							
	Creek	0	tornado: closed		2008, 2009, 2010	1		
90	Jones							
	Creek B	**1**	**1**	0	2009, 2010, 2011	1	1	1
72	Waters							
	Creek A	0	**4**		2008, 2009, 2010, 2011	1	1	
72	Waters							
	Creek B	0	0		2009, 2011	1	2	
91	Cochran							
	Creek	**2**	road closed		2009, 2010, 2011	1		
78	Canada							
	Creek 1			**20**	2008, 2009			**3**
74	Coopers							
	Creek		0	0	2008, 2009		2	**3**
73	Dockery							
	Lake	**139**	**32**	**19**	2008, 2009, 2010	1	1	2

77	Canada Creek 2	**8**	0	0	2008, 2009	1	2	**3**
140	Mart Helton		0	0	2008, 2009		2	**3**
88	Noontootla			**138**	2009, 2010			2
95	Jacks River	0	0	0	2009, 2010, 2011	1	1	1
84	Blackwell Creek Slaughter	0	**27**	**27**	2010, 2011	-	1	1
71	Creek Wolf Pen		**8**	**50**	2006, 2007, 2008, 2009		2	**3**
63	Gap		**27**	**53**	2008, 2009		2	**3**
	Total	**153**	**100**	**312**				

Table 2.4: Recovery of *Laricobius nigrinus* and *L. nigrinus* x *L. rubidus* hybrids during 2010-2012. Year of release indicates when *L. nigrinus* was released in the HCA. Years (Yrs.) post release indicates how long it has been since the last *L. nigrinus* release in the area prior to sampling with (-) for no prior releases and 0 if a release was made prior to sampling.

HCA #	Site	No. of *L. nigrinus* and hybrids Recovered						*L. nigrinus* Releases	Yrs. Post Release		
		2010		2011		2012					
		Ln	Ln x Lr	Ln	Ln x Lr	Ln	Ln x Lr		2010	2011	2012
29	Lower Panther A					0	0	2008, 2009, 2010*			1
29	Lower Panther B	0	0			0	0	2008, 2010, 2011	2		1
29	Upper Panther	**9**	**2**			0	0	2009, 2010*	2		1
145	Soque River	0	0	0†	0	**1**	0	2007, 2008, 2010*, 2011	2	0	1

115	Yahoola										
	Creek	**1**	0	0	0	0	0	2009, 2010	1	1	2
90	Jones										
	Creek A	0	0	0	0	0	0	2009, 2010, 2011		1	2
68	Boggs										
	Creek	0	0	tornado: closed				2008, 2009, 2010	0		
90	Jones										
	Creek B	0	0	0	0	0	0	2009, 2010, 2011	1	1	1
72	Waters										
	Creek A	0	0	0	**1**				-	-	
72	Waters										
	Creek B	0	0	0	0			2010	-	1	
91	Cochran										
	Creek	0	0	road closed				2009, 2010, 2011	1		
78	Canada										
	Creek 1					**1**	0	2008, 2009			**3**
74	Coopers										
	Creek			0	0	0	0	2009		2	**3**

73	Dockery										
	Lake	**12ŧ**	**20**	**4**	0	**5**	0	2008, 2009, 2010	0	1	2
77	Canada										
	Creek 2	0	0	0	0	0	0	2008	2	**3**	**4**
140	Mart Helton			0ŧ	0	0	0	2009, 2010*****		0	1
88	Noontootla					**44**	**11**	2009, 2010			2
95	Jacks River	0	0	0	0	0	0	2010	-	1	2
84	Blackwell										
	Creek	**3**	0	**13ŧ**	**7**	**78**	**2**	2008, 2009, 2010*****	1	0	1
71	Slaughter										
	Creek			0	0	0	**5**	2007, 2008		**3**	**4**
63	Wolf Pen										
	Gap			**41ŧ**	**7**	**17**	**1**	2008, 2010*, 2011		0	1
	Total	**25**	**22**	**58**	**15**	**146**	**19**				

ŧ indicates releases occurred in the area prior to sampling.
*** indicates winter releases which would be a part of the following year release season.**

Table 2.5: *Laricobius rubidus* collected in predatory beetle release sites during 2010-2012.

		No. of *L. rubidus*		
HCA #	**Site**	**2010**	**2011**	**2012**
29	Lower Panther A			0
29	Lower Panther B	0		**1**
29	Upper Panther	**1**		0
145	Soque River	**2**	0	**6**
115	Yahoola Creek	0	0	0
90	Jones Creek A	0	**3**	0
68	Boggs Creek	0		
90	Jones Creek B	0	0	0
72	Waters Creek A	**1**	**22**	
72	Waters Creek B	0	**2**	
91	Cochran Creek	0		
78	Canada Creek 1			**8**
74	Coopers Creek		0	0
73	Dockery Lake	**16**	**35**	**25**
77	Canada Creek 2	0	0	0
140	Mart Helton		**7**	0
88	Noontootla			0
95	Jacks River	0	0	0
84	Blackwell Creek	0	**27**	0
71	Slaughter Creek		**1**	**15**
63	Wolf Pen Gap		**24**	**10**
	Total	**20**	**121**	**65**

Fig. 2.1: Hemlock conservation areas (HCAs) in the Chattahoochee National Forest in Georgia. The HCAs are outlined in grey and those sampled for predatory beetles are represented by red dots. The numbers listed above the dots correspond to site location in Table 2.1.

Fig. 2.2: Modified rearing tents to allow access to dropping *Laricobius* larvae.

Fig. 2.3: Modified floral foam blocks.

Fig. 2.4: Small tote set-up for samples with less than 30 twigs.

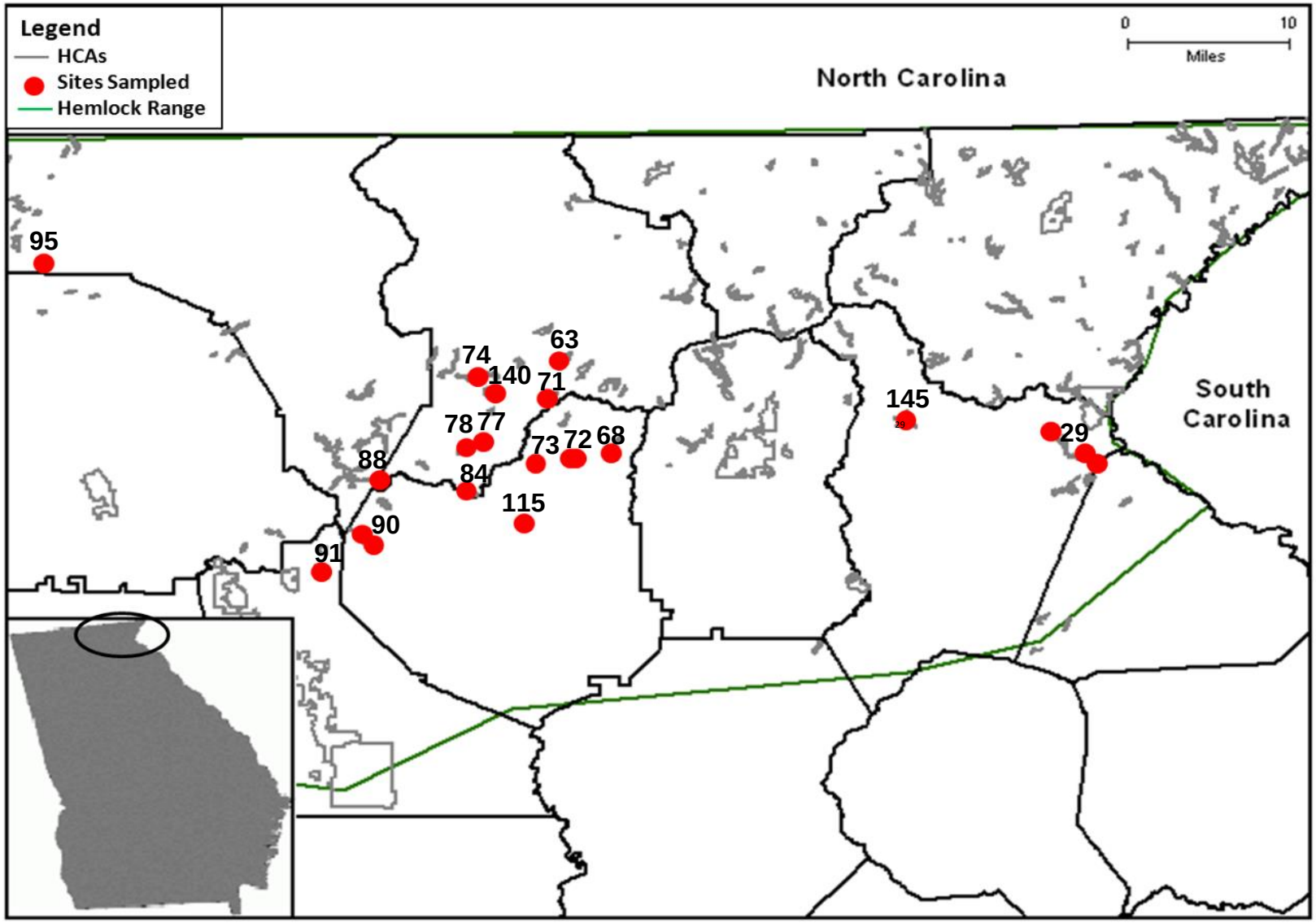
Legend
HCAs
Sites Sampled
Hemlock Range
0
10
Miles
North Carolina
South Carolina
95
74
140
63
71
78
77
73
72
68
88
84
115
90
91
145
29

