5-2013

# Assessment of Factors Affecting Establishment of Biological Control Agents of Hemlock Woolly Adelgid on Eastern Hemlock in the Great Smoky Mountains National Park

Abdul Hakeem
ahakeem@utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Abdul Hakeem entitled "Assessment of Factors Affecting Establishment of Biological Control Agents of Hemlock Woolly Adelgid on Eastern Hemlock in the Great Smoky Mountains National Park." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plants, Soils, and Insects.

Jerome F. Grant, Major Professor

We have read this dissertation and recommend its acceptance:

Paris Lambdin, David Buckley, Frank Hale

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

# Assessment of Factors Affecting Establishment of Biological Control Agents of Hemlock Woolly Adelgid on Eastern Hemlock in the Great Smoky Mountains National Park

**A Dissertation Presented for the**

**Doctor of Philosophy**

**Degree**

**The University of Tennessee, Knoxville**

**Abdul Hakeem**

**May 2013**

# DEDICATION

**This dissertation is dedicated to my parents**

**for their love, endless support**

**and encouragement**.

# ACKNOWLEDGEMENTS

To all my friends, thank you for your understanding and encouragement. I am unable to list all the names here, but you are always in my mind and prayers.

This dissertation could not have been finished without the help, support and guidance from many professors, research staff, graduate students, colleagues and my family. It is my pleasure to acknowledge those who have had a significant impact on my achievements of this goal.

First of all, I would like to thank my major advisor, Dr. Jerome Grant, for his invaluable support, patience and guidance throughout this project and beyond. His positive and welcome attitude helped me to meet this goal. I extend my heartiest thanks to my committee members, Dr. Paris Lambdin, Dr. Frank Hale, Dr. David Buckley and Rusty Rhea, for their input, valuable suggestions and guidance. I am thankful to Dr. Greg Wiggins, for his help, guidance, positive criticism and readiness to listen and make efforts to address the issues. I am thankful to Ashley Lamb, Dave Paulsen, Elizabeth Benton, Josh Grant, Monica MacCarroll, Renee Follum, Rudi Hendricks and Scott Sherry for helping in the field. I would also like to thank Becky Nichols, Glenn Taylor, Jesse Webster, Jim Renfro, Kristine Johnson and Tom Colson (Great Smoky Mountains National Park), James Vogt (JT) (Forest Inventory Analysis), Willium Hargrove and Bill Christie (USDA Forest Service) for providing data and professional advice. I am thankful to Ann Reed for verifying statistical analysis. I would like to thank my parents, family members, my wife, Muke, my son, Aetizaz, and my daughters, Anumta and Sameeha, for their good behavior and support. Funding for this research was provided by USDA Forest Service, Southern Region and USDA APHIS.

## ABSTRACT

Eastern hemlock in the Great Smoky Mountains National Park (GRSM) is threatened by hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). To manage this invasive pest in GRSM, ca. 550,000 *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) and 7,857 *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) have been released. Limited information is available on their establishment in GRSM. To fill this gap, a study was initiated to assess establishment of these predators and their impact on hemlock health. To determine factors affecting establishment of these predators, 65 release sites were sampled from 2008 to 2012. Several factors were evaluated for their association with establishment and recovery of *S. tsugae*. Predatory beetle release information, topographic features, and temperature data were obtained from GRSM personnel. These factors were evaluated using stepwise logistic regression and Pearson correlation. High resolution digital imagery was used in conjunction with field-conducted tree-health surveys to test association between *S. tsugae* and tree health. *S. tsugae* were recovered from 13 of 65 sites (20%); recovery was significantly associated with older release sites which indicates that *S. tsugae* may require more time to reach readily detectable levels. Regression analysis indicated that establishment was positively associated with the average maximum temperature seven days following release and elevation. Several significant correlations were found between presence of *S. tsugae,* and year of release, season of release, and temperature variables. These results indicate that *S. tsugae* should be monitored for establishment for at least five years following releases to enhance knowledge of establishment. Coexistence of three predatory beetles, *S. tsugae, L. nigrinus* and *L. rubidus,* was observed on the same

hemlock trees. Significant differences between normalized difference vegetation index values and *S. tsugae* presence was observed. For understory and overstory hemlock, percent crown transparency, percent live crown, and percent branch dieback was also significant with presence of *S. tsugae*. Coexistence of *S. tsugae* and *L. nigrinus* on eastern hemlock may provide prolonged feeding on HWA. Predictive models would assist land managers in selecting appropriate times and sites for future releases. Application of digital imagery could be used to evaluate other natural enemies in forest landscapes.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# Introduction and Review of Literature

## Eastern Hemlock

*Tsuga* species (i.e., hemlocks) are found worldwide. Of the 14 known *Tsuga* species, most are found in Asia. Four hemlocks are native to the United States. Mountain hemlock, *Tsuga mertensiana* Carrière, and western hemlock, *T. heterophylla* Sargent, occur in the western United States. Carolina hemlock, *T. caroliniana* Engelmann, is found in North Carolina and South Carolina, and eastern hemlock, *Tsuga canadensis* (L.) Carrière, is found in the eastern United States and adjacent Canadian provinces (Welch and Haddow 1993).

Eastern hemlock is a slow-growing long-lived common conifer found throughout northeastern North America (Brooks 2001, Godman and Lancaster 2003). Eastern hemlock ranges from Nova Scotia to Georgia and westward to Minnesota (Ward et al. 2004) (Fig. 1). In the southern Appalachians, eastern hemlock is mostly found from 610 to 1,520 m (Godman and Lancaster 2003) and restricted to coves and north- and east facing lower slopes (Ward et al. 2004). Great Smoky Mountains National Park (GRSM), which is located in the southern Appalachians, comprises 210,876 ha (NPS 2012); hemlock resources cover ca. 55,442 ha with more than 5,665 ha of hemlock-dominated forests (Yost et al. 1994, Soehn et al. 2005, Webster 2010). In Tennessee, hemlock woolly adelgid is found in 36 of 95 counties (TDA 2013, USDA 2013) (Fig. 2).

Eastern hemlock grows better in moist and well drained soils, but also grows in other soil types, i.e. shallow and bedrock drained soils (Yamasaki et al. 1999). This native species grows in cool climates (-12.2 C$^{o}$ in winter and 16.0 C$^{o}$ in summer) with precipitation ranges from 740 mm to 1,270 mm per year (Godman and Lancaster 2003, Ward et al. 2004.

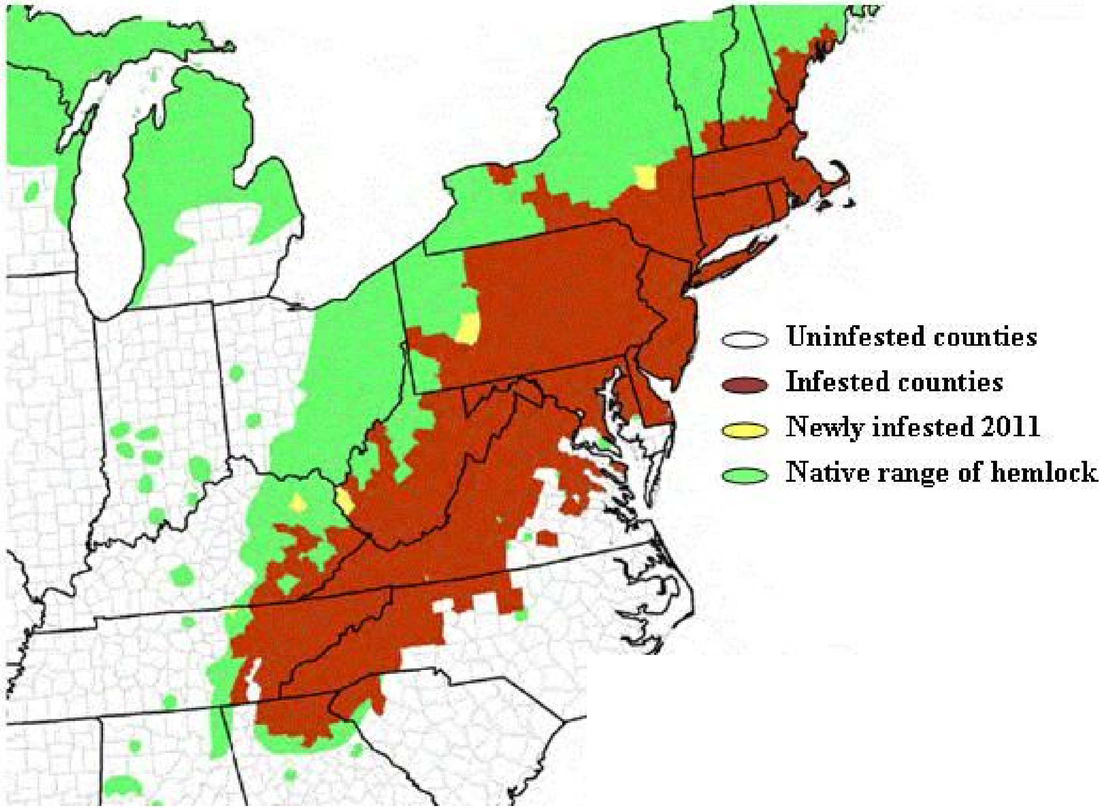


**Figure 1. Distribution of eastern hemlock in North America; counties with established populations of hemlock woolly adelgid, 2011 (Source:http://na.fs.fed.us/fhp/hwa/maps/2011.pdf).**

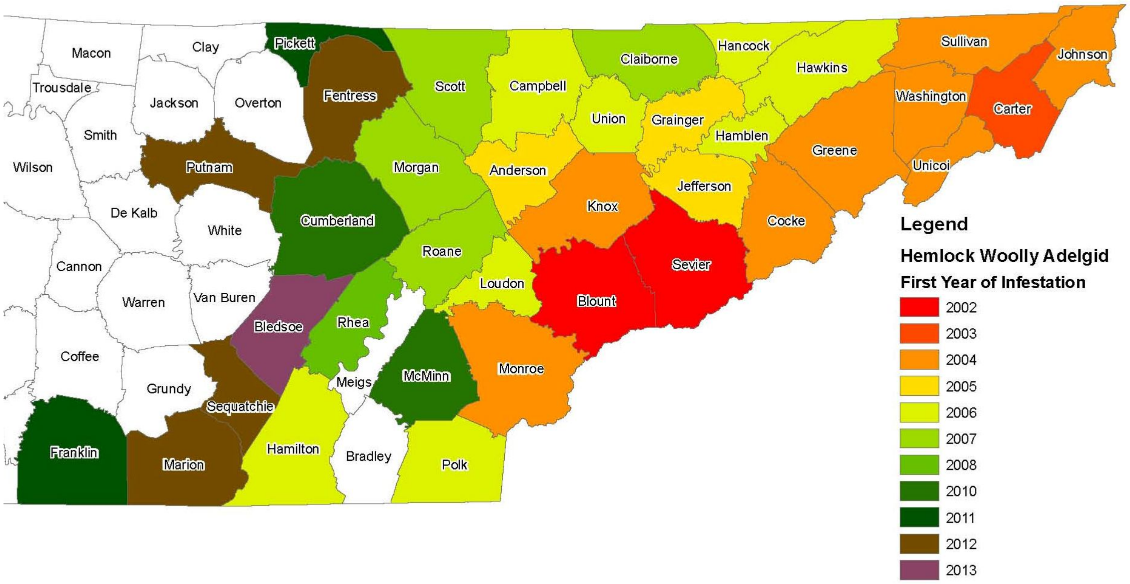


**Figure 2. Distribution of hemlock woolly adelgid in Tennessee (Source: www.tn.gov/agriculture/regulatory/hwa.shtml).**

In North America, eastern hemlock is found in 29 forest cover types (Eyre 1980, Godman and Lancaster 2003). In the northern forest region, white pine-hemlock (Society of American Foresters Type 22), eastern hemlock (Type 23), and hemlock-yellow birch (Type 24) forest cover types are present, while yellow-poplar-eastern hemlock (Type 58) are present in the central forest region. Eastern hemlock is also common in seven forest cover types of the northern forest region: white pine-northern red oak-red maple (Type 20), eastern white pine (Type 21), red spruce-yellow birch (Type 30), red spruce-sugar maple-beech (Type 31), red spruce (Type 32), red spruce-balsam fir (Type 33), and red spruce-fraser fir (Type 34). Eastern hemlock also occurs as a minor species in balsam fir, pine cherry, and paper birch (Godman and Lancaster 2003).

## Uses of Eastern Hemlock

Eastern hemlock is used primarily for lumber and paper pulp. Historically, it has been used as an important source of tannin for the leather industry. Hemlock wood has been used to build light framing, roofing, boxes and crates (Brisbin 1970, McWilliams and Schmidt 2000, Nesom 2002). Eastern hemlock also has been used in the toy industry. American Indians used the cambium as base for breads and soaps or mixed it with dried fruit and animal fat for pemmican (concentrated mixture of fat and protein used as a nutritious food). Native Americans used hemlock leaves to make tea due to its high vitamin C content (Godman and Lancaster 2003).

## Ecological and Aesthetic Value of Eastern Hemlock

Eastern hemlock has a relatively low economic value as a timber, but it has a great ecological value (Snyder et al. 2002, Ward et al. 2004). Eastern hemlock is among the conifers grown as an ornamental tree due to its aesthetic appeal. The hemlock canopy provides a unique dark and cool place (Jordan and Sharp 1967). Dense and evergreen canopy of hemlock forests provides areas for outdoor recreation and a unique habitat for several fauna and flora, such as animals, wildlife, fish, turkey, ruffed grouse, and shade tolerant plants, etc. During winter season, the foliage of hemlock makes it suitable forage and habitat for deer (Lapin 1994). More than 120 vertebrate and many aquatic species, such as brook trout, *Salvelinus fontinalis* (Mitchill), are associated with cool streams sheltered by eastern hemlock (Godman and Lancaster 2003, Nesom 2002). In Connecticut, 90 species of birds use hemlock as food, nesting sites or roosting sites (Lapin 1994). Black-throated green warbler, *Dendroica virens* (Gmelin), blue-headed vireo, *Vireo solitarius* (Wilson), and the northern goshawk, *Accipiter gentilis* (L.), are

hemlock obligates (DeGraaf and ChadWick 1987, Benzinger 1994a, b, c). Absence of riparian vegetation within 25 m of the stream may cause stream temperatures to rise 6 to 9 $C^0$ (Lapin 1994) which may be devastating to cool climate brook trout. Hemlock growing along streams and lakes provide an ideal environment for outdoor recreational habitats (Burnham et al.1947). It also helps to clean water resources by providing cover to soil, which reduces erosion.

**Hemlock Woolly Adelgid in North America**

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), an exotic insect from Japan was first discovered in the eastern United States near Richmond, Virginia, in 1951 (Orwig et al. 2002, Ward et al. 2004). The invasive HWA is a small (ca 1.5 mm long), dark reddish-brown to purplish-black insect with piercing-sucking mouthparts. HWA inserts its stylet at the base of needles and feeds on sap. Feeding disrupts flow of sap which eventually causes death of hemlock trees, if infestations are high and prolonged. Nymphs and wingless adults produce a white woolly secretion or mass, which protects eggs and adelgids from desiccation, natural enemies, pesticides, and external environmental factors, such as low and high temperature, rain, etc. Eggs hatch into first instars (crawlers), which disperse to locate feeding sites where they insert their stylets into the plant at the base of needles and feed on sap. Due to excessive and prolonged feeding of HWA, eastern hemlock populations have rapidly declined in the eastern United States. Among all 14 *Tsuga* hemlock species, eastern and Carolina hemlock are more susceptible to HWA while other hemlock species have either resistance against HWA or sufficient predator populations to suppress HWA and prevent tree mortality (Cheah and McClure 1996, Montgomery and Lyon 1995). In western North

America, HWA does not cause mortality to western hemlock. Phylogenetic analyses revealed that adelgids on eastern hemlock are likely a population of HWA found on Japanese hemlock, *T. sieboldii* Carrière, in southern Japan (Havill 2006).

## Hemlock Woolly Adelgid Lifecycle

All HWA are female and develop parthenogenetically. HWA undergoes two overlaping generations [sistens (overwintering generation) and progrediens (spring generation)] each with six development stages: egg, four nymphal instars and adult (Fig. 3). Sistens produce ca. 300 eggs per adult, while progrediens produce ca. 75 eggs per adult (Cheah et al. 2004).

In the southeastern United States, the HWA lifecycle is slightly different from that found in the northeastern United States. In the southeast, sistens are present all year round except May (Fig 3), and adults begin to lay eggs in February. In March and April, reddish-brown crawlers settle on new growth and mature in about one month. However, in the northeast, sistens are present throughout the year except May and June, and begin to lay eggs in March; progrediens begin to lay eggs in June. Both in the northeast and southeast, sistens aestivate from July to October (Deal 2007, Ward et al. 2004).

## Hemlock Woolly Adelgid Dispersal

Six decades after its accidental introduction into eastern United States, HWA has spread into 19 states and is currently found in more than one-half of the range of eastern hemlock (Fig. 1). HWA expansion has been more rapid (20 to 30 km per year) in the southern than northern range of eastern hemlock (McClure et al. 2001, Ward et al. 2004, Trotter et al. 2008). In GRSM, HWA was first reported in 2002, but it may have been present for several years before it was discovered (Soehn et al. 2005). As of March 2013,

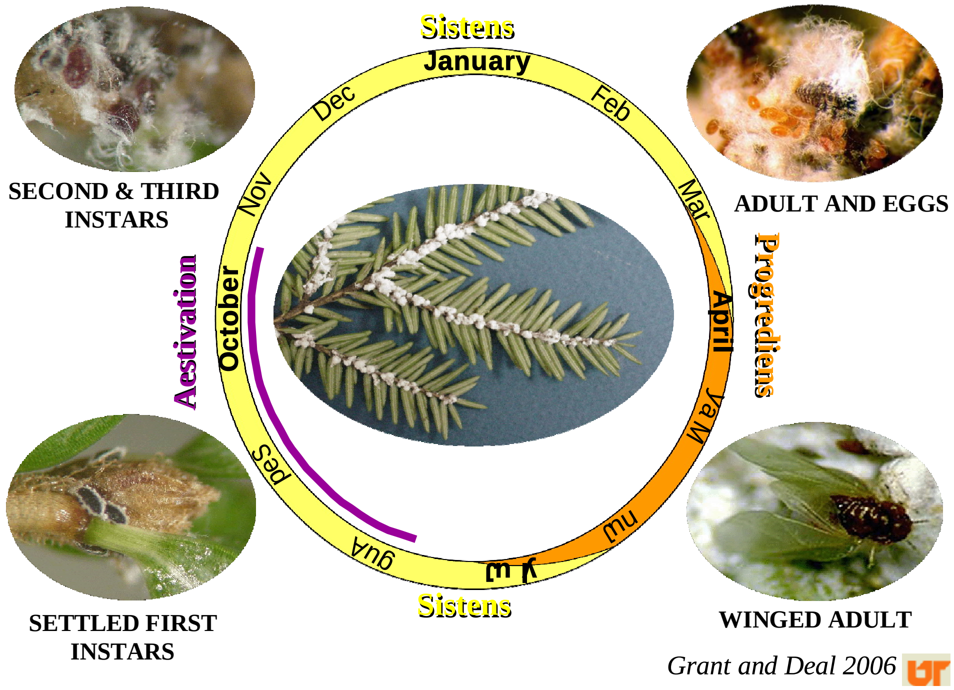


**Figure 3. Hemlock woolly adelgid lifecycle on eastern hemlock in North America (Deal 2007).**

HWA has spread into 36 counties in Tennessee (TDA 2013). HWA is covered in a wool-like material secreted by the developing adelgid, which can adhere to animals (such as birds), or be blown by air currents. HWA eggs and crawlers are spread by wildlife, humans and through movement of infested nursery stock (Ward et al. 2004). High winds also may contribute to rapid and long distance dispersal of this invasive pest into non-infested areas.

## Implications of Eastern Hemlock Decline

Due to hemlock decline, gaps have formed in the forest which may cause Increase in water temperatures. These elevated temperatures may have long-term negative effects on brook trout and other organisms which are associated with eastern hemlock-sheltered streams. Eastern hemlock mortality has been swift and widespread which has led to a decline of eastern hemlock from its native range. At present, HWA has spread to more than 50% of its hemlock range. A landscape that was once dominated with eastern hemlock is being replaced by several plant species, including rhododendron, *Rhododendron maximum* L. Expansion of rhododendron would also be detrimental to hardwood species because it restricts expansion of hardwood recruitment. Due to extensive tree mortality, rhododendron forms a thick and continuous subcanopy known as "laurel slicks" or "laurel hells" (Dighton and Coleman 1992, Baker and Van Lear 1998, Clinton 2004, Roberts et al. 2009).

In 2010, 9.4 million people visited GRSM, which is among the most visited National Parks in the United States. The Park provides ca. 1,288 km backcountry trails, 10 campgrounds totaling 1,000 sites, and 3,403 km of streams. The Park generates income of $718 million per year for surrounding communities (NPS 2013b). Hemlock decline due to HWA will cause tremendous losses in term of revenue and resources. Dead or dying trees pose hazards to safety of hikers, bikers, visitors, employees, and other people who are associated with the Park. Poor and dangerous conditions caused by these dead and leaning trees may have negative impacts on the local economy. Tree removal also will increase economic expenses associated with HWA.

