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Assessing The Efficacy Of Two Species Of Silver Fly, Leucopis Argenticollis And L. Piniperda, As Biological Control Agents Of Hemlock Woolly Adelgid, Adelges Tsugae

Kyle Motley
University of Vermont

ASSESSING THE EFFICACY OF TWO SPECIES OF SILVER FLY, *LEUCOPIS ARGENTICOLLIS* AND *L. PINIPERDA*, AS BIOLOGICAL CONTROL AGENTS OF HEMLOCK WOOLLY ADELGID, *ADELGES TSUGAE*

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Thesis Examination Committee:

Kimberly F. Wallin, Ph.D., Advisor
Scott C. Merrill, Ph.D., Chairperson
Anthony W. D'Amato, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

ABSTRACT

Adelges tsugae Annand is a non-native invasive insect threatening the survival of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana)*. *A. tsugae* is established in over half of the total range of eastern hemlock and the entire range of Carolina hemlock. Its continued spread, establishment and associated hemlock mortality make research into biological control of *A. tsugae* crucial. Field surveys of predators associated with *A. tsugae* in the Pacific Northwest identified a strong correlation between *A. tsugae* abundance with *Laricobius nigrinus* and two species of silver fly, *Leucopis argenticollis* and *Leucopis piniperda*. Flies in the genus *Leucopis* are known specialist predators of adelgids and recent studies have shown a strong synchronization between the lifecycles of *Leucopis* spp. and *A. tsugae*. The purpose of this study was to test the potential establishment of *Leucopis* spp. at the southern and northern extent of *A. tsugae* infested eastern hemlock in eastern United States. In 2015 and 2016, western *Leucopis* spp. adults were released at two different densities into enclosed branches of *A. tsugae* infested *T. canadensis* in Tennessee and New York. *A. tsugae* on the branches were counted before putting on the enclosure. Four weeks after set-up, all of the enclosures were collected. The number of *Leucopis* spp. offspring were counted and then stored in ethanol. The number of *Leucopis* spp. offspring collected were positively related to adelgid density, but did not differ by the number of adult flies per enclosure. Flies collected from enclosures and from the source colony were identified as *L.argenticollis* and *L. piniperda* using DNA barcoding. These results show that *Leucopis* spp. from the Pacific Northwest feed and develop to the adult stage on *A. tsugae* in the eastern USA. They are able to tolerate environmental conditions during late spring and early summer at the southern and northern extent of the area invaded by *A. tsugae* in the eastern USA.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Invasive species are among the greatest threats to ecosystems and biodiversity worldwide and invasions are expected to continue, if not accelerate, in the future (Levine & D'Antonio, 2003). Aukema et al. (2011) found that the costs of damage done by invasive insects in the US is nearly $1.7 billion in local government expenditure and approximately $830 million in lost property values, totaling over $2.5 billion in local scale management alone. The hemlock woolly adelgid, *Adelges tsuae* Annand (Hemiptera: Adelgidae), is one example of an invasive insect threatening the structure, function, composition and dynamics of ecosystems in eastern North America.

Adelges tsugae in North American

Adeges tsugae is an introduced insect on two species of native hemlock trees in the eastern United States: eastern hemlock, *Tsuga canadensis,* and Carolina hemlock, *Tsuga caroliniana*. Introduced from Japan and first found in the eastern United States in Richmond, VA in 1951, *A. tsugae* has spread through hemlock ecosystems and is now present in 19 states on the east coast of the United States (McClure 1987; Havill et al. 2014). Dispersed by wind, human and animal activity and with few natural enemies, *A. tsugae* can spread at a rate of 8.1 km per year in the northern portion of its eastern U.S. range and 15.6 km per year in the southern portion (McClure 1990; Shields & Cheah 2005). Currently, *A. tsugae* northern expansion is being limited by winter temperatures below -26º (Cheah 2017). No known such limit exists in it's southern range (Evans and Gregoire 2006).

Eastern hemlock is found throughout eastern North America, from Canada to Alabama and as far west as Minnesota (Burns & Honkala 1990). The range of Carolina hemlock is restricted to the Appalachian highlands from northern Georgia to southwest Virginia (Burns & Honkala 1990). Both of these species are vital components of forested ecosystems in eastern North America and act as foundation species (Burns & Honkala 1990). Shade-tolerant and shade creating, hemlock stands create a cool, damp microclimate that influences species diversity and ecosystem conditions (Ellison et al. 2005; Orwig & Foster 1998). The loss of hemlocks in eastern forests has caused dramatic shifts in understory light levels, soil and stream temperatures, understory plant and animal diversity, and riparian discharge (Eschtruth et al. 2006; Tingley et al. 2002; Brantley et al. 2013). Increased biological invasions have also been associated with hemlock loss, leading to further disturbance of these unique ecosystems (Eschtruth et al. 2006).

Hemlock adelgids are native to Japan, China, Taiwan, South Korea and western North America and have been found on all species of hemlocks native to these ranges (Havill et al. 2006). Havill et al. (2006) found that *A. tsugae* in the eastern United States share an identical haplotype with *A. tsugae* collected from southern Japan and concluded this as the source population of the introduction. In its native range, *A. tsugae* only causes significant damage when trees are stressed or diseased. Natural enemies and host tree resistance are thought to be the main factors regulating *A. tsugae* populations from reaching damaging densities.

A. tsugae Biology

A minute (0.4-1.4mm), sucking and predominantly sessile insect, *A. tsugae* feeds on ray parenchyma cells by inserting its stylet bundle into the base of hemlock needles (Young et al. 1995; McClure et al. 2001). Feeding by *A. tsugae* is mostly done in early spring and late fall, allowing access to the highest nutrient reserves (McClure et al. 2001).

In North America, *A. tsugae* has two generations per year: the progredien and sisten generation. The progredien generation emerges in early spring and seeks out suitable feeding sites at the base of hemlock needles (McClure 1989). The first instar nymph crawlers either settle permanently to feed or are passively dispersed via wind, birds, deer or human activity (McClure 1990). Progredien nymphs develop through four instars and produce the waxy secretions that become an ovisac for the adult (McClure 1987). These adult progrediens oviposit eggs that become the sisten generation in early summer (McClure 1987). Sisten nymphs enter a two to four-month aestivation in late summer (McClure 1987). Nymphs break aestivation in late fall and develop through out the winter months until adults lay eggs in late winter (McClure 1987). These eggs hatch into the progredien generation and complete the life cycle (McClure 1989).

A. tsugae is highly fecund, with each progredien female ovipositing between 25 and 125 eggs and each sisten female between 50 and 175 eggs (McClure et al. 2001). In North America, *A. tsugae* does not undergo sexual reproduction; all individuals are reproduced parthenogenetically. In Asia and North America, eggs laid by the sistens generation will develop into sessile progrediens adults or flying seuxparae (McClure 1991). Sexuparae fly to *Picea* spp. where they produce eggs that become the sexual

generation known as sexuales (McClure 1991). In North America, there is no suitable *Picea* species for development and therefore sexuales do not survive past the first instar (McClure 1991). Sexual reproduction only occurs in Asia where *Picea* species support the sexuales (McClure 1991). Sexuparae abundance is positively related to *A. tsugae* density on hemlock (McClure 1991). Despite the loss of sexuparae, *A. tsugae* populations grow rapidly in North America.

A. tsugae collected in the eastern United states were found to have a minimum temperature threshold for development of 3.9ºC (Salom et al. 2002), but cold tolerance of *A. tsugae* varies with geography. Individuals from the eastern United States were able to survive for several hours at -30ºC, but the real limit to cold tolerance is believed to be closer to -25ºC. Populations from the mountainous regions of Japan are able to survive temperatures up to -40ºC (Skinner et al. 2003). Shields and Cheah (2005) reported that average mortality of *A. tsugae* in New England to be as high as 93% and found a positive correlation between latitude and *A. tsugae* mortality. Recent research by Cheah (2017) suggests populations of *A. tsugae* that survive winter temperatures in New England are developing cold adaptations that will allow *A. tsugae* to spread further north.

Effects of *A. tsugae* on Hemlock Ecosystems

Because of their parthenogenetic reproduction, *A. tsugae* infestations can begin with a single individual. *A. tsugae* crawlers preferentially infest new growth, but will move to older branches and eventually the entire tree when populations increase (McClure 1987).

A. tsugae feed on all sizes and ages of hemlock trees. Feeding during late winter and early spring affects new shoot growth and bud break (McClure 1991). By feeding on xylem ray parenchyma cells, *A. tsugae* depletes stored nutrients, causing desiccation, needle drop and branch dieback (McClure 1991). *A. tsugae* herbivory also activates a hypersensitive response in hemlocks, characterized by false growth rings around the feeding site that restrict water and solute transfer (Domec et al. 2013). This induced response to feeding diminishes the ability of hemlock trees to respond to stressful environmental conditions (Young et al. 1995). *A. tsugae* infested hemlock trees can be killed within four years if there are additional environmental stressors but are usually dead within ten years of initial *A. tsugae* colonization, even under ideal conditions (McCure 1987).

The long-term effects of *T. canadensis* hemlock decline caused by *A. tsugae* are still relatively unknown. Because there is no functionally similar species to replace *T. canadensis* and *T. caroliniana* in eastern North America, the removal of these two species can cause dramatic changes to the microclimate, soil conditions, flora and fauna even over short periods of time.

The loss of hemlocks in riparian habitats has caused shifts in stream discharge and increased water temperature in the southern Appalachians, leading to decreased native macroinvertebrates (Brantley et al. 2013). Eastern and Carolina hemlock provide habitat for many bird species and Tingley et al. (2002) found three bird species in New England that had a 60% mortality rate as a result of hemlock decline. Hemlock decline in the Delaware Water Gap National Recreation Area resulted in a doubling of the amount

of light reaching the understory, a fourfold increase in the cover of understory species and increased densities of understory invasive species (Eschtruth et al. 2005).