Fig. 2.1

Fig. 2.2

Fig. 2.3

Fig. 2.4

Appendix 2.1: Predatory beetle releases in the North Georgia mountains

HCA #	Location Name	Size (hectares)	Elev. (m) (avg)	Group(s)	Release Year(s)	TOTAL Beetles (Adult & Eggs)	*L. nigrinus*		*S. tsugae*		*S. sinuanodulus*	
							Adults	Eggs	Adults	Eggs	Adults	Eggs
2	Burrells Ford 2	3		CU	04	1856			1856			
4	Hicks/Ridley/Reed	49		Eco	04	5550			5400		150	
6	Red Mill Creek	522		CU	04,05	15162	150		14462		550	
7	Mose Branch	25		CU	04	3000			3000			
8	Holcomb Creek	59		CU	04,05	18658			18658			
9	Billingsley Creek	68		CU	04,05	4645			4645			
11	Walnut Fort	34		CU	05	5216	150		5066			
18	Timpson Crk	80	782	CU	06,08	10438	900		7924	1614		
20	Upper Cliff Creek	19		CU	06	5000			5000			
22	Muscadine Branch	19	425	CU	06,08	13000			13000			
23	Camp/Cliff/Wolf Crk	161	565	CU	06,07,08	12853	300		12553			
24	Stonewall	8		CU	06	5119	300		2500	2319		
26	Slick Shoals	20		CU	06	2500			2500			

27	Crow Crk	53	621	CU, PNW	06,07,08	15773	600		15173			
28	Falls Branch	42		CU	06	2500			2500			
29	Upper Panther Crk	117	451	UGA, CU	07,08,09,10	63814	839	22407	39809		89	670
29	Lower Panther Crk		251	UGA, CU,NGC	07,08,09,10	42488	746	18817	20048	1413	49	1415
31	Davidson Crk	88	360	UGA, CU,NGC	08,09,10	79462	1711	33618	40982		75	3076
34	Coleman River	94		CU	06	2403			2403			
37	Tallulah River	326	757	CU	06,08	17020			15600	1420		
38	Nicholson Branch	36		CU	06	4779			2500	2279		
40	Scataway	17	715	CU, YH	06,07	6349			6349			
42	Swallows Crk	23	651	CU, YH	06,07	9110			5155	3955		
46	Hemlock Falls	70		CU	06	2500			2500			
48	Corbin Crk	138	795	CU, YH, NGC, YH	06,07,11	14873	177	915	9704	3817		260
49	Hickory Nut Mnt.	30		CU	06	7500			7500			
51	York Creek	97		CU	06	5000			5000			
52	High Shoals	123	791	YH, UGA, CU, NGC	06,07,11	18512		2168	6333	9448		563
53	Soapstone	70	708	CU, YH	05,06,07	9247	182		5704	3361		
54	Big Bald Cove	18	876	CU, YH	06,07	7555			7,555			
56	Ivylog	58	735	CU, YH	06,07	15413			7650	7763		
58	Bob Crk	129	962	CU, YH, UGA, NGC	06,07,11	17640	200	784	12895	3567		194
59	Chattahoochee River	308		CU	05,06	5245	245		5000			
60	Dukes Crk Conserv.	31	528	CU	06,07,08	14402	574		13828			
62	Bowers Cove	69	767	CU, YH	06,07	15956			8878	7078		
63	Wolf Pen Gap/ Crk	68	983	UGA,CU,YH,NGC	05,06,07,08,09,10	34150	1314	6602	15049	9928	136	1121
64	Helton Crk	111	480	CU, YH	06,07	9693			6070	3623		

65	Alex Cove	19	735	CU, YH	06,07	5790			5790			
66	Lordamercy Cove	21	738	CU, YH, UGA	06,07,11	16678	1139	3412	7101	4281		745
68	Boggs Crk	49	550	UGA,CU,YH,NGC	06,08,09,10	29021	415	4830	19411	2610		1755
69	Blood Mtn Cove	36	670	UGA,YH,NGC	07,08,09	5462	186	480	2339	2302	107	48
70	Tigue Branch	33	936	CU, YH	06,07,08	6235	300		5935			
71	Slaughter Crk/ Winfield Scott	33	952	UGA,CU,YH,NGC	06,07,08,09,10	42545	798	3672	30692	6975	30	378
72	Waters/Dicks Crk	13	552	UGA,CU,YH,NGC	07,08,09,10	37396	162	4706	26621	4744		1163
73	Dockery Lake	17	730	UGA,CU,YH,NGC	08,09,10	25987	946	8385	14737			1919
74	Coopers Crk	105	739	UGA,CU,YH,NGC	07,08,09,10	33954	167	6731	16969	9410	67	610
75	Mulky Crk	62	672	CU, YH	07,08	10795			7810	2985		
77	Canada Crk 2 (upper)	48	741	UGA,CU,NGC	08,09,10	18240	118	6045	8910	2454	13	700
78	Canada Crk 1 (lower)	104	730	UGA,CU,YH,NGC	08,09	24499	426	3768	19017		16	1272
79	Sea Crk/Clements Bridge	36	702	CU, YH	08,	7938	150		1303	6485		
80	Toccoa River/ Benton-Mackaye	105	619	UGA,CU,YH,NGC	08,09,10	17077	178	4564	9903			2432
81	Little Rock Crk	51	721	CU,YH	07,08	8708			8708			
82	Rock Crk/Fish Hatchery	64	710	UGA,CU,YH,NGC	08,09,10	27292	660	7434	17449		21	1728
84	Blackwell Crk	53	874	UGA,CU,NGC	08,09,10	15521	829	3985	9443		71	1193
85	Flat Crk	80	588	UGA,CU,YH,NGC	09,10	14982	212	2159	8855	3003		753
86	Stanley Crk	45	657	UGA,CU, YH	08,09,10	25721	50	5358	12436	6681		1196

88	Noontootla	447	845	UGA,CU,YH,NGC	08,09,10	39634	440	10043	24857	1318	49	2927
89	Montgomery Crk	45	575	UGA,CU,NGC	08,09,10	20410	205	5216	13936	227		826
90	Lance/Jones Crk	92	539	UGA,CU,YH,NGC	09,10	29145	318	5968	19839	549	67	2404
91	Cochran Falls	29	554	UGA,CU,NGC	09,10	28388	316	4881	22459			732
92	Anderson Crk	34	743	UGA, NGC	10	12560		6266	4191	726	245	1132
95	S. Fork Jacks River	10	882	UGA,NGC	09,10	9583	50	3285	5365			883
97	Bukes Branch	15		UGA	10	2195		1617				578
103	Jacks Junction	55		UGA	11	424		424				
105	Sumac Crk	15	590	UGA, CU,NGC	10	5076	169	2772	1585			550
109	Emery Crk	104	643	UGA, CU,NGC	10	17960		7983	8335		586	1056
110	Lake Conasauga	51	986	UGA, CU,NGC	09,10	20482	2591	9727	5870		199	2095
112	Holly Crk	21	429	UGA, CU, NGC	10,11	17940	1069	3400	11734		47	1690
115	Yahoola Crk	15	470	UGA,CU,NGC	08,09,10	21893	474	2319	15694	2796		610
124	Cashes Valley/ Fightingtown Crk	11	752	UGA,NGC	09,10	5149		2593	2118			438
126	Rice Cabin Crk	17	518	CU	08,	5456			5456			
128	Upper Mill Creek	6		UGA, CU, NGC	11	15209	503	4503	9793		110	300
129	Tatum Lead	17		UGA	11	520		520				
132	Laurel Creek	62		CU	05	6879			6879			
133	Willis Knob	221	392	CU	08,09,	8155	273		7882			
134	Licklog Crk	73	471	CU	08,	5460			5460			
135	Seals Creek	25		CU	06	5000			5000			
137	Wolfpen Branch	66		CU	06	2500			2500			
140	Mark Helton	29	814	UGA,YH,NGC	08,09	6926		936	5810			180
142	Chestnut Crk	27	817	UGA, CU,NGC	10	6599		3268	3030			301

143	Birch Crk	16	828	UGA, CU	10	1303		771	276			256
144	Pounding Mill Creek	28		CU	05	2500			2500			
145	Soque River		468	UGA,CU,NGC	06,07,09,10	23037	864	8244	10806		134	2989
	Two Run Creek		516	NGC	09,	2350			2350			
	Chattooga River Corridor			UGA, CU, NGC	08,09,10,11	9130	1862	1672	4634		522	440
	Dawson Forest: Wildcat Track			UGA, NGC, YH	09,10,11	7445	90	1179	2852	2774		550
	near GA Mnt. Experi. Stat.			YH	06	1817			1015	802		
	Big Creek			CU	04,05	5012			5012			
	Tallulah Gorge			CU	07,08	20360			20360			
11-144	Between HCA 11 and 144			CU, UGA	05,06	9750	150		9600			
12-11	Between HCA 12 and 11			CU	05	5057			5057			
133-134	Btwn HCA 133 and 134			CU	05	7652	93		7559			
15/11/13	Btwn HCA 15, 11, and 13			CU	05,06	5511			5511			
42-122	Btwn HCA 42 and 122			CU,YH	06	7821			3866	3955		
5-9	Btwn HCA 5 and 9			CU, Eco	04	5700			5700			
7-132	Btwn HCA 7 and 132			CU	04	300			300			

Totals	**1306510**	**24591**	**238427**	**869369**	**126662**	**3333**	**44128**

Groups are CU (Clemson University), YH (Young Harris College), UGA (University of Georgia), NGC (North Georgia College and State University), VT (Virginia Tech) Eco (Ecoscientic Solutions), and PNW (Pacific Northwest wild caught)**.** Data obtained from CU, YH, UGA, NGC rearing labs and Mausel et al., 2010

CHAPTER 3

EMERGENCE OF *LARICOBIUS NIGRINUS* (FENDER) (COLEOPTERA: DERODONTIDAE) IN THE NORTH GEORGIA MOUNTAINS

Jones, C.E., Hanula, J.L., and Braman, S. K. To be submitted to the *Journal of Entomological Science*.

