Forest Health Technology Enterprise Team

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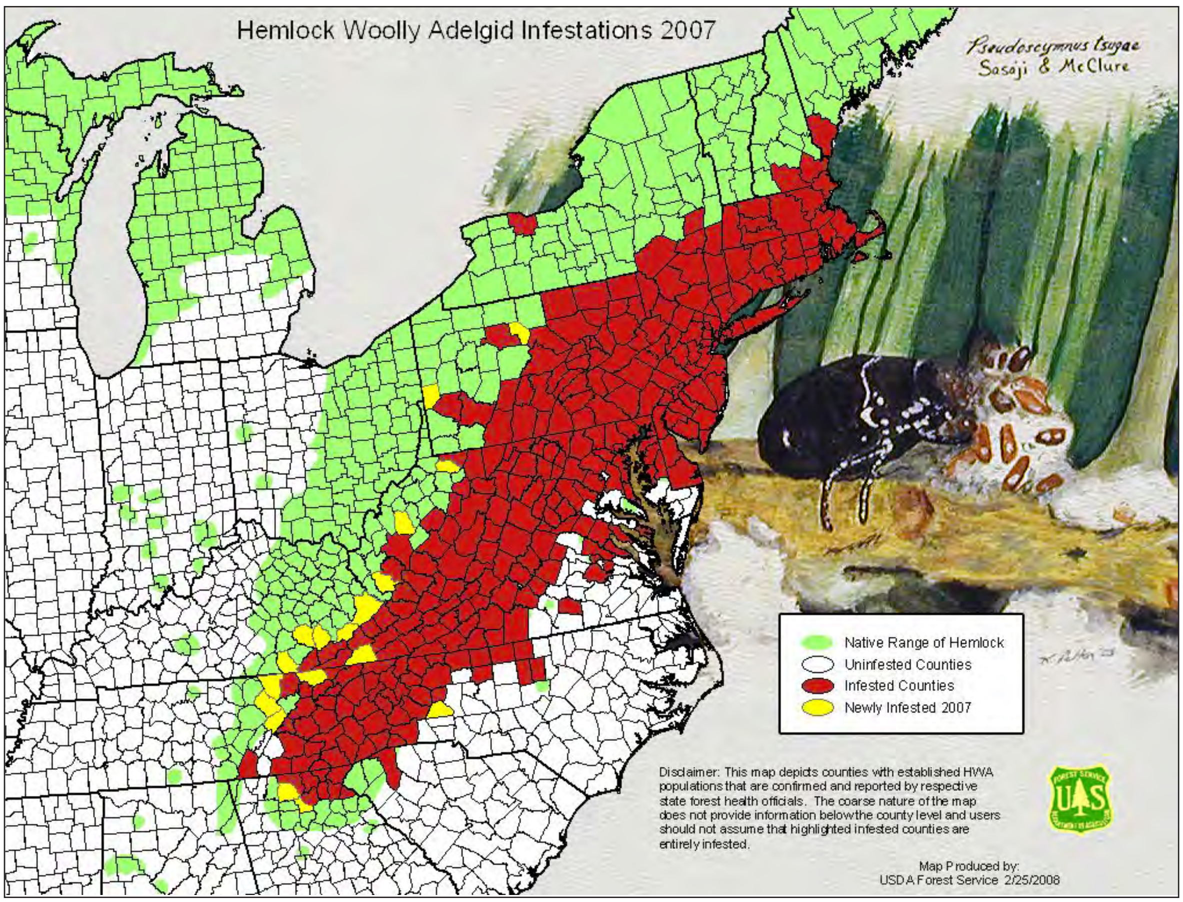
Hemlock Woolly Adelgid

Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States

Hartford, Connecticut
February 12-14, 2008



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Foreword

At the request of the National Association of State Foresters and the National Plant Board, the USDA Forest Service initiated a program that would accelerate development and implementation of management options to reduce the spread and impact of hemlock woolly adelgid. From 2003-2007, the USDA Forest Service implemented the "Hemlock Woolly Adelgid Initiative," which involved scientists and resource managers from four federal agencies, 20 state agencies, 24 universities, seven institutions in China and Japan, and more than nine private companies. Accelerated research and technology development during this five-year period resulted in a significant increase in our knowledge of hemlock woolly adelgid, its impact on hemlock resources, and the development of new management strategies. The manuscripts and posters presented at the Fourth Symposium on Hemlock Woolly Adelgid reflect much of this collective knowledge.