In addition to changing ecosystem processes, hemlock loss associated with *A. tsugae* affects social and economic systems. Eastern hemlock is an important ornamental tree represented by more than 247 cultivars in the nursery trade (Quimby 1996). In 1995, eastern hemlock nursery stock was valued at approximately $34 million (Bentz et al. 2002). *A. tsugae* limits the practicality of hemlock ornamental use within the range of eastern and Carolina hemlock (Woodsen 2001). Land values have also decreased as a result of hemlock decline. A study of a residential area in New Jersey suggested that average property value was $7,000 lower in *A. tsugae* infected areas than *A. tsugae* free areas (Holmes et al. 2005). Li et al. (2014), found that hemlock decline has led to a loss of at least $24.6 million in Connecticut and Massachusetts real estate.

In 2013, eastern hemlock was added to the IUCN Red List of Threatened Species as a Near Threatened species (Farjon 2013). In the face of continued spread of *A. tsugae* and the resulting ecological, social and economic losses, research into controlling *A. tsugae* is crucial.

Management of *A. tsugae*

While there has been some success suppressing *A. tsugae* populations with integrated management involving pesticides and host resistance, these strategies aren't applicable over larger landscape. Large scale chemical treatments are often cost inefficient (Cowles et al. 2006). Furthermore, forested regions can be inaccessible to the equipment needed for adequate chemical application, while certain forest habitats can be

sensitive to widespread chemical treatment. Additionally, the protective woolly wax produced by *A. tsugae* can reduce contact with the pesticide and reduce its effectiveness (Mills 1990).

Current control efforts for *A. tsugae* is the eastern United States are focused on developing a classical biological control program. Several native predators have been identified as associated with *A. tsugae* in eastern North America, but were generalist predators and found at densities too low to impact *A. tsugae* populations (McClure 1987; Wallace & Hain 2000). Because of this, biological control efforts have focused on natural enemies from Japan, China and western North America. There are no known parasitoids of any adelgid species, so biological control efforts are limited to the use of predators and entomopathogens.

Chemical Control of *A. tusgae*

Insecticides are effective for controlling *A. tsugae* populations on individual trees, but are not easily scaled up to ecosystems. In nursery and horticultural settings, insecticidal soap and horticultural oils provide successful control, but must be applied to the entire plant surface to work (McClure et al. 2001). Insecticidal soaps and horticultural oils also have little residual effect and pest control is limited in the long-term (McClure et al. 2001).

A systemic neo-nicotinoid insecticide, imidacloprid is highly effective in controlling *A. tsugae* populations. It can be applied as a foliar spray, or through trunk and soil injections where the chemical is absorbed by the tree and transmitted to insects during feeding (Silcox 2002). While used successfully, imidacloprid has several

limitations beyond the inability to be used over large forested areas. Imidacloprid is highly toxic in riparian systems, limiting its use in areas where hemlocks thrive (Cowles et al. 2006). Additionally, imidacloprid can impact natural enemies through both direct and indirect exposure, but these non-target impacts may be negligible in field settings (Eisenback et al. 2010).

Breeding Resistant Hemlocks

Breeding and planting resistant trees are important parts of pest management. In the native range of *A tsugae*, hemlock trees are rarely killed by the insect. Havill and Montgomery (2008) propose that these hemlocks have evolved in tri-trophic relationship between hemlock, *A. tsugae* and predators of *A. tsugae*. Because of this stability in the native range of *A. tsugae*, efforts are currently underway to develop resistant hybrids of *T. caroliniana* with resistant hemlock species from China and the Pacific Northwest (Montgomery et al. 2009a). Some degree of resistance has been demonstrated by a hybrid of *T. caroliniana* and *T. chinensis* and more research is underway to assess its potential for use in landscape settings (Montgomery et al. 2009a).

Biological Control of *A. tsugae*

The limited options for controlling *A. tsugae* populations and their impacts on hemlock ecosystems in the eastern United States has focused control efforts on identifying natural enemies and implementing classical biological control methods. In forest ecosystems, classical biological control is the most frequently used approach for controlling insect pests (Dahlsten & Mills 1999). Most of these biological control agents

are parasitoids and have been employed against homopteran pests (Dahlsten & Mills 1999).

Surveys of predators associated with *A. tsugae* in the eastern United States have found native species of Syrphidae, Ceciomyiidae and Chrysopidae established on *A. tsugae* in Connecticut, North Carolina and Virginia (McClure 1987; Montgomery & Lyon 1996; Wallace & Hain 2000). All predators identified were generalists and were found at densities too low to reduce *A. tsugae* populations.

Classical biological control efforts using predators identified from western North America and Asia have been underway since the early 1900s. In this time, several predators have been investigated and released on populations of *A. tsugae* in the eastern United States.

Current Biological Control of *A. tsugae*

Entomopathogens

Several fungal pathogens from China and the United States have been isolated from *A. tsugae* and are being evaluated for potential biological control. Current focus of these efforts is on single strains of *Metarhizium anisopliae* and *Verticillum lecanni* and two strains of *Beauveria bassiana* (Costa et el. 2005; Reid et al. 2010). These fungi have been shown to cause significant mortality to *A. tsugae* in laboratory settings, but field potential and non-target effects are still currently being evaluated (Cheah et al. 2004; Reid et al. 2010).

Sasajiscymnus tsugae

Discovered in Japan in 1992, this coccinellid beetle belongs to the Tribe Scymini, a group of specialist predators of adelgids, mealybugs and aphids. While *A. tsugae* is the preferred host, *S. tsugae* will feed on several other species of adelgid (Butin et al. 2004). Since 1995, over 1 million individuals of *S. tsugae* have been released in more than 100 sites in 15 states along the east coast of the United States (Cheah et al. 2004). *S. tsugae* were identified as biological control agents because both adults and larvae of the beetle are highly mobile, display good searching and dispersal abilities and feed on all life stages of *A. tsugae* (Cheah & McClure 1996). Furthermore, *S. tsugae* exhibit a high degree of synchrony with the life cycle of *A. tsugae* and released individuals have shown the ability to successfully reproduce, establish and overwinter on *A. tsugae* in field conditions (Cheah & McClure 2000). Inconsistent recovery at release sites reported by Cheat et al. (2005) coupled with studies indicating that *S. tsuage* may have negligible effects on *A. tsugae* populations indicate that this predator may not be the ideal control agent for *A. tsugae* (Butin et al. 2003; Asaro et al. 2005)

***Scymnus* spp.**

Several species of coccinellid beetles have been found associated with *A. tsugae* on hemlock trees in China. Within this genus, three species have been studied for release on *A. tsugae* in eastern North America: *Scymnus camptodromus*, *S. sinuanodulus* and *S. ningshanensis* (Yu et el. 2000). All three of these species are univoltine and coincide with the progredien generation *A. tsugae* (Yu et al. 2000). Although *Scymnus* spp. adults feed

on all life stages of *A. tsugae* and other adelgid species, first instar larvae will only survive on the eggs of *A. tsugae* (Montgomery et al. 2002).

S. camptodromus exhibited the desired characteristics described above, but challenges in laboratory rearing have prevented further research into its potential as a biological control agent (Montgomery et al 2009b). *S. sinuanodulus* and *S. ningshanensis* have both been successfully released in the eastern United States. Releases of *S. ningshanensis* began in Connecticut and Massachusetts in 2007 while *S. sinuanodulus* was first released in Georgia in 2004 (Montgomery et al. 2009b). Field studies done by Butin et al. (2003) suggest that *S. sinuanodulus* can reduce the rate of *A. tsugae* population increase and that *S. ningshanensis* can augment *Sa.tsugae* by suppressing *A. tsugae* population growth where *Sa. tsugae* was unable to do so. Unfortunately, the ability of *S. sinuanodulus* and *S. ningshanensis* to reduce high levels of *A. tsugae* infestations has not been exhibited in the eastern United States, despite their potential for reducing population growth.

***Laricobius* spp.**

Laricobius nigrinus Fender is a derodontid beetle native to western North America. It was first found on *A. tsugae* on western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in British Columbia and has since undergone extensive study (Zilahi-Balogh et al. 2003; Mausel et al. 2011; Mayfield et al. 2015; Wallin et al. 2011). *L. nigrinus* is considered very host specific in that it can only complete development on *A. tsugae,* despite being able to feed on other adelgid species in laboratory settings (Zilahi-Balogh et al. 2002a). The life cycle of *L. nigrinus* is highly synchryonized with *A. tsugae*, with *L.*

nigrinus egg-laying coinciding with egg-laying of the *A. tsugae* sisten generation (Zilahi-Balogh et al. 2003). *L. nigrinus* eggs are laid directly in *A. tsugae* ovisacs, where hatched larvae feed on *A. tsugae* eggs (Zilahi-Balogh et al. 2003).

Between 2003 and 2010, several hundred thousand *L. nigrinus* adults and eggs were released at sites in 14 eastern states (Mausel et al. 2011). This predator has become established at numerous sites in the eastern United States and has been shown to reduce densities of the *A. tsugae* winter generation (Mayfield et al. 2015). An additional derodontid species from Japan, *Laricobius osakensis*, is also beginning to be released in the eastern United States (Mooneyham et al. 2016). Despite successful release and establishment, so far there is no evidence that *Laricobius* spp. are reducing the rate of hemlock mortality.

***Leucopis* spp.**

A beat sheet sampling method was used to survey 116 *A. tsugae* infested western hemlocks across 16 sites in western Oregon and Washington over two years identified three adelgid specific predators: *Laricobius nigrinus*, *Leucopis argenticollis* and *Leucopis piniperda* (Diptera: Chamaemyiidae) (Kohler et al. 2008). *La. nigrinus* was found to be the most abundant, comprising 43% of all predators collected (Kohler et al. 2008). Collectively, the two species of *Leucopis* were the second most abundant predators comprising 16% of the total (Kohler et al. 2008). A more recent study in Oregon and Washington found three times more *Leucopis* spp. than *La. nigrinus* associated with *A. tsugae* after sampling and dissecting branches over a year (Kohler et al. 2016). The ratio of immatures to adults was over three times higher for *Luecopis* spp.