## Management of Hemlock Woolly Adelgid

Since the introduction of HWA in GRSM, trees have died, and HWA has spread quickly from infested areas that make management difficult. Most of the hemlocks in GRSM are located in inaccessible areas which makes it difficult to manage HWA. To reduce populations of HWA in the Park, GRSM personnel have applied management strategies, including chemical, cultural and biological control. Resistance of existing hemlock to HWA may be an important tactic once resistant trees are formed.

### Chemical Control

Imidacloprid, a neonicotinoid, is used against HWA as a soil drench, soil injection, or tree injection (Cowles et al. 2006). Imidacloprid is the primary chemical insecticide used to control HWA in GRSM. Formulations of imidacloprid include Merit® 75 WP, Imicide® and Core Tect®. Merit 75 WP can be used as a soil drench, or a foliar spray, while Imicide® can be used as a trunk injection (Charles 2002). Timing of imidacloprid application is not important for its effectiveness, as both fall and spring treatments provide effective control of HWA (Dilling et al. 2010). Imidacloprid metabolizes into other compounds, such as olefin, which may also provide long-term control of HWA. Safari® (dinotefuran) is also used against HWA, which has a faster uptake than imidacloprid. Safari can be used as soil drench or bark spray (Sidebottom 2009).

Application of any type of chemical to control HWA in forest landscapes is expensive, time consuming and impractical. Chemicals applied on eastern hemlock can leach into groundwater which may contaminate water resources. It can also be devastating for fish and other aquatic inhabitants. On eastern hemlock, significantly

higher densities of predators were recorded in control treatments (i.e., no insecticide application) than those with imidacloprid treatments, which indicate a short-term non-target impact of imidacloprid (Hakeem 2008). However, long-term suppression of predator populations was not expected.

## Cultural Control

Cultural control is limited to small trees, primarily in a newly-infested area with few hemlock trees. On a limited area, branches containing HWA can be trimmed or HWA can be crushed which may reduce spread and further infestation. Cultural control is not feasible in forest landscapes due to inaccessibility, volume of canopy surface area and practical constraints.

## Resistance against Hemlock Woolly Adelgid

Eastern hemlock is susceptible to HWA while Chinese hemlock, *Tsuga chinensis* (Franch.) E. Pritz, Japanese hemlock and western hemlock are resistant to HWA. Breeding to develop a resistant cultivar is in progress. Chinese hemlock has been proven to be resistant against HWA and may be a suitable replacement for eastern hemlock due to its fast growth and shade tolerance (Tredici and Kitajima 2004). Hybrid hemlocks are growing well and may be selected for mass propagation in the future (Montgomery et al. 2009).

## Biological Control

Hemlock forests are widespread which makes it more difficult to control HWA using conventional pest elimination techniques. Biological control is the only method which is feasible, self-perpetuating, long-term, economical and has limited known non-target effects on other organisms. Biological control is “the action of parasites, predators,

and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach 1964). Natural enemies can play key roles in suppressing pest species (Bellows and Fisher 1999). For example, 55 predator species in 14 families are associated with western hemlock (Britannica, 2013, Kohler et al. 2008), 54 species of lady beetles are present on Chinese hemlock (Yu et al. 2000), and 27 predaceous species are associated with Japanese hemlock (Shiyake et al. 2008). However, few predators are associated with eastern hemlock in the eastern United States, and no native predatory beetle species is known to suppress HWA in the eastern United States (Montgomery and Lyon 1995, Wallace and Hain 2000). Since 2002, tens of thousands of hemlock trees have either died or are declining in GRSM (Lambdin et al. 2006, AH personal observation) due to HWA infestation. To address long-term management of HWA in the Park, a classical biological control program, including releases of *S. tsugae* beginning in 2002 and *L. nigrinus* beginning in 2004, was initiated. Since the start of this program, about 550,000 *S. tsugae* and 7,857 *L. nigrinus* have been released in GRSM (Eisenback et al. 2010, Webster 2010).

Infestation of HWA does not cause severe damage on Asian hemlock species, where a complex of predators of HWA is present. However, no specialist predator of HWA is present in the eastern United States. To fill this gap, several predatory beetle species were imported into the eastern United States from other countries. These predatory beetles include *Sasajiscymnus* (*Pseudoscymnus*) *tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), *Scymnus* (*Neopullus*) *camptodromus* Yu and Liu (Coleoptera: Coccinellidae), the conifer lady beetle, *Scymnus (Pullus) coniferarum* Crotch (Coleoptera: Coccinellidae), *Scymnus ningshanensis* Yu and Yao (Coleoptera:

Coccinellidae), and *Scymnus* (*Neopullus*) *sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae). At least 20 *Scymnus* species have been associated with hemlocks in China (Yu et al. 1997, 2000), where *S. ningshanensis* is one of 60 different species of natural enemies of HWA (Butin et al. 2004, Montgomery et al. 2005). Two predatory beetles, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), native to western North America, and *L. osakensis* Montgomery and Shiyake (Coleoptera: Derodontidae), imported from Japan, were also introduced on eastern hemlock against HWA in the eastern United States. Description of each species is provided below.

### ***Sasajiscymnus* (*Pseudoscymnus*) *tsugae***

*Sasajiscymnus* (*Pseudoscymnus*) *tsugae* (Fig. 4), native to Japan, was introduced against HWA in 1994. Adult *S. tsugae* are black with brownish antennae and less than 2 mm long; both larvae and adults feeds on all life stages of HWA. Without adequate magnification, it is difficult to differentiate this predatory beetle from other beetles found on eastern hemlock. Elytra pubescence is fine, silvery-white and arranged backwards which makes it distinctly different from other coccinellids found on eastern hemlock (Cheah et al. 2004). Eggs of *S. tsugae* are oval-shaped, reddish-brown, about 0.48 mm long and 0.25 mm wide. Eggs are laid singly or in small groups. Larvae (1.1 to 2.7 mm) are reddish-brown to gray. The pupa is also reddish-brown and ca. 1.9 mm long and 1.1 mm wide (Cheah 2010).

*Sasajiscymnus tsugae* was the first predator introduced from Japan against HWA in the United States (Cheah et al. 2005) (Fig. 4). Since its initial release, more than 3.5 million *S. tsugae* have been released in the eastern United States. Of these, 550,000 *S. tsugae* have been released at more than 200 sites in GRSM (Eisenback et al. 2005, 2010,

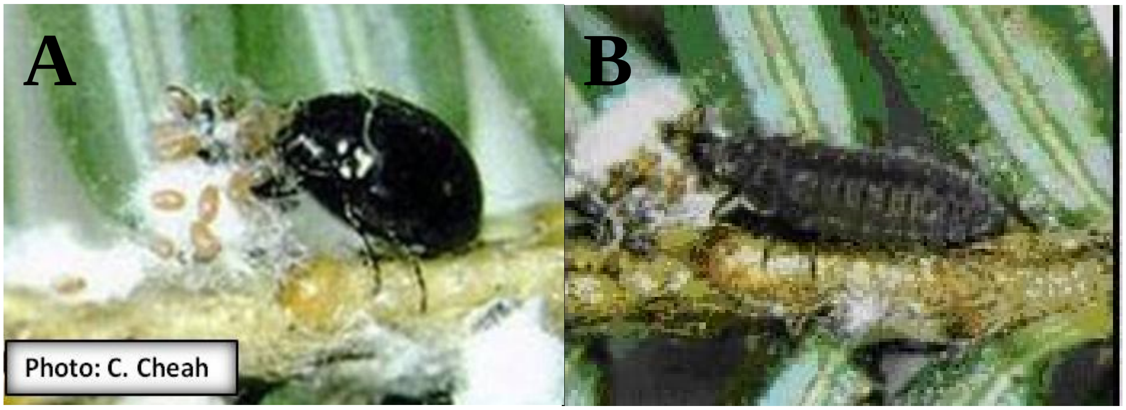


**Figure 4. Adult (A) and (B) larva *Sasajiscymnus tsugae* feeding on hemlock woolly adelgid.**

Grant et al. 2010c, Webster 2010). *S. tsugae* is active throughout the year. In Georgia, augmentative releases of *S. tsugae* have been made (Cera Jones, personal communication) while in GRSM, no augmentative releases have been made. *S. tsugae* has been reared in various rearing facilities, including Clemson University, Connecticut Experimental and Research Station, Lindsay Young Beneficial Insects Laboratory (LYBIL) at the University of Tennessee, North Carolina Department of Agriculture, North Georgia College and State University, and University of Georgia. A new strain of *S. tsugae*, which may provide fresh genetic stock, is now reared at the University of Tennessee (Grant, personal communication).

Recoveries of *S. tsugae* from GRSM is ca. 20% which is encouraging considering the size of the forest system compared to the number of *S. tsugae* released and the reproductive capacity of HWA. *S. tsugae* recoveries are associated with older release sites which indicate that they require more time to increase to detectable levels (Hakeem 2010). In many sites, *S. tsugae* was not recovered during the first sampling visit, but *S.*

*tsugae* was recovered in subsequent sampling. Thus, if *S. tsugae* is not recovered from a site, it should not be considered that these predators are not established. Rather, continued sampling for several years is necessary to adequately assess establishment.

### ***Scymnus camptodromus***

*Scymnus* (*Neopullus*) *camptodromus,* native to China, has a broad geographic range in its native country. Adults are small, ca. 3 mm long, covered with short hairs, and have reddish-brown elytra with black margins (Whitehead 1967, Gordon 1976) (Fig. 5). Eggs of *S. camptodromus* require diapause, which makes this species difficult to rear in the laboratory. In choice tests, *S. camptodromus* fed exclusively on adelgid eggs (Keena 2006). Oviposition begins one month after eclosion, and eggs enter aestivo-hibernal diapause and hatch the following spring (Keena et al. 2012).

### ***Scymnus (Pullus) coniferarum***

The conifer lady beetle is distributed west of the Mississippi River (Fig. 6). A similar species, *S. (Pullus) suturalis* Thunberg, is found in Europe. Both species are associated with pine infested with adelgids. In the laboratory, *S. coniferarum* successfully completed its life cycle on HWA. *S. coniferarum* favored HWA and pine adelgids (Montgomery et al. 2009).

HWA populations significantly as compared to control cages (Cheah et al. 2004). In choice tests, *S. ningshanensis* preferred HWA to blade spruce gall adelgid, *Adelges laricis* Vallot. In China, *S. ningshanensis* is one of 60 different species of natural enemies of HWA (Butin et al. 2004, Montgomery et al. 2005).


**Figure 5. Adult (A) and larva (B) *Scymnus camptodromus.***

**Figure 6. Adult *Scymnus coniferarum* feeding on hemlock woolly adelgid.**

### ***Scymnus ningshanensis***

*Scymnus (Neopullus) ningshanensis*, native to China, is a univoltine species. Adults have a black head and black spots on the hind elytra (Fig. 7). *S. ningshanensis* was evaluated in sleeve cages, and preliminary results indicated that *S. ningshanensis* reduced HWA populations significantly as compared to control cages (Cheah et al. 2004). In choice tests, *S. ningshanensis* preferred HWA to blade spruce gall adelgid, *Adelges laricis* Vallot. In China, *S. ningshanensis* is one of 60 different species of natural enemies of HWA (Butin et al. 2004, Montgomery et al. 2005).

### ***Scymnus sinuanodulus***

*Scymnus* (*Neopullus*) *sinuanodulus* is native to China. Adult beetles are small (1.5 mm) and brown-reddish (Fig. 8). Since 2005, ca. 25,000 *S. sinuanodulus* have been released in 11 eastern states against HWA (Salom et al. 2008). Eggs hatch in 10 days and newly-emerged larvae feed on adelgid eggs and nymphs for three weeks and pupate (Lu and Montgomery 2001, Salom et al. 2008, USDA 2010). In the laboratory, *S. sinuanodulus* completes its development on all stages of HWA but larval development is faster on HWA eggs (Lu and Montgomery 2001).

In Tennessee, *S. sinuanodulus* was released in large field cages (ca. 8 m tall) and recovered one year after initial release; however, they were not recovered in the following years. Non-recovery of *S. sinuanodulus* indicates that this beetle has not established in Tennessee and may not be a good candidate for southern climates (Hakeem et al. 2011).

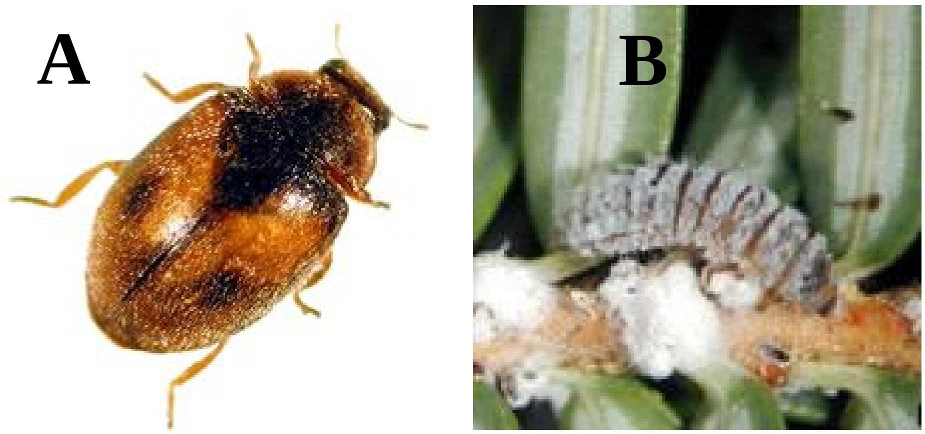


**Figure 7. Adult (A) and larva (B) *Scymnus ningshanensis.***

### *Laricobius nigrinus*

Members of the genus *Laricobius* (Coleoptera: Derodontidae) are exclusive predators of adelgids. Four described species of *Laricobius* are present in North America: *L. erichsonii* Rosenhauer, *L. laticollis* Fall, *L. nigrinus,* and *L. rubidus* LeConte. *L. nigrinus,* native to Pacific Northwest, is a specialist predator of HWA with a narrow host range. Adult *L. nigrinus* are dark black and the elytra have typical fringes (Fig. 9). Both larvae and adults feed on all stages of HWA. Releases of *L. nigrinus* began in 2004 to reduce populations of HWA in the eastern United States. Since 2004, ca. 102,000 *L. nigrinus* have been released in the eastern United States. Of these, ca. 7,857 have been released in the GRSM (DCNR 2007, Eisenback et al. 2010, Lamb et al. 2006, USDA 2012, Webster 2010, Zilahi-Balogh et al. 2006). *L. nigrinus* is a univoltine species and actively feeds on adelgids throughout the fall and winter. *L. nigrinus* oviposits single eggs, pale greenish-yellow, in adelgid ovisacs. Newly-emerged larvae feed on HWA eggs and complete development in four larval instars (Zilahi-Balogh et al. 2002, 2003).

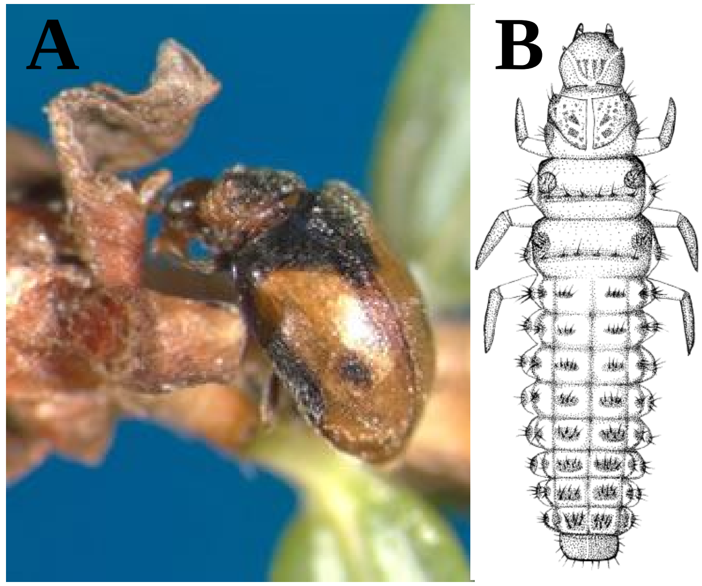


**Figure 8. Adult (A) and larva (B) *Scymnus sinuanodulus*.**

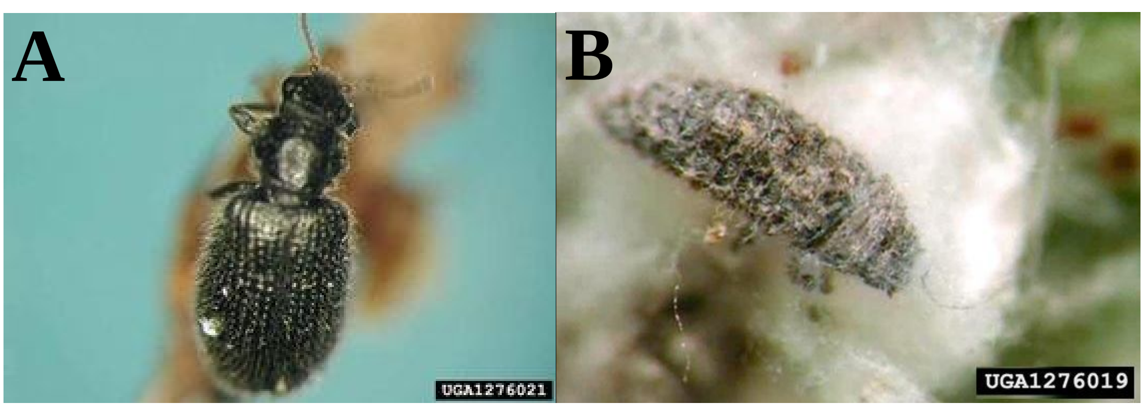


**Figure 9. Adult (A) and larva (B) *Laricobius nigrinus.***

### *Laricobius rubidus*

*Laricobius rubidus*, native to the eastern United States, is a predator of pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae), on white pine, *Pinus strobus* L. (Clark and Brown 1960). *L. rubidus* feeds exclusively on adelgids and can complete development on HWA (Zilahi-Balogh et al. 2006). *L. rubidus* is reported to hybridize with *L. nigrinus* (Havill et al. 2006). Consequences of hybridization are not yet clear. In the southern Appalachians, *L. rubidus* has been recovered from emergence cages and beat sheets. *L. rubidus* also coexisted with two other predatory species, *L. nigrinus* and *S. tsugae*, on the same tree and even on the same beat sheet (Hakeem et al. 2011).

### *Laricobius osakensis*

*Laricobius osakensis* Montgomery and Shiyake (Coleoptera: Derodontidae), a potential predator of HWA was imported from Japan. Host range testing was conducted which indicated that *L. osakensis* is a specific predator of HWA and is not a threat to non-target organisms (Fig. 10) (Vieira et al. 2011). *L. osakensis* is currently reared at LYBIL and Virginia Polytechnic Institute and State University and will soon be released against HWA.

## Factors Affecting Establishment of *S. tsugae* on Eastern Hemlock

In GRSM, *S. tsugae* was recovered from 20% of the release sites sampled (Hakeem et al. 2010, 2011, 2013). These recoveries of *S. tsugae* resulted from one-time releases, and no repeated releases at the site were made. *L. nigrinus* was recovered from 59% of the release sites sampled in the eastern United States, and its establishment was positively associated with minimum winter temperature and number of beetles released


**Figure 10. Adult *Laricobius osakensis.***

(Mausel et al. 2010). No such model exists to predict factors affecting establishment and recovery of *S. tsugae* on eastern hemlock. Several abiotic and biotic factors (such as temperature, time of release and elevation) may influence establishment and recovery of populations of *S. tsugae*. Climatic conditions in its native range are different than in introduced areas, therefore, it is important to study climatic conditions which favor long-term establishment of these introduced predators. Elevation is important because temperature fluctuates at different elevations; lower temperature is expected at higher elevations and vice versa. Temperature at the time of release is important because these predatory beetles are reared in insectaries at higher temperatures than normal field

temperatures. Their ability to withstand sudden exposure to lower temperatures is important for long-term establishment. Knowledge of these factors would enhance current biological control programs against HWA by increasing the probability of establishment of *S. tsugae*. Improved release strategies using these recommendations will enhance overall management of HWA.

## Cluster and Multiple Releases of *S. tsugae* on Hemlock Woolly Adelgid

Predatory beetles tend to disperse upon release and may not find mates. Cluster or multiple releases in close vicinity may enhance their ability to find mates and reproduce. Of ca. 200 *S. tsugae* release sites in GRSM, many sites were located in close proximity. Three sites were near Laurel Falls, three sites on Gregory Ridge Trail, five sites near Buckhorn Gap, six sites near Buckeye Creek, and seven sites near Brushy Mountain. These sites are located within an area of 0.5 to 1.5 km of each other. Recovery of *S. tsugae* from cluster or multiple releases may indicate that multiple or cluster releases in adjacent areas can aid these predatory beetles in finding mates and prey, promote reproduction, and increase the likelihood of establishment and recovery.