Abstract

Hemlock woolly adelgid, *Adelges tsugae*, is currently found throughout most of the range of eastern hemlock, *Tsuga canadensis*. Biological control agents have been released in attempts to control it but how different climates influence their efficacy and survival has not been studied. One predatory beetle of *A. tsugae*, *Laricobius nigrinus*, is native to the Pacific Northwest and, therefore, experiences a much different summer climate in the North Georgia mountains. To better understand survival of this predator as it aestivates in the soil, five mesh cages were set up at each of 16 sites with 4 sites located at an elevation below 549 m, 4 sites between 549 m – 732 m, 5 sites between 732 m – 914 m, and 3 sites over 914 m. At each site 30 larvae were placed inside one of the cages during March, April, or May on infested hemlock twigs and emergence of adults was monitored in the fall. Of the 1,440 larvae placed at the 16 sites only 4 adult beetles emerged between October 6, 2012 and November 5, 2012. The overall success rate is still unknown and more research is needed to assess the efficacy of *L. nigrinus* as a biological control agent in Georgia.

Keywords Hemlock woolly adelgid, *Adelges tsugae*, biological control, emergence

Introduction

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an invasive, aphid-like insect that feeds on the sap of hemlock trees and is devastating eastern (Canadian) hemlock, *Tsuga canadensis* (L.) Carrière and Carolina hemlocks, *Tsuga caroliniana* Engelmann in the Eastern United States. *Adelges tsugae* can be found as far north as Maine, south to Georgia, and west into Tennessee and Kentucky. Hemlock mortality occurs in 4-6 years (Mayer et al., 2002, McClure, 1991) and faster in the southern range due to other factors such as drought stress (Ward et al., 2004) and mild winters that favor *A. tsugae* survival (Trotter and Shields, 2009).

To save a portion of the mature hemlock trees in North Georgia, the U.S. Forest Service created 114 Hemlock Conservation Areas (HCAs) within the Chattahoochee National Forest to be treated either with biological controls and/or insecticides (Fig. 3.1). One of the major biological control agents reared and released in Georgia (since 2007) is *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae), a small black beetle native to the Pacific Northwest where it feeds on *A. tsugae* (Zilahi-Balogh et al., 2002). It has one generation a year, with winter active adults laying eggs when overwintering *A. tsugae* sisten egg laying occurs. The beetle larvae feed on the adelgid eggs and, upon maturation, drop to the soil to pupate (Zilahi-Balogh et al., 2002).

While *L. nigrinus* has been studied for cold tolerance (Mausel et al., 2010 and 2011) and North Georgia is comparable to Seattle, Washington in terms of cold hardiness zone, the effect of heat/moisture tolerance of pupae/adults in the soil has never been considered. The summer climate of Seattle WA, (mean high of 23 ± 3°C in

August 2012; source www.farmersalmanac.com/) is quite different from that of North Georgia [mean high of 28.3°C in Blairsville, GA (elevation 590 m] in August 2012; source www.georgiaweather.net). Soil temperatures in a *Pseudotsuga menziesii* (Mirb.) Franco/*Tsuga heterophylla* (Raf.) Sarg. stand in southwestern Washington (elev. 300 m) did not rise above 16°C throughout the year (Devine and Harrington, 2007). The annual precipitation also differs with the Pacific Northwest receiving an average annual precipitation of 988 ± 139 mm and Seattle, WA receiving 918 mm. The area has dry summers (monthly avg. 53.2 ± 27 mm, March through October) and wet winters (monthly avg. 123.1 ± 28 mm, November through February; source www.weatherdb.com/). North Georgia receives an average annual rainfall of 1426 ± 81 mm with Blairsville, GA annual precipitation being 1,510 mm that is consistent throughout the year with a monthly average of 120.5 ± 17 mm from March through October and 136.5.8 ± 14 mm from November through February (source www.weatherdb.com/).

The phenology of *A. tsugae* in Georgia also varies from the Pacific Northwest in that sisten adults are found two months later, the occurrence of progredien adults and their sisten eggs ends one month earlier, and the end of the sisten generation aestivation period occurs a month later in Georgia (Joseph et al., 2011, Zilahi-Balogh et al., 2003a). In a laboratory study *L.* nigrinus prepupae were unable to complete development when temperatures were higher than 21°C (Zilahi-Balogh et al., 2003b). Based on average temperatures daily temperatures in Georgia, *L. nigrinus* would need to complete development by Mid-May. In Georgia, it is thought that *L. nigrinus* larvae start dropping to the ground to pupate in March, and with their life cycle being

synchronized with *A. tsugae*, adult *L. nigrinus* emerge when *A. tsugae* breaks its summer aestivation in November. Thus, *L. nigrinus* must survive two months longer in Georgia than in their native range under both soil moisture and temperature conditions much different from where it evolved in the Pacific Northwest.

In one study on factors influencing aestivation of *L. nigrinus*, fewer adults emerged when aestivating adult were stored at 20°C (26.7%) compared to 15°C (37.3%) and 10°C (44.4%) in a laboratory setting, and temperature had a significant influence on time spent in the soil, with those at higher temperatures remaining in the soil longer (Lamb et al., 2007). It was also found that moisture did not influence time spent in soil, however, a significantly greater number emerged when moisture content was at 45% compared to 30% (Lamb et al., 2007). At a western hemlock [*T. heterophylla* (Raf.) Sarg.] site in southwestern Washington, with annual precipitation averaging 2,260 mm, the percent volumetric soil moisture over two years between May and October was 34.6% (S.E.=0.13) and 38.0% (S.E.=0.22) respectively (Roberts et al., 2005). However, June through September is much dryer in southwestern Washington (monthly avg. 2.1 ± 0.86 mm) compared to North Georgia (monthly avg. 4.5 ± 0.13 mm; source www.weatherdb.com). While soil moisture levels up to 45% benefit *L. nigrinus* development, it is unclear if there is a point where extremely high soil moisture would have a negative influence and what moisture levels are reached on the east coast.

The objective of this study was to determine how timing of *L. nigrinus* pupation in the spring and soil temperatures during their soil aestivation period in Georgia affects their survival prior to adult emergence in the fall.

Materials and Methods

Preliminary Study. During 2010, air and soil temperatures were monitored at 5 sites ranging in elevation from 497 m – 919 m using HOBO® Micro Station Data Loggers (H21-002) with a temperature/relative humidity data logger (U23-002) and temperature sensor (U23-004), (Onset, Bourne, MA). Air temperature was recorded at a height of about 3 m near the bole of the tree and soil measurements were taken from a depth of 5 cm.

In 2011 a trial run of soil emergence boxes was set up in Dahlonega, GA (elv. 442 m) and Young Harris, GA (elv. 586 m). The trial consisted of five aluminum boxes, with no top or bottom, (25.4 x 18 x 30.5 cm; L x W x H) placed in the ground 15-20 cm deep at each location in January, because all *Laricobius spp.* should have emerged by that time, and the boxes were covered with no-see-um netting (Mosquito Curtains, Inc., Atlanta, GA) to prevent new larvae from dropping in. A HOBO® Micro Station Data Logger with a temperature/relative humidity data logger and temperature sensor were used at each site to record soil (depth 5 cm) and air temperatures (height 2 m) near the cages from August through November.

The boxes at each site were randomly assigned one of the following treatments: 1) no beetles (control); 2) 55 prepupae during mid-late March; 3) 55 prepupae during early April; 4) 55 prepupae during late April/early May; or 5) 55 prepupae during late May. The prepupae were obtained from the University of North Georgia (Dahlonega, GA) rearing lab, where they were collected as they dropped from infested foliage using a similar funnel design as described in Salom et al. (2012). The containers were

checked twice a day for prepupae which were immediately placed in a moist 1:1 peat/sand mixture and then placed in the field within 36 hours. Once the larvae were placed in the soil boxes a rectangular lid was attached (Fig. 3.2) that was made out of no-see-um netting and 1.27 cm hardware cloth for support. An inner pyramid (Fig. 3.2) was created to direct emerging adults into a collection area.

The containers were checked once a month until October. In October they were checked twice a month, weekly in November, and then twice a month again in December before the site was dismantled.

2012 Emergence Study. Sixteen sites in Hemlock Conservation Areas (HCAs) were selected with 4 sites located at an elevation below 549 m, 4 sites between 549-732 m, 5 sites between 732-914 m, and 3 sites over 914 m (Fig. 3.1, Table 3.1). Areas that had previous releases were selected in order to compare emergence data to recovery of the larval stage from hemlock foliage in the same areas (Chapter 2).

Four hundred wild caught *L. nigrinus* adults were obtained from field insectaries in Boone, N.C. in order to collect eggs and rear larvae at the University of North Georgia (Dahlonega, GA) rearing facility. Oviposition jars, containing 20 *L. nigrinus* adults and a bouquet of 10 *A. tsugae* infested hemlock twigs were held at 6°C, 65% R:H, and 12:12h L:D. After 1 week, the adults were relocated to a new jar and the bouquets were collected and placed, with an additional 20 infested twigs, in 64.4 liter clear plastic totes with locking lid (68 x 45 x 30 cm, 292010, The Container Store, Atlanta, GA) that were modified by cutting 38 x 23 cm holes in the longer sides and lid, and then attaching no-see-um netting using hot glue to cover the hole. These rearing totes were held at 10°C,

65% RH, and 13:11h L:D for one week, 14°C the second week and 16°C for the remainder of the rearing period. After three weeks, fresh bouquets of 20 adelgid infested twigs (15-20 cm long stuck in parafilm wrapped floral foam) to which 30 *L. nigrinus* larvae were added were taken to the field and placed in the designated cages at the appropriate times. The whole bouquet was placed inside the cage on a stand made of hardware cloth to elevate the twigs off the ground (Fig. 3.3d-e), and to allow the larvae to drop naturally to the soil. Bouquets remained in the cages for a month.