(9.2) compared to the derodontids (2.6) suggesting that beat sheet sampling method was less effective at collecting adult chamaemyiids than whole branch sampling method (Kohler et al. 2008).

Laboratory, no-choice feeding trials with *Leucopis* spp. larvae collected from *A. tsugae* infested western hemlock indicated that both species can feed, survive, and develop to the adult stage on other adelgid species, although survival was always highest on *A. tsugae* (Grubin et al. 2011). Grubin et al. (2011) also found a high degree of synchronization between the species, with *Leucopis* spp. displaying two annual peak abundances coinciding with both generations of *A. tsugae.* Chamaemyiid predators have been used as biological control for other adelgid pests with varying degrees of success, justifying the potential of these two species as biological control agents of *A. tsugae* (Zilahi-Balogh et al. 2002b)

Biological Control with Chamaemyiidae

Zilahi-Balogh et al. (2002b) reviewed the biological control programs for the family Adelgidae worldwide and concluded that the most effective predators at controlling adelgid populations have been members of the Chamaemyiidae. All larvae in the Chamaemyiid family prey on adelgids, aphids, scales and other homopterans (Gaimari & Turner 1996). Adelgid specialists can be found in several genera of the chamaemyiid family including *Neoleucopis*, *Lipoleucopis* and *Cremifana*, but the majority belong to the genus *Leucopis* (McAlpine 1971; McLean 1992; Hagen et al. 1999).

Several species of the *Leucopis* genus have been used in classical biological control efforts throughout the world. In efforts to control the balsam woolly adelgid, *Adelges piceae* Ratzenburg, four chamaemyiid species have been released in North America: *Creminfania nigrocellulata*, *Leucopis atratula*, *L. hennigrata* and *L. obscura* (Mitchell and Wright 1967, Schooley et al. 1984). None of these species has resulted in control of *A. piceae*, despite their ability to establish on *A. piceae* in field settings (Mitchell & Wright 1967).

Several chamaemyiid predators control *Pineus boerneri* and *Pineus pini*, two species of pine bark adelgid, in New Zealand, Hawaii and Chile (Greathead 1995; Hagen et al. 1999; Mills 1990). *P. pini* in New Zealand and *P. boerneri* in Hawaii were controlled by the introduction of *Leucopis tapiae* (Greathead 1995). *Leucopis obscura* controlled *P. pini* populations in Chile (Mills 1990). Based on this success, Mills (1990) suggests *Leucopis argenticollis* for controlling *P. pini* in parts of Africa where other chamaemyiid predators have been unsuccessful.

Conclusion

Research into and release of predators for *A. tsugae* have not achieved the desired results. Successful management of adelgid pests have been achieved in Hawaii, New Zealand and Chile by using members of the Chamaemyiid family, particularly the *Leucopis* genus (Greathead 1995; Mills 1990; Hagen et al. 1999). Zilahi-Balogh et al. (2002b) suggested that further exploration of natural predators of *A. tsugae* should include Chamaemyiids. A study done by Kohler et al. (2008) in western North America identified *L. argenticollis* and *L. piniperda* as adelgid specialists associated with *A.*

tsugae. Grubin et al. (2011) found *L. argenticollis* and *L. piniperda* to be highly synchronized with the bivoltine life cycle of *A. tsugae*. These two species of *Leucopis* are excellent potential candidates for biological control of *A. tsugae* in the eastern united states.

CHAPTER 2: FEEDING BY *LEUCOPIS ARGENTICOLLIS* AND *LEUCOPIS PINIPERDA* (DIPTERA: CHAMAEMYIIDAE) FROM THE WESTERN USA ON *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE) IN THE EASTERN USA

Kyle Motley[1], Nathan P. Havill[2], Arielle L. Arsenault-Benoit[1], Albert E. Mayfield[3], Daniel S. Ott[4], Darrell Ross[4], Mark C. Whitmore[5], and Kimberly F. Wallin[1,6]

[1]Rubenstein School of Environment and Natural Resources, The University of Vermont, Burlington, VT 05405

[2]USDA Forest Service, Northern Research Station, Hamden, CT

[3]USDA Forest Service, Southern Research Station, Asheville, NC 28804

[4]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331

[5]Department of Natural Resources, Cornell University, Ithaca, NY 14853

[6]USDA Forest Service, Northern Research Station, Burlington, VT 05403

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Abstract

Leucopis argenticollis (Zetterstedt) and *Le. piniperda* (Malloch) are known to feed on the lineage of *Adelges tsugae* Annand that is native to western North America, but it is not known if they will survive on the lineage that was introduced from Japan to the eastern United States. In 2014, western *Leucopis* spp. larvae were brought to the laboratory and placed on *A. tsugae* collected in either Washington (North American *A. tsugae* lineage) or Connecticut (Japanese lineage). There were no significant differences in survival or developmental times between flies reared on the two different adelgid lineages. In 2015 and 2016, western *Leucopis* spp. adults were released at two different densities onto enclosed branches of *A. tsugae* infested eastern hemlock (*Tsuga canadensis* (L.) Carr.) in Tennessee and New York. Cages were recovered and their contents examined four weeks after release at each location. *Leucopis* spp. larvae and puparia of the F1 generation were recovered at both release locations and adults of the F1 generation were collected at the Tennessee location. The number of *Leucopis* spp. offspring collected increased with increasing adelgid density, but did not differ by the number of adult flies released. Flies recovered from cages and flies collected from the source colony were identified as *Le.argenticollis* and *Le. piniperda* using DNA barcoding. These results demonstrate that *Leucopis* spp. from the Pacific Northwest are capable of feeding and developing to the adult stage on *A. tsugae* in the eastern USA and they are able to tolerate environmental conditions during late spring and early summer at the southern and northern extent of the area invaded by *A. tsugae* in the eastern USA.

KEY WORDS Silver flies, hemlock woolly adelgid, biological control, *Tsuga canadensis*

Introduction

The hemlock woolly adelgid (*Adelges tsugae* Annand) was introduced to the eastern USA from Japan sometime before 1951 when it was first documented in Virginia (Stoetzel, 2002; Havill et al., 2006). In the 1980s, it began spreading rapidly throughout the range of hemlock causing high levels of tree mortality. It is now present in 19 eastern USA states from Georgia to southern Maine where it damages two native hemlock species, eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.) (Havill et al., 2011). The first efforts to develop and implement classical biological control for *A. tsugae* began in the early 1990s, but increased dramatically in the early 2000s with the formation of the Hemlock Woolly Adelgid Initiative, a cooperative research and development program involving federal and state government agencies and other partners (Onken & Reardon, 2011). To date, the biological control program has focused mostly on two predators, *Sasajiscymnus tsugae* (Sasaji and McClure), a coccinelid imported from Japan, and *Laricobius nigrinus* Fender, a derodontid imported from western North America where *A. tsugae* is also native. Between 1995 and 2010, over 2 million *S. tsugae* were released at more than 400 sites in 16 eastern states (Cheah, 2011). Between 2003 and 2010, several hundred thousand *L. nigrinus* adults and eggs were released at sites in 14 eastern states (Mausel et al., 2011); this predator has become established at numerous eastern USA sites, reduces densities of the *A. tsugae* winter generation (Mayfield et al. 2015), and continues to be released. An

additional derodontid species from Japan, *Laricobius osakensis*, is also beginning to be released in the eastern USA (Mooneyham et al., 2016). Despite the coordinated effort to control *A. tsugae* with *S. tsugae* and *La. nigrinus*, there is, so far, no indication that they are reducing the rate of hemlock mortality. Consequently, efforts have continued to identify additional biological control agents in Asia and western North America (Onken & Reardon, 2011) where there are endemic lineages of *A. tsugae* (Havill et al., 2016).

A beat sampling survey of 116 *A. tsugae* infested western hemlocks (*Tsuga heterophylla* (Raf.) Sarg.) across 16 sites in western Oregon and Washington over 23 months resulted in the collection of over 6,000 adult and immature predators representing 55 species from 43 genera, 14 families, and 4 orders (Kohler et al., 2008). *La. nigrinus* was found to be the most abundant comprising 43% of all predators collected. Collectively, two species of *Leucopis* (Diptera: Chamaemyiidae), *Le. argenticollis* and *Le. piniperda* (misidentified as *Le. atrifacies*, see Grubin et al., 2011) were the second most abundant predators comprising 16% of the total. However, the ratio of immatures to adults was over three times higher for the chamaemyiids (9.2:1) compared to the derodontids (2.6:1) or hemerobiids (3.1:1), the third most abundant group, suggesting that beat sampling was less effective at collecting adult chamaemyiids and that their relative abundance may be higher than indicated by counts from beat sampling. *La. nigrinus*, *Le. argenticollis*, and *Le. piniperda* were the only adelgid-specific predators that were both frequently encountered and abundant in the survey. This was the first record of either *Le. argenticollis* or *Le. piniperda* collected from *A. tsugae*, although both species have been collected in association with other *Pineus* and *Adelges* species in other parts of North

America (Ross et al., 2011). A more recent study in Oregon and Washington found 2.3-3.5 times more *Leucopis* spp. than *La. nigrinus* after sampling and dissecting branches over a year (Kohler et al., 2016). Laboratory, no-choice feeding trials with *Leucopis* spp. larvae collected from *A. tsugae* infested western hemlock indicated that both species can feed, survive, and develop to the adult stage on other adelgid species, although survival was always highest on *A. tsugae* (Grubin et al., 2011).

The objective of the studies reported in this paper was to determine whether *Leucopis* spp. from the Pacific Northwest (PNW) could feed and complete their development on Japanese *A. tsugae* introduced to the eastern USA in the laboratory and under field conditions.