## Monitoring Biological Control Efforts using Spatial Analysis

Monitoring the effectiveness of biological control agents against HWA is an expensive and time-consuming task. Predatory beetles tend to disperse and usually do not remain at the release site which makes it difficult to determine their establishment and effectiveness. Spatial analysis using large to small scale digital images is an alternate method to evaluate natural enemies. Landsat, MODIS, Composite Infra-Red (CIR) and National Agricultural Inventory Program (NAIP) data are examples of spatial data. High resolution spatial data provide crisp and realistic overviews of the forest. Analyzing

spatial data over a period of time can detect forest structure changes, such as those caused by pests and disease outbreaks. Such changes can be studied to determine negative impacts of pests and diseases, and the effectiveness of biological control efforts. Spatial analysis may be a useful fool for an early warning system.

NAIP has acquired digital images of various resolutions which could be used to evaluate natural enemies released on eastern hemlock as well as monitor hemlock health over a period of time. This type of spatial data acquired over a period of time could be compared to detect potential changes in forest landscapes. Foliar discoloration or defoliation due to pest and diseases can be quickly detected using high resolution spatial data. NAIP digital imagery contains 4 bands [red, blue, green and near-infrared (NIR)]. Each band represents a digital number (0-255). Using red and NIR, normalized difference vegetation index (NDVI) is calculated; this value represents the health of vegetation. NDVI is light reflectance from vegetation. Higher NDVI values represent healthier vegetation.

## Research Objectives

Despite releases of these predatory beetles in GRSM, hemlock mortality occurs. Little data on establishment and recovery of these predatory beetles at release sites, factors that may contribute to establishment, and associations between predator establishment and forest health are available. To address these issues, a study was initiated in 2008 with the overall goal of assessing releases of biological control agents and their impact on hemlock health. The specific objectives of this study are to:

1) Assess establishment and recovery of *S. tsugae* and *L. nigrinus* on eastern hemlock in the GRSM,

2) Model abiotic and biotic factors affecting establishment of *S. tsugae* released against HWA in the GRSM, and

3) Develop a novel approach to assess impact of introduced biological control agents of HWA using remotely-sensed digital imagery.

The hypotheses are that: 1) *S. tsugae* and *L. nigrinus* released against HWA are established on eastern hemlock in the GRSM, 2), several abiotic and biotic factors affect establishment of *S. tsugae* released against HWA in the GRSM, and 3) remotely-sensed digital imagery can be used to determine the impact of introduced biological control agents of HWA on hemlock health.

By addressing these objectives, this study will generate information for establishment of HWA predators released in GRSM. This study will also provide significance of abiotic and biotic factors that influence establishment of predatory beetles on HWA. Spatial analysis will enable us to evaluate natural enemies using high resolution digital imagery. Analysis of NDVI may be helpful to identify tree health condition which may be used to evaluate natural enemies using high resolution spatial data. Visual interpretation of digital imagery will provide a better mean to estimate hemlock mortality due to HWA in GRSM. The findings of this study will facilitate land managers devise pest management strategies against HWA.

# CHAPTER I

## Recovery of *Sasajiscymnus tsugae*, Released against Hemlock Woolly Adelgid, *Adelges tsugae*, in the Southern Appalachians

A version of this chapter was originally published by Abdul Hakeem, Jerome F. Grant, Paris L. Lambdin, David S. Buckley, Frank A. Hale, James R. Rhea, Gregory J. Wiggins and Glenn Taylor:

Abdul Hakeem, Jerome F. Grant, Paris L. Lambdin, David S. Buckley, Frank A. Hale, James R. Rhea, Gregory J. Wiggins and Glenn Taylor. "Recovery of *Sasajiscymnus tsugae*, released against hemlock woolly adelgid, *Adelges tsugae*, in the southern Appalachians." *Biocontrol Science and Technology* 20 (2010): 1069-1074.

## Abstract

Eastern hemlock in the Great Smoky Mountains National Park is currently threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). As part of a management plan against this invasive insect pest, about 350,000 adults of the predatory beetle *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) were released at ca. 150 sites in the Park from 2002 to 2007. Of these adult release sites, 33 were sampled in 2008 and 2009 using beat sheet sampling for 4 man-hours. *Sasajiscymnus tsugae* adults ($n$ = 78) and/or larvae ($n$ = 145) were recovered from seven sites (21.2% of the release sites sampled). Recovery of *S. tsugae* was significantly associated with older release sites, with the most beetles recovered from 2002 release sites. These results indicate that *S. tsugae* may require more time (i.e., 5-7 years) than anticipated for population densities to reach readily detectable levels in some areas.

# Introduction

## Eastern Hemlock

Populations of eastern hemlock, *Tsuga canadensis* (L.) Carriere, have experienced widespread mortality throughout the eastern United States because of excessive feeding by hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Vose et al. 2008). Since its introduction from Asia, HWA has gradually spread into 17 states in the eastern United States (Trotter et al. 2008) and since it was first documented in the Great Smoky Mountains National Park in 2002, thousands of eastern hemlocks have died (JFG, Personal observation).

## Biological Control of Hemlock Woolly Adelgid

As part of a classical biological control program to introduce a complex of natural enemies against HWA, *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), which is native to Japan, was the first introduced species of natural enemy selected for mass rearing and release against HWA in the United States beginning in Connecticut in 1995 (Cheah et al. 2005). Since 2002, more than two million *S. tsugae* beetles have been reared in insectaries in several states and released throughout the eastern United States, with many of the releases made in the southeastern United States (Grant 2008, Salom et al. 2008). Of these, approximately 350,000 were released at ca. 150 sites in the Great Smoky Mountains National Park from 2002 to 2007. However, limited information exists on the establishment of these introduced natural enemies in areas of the releases in the eastern or southeastern United States (McClure and Cheah 1998; Cheah and McClure 2000; Grant 2008; McDonald et al. 2008).

In 2008, a study was initiated to evaluate the establishment of *S. tsugae* released against HWA in the Great Smoky Mountains National Park. The objective of this study was to determine the presence and extent of established populations of this introduced predatory species at multiple release sites in the National Park.

## Materials and Methods

Sampling was conducted in 2008 and 2009 in the Great Smoky Mountains National Park, which is located in the southern Appalachians spanning the border of North Carolina and Tennessee. Eastern hemlock trees at each release site were sampled using beat sheets for *S. tsugae*. Coordinates for all *S. tsugae* release sites were obtained from Park personnel, and sites were located using a Garmin GPS map 60 CSx6 GPS unit. Original release trees, which were identified by an aluminum tag, at each site were sampled where possible. Each site was sampled once except Laurel Falls 1, Laurel Falls 2, Laurel Falls 3 and Anthony Creek; each of these four sites was sampled in both years. Sampling was conducted from 5 May to 7 July 2008 and 25 February to 16 June 2009 (this period coincided with expected activity of *S. tsugae*). At each site, beat-sheet sampling was conducted for 4 man-hours. On all hemlock trees in the release site area, accessible branches from ground level to a height of 2.5 m were sampled by tapping them five to eight times with a wooden rod while holding a white beat sheet (71 x 71 cm) beneath the branch to catch dislodged predators. Depending on the size of the tree, one to three beat-sheet samples were collected per tree. All *S. tsugae* observed during beat-sheet sampling were recorded, and representative specimens of suspected adults and larvae of *S. tsugae* were taken to the laboratory, where larvae were reared in glass jars (2.64 liters) on HWA-infested hemlock until pupation and emergence as adults. Field-collected and

laboratory-reared adults were identified in the laboratory (GJW), and species identification was confirmed by James Parkman at the Lindsay Young Beneficial Insects Laboratory (University of Tennessee). Voucher specimens are housed in the Integrated Pest Management and Biological Control Laboratory at the University of Tennessee.

**Statistical Analysis**

The number of larvae, adults, and both stages of *S. tsugae* (i.e., larvae and adults) recovered from all sites sampled were compared among years using a Chi-square ($X^2$) test. Pearson's correlation was performed to qualify the relationship (i.e., positive or negative) between year of release and number of larvae, adults, and both stages of *S. tsugae* recovered. All alpha levels were $P \leq 0.05$ and analyses were conducted using the statistical package SPSS 17.0 (SPSS, Chicago, IL, USA).

## Results and Discussion

Thirty-three *S. tsugae* release sites (ca. 3,500 trees) were sampled in 2008 and 2009 (Fig. 11). These sites represented releases made in 2002 (nine sites), 2003 (one site), 2004 (four sites), 2005 (three sites), 2006 (10 sites), and 2007 (six sites). During this study, *S. tsugae* adults ($n$ = 78) and/or larvae ($n$ = 145) were recovered from seven (21.2%) of these release sites (Fig. 11, Table 1, see Appendix). The greatest numbers of adult *S. tsugae* were recorded from Buckthorn Gap ($n$ = 22) (Site B) and Laurel Falls 2 ($n$ = 15; sampled 2009) (Site D) while only one *S. tsugae* was recovered from Ramsey Cascades (Site F) (Fig. 11, Table 1). The greatest numbers of larvae were recovered from Laurel Falls 2 ($n$ = 83; sampled 2008) (Site D), Laurel Falls 1 ($n$ = 40; sampled 2008) (Site C), and Buckthorn Gap ($n$ = 18) (Site B) (Fig. 11, Table 1). The percentages of release sites that were positive for the recovery of *S. tsugae* was influenced by the year of

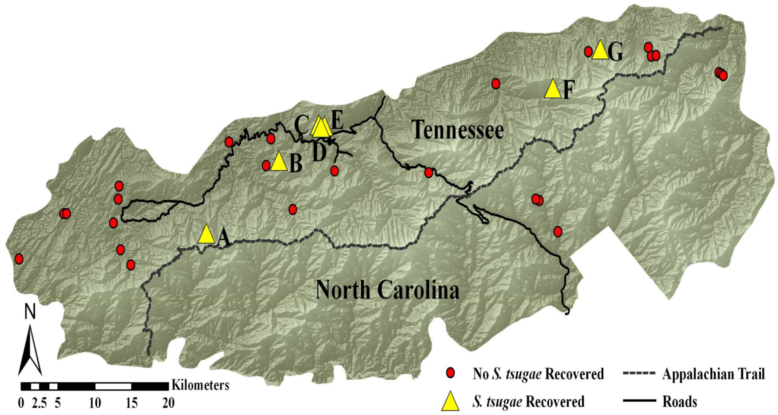


**Figure 11. Release (*n* = 33 sites) and recovery (*n* = seven sites) of *Sasajiscymnus tsugae* from eastern hemlock in the Great Smoky Mountains National Park, 2008 - 2009 (A, Anthony Creek; B, Buckthorn Gap; C, Laurel Falls 1; D, Laurel Falls 2; E, Laurel Falls 3; F, Ramsey Cascades; and G, Buckeye Creek) (symbols indicate sites where *S. tsugae* have been released and sites where *S. tsugae* have been recovered).**

the release. *Sasajiscymnus tsugae* was recovered from 55.5% (5 of 9) of the 2002 release sites, but recovered from only 10.0% (1 of 10) and 16.6% (1 of 6) of the 2006 and 2007 release sites, respectively (Table 1). Adult *S. tsugae* were recorded from 2002 (*n* = 75), 2006 (*n* = 2), and 2007 (*n* = 1) release sites. Larvae of *S. tsugae* were found at only 2002 (*n* = 145) release sites. These results indicate that significantly greater numbers of larvae ($X^2$ = 16.892, df =1, $P$ < 0.001), adults ($X^2$ = 16.676, df = 1, $P$ = 0.005), and total *S. tsugae* ($X^2$ = 25.973, df =1, $P$ = 0.001) were recovered from sites where releases were made in 2002 than all other sites sampled. Additionally, a significant inverse relationship between year of release and number of larvae (Pearson = -0.316; $P$ = 0.029), adults

**Table 1. Recovery of *Sasajiscymnus tsugae* (St) on eastern hemlock, *Tsuga canadensis*, at seven of 33 release sites in the Great Smoky Mountains National Park (recoveries were documented in 2008- 2009).***

| Location | Date of Release | Date Recovered | No of St Beetles Released | No of St Adults Recovered | No of St Larvae Recovered |
|---|---|---|---|---|---|
| Anthony Creek (A)** | 26 Jun 2002 | 13 Jun 2008 | 2,923 | 0 | 1 |
| | | 23 Apr 2009 | | 13 | 0 |
| | | | | 6.5/site | 0.5/site |
| Buckthorn Gap (B) | 25 Jun 2002 | 21 May 2009 | 3,118 | 22 | 18 |
| Laurel Falls 1 (C) | 03 Jun 2002 | 11 June 2008 | 2,039 | 0 | 40 |
| | | 08 June 2009 | | 13 | 0 |
| | | | | 6.5/site | 20.0/site |
| Laurel Falls 2 (D) | 03 Jun 2002 | 11 Jun 2008 | 1,940 | 0 | 83 |
| | | 08 Jun 2009 | | 15 | 2 |
| | | | | 7.5/site | 42.5/site |
| Laurel Falls 3 (E) | 10 Jul 2002 | 08 Jun 2009 | 848 | 12 | 1 |
| Ramsey Cascades (F) | 03 May 2007 | 10 Mar 2009 | 2,386 | 1 | 0 |
| Buckeye Creek (G) | 22 Feb 2006 | 04 May 2009 | 2,045 | 2 | 0 |
| Total | | | | 78 | 145 |

**Sasajiscymnus tsugae* was recovered at seven of 33 release sites; only information for the seven release sites where *S. tsugae* was recovered is provided. The 33 release sites represented releases made in 2002 (nine sites), 2003 (one site), 2004 (four sites), 2005 (three sites), 2006 (10 sites), and 2007 (six sites).

**Letters in parentheses correspond to locations on the release and recovery map shown in Figure 11.

(Pearson = -0.447; *P* = 0.003), and total *S. tsugae* (Pearson = -0.440; *P* =0.003) was documented.

Previous efforts to recover and/or document establishment of *S. tsugae* on HWA have yielded mixed results. For example, low numbers of *S. tsugae* were recovered three months after their initial releases in the Great Smoky Mountains National Park in 2002 from 3 of 10 sites. These sites included Laurel Falls 2 (*n* = 2 adults) (Site D), Laurel Falls 3 (*n* = 1 adult) (Site E), and Anthony Creek (*n* = 1 adult) (Site A) (Fig. 11) (Buck et al. 2005, Lambdin et al. 2006). During 2003 (one year after these initial releases), *S. tsugae* was not recovered from any of the 10 release sites (Lambdin et al. 2006). In the current study, however, all three of these release sites were documented as positive recovery sites in 2008 and/or 2009. In similar studies on hemlock in Connecticut and North Carolina, *S. tsugae* were recovered 1-6 years after their initial releases (Cheah and McClure 2000; Cheah et al. 2005; McDonald et al. 2008). In Georgia, however, no *S. tsugae* were recovered from six sites two years following releases (M. Dalusky, personal Communication).

Despite the seemingly large numbers (more than 350,000) of beetles that have been released in the National Park (ca. 210,000 ha) since 2002, this number equivocated to only 1.8 *S. tsugae*/ha in the Park (Grant 2008). If only the area of hemlock-dominated forest (ca. 36,000 ha) is considered (GRSM 2010), *S. tsugae* has been released at a density of 10.8 beetles/ha. In many biological control programs, predators are released at much greater densities, usually to attain a rapid reduction in pest populations. As compared to *S. tsugae*, HWA can increase its population rapidly. For example, the parthenogenic HWA undergoes two generations each year with about 300 and 50

progeny per female for the first and second generations, respectively (all progeny are female). On the other hand, *S. tsugae* undergoes one generation each year under field conditions with about 100 - 200 progeny (males and females) per female. These reproductive and generational differences, combined with the relatively low numbers released per hectare, partially explain the slow increase in predator populations.

Sampling efforts during this study were restricted by necessity to the canopy closest to the ground (within 3 m). However, many of the hemlocks sampled were between 5 and 20 m tall, and some hemlocks were more than 30 m tall. *Sasajiscymnus tsugae* has been found in the upper canopy shortly after release (Cheah et al. 2005), and heavily-infested hemlocks provide ample prey material throughout the canopy. Therefore, the detection of low numbers of *S. tsugae* when sampling a fraction of the potential habitat on a single tree is promising. If *S. tsugae* were detected in the lower one-third to one-fourth of the canopy and larger numbers are expected in the upper canopy, then the established population of *S. tsugae* in the National Park is greater than that estimated in our research. However, recovery of predatory beetles in release sites in the National Park does not necessarily indicate improved tree health. Although beetles have established in several areas of the Park, their populations may not have increased sufficiently to reduce infestations of HWA to non-damaging levels.

This ongoing study documents the presence of *S. tsugae* persisting at seven locations in the Great Smoky Mountains National Park. Populations of *S. tsugae* are well established (present 6+ years after release and adults and larvae recovered) at five of these sites. Results indicate that *S. tsugae* may take longer (as many as 5-7 years) than anticipated for populations to establish and attain readily measurable levels, as relatively

greater numbers of *S. tsugae* were recovered from releases made in 2002 compared to releases made in subsequent years (Table 1). Although *S. tsugae* was the first introduced predator species to be released en masse against HWA, it is one of several introduced predators, including species in the genera *Laricobius*, *Scymnus* and others, that have been released, or are being considered for release, as part of a natural enemy complex (Asaro et al. 2005, Salom et al. 2005). Therefore, establishment of *S. tsugae* on HWA can only enhance future efforts involving the releases of new biological control agents.

Additional release sites will be sampled to fully assess establishment of *S. tsugae* in the Great Smoky Mountains National Park and investigate the conditions which may contribute to its establishment. Concurrently, tree health and HWA population data will be obtained by Park personnel and others. These findings will be combined and analyzed to assess the impact of *S. tsugae* on HWA and to develop a predictive model to help to understand factors that influence establishment of *S. tsugae* on eastern hemlock.

# CHAPTER II

## Establishment and Coexistence of Two Predators, *Laricobius nigrinus* and *Sasajiscymnus tsugae*, Introduced against Hemlock Woolly Adelgid on Eastern Hemlock

A version of this chapter was originally published by Abdul Hakeem, Jerome F. Grant, Gregory J. Wiggins, Paris L. Lambdin, and James R. Rhea:

Abdul Hakeem, Jerome F. Grant, Gregory J. Wiggins, Paris L. Lambdin, and James R. Rhea. "Establishment and coexistence of two predators, *Laricobius nigrinus* and *Sasajiscymnus tsugae*, introduced against hemlock woolly adelgid on eastern hemlock." *Biocontrol Science and Technology* 21 (2011): 687-691.

## Abstract

The coexistence of two introduced predatory species, *Laricobius nigrinus* Fender and *Sasajiscymnus tsugae* (Sasaji and McClure), and a native predator, *L. rubidus* LeConte, on eastern hemlock was documented for the first time. Details of their coexistence and implications to management of hemlock woolly adelgid, *Adelges tsugae* Annand, are discussed.

**Keywords:** Coexistence, *Laricobius nigrinus, Laricobius rubidus, Sasajiscymnus tsugae, Adelges tsugae, Tsuga canadensis.*

## Introduction

Eastern hemlock, *Tsuga canadensis* (L.) Carriere, is an important component of southern Appalachian forest ecosystems (Schmidt and McWilliams 1996). This ecologically and aesthetically important tree species is currently threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), an invasive insect species introduced into eastern North America from Asia in 1951 (Ward et al. 2004). The adelgid has devastating impacts on eastern hemlock (Kimple and Schuster 2002) and can cause tree mortality within 2-6 years after heavy infestation (Mayer et al. 2002, NPS 2010). To reduce the spread and impact of *A. tsugae*, several natural enemies

of this forest pest have been released as biological control agents. *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), native to Japan, has been released throughout the eastern United States beginning in Connecticut in 1994 (Cheah et al. 2004). Adults and larvae of *S. tsugae* feed on all stages of *A. tsugae*. *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is native to western North America and has been released in the eastern United States beginning in 2003 (Zilahi-Balogh et al. 2006). Adult *L. nigrinus* feed on nymphs and adults of *A. tsugae*, while larvae feed on eggs of *A. tsugae* (Zilahi-Balogh et al. 2002, 2003, Kohler et al. 2008). Another predatory beetle, *Scymnus sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae), which is native to China and feeds on aphids and adelgids, has been introduced in small numbers in the southern Appalachians beginning in 2004 (Cheah et al. 2004).

In 2002, hemlock woolly adelgid was first documented in the Great Smoky Mountains National Park (Lambdin et al. 2006, Grant 2008). As part of an integrated management plan, about 550,000 adult *S. tsugae* and ca. 7,857 adult *L. nigrinus* have been released on adelgid-infested eastern hemlock in this National Park (Grant 2008, J. Webster, personal communication). These predatory species have been individually recovered in some of their respective release sites (Hakeem et al. 2010, A. Hakeem, unpublished data), but no published reports exist of co-occurrence of established populations of these introduced predator species at the same location or on the same trees in the eastern United States. Thus, little is known of the coexistence of established populations of these species in natural settings.

## Materials and Methods

In 2007, a study involving releases of *L. nigrinus*, *S. tsugae*, and *S. sinuanodulus* was initiated to assess the feasibility of the use of whole-tree enclosures (i.e., cages) to evaluate and establish predators of *A. tsugae*. After the removal of cages, insight was sought on the establishment, incidence, and coexistence (i.e., occurrence on the same tree) of these introduced beetle species. The intent of this paper is to report coexistence of *L. nigrinus* and *S. tsugae* on eastern hemlock.

Fifteen eastern hemlock trees (ca. 8 m tall) were selected at a site (ca. 0.5 ha) at Blackberry Farm near Walland, Blount County, Tennessee. No predatory beetles had been released in this area before this study was initiated. In addition, sampling of trees prior to the study yielded none of the predatory species used in this research.