In January 2012, four rectangular aluminum enclosures, 30 x 41 x 10 cm (L x W x H), were inserted 5-8 cm into the ground at each of the 16 sites, using an edger (Hound Dog "Edge Hound"®, Griffon Corp., New York, NY) to initially cut a slit into the ground without disturbing the duff layer. A lid, made from 1.27 cm mesh hardware cloth covered with no-see-um netting, was placed over each enclosure to prevent any *Laricobius* spp. in the area from dropping into the box (Fig. 3.3a). During the emergence period a slightly larger rectangular cage, 31 x 41 x 25 cm (L x W x H), also made out of 1.27 cm mesh hardware cloth with no-see-um netting lining the inside and secured with hot glue (Fig. 3.3b, c & d), was placed over the box and the lid was flipped over and used to cover the top of this cage (Fig. 3.3f). The no-see-um netting was at least 10 cm longer at the top and bottom in order to secure them to the ground, with 15 cm long nails, and seal the top with the sides of the cages by rolling the excess material from the lid with that of the sides to create a tight seam that was held together with binder clips (Fig. 3.3f). White Outdoor Duck Tape® (1.88 in x 30 yd., Shurtech Brands, Avon, OH) was used to seal the no-see-um netting of the cage to the aluminum enclosure (Fig.

3.3c, d, & e). The 4 cages at each were encircled with wire fencing to discourage disturbance.

Boxes at each site were randomly selected to receive *L. nigrinus* larvae in: 1) March, 2) April, 3) May, or 4) not at all (control). The introduction of *L. nigrinus* to the box occurred within the third week of each month (March-May) and the cages were checked for emerging beetles monthly until October when they were checked twice. On the second visit, bouquets of 20 *A. tsugae* infested hemlock twigs were placed in each container to provide a substrate for any emerging beetles (Fig. 3.3e). Cages were then visited weekly to collect and replace the bouquets until the second week of December. During each visit the netting was checked for beetles and the bouquets were collected by quickly placing them into 7.6 liter plastic Ziploc bags, returned to the lab, and examined for adult *L. nigrinus*. Any beetles found were placed into 95% EtOH and stored in the freezer.

A variety of site parameters were measured to determine if they might be important factors in explaining where *L. nigrinus* was most likely to be successful. Soil temperature and moisture were measured at 5 cm depth using HOBO® Micro Station Data Loggers with temperature sensor and soil moisture sensor (S-SMD-M005). Additional HOBO Pendant® Temperature/Alarm Data Loggers (UA-001-64) were placed 2 meters above the emergence cages on the closest branch at each site to record air temperature. Measurements were taken every 4 hours.

In December, two leaf litter samples were obtained at each site by placing a rectangular aluminum enclosure (30 x 41 x 10 cm) on the ground in the vicinity of the

cages, and collecting all identifiable leaf/needle material from within. The samples were then placed into paper bags and oven dried at 100°C for 72 hours and then immediately weighted. After the leaf litter was removed in the field a soil core (5 x 10 cm) was obtained using an AMS soil core sampler with hammer attachment (77455, Forestry Suppliers Inc., Jackson, MS) and the organic layer was measured. Two additional soil samples were collected into 118 ml Glad® plastic containers with snap on lids. Upon returning to the lab, 5 *Galleria mellonella* larvae were placed inside each of the 118 ml containers and held at room temperature for 7-10 days to determine the prevalence of parasitic fungi and nematodes. After that time the larvae were located and assessed as living or dead and for the presence of fungi or nematodes. Subsamples were sent off for pathogen identification (W. Gardner, Univ. of Georgia, Griffin).

Cage Design Test. In December 2012, twenty wild caught *L. nigrinus* adults were placed into the control cage at 12 sites on an *A. tsugae* infested bouquet of hemlock in order to test how well the cages held beetles for collection. After 7 days the sides and lid of the cages were examined, adults were aspirated into vials, and the bouquets were quickly placed into 7.6 liter plastic Ziploc bags and examined back at the lab.

Statistical Analyses. Emergence success of *L. nigrinus* was analyzed using logistic regression to compare the likelihood of emergence with site parameters (elevation, maximum and minimum soil moisture and temperature, leaf litter weight, and depth of organic layer). Additional analysis using Poisson regression was conducted to

compare site parameters with number of beetles recovered (Chapter 2). The data were overdispersed and were corrected using the “scale=deviance” option.

Results

Preliminary Study: Average daily soil temperatures in 2010 exceeded 20°C 110 days (June through October, max 28°C) at the lowest elevation and 43 days at the highest (end of July through August, max 23°C).

During the week 4-11 November 2011, four adults were collected from both site (8 out of 440 total) and in each case they were collected from cages containing prepupae placed in March and early April. Average air temperature during the week of emergence was 9.5 ± 5°C.

***L. nigrinus* emergence 2012.** Four beetles were recovered from emergence cages between 30 October and 1 November 2012. One beetle from the March treatment was found at Waters Creek (HCA# 72) a low elevation site < 548.64 m above sea level (Table 3.1, Fig. 3.1). Two beetles were recovered from sites between 548.64 m – 731.52 m elevation, one from the April treatment at the Coopers Creek A site (HCA# 74) and one from the May treatment at Noontootla (HCA# 88). One additional beetle from the April treatment was recovered at the Blackwell Creek site (HCA# 84) located between 731.52 m – 914.4 m elevation. Elevation of the site was not a significant predictor for *L. nigrinus* emergence.

Average monthly air temperature in 2012 at the lowest and highest elevation sites during *L. nigrinus* larval development were 15 ± 0.38°C (Mean ± SD) and 14 ± 0.14°C in April, 19 ± 0.22°C and 17 ± 0.15°C in May, and 21 ± 0.44°C and 19 ± 0.13°C in June (Fig. 3.5). Air temperatures reached an average maximum of 36 ± 0.22°C at the low elevation sites and 32 ± 0.87°C at the highest elevations between June 29, 2012 and July 1, 2012 (Fig. 3.5).

Average 2012 monthly soil temperature at the lowest elevation sites during *L. nigrinus* pupation through adult emergence were at 14.5 ± 0.61°C in April, reaching the highest point in July at 22.5 ± 0.79°C, and dropping to 9.3 ± 2.1°F in November (Fig. 3.6). Average daily temperatures reached above 20°C starting 25 May 2012 and reached these temperatures 67 to 110 days at the lower elevation sites, not going above 20°C after 22 September 2012. At the highest elevation sites the average monthly soil temperatures were at 12.16 ± 0.52°C in April, reaching the highest point in July at 19.64 ± 0.36°C, and dropping to 8.18 ± 1°C in November (Fig. 3.6). Average daily temperatures reached above 20°C starting 29 June 2012 and reached these high temperatures eight to 38 days at the high elevation sites, not going above 20°C after 8 September 2012. Comparing air and soil temperature data obtain in 2010 and 2012 there was no difference in monthly averages from April through November. Minimum and maximum soil temperatures were not significant in predicting emergence of *L. nigrinus*.

Average soil moisture between April and December 2012 was 0.13 ± 0.02m^3/m^3 at the low elevations and 0.13 ± 0.01m^3/m^3 at the highest elevations. The average

monthly soil moistures (Fig. 3.4) were similar at high and low elevation sites during *L. nigrinus* pupation through adult emergence (April through November) and varied between $0.09 \pm 0.04 m^3/m^3$ and $0.19 \pm 0.07 m^3/m^3$ with the driest month in July. However, the low elevation sites had the wettest months in April and May, while the higher elevation sites had their wettest month in November (Fig. 3.4). Minimum and maximum soil moisture was not significant in predicting emergence of *L. nigrinus*.

Leaf litter samples weighted 56.7 ± 15 g and the organic layer depth was 1.72 ± 0.77 cm. Leaf litter weight and organic layer thickness was not significant for predicting *L. nigrinus* emergence. However, analysis of the organic layer depth using Poisson regression corrected for overdispersal was significant (P= 0.001) in regards to recovery of *L. nigrinus* (Chapter 2) suggesting that it is more likely to recover higher numbers of *L. nigrinus* as the organic layer gets deeper.

Fungal samples from *G. mellonella* larvae were sent to Dr. Wayne A. Gardner (University of Georgia-Griffin) who identified them as *Metarhizium anisopliae*, a common soil fungus that is saprophytic as well as pathogenic. Other *G. mellonella* larvae were found to have thousands of predatory nematodes inside their body.

Cage Design. Of the 12 sites that had adult beetles placed into the control cage, 11 returned beetles with an average of 4.3 ± 3 beetles. The majority of the beetles recovered (78%) were recovered from the bouquet that was placed in the cage. Two of the sites were also disturbed with cages smashed in or turned over by bears.

Discussion

The cage design test showed that while they were not perfect they were able to hold and collect 22% of beetles so the fact that only 4 out of 1,440 *L. nigrinus* beetles were recovered raises a lot of questions. The emergence of the 4 adults occurred about 163-221 days after the larvae were placed into the cages. The emergence time was consistent with the preliminary trial conducted in 2011. A very similar study conducted at the same time in Tennessee (A. Lamb, personal communication) also resulted in a low emergence rate of 4% which occurred between October 6, 2012 and November 5, 2012 the same time as our beetles emerged. Another emergence study conducted in 2007 in Georgia resulted in a 1.7% emergence rate in the field and 12.3% emergence in the lab (T. Coleman, personal communication). These results suggest that the low emergence we observed was not due to cage design but to poor survival of aestivating *L. nigrinus*.

Initially, low emergence was thought to be due to the unusually warm winter and spring in 2012, but comparing air and soil temperature data obtain in 2010 showed no difference in monthly averages from April through November and in both years each trial resulted in very low emergence. In the lab *L. nigrinus* larvae cannot complete development at temperatures above 21°C (Zilahi-Balogh et al., 2003b). Whether similar high temperatures affect aestivating pupal and adult stages while dormant in the soil or if the critical maximum temperature of 21°C only applies to the developing larvae are unknown.