Materials and Methods

Laboratory Feeding Experiment

In March 2014, western hemlock branches with *A. tsugae* infestations were collected in Olympia and Tacoma, WA. The branches were placed in plastic bags and shipped overnight to the USDA Forest Service, Northern Research Station (USDA-FS-NRS) laboratory in Hamden, CT. Interstate movement of this material was regulated under USDA-APHIS permit number P526P-13-03488 issued to N. Havill. Branches were examined under a dissecting microscope and *Leucopis* spp. larvae were removed, their length measured using an ocular micrometer calibrated with a 2mm stage micrometer (American Optical Company, Buffalo, New York), and alternately placed into one of two treatment groups. One group received western *A. tsugae* on *T. heterophylla*, and the

other received eastern (Japanese) *A. tsugae* on *T. canadensis*. Flies were placed on 5-cm-long branch tips with at least three undisturbed adelgid ovisacs with eggs. Each infested branch and fly larva was placed in a 60-ml plastic cup with a moist filter paper on the bottom. The lid of each cup had a 2-cm diameter hole covered with fine mesh.

Flies were held in a walk-in environmental chamber at 25°C, 60% relative humidity, and a photoperiod of 12:12 (L:D) h. Flies were observed every 1-3 days until they died or pupariated. Puparia were removed from the foliage and placed individually in 5-cm diameter petri dishes and provided with a 50:50 Wheast (Planet Natural, Bozeman, Montana) and honey paste spotted onto a small square of filter paper to provide nutrition for the adult fly upon emergence. The dates that flies died, pupariated, and/or emerged as adults were recorded. Puparia that did not yield an adult fly or parasitoid were dissected to determine whether a fly or parasitoid died during development.

Branch Enclosure Study

T. heterophylla branches with *A. tsugae* infestations were collected in April and May 2015 and 2016 from several locations in Olympia, Tacoma, Vashon Island, and Whidbey Island, WA. The branches were sealed in plastic bags and shipped overnight to the USDA-FS-NRS laboratory in Hamden, CT in 2015 and transported directly to the Oregon State University, Department of Forest Ecosystems & Society in Corvallis, OR in 2016.

Foliage was held in cages to monitor for adult fly eclosion. Two types of cages were used: 60x60x60 cm tent-style fine mesh bugdorms (Item number BD2120, MegaView Science, Taiwan), or custom built 50x45x45 cm plexiglass cages with mesh insets on the top and side. Upon arrival, foliage was clipped into pieces that would fit in the cages and the stems were inserted in 22.5x10.5x8.0 cm floral foam blocks held in Sterilite® plastic shoeboxes (31x19x10 mm). The floral foam was saturated with deionized water, with additional water left standing in the bottom of the shoe box to compensate for evaporation. Two shoeboxes with foliage were placed into each cage. A paste of honey and Wheast was spread on strips of yellow paper, which were taped to the inside wall of each cage. A combination of vials containing deionized water, dilute honey water, and dilute honey-Wheast water were stopped with a cotton wick and placed in each cage as well (based on Giamari and Turner, 1996). Water in the shoeboxes and the food and water vials were replenished 1-2 times per week as needed. Once cages were prepared, they were held in two walk-in environmental chambers with a photoperiod of 16:8 (L:D) h at either 15°C or 17°C in 2015 and in a laboratory at room temperature in 2016.

Cages were checked every one or two days. During these checks, any arthropods other than *Leucopis* spp. (especially predators that might prey on emerging flies) were removed. Adult *Leucopis* spp. found in the cages were collected with an aspirator and moved to a collective adult cage which was similar to the rearing cages, except a small amount of uninfested *Tsuga canadensis* foliage was used instead of infested *T. heterophylla* foliage. Also, the foliage was placed into a 1000 ml flask filled with water and covered with parafilm® instead of floral foam in a shoe box to prevent flies from

becoming trapped in the water. Consequently, flies had foliage to alight on, but they did not have a prey source on which to lay their eggs. Adult cages were held at 15°C with a photoperiod of 16:8 (L:D) h.

For two nights prior to a field release in 2015, cages with adults were removed from the environmental chambers. They were placed in a room (~23°C) near a window in case the dawn and/or dusk periods were required to stimulate mating behavior. Each day, they were removed from the chamber at approximately 04:15 p.m. and returned to the chamber at approximately 07:15 a.m. the next morning. No attempts were made to expose the adult flies to dawn or dusk lighting in 2016.

On the day of shipment to field sites, adult flies were sorted by sex based on dimorphism of the abdomens, viewed under a dissecting microscope with individual flies in 5cm petri dishes. Flies were then placed in separate female and male cages with vials of water, honey water, and honey-Wheast water, but no foliage. The required number of females and males for field experiments onto caged branches could then be drawn from each cage. It was not possible to determine the species of the flies prior to release because the character used to distinguish them could not be seen on live flies. *L. argenticollis* have several long setulae on the postpronotum, medial from the postpronotal seta, while *L. piniperda* have no such setulae (S. Gaimari, personal communication, 2015).

In preparation for shipment to experimental field sites, plastic aspirator vials were prepared similarly to Giamari and Turner (1996). Adult flies were aspirated into the vials

in specific sex ratios according to the experimental design. Flies were in transport to experimental field sites in insulated boxes with ice for less than 24 hours.

Enclosed branch experiments were performed at two locations, near Grandview, TN (35.74853, -84.82871) and Skaneateles Lake, Niles, NY (42.80186, -76.30139), located near the southern and northern edges, respectively, of the invasive range of *A. tsugae* in the eastern USA. Flies were placed on enclosed branches in TN on 12 May 2015 and 10 May 2016. Flies were placed on enclosed branches in NY on 5 June 2015 and 27 May 2016. Ambient conditions during releases in TN and NY were 20-23°C and sunny and 22-24°C and partly cloudy, respectively.

The date for each experimental field release was timed to coincide with *A. tsugae* entering the progrediens nymph stage, so that if flies reproduced, the larvae could feed on eggs of the next generation (sistentes). All live *A. tsugae* progrediens nymphs (evidenced by fresh woolly ovisac production) 50 cm from the terminal end on each branch were counted and recorded. Prior to enclosure, treatments were assigned at random to infested *T. canadensis* branches. There were four treatments, each replicated on six branches in 2015 and seven branches in 2016. The treatments were 1) enclosed branch with 2F:2M *Leucopis* spp., 2) enclosed branch with 6F:4M *Leucopis* spp. in 2015 and 5F:5M *Leucopis* spp. in 2016, 3) enclosed control branch without *Leucopis* spp. and 4) non-enclosed control branch without *Leucopis* spp. All branches were tapped along their length 20 times to dislodge predators prior to enclosing.

Branch enclosures were 71x48 cm bags made of fine mesh nylon netting (Item number DC3148, MegaView Science Co., Taiwan). To secure enclosures to branches, a piece of foam pipe insulation was wrapped onto the branch 50 cm from the end. The open end of the enclosure was secured around the pipe insulation with two zip ties. Flies were added to the enclosures through the zipper.

Branches from the 2015 study were collected 28 days (9 June) after experimental release in TN, and 33 days (8 July) after release in NY. Branches from the 2016 study were collected 29 days (7 June) after experimental release in TN, and 26 days (22 June) after release in NY. Branches were collected by placing a large plastic bag around each branch, clipping the branch, and sealing the plastic bag. In 2015, branches were shipped overnight with ice packs to the USDA Forest Service laboratory in Hamden, CT, where they were kept at 7°C until processed. Branches collected in 2016 were shipped overnight with ice packs to the USDA Forest Service George D. Aiken Forestry Sciences Laboratory in Burlington, VT and stored at 7°C until processed. Branches were clipped into small pieces approximately 10 cm long. All *A. tsugae* ovisacs, settled adults, and new *T. canadensis* growth were recorded. Each branch was thoroughly searched for fly offspring under a dissecting microscope. The entire contents of each mesh enclosure was thoroughly searched for fly offspring and the number of offspring in each life stage was recorded. Larvae and adults were collected into 95% ethanol and stored at -20°C. Puparia were held in individual 5 cm petri dishes until eclosion of adults, which were then placed into 95% ethanol and stored at -20°C.

Up to 20 larvae and/or adult flies per enclosure were identified using DNA barcoding. DNA was extracted using the Mag-Bind Blood & Tissue Kit (Omega Bio-Tek, Norcross, Georgia). DNA was extracted from adults after grinding 3 legs removed from one side of the specimen with the remainder saved as a voucher. Larvae underwent non-destructive extraction by cutting a small slit in the side of the specimen, incubating with proteinase for at least one hour in a microcentrifuge tube, and then spinning at 14,000 rpm to squeeze the body contents into solution. The cuticle was removed before resuming extraction and was later slide mounted as a voucher. All vouchers are deposited at the Yale Peabody Museum of Natural History. The 658 bp portion of the mitochondrial cytochrome oxidase I gene used for DNA barcoding animals was amplified and sequenced using standard protocols (deWaard et al., 2008).

Statistical Analyses

Differences in mean initial larval length, time to pupariation, and puparial duration between eastern (Japanese) versus western *A. tsugae* were tested using unpaired t-tests. Differences in percent survival to pupariation, percent survival to adult, and percent parasitism of *Leucopis* spp. were compared using chi-square tests to compare the equality of proportions between treatments. Total number of *Leucopis* spp. offspring per enclosure verses initial *A. tsugae* ovisac populations were tested using a linear regression. Differences in the total number of *Leucopis* spp. offspring between enclosed densities were analyzed using a one-way analysis of variance. Statistical analyses were performed using R version 3.1.1 and RStudio v2.1 (R Core Team, 2014)

Results

Laboratory Feeding Experiment

Of the 102 *Leucopis* spp. larvae that were collected from the infested foliage and used in the experiment, 53 were reared on eastern *A. tsugae* and 49 were reared on western *A. tsugae*. There were no significant differences in initial larval size, time to pupariation, puparial duration, time to adult, percent survival to adult, or percent parasitism among the two groups reared on different populations of *A. tsugae* (Table 1). All parasitoids emerged during the puparial stage. These flies would have been parasitized during the egg or larval stage in the field.

Branch Enclosure Study

For both years at the TN site, the mean number of *Leucopis* spp. offspring was 9.1 in 2F:2M treatments and 15.2 in 6F:4M/5F:5M treatments. These values were not statistically different ($F = 1.36$; $P = 0.251$). The mean numbers of larvae, puparia, and adult offspring recovered per enclosure were 3.9, 5.0, and 0.15, respectively, for the 2F:2M treatment and 5.2, 8.3, and 0.5, respectively, for the 6F:4M/5F:5M density treatment.