Twelve trees were caged using whole-tree enclosures (dimensions: ca. 8.26 m tall, 6.35 m basal diameter, made of lightweight Nylon netting and constructed by Camel Manufacturing, Pioneer, TN). Releases of *L. nigrinus* (*n* = 190/caged tree, three trees) were conducted in December 2007 and January 2008. Releases of *S. tsugae* (*n* = 300/caged tree, three trees) and *S. sinuanodulus* (*n* = 90/caged tree, three trees) were conducted in March 2008. Three trees were caged with no beetles as a caged control, and three trees were left uncaged and included in the study as control trees. Adults of *S. tsugae* were obtained from Lindsay Young Beneficial Insects Laboratory, University of Tennessee, and adults of *S. sinuanodulus* were obtained from University of Georgia and the United States Department of Agriculture Forest Service, Northern Research Station, Connecticut. Adults of *L. nigrinus* were obtained from Lindsay Young Beneficial Insects Laboratory, as well as from field collections from Seattle, Washington. Cages were

removed from trees in July 2009, and beat-sheet sampling (five beats per tree, 15 study trees) was conducted every seven to 14 days from January through July 2010 to assess establishment of these introduced predatory beetles. For all samples, predatory beetles were identified and recorded, and voucher specimens are maintained in the Integrated Pest Management and Biological Control Laboratory, University of Tennessee.

## Results and Discussion

Adults of *L. nigrinus* (*n* = 33; F2), *S. tsugae* (*n* = 48; F2 to F4), and a native species *Laricobius rubidus* (LeConte) (Coleoptera: Derodontidae) (*n* = 9), as well as larvae of *Laricobius* spp. (*n* = 36; F3, *L. nigrinus*) and *S. tsugae* (*n* = 11; F3 to F5), were collected from beat-sheet samples from the eastern hemlock study trees following removal of whole-tree enclosures (Fig. 12). The generations of each species were approximated based on the time since release and their biologies documented in the literature (Cheah and McClure 2000,  Zilahi-Balogh et al. 2003, Cheah et al. 2004). Hybridization of *L. nigrinus* and *L. rubidus* among collected individuals was not determined. *Scymnus sinuanodulus* was not recovered during sampling and may not have established in the study site.

During 2010, *A. tsugae* were present on all trees sampled; however, adelgid densities among trees varied and were greatest (3.53 woolly masses/cm, 24 March) on the tree where all three predator species were found (caged control tree 1) (Fig. 12). Average adelgid density on all other trees was low (<3.53 woolly masses/cm). Either adults or larvae of *S. tsugae* were collected from 12 study trees, while adult *L. nigrinus* and/or larval *Laricobius* species were collected from four of the 15 study trees (Fig. 12). Both *L. nigrinus* and *S. tsugae* were found on these four study trees, and *L. rubidus* also was

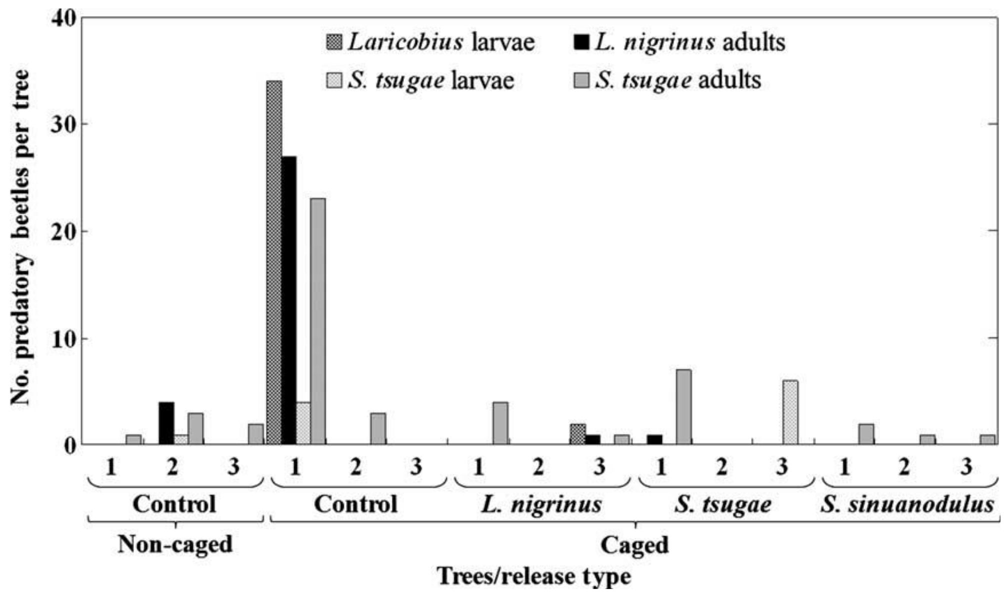


**Figure 12. Establishment and coexistence of *Laricobius nigrinus* and *Sasajiscymnus tsugae* from hemlock woolly adelgid-infested hemlock, January - July 2010 (6-12 months after removal of whole-tree enclosures), Blackberry Farm, Walland, TN. Labels along the X-axis represent replicate trees for each predator release type.**

found on one of these four trees (caged control tree 1) (Fig. 12). Adults and/or larvae of *L. nigrinus* were collected from January to early June, and adults and larvae of *S. tsugae* were collected from March to July. Both *L. nigrinus* and *S. tsugae* dispersed throughout the study site (0.5 ha area) within 1 year after removal of cages. One adult *L. nigrinus* also was recovered from an eastern hemlock outside of the study site (a distance of ca. 50 m). Adult *L. nigrinus*, *L. rubidus,* and *S. tsugae* were recovered on the same tree (caged control tree 1) on three sampling dates (24 March, 2 April, and 20 April), but these three species were not recovered from any of the same beat-sheet samples. *Laricobius nigrinus* and *L. rubidus* were recovered on the same beat-sheet sample on two sampling dates (24

March and 2 April), *L. nigrinus* and *S. tsugae* were recovered on the same beat-sheet sample on four sampling dates (24 March, 2 April, 9 April, and 20 April), and *L. rubidus* and *S. tsugae* were recovered from the same beat-sheet sample on one sampling date (20 April). The co-occurrence of these predatory beetle species on the same tree on several sampling dates reflects a partial temporal overlap of their life stages. The co-occurrence of these species on the same beat-sheet samples indicates individuals can exist in close spatial proximity on hemlock trees infested with hemlock woolly adelgid.

In previous studies in Great Smoky Mountains National Park, many of the recoveries of *S. tsugae* have been from older (5 - 6 years) release sites (Hakeem et al. 2010). However, *S. tsugae* released on caged trees were recovered at this location 2 years after the initiation of the study. The whole-tree cages may have enhanced the ability of these introduced predators to find food and mate or prevented their dispersal from the release tree and/or site. Thus, releases of these natural enemies, especially *S. tsugae*, in large whole-tree cages, placed over hemlocks in a forest, may better acclimate the released beetles to field conditions, increasing their establishment and potential effectiveness. *Laricobius nigrinus* and *S. tsugae* were successfully established at the same location and recovered from trees other than those on which they had been released.

Both introduced predators and the native species colonized the tree having the heaviest adelgid infestation (Fig. 12), demonstrating their ability to seek and find suitable prey. The coexistence of the two introduced predators, *L. nigrinus* and *S. tsugae*, and a native predator species, *L. rubidus*, on eastern hemlock is encouraging, especially with minimal temporal generational overlap of the introduced species. *Laricobius nigrinus* emerges in the fall and is active primarily through April, while *S. tsugae* is active from

March through summer. Establishment of these multiple predator species may result in prolonged predation, and their coexistence may enhance existing biological control efforts against hemlock woolly adelgid.

# CHAPTER III

## Factors Affecting Establishment and Recovery of *Sasajiscymnus tsugae*, an Introduced Predator of Hemlock Woolly Adelgid, in the Great Smoky Mountains National Park

A version of this chapter was submitted by Abdul Hakeem, Jerome F. Grant, Gregory J. Wiggins, Paris L. Lambdin, Frank A. Hale, David S. Buckley, James R. Rhea, James P. Parkman and Glenn Taylor:

Abdul Hakeem, Jerome F. Grant, Gregory J. Wiggins, Paris L. Lambdin, Frank A. Hale, David S. Buckley, James R. Rhea, James P. Parkman and Glenn Taylor. “Factors affecting establishment and recovery of *Sasajiscymnus tsugae*, an introduced predator of hemlock woolly adelgid, in the Great Smoky Mountains National Park.” *Environ. Entomol.* (*In press*).

## Abstract

To reduce populations of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), more than 550,000 *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) have been released at more than 200 sites in the Great Smoky Mountains National Park (GRSM) since 2002. To determine factors affecting establishment and recovery of these predatory beetles, 65 release sites were sampled using beat sheets from 2008 to 2012. Several biotic and abiotic factors were evaluated for their association with establishment and recovery of *S. tsugae*. Information on predatory beetle releases (location, year of release, number released, and season of release), topographic features (elevation, slope, Beers transformed aspect, and topographic relative moisture index), and temperature data (minimum and maximum temperatures one day following release, and average minimum and maximum temperatures seven days following release) were obtained from GRSM personnel. These factors were evaluated using stepwise logistic regression and Pearson correlation. *S. tsugae* was recovered from 13 sites two to 10 years after release, and the greatest number was recovered from 2002

release sites. Regression indicated establishment and recovery were negatively associated with year of release and positively associated with the average maximum temperature seven days following release and elevation. Several significant correlations were found between presence, as well as the number, of *S. tsugae* and year of release, season of release, and temperature variables. These results indicate that releases of *S. tsugae* should be made in warmer temperatures and monitored for establishment for at least five years following releases to enhance establishment and recovery efforts.

**KEYWORDS** *Sasajiscymnus tsugae*, *Adelges tsugae,* temperature, establishment, biological control.

## Introduction

Eastern hemlock, *Tsuga canadensis* Carrière, is an important component of many eastern forest types (Eyre 1980) and has been designated as a foundation species. Eastern hemlock provides fundamental structure to the forest ecosystem, facilitates communities of organisms through habitat creation, is irreplaceable by another species, and sustains ecosystem services (i.e., habitat for fish, reduced erosion, water filtration, and timber production) (Dayton 1972, Ellison et al. 2005, Sackett et al. 2011). Healthy hemlock stands also regulate forest floor moisture, decomposition and nitrogen cycling, and restrict vines and shrubs by limiting light and other resources (Kizlinski et al. 2002). A large number of vertebrates, birds, fish, and insects are associated with eastern hemlock and the forests of which they are a component (Reay et al. 1990, DeGraaf et al. 1992, Buck et al. 2005, Dilling et al. 2007).

Eastern hemlock is distributed throughout the northeastern and Appalachian regions of North America extending from Nova Scotia to Georgia and westward to

Minnesota (Ward et al. 2004, USDA 2012). The Great Smoky Mountains National Park (GRSM) is located in the southern Appalachians and comprises 210,876 ha (NPS 2012). Within GRSM, the total hemlock resources cover ca. 55,442 ha with more than 5,665 ha of hemlock-dominated forests (Yost et al. 1994, Soehn et al. 2005, Webster 2010).

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), was first reported in the GRSM in 2002, but may have been present and undetected for several years (Soehn et al. 2005). This invasive pest species feeds at the base of needles on stored sugars in xylem ray parenchyma cells (Young et al. 1995). Due to prolonged feeding by HWA, hemlock mortality is more rapid in southern than northern forests (Nuckolls et al. 2009, Spaulding and Rieske 2010). In the southern Appalachians, hemlock tree mortality occurs 3 to 6 years after infestation of HWA, while in the northeastern United States, hemlock mortality occurs 4 to 10 years after infestation (NPS 2005, Spaulding and Rieske 2010, Ford et al. 2012). Extensive mortality of eastern hemlock may be due to tree susceptibility, high numbers of HWA, and low populations of native predators of HWA (Cheah and McClure 1996).

A suite of predatory insect species, some of which feed on HWA, has been collected from various hemlock species worldwide. For example, 54 lady beetle species were collected in China from three hemlock species, *T. dumosa* (D. Don) Eichler, *T. forrestii* Downie and *T. chinensis* (Franchet) Pritzel (Yu et al. 2000). Also, 55 predatory species representing 14 families were recovered from western hemlock, *T. heterophylla* (Raf.) Sargent, in the western United States. The abundances of larval *Laricobius* spp., adult *L. nigrinus* Fender (Coleoptera: Derodontidae), and adult *Leucopis argenticollis* Zetterstedt (Diptera: Chamaemyiidae) on western hemlock were found to be positively

correlated to HWA, and considered to be HWA specialist predators (Kohler et al. 2008). In the eastern United States, more than 60 generalist predatory species belonging to 21 families in five orders have been documented from eastern hemlock (Wallace and Hain 2000, Buck et al. 2005). However, none of the species found on eastern hemlock is a specialist predator of HWA.

To increase populations of natural enemies of HWA in the eastern United States, a biological control program was initiated in 1993. As part of the biological control program against HWA, more than 3.5 million *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) and 102,000 *L. nigrinus* have been released in the eastern United States. Of these, ca. 550,000 *S. tsugae* and ca. 7,857 *L. nigrinus* were released in the GRSM from 2002 to 2007 and 2004 to 2007, respectively (Grant et al. 2010c, Webster 2010, Jetton et al. 2011).

Periodic recoveries of these two predatory species in areas of release are encouraging. In a study examining establishment of *L. nigrinus* throughout the eastern United States, *L. nigrinus* was recovered from 13 of 22 release sites, and its establishment was positively associated with minimum winter temperature and number of beetles released (Mausel et al. 2010). Additionally, *L. nigrinus* was recovered from three sites (27.3%; 3 of 11 sampled sites) from GRSM (Grant et al. 2010b), and the number of adult *L. nigrinus* collected at one site in North Carolina increased from ca. 50 in November 2009 to more than 550 in April 2010 (McDonald et al. 2010).

Likewise, *S. tsugae* has been recovered in several areas of release in the United States. In the northeastern United States, adults and larvae of *S. tsugae* were recovered from several sites five years after release and had dispersed 0.93 km from the original

release site (Blumenthal 2002, McClure and Cheah 2002, Cheah et el. 2005). In North Carolina, adult *S. tsugae* were recovered from several release sites one to two years following releases (McDonald et al. 2008). In a study conducted in Tennessee near GRSM, *S. tsugae* released in whole-tree canopy enclosures were recovered for several consecutive years after the enclosures were removed (Grant et al. 2010a, Hakeem et al. 2010, Wiggins et al. 2010). Furthermore, coexistence of *S. tsugae* and *L. nigrinus* has been documented in some release sites (Hakeem et al. 2010, 2011), which indicates that these predatory beetles are compatible and may enhance biological control efforts against HWA.

Despite the widespread releases of *S. tsugae* and its sporadic recovery in several areas of the eastern United States, little is known about the abiotic and biotic factors that may influence establishment and recovery of populations of *S. tsugae*. Knowledge of these factors would enhance current biological control programs against HWA by increasing the probability of establishment of *S. tsugae*. In 2008, a study was initiated in GRSM to determine the incidence of *S. tsugae* at release sites and to identify abiotic and biotic factors that affect establishment and recovery of these introduced predatory beetles.

## Materials and Methods

**Assessment of Establishment of *S. tsugae*.** To evaluate the extent of establishment of *S. tsugae* released against HWA between 2002-2007 in GRSM, 65 beetle release sites were sampled from 2008 to 2012. Location and release information, such as release date and number released, for each site were obtained from GRSM personnel, and sites were located using a Garmin GPS map 60 CSx GPS unit. In the sites examined during this study, releases of *S. tsugae* were made from 2 June to 10 July 2002, 9 April to 5 June

2003, 24 February to 24 May 2004, 24 January to 30 June 2005, 26 January to 22 June 2006, and 24 January to 9 May 2007. Sampling was conducted from 1 May to 17 July 2008, 24 February to 16 June 2009, 12 April to 8 July 2010, 4 May to 14 June 2011, and 12 June to 26 June 2012, and some sites were sampled multiple (as many as three) times. These sampling dates coincided with expected activity of *S. tsugae* in the field (Wiggins et al. 2010). At each site, beat-sheet sampling was conducted for four person-hours. Sampling was conducted within 100 m of the release site, and beat-sheet sampling was performed by striking accessible branches from ground level to a height of ca. 2.5 m five to eight times with a wooden rod while holding a canvas beat sheet (71 cm x 71 cm) beneath the branch to catch adults and larvae of dislodged predators. Depending on the size of the tree, one to three beat-sheet samples were collected per tree. Voucher specimens were placed in 2-ml vials and taken to the laboratory for identification and confirmation. Vouchers of suspected larvae were maintained in a jar (2.64 l) with hemlock twigs (10-15 cm) infested with HWA until emergence as an adult. Specimens were identified and confirmed by the authors (AH, GJW) and staff at the Lindsay Young Beneficial Insects Laboratory (JPP and other personnel) (University of Tennessee). Voucher specimens are stored at the Integrated Pest Management and Biological Control Laboratory at the University of Tennessee.

**Assessment of Abiotic and Biotic Factors Affecting Establishment and Recovery of *S. tsugae*.** Several factors associated with releases of *S. tsugae* were assessed to evaluate their influence on establishment and recovery of predatory beetle populations. The number of adult *S. tsuage* released, the year of release, and the season of release, which was designated by release date [Winter: 24 January – 17 March (season = 1), Spring: 22

March – 16 June (season = 2), and Summer: 25 June – 10 July (season = 3); no releases from 11 July to 23 January were assessed], were evaluated for their influence on establishment. Topographic variables that characterize physical site conditions were obtained from GRSM personnel for each site. Elevation data (10 m resolution) were included, and slope was calculated from the elevation data for all release sites in ArcMap 10 (ESRI 2010). Aspect was transformed using Beers method (Beers et al. 1966), which provides a weighted value to indicate site exposure to sunlight, and was analyzed for influence on establishment of *S. tsugae*. Topographic relative moisture index (TRMI) (Parker 1982), which is a measure of potential soil moisture calculated from elevation and slope, also was analyzed for influence on establishment of *S. tsugae*. Climate data are continually collected from several weather stations throughout GRSM, and temperature data for the release period of each study year were obtained from 10 weather stations from GRSM personnel (Fig. 13). Temperature data, including minimum and maximum temperatures for the day following releases and the average minimum and maximum temperatures for the seven-day period following releases, were acquired from the weather station closest to each release site.

**Data Analysis.** Data were analyzed using logistic regression and Pearson correlation in SPSS 18.0 (SPSS 2009). All repeated samples were entered as a separate sampling date. Backward stepwise logistic regression was used to select significant variables ($P = 0.05$) associated with establishment and recovery of *S. tsugae*. The dependent variable was presence of *S. tsugae* (i.e., recovery at a release site) and the independent variables were year of release, number of *S. tsugae* released, season of release, elevation, slope, Beers

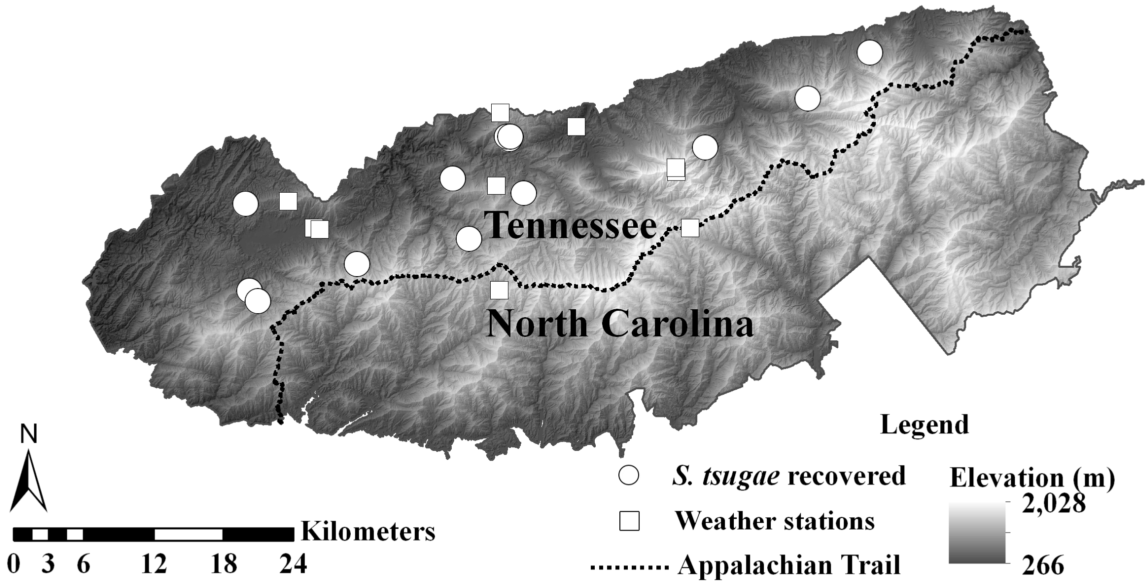


**Figure 13. Locations of weather stations (*n* = 10) and *Sasajiscymnus tsugae* recovery sites (*n* = 13) in the Great Smoky Mountains National Park, 2008 to 2012.**

transformed aspect, TRMI, minimum and maximum temperatures one day following release, and average minimum and maximum temperatures for the seven-day period following release. To further evaluate the association of these factors with establishment of *S. tsugae*, Pearson correlations were performed between the independent variables listed above and presence of *S. tsugae*, number of adult *S. tsugae*, number of larval *S. tsugae*, and total number of *S. tsugae* at recovery sites.