There were a tremendous number of accomplishments in the past five years, but the job is not done. Two general areas that require further effort are: 1) effective and efficient landscape-level treatments to minimize hemlock woolly adelgid damage; and 2) strategies for rehabilitation of damaged areas. As we move forward with the Initiative, continued cooperation and the sharing of ideas and information among the research and management communities will be essential if we hope to address these areas successfully in the shortest term possible. The progress thus far brings us much closer to a solution.

There have been three previous symposia addressing all aspects of hemlock woolly adelgid: in 1995, First Hemlock Woolly Adelgid Review, Charlottesville, Virginia; in 2002, Hemlock Woolly Adelgid in the Eastern United States Symposium, East Brunswick, New Jersey; and in 2005, Third Symposium on Hemlock Woolly Adelgid in the Eastern United States, Asheville, North Carolina. Proceedings from these and the Fourth Symposium on Hemlock Woolly Adelgid held in Hartford, Connecticut, February 12-14, 2008 is now available on a single CD ROM or separately on the web at: http://na.fs.fed.us/fhp/hwa.

Welcoming Address

Donald Smith
Connecticut State Forester

Connecticut is honored to host the Fourth Hemlock Woolly Adelgid Symposium and to be, at least for a short while, the focal point of the hemlock woolly adelgid research world. After all, this state has over 370 years of experience with non-native invasives. For the forests of Connecticut, it all began in the year 1636, when English settlers (the first non-native invasive species) arrived and settled the plantations of Windsor, Hartford, and Wethersfield alongside the Connecticut River. As has been the case with many invasive species, it has taken many, many years for the forest environment to adapt to the new presence and establish an uneasy balance.

In this case, it took about 200 years for the two-legged invaders to drive the forest out of about 85% of the state for agriculture and industry. It then it took the next 150 years for the forest to reclaim lost ground and return to cover 60% of the state. Over the past two decades or so, the two have tried to co-exist responsibly, but, of late, there have been signs that the forest is losing ground once again.

I have been looking forward to this opportunity to welcome you all to Connecticut. There are 47 other states in the Union that are larger than Connecticut; we may be a small state, but what we lack in size, we make up for in complexity. We occupy just 5,565 square miles, and 12.6% of that is covered by water, so we've really only got about 4,865 square miles of dry land. But we put that dry land to real good use as we pack an average of 722 people onto every acre—and there are only three other states that pack more people onto each square mile.

Because we're squeezed in between New York City and Boston, lots of folks around the rest of the nation assume we must be all concrete, asphalt, and buildings and that we have no trees—or that any trees that may be here are being held hostage in a few city parks. My wife was one of those people. In 1972, when she made her first drive from Maine to Connecticut to visit my family, she was astounded to find forests and fields and—my God!—dirt roads! Like so many others, she thought we'd been paved over long ago.

Yes, people are shocked as they look out the airplane windows on the descent into Bradley International Airport. They see acres and acres of green forest and agricultural fields. Admittedly, when the leaves are off the trees at this time of year, you do see houses under many of those trees, but, in the summer, with the tree canopies all greened up, it's pretty impressive. And it is impressive, because Connecticut is now about 57% forest.

When you do the comparison, you find that there are only 12 states that have a higher percentage of forest cover than Connecticut. States like Michigan, Wisconsin, Pennsylvania, Washington, Alaska, Oregon, California, Minnesota, and Colorado may have lots of forest, but Connecticut, the green jewel flanked by the major metropolitan centers of Boston and New York, has a higher percentage of forest cover than any of those states.

Connecticut is ranked thirteenth in percentage of forest cover and yet fourth in population density. There are few places on Earth where so many people live, work, and play amidst so much forest. The forest here is the backdrop to our lives. It is a key factor in the quality of life in this state.

Intuitively, people who live here know that—and that's why people here are so concerned when a new non-native invasive insect or disease threatens their forest. People here fear the approach of new threats such as sudden oak death, emerald ash borer, and Asian long-horned beetle. They fear it because they have experienced the impact of chestnut blight in the early 1900s, Dutch elm disease in the 1930s, red pine scale in the mid- to late-1900s, gypsy moth throughout the 1900s, and now hemlock woolly adelgid.