For both years at the NY site, the mean number of *Leucopis* spp. offspring was 4.2 in 2F:2M treatments and 7.7 in 6F:4M/5F:5M treatments. These values were also not statistically different ($F = 1.022$; $P = 0.322$). The mean numbers of larvae, puparia and adult offspring recovered per enclosure were 0.9, 3.2 and 0.1, respectively, for the 2F:2M treatment and 2.2, 5.4 and 0.2, respectively, for the 6F:4M/5F:5M treatment.

At both the TN and NY sites, the number of *Leucopis* spp. offspring were linearly correlated to the number of initial *A. tsugae* ovisacs (R^2=0.22 and R^2=0.54, respectively) (Figure 1).

Both *L. argenticollis* and *L. piniperda* were recovered from the TN site, with only *L. argenticollis* found in 36.8% of the enclosed branches, only *L. piniperda* found in 47.3% and both species found in 15.7% of the enclosed branches. Only *L. argenticollis* was recovered from the NY site (Table 2).

Discussion

The results of these experiments demonstrate that, under both laboratory and field conditions, *Leucopis* spp. from the PNW are capable of feeding and developing on a diet of the Japanese lineage of *A. tsugae* that was introduced to the eastern United States. There were no significant differences in survival or developmental times in the laboratory experiment between *Leucopis* spp. reared on *A. tsugae* from the two different geographic regions. This suggests that *Leucopis* spp. from the PNW would have suitable prey if released in the eastern USA as biological control agents for *A. tsugae*.

Propagule pressure, defined as the number of individuals released and the number of releases, is a key component of establishment success (Lockwood et al., 2015). Because the number of *Leucopis* spp. offspring collected did not differ significantly by the number of adult flies released, we could not draw conclusions about an optimal release density

based on these experiments. This lack of significance could be explained by the relatively small difference in number of individuals between the treatments. Future work should increase the difference between the number of individuals released in each treatment, and increase the number of treatments, to better understand this relationship.

It is important for biological control agents to be able to establish on both high and low densities of their prey (Debach & Rosen, 1991). The linear relationship between *Leucopis* spp. offspring and initial *A. tsugae* populations (Figure 1) indicates that *Leucopis* spp. exhibit this characteristic. *Leucopis* spp. were able to survive and reproduce on the relatively low number of live ovisacs per enclosed branch (an average of 6 ovisacs per branch) found in NY during the 2016 field release.

The difference in species recovered from enclosures between TN and NY suggests that there is a temporal difference in life cycles of *L. argenticollis* and *L. piniperda*. Since both species were recovered at the TN site, but only *L. argenticollis* was recovered at the NY site, *L. piniperda* may complete its development earlier than *L. argenticollis* in the PNW (adults released in TN were collected earlier than the adults released in NY). This difference could be a function of niche partitioning, but more work is needed to understand phenological differences between the species in the PNW.

Because *A. tsugae* has two generations per year in its invaded range, it is critical that biological control efforts address both. Kohler et al. (2016) found that *Leucopis* spp. exhibit peak abundances coinciding with both *A. tsugae* progrediens and sistens egg

stages in the PNW. While this study shows that *Leucopis* spp. can establish and reproduce during the progrediens egg stage in the invaded range of *A. tsugae*, it is yet unknown whether *Leucopis* spp. can survive and reproduce during both generations of *A. tsugae* in the eastern USA. Future work will focus on feeding, reproduction, and survival of *Leucopis* spp. during the different generations of *A. tsugae* in the eastern USA, particularly during *A. tsugae* aestivation and sistens egg stages.

For biological control to be effective, biological control agents must be able to establish and spread under conditions throughout the year and geographical extent of their target's invaded range. Results from the branch enclosure study in the field indicate that environmental conditions at both the northern and southern extremes of the area invaded by *A. tsugae* are within the environmental thresholds of *Leucopis* spp. from the PNW during the late spring and early summer. It remains to be seen whether western *Leucopis* spp. can tolerate environmental conditions throughout the year in the eastern USA. However, the fact that different populations of both species are already present in the eastern USA (McAlpine & Tanasijtshuk, 1972) suggests that the *Leucopis* spp. from the PNW might also be able to tolerate conditions in eastern USA throughout the year.

There is a growing body of evidence that *Leucopis* spp. have a high potential for impacting *A. tsugae* populations in their invaded range. *Leucopis* spp. are the only examples of successful biological control of adelgids worldwide and have been used effectively in Hawaii, New Zealand, and Chile (Rawlings, 1958; Franke-Grossman, 1963; Zúñiga 1985; Culliney et al., 1988; Zondag & Nutall, 1989). A recent publication of data

from the PNW demonstrates that *Leucopis* spp. larvae are more abundant and present for a much longer period of time than *La. nigrinus* larvae in their native ranges (Kohler et al., 2016). The data reported here add to the evidence that *Leucopis* spp. warrant increased and continued study as potential biological control agents of *A. tsugae* in the eastern USA.

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References Cited

DeBach, P. & Rosen, D. (1991) *Biological Control by Natural Enemies* (2nd Edition). New York, NY, USA: Cambridge University Press.

Cheah, C.A. (2011) *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae*, pp. 43-52. *In* B. Onken and R. Reardon (eds.), Implementation and status of biological control of the hemlock woolly adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. FHTET-2011-04.

Culliney, T.W., Beardsley Jr., J.W. & Drea, J.J. (1988) Population regulation of the Eurasian Pine Adelgid (Homoptera: Adelgidae) in Hawaii. *Journal of Economic Entomology* **81**(1), 142–147.

Francke-Grosmann, H. (1963) Einige Beobachtungen über Schäden und Schädlinge in *Pinus-radiata*-Anpflanzungen bei Valparaiso. *Zeitschrift für Angewandte Entomologie* **51**, 335- 345.

Gaimari, S.D. & Turner, W.J. (1996) Methods for rearing aphidophagous *Leucopis* spp. (Diptera: Chamaemyiidae). *Journal of the Kansas Entomological Society* **69**, 363-369.

Grubin, S.M., Ross, D.W. & Wallin, K.F. (2011) Prey suitability and phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) associated with hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology* **4,** 1410-1416.

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

Havill, N.P., Montgomery, M.E. & Keena, M.A. (2011) Hemlock woolly adelgid and its hemlock hosts: a global perspective, pp. 3-14. *In* B. Onken and R. Reardon (eds.), Implementation and status of biological control of the hemlock woolly adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. FHTET-2011-04.

Kohler, G.R., Stiefel, V.L., Wallin, K.F. & Ross, D.W. (2008) Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomolology* **37,** 494-504.

Kohler, G.R., Wallin, K.F. & Ross, D.W. (2016) Seasonal phenology and abundance of *Leucopis argenticollis*, *Leucopis piniperda* (Diptera: Chamaemyiidae), *Laricobius nigrinus* (Coleoptera: Deridontidae) and *Adelges tsugae* (Hemiptera: Adelgidae) in the Pacific Northwest USA. *Bulletin of Entomological Research* **106**(4), 546-550.

L **Lockwood, J. L., Cassey, P. & Blackburn, T.** (2005) The role of propagule pressure in explaining species invasions. *Special Issue: Invasions, Guest Edited by Michael E. Hochberg and Nicholas J. Gotelli* **20**(5), 223–228.

Mausel, D.L., Davis, G.A., Lamb, A.S., Zilahi-Balogh, G.M.G., Kok, L.T. & Salom, S.M. (2011) *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), pp. 77-89. *In* B. Onken and R. Reardon (eds.), Implementation and status of biological control of the hemlock woolly adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. FHTET-2011-04.

Mayfield, A.E., III, Reynolds, B.C., Coots, C.I., Havill, N.P., Brownie, C., Tait, A.R., Hanula, J.L., Joseph, S.V. & Galloway, A.B. (2015). Establishment, hybridization and impact of Laricobius predators on insecticide-treated hemlocks: Exploring integrated management of the hemlock woolly adelgid. Forest Ecology and Management 335: 1-10.

McAlpine, J.F. & Tanasijtshuk, V.N. (1972) Identity of *Leucopis Argenticollis* and description of a new species (Diptera: Chamaemyiid). *The Canadian Entomologist* **104**(12), 1865–1875.

Mooneyham, K.L., Salom, S.M. & Kok, L.T. (2016) Release and colonization of *Laricobius osakensis* (Coleoptera: Derodontidae), a predator of the hemlock woolly adelgid, *Adelges tsugae*. *Northeastern Naturalist* **23,** 141-150.

Onken, B. & Reardon, R. (eds.). (2011) Implementation and status of biological control of the hemlock woolly adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. Publication FHTET-2011-04.

R Core Team (2014) R: A Language and Environment for Statistical Computing. http://www.R-project.org. Foundation for Statistical Computing, Vienna, Austria.

Rawlings, G.B. (1958) Problems of forest entomology in exotic forests in New Zealand. Proceedings, *Tenth International Congress of Entomology* **4**, 241-146.

Ross, D.W., Gaimari, S.D., Kohler, G.R., Wallin, K.F. & Grubin, S.M. (2011) Chamaemyiid predators of the hemlock woolly adelgid from the Pacific Northwest. *In* B. Onken and R. Reardon (eds.), Implementation and status of biological control of the hemlock woolly adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. FHTET-2011-04.

Stoetzel, M.B. (2002) History of the introduction of *Adelges tsugae* based on voucher specimens in the Smithsonian Institute National Collection of Insects, p. 12. *In* B. Onken, R. Reardon, and J. Lashomb, Proceedings: Hemlock Woolly Adelgid in Eastern North America Symposium, 5-7 February 2002, East Brunswick, NJ. USDA Forest Service and State Univ. of N.J. Rutgers, East Brunswick, New Jersey.

deWaard J.R., Ivanova, N.V., Hajibabaei, M. & Hebert, P.D.N. (2008) Assembling DNA barcodes: analytical protocols, pp. 275–293. *In* C.C. Martin (ed.), Environmental Genomics, Methods in Molecular Biology, vol. 410, Humana Press, Totowa, New Jersey.