## Results and Discussion

**Assessment of Establishment of *S. tsugae*.** From 2008-2012, 614 *S. tsugae* (*n* = 316 adult and *n* = 298 larvae) were recovered in beat-sheet sampling from 13 of 65 (20%) sites in GRSM (Fig. 13, Table 2, see Appendix). Sites where releases were made during

**Table 2. Sites (*n* = 13) from where *Sasajiscymnus tsugae* were recovered after releases between 2002 and 2007, Great Smoky Mountains National Park.**

| Site Name | Date of Release | Year of Release | No. Released | Date Recovered | No. *S. tsugae* Recovered | |
|---|---|---|---|---|---|---|
| | | | | | Adults | Larvae |
| Anthony Creek | 26 Jun | 2002 | 2,923 | 13 Jun 08 | 0 | 1 |
| | | | | 23 Apr 09 | 13 | 0 |
| Brushy Mountain | 22 Mar | 2007 | 2,804 | 29 Jun 10 | 1 | 0 |
| Buckeye Creek | 22 Feb | 2006 | 2,045 | 4 May 09 | 2 | 0 |
| Buckhorn Gap | 25 Jun | 2002 | 3,118 | 17 Jul 08 | 0 | 0 |
| | | | | 21 May 09 | 22 | 18 |
| Gregory Ridge 1 | 25 Jun | 2002 | 3,396 | 19 Jun 08 | 0 | 0 |
| | | | | 22 Jun 10 | 4 | 9 |
| Gregory Ridge 2 | 09 Feb | 2003 | 2,500 | 15 Jun 10 | 22 | 4 |
| Jakes Creek | 24 Feb | 2003 | 4,000[a] | 14 May 08 | 0 | 0 |
| | | | | 14 June 11 | 2 | 0 |
| Laurel Falls 1 | 03 Jun | 2002 | 2,039 | 11 Jun 08 | 0 | 40 |
| | | | | 8 Jun 09 | 13 | 0 |
| | | | | 13 Jun 12 | 77 | 55 |
| Laurel Falls 2 | 03 Jun | 2002 | 1,940 | 11 Jun 08 | 0 | 83 |
| | | | | 8 Jun 09 | 15 | 2 |
| | | | | 13 Jun 12 | 85 | 58 |
| Laurel Falls 3 | 10 Jul | 2002 | 848 | 11 Jun 08 | 0 | 0 |
| | | | | 8 Jun 09 | 12 | 1 |
| | | | | 13 Jun 12 | 36 | 25 |
| Lynn Camp | 25 Jun | 2002 | 3,434 | 5 Jun 08 | 0 | 0 |
| | | | | 18 Jun 12 | 8 | 2 |
| Ramsey Cascades | 03 May | 2007 | 2,386 | 10 Mar 09 | 1 | 0 |
| Stony Branch | 02 Jun | 2002 | 3,953 | 19 Jun 08 | 0 | 0 |
| | | | | 19 Jun 12 | 3 | 0 |

2002 had the greatest percent recovery of *S. tsugae* (8 of 10 sites, 80%), followed by 2003 (2 of 5 sites, 40%), 2006 (1 of 15 sites, 7%), and 2007 (2 of 19 sites, 11%) (Table 2). No recoveries were made from 2004 ($n$ = 7) or 2005 ($n$ = 9) release sites.

These findings underscore the importance of continued and repeated monitoring of biological control agents following releases. Recoveries of *S. tsugae* were made two to 10 years following releases, illustrating the importance of monitoring several years following releases. Also, *S. tsugae* was not recovered from six of the 13 recovery sites the first time they were sampled, but was recovered from all six of these sites after a second sampling was conducted, illustrating the importance of repeated sampling at the same site. Of the sites where *S. tsugae* was not recovered, four release sites were sampled twice, and all others were sampled once. Lack of recovery of *S. tsugae* from these sites does not mean that *S. tsugae* is not established, and continued sampling at these sites may yield *S. tsugae* in the future.

This study also documented several recoveries of *S. tsugae* in areas where multiple or clusters of release sites occurred. Three release sites near Laurel Falls were in close proximity to each other (ca. 500 m), and the total number of *S. tsugae* released in 2002 at these sites was 4,827 (Table 2). In 2008, *S. tsugae* was recovered from two of these sites, and *S. tsugae* were recovered from all three sites in 2009 and 2012. Additionally in 2012 sampling, the numbers of *S. tsugae* collected were 37% higher ($n$ = 336) than in 2008 ($n$ = 123) (Fig. 14), which suggests that populations of *S. tsugae* are increasing over time (Table 2, Fig. 14). In addition to the three Laurel Falls sites, larvae and adults of *S. tsugae* also were recovered from two of three release sites along the Gregory Ridge Trail (within ca. 1 km of each other) and one of four release sites near

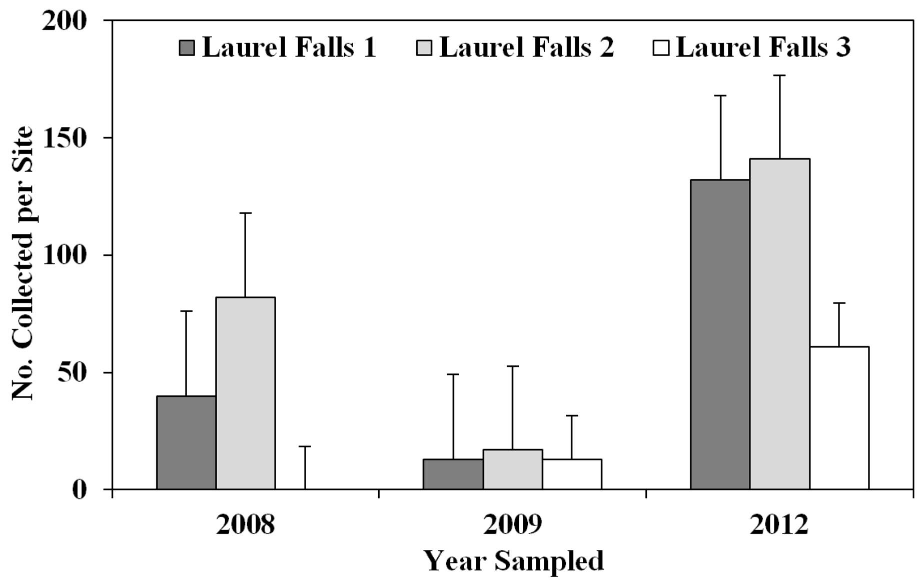


**Figure 14. Recovery of *Sasajiscymnus tsugae* on eastern hemlock from repeated sampling in release sites near Laurel Falls, Great Smoky Mountains National Park, 2008 to 2012.**

Buckhorn Gap (within 1.5 km of each other). Documentation of *S. tsugae* in these areas of multiple releases may indicate that multiple or clusters of releases in close proximity can aid these beetles in finding mates and prey, promote reproduction, and increase the likelihood of establishment and recovery of *S. tsugae*.

**Assessment of Abiotic and Biotic Factors Affecting Establishment and Recovery of *S. tsugae*.** Several factors were identified as important to establishment and recovery of *S. tsugae* in GRSM. Backward stepwise logistic regression selected the year of release ($P$ = 0.001), average maximum temperature seven days following release ($P$ = 0.035), and elevation ($P$ = 0.031) as significant variables associated with establishment and recovery of *S. tsugae* on eastern hemlock in GRSM (Table 3). This model explains 58%

**Table 3. Factors which influenced establishment and recovery of *Sasajiscymnus tsugae* in the Great Smoky Mountains National Park selected by backward stepwise logistic regression ($R^2$ = 0.580).**

| Variables | Estimate | S. E* | Wald Coef.** | Probability |
|---|---|---|---|---|
| Intercept | 2059.891 | 622.194 | 10.961 | 0.001 |
| Avg. maximum temperature seven days following release | 0.245 | 0.116 | 4.469 | 0.035 |
| Elevation | 0.004 | 0.002 | 4.663 | 0.031 |
| Year of release | -1.032 | 0.311 | 11.020 | 0.001 |

*Standard error
**Tests the significance of each model variable by comparing the Wald coefficient to an expected value.

($R^2$ = 0.580) of *S. tsugae* recovery on eastern hemlock in GRSM. No other variables were significant.

The significant inverse relationship between year of release and presence of *S. tsugae* suggests that the likelihood of detecting establishment of *S. tsugae* increases as the duration of time since release increases. A similar association between year of release and presence of *S. tsugae* was previously documented (Hakeem et al. 2010). When coccinellid beetles, such as *S. tsugae*, are released, they may disperse and have difficulty finding mates. This dispersal may slow the rate of population growth, thus, requiring a longer period of time for populations to grow and attain detectable levels. For example,

no *S. tsugae* was recovered from the 2002 release sites during sampling conducted in 2003 (Lambdin et al. 2006), yet *S. tsugae* was recovered from eight of these sites six to 10 years after releases. Additionally, *S. tsugae* was not recovered from Lynn Camp (2002 release site) in 2008 sampling, but was recovered in 2012 sampling, which suggests that *S. tsugae* may have been present in 2008, but population levels were undetectable.

The significance of the average maximum temperature seven days following release indicates that warmer temperatures in the field are more conducive to establishment. This finding also may illustrate the necessity of acclimatizing beetles to field conditions prior to release. Insectaries rear and maintain colonies of *S. tsugae* at warm (ca. 24˚C to 30˚C) temperatures, and temperatures in the field at the time of release that are similar to rearing conditions may enhance initial survival of *S. tsugae*. Early-season releases may require acclimatization to minimize inactivity and/or mortality due to sudden exposure to low temperatures. Releases made in April through June may not require acclimatization, as temperatures in the field during this time of year may be similar to rearing conditions, allowing predators to adjust to any minor temperature differences between insectaries and the field.

The significance of elevation in the regression model suggests that certain elevations may be more suitable for establishment of *S. tsugae*. However, no significant associations were identified between establishment or recovery of *S. tsugae* and elevation using Pearson correlation (Table 4). Warmer temperatures contribute to establishment of *S. tsugae,* and interactions between temperature and elevation of certain release sites may have enhanced establishment. For example, releases in all but one recovery site were made in the spring or summer of the year, and elevations at these sites ranged from 670 to

**Table 4. Correlation between abiotic and biotic factors and *Sasajiscymnus tsugae* recovery and establishment in the Great Smoky Mountains National Park, 2008-2012.**

| Variables | Presence of *S. tsugae* | | No. *S. tsugae* adults | | No. *S. tsugae* larvae | | Total No. *S. tsugae* (adults & larvae) | |
|---|---|---|---|---|---|---|---|---|
| | Correlation coefficient | P-value | Correlation coefficient | P-value | Correlation coefficient | P-value | Correlation coefficient | P-value |
| Release variables | | | | | | | | |
| Year of release | -0.541 | 0.001***[a] | -0.374 | 0.001*** | -0.373 | 0.001*** | -0.413 | 0.001*** |
| Season of release | 0.446 | 0.001*** | 0.198 | 0.080 | 0.146 | 0.199 | 0.190 | 0.093 |
| Number released | -0.095 | 0.403 | -0.149 | 0.191 | -0.153 | 0.177 | -0.167 | 0.141 |
| Temperature variables | | | | | | | | |
| Avg. minimum seven days following release | 0.378 | 0.001*** | 0.219 | 0.053 | 0.210 | 0.064 | 0.237 | 0.036* |
| Avg. maximum seven days following release | 0.375 | 0.001*** | 0.240 | 0.039* | 0.276 | 0.014* | 0.293 | 0.009** |
| Minimum one day following release | 0.378 | 0.001*** | 0.226 | 0.045* | 0.236 | 0.036* | 0.256 | 0.023* |
| Maximum one day following release | 0.355 | 0.001*** | 0.223 | 0.039* | 0.267 | 0.017* | 0.276 | 0.014* |
| Topographic variables | | | | | | | | |
| Beers[b] | 0.183 | 0.110 | 0.079 | 0.489 | 0.061 | 0.596 | 0.078 | 0.499 |
| Elevation | 0.048 | 0.674 | 0.038 | 0.742 | 0.066 | 0.563 | 0.057 | 0.616 |
| Slope | -0.078 | 0.495 | -0.129 | 0.257 | -0.153 | 0.179 | -0.156 | 0.171 |
| TRMI[c] | -0.001 | 0.992 | -0.009 | 0.936 | 0.059 | 0.606 | 0.038 | 0.742 |

[a] Probabilities: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; if no symbol is present, no significant correlation was found.

[b] Beers transformed aspect is a measure of exposure to direct sunlight based on original aspect (Beers et al. 1966).

[c] Topographic relative moisture index is an estimate of soil moisture based on topography (Parker 1982).

1,086 m. The temperatures during this time of year at the corresponding elevations may have been more conducive to survival of *S. tsugae* than other temperature-elevation interactions and led to population establishment. Further research is needed to determine more precisely the role of elevation in establishment of introduced predators of HWA.

Pearson correlation analysis supports the results obtained from regression analysis and identified several other abiotic and biotic factors significantly associated with presence and numbers of *S. tsugae* at release sites (Table 4). A negative association was documented between presence of *S. tsugae* and year of release ($P$ = 0.001), and a positive association was documented between presence of *S. tsugae* and season of release ($P$ = 0.001). Temperature [average minimum ($P$ = 0.001) and maximum ($P$ = 0.001) temperatures seven days following release and minimum ($P$ = 0.001) and maximum ($P$ = 0.001) temperatures one day following release] also was positively associated with presence of *S. tsugae*. The number of adult *S. tsugae* recovered was negatively associated with year of release ($P$ = 0.001) and positively associated with the average maximum ($P$ = 0.039) temperature seven days following release and the minimum ($P$ = 0.045) and maximum ($P$ = 0.039) temperatures one day following release. The number of larval *S. tsugae* recovered was negatively associated with year of release ($P$ = 0.001) and positively associated with the average maximum ($P$ = 0.014) temperature seven days following release and the minimum ($P$ = 0.036) and maximum ($P$ = 0.017) temperatures one day following release. The total number of *S. tsugae* (adults and larvae) recovered was negatively associated with year of release ($P$ = 0.001) and positively associated with the average minimum ($P$ = 0.036) and maximum ($P$ = 0.009) temperatures seven days following release, as well as the minimum ($P$

= 0.023) and maximum (*P* = 0.014) temperature one day following release. No other significant associations were documented (Table 4).

These findings further suggest that temperature following releases significantly influences establishment and time since release significantly increases the ability to detect *S. tsugae*. The significant negative relationship between year of release and the presence and number of *S. tsugae* supports the finding that a longer time period is required for populations of *S. tsugae* to increase to detectable levels than previously anticipated. The positive associations among various measures of *S. tsugae* and temperature at release sites further indicate that warmer field temperatures within one week of release increase the likelihood of establishment. The association between the presence of *S. tsugae* and the season of release also indicates that releases made in warmer seasons are conducive to establishment of *S. tsugae*. The importance of temperature following beetle releases seems intuitive, and this finding may be the most important factor for successful establishment of *S. tsugae*. The general activity (feeding, mating, predator avoidance, etc.) of most predatory beetles may be limited by cold temperatures. In a field cage study in New England, adults of *S. tsugae* were documented to survive temperatures consistently between -4 to 1˚C (Cheah and McClure 2000) and were able to withstand minimum temperatures as low as -21.6˚C (Cheah et al. 2004); however, all *S. tsugae* observed were inactive during these cold periods. Additionally, these beetles were not released in cold temperatures, rather they were released in warmer seasons and were monitored throughout the winter months. If cold temperatures prompt *S. tsugae* to remain inactive for too long after being accustomed to artificial rearing conditions, these beetles may starve or die due to sudden exposure, despite their purported ability to withstand cold temperatures. In the current study, in all but one site *S. tsugae*

established from releases made when the average minimum and maximum temperatures seven days following releases were at or above 7.5˚C and 28.6˚C, respectively. The one exception was a release with average temperatures seven days following release ranging from -1.3˚C to 6.1˚C, but the coldest of these temperatures occurred five to seven days following releases (minimum and maximum temperatures the day after release were 3.9˚C and 7.1˚C, respectively), possibly giving the beetles time to feed and/or seek shelter before sub-zero (˚C) temperatures occurred. These findings may have implications for releases of other biological control agents of HWA. Keena and Montgomery (2010) found that larvae of *Scymnus camptodromus* (Yu and Liu) (Coleoptera: Coccinellidae), a potential predator of HWA imported from China and studied in quarantine, may develop at temperatures as low as 10˚C but may survive best at 15-20˚C. Based on current findings, field studies incorporating releases at different temperature ranges should be conducted to document the optimum conditions for the survival of this species in the field following release.

Reduction of impacts of HWA to eastern hemlock is important, as no other tree species is available to replace this long-lived, evergreen and shade-tolerant tree. Loss of eastern hemlock due to HWA may have negative impacts on riparian and terrestrial communities associated with eastern hemlock. Area-wide application of pesticides on eastern hemlock against HWA is not feasible due to high cost, inaccessibility, and practical constraints. The most feasible and economical long-term option to manage HWA is through release of biological control agents, such as *S. tsugae* and other predators, that have limited non-target effects. In general, abiotic and biotic factors play an important role in the success of biological control programs. This research demonstrated that several temperature variables affect establishment and recovery of these

introduced predatory beetles. Temperature at the time of release is critical for long-term establishment of *S. tsugae*, and releases may be most successful if made when temperatures average 10˚C to 25˚C seven days following release. Accordingly, establishment may be enhanced by releasing in spring and early summer when temperatures are consistently warmer.

These findings may assist land managers, foresters, and other professionals engaged in biological control of HWA to determine the most effective time of year to release predatory beetles to increase their survival in the field. The efficacy and survival of *S. tsugae* in the eastern United States will be enhanced by these findings, and will lead to better management of HWA in GRSM and elsewhere. Long-term monitoring and repeated sampling at release sites will provide greater estimations of establishment of *S. tsugae*, and continued monitoring in GRSM will provide a better understanding of the factors influencing establishment of *S. tsugae*.

# CHAPTER IV

## High Resolution Digital Imagery: A Novel Tool For Monitoring Effectiveness of Biological Control Agents of Hemlock Woolly Adelgid

This article has not been published anywhere, nor will it be before I submit the final version of my Electronic Thesis and Dissertation, so I did not include a publication statement.

## Abstract

The National Agricultural Imagery Project (NAIP) involves remotely-sensed digital imagery, which is comprised of imagery with spatial resolution of 0.3 m. NAIP imagery was used to assess hemlock mortality and the effectiveness of introduced natural enemies released against hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, in the Great Smoky Mountains National Park (GRSM). To assess hemlock mortality, visual interpretation of the digital imagery was conducted. Near-infrared (NIR) values were extracted from the imagery, and Normalized Difference Vegetation Index (NDVI) values were calculated. Ground truth data were collected in winter 2011 and 2012 from *S. tsugae* release sites (both recovery and non-recovery sites). Independent-samples t- test was performed to detect differences between NDVI values and number of dead hemlock in *S. tsugae* recovery and non-recovery sites, as well as between percent live crown, percent crown transparency, percent branch dieback, tree vigor, and *S. tsugae* recovery and non-recovery sites in GRSM. Visual interpretation of the digital imagery indicated that hemlock mortality was lower (6.1 trees/ha) in sites where *S. tsugae* has been established as compared to sites where *S. tsugae* has not been established (9.5 trees/ha). Significant differences ($P$ = 0.001) in NDVI values between *S. tsugae* recovery and non-recovery sites were documented. For understory hemlock, significant differences were observed between percent live crown ($P$ = 0.001) and percent crown transparency ($P$ = 0.016), and *S. tsugae* recovery and *S. tsugae* non-recovery sites. For overstory trees, significant differences between percent crown transparency ($P$ = 0.019), branch dieback ($P$ = 0.020), and tree vigor ($P$ = 0.037), and *S. tsugae*

recovery sites and *S. tsugae* non-recovery sites were observed. Comparisons between dead and/or live trees observed in the field with dead and/or live trees detected in the NAIP imagery yielded high agreement (73.4%). These results suggest that high resolution digital imagery may be helpful to detect tree decline and evaluate natural enemies released against HWA. This technique could be applied to evaluate other biological control efforts in forest landscapes. This research will provide information on site quality improvements from predator release sites (i.e., are hemlocks declining or recovering since predators were released?). These findings will facilitate land managers to estimate hemlock decline, and determine effectiveness of predatory beetles released against HWA. These findings could also be used for early detection of tree decline in other forest systems.