Although it is unclear why emergence was so low in this study, we suspect the overall high temperatures in Georgia are an important factor. Since there is no data

indicating survival rate of each developmental stage of *L. nigrinus* in its native range it is impossible to estimate how well it survives in its introduced range. Average lab mortality data from 2001 – 2004 indicate that there is roughly 29.5% mortality of mature larvae, 35.5% mortality of pupae, and 18.5% mortality of adults emerging from aestivation (Salom et al., 2012). This mortality occurred at optimal temperatures which are not experienced under field conditions in North Georgia. Using these survival rates and applying them to our study we expect that out of the 30 larvae put into an emergence cage only 8.9 would become prepupae, 3.1 would pupate, and 0.6 adults would emerge in the fall. Since other factors could affect survival in the field such as pathogenic microorganisms, damage during handling and placement in the field, or variable soil temperatures and moistures, our results were not totally unexpected.

In another study assessing establishment of *L. nigrinus* from 2010-2012 (Chapter 2), *L. nigrinus* was found one to three years post-release at various sites throughout North Georgia. Therefore, we know it can survive throughout the year and establish, but the annual survival rate is still unknown. More research is needed to assess the efficacy of *L. nigrinus* as a biological control agent in Georgia.

Laricobius osakensis Montgomery and Shiyake is a predatory beetle of *A. tsugae* from Japan, the same origin as *A. tsugae* introduced to Eastern North America (Montgomery et al., 2011). Just like *L. nigrinus*, this predator is also specific to *A. tsugae* (Vieira et al., 2011), however *L. osakensis* laid significantly more eggs and the larvae fed more voraciously on *A. tsugae* compared to *L. nigrinus* (Vieira et al., 2012). Another potential benefit of *L. osakensis* is that it is in a separate Asian clade, while *L. nigrinus*

and the native *Laricobius rubidus* (LeConte), a native predator of pine bark adelgid [*Pineus strobi* (Hartig)] are sister species in the North American clade (Montgomery et al., 2011) that hybridize (Havill et al., 2012). Finally, with Japan being more closely related in regards to summer temperature and rainfall, and that *L. osakensis* doesn't emerge until November (Vieira et al., 2013), which is when *A. tsugae* breaks aestivation in Georgia, this predator has potential to be more suitable to the North Georgia mountains than *L. nigrinus*.

Acknowledgements

We thank James Wentworth (US Forest Service- Blue Ridge Ranger District), James Sullivan (Georgia Forestry Commission), and Ryan Kelly for their assistance in setting up the field sites. Scott Horn, Michael Cody (US Forest Service- Southern Research Station) Yanzhuo Zhang, Jesse Kalina, and Lake Maner (University of Georgia) for field assistance. Richard McDonald for collecting *L. nigrinus* adults for the colony. Kamal Gandhi and Angela Mech (University of Georgia) for providing valuable input with this experiment. Dr. K. Love-Meyer, UGA Statistical Consulting Service, provided assistance with statistical analyses. Research was supported through the University of North Georgia, the University of Georgia, and the USDA Forest Service, Southern Research Station work unit SRS 4552, Insects, Diseases and Invasive Plants.

Literature Cited

Devine, W. D. and C. A. Harrington. 2007. Influence of harvest residues and vegetation on microsite soil and air temperatures in a young conifer plantation Agric. For. Meteorol. 145: 125-138.

Havill, N. P., G. Davis, D. L. Mausel, J. Klein, R. McDonald, C. Jones, M. Fischer, S. Salom and A. Caccone. 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biol. Control. 63: 359-369.

Joseph, S. V., A. E. M. III, M. J. Dalusky, C. Asaro and C. W. Berisford. 2011. Phenology of the hemlock woolly adelgid (Hemiptera: Adelgidae) in northern Georgia. J. Entomol. Sci. 46: 315-324.

Lamb, A. B., S. M. Salom and L. T. Kok. 2007. Factors influencing aestivation in *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of *Adelges tsugae* (Hemiptera: Adelgidae). Can. Entomol. 139: 576-586.

Mausel, D. L., R. G. V. Driesche and J. S. Elkinton. 2011. Comparative cold tolerance and climate matching of coastal and inland *Laricobius nigrinus* (Coleoptera: Derodontidae), a biological control agent of hemlock woolly adelgid. Biol. Control. 58: 96-102.

Mausel, D. L., S. M. Salom, L. T. Kok and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39: 440-448.

Mayer, M., R. Chianese, T. Scudder, J. White, K. Vongpaseuth and R. Ward. 2002. Thirteen years of monitoring the hemlock woolly adelgid in New Jersey forests, pp. 50-60. *In* B. Onken, R. C. Reardon and J. Lashcomb [eds.]. Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium New Brunswick, NJ: Rutgers University.

McClure, M. S. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environ. Entomol. 20: 258-264.

Montgomery, M. E., S. Shiyake, N. P. Havill and R. A. B. Leschen. 2011. A new species of *Laricobius* (Coleoptera: Derodontidae) from Japan with phylogeny and a key for native and introduced congeners in North America. Ann. Entomol. Soc. Am. 104: 389-401.

Roberts, S. D., C. A. Harrington and T. A. Terry. 2005. Harvest residue and competing vegetation affect soil moisture, soil temperature, N availability, and Douglas-fir seedling growth For. Ecol. Manage. 205: 333-350.

Salom, S. M., L. T. Kok, A. B. Lamb and C. Jubb. 2012. Laboratory rearing of *Laricobius nigrinus* (Coleoptera: Derodontidae): a predator of the hemlock woolly adelgid (Hemiptera: Adelgidae). Psyche. 2012: 9 p

Trotter, R. T. and K. S. Shields. 2009. Variation in winter survival of the invasive hemlock woolly adelgid (Hemiptera: Adelgidae) across the eastern United States. Environ. Entomol. 38: 577-587.

Vieira, L. C., A. B. Lamb, S. Shiyake, S. M. Salom and L. T. Kok. 2013. Seasonal abundance and synchrony between *Laricobius osakensis* (Coleoptera: Derodontidae) and its prey, *Adelges tsugae* (Hemiptera: Adelgidae), in Japan. Ann. Entomol. Soc. Am. 106: 249-257.

Vieira, L. C., T. J. McAvoy, J. Chantos, A. B. Lamb, S. M. Salom and L. T. Kok. 2011. Host range of *Laricobius osakensis* (Coleoptera: Derodontidae), a new biological control agent of hemlock woolly adelgid (Hemiptera: Adelgidae). Environ. Entomol. 40: 324-332.

Vieira, L. C., S. M. Salom and L. T. Kok. 2012. Functional and numerical response of *Laricobius spp*. predators (Coleoptera: Derodontidae) on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). Biol. Control. 61: 47-54.

Ward, J. S., M. E. Montgomery, C. A. S.-J. Cheah and R. S. Cowles. 2004. Eastern hemlock forest: guidelines to minimize the impacts of hemlock woolly adelgid. U.S. Department of Agriculture. Forest Service, Washington, DC.

Zilahi-Balogh, G. M. G., L. M. Humble, A. B. Lamb, S. M. Salom and L. T. Kok. 2003a. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). Can. Entomol. 135: 103-115.

Zilahi-Balogh, G. M. G., L. T. Kok and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Biol. Control. 24: 192-198.

Zilahi-Balogh, G. M. G., S. M. Salom and L. T. Kok. 2003b. Temperature-dependent development of the specialist predator *Laricobius nigrinus* (Coleoptera: Derodontidae). Environ. Entomol. 32: 1322-1328.

Table 3.1: *L. nigrinus* soil aestivation monitoring sites in North Georgia with 4 sites located at an elevation below 549m, 4 sites between 549m - 732m, 5 sites between 732m - 914m, and 3 sites over 914m.

HCA #	Site	Latitude	Longitude	Altitude (m)
29	Lower Panther	34.66906	-83.36742	225
29	Upper Panther	34.69642	-83.41708	449
145	Soque River	34.71582	-83.57611	457
72	Waters Creek	34.67639	-83.94282	526
88	Noontootla A	34.69694	-84.20783	654
78	Canada Creek 1	34.68727	-84.05750	718
74	Coopers Creek A	34.74991	-84.03668	724
73	Dockery Lake	34.67544	-83.97596	731
77	Canada Creek 2	34.68952	-84.04485	739
74	Coopers Creek B	34.75446	-84.03966	757
140	Mart Helton	34.74240	-84.02956	775
88	Noontootla B	34.64995	-84.17449	841
84	Blackwell Creek	34.64338	-84.04360	856
71	Slaughter Creek	34.74132	-83.95564	974
110	Lake Conasauga	34.85547	-84.64712	992
63	Wolf Pen Gap	34.76447	-83.95298	1024

Fig. 3.1: A map of *L. nigrinus* soil aestivation sites in North Georgia displaying 2011 sites, 2012 sites separated by elevation bracket, hemlock conservation areas (HCAs) in Georgia, and the location of Blairsville, GA where temperature data was compared with Seattle, WA.

Fig. 3.2: 2011 *L. nigrinus* emergence cage design with an inner pyramid to funnel beetles into the rectangular collection area.

Fig. 3.3: 2012 *L. nigrinus* emergence cage design. The site was covered by a lid (a) to prevent any *Laricobius spp.* from dropping in. During the treatment time a rectangular box made of hardware cloth and no-see-um netting (b) was placed on top and secured with weather-proof duct tape (c). A stand made of hardware cloth (d) was set inside to elevate the bouquet (e). The lid was then flipped over and used as the lid for the box and secured with binder clips and the site was surrounded by a fence (f).

Fig. 3.4: Average monthly 2012 soil volumetric water content (m^3/m^3) at lowest and highest elevation sites sampled during *L. nigrinus* pupation to adult emergence (April-November). Error bars = St. Dev.