Zondag, R. & Nuttall, M.J. (1989) *Pineus laevis* (Maskell), pine twig chermes or pine woolly aphid (Homoptera: Adelgidae), pp. 295-297. *In* P.J. Cameraon, R.L. Hill, J. Bain, and W.P. Thomas, (eds.), Review of biological control of invertebrate pests and weeds in New Zealand 1874 to 1987. Technical Communication No. 10. Wallingford, Oxon, United Kingdom, CAB International Institute of biological Control.

Zúñiga, S.E. (1985) Eighty years of biological control in Chile. Historical review and evaluation of the projects undertaken (1903-1983). Agricultura Tecnica **45**, 175-183.

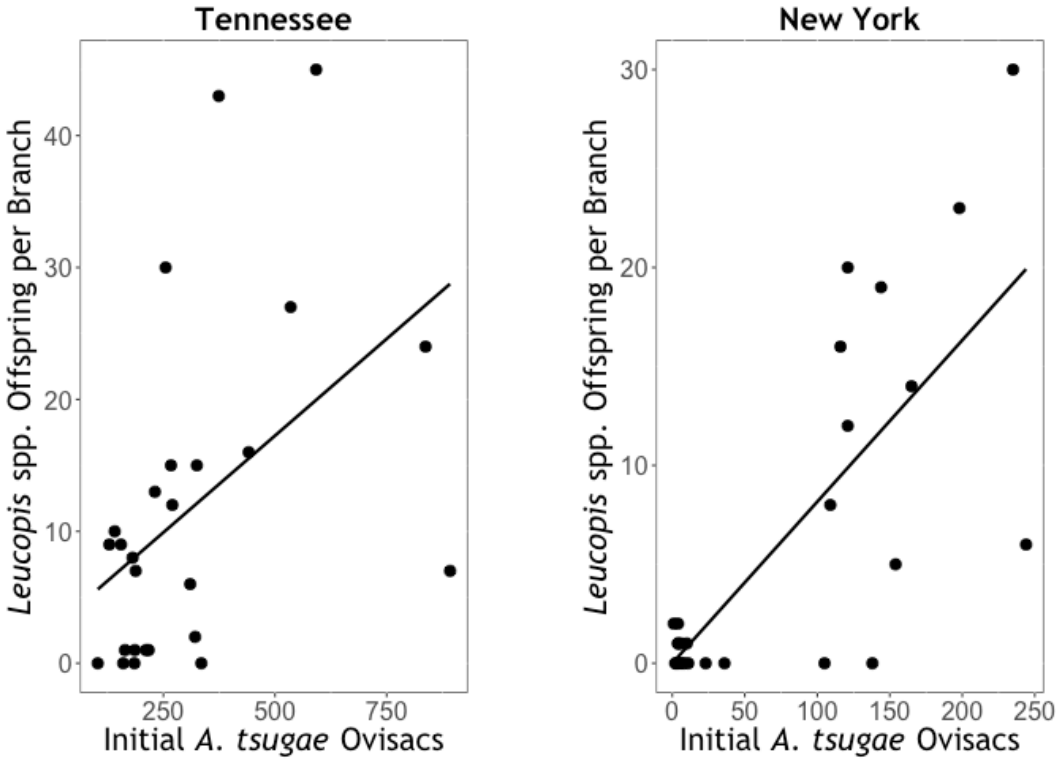

Figure 1: Number of *Leucopis* offspring collected verses the initial *A. tsugae* populations in each enclosure in TN and NY. Data are pooled for 2015 and 2016.

Table 1: Survival parameters for *Leucopis* spp. larvae reared on reared on eastern and western USA populations of *Adelges tsugae* under laboratory conditions

Growth and survival parameters	Larvae reared on eastern USA (Japanese) *A. tsugae* (N=53)	Larvae reared on western USA *A. tsugae* (N=49)	Test statistics and *p*-value		
Initial larval length (mm)	2.37	2.30	t =-0.46	df =1, 90	P =0.652
Time to pupariation (days)	4.48	4.21	t =0.53	df =1, 50	P =0.597
Puparial duration (days)	10.53	9.53	t =1.1	df =1, 29	P =0.279
% Survival to pupariation	50.0	63.0	X^2 =1.59	df =1	P =0.21
% Survival to adult	30.4	37.0	X^2 =0.40	df =1	P =0.51
% Parasitized puparia	2.86	2.24	X^2 =0.03	df =1	P =0.75

Table 2: Number of branches with *Leucopis* spp. offspring from enclosed branch studies in TN and NY in 2015

	L. argenticollis	*L. piniperda*	*Both*
Tennessee	7	9	3
New York	13	0	0

CHAPTER 3: CONCLUSION

The overall objective of this research was to investigate *L. argenticollis* and *L. piniperda* from the western United States as potential biological control agents of *A. tsugae* in the eastern United States. To do this, we evaluated the reproduction and establishment of *Leucopis* spp. in field conditions in the eastern United states using enclosed eastern hemlock branches infested with *A. tsugae*. We found that both species of *Leucopis* can survive, reproduce and establish on *A. tsugae* in field conditions in the eastern United States. In both Tennessee and New York field sites, the number of *Leucopis* spp. offspring recovered was linearly correlated with with initial number of *A. tsugae* ovisacs. Furthermore, we found that *Leucopis* spp. can survive and reproduce on both low and high numbers of initial *A. tsugae* ovisacs. In addition, we found suggestions of a temporal difference in life cycles of *L. argenticollis* and *L. piniperda*, with both species recovered from Tennessee but only *L. argenticollis* recovered from New York.

In summation, *Leucopis* spp. will continue as a focus for biological control of *A. tsugae* in eastern North America. One limitation of this study was the inability to sample at several points over the course of a year. To effectively control *A tsugae* in the eastern United States, *Leucopis* spp. will need to survive, search for and prey on *A. tsugae* throughout the year. Future research will use whole-tree enclosures so that branches can be collected at regular intervals to assess *Leucopis* spp. annual life cycle. Another limitation of this study was the lack of temperature and climate data from branch enclosure field sites in the eastern United States and branch collection sites in the Pacific Northwest. Future studies should use temperature data loggers and available climate data to compare *Leucopis* spp. development with environmental conditions. This study

showed that there was no significant difference in the number of *Leucopis* spp. offspring recovered between the two release densities, but propagule pressure is considered the most important factor in establishment (Lockwood et al. 2015). Future studies might enlarge the difference in the number of individuals released between densities to better understand an ideal release density.

In addition, further research into the life cycle and biology of *Leucopis* spp. is needed to better understand developmental thresholds and any temporal or behavioral stratification between the two species. In this study, branches selected for treatments where all at ground level. Future studies might select branches over the entire tree height to test whether *L. argenticollis* and *L. piniperda* separate across this micro-habitat. Future use of temperature data loggers might also provide data to explain the difference in species recovered between New York and Tennessee. Additionally, future work into peak activity times between these two species might also give a clue into how *L. argenticollis* and *L. piniperda* differ in behavior.

COMPREHENSIVE BIBLIOGRAPHY

Asaro, C., Berisford, M. E., Montgomery, M. E., Rhea, J., & Hanula, J. (2005). Biological control of the hemlock woolly adelgid in the southern Appalachians. *Third Symposium on Hemlock Woolly Adelgid in the Eastern United States*, *FHTET-2005-01*, 248–251.

Aukema, J. E., Leung, B., Kovacs, K., Chivers, C., Britton, K. O., Englin, J., … Von Holle, B. (2011). Economic Impacts of Non-Native Forest Insects in the Continental United States. *PLOS ONE*, *6*(9), e24587. https://doi.org/10.1371/journal.pone.0024587

Bentz, S. ., Riedel, L., Pooler, M. R., & Townsend, A. M. (2002). Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (Tsuga) species. *Journal of Arboriculture*, *28*, 200–205.

Bentz, S. E., Griesbach, R. J., Pooler, M. R., & Townsend, A. M. (2007). Tsuga chinensis as a source of host resistance to the Hemlock Woolly Adelgid. Retrieved from http://www.treesearch.fs.fed.us/pubs/12453

Brantley, S., Ford, C. R., & Vose, J. M. (2013). Future species composition will affect forest water use after loss of eastern hemlock from southern Appalachian forests. *Ecological Applications*, *23*(4), 777–790.

Burns, R. M., & Honkala, B. H. (1990). *Silvics of North America* (Vol. 1). USDA Forest Service.

Butin, E. E., Havill, N. P., Elkinton, J. S., & Montgomery, M. E. (2004). Feeding preference of three lady beetle predators of the hemlock woolly adelgid (Homoptera: Adelgidae). *Journal of Economic Entomology*, *97*(5), 1635–1641.

Butin, E., Elkinton, J., Havill, N., & Montgomery, M. (2003). Comparison of numerical response and predation effects of two coccinellid species on hemlock woolly adelgid (Homoptera: Adelgidae). *Journal of Economic Entomology*, *96*(3), 763–767.

Cheah, C. A. S., & McClure, M. S. (1998). Life history and development of Pseudoscymnus tsugae (Coleoptera: Coccinellidae), a new predator of the hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology*, *27*(6), 1531–1536.

Cheah, C. A. S.-J. (2011). Sasajiscymnus (=Pseudoscymnus) tsugae. *Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. US Department of Agriculture, Forest Service, Morgantown, WV.*, *FHTET-2011-044*, 43–52.

Cheah, C. A. S.-J. (2017). Predicting Hemlock Woolly Adelgid Winter Mortality in Connecticut Forests by Climate Divisions. *Northeastern Naturalist*, *24*(sp7), B90–B118. https://doi.org/10.1656/045.024.s713

Cheah, C. A. S.-J., Mayer, M. A., Palmer, D., Scudder, T., & Chianese, R. (2005). Assessments of biological control of hemlock woolly adelgid with Sasajiscymnus tsugae in Connecticut and New Jersey. *Third Symposium on Hemlock Woolly Adelgid in the Eastern United States*, *FHTET-2005-01*, 116–130.