Eastern hemlock is an important component of Connecticut's mix of tree species. It is the seventh-most common tree in the state and contributes significantly to air quality, water quality, and aesthetic values. Hemlock is one of few conifers that persist in shaded conditions, and it provides a crucial element within forest ecosystems across the state. For homeowners, hemlock has long been a favored selection within the residential landscape for screening, windbreaks, and specimen trees.

Hurricane Gloria hammered the east coast in September of 1985. In the following growing season, Dr. Mark McClure of the Connecticut Agricultural Experiment Station found the adelgid along the southern Connecticut coastline, on higher elevations inland, and along the banks of the Housatonic and Connecticut Rivers. In short order, we began seeing hemlock mortality in forested environments. When it arrived in Connecticut, the hemlock woolly adelgid immediately established itself as much, much more than simply an annoying but easily controlled ornamental pest.

It was in Connecticut that this pest crossed the threshold from homeowners' yards to the realm of the forest – with dire environmental and ecological implications for New England and the Canadian Maritimes. It is not too late to respond. The adelgid has been in Connecticut forests for 20 years, and while hemlock is having a tough time in some areas, it's still here. Barring some natural catastrophe, eastern hemlock as a species will not become extinct: it will remain a part of the forest throughout its natural range for at least a few more decades.

So, while every passing day does raise the ante, we still have time. Rather, you have time. I say you have time, because to a great extent, the fate of eastern hemlock lies in your hands. You—your minds, your commitment to good science, your determination, your . . . hearts—hold the key that will unlock the secrets of stopping the deadly march of hemlock woolly adelgid. Some day, one of you—or one who will come after you—will find the control or the management tool that will allow the forest environment to adapt, once again, to a non-native invader and establish a balance—however uneasy—once more. The people of Connecticut and all who understand the vital place of eastern hemlock throughout its range pray that the day will come soon.

So welcome to Connecticut—and now, to work!

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The Historical and Future Impacts of Exotic Insects and Diseases on Connecticut's Forests

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Nearly 60 percent of Connecticut is a forested mosaic of oaks and pines, maples and birches, and hemlocks and ashes. The Nutmeg State also has extensive forest cover in our cities, towns, and suburbs. These forests filter drinking water, support diverse wildlife habitats, provide opportunities for outdoor recreation, and supply timber for a vibrant forest products industry. Responsible stewardship of our forest by this generation will provide future generations with healthy, sustainable forests.

Since the arrival of humans in the western hemisphere over 10,000 years ago, exotic species have had a profound impact on the ecosystems. Increases in the speed and volume of trade between continents have accelerated the introduction of insects and diseases. Several of these exotic pests, such as gypsy moth (*Lymantria dispar*), have killed and damaged millions of trees in both managed landscapes and natural forests. Our responsibility—and hopefully our passion—is to develop effective cultural, biological, and chemical treatments that eliminate, or at least mitigate, the effects exotic pests have once established.

National estimates of the number of introduced insects and diseases have not been updated for over a decade (Pimentel et al. 2005). Mattson et al. (1994) listed 368 introduced insect species in the United States, a list that does not include two species, Asian longhorned beetle (*Anoplophora glabripennis*) and emerald ash borer (*Agrilus planipennis*), which will likely be found in Connecticut within several years. Haack and Byler (1993) reported that 20 exotic diseases found in North America attacked woody plants, many of which are in Connecticut. Although no Connecticut estimates are available, annual forest productivity losses in the United States due to exotic insect and diseases are approximately $4.2 billion dollars (Pimentel et al. 2005). This does not include losses caused by damage to shade and street trees, nor the money expended to protect them from pests.

At least 17 exotic insects and eight exotic diseases that attack forest trees have been found in Connecticut (Table 1). Some are cryptic and rarely found, others have caused widespread damage, and a few have completely changed the character of Connecticut's natural and urban forests. I will begin by highlighting the lasting changes caused by chestnut blight, gypsy moth, and hemlock woolly adelgid (*Adelges tsugae*). I will conclude by offering my opinions on the potential damage if emerald ash borer and Asian longhorned beetle become established in Connecticut and if Ramorum blight (*Phytophthora ramorum*) can survive in southern New England.

Table 1. Exotic insects and diseases that have been found in Connecticut and those established in the United States that are a risk for Connecticut's trees.