**KEYWORDS** Spatial analysis, digital imagery, hemlock mortality, NDVI, predator, *Sasajiscymnus tsugae, Adelges tsugae.*

## Introduction

Eastern hemlock, *Tsuga canadensis* Carrière, is an important component of southern Appalachian forest ecosystems. Since the introduction of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), in the Great Smoky Mountains National Park (GRSM), tens of thousands of hemlock trees have perished due to excessive feeding of HWA (Lambdin et al. 2006, Roberts et al. 2009, Webster 2010). In the United States, exotic pests cause overall economic losses of forest products estimated at 2 billion dollars annually (Pimentel et al. 2000). Estimation of such losses due to pests and diseases in the field is not an easy task. For example, detection and estimation of hemlock decline or mortality in the field requires time and

resources. Besides on-site data collection, other methods are employed to estimate defoliation and mortality of trees in the forest landscapes. Orthophotomaps, which are based on georeferened uniform-scale aerial photographs, can be used to detect foliar changes and defoliation (Doneus 2000, USGS 2013). For example, orthophotomaps depicted ca. 84% foliage defoliation of pine, spruce and birch (Lee et al. 1983, Eigirdas et al. 2013). Moderate resolution imaging spectroradiometer (MODIS) are multispectral and multi band with varying spatial resolution. Spruce et al. (2011) assessed the effectiveness of MODIS time-series data (250 m resolution) to detect defoliation by European gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). Accuracy of MODIS data to detect forest pests is ca. 88%. Color infrared (CIR) images comprise visible and invisible light. CIR images contain three to four bands [red, green, blue and near-infrared (NIR)] and can also be used to detect pests in forest systems. These bands are used to detect any abnormal changes in the canopies of forests or cropping systems. For example, Kautz et al. (2011) utilized CIR images to determine spatio-temporal dispersal of spruce bark beetles, *Ips typographus* (L.) (Coleoptera: Curculionidae). Analysis of patches of dead Norway spruce, *Picea abies* (L.) Karst, indicated that infestation was distance dependent and significantly greater within 100 m of previous year's infestation using dispersion rates. Also, results on the spatial distribution of beech moth, *Cydia fagiglandana* (Zeller) (Lepidoptera: Tortricidae), a serious pest of fruits of beech, oak and chestnut trees, revealed that the larval infestation rate and larval density of beech moth decreased progressively (Jimenez-Pino et al. 2011).

In the GRSM, hemlock mortality is widespread. Predatory beetles released against HWA were found at 20% of the release sites sampled (Hakeem 2010, 2011, 2013). Despite

documentation of recovery and establishment of *S. tsugae*, effectiveness of these predatory beetles to manage HWA, and their association with tree health, are unclear. In 2011, a study was initiated to investigate the use of digital spatial imagery to assess tree health and tree mortality. The objectives of this study are to 1) investigate if remotely-sensed digital imagery can be used for detection and evaluation of hemlock mortality, 2) utilize spatial imagery to assess tree health in release sites of *S. tsugae*, and 3) conduct conventional on-site tree health assessments to compare with spatial imagery assessment.

## Materials and Methods

**Study Sites.** Establishment of *S. tsugae* was assessed in 65 sites where *S. tsugae* was released in GRSM. *S. tsugae* were recovered in 13 (20%) of these sites. For more details, see Chapters 1 and 3. Of these sites, 10 were used to test the accuracy of digital spatial imagery to delineate dead trees, six (three *S. tsugae* recovery and three *S. tsugae* non-recovery) were used in on-site tree evaluations and digital imagery assessment of hemlock health, 22 (11 recovery and 11 non-recovery sites) sites were used to evaluate hemlock mortality (see Appendix).

**Acquisition of digital images.** To assess eastern hemlock mortality and effectiveness of biological control agents, such as *S. tsugae* released against HWA in GRSM, remotely-sensed digital imagery (0.3 m pixel resolution) which covers the entire GRSM was acquired from National Park Service personnel (Fig. 15). These images were taken aerially in Fall 2011 by U.S. Department of Agriculture, National Agricultural Imagery Project (NAIP). Images were comprised of four bands (red, green, blue, and NIR), and each pixel of the imagery had a value ranging from 0 to 255 for each band (Red 0 to 255, blue 0 to 253 and green 0 to 248, and NIR 0

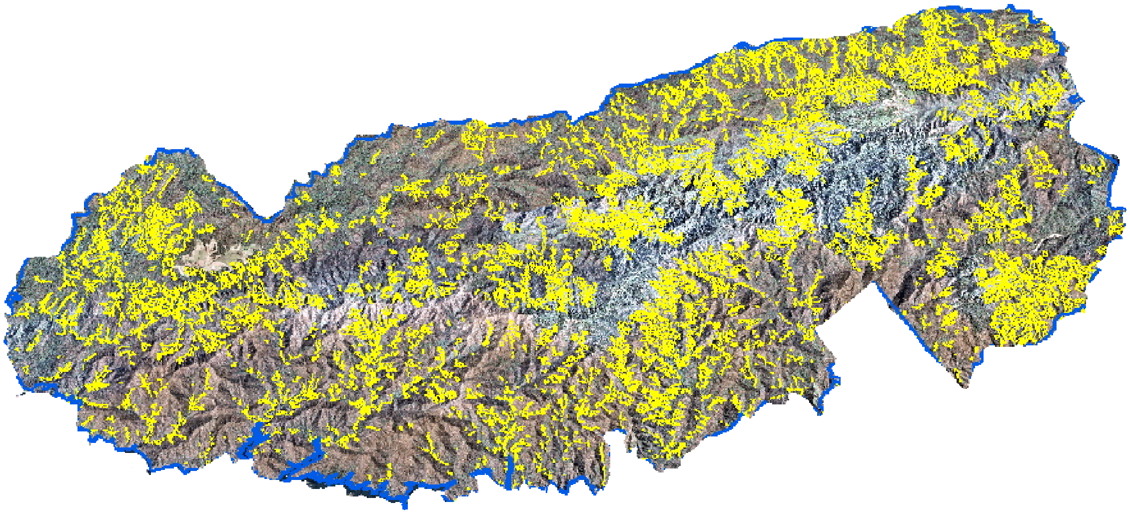

**Figure 15. National Agricultural Imagery Project data of the Great Smoky Mountains National Park with eastern hemlock distribution highlighted in yellow, 2011.**

to 255). This imagery was used alone and in conjunction with field-collected data for all studies described below.

**Field observed vs. imagery depicted agreement.** Ten sites were used to test ability of digital imagery to delineate live from dead hemlocks (see Appendix). Sites were located using a Garmin GPS 60 Csx unit. To reduce GPS signal obstruction, tree locality data were collected during winter from all sites, when deciduous trees had no leaves. From each site, 50 trees (25 live, 25 dead) were georeferenced and classified as live or dead. These data were combined with the digital imagery in the laboratory using ArcMap 10. Percent agreement between live or dead trees observed in the field and live or dead trees detected on the imagery was calculated.

**Tree health assessment.** Normalized difference vegetation index (NDVI) is a measurement of light reflected from different types of vegetation which indicates health of particular vegetation.

Higher NDVI values indicate healthy (i.e., greener) vegetation and lower NDVI values indicate less-green vegetation (NASA 2013) (Fig. 16). NDVI values generally fall between -1 and 1, and NDVI is calculated as follows:

$$NDVI = (NIR - Red)/(NIR + Red).$$

In this study, average NDVI values were compared between *S. tsugae* recovery and non-recovery sites using the digital imagery described above. Three *S. tsugae* recovery sites and three *S. tsugae* non-recovery sites were used to calculate NDVI. Thousands of hemlock trees throughout GRSM have been treated with imidacloprid (some in areas where *S. tsugae* has been released). To reduce the influence of imidacloprid treatments on assessment of associations between *S. tsugae* and tree health, these sites were selected which had low numbers of treated trees with imidacloprid. Imagery used for this study was reclassified from 0.3 m to 5.0 m resolution, resulting in pixels that are similar in size to hemlock canopies. A 500 m buffer was placed around each site, and within this area, hemlock forests were delineated using the vegetation classification data generated from CIR imagery from 1997 and 1998, in combination with topographic data, such as slope, aspect and elevation (Welch et al. 2002) (Fig. 17). Red and NIR bands were extracted within the area containing hemlock using Arc Toolbox in ArcMap. NDVI was calculated for each pixel in the three *S. tsugae* recovery sites and three *S. tsugae* non-recovery sites using the formula above in Microsoft Excel, and the overall mean NDVI for recovery and non-recovery sites was compared.

To assess the impact of introduced predators on hemlock health, tree health characteristics were collected from these same three *S. tsugae* recovery sites and three *S. tsugae*

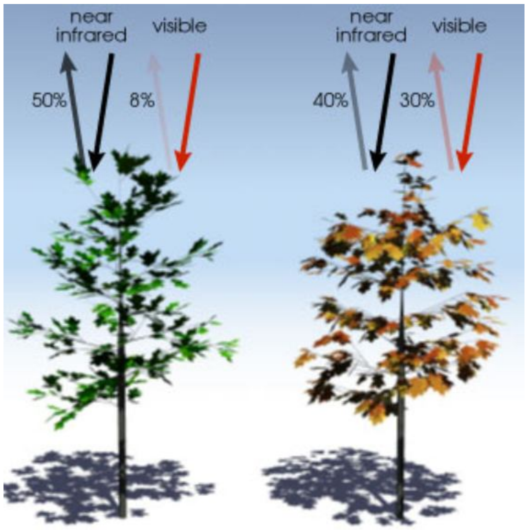


**Figure 16. Detection of tree decline using normalized difference vegetation index (NDVI) (www.nasa.gov).**

**Figure 17. Digital imagery depicting hemlock vegetation (between green lines) clipped from The Laurel Falls 1 site using vegetation classification data developed by Welch et al. (2002).**

non-recovery site, and used to compare NDVI (see Appendix). At each site, nine understory (<25 cm dbh) and nine overstory (>25 cm dbh) hemlock were evaluated for various attributes of tree health, such as percent crown transparency, percent live crown, percent branch dieback, and tree vigor. Tree vigor ratings used were 1) healthy, 2) slight decline, 3) moderate decline, 4) severe decline, 5) dead standing, and 6) dead fallen, following U.S Department of Agriculture Forest Service field data collection procedures (USDA FS 2005).

**Hemlock mortality assessment.** To assess hemlock mortality between *S. tsugae* recovery sites and *S. tsugae* non-recovery sites in GRSM, visual interpretation was conducted at 22 release sites of *S. tsugae* (11 recovery, 11 non-recovery sites) (see Appendix). A 78.5 h-area surrounding each of 22 sites was selected for assessment. Within this site hemlock stands were delineated using vegetation classification data (Welch et al. 2002). Visual interpretation was conducted within this area to designate dead hemlock, and the number of dead hemlock within hemlock stands in *S. tsugae* recovery and non-recovery sites were recorded (Fig. 18). The number of dead hemlock per ha were calculated for each site, and compared between *S. tsugae* recovery and non-recovery sites.

**Data analysis.** Differences in mean tree health characteristics (percent crown transparency, percent live crown, percent branch dieback, and tree vigor), NDVI values, and number of dead hemlock per ha between *S. tsugae* recovery and non-recovery sites were analyzed using independent-samples t-tests. Levene's test of homogeneity of variance was used to verify that data conformed to equal variance assumption. All alpha levels were $P \leq 0.05$ and analyses were conducted using the statistical package SPSS 17.0 (SPSS, Chicago, IL, USA).

**Figure 18. Dead hemlock (between green lines) delineation using National Agricultural Imagery Project 2011 data (0.3 m pixel resolution).**

## Results and Discussion

**Field observed vs. imagery depicted agreement.** Overall agreement between field-observed and imagery-depicted live and dead trees was 73.4% (Table 5). Greater agreement between field and imagery data was observed with dead trees (ca. 82%), possibly due to less GPS signal disturbance under dead trees (less dense) canopies (Fig. 18). These results indicate that digital imagery can be used to delineate between live and dead hemlocks with greater than 73% confidence. Remotely-sensed digital images provide vital data about landscape changes on a broad scale which span over time. By observing these digital images, tree mortality in a particular location could be easily identified. This study provides a baseline to compare hemlock mortality between *S. tsugae* recovery and *S. tsugae* non-recovery sites in other areas of releases.

**Table 5. Number of hemlocks identified in the field compared to those depicted by USDA National Agricultural Imagery Project digital imagery and percent agreement between the two data sets.**

| Number of Dead Trees | | | Number of Live Trees | | |
|---|---|---|---|---|---|
| Observed in the field | Detected in imagery | Percent detection | Observed in the field | Detected in imagery | Percent detection |
| 277 | 229 | 82.67 | 270 | 173 | 64.07 |
| Total Detection | | | | | 73.4 |

*****Visual observation of trees within 500 m of release site and control sites.

**Tree health assessment.** The overall mean NDVI value was significantly (*P* = 0.001) greater in *S. tsugae* recovery than in non-recovery sites, which indicates healthier canopies in areas where *S. tsugae* is present (Fig. 19). NDVI values of *S. tsugae* recovery sites ranged from 0 to 0.630 with an average of 0.327, while NDVI values of *S. tsugae* non-recovery sites ranged from 0 to 0.592 with an average of 0.312. Higher NDVI values from *S. tsugae* recovery sites indicate greener (i.e., healthier) vegetation in these sites than in non-recovery sites. By using NDVI values from study sites, tree health, as well as effectiveness of other natural enemies of HWA, could be assessed on a landscape scale and confirmed by on-site data collection.

**Health characteristics of understory hemlock.** Analysis of understory hemlock health data indicated significantly lower percent live crowns (*P* = 0.001) and percent crown transparencies

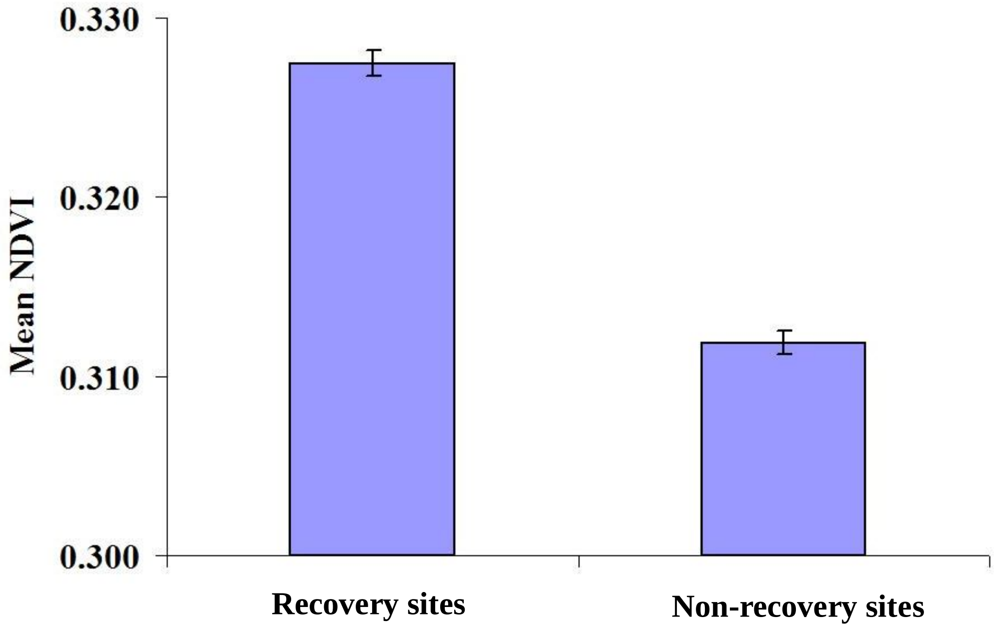


**Figure 19. Average normalized difference vegetation index (NDVI) values observed from random samples within hemlock vegetation layer from *Sasajiscymnus tsugae* recovery and non-recovery sites in the Great Smoky Mountains National Park.**

($P$ = 0.016) in *S. tsugae* recovery sites than in non-recovery sites. No significant differences were found in other tree health characteristics between *S. tsugae* recovery sites and non-recovery sites (Table 6). Percent live crown reflects the percent of total tree height which supports live foliage, and trees with high percent live crowns are often associated with more open habitats. Although percent live crown was greater in non-recovery sites, trees at these sites may have been in more open settings (such as along trails, creeks, old road beds, etc.). Percent crown transparency is a more direct measure of tree health. Percent crown transparency depicts the percent of light disseminating through the defoliated portion of the live crown. Therefore, lower crown

**Table 6. Hemlock tree health characteristics from understory trees in sites where *Sasajiscymnus tsugae* has been released (n = 6)* in the Great Smoky Mountains National Park.**

| Tree Health Characteristics** | *S. tsugae* Release Sites (mean±S.E.†) | | t-value | Probability |
|---|---|---|---|---|
| | Recovery | Non-Recovery | | |
| Percent live crown | 54.74 ± 4.20 | 76.11 ± 3.25 | -4.021 | 0.001 |
| Percent crown transparency | 46.85 ± 5.76 | 65.00 ±4.41 | -2.498 | 0.016 |
| Percent branch dieback | 30.19 ± 3.72 | 37.22 ± 3.00 | -1.472 | 0.147 |
| Tree vigor | 3.04 ± 0.17 | 3.07 ± 0.15 | -0.162 | 0.872 |

*Sites used in this study are listed in Appendix.
**Tree health characteristics were evaluated based on guidelines described in USDA FS (2005).
†Standard error.

transparencies in understory trees at recovery sites indicate denser and healthier canopies than at non-recovery sites.

**Health characteristics of overstory hemlock.** Analysis of overstory hemlock health data indicated significantly lower percent crown transparencies ($P$ = 0.019), percent branch dieback ($P$ = 0.002) and tree vigor ($P$ = 0.037) at *S. tsugae* recovery than non-recovery sites (Table 7). As with understory trees, overstory trees in recovery sites had less transparent, denser canopies, indicating healthier trees. Branch dieback is the percent live crown that has dead or non-foliated branches. Greater branch dieback in sites where *S. tsugae* was not recovered further indicates that hemlock at these sites are in poorer health than in the recovery sites. Likewise, lower tree vigor ratings indicate less decline, and low ratings at *S. tsugae* recovery sites indicate that trees are healthier at these sites than at non-recovery sites. Therefore, hemlock trees in *S. tsugae*

**Table 7. Hemlock tree health characteristics from overstory trees in sites where *Sasajiscymnus tsugae* has been released (n = 6)* in the Great Smoky Mountains National Park.**

| Tree Health Characteristics** | *S. tsugae* Release Sites (mean±S.E.†) | | t-value | Probability |
|---|---|---|---|---|
| | Recovery | Non-Recovery | | |
| Percent live crown | 63.52 ± 4.22 | 67.59 ± 4.65 | -0.648 | 0.520 |
| Percent crown transparency | 47.59 ± 5.05 | 63.33 ± 4.06 | -2.427 | 0.019 |
| Percent branch dieback | 33.89 ± 3.15 | 48.89 ± 3.23 | -3.320 | 0.002 |
| Tree vigor | 2.70 ± 0.16 | 3.19 ± 1.51 | -2.135 | 0.037 |

*Sites used in this study are listed in Appendix.
**Tree health characteristics were evaluated based on guidelines described in USDA FS (2005).
†Standard error.

recovery sites had less transparent crowns, fewer dead branches and more vigorous trees compared to non- recovery sites, which indicate that *S. tsugae* had a significant impact on overall health of eastern hemlock at the three recovery sites examined.

**Hemlock mortality assessment.** No significant difference ($P$ = 0.371) in the mean number of dead trees per ha between recovery ($n$ = 6.1±2.0) and non-recovery ($n$ = 9.5±3.1) sites was documented (Fig. 20). Although no significant differences were observed between *S. tsugae* recovery and non-recovery sites, the number of dead trees in *S. tsugae* non-recovery sites was numerically greater than in *S. tsugae* recovery sites. HWA was first detected in GRSM in 2002 but was most likely present in many areas a few years before it was detected. Despite releases of biological control agents and application of pesticides, hemlock mortality is still high. Older (i.e.,

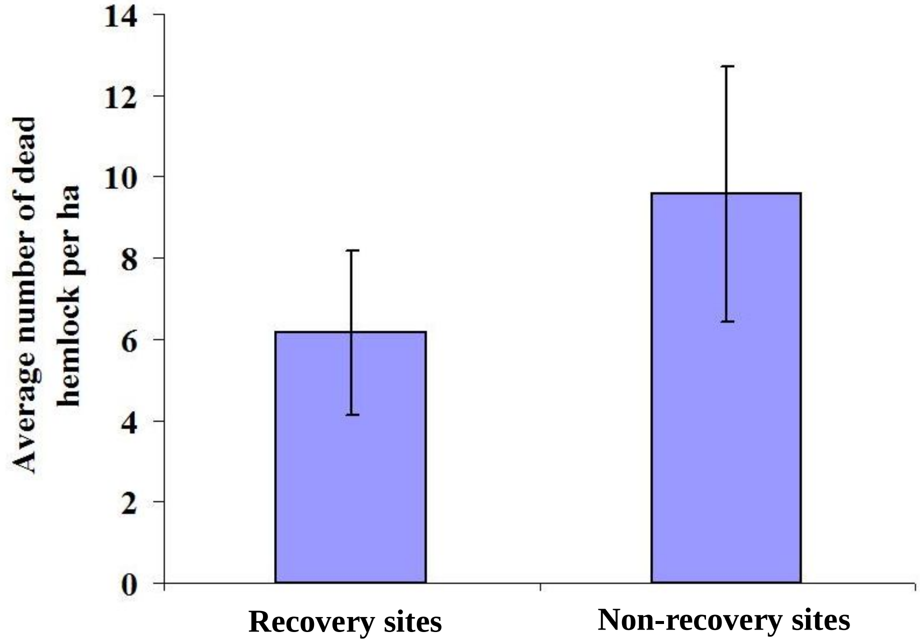


**Figure 20. Average number of dead hemlock from *Sasajiscymnus tsugae* recovery (n = 11) and non-recovery sites (n = 11) in the Great Smoky Mountains National Park**.

dominant) trees are more susceptible to HWA feeding, and many of the dead trees observed at sites may have been dead or dying at the time of releases of predatory beetles.