Cheah, C. A. S.-J., & McClure, M. S. (1996). Exotic natural enemies of Adelges tsugae and their prospect for biological control. *Proceedings: First Hemlock Woolly Adelgid Review*, *FHTET-96-10*, 103–112.

Cheah, C. A. S.-J., & McClure, M. S. (2000). Seasonal synchrony of life stages between the exotic predator, Pseudoscymnus tsugae (Coleoptera: Coccinellidae) and its prey the hemlock woolly adelgid, Adelges tsugae (Homoptera: Adelgidae). *Agricultural and Forest Entomology*, *2*, 241–251.

Cheah, C., Reardon, R. C., & Onken, B. (2004). *Biological control of hemlock woolly adelgid*. US Department of Agriculture, Forest Service, FHTET. Retrieved from http://www.researchgate.net/profile/Carole_Cheah/publication/242112534_BIOLOGICAL_CONTROL_OF_HEMLOCK_WOOLLY_ADELGID_Forest_Health_Technology_Enterprise_Team/links/54217b640cf274a67fea8e38.pdf

Costa, S. D., Parker, B. L., Gouli, V., Brownbridge, M., Skinner, M., & Gouli, S. (2005). Insect-killing fungi as a component of hemlock woolly adelgid inegrated pest management. *Third Symposium on Hemlock Woolly Adelgid in the Eastern United States*, *FHTET-2005-01*, 155–160.

Cowles, R. S., Montgomery, M. E., & Cheah, C. A. S.-J. (2006). Activity and Residues of Imidacloprid Applied to Soil and Tree Trunks to Control Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in Forests. *Journal of Economic Entomology*, *99*(4), 1258–1267. https://doi.org/10.1603/0022-0493-99.4.1258

Culliney, T. W., Beardsley Jr., J. W., & Drea, J. J. (1988). Population regulation of the Eurasian Pine Adelgid (Homoptera: Adelgidae) in Hawaii. *Journal of Economic Entomology*, *81*(1), 142–147.

Dahlsten, D. L., & Mills, N. J. (1999). Biological control of forest insects. *Handbook of Biological Control*, 761–788.

DeBach, P., & Rosen, D. (1991). *Biological Control by Natural Enemies* (2nd Edition). New York, NY, USA: Cambridge University Press.

deWaard, J. R., Ivanova, N. V., Hajibabaei, M., & Hebert, P. D. N. (2008). Assembling DNA barcodes: analytical protocols. *In C.C. Martin (Ed.), Environmental Genomics, Methods in Molecular Biology*, *410*.

Domec, J.-C., Rivera, L. N., King, J. S., Peszlen, I., Hain, F., Smith, B., & Frampton, J. (2013). Hemlock woolly adelgid (*Adelges tsugae*) infestation affects water and carbon relations of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). *New Phytologist*, *199*(2), 452–463. https://doi.org/10.1111/nph.12263

Eisenback, B. M., Salom, S. M., Kok, L. T., & Lagalante, A. F. (2010). Lethal and sublethal effects of imidacloprid on hemlock woolly adelgid (Hemiptera: Adelgidae) and two introduced predator species. *Journal of Economic Entomology*, *103*, 1222–1234.

Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., … Webster, J. R. (2005). Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment*, *3*(9), 479–486. https://doi.org/10.1890/1540-9295(2005)003[0479:LOFSCF]2.0.CO;2

Ellison, A. M., Barker-Plotkin, A. A., Foster, D. R., & Orwig, D. A. (2010). Experimentally testing the role of foundation species in forests: the Harvard Forest Hemlock Removal Experiment: Foundation species experiments. *Methods in Ecology and Evolution*, *1*(2), 168–179. https://doi.org/10.1111/j.2041-210X.2010.00025.x

Eschtruth, A. K., Cleavitt, N. L., Battles, J. J., Evans, R. A., & Fahey, T. J. (2006). Vegetation dynamics in declining eastern hemlock stands: 9 years of forest response to hemlock woolly adelgid infestation. *Canadian Journal of Forest Research*, *36*(6), 1435–1450. https://doi.org/10.1139/x06-050

Evans, A. M., & Gregoire, T. G. (2006). A geographically variable model of hemlock woolly adelgid spread. *Biological Invasions*, *8*, 1–14.

Farjon, A. (2013). *Tsuga canadensis* (The IUCN Red List of Threatened Species 2013). International Union for Conservation of Nature. Retrieved from http://www.iucnredlist.org/details/42431/0

Francke-Grosmann, H. (1963). Einige Beobachtungen über Schäden und Schädlinge in Pinus-radiata-Anpflanzungen bei Valparaiso. *Zeitschrift Für Angewandte Entomologie*, *51*, 335–345.

Gaimari, S. D., & Turner, W. J. (1996). Larval feeding and development of Leucopis ninae Tanasijtshuk and two populations of Leucopis gaimarii Tanasijtshuk (Diptera: Chamaemyiidae) on Russian wheat aphid, Diuraphus noxia (Mordviko) (Homoptera: Aphididae), in Washington. *Proc. Entomol. Soc. Wash.*, *98*, 667–676.

Greathead, D. J. (1995). The Leucopis spp. (Diptera: Chamaemyiidae) introduced for biological control of Pineus sp. (Homoptera: Adelgidae) in Hawaii: implications for biological control of Pineus ?boerneri in Africa. *Entomologist*, *114*(2), 83–90.

Grubin, S. M., Ross, D. W., & Wallin, K. F. (2011). Prey Suitability and Phenology of Leucopis spp. (Diptera: Chamaemyiidae) Associated With Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology*, *40*(6), 1410–1416. https://doi.org/10.1603/EN11127

Hagen, K. S., Mills, N. J., Gordh, G., & McMurty, J. A. (1999). Terrestrial arthropod predators of insect and mite pests. *Handbook of Biological Control*, 383–503.

Havill, N. P., & Montgomery, M. E. (2008). The role of arboreta in studying the evolution of host resistance to the hemlock woolly adelgid. *Arnoldia*, *65*, 2–9.

Havill, N. P., Montgomery, M. E., & Keena, M. A. (2011). Hemlock woolly adelgid and its hemlock hosts: a global perspective. *In B. Onken and R. Reardon (Eds.), Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. US Department of Agriculture, Forest Service, Morgantown, WV.*, *FHTET-2011-04*, 3–14.

Havill, N. P., Montgomery, M. E., Yu, G., Shiyake, S., & Caccone, A. (2006). Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America*, *99*(2), 195–203.

Havill, N. P., Shiyake, S., Lamb Galloway, A., Foottit, R. G., Yu, G., Paradis, A., … Caccone, A. (2016). Ancient and modern colonization of North America by hemlock woolly adelgid, Adelges tsugae (Hemiptera: Adelgidae), an invasive insect from East Asia. *Molecular Ecology*, *25*(9), 2065–2080. https://doi.org/10.1111/mec.13589

Havill, N. P., Vieira, L. C., & Salom, S. M. (2014). Biology and control of hemlock woolly adelgid. *USDA Forest Service, FHTET*, *5*. Retrieved from http://www.dcnr.pa.gov/cs/groups/public/documents/document/dcnr_20029649.pdf

Holmes, T. P., Murphy, E., & Royle, D. D. (2005). The Economic Impacts of Hemlock Woolly Adelgid on Residential Landscape Values: Sparta, New Jersey Case Study. *Third Symposium on Hemlock Woolly Adelgid in the Eastern United States*. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.65.638&rep=rep1&type=pdf

Kohler, G. R., Stiefel, V. L., Wallin, K. F., & Ross, D. W. (2008). Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology*, *37*, 494–504.

Kohler, G. R., Wallin, K. F., & Ross, D. W. (2016). Seasonal phenology and abundance of Leucopis argenticollis, Leucopis piniperda (Diptera: Chamaemyiidae), Laricobius

nigrinus (Coleoptera: Derodontidae) and Adelges tsugae (Hemiptera: Adelgidae) in the Pacific Northwest USA. *Bulliten of Entomological Research*, *106*(4), 546–550.
Levine, J. M., & D'Antonio, C. M. (2003). Forecasting Biological Invasions with Increasing International Trade. *Conservation Biology*, *17*(1), 322–326.
Li, X., Preisser, E. L., Boyle, K. J., Holmes, T. P., Liebhold, A., & Orwig, D. (2014). Potential social and economic impacts of the hemlock woolly adelgid in southern New England. *Southeastern Naturalist*, *13*(6), 130–146.
Lockwood, J. L., Cassey, P., & Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. *Special Issue: Invasions, Guest Edited by Michael E. Hochberg and Nicholas J. Gotelli*, *20*(5), 223–228. https://doi.org/10.1016/j.tree.2005.02.004
Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2013). *Invasion Ecology* (2nd ed.). West Sussex, UK: Blackwell Publishing.
Mausel, D. L., Davis, G. A., Lamb, A. S., Zilahi-Balogh, G. M. G., Kok, L. T., & Salom, S. M. (2011). Laricobius nigrinus Fender (Coleoptera: Derodontidae). *In B. Onken and R. Reardon (Eds.), Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. FHTET-2011-04.*, 77–89.
Mausel, D. L., Salom, S. M., & Kok, L. T. (2011). Visual ability and searching behavior of adult Laricobius nigrinus, a hemlock woolly adelgid predator. *Journal of Insect Science*, *11*(1), 111.
Mayfield, A. E., Reynolds, B. C., Coots, C. I., Havill, N. P., Brownie, C., Tait, A. R., … Galloway, A. B. (2015). Establishment, hybridization and impact of Laricobius predators on insecticide-treated hemlocks: Exploring integrated management of the hemlock woolly adelgid. *Forest Ecology and Management*, *335*, 1–10. https://doi.org/10.1016/j.foreco.2014.09.021
McAlpine, J. F. (1971). A revision of the subgenus Neoleucopis. *The Canadian Entomologist*, *103*, 1851–1874.
McAlpine, J. F., & Tanasijtshuk, V. N. (1972). Identity of Leucopis Argenticollis and description of a new species (Diptera: Chamaemyiid). *The Canadian Entomologist*, *104*(12), 1865–1875. https://doi.org/10.4039/Ent1041865-12
McClure, M. S. (1987). *Biology and control of hemlock woolly adelgid* (Vol. 851). Connecticut Agricultural Experiment Station New Haven, CT. Retrieved from http://www.na.fs.fed.us/fhp/hwa/pdfs/mcclure_hwa.pdf
McClure, M. S. (1989). Evidence of a polymorphic life cycle in the hemlock woolly adelgid Adelges tsugae (Homoptera: Adelgidae). *Annals of the Entomological Society of America*, *82*(1), 50–54.
McClure, M. S. (1990). Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology*, *19*(1), 36–43.
McClure, M. S. (1991). Pesticides will protect ornamentals from hemlock woolly adelgid. *Fronteirs of Plant Science*, *44*(1), 2–3.
McClure, M. S., Salom, S. M., & Shields, K. S. (2001). Hemlock Woolly Adelgid. *USDA Forest Service, FHTET*, (3).
McLean, I. F. . (1992). Behaviour of larval and adult Leucopis (Diptera: Chamaemyiidae). *British Journal of Entomology and Natural History*, *5*, 35–36.