Common Name	Latin Name	Host(s)
Insects		
Balsam woolly adelgid	*Adelges piceae*	Abies
Hemlock woolly adelgid	*Adelges tsugae*	*Tsuga*
Lesser Japanese cedar longhorned beetle	*Callidiellum rufipenne*	Thuja
Violet tanbark beetle	*Callidium violaceum*	Conifers
Beech scale	*Cryptococcus fagisuga*	Fagus
Hemlock elongate scale	*Fiorinia externa*	*Tsuga*
Satin moth	*Leucoma salicis*	Populus
Gypsy moth, European	*Lymantria dispar*	Many
Red pine scale	*Matsucoccus matsumurae*	*Pinus*
Circular hemlock scale	*Nuculaspis tsugae*	Conifers
Winter moth	*Operophtera brumata*	Many
Red pine adelgid	*Pineus borneri*	*Pinus*
Elm leaf beetle	*Pyrrhalta luteola*	*Ulmus*
Viburnum leaf beetle	*Pyrrhalta viburni*	Viburnum
European Pine Shoot Moth	*Rhyacionia buoliana*	*Pinus*
European elm bark beetle	*Scolytus multistriatus*	*Ulmus*
Banded elm bark beetle	*Scolytus schevyrewi*	*Ulmus*
Diseases		
Dutch elm disease	*Ceratocystis ulmi*	*Ulmus*
White pine blister rust	*Cronartium ribicola*	*Pinus*
Chestnut blight	*Cryphonectria parasitica*	Castanea
Dogwood anthracnose	*Discula destructive*	Cornus
Red band needle blight	*Mycosphaerella pini*	*Pinus*
Butternut canker	*Sirococcus clavigignenti-juglandacearum*	Juglans
Willow scab fungus	*Venturia saliciperda*	*Salix*
X-disease	X-*phytoplasma*	Prunus
Future threats		
Emerald ash borer	*Agrilus planipennis*	Fraxinus
Asian longhorned beetle	*Anoplophora glabripennis*	Acer
Oriental chestnut gall wasp	*Dryocosmus kuriphilus*	Castanea
Gypsy moth, Asian	*Lymantria dispar* sp.	Many
Ramorum blight	*Phytophthora ramorum*	*Quercus*
Siricid woodwasp	*Sirex noctilio*	*Pinus*
Diplodia blight	*Sphaeropsis sapinea*	*Pinus*
Eurasian pine shoot beetle	*Tomicus piniperda*	*Pinus*

CHESTNUT BLIGHT

While not the first exotic pest to arrive in North America, chestnut blight (*Cryphonectria parasitica*) has arguably had the greatest impact on the eastern deciduous forest. (Note: Much of the beginning of this section was heavily borrowed from Anagnostakis 2007.) American chestnut (*Castanea dentata*) was described as "the best tree that any one can plant for timber and other purposes" in the 1877 Report of the Connecticut Board of Agriculture. Because of its fast growth rate, high-quality timber, and predominance in Connecticut, the first forest management experiments established in 1905 by scientists at The Connecticut Agricultural Experiment Station focused on chestnut. Two years later, in 1907, chestnut blight was first reported in Connecticut. The majority of American chestnut trees succumbed before 1920. It is hard to fathom the changes wrought on the forest by chestnut blight. Prior the appearance of the disease, the 130 million chestnuts accounted for 25 to 40 percent of trees in Connecticut. Now, this former stately giant is relegated to the status of an understory shrub. Chestnuts were not only an important component of the forest products industry, but provided a reliable source of high quality mast for forest animals.

Austin Hawes (1907), a forester at The Connecticut Agricultural Experiment Station, estimated that 50-yr-old chestnut plantations could produce 23,000 board feet, roughly twice that now found in 100-yr-old oak stands. In other words, chestnut grew at rates comparable to, albeit less, than those of southern pines. Forest management in Connecticut and the entire eastern hardwood forest might have been very different if chestnut blight had never arrived. Because chestnut is a very fast-growing tree with an attractive wood that is extremely rot resistant, it is easy to imagine that a large proportion of our forests would have converted to nearly pure chestnut stands, a hardwood mirror of the southern pine plantations.

The demise of the chestnut allowed oaks to become the dominant species in Connecticut. By 1938, twenty-one years after the arrival of chestnut blight, oaks were the predominant species on 75 percent of Connecticut's forest (Ward and Barsky 2000). The impact to wildlife was somewhat minimized because oaks, like chestnut, produce a hard mast high in protein and fats that is readily utilized by both mammals and birds. It has been suggested that gypsy moth was not a problem until after chestnut's decline because chestnut is not a favored food of gypsy moth (Smith 1976). Thus, the effect of one exotic disease (chestnut blight) set the stage for an exotic insect (gypsy moth). As will be discussed later, the mortality that followed gypsy moth defoliation has been at least partly responsible for the increasing dominance of red maple (*Acer rubrum*) and black birch (*Betula lenta*).