Fig. 3.5: Average monthly 2010 air temp. (°C) and 2012 air temp. at lowest and highest elevation sites sampled during *L. nigrinus* larval development (April- July). Error bars = St. Dev.

Fig. 3.6: Average monthly 2010 soil temp. (°C) and 2012 soil temp. at lowest and highest elevation sites sampled during *L. nigrinus* pupation to adult emergence (April-November). Error bars = St. Dev.

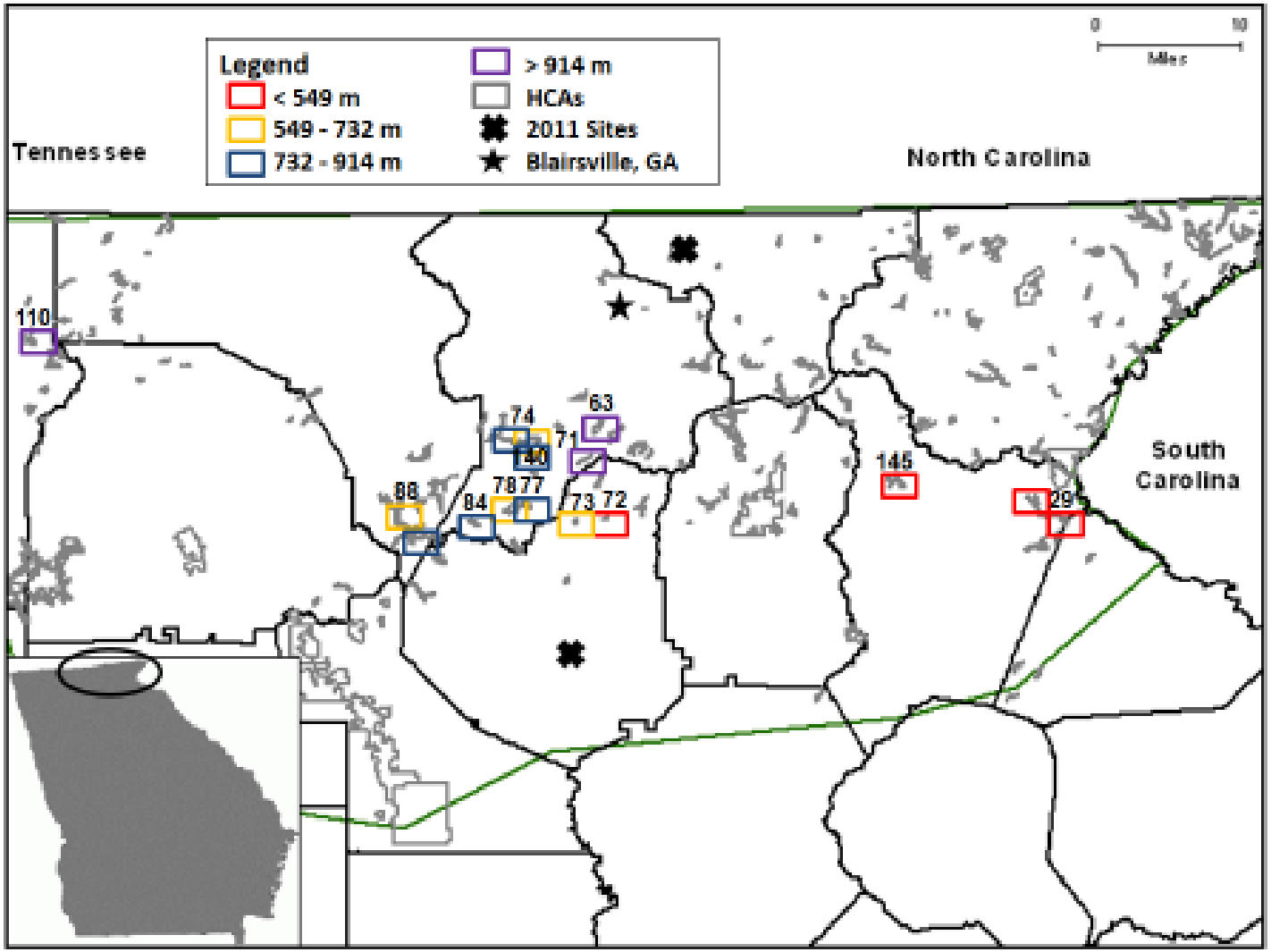
Legend
< 549 m
549 - 732 m
732 - 914 m
> 914 m
HCAs
2011 Sites
Blairsville, GA
Tennessee
North Carolina
South Carolina
110
74
63
71
88
84
78
77
73
72
145
29

Fig. 3.1

Fig. 3.2

Fig. 3.3

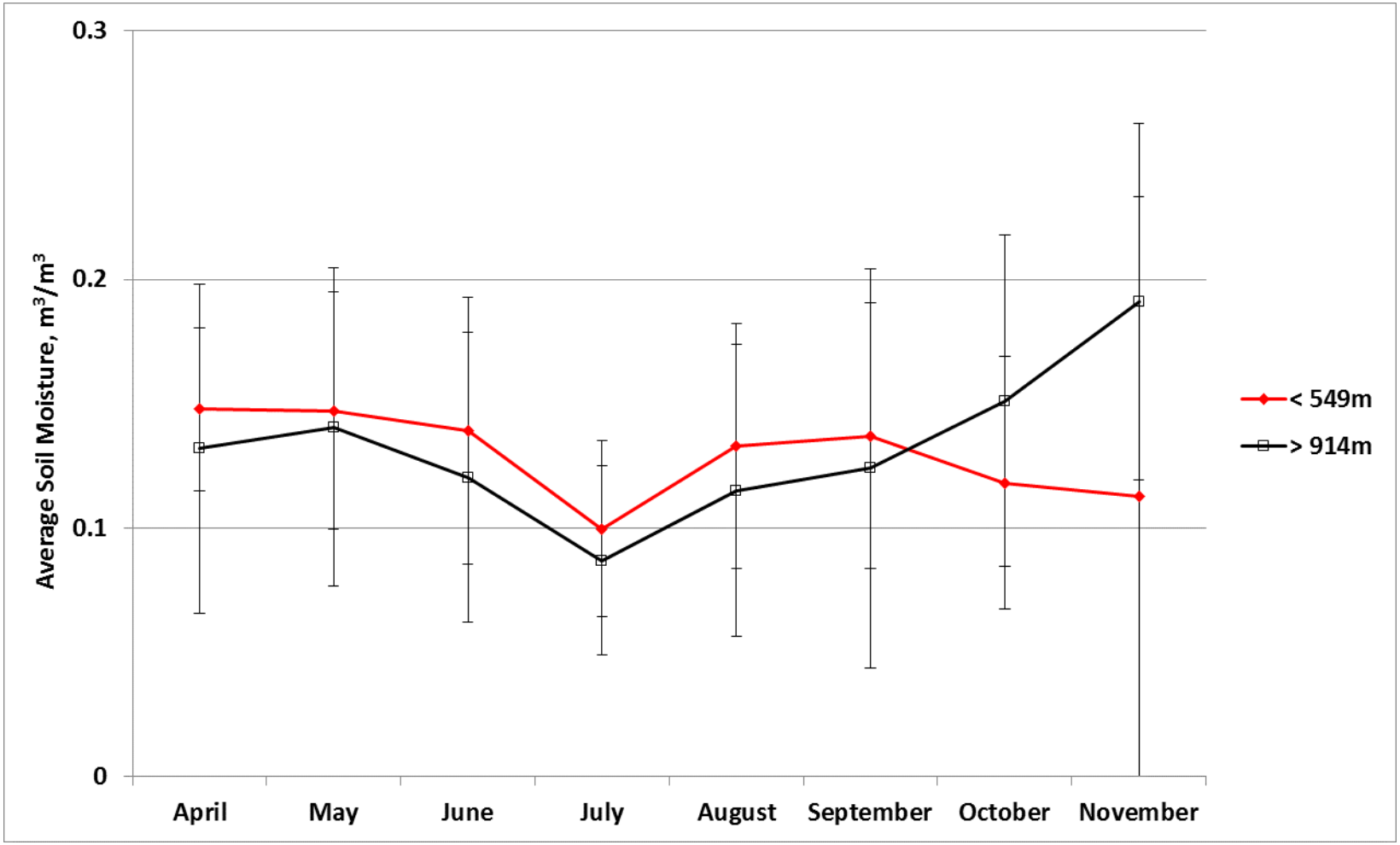
0.3
0.2
0.1
0
Average Soil Moisture, m³/m³
April
May
June
July
August
September
October
November
< 549m
> 914m