Mills, N. J. (1990). Biological control of forest pests in Africa. *Bulliten of Entomological Research*, *80*, 31–36.
Mitchell, R. G., & Wright, K. H. (1967). Foreign predator introductions for control of the balsam woolly aphid in the Pacific Northwest. *Journal of Economic Entomology*, *60*, 140–147.
Montgomery, M. E., Bentz, S. ., & Olsen, R. T. (2009). Evaluation of hemlock (Tsuga) species and hybrids for resistance to Adelges tsugae (Hemiptera: Adelgidae) using artificial infestation. *Journal of Economic Entomology*, *102*, 1247–1254.
Montgomery, M. E., & Lyon, S. M. (1995). Natural enemies of adelgids in North America: their prospect for biological control of Adelges tsugae (Homoptera: Adelgidae). Retrieved from http://www.treesearch.fs.fed.us/pubs/35505
Montgomery, M. E., & Lyon, S. M. (1996). Natural enemies of adlgids in North America: their prospect for biological control of Adelges tsugae (Homoptera: Adelgidae). *Proceedings: First Hemlock Woolly Adelgid Review*, *FHTET-96-10*, 89–102.
Montgomery, M. E., Salom, S. M., Kok, L. T., Mausel, D. L., Lamb, A. B., & Elkinton, J. S. (2009). Hemlock Woolly Adelgid. *Biological Control of Pests in Forests of Eastern United States: UMass Amherst*.
Montgomery, M. E., Wang, H., Yao, D., Lu, W., Havill, N., Li, G., & others. (2002). Biology of Scymnus ningshanensis (Coleoptera: Coccinellidae): a predator of Adelges tsugae (Homoptera: Adelgidae). In *Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium* (pp. 181–188). Retrieved from http://na.fs.fed.us/fhp/hwa/pubs/proceedings/2002_proceedings/biology.pdf
Mooneyham, K. L., Salom, S. M., & Kok, L. T. (2016). Release and colonization of Laricobius osakensis (Coleoptera: Derodontidae) a predator of hemlock woolly adelgid, Adelges tsugae. *Northeastern Naturalist*, *23*, 141–150.
Onken, B., & Reardon, R. (2011). Implementation and status of biological control of the hemlock woolly adelgid. *US Department of Agriculture, Forest Service, Morgantown, WV. Publication FHTET-2011-04*.
Orwig, D. A., & Foster, D. R. (1998a). Ecosystem Response to an Imported Pathogen: The Hemlock Woolly Adelgid. *Arnoldia*, *58*(2), 41–44. https://doi.org/10.2307/42954554
Orwig, D. A., & Foster, D. R. (1998b). Forest Response to the Introduced Hemlock Woolly Adelgid in Southern New England, USA. *Journal of the Torrey Botanical Society*, *125*(1), 60. https://doi.org/10.2307/2997232
Quimby, H. W. (1996). Value and importance of hemlock ecosystems in eastern United States. *First Hemlock Woolly Adelgid Review*, 1–8.
R Core Team. (2014). *R: A Language and Environment for Statistical Computing. www.R-project.com*. Foundation for Statistical Computing, Vienna, Austria.
Rawlings, G. B. (1958). Problems of forest entomology in exotic forests in New Zealand. *Proceedings: Tenth International Congress of Entomology*, *4*, 141–146.
Reid, W. R., Parker, B. L., Gouli, S., Skinner, M., Gouli, V., & Teillon, H. B. (2010). Fungi associated with the hemlock woolly adelgid, Adelges tsugae, and assessment of entomopathogenic isolates for management. *Journal of Insect Science*, *10*, 1–18.
Ross, D. W., Gaimari, S. D., Kohler, G. R., Wallin, K. F., & Grubin, S. M. (2011). Chamaemyiid predators of the hemlock woolly adelgid from the Pacific Northwest. *In B. Onken and R. Reardon (Eds.), Implementation and Status of Biological Control of the*

Hemlock Woolly Adelgid. US Department of Agriculture, Forest Service, Morgantown, WV. FHTET-2011-04.

Salom, S. M., Sharov, A. A., Mays, W. T., & Gray, D. R. (2002). Influence of temperature on development of hemlock woolly adelgid. *Journal of Entomological Science*, *37*, 166–176.

Sasaji, H., & McClure, M. S. (1997). Description and distribution of Pseudoscymnus tsugae sp. nov.(Coleoptera: Coccinellidae), an important predator of hemlock woolly adelgid in Japan. *Annals of the Entomological Society of America*, *90*(5), 563–568.

Schooley, H. O., Harris, J. W. E., & Pendrel, B. (1984). Adelges piceae (Ratz.), balsam woolly adelgid (Homoptera: Adelgidae). *Biological Control Programmes against Insects and Weeds in Canada 1969-1980*, 229–234.

Shields, K. S., & Cheah, C. A. S.-J. (2005). Winter mortality in Adelges tsugae populations in 2003 and 2004. *Third Symposium on Hemlock Woolly Adelgid in the Eastern United States*, 354–356.

Silcox, C. A. (2002). Using imidacloprid to control hemlock woolly adelgid. *Proceedings: Hemlock Woolly Adelgid in Eastern North America Symposium*, 280–287.

Skinner, M., Parker, B. L., Gouli, S., & Ashikaga, T. (2003). Regional responses of hemlock woolly adelgid (Homoptera: Adelgidae) to low temperatures. *Environmental Entomology*, *32*(3), 523–528.

Stoetzel, M. B. (2002). History of the introduction of Adelges tsugae based on voucher specimens in the Smithsonian Institute National Collection of Insects. In *Proceedings: hemlock woolly adelgid in the eastern United States symposium*. East Brunswick, NJ: Rutgers University.

Tingley, M. W., Orwig, D. A., Field, R., & Motzkin, G. (2002). Avian response to removal of a forest dominant: consequences of hemlock woolly adelgid infestations. *Journal of Biogeography*, *29*(10–11), 1505–1516.

Wallace, M. S., & Hain, F. P. (2000). Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. *Environmental Entomology*, *29*(3), 638–644.

Wallin, K. F., Latty, T. M., & Ross, D. W. (2011). Orientation Behavior of the Predator *Laricobius nigrinus* (Coleoptera: Derodontidae) to Hemlock Woolly Adelgid and Host Tree Odors in a Multi-Chambered Olfactometer. *Environmental Entomology*, *40*(4), 864–872. https://doi.org/10.1603/EN10287

Woodsen, M. M. (2001). Forest invaders: Insects that invade hemlock trees. *American Forests*, *4*, 12–13.

Young, R. F., Shields, K. S., & Berlyn, G. P. (1995). Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America*, *88*(6), 827–835.

Yu, G., Montgomery, M. E., & Yao, D. (2000). Lady Beetles (Coleoptera: Coccinellidae) from Chinese Hemlocks Infested with the Hemlock Woolly Adelgid, Adelges tsugae Annand (Homoptera: Adelgidae). *The Coleopterists Bulletin*, *54*(2), 154–199. https://doi.org/10.2307/4009532

Zilahi-Balogh, G. M. G., Humble, L. M., Lamb, A. B., Salom, S. M., & Kok, L. T. (2003). Seasonal abundance and synchrony between Laricobius nigrinus (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). *The Canadian Entomologist*, *135*(01), 103–115.

Zilahi-Balogh, G. M. G., Kok, L. T., & Salom, S. M. (2002). Host specificity of Laricobius nigrinus Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, Adelges tsugae Annand (Homoptera: Adelgidae). *Biological Control*, *24*(2), 192–198.

Zilahi-Balogh, G. M. G., Kok, L. T., Salom, S. M., Onken, B., Reardon, R., & Lashomb, J. (2002). A review of world-wide biological control efforts for the family Adelgidae. In *Proceedings, Symposium on Hemlock Woolly Adelgid in the Eastern United States* (pp. 129–140). Citeseer. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.365.4486&rep=rep1&type=pdf

Zilahi-Balogh, G. M. G., Loke, K. T., & Salom, S. M. (2002). A review of world-wide biological control efforts for the family Adelgidae. *Proceedings: Hemlock Woolly Adelgid in Eastern North America Symposium*, 129–140.

Zondag, R., & Nuttall, M. J. (1989). Pineus laevis (Maskell), pine twig chermes or pine woolly aphid (Homoptera: Adelgidae). *In P.J. Cameraon, R.L. Hill, J. Bain, and W.P. Thomas, (Eds.), Review of Biological Control of Invertebrate Pests and Weeds in New Zealand 1874 to 1987. Technical Communication No. 10. Wallingford, Oxon, United Kingdom, CAB International Institute of Biological Control.*, 295–297.

Zúñiga, S. E. (1985). Eighty years of biological control in Chile. Historical review and evaluation of the projects undertaken. *Agricultura Tecnica*, *45*, 175–183.