The future of American chestnut is promising. Research in Connecticut and elsewhere is producing hybrids with the form of American chestnut and the disease resistance of the Asian species (Kubisiak et al. 1997). It is probable that future generations of foresters and natural resource managers will re-introduce this species back into our forests – perhaps returning American chestnut to its former preeminence (Anagnostakis 2001).

GYPSY MOTH

Since its accidental introduction outside of Boston in the late 1800s, gypsy moth has spread to at least 17 eastern states (Morin et al. 2005). It was first reported in southeastern Connecticut in 1905. However, the first large-scale defoliation (1,000 acres) did not occur until several decades later, in 1938. A wide range of control measures—from painting egg masses to spraying trees to smashing caterpillars—were tried to eradicate gypsy moth, measures that were, as we now know, unsuccessful.

During the 1960s and 1970s, there were multi-year episodes in which hundreds of thousands of acres were defoliated. The most severe outbreak occurred in 1981, when nearly 1.5 million acres (80% of 1.86 acres of forested land) had some degree of defoliation (Figure 1). Another episode of large-scale gypsy moth defoliation appeared to be imminent in 1989 when a gypsy moth fungus (*Entomophaga maimaiga*), discovered by scientists at The Connecticut Agricultural Experiment Station, killed nearly all caterpillars (Andreadis and Weseloh 1990). Since then, there have been minor defoliations, most notably in 2006, but none of the scale or duration of earlier outbreaks.

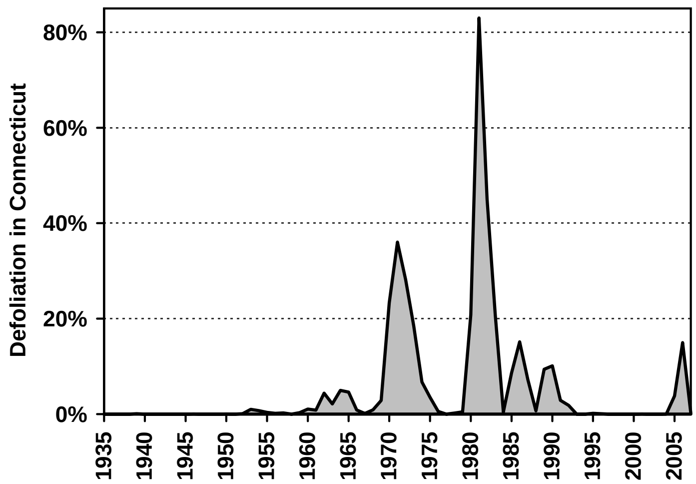

Figure 1. Forest defoliation in Connecticut from 1935-2007. Sources: 1935-1980, Anderson (1980); 1981-2007, Department of Entomology, The Connecticut Agricultural Experiment Station.

Our research on permanent plots established in 1926-1927 and monitored at 10-year intervals allowed us to examine both the immediate and long-term impact of defoliation on Connecticut's forests (Stephens 1971, Ward et al. 1999, Ward 2007). The immediate effect of multiple years of defoliation was increased mortality of oaks (*Quercus*) and hickories (*Carya*), especially those growing in the subcanopy. Mortality of subcanopy oak peaked at over 80 percent between 1957 and 1967. Much of the mortality following defoliation was caused by secondary agents, such as twolined chestnut borer (*Agrilus bilineatus*) and shoestring root rot (*Armillaria mellea*), which attack weakened trees (Dunbar and Stephens 1975).

Stand basal area, an indicator of total biomass, actually decreased following the initial defoliation events and allowed a pulse of ingrowth. When trees die, the growing space they occupied is released. Growth and survival of understory red maple increased during defoliation episodes (Collins 1961). The number of new birch, maple, and beech (*Fagus grandifolia*) nearly tripled in response to the now-available growing space. American chestnut, sassafras (*Sassafras albidum*), and flowering dogwood (*Cornus florida*) ingrowth also increased dramatically.

The longer-term impact of defoliation has been an acceleration of succession in our forests. While the oaks would have eventually been replaced by maple, birch, and beech without the mortality initiated by repeated defoliation, this process was advanced at least several decades.