Fig. 3.4

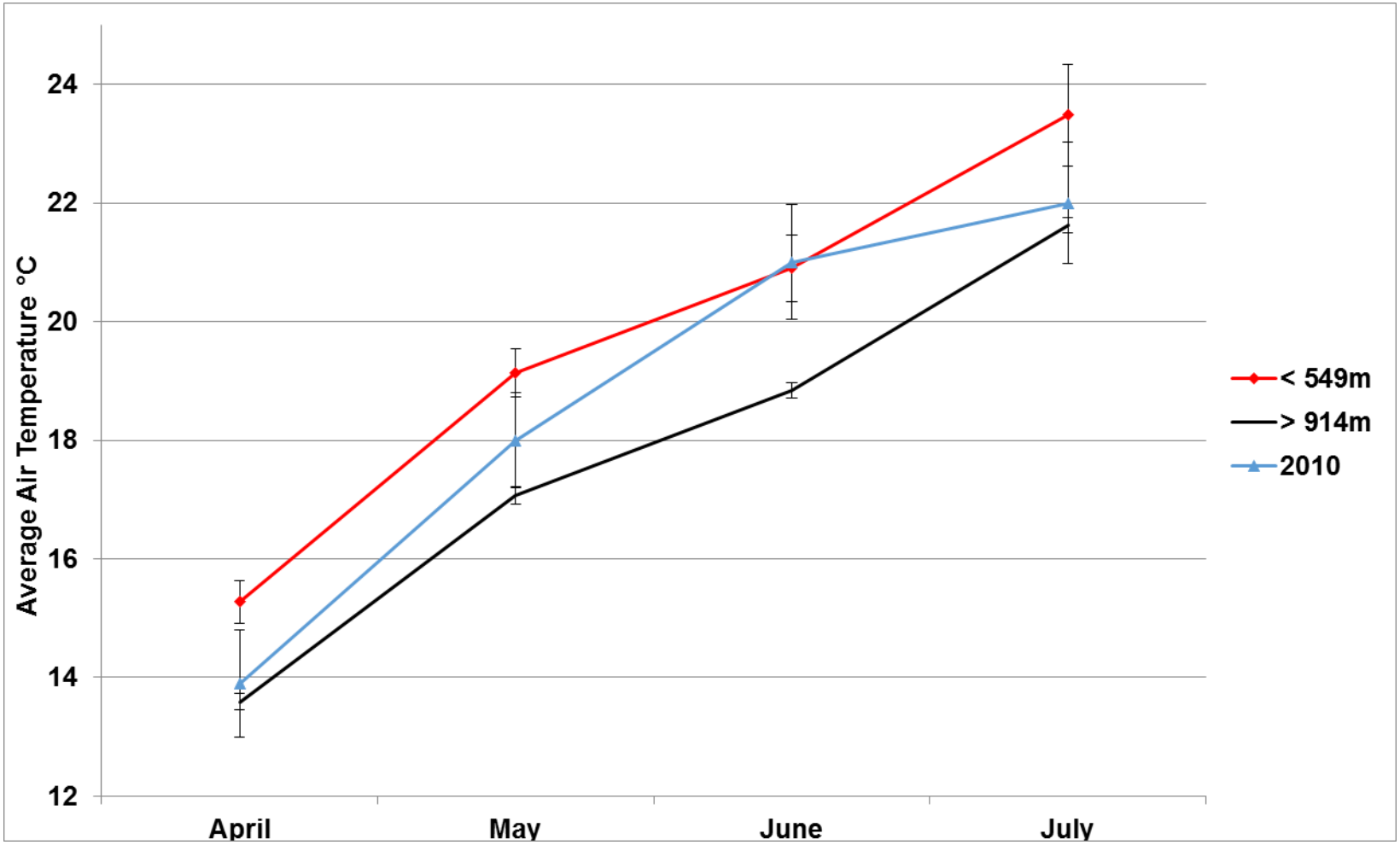
Average Air Temperature °C
24
22
20
18
16
14
12
April
May
June
July
< 549m
> 914m
2010

Fig. 3.5

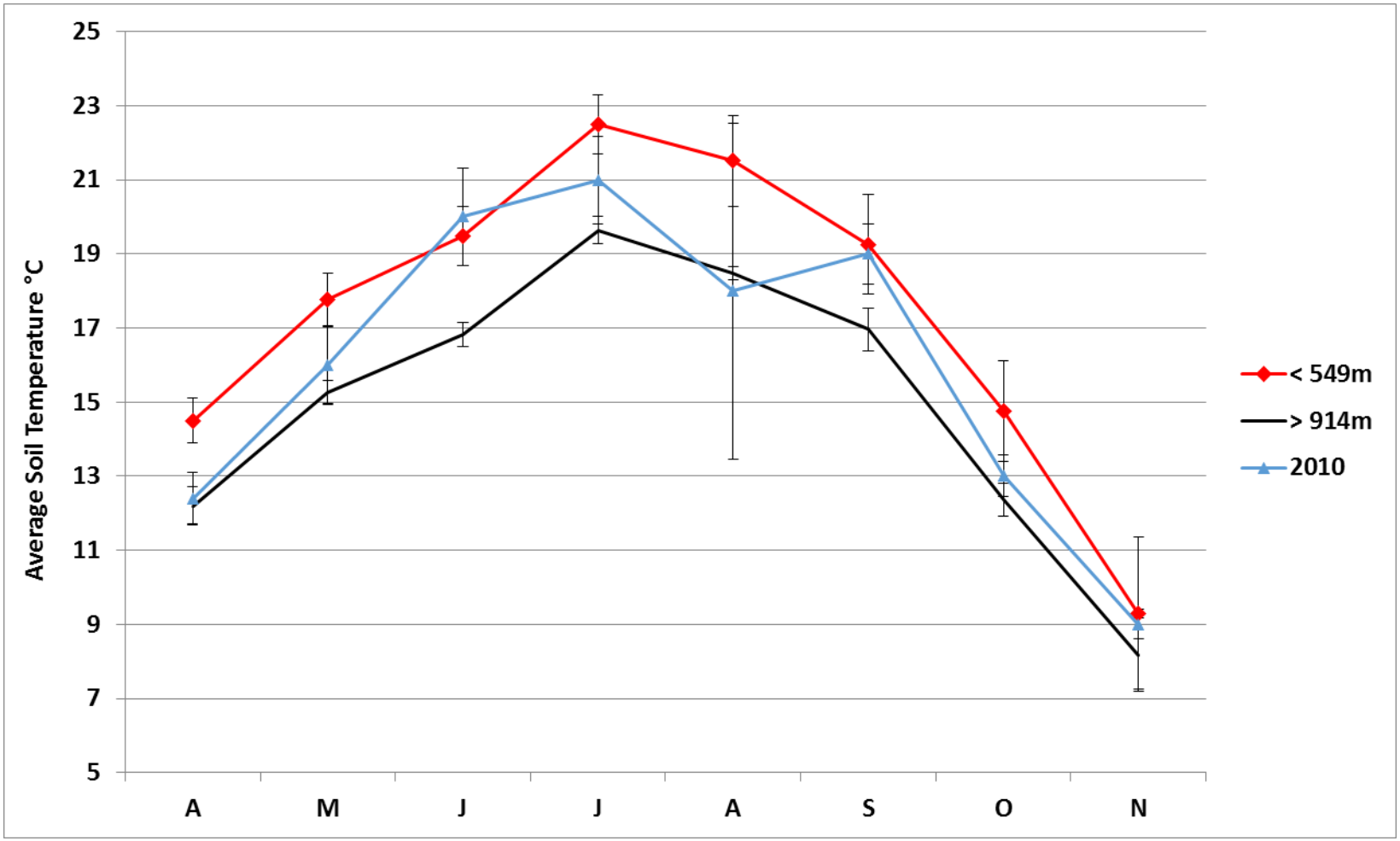
Average Soil Temperature °C
25
23
21
19
17
15
13
11
9
7
5
A
M
J
J
A
S
O
N
< 549m
> 914m
2010

Fig. 3.6

CHAPTER 4:

DISCUSSION

Tsugae canadensis is a long-lived, extremely shade tolerant, and late-successional evergreen that regulates stand microclimates and acidic soil conditions (Eschtruth et al., 2006). Mortality of *T. canadensis* has been estimated at as little as one to four years (McClure, 1991a, 1991b) but stands can harbor infestations for over 15 years in the northern states, resulting with an average stand survival of 73% (Eschtruth et al., 2013). Predicting mortality rates on a large scale works best when factors such as number of years *Adelges tsugae* has infested the area, mean winter temperature from the prior year of tree death, and the current year summer water deficiency are incorporated into the model (Eschtruth et al., 2013). However, additional factor like severity of tree infestation, genetic or physiological resistance, and nutrient availability, especially nitrogen, could help account for variation of mortality seen between sites (Eschtruth et al., 2013). Nitrogen availability strongly impacts level of infestation with *A. tsugae* positively influenced by higher levels of foliar nitrogen and low levels limiting the population (Joseph et al., 2011, McClure, 1991b). Hemlock health is important for establishment of biological control agents to allow enough time for the population to grow so that dispersal, in search for healthier *A. tsugae* populations, doesn't decrease the chances of finding a mate or adequate food. Therefore, stands should be considered on site specific factors suggested in Eschtruth et al. (2013) to determine release areas that will be healthy enough to support beetle establishment.

With almost 10 years of biological control agents *Laricobius nigrinus* and *Sasajiscymnus tsugae* released in north Georgia, research needs to be conducted to evaluate their effectiveness. In order to evaluate biological control agents, an understanding of the mechanisms used to find and recognize the target prey is required (Cortesero et al., 2000). Antennal morphology of *L. nigrinus* and *S. tsugae* suggest that *L. nigrinus* is adapted for long range host finding, while *S. tsugae* sensilla is more adapted for contact chemoreception (Broeckling and Salom, 2003a) and relies more on vision (Flowers et al., 2007). While olfaction plays a role in prey searching and detection for *L. nigrinus*, a combination of host searching stimuli may be used to locate the host (Joseph, 2010) and volatile concentrations too high in an enclosure might act as a repellant. In a field environment different responses could also be triggered (Broeckling and Salom, 2002). In an olfactometer arena, *L. nigrinus* did not display a response to odors from *A. tsugae* alone, but did respond to odors of host plants associated with *A. tsugae*, suggesting that *L. nigrinus* locates its prey based on cues from the host plant (Wallin et al., 2011) which is thought to be a common cue that predators and parasites use to locate prey (Cortesero et al., 2000). Though *A. tsugae* infestation increases monoterpene concentrations (Broeckling and Salom, 2003b) the presence of *A. tsugae* did not increase the attraction to a host plant, which would indicate that *L. nigrinus* is using general volatiles caused by wounding (branches were cut prior to testing) rather than a response to *A. tsugae* feeding (Wallin et al., 2011), or that volatile concentrations were too high to show a difference between *A. tsugae* feeding damage and mechanical wounding (Broeckling and Salom, 2002).