These results indicate that application of spatial analysis, in conjunction with conventional on-site tree evaluations, can be a feasible means to determine effectiveness of biological control agents. High resolution digital imagery was effectively used to estimate tree mortality in forest landscapes, and NDVI values corroborated on-site overstory tree health evaluations. Spatial analysis may be useful to determine impacts of invasive species of forests, which will facilitate land managers to plan scouting and devise appropriate management strategies. Future analyses could include evaluating NDVI values in areas treated with

imidacloprid or other management strategies. These analyses will enable land managers to evaluate effectiveness of treatments against HWA and monitor tree health across the landscape.

# CONCLUSIONS

Eastern hemlock, *Tsuga canadensis* (L.) Carrière, in its native range of the eastern United States, especially in the Great Smoky Mountains National Park (GRSM), is currently threatened by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). To reduce populations of HWA on eastern hemlock, more than 3.5 million *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) have been released in the eastern United States. Of these, ca. 550,000 *S. tsugae* have been released at more than 200 sites in GRSM since 2002. The overall goal of this research was to determine establishment in release sites, identify factors affecting establishment of biological control agents released against HWA, and assess the application of high resolution digital imagery to evaluate hemlock health from *S. tsugae* recovery and non-recovery sites.

Little information on the establishment of *S. tsugae* is known. Therefore, from 2008 to 2012, 65 of these adult release sites were sampled using beat sheets for four person-hours. *S. tsugae* adults ($n$ = 316) and/or larvae ($n$ = 298) were recovered from 13 sites (20% of the release sites sampled). *S. tsugae* was recovered from 13 sites two to 10 years after release, and the greatest number was recovered from 2002 release sites.

Several abiotic and biotic factors were evaluated for their association with establishment and recovery of *S. tsugae*. Information on predatory beetle releases (location, year of release, number released, and season of release), topographic features (elevation, slope, Beers transformed aspect, and topographic relative moisture index), and temperature data (minimum and maximum temperatures one day following release, and average minimum and maximum temperatures seven days following release) were obtained from GRSM personnel. These factors were evaluated using stepwise logistic regression and Pearson correlation. Regression indicated

establishment and recovery were negatively associated with year of release and positively associated with the average maximum temperature seven days following release and elevation. Several significant correlations were found between presence, as well as the number of *S. tsugae*, and year of release, season of release, and temperature variables. These results indicate that *S. tsugae* may require more time (i.e., 5-7 years) than anticipated for population densities to reach readily detectable levels in some areas. Also, these results indicate that releases of *S. tsugae* should be made at appropriate temperatures and monitored for establishment for at least five years following releases to enhance establishment and recovery efforts.

Multiple or cluster releases had a significant impact on recovery and establishment of *S. tsugae*. Relatively more *S. tsugae* were recovered from sites where more releases were made in close proximity (ca. 1.5 km). Thus, multiple releases in close proximity may also enhance establishment of biological control agents, by increasing their ability to find mates.

*Laricobius nigrinus* Fender (Coleoptera: Derodontidae) is native to western North America. Since 2004, ca. 7,857 *L. nigrinus* have been released on eastern hemlock in GRSM. *L. nigrinus* has been recovered from three (20%) of 15 release sites. For the first time, coexistence of two introduced predators, *L. nigrinus* and *S. tsugae*, and a native predator, *L. rubidus* LeConte, on eastern hemlock was documented. Coexistence of these introduced predators indicates that they are compatible and may enhance biological control efforts against HWA. *L. nigrinus* and *L. rubidus* are producing hybrids, and consequences of hybridization is unclear.

In GRSM, remotely-sensed digital imagery (National Agricultural Imagery Project (NAIP), 0.3 m resolution) was used alone and in conjunction with field-conducted tree health assessments to assess hemlock mortality and the association of introduced natural enemies

released against HWA to tree health. Visual interpretation of the digital imagery was conducted to assess mortality of hemlocks in release sites. Near-infrared (NIR) and red values were extracted from the imagery, and normalized difference vegetation index (NDVI) values were calculated from three *S. tsugae* recovery and three *S. tsugae* non-recovery sites. Differences in mean tree health characteristics (percent crown transparency, percent live crown, percent branch dieback and tree vigor), NDVI values, and tree mortality in *S. tsugae* recovery and non-recovery sites were determined using independent-samples t-tests. Visual interpretation of the digital imagery indicated higher but non-significant hemlock mortality (9.5 trees/ha) in sites where *S. tsugae* has not been established as compared to sites where *S. tsugae* has been established (6.1 trees/ha).

Significantly higher ($P = 0.001$) NDVI values were found from *S. tsugae* recovery than non-recovery sites. For understory hemlock health characteristics, significantly higher live crown ($P = 0.001$) and lower crown transparency ($P = 0.016$) was found in *S. tsugae* recovery sites compared to non-recovery sites. For overstory trees, significantly lower crown transparency ($P = 0.019$), lower branch dieback ($P = 0.002$), and higher tree vigor ($P = 0.037$) was found between *S. tsugae* recovery than non-recovery sites.

Significant differences between these variables and establishment of *S. tsugae* indicated that hemlocks are healthier in *S. tsugae* established sites than non-established sites. A strong agreement (73.4%) was found between dead and/or live trees observed in the field with dead and/or live trees detected in the NAIP imagery. This agreement indicates that high resolution digital imagery may be useful to detect defoliation or tree decline and also evaluate natural

enemies released against HWA. This technique could be applied to evaluate other biological control agents or management strategies against HWA and other pests in forest landscapes.

This research demonstrated that coexistence of multiple predators of HWA may provide better options to manage HWA by providing prolonged feeding. Cluster and multiple releases enhance establishment of *S. tsugae* in GRSM. Predictive models indicated that daily minimum and maximum temperatures following release, and average seven days minimum and maximum temperatures following release of *S. tsugae,* influenced establishment of *S. tsugae*. These findings will assist land managers with selecting appropriate times and sites for future releases of *S. tsugae*. Analysis of high resolution digital imagery indicated that associations between tree health and biological control agents could be evaluated using high resolution digital imagery. High resolution digital imagery could also provide reliable estimates of tree mortality in forest landscapes. Analysis of high resolution digital imagery to also could lead to early detection of forest pests, enabling land managers to plan scouting and devise appropriate management strategies.

# LIST OF REFERENCES

**Asaro, C., C. W. Berisford, M. E. Montgomery, J. Rhea, and J. Hanula. 2005.** Biological control of the hemlock woolly adelgid in the southern Appalachians, pp. 241-251. *In* B. Onken and R. Reardon (eds.), Third Symposium on Hemlock Woolly Adelgid in The Eastern United States, 1-3 February 2005, Asheville, NC.

**Baker, T., and D. H. Van Lear, 1998.** Relations between density of rhododendron thickets and diversity of riparian forests. For. Ecol. and Manag. 109: 21-32.

**Beers, T. W., P. E. Dress, and L. C. Wensel. 1966.** Aspect transformation in site productivity research. J. For. 64: 691-692.

**Bellows, T., and T. Fisher. 1999.** Handbook of Biological Control, Academic Press, San Diego, CA. pp.140.

**Benzinger, J. 1994a.** Hemlock decline and breeding birds. I. Hemlock ecology. Rec. N. J. Birds. N. J. Audubon Soc. 20: 2-12.

**Benzinger, J. 1994b.** References for hemlock decline and breeding birds. I. Introduction. Rec. N. J. Birds. N. J. Audubon Soc. 20: 1-8.

**Benzinger, J. 1994c.** Hemlock decline and breeding birds. H. Effects of habitat changes. Rec. N. J. Birds. N. J. Audubon Soc. 20: 34-51.

**Blumenthal, E. M. 2002.** *Pseudoscymnus tsugae* in Pennsylvania Forests, pp. 166-169. *In* B. Onken, R. Reardon and J. Lashomb (eds.), Proceedings: Hemlock Woolly Adelgid in Eastern United States Symposium, 5-7 February 2002, Rutgers University, East Brunswick, NJ.

**Brisbin, R. L. 1970**. Eastern hemlock, *Tsuga canadensis* (L.) Carr.). http://www.fpl.fs.fed.us/documnts/usda/amwood/239hemlo.pdf.

**Britannica. 2013**. http://www.britannica.com/EBchecked/topic/260886/hemlock.

**Brooks, R. T. 2001.** Effects of the removal of overstory hemlock from hemlock-dominated forests on eastern redback salamanders. For. Ecol. Manag. 149: 197-204.

**Buck, S. L., P. Lambdin, D. Paulsen, J. Grant, and A. Saxton. 2005.** Checklist of insect species associated with eastern hemlock in the Great Smoky Mountains National Park and environs. Tenn. Acad. Sci. 80: 1-10.

**Burnham, C. F., M. J. Ferree, and F. E. Cunningham. 1947.** The white pine-hemlock forests of the Anthracite Region, U. S. Department of Agriculture, Forest Service. 23 pp.

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

**Charles, A. S. 2002.** Using imidacloprid to control hemlock woolly adelgid, pp. 280-287. *In* B. Onken, R. Reardon and J. Lashomb (eds.), Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium, 5-7 February 2002, Rutgers University, East Brunswick, NJ.

**Cheah, C. A. S.-J. 2010.** *Sasajiscymnus* (formerly *Pseudoscymnus) tsugae* (Coleoptera: Coccinellidae). http://www.biocontrol.entomology.cornell.edu/predators/sasajiscymnus.html

**Cheah, C.A.S.-J., and M. S. McClure. 1996**. Exotic natural enemies of *Adelges tsugae* and their prospect for biological control, pp. 103-112. *In* S. M. Salom, T. C. Tigner, and R. C. Reardon (eds.), Proceedings: First Hemlock Woolly Adelgid Review, 12 October 1995, U.S. Department of Agriculture Forest Service, Morgantown, WV.

**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). Agr. For. Entomol. 2: 241-251.

**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. 116-122. *In* B. Onken and R. Reardon (eds.), Third Symposium on Hemlock Woolly Adelgid in Eastern United States, 1-3 February 2005, U.S. Department of Agriculture, Forest Service, Asheville, NC.

**Cheah, C., M. E. Montgomery, S. M. Salom, B. L. Parker, S. Costa, and M. Skinner. 2004.** *Sasajiscymnus* lady beetle from Japan, pp. 8-10. *In* R. Reardon and B. Onken (eds.), Biological Control of Hemlock Woolly Adelgid, U.S. Department of Agriculture, Forest Service, FHTET-2004-04, 22 pp.

**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. Entomol. 92: 237-240.

**Clinton, B. D. 2004.** *Rhododendron maximum,* pp. 623-624. *In* J. Francis (ed.), Wildland Shrubs of the United States and its Territories. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

**Cowles, R. S., M. E. Montgomery, and C.A.S.-J. Cheah. 2006.** Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. J. Econ. Entomol. 99: 1259-1267.

**Dayton, P. K. 1972.** Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica, pp. 81-95. *In* B. Parker (ed.), Proceedings of the Colloquium on Conservation Problems in Antarctica. Lawrence, Kansas, Allen Press.

**Deal, I. K. 2007**. Life history of hemlock woolly adelgid, *Adelges tsugae* Annand, on eastern hemlock, *Tsuga canadensis* (L.) Carriere, in the southern Appalachians and assessment of egg release of *Sasajiscymnus tsugae* (Sasaji and McClure) for its management. M. S. thesis, University of Tennessee, Knoxville, 67 pp.

**DeBach, P. (ed.) 1964**. Biological control of insects pests and weeds. Chapman and Hall, London. 844 pp.

**DeGraaf, R. M., and N. L. Chadwick. 1987**. Forest type, timber size class, and New England breeding birds. J. Wildlife Manag. 51: 212-217.

**DeGraaf, R. M., M. Yamasaki, W. B. Leak, and J. W. Lanier. 1992.** New England wildlife: Management of forested habitats. U.S. Department of Agriculture, Forest Service, Northeastern Research Station, General Technical Report, Rador, PA. 271 pp.

**Dighton, J., and D. C. Coleman. 1992**. Phosphorus relations of roots and mycorrhiza of *Rhododendron maximum* L. in the southern Appalachians, North Carolina. Mycorrhiza. I: 175-184.

**Dilling, C., P. Lambdin, J. Grant, and L. Buck. 2007.** Insect guild structure associated with eastern hemlock in the southern Appalachians. Environ. Entomol. 36: 1408-1414.

**Doneus, M. 2000.** Interpretation and mapping of vertical and oblique photographs using digital photogrammetry and GIS. AARGnews 20: 9 pg.

**Eigirdas M., A. Augustaitis, and G. Mozgeris. 2013**. Predicting tree crown defoliation using color-infrared orthophoto maps. iForest. 6: 23-29.

**Eisenback, B. M., J. G. Fidgen, S. M. Salom, and L. T. Kok. 2005.** Effects of systemic, sublethal doses of imidacloprid on two predators of hemlock woolly adelgid, pp. 278-279. *In* B. Onken and R. Reardon (eds.), Third Symposium on Hemlock Woolly Adelgid in the Eastern United States, 1-3 February 2005, U.S. Department of Agriculture, Forest Service, Asheville, N.C.

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

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

**ESRI. (Environmental Systems Resource Institute). 2010.** ArcMap 10. ESRI, Redlands, CA.

**ESRI. (Environmental Systems Resource Institute).** 2007. GIS Best Practices: Mobile resource planning and logistics. http://www.esri.com/library/bestpractices/mobile-resource-planning.pdf.

**Eyre, F. 1980.** Forest cover types of the United States and Canada, Soc. Am. Foresters, Washington, DC. 148 pp.

**Ford, C. R., K. J. Elliott, B. D. Clinton, B. D. Kloeppel, and J. M. Vose. 2012.** Forest dynamics following eastern hemlock mortality in the southern Appalachians. Oikos 121: 523-536.

**Godman, R. M., and K. Lancaster. 2003.** Eastern Hemlock-*Tsuga canadensis* Pinaceae-Pine family. http://www.na.fs.fed.us/pubs/silvics_manual/Volume_1/tsuga/canadensis.htm

**Gordon, R. D. 1976.** The Scymnini (Coleoptera: Coccinellidae) of the United States and Canada: Key to genera and revision of *Scymnus*, *Nephus*, and *Diomus*. Bull. Buffalo Soc. Nat. Sci. 28: 1-71.

**Grant, J. F. 2008.** Establishing *Sasajiscymnus tsugae* in the South, pp. 39-44. *In* B. Onken and R. Reardon (eds.), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 12-14 February 2008, U.S. Department of Agriculture, Forest Service, Hartford, CT.

**Grant, J. F., A. Hakeem, G. Wiggins, R. Rhea, and P. L. Lambdin. 2010a.** Assessment of introduced predator species against hemlock woolly adelgid on eastern hemlock using whole-tree canopy enclosures, pp. 53-56. *In* B. Onken and R. Reardon (eds.), Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 17-19 August 2010, U.S. Department of Agriculture, Forest Service, Asheville, NC.

**Grant, J. F., A. Hakeem, R. Rhea, G. Wiggins, P. L. Lambdin, and G. Taylor. 2010b.** Recovery and establishment of introduced predators of hemlock woolly adelgid in the southern Appalachians, pp. 21-23. *In* B. Onken and R. Reardon (eds.), Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 17-19 August 2010, U.S. Department of Agriculture, Forest Service, Asheville, NC.

**Grant, J. F., A. Hakeem, G. J. Wiggins, and P. L. Lambdin. 2010c**. Recovery of introduced biological control agents on southern hemlocks: So what?, pp 25-26. *In* K. Klepzig and F. Stephen (eds.), Proceedings of the 53rd Southern Forest Insect Work Conference, 20-23 July 2010, Wilmington, NC.

**(GRSM) Great Smoky Mountains National Park. 2010.** Hemlock Woolly Adelgid. www.nps.gov/grsm/naturescience/hemlock-woolly-adelgid.htm.

**Hakeem, A. 2008.** Non-target effect of imidacloprid on the predatory arthropod guild on eastern hemlock, *Tsuga canadensis* (L.) Carriere, in the southern Appalachians. M.S. thesis, University of Tennessee, Knoxville, 78 pp.

**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. Technol. 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. Technol. 21: 687-691.

**Hakeem, A., J. F. Grant, G. J. Wiggins, P. L. Lambdin, F. A. Hale, D. S. Buckley, J. R. Rhea, J. P. Parkman, and G. Taylor. 2013**. Factors affecting establishment and recovery of *Sasajiscymnus tsugae*, an introduced predator of hemlock woolly adelgid, in the Great Smoky Mountains National Park. Environ. Entomol. (In press).

**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.

**Jetton, R. M., J. F. Monahan, and F. P. Hain. 2011.** Laboratory studies of feeding and oviposition preference, developmental performance, and survival of the predatory beetle, *Sasajiscymnus tsugae* on diets of the woolly adelgids, *Adelges tsugae* and *Adelges piceae*. J. Insect Sci. 11: 1-14.

**Jimenez-Pino, A., L. Maistrello, M. A. Lopez-Martinez, M. E. Ocete-Rubio, and F. J. Soria-Iglesias. 2011**. Spatial distribution of *Cydia fagiglandana* (Zeller) in an exploited holm oak (*Quercus ilex* L.) forest. Spanish J. Agric. Res. 9: 570-579.

**John, D., and D. C. Coleman. 1992.** Phosphorus relations of roots and mycorrhizas of *Rhododendron maximum* L. in the Southern Appalachians, North Carolina. Mycorrhiza. 1: 175-184.

**Jordan, J. S., and W. M. Sharp 1967.** Seeding and planting hemlock for ruffed grousecover. U.S. Department of Agriculture, Forest Service. 17 pp.

**Kautz, M., K. Dworschak, A. Gruppe, and R. Schopf. 2011**. Quantifying spatio-temporal dispersion of bark beetle infestations in epidemic and non-epidemic conditions. For. Ecol. Manag. 262: 598-608.

**Keena, M. 2006.** Work on third *Scymnus* beetle progressing, pg. 13. *In* B. Onken and S. Dennis (eds.), Hemlock Woolly Adelgid Newsletter, Issue No. 7, U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry, Forest Health Protection. Hamden, CT.

**Keena, M., and M. Montgomery. 2010.** Effects of temperature on the eggs and larvae of *Scymnus camptodromus,* pp. 25-28. *In* B. Onken and R. Reardon (eds.), Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 17-19 August 2010, U.S. Department of Agriculture, Forest Service, Asheville, NC.

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

**Kimple, A., and W. Schuster. 2002.** Spatial pattern of HWA damage and impacts on tree physiology and water use in the Black Rock Forest, southern New York, pp 344-350. *In* B. Onken, R. Reardon, and J. Lashomb (eds.), Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium, Agricultural Experiment Station, Rutgers University, East Brunswick, NJ.

**Kizlinski, M. L., D. A. Orwig, R. C. Cobb, and D. R. Foster. 2002.** Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. J. Biogeogr. 29: 1489-1503.

**Kohler, G. R., V. L. Stiefel, K. F. Wallin, and D. W. Ross. 2008.** Parasitoids reared from predators of hemlock woolly adelgid (Hemiptera: Adelgidae), and the hymenopterous parasitoid community on western hemlock in the Pacific northwest. Environ. Entomol. 37: 1477-1487.

**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.

**Lambdin, P., J. Grant, D. Paulsen, and A. Saxton. 2006.** Invasion of hemlock woolly adelgid on eastern hemlock in the Great Smoky Mountains National Park. Tenn. Acad. Sci. 81: 49-54.

**Lapin, B. 1994.** The impact of hemlock woolly adelgid on resources in the Lower Connecticut River Valley. U. S. Department. of Agriculture, Forest Service. Hamden, CT. 45 pp.

**Lee, Y., R. Alfaro, and G. Van Sickle. 1983**. Tree-crown defoliation measurement from digitized photographs. Can. J. For. Res. 13: 956-961.

**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.

**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. Reardon, and J. Lashomb (eds.), Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium, Rutgers University, New Brunswick, NJ.

**McClure, M. S., and C. A. S.-J. Cheah. 1998.** Released Japanese ladybugs are multiplying and killing hemlock woolly adelgids. Frontiers of Plant Sci. 50: 6-8.

**McClure, M. S., and C. A. S.-J. Cheah. 2002.** Establishing *Pseudoscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) for biological control of hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), in the eastern United States, pp. 351-352. *In* B. Onken, R. Reardon, and J. Lashomb (eds.), Proceedings of the Hemlock Woolly Adelgid in the Eastern United States, 5-7 February 2002, East Brunswick, NJ.

**McClure, M. S., S. M. Salom, and K. S. Shields. 2001.** Hemlock woolly adelgid, U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, 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.), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 12-14 February, 2008, U.S. Department of Agriculture, Forest Service, Hartford, CT.

**McDonald, R., D. Mausel, S. Salom, L. Kok, S. Steury, C. Vinson, G. Davis, S. Skeate, G. Spears, and R. Rhea. 2010**. Collection and redistribution of 581 adults of the HWA predator, *Laricobius nigrinus* Fender, from the Hemlock Hill Area, Lees-Mcrae College, Banner Elk, North Carolina, during November 2009 and April 2010, pg. 170. *In* B. Onken and R. Reardon (eds.), Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 17-19 August 2010, U.S. Department of Agriculture, Forest Service, Asheville, NC.