While volatiles might assist in prey location at long distances (Broeckling and Salom, 2003a), visual cues appear to be an important factor for *L. nigrinus* searching at a close range. Adult beetles were 2.3 to 3.4 times more likely to find a host tree during a simulated day time than during night in which there was random straight line searching until the beetle was one centimeter away from the tree (Mausel et al., 2011b). Beetles that were given an *A. tsugae* infested *T. canadensis* all fed on *A. tsugae*, while 90% of those given an uninfested *T. canadensis* host tree flew away after searching (Mausel et al., 2011b).

For biological control to be successful it is also necessary to track population dynamics of both the predator and prey species (Broeckling and Salom, 2002). Both *L. nigrinus* and *S. tsugae* adults released in to the field can more often be found in the upper canopy than on the lower branches (Cheah et al., 2005, Davis et al., 2012). The larval stage is the most easily detected, however this life stage requires additional sampling steps and resources to determine species (Davis et al., 2012, Mausel et al., 2010). Additional techniques may prove to be more beneficial and continued research on *Tsuga spp*. and predator volatiles could provide a lure to assist in capturing beetles as accessing the canopy is not always possible in a forest setting. Targeting certain life cycle events, such as mate location, oviposition (Chapter 2), and emergence (Chapter 3) may help to determine density and survival. Flight by *L. nigrinus* was observed in an olfactometer arena in February, which coincides with the production of *A. tsugae* eggs and oviposition of *L. nigrinus* (Zilahi-Balogh et al., 2003), possibly indicating a time of dispersal or mate location (Wallin et al., 2011) and could be targeted for trapping. If *L. nigrinus* climbs up the bole of the hemlock upon emergence, as seen in Mausel et al.

(2011b), then bole traps (Hanula and Kirsten, 1996) could be employed to capture newly emerged adults.

With the use of a multiple predator approach for obtaining year round control with the winter active *Laricobius* spp. and the spring/summer Coccinellids (*Scymnus spp*. and *S. tsugae*), it is important to understand behavioral interactions to ensure these efforts are not hindering the cause. Research has showed that an increase in conspecific interactions had no significant difference in behaviors (feeding, oviposition, searching, resting) of *S. tsugae*, while *L. nigrinus* showed an increase in searching behavior and decrease in resting (Flowers et al., 2007). Interspecific interactions had no significant influence and effects on spatial separation only occurred as a direct contact response and therefore, these predators can coexist without any significant interspecific competition (Flowers et al., 2006, 2007). Conspecific and congeneric interactions with *L. nigrinus*, the native *L. rubidus*, and *L. osakensis* (from Japan) show that while *L. rubidus* lays significantly less eggs than the others, there is no significant difference in means r eggs produced and consumption of *A. tsugae* eggs when predators were increased to either three conspecifics or one of each in an assay (Story et al., 2012). They also found that consumption of conspecific and congeneric eggs was not significantly different and overall findings suggest that these three predators can co-exist. However, with hybridization broadly occurring between *L. nigrinus* and *L. rubidus* (Havill et al., 2012), concerns of hybrid fitness needs to be evaluated to document changes in fecundity, host preference and consumption rate, and host seeking ability to better understand the influence this hybridization will have on biological control and if there will be a complete admixture of genes or if reproductive isolation will continue (Fischer et al., 2010, Havill

et al., 2012). It is also possible that this hybridization will benefit *L. nigrinus* establishment by providing additional mates and allelic combinations that could increase genetic variation and possibly improve survival and predation by the hybrids on the east coast in comparison to *L. nigrinus* or *L. rubidus* (Havill et al., 2012). In addition, *L. osakensis* needs to be evaluated to determine if mating occurs with the other two *Laricobius* species and whether fertilization occurs.

In conclusion, efforts should be made to not only determine presence of establishment (Chapter 2), but to determine population size and effectiveness of the predators. It would also be beneficial to sample areas where initial releases were conducted for establishment and to estimate hemlock health. In addition, if *L. nigrinus* survival and emergence in North Georgia is as poor as seen in the field (Chapter 3), then efforts should be made to find a biological control agent suited to this environment. With an inland variety of L. nigrinus being found to exhibit a higher cold tolerance and potentially more adapted to the New England states environment (Mausel et al., 2011a), it could be that *L. osakensis* from Japan is more suited to the hot summers experienced in the southern United States (Vieira et al., 2013). Overall, research needs to be continued on these predators released in North Georgia.

Literature Cited

Broeckling, C. D. and S. M. Salom. 2002. Behavioral responses of two hemlock woolly adelgid specialist predators using olfaction to locate host or host habitat, pp. 235-247. *In* B. Onken, R. C. Reardon and J. Lashcomb [eds.]. Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. New Brunswick, NJ: Rutgers University.

Broeckling, C. D. and S. M. Salom. 2003a. Antennal morphology of two specialist predators of hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. . 96: 153-160.

Broeckling, C. D. and S. M. Salom. 2003b. Volatile emissions of eastern hemlock, *Tsuga canadensis*, and the influence of hemlock woolly adelgid. Phytochemistry. 62: 175-180.

Cheah, C. A. S.-J., M. A. Mayer, D. Palmer, T. Scudder and R. Chianese. 2005. Assessments of biological control of hemlock woolly adelgid with *Sasajiscymnus tsugae* in Connecticut and New Jersey, pp. 116-130. *In* B. Onken and R. Reardon [eds.]. Proceedings of the Third Hemlock Woolly Adelgid Symposium USDA For. Serv. FHTET-2005-01.

Cortesero, A. M., J. O. Stapel and W. J. Lewis. 2000. Understanding and manipulating plant attributes to enhance biological control. Biol. Control. 17: 35-49.

Davis, G. A., S. M. Salom, C. C. Brewster, B. P. Onken and L. T. Kok. 2012. Spatiotemporal distribution of the hemlock woolly adelgid predator *Laricobius nigrinus* after release in eastern hemlock forests. Agric. For. Entomol. 14: 408-418.

Eschtruth, A. K., N. L. Cleavitt, J. J. Battles, R. A. Evans and T. J. Fahey. 2006. Vegetation dynamics in declining eastern hemlock stands: 9 years of forest response to hemlock woolly adelgid infestation. Can. J. For. Res. 36: 1435-1450.

Eschtruth, A. K., R. A. Evans and J. J. Battles. 2013. Patterns and predictors of survival in *Tsuga canadensis* populations infested by the exotic pest *Adelges tsugae*: 20 years of monitoring. For. Ecol. and Manag. 305: 195 - 203.

Fischer, M. J., S. M. Salom, L. T. Kok, N. P. Havill, C. Brewster and B. Opell. 2010. Evaluation of hybridization among three *Laricobius* species, predators of hemlock woolly adelgid (Adelgidae), pp. 153. *In* B. Onken and B. Reardon [eds.]. Proceedings of the Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA For. Serv. FHTET-2010-07.

Flowers, R. W., S. M. Salom and L. T. Kok. 2006. Competitive interactions among two specialist predators and a generalist predator of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae) in south-western Virginia. Agric. For. Entomol. 8: 253-262.

Flowers, R. W., S. M. Salom, L. T. Kok and D. E. Mullins. 2007. Behavior and daily activity patterns of specialist and generalist predators of the hemlock woolly adelgid, *Adelges tsugae*. J. Insect Sci. 7: 1-20.

Hanula, J. L. and C. P. Kirsten. 1996. A trap for capturing arthropods crawling up tree boles. USDA For. Ser. Res. Note, SRS-3.

Havill, N. P., G. Davis, D. L. Mausel, J. Klein, R. McDonald, C. Jones, M. Fischer, S. Salom and A. Caccone. 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biol. Control. 63: 359-369.

Joseph, S. V. 2010. Olfactory response of hemlock woolly adelgid (Hemiptera: Adelgidae) predators to hemloc species (Tsugae spp.).

Joseph, S. V., S. K. Braman and J. L. Hanula. 2011. Effects of fertilization of four hemlock species on Adelges tsugae (Hemiptera: Adelgidae) growth and feeding preference of predators. J. Econ. Entomol. 104: 288.

Mausel, D. L., R. G. V. Driesche and J. S. Elkinton. 2011a. Comparative cold tolerance and climate matching of coastal and inland *Laricobius nigrinus* (Coleoptera: Derodontidae), a biological control agent of hemlock woolly adelgid. Biol. Control. 58: 96-102.

Mausel, D. L., S. M. Salom and L. T. Kok. 2011b. Visual ability and searching behavior of adult *Laricobius nigrinus*, a hemlock woolly adelgid predator. J. Insect Sci. 11: 9 pps.

Mausel, D. L., S. M. Salom, L. T. Kok and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39: 440-448.

McClure, M. S. 1991a. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environ. Entomol. 20: 258-264.

McClure, M. S. 1991b. Nitrogen fertilization of hemlock increases susceptibility to hemlock woolly adelgid. J. Arboriculture. 17: 227-230.

Story, H. M., L. C. Vieira, S. M. Salom and L. T. Kok. 2012. Assessing performance and competition among three *Laricobius* (Coleoptera: Derodontidae) species, predators of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). Environ. Entomol. 41: 896-904.

Vieira, L. C., A. B. Lamb, S. Shiyake, S. M. Salom and L. T. Kok. 2013. Seasonal abundance and synchrony between *Laricobius osakensis* (Coleoptera: Derodontidae) and its prey, *Adelges tsugae* (Hemiptera: Adelgidae), in Japan. Ann. Entomol. Soc. Am. 106: 249-257.

Wallin, K. F., T. M. Latty and D. W. Ross. 2011. Orientation behavior of the predator *Laricobius nigrinus* (Coleoptera: Derodontidae) to hemlock woolly adelgid and host tree odors in a multi-chambered olfactometer. Environ. Entomol. 40: 864-872.

Zilahi-Balogh, G. M. G., L. M. Humble, A. B. Lamb, S. M. Salom and L. T. Kok. 2003. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). Can. Entomol. 135: 103-115.