**McWilliams, W. H., and T. L. Schmidt. 2000.** Composition, structure, and sustainability of hemlock ecosystems in Eastern North America, pp. 5-10. *In* K. McManus, K. Shields, and D. Souto (eds.), Proceedings, Symposium on sustainable management of hemlock ecosystems in Eastern North America, 22-24 June 1999, U.S. Department of Agriculture, Forest Service, Northeastern Research Station, Durham, NH.

**Montgomery, M. E., and S. M. Lyon. 1995**. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pp. 89-102. *In* S. M. Salom, T. C. Tigner, and R. C. Reardon (eds.), Proceedings of the First Hemlock Woolly Adelgid Review, Charlottesville, VA, U.S. Department of Agriculture. Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

**Montgomery, R. McDonald, and L. Schwartzberg. 2009**. *Scymnus (Pullus) coniferarum* (Coleoptera: Coccinellidae): An adelgid predator native to the western United States, pg. 89. *In* K. McManus, and K. Gottschalk (eds.), Proceedings, 20th U.S. Department of

Agriculture Interagency Research Forum on Invasive Species, 13-16 January 2009, Annapolis, MD.

**Montgomery, M., W. Hongbin, E. Butin, Y. Defu, L. Wenhau, and H. Nathan. 2005**. *Scymnus ningshanensis* Yu Et Yao (Coleoptera: Coccinellidae) for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pg. 63. *In* G. Kurt (ed.), Proceedings, XV U.S. Department of Agriculture Interagency Research Forum on Gypsy Moth and other Invasive Species, 13-16 January 2005, Annapolis, MD.

**(NASA) National Aeronautics and Space Administration. 2013**. http://earthobservatory.nasa.gov/Features/MeasuringVegetation/measuring_vegetation_2.php.

**(NCGICC) North Carolina Geographic Information Coordinating Council. 2011**. Using Color Infrared Imagery: A guide for understanding, interpreting and benefiting from CIR Imagery. http://www.ncgicc.com/portals/3/documents/using_color_infrared_imagery_20110810.pdf.

**Nesom, G. 2002.** North Carolina botanical garden, University of North Carolina, Chapel Hill, North Carolina. http://plants.usda.gov/plantguide/pdf/cs_tsca.pdf.

**(NPS) National Park Service. 2005.** Finding of no significant impact. http://www.nps.gov/grsm/parkmgmt/upload/FONSI.doc.

**(NPS) National Park Service. 2012.** Great Smoky Mountains. http://www.nps.gov/grsm/parkmgmt/statistics.htm.

**(NPS) National Park Service. 2013**. Hemlock woolly adelgid. http://www.nps.gov/grsm/naturescience/hemlock-woolly-adelgid.htm.

**(NPS) National Park Service. 2013b.** Park Statistics. http://www.nps.gov/grsm/parkmgmt/statistics.htm

**Nuckolls, A., N. Wurzburger, C. Ford, R. Hendrick, J. Vose, and B. Kloeppel. 2009.** Hemlock declines rapidly with hemlock woolly adelgid infestation: Impacts on the carbon cycle of southern Appalachian forests. Ecosystems 12: 179-190.

**Orwig, D. A., D. R. Foster, and D. L. Mausel. 2002.** Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. J. Biogeog. 29: 1475-1487.

**Parker, A. J. 1982.** The topographic relative moisture index: An approach to soil-moisture assessment in mountain terrain. Phys. Geog. 3: 160-168.

**Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000.** Environmental and economic costs associated with non-indigenous species in the United States. Biosci. 50: 53-65.

**(DCNR) Pennsylvania Department of Conservation and Natural Resources. 2007.** DCNR management of hemlock woolly adelgid. Department of Conservation and Natural Resources. Forest Health Fact Sheets. http://www.dcnr.state.pa.us/forestry/woollyadelgid/pestmanagement.aspx

**Reay, R. S., D. W. Blodgett, B. S. Burns, S. J. Weber, and T. Frey. 1990.** Management guide for deer wintering areas in Vermont. Vermont Fish and Wildlife Department, VT. 35 pp.

**Roberts, S., R. Tankersley, and K. Orvis. 2009.** Assessing the potential impacts to riparian ecosystems resulting from hemlock mortality in Great Smoky Mountains National Park. Environ. Manag. 44: 335-345.

**Sackett, T. E., S. Record, S. Bewick, B. Baiser, N. J. Sanders, and A. M. Ellison. 2011.** Response of macroarthropod assemblages to the loss of hemlock (*Tsuga canadensis*), a foundation species. Ecosphere 2: 1-16.

**Salom, S. M., L. Kok, A. Lamb, C. Jubb, and B. Onken. 2008.** Biological control of hemlock woolly adelgid: What is it going to take to make it work?, pp. 11-17. *In* B. Onken and R. Reardon (eds.), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States, Hartford, CT.

**Salom, S., L. Kok, B. Eisenback, J. Fidgen, R. Flowers, H. Gatton, A. Lamb, T. McAvoy, D. Mausel, and G. Zilahi-Balogh. 2005.** Overview of HWA biological control activities with *Laricobius* spp, pp. 131-133. *In* B. Onken and R. Reardon (eds.), Third Symposium on Hemlock Woolly Adelgid in the Eastern United States. U.S. Department of Agriculture, Forest Service, Asheville, NC.

**Schmidt, T. L., and W. H. McWilliams. 1996.** Status of eastern hemlock in the northern U.S., pp. 61-72. *In* G. Mroz and J. Martin (eds.), Proceedings of Regional Conference on Ecology and Management of Eastern Hemlock, University of Wisconsin, Madison, WI.

**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.), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 12-14 February 2008, U.S. Department of Agriculture, Forest Service, Hartford, CT.

**Sidebottom, J. R. 2009**. Recommendations for hemlock woolly adelgid control in the landscape. http://www.ces.ncsu.edu/depts/ent/notes/O&T/trees/note119a/note119a.htm.

**Snyder, C. D., J. A. Young, D. P. Lemarle, and D. R. Smith. 2002.** Influence of eastern hemlock (*Tsuga canadensis*) forests on aquatic invertebrate assemblages in headwater streams. Can. J. Fish. Aquat. Sci. 59: 262.

**Soehn, D., G. Taylor, T. Remaley, and K. Johnson. 2005.** Draft environmental assessment of hemlock woolly adelgid control strategies in Great Smoky Mountains National Park. http://www.nps.gov/grsm/parkmgmt/upload/Hemlock-Woolly-Adelgid-Control-EA%5B1%5D.pdf.

**Spaulding, H. L., and L. K. Rieske. 2010.** The aftermath of an invasion: Structure and composition of central Appalachian hemlock forests following establishment of the hemlock woolly adelgid, *Adelges tsugae*. Biol. Invasions 12: 3135-3143.

**Spruce, J. P., S. Sader, R. E. Ryan, J. Smoot, P. Kuper, K. Ross, D. Prados, J. Russell, G. Gasser, R. McKellip, and W. Hargrove.** 2011. Assessment of MODIS NDVI time series data products for detecting forest defoliation by gypsy moth outbreaks. Remote Sens. Environ. 115: 27-437.

**SPSS. 2009**. SPSS for Windows, release 19.0 computer program, version 19.0. SPSS, Chicago, IL.

**(TDA) Tennessee Department of Agriculture. 2013**. Hemlock woolly adelgid. http://tn.gov/agriculture/regulatory/hwa.shtml

**Tredici, P. D., and A. Kitajima. 2004.** Introduction and cultivation of Chinese hemlock (*Tsuga chinensis*) and its resistance to hemlock woolly adelgid (*Adelges tsugae*). J. Arbor. 30: 282-287.

**Trotter, R. T., A. W. Courter, R. M. Turcotte, and B. Onken. 2008.** Incorporating hemlock woolly adelgid impacts into the forest vegetation simulator model, pp. 114-117. *In* B. Onken and R. Reardon (eds.), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 12-14 February 2008, U.S. Department of Agriculture, Forest Service, Hartford, CT.

**(USDA) U.S. Department of Agriculture. 2012.** Eastern hemlock, *Tsuga canadensis* (L.) Carrière. http://plants.usda.gov/plantguide/pdf/cs_tsca.pdf.

**(USDA) U.S. Department of Agriculture. 2013.** *Tsuga canadensis* (L.) Carrière -eastern hemlock. http://plants.usda.gov/java/county?state_name=Tennessee&statefips=47&symbol=TSCA

**(USDA FS) U.S. Department of Agriculture Forest Service. 2005.** Forest Inventory and Analysis National Core Field Guide: Field data collection procedures for Phase 2 plots. Version 3.0. http://www.srs.fs.usda.gov/fia/data_acquisition/field_guide/Final/SRS_East_Ver_3_2006.pdf

**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.

**Vose, J. M., C. R. Ford, B. D. Clinton, B. D. Kloeppel, K. J. Elliott, and J. D. Knoepp. 2008.** Effects of eastern hemlock mortality on riparian ecosystems in the southern Appalachians, pp. 178. *In* B. Onken and R. Reardon (eds.), Fourth Symposium on

Hemlock Woolly Adelgid in the Eastern United States, 12-14 February 2008, U.S. Department of Agriculture, Forest Service, Hartford, CT.

**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, B. P. Onken, and R. S. Cowles. 2004.** Eastern hemlock forests: Guidelines to minimize the impacts of hemlock woolly adelgid. U.S. Department of Agriculture, Forest Service, Morgantown, WV. pp. 27.

**Webster, R. J. 2010.** Management of hemlock woolly adelgid in Great Smoky Mountains National Park, pp. 57-60. *In* B. Onken and R. Reardon (eds.), Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 17-19 August 2010, U.S. Department of Agriculture, Forest Service, Asheville, NC.

**Welch, H., and G. Haddow. 1993.** The world checklist of conifers. Landsman's Bookshop Ltd., Herefordshire, England.

**Welch, R., M. Madden, and T. Jordan. 2002.** Photogrammetric GIS techniques for the development of vegetation database of mountains areas: Great Smoky Mountains National Park. J. Photogrammetry Remote Sens. 57: 53-68.

**Whitehead, V. B. 1967.** The validity of the higher taxonomic categories of the tribe Scymnini (Coleoptera: Coccinellidae). Ph.D. Thesis. University of California, Berkley. 215 pp.

**Wiggins, G., A. Hakeem, R. Follum, D. Paulsen, R. Rhea, P. L. Lambdin, and J. F. Grant. 2010.** Seasonality of established populations of *Sasajiscymnus tsugae* on eastern hemlock

in the southern Appalachians, pp. 203-205. *In* B. Onken and R. Reardon (eds.), Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 17-19 August 2010, U.S. Department of Agriculture, Forest Service, Asheville, NC.

**Yamasaki, M., R. M. DeGraaf, and J. W. Lanier**. 1999. Wildlife habitat associations in eastern hemlock - birds, smaller mammals, and forest carnivores, pp. 135-143. *In* K. A. McManus, K. S. Shields and D. R. Souto (eds.), Proceedings, Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America, 22-24 June 1999, U.S. Department of Agriculture, Forest Service, Durham, NH.

**Yost, E. C., K. S. Johnson, and W. F. Blozan. 1994.** Old-growth project stand delineation and disturbance rating in Great Smoky Mountains National Park. Technical Report NPS/SERGRSM/NRTR.

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

**Yu, G., D. Yao, and H. Liu. 1997.** The Coccinellidae collected from *Tsuga* with *Adelges tsugae* Annand (Homoptera: Adelgidae). Sci. Silvae Sin. 33: 432-440.

**Yu, G., M. E. Montgomery, and D. Yao. 2000.** Lady beetles (Coleoptera: Coccinellidae) from Chinese hemlocks infested with the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Coleopts. Bull. 54: 154-199.

**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). Bio. Control 24: 192-198.

**Zilahi-Balogh, G. M. G., S. M. Salom, and L. T. Kok. 2003**. Development and reproductive biology of *Laricobius nigrinus*, a potential biological control agent of *Adelges tsugae*. Bio. Control. 48: 293-306.

**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. Entomol. 138: 595-601.

# APPENDIX

**Release sites of *Sasajiscymnus tsugae* (*n* = 65) sampled between 2008 and 2012, Great Smoky Mountains National Park.**

| Site Name | Year Released | No. Released | Date Released | Date Sampled | Research Usage* |
|---|---|---|---|---|---|
| Anthony Creek | 2002 | 2,923 | 26-Jun | 13-Jun-08 | 1, 2, 3, 5 |
| Buckhorn Gap 1 | 2002 | 3,118 | 25-Jun | 17-Jul-08 | 1, 2, 3, 5 |
| Gregory Ridge 1 | 2002 | 3,396 | 25-Jun | 19-Jun-08 | 1, 2, 3, 4, 5 |
| Horse Pen Ridge | 2002 | 5,326 | 01-Jun | 18-Jun-12 | 1, 2 |
| Laurel Falls 1 | 2002 | 2,039 | 03-Jun | 11-Jun-08 | 1, 2, 3, 5 |
| Laurel Falls 2 | 2002 | 1,940 | 03-Jun | 11-Jun-08 | 1, 2, 5 |
| Laurel Falls 3 | 2002 | 848 | 10-Jul | 11-Jun-08 | 1, 2, 5 |
| Lynn Camp | 2002 | 3,434 | 25-Jun | 5-Jun-08 | 1, 2, 3 |
| Panther Creek | 2002 | 2,998 | 03-Jun | 15-Jun-09 | 1, 2, 3, 4, 5 |
| Stony Branch | 2002 | 3,953 | 02-Jun | 19-Jun-08 | 1, 2, 3 |
| Gregory Ridge 2 | 2003 | 2,500 | 09-Apr | 15-Jun-10 | 1, 2, 3, 4, 5 |
| Jakes Creek | 2003 | 4,000 | 24-Feb | 14-May-08 | 1, 3, 5 |
| Rush Branch | 2003 | 2,500 | 24-Apr | 1-May-08 | 1, 2, 3, 5 |
| Tunnel Ridge | 2003 | 2,546 | 05-Jun | 9-Jun-11 | 1, 2 |
| Allnight Ridge | 2004 | 2,489 | 04-Apr | 4-Jun-10 | 1, 2 |
| Arbutus Ridge | 2004 | 2,571 | 14-May | 1-May-08 | 1, 2 |
| Beech Flats | 2004 | 2,785 | 24-May | 12-May-09 | 1, 2, 3, 5 |
| Boring Ridge | 2004 | 2,500 | 22-Apr | 28-May-09 | 1, 2 |
| Bull Head | 2004 | 2,500 | 02-Mar | 7-Jun-10 | 1, 2 |
| Gregory Ridge 3 | 2004 | 2,500 | 24-Feb | 28-Apr-09 | 1, 2, 3, 5 |
| Trillium Gap 1 | 2004 | 2,663 | 02-Mar | 23-Jun-10 | 1, 2 |
| Bradley Fork 1 | 2005 | 1,125 | 27-Jan | 16-Jun-09 | 1, 2, |
| Bradley Fork 2 | 2005 | 1,035 | 27-Jan | 15-Jun-09 | 1, 2, |
| Bunker Hill Lead | 2005 | 3,460 | 16-Jun | 8-Jun-10 | 1, 2 |
| Chasteen Creek | 2005 | 2,370 | 19-May | 16-Jun-09 | 1, 2 |
| Pine Ridge | 2005 | 1,953 | 17-Mar | 8-Jun-10 | 1, 2 |
| Rabbit Ridge | 2005 | 2,376 | 17-Feb | 18-Jun-10 | 1, 2 |
| Ramsey Branch | 2005 | 1,000 | 24-Jan | 4-May-11 | 1, 2 |
| Sag Branch | 2005 | 1,014 | 30-Jun | 18-Jun-10 | 1, 2 |
| Trillium Gap 2 | 2005 | 1,502 | 31-Mar | 29-Jun-10 | 1, 2 |
| Balsam Point | 2006 | 2,500 | 30-Mar | 3-Jun-10 | 1, 2, 3, 5 |
| Baxter Creek Trail 1 | 2006 | 1,500 | 12-Jun | 19-May-09 | 1, 2 |
| Baxter Creek Trail 2 | 2006 | 2,956 | 09-Feb | 19-May-09 | 1, 2 |

| Site Name | Year Released | No. Released | Date Released | Date Sampled | Research Usage |
|---|---|---|---|---|---|
| Bloody Branch 1 | 2006 | 2,500 | 07-Apr | 21-May-09 | 1, 2, 3, 4, 5 |
| Buckeye Creek 1 | 2006 | 2,000 | 13-Apr | 4-May-09 | 1, 2 |
| Buckeye Creek 2 | 2006 | 2,045 | 22-Feb | 4-May-09 | 1, 2, 3, 5 |
| Chimney Tops | 2006 | 1,731 | 26-Jan | 6-May-10 | 1, 2 |
| Dry Branch 1 | 2006 | 2,062 | 22-Feb | 20-Mar-09 | 1, 2 |
| Dry Branch 2 | 2006 | 2,500 | 06-Apr | 2-Jun-09 | 1, 2 |
| Jones Branch | 2006 | 1,946 | 23-Mar | 24-Feb-09 | 1, 2 |
| Mountain Grove School | 2006 | 2,450 | 09-Feb | 8-Jul-10 | 1, 2, 3, 5 |
| Ramp Creek | 2006 | 2,500 | 09-Mar | 27-May-10 | 1, 2 |
| Rocky Spur | 2006 | 2,588 | 22-Jun | 23-Jun-10 | 1, 2 |
| Scott Gap 1 | 2006 | 2,500 | 23-Mar | 13-May-09 | 1, 2 |
| Trillium Branch 1 | 2006 | 2,500 | 16-Jun | 29-Jun-10 | 1, 2, 3, 5 |
| Baxter Creek Trail 3 | 2007 | 2,753 | 08-Feb | 19-May-09 | 1, 2 |
| Bloody Branch 2 | 2007 | 2,356 | 09-May | 21-May-09 | 1, 2 |
| Bloody Branch 3 | 2007 | 3,168 | 28-Feb | 21-May-09 | 1, 2 |
| Brushy Mountain | 2007 | 2,804 | 22-Mar | 29-Jun-10 | 1, 2, 3, 4, 5 |
| Buckhorn Gap 2 | 2007 | 1,397 | 09-May | 17-Jul-08 | 1, 2, 3, 5 |
| Cat Stairs | 2007 | 3,032 | 14-Mar | 25-May-11 | 1, 2 |
| Clontz Branch | 2007 | 2,492 | 22-Feb | 18-Jun-10 | 1, 2 |
| Cole Creek | 2007 | 2,592 | 02-May | 21-Mar-10 | 1, 2 |
| Cooks Creek | 2007 | 2,821 | 02-May | 24-May-11 | 1, 2 |
| Cosby Knob | 2007 | 2,461 | 22-Feb | 6-Apr-11 | 1, 2 |
| Double Gap Ridge | 2007 | 2,508 | 22-Feb | 17-Jun-10 | 1, 2 |
| Hurricane Creek | 2007 | 2,462 | 06-Mar | 17-Jun-10 | 1, 2 |
| Leatherwood Branch | 2007 | 2,365 | 08-Mar | 13-May-10 | 1, 2 |
| Little Bird Branch | 2007 | 2.365 | 31-Jan | 12-May-09 | 1, 2, 3, 4, 5 |
| Little Rock Creek | 2007 | 2,451 | 24-Jan | 12-Apr-10 | 1, 2, 3, 5 |
| Ramsey Cascades | 2007 | 2,386 | 03-May | 10-Mar-09 | 1, 2, 3, 5 |
| Scott Gap 2 | 2007 | 2,535 | 14-Mar | 13-May-09 | 1, 2 |
| Scratch Britches | 2007 | 2,373 | 03-May | 3-Jun-10 | 1, 2 |
| Sugar Cove Campsite 1 | 2007 | 2,533 | 02-May | 25-May-10 | 1, 2 |
| Sugar Cove Campsite 2 | 2007 | 2,672 | 02-May | 26-Mar-10 | 1, 2 |

*Research usage definitions: 1) evaluated for establishment of *S. tsugae* (Chapter 1), 2) included in analysis of factors contributing to establishment of *S. tsugae* (Chapter 3), 3) included in evaluation of agreement between field-observed and imagery-depicted hemlock (Chapter 4), 4) included in assessment of hemlock health (Chapter 4), and 5) included in evaluation of hemlock mortality (Chapter 4).

# Vita

Abdul Hakeem was born and raised in Gilgit where he received his primary and secondary education. He received a Master of Science Degree in Agriculture from North West Frontier Province (NWFP) Agricultural University Peshawar. Abdul Hakeem worked with North South Seeds, Aga Khan Rural Support Program Gilgit for three years. He has worked as an Agriculture Officer in Department of Agriculture, Gilgit since 2001. He received a Fulbright scholarship to pursue Master of Science Degree in Entomology and Plant Pathology. He received Master of Science Degree at the University of Tennessee in Department of Entomology and Plant Pathology under supervision of Dr. Jerome Grant. Abdul Hakeem received his Ph.D. degree in Plants, Soils and Insects from the University of Tennessee. During his M.S. and Ph.D. programs at the University of Tennessee, he presented several oral and poster presentations and received six poster and three other awards. Abdul Hakeem is a member of the Entomological Society of America, Tennessee Entomological Society, Gamma Sigma Delta Agricultural Honor Society, Fulbright Association, and University of Tennessee Alumni Association.