These changes will affect, not only the quality and makeup of forest products available to future generations, but the quality and variety of wildlife habitats. The resulting flush of ingrowth also created brushy understories with abundant succulent foliage. Perhaps it just a coincidence, but the white-tailed deer (*Odocoileus virginianus*) population in Connecticut began its dramatic population explosion during this period (Ward 1996). This increase in deer population has exacerbated the difficulty in obtaining adequate oak regeneration. Through a complicated feed-back loop, gypsy moth populations may also play a role in the incidence of Lyme disease (Jones et al. 1998). Thus, the impact of exotic insects such as gypsy moths can be felt across multiple trophic levels, including trees, mammals, ground layer regeneration, ticks, and human disease.

HEMLOCK WOOLLY ADELGID

Eastern hemlock (*Tsuga canadensis*) comprises the largest component (41 percent) of conifers in the Connecticut forest (Miles 2008). Although hemlock can be found from ridgetops to swamps, the best development is on moist, fertile soils with a northern aspect. The economic value of hemlock as a timber species is secondary to its importance in protecting watersheds, enhancing habitat diversity, and its esthetic appeal. The deep, dense canopy of eastern hemlock provides vertical structure heterogeneity, thermal cover, forage, and habitat for a variety of mammals and birds. Hemlock stands regulate stream flow and moderate water temperature, resulting in unique aquatic habitats.

Hemlock woolly adelgid was first reported in Connecticut in 1985 (McClure 1987) and can now be found throughout the state. Many hemlock stands in the warmer southern counties have been completely altered by the loss of all, or significant proportions, of hemlock. Stand-wide mortality is less frequent in the northern part of the state, but large numbers of individual trees have died as the result of infestation, especially those in the larger diameter classes (Figure 2). The crowns of surviving trees infested with adelgid have become transparent and short. Decreased hemlock foliage has resulted in increased light levels on the forest floor.

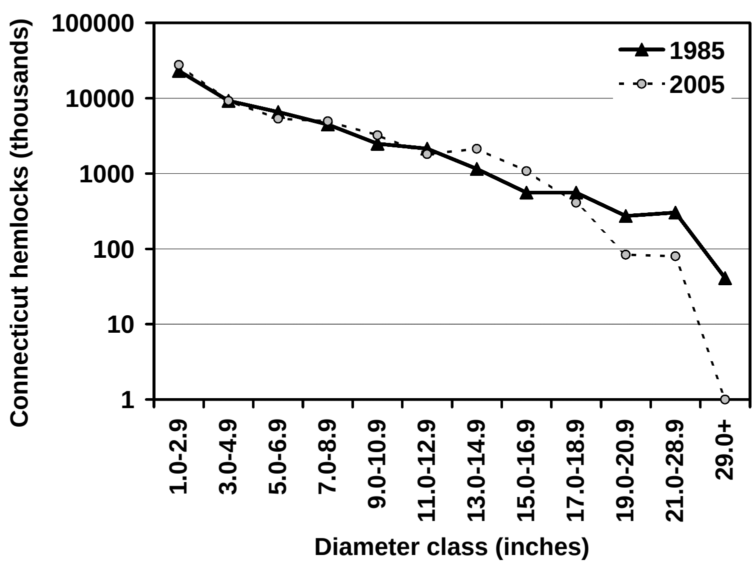

Figure 2. Number of eastern hemlocks in Connecticut prior to hemlock woolly adelgid (1985) and twenty years later (2005). Sources: Dickson and McAfee (1988) and Miles (2008).

The increased light initiated a flush of deciduous tree and shrub ingrowth. Black birch and red maple commonly replace hemlock in areas where the deer populations are low (Orwig 2002). The ecological and esthetic values associated with hemlock stands were lost when hemlock was replaced by these deciduous hardwoods.

More problematic are the vegetation communities that develop in areas with high deer populations. Nearly impenetrable thickets of Japanese barberry (*Berberis thunbergii*) and multiflora rose (*Rosa multiflora*) interwoven with vines of Oriental bittersweet (*Celastrus orbiculatus*) have developed in areas where deer are protected from hunting. These assemblages of invasive shrubs are not only associated with a paucity of forest regeneration and herbaceous plants, but as our recent work has found, these areas have blacklegged tick (*Ixodes scapularis*) densities that are nearly an order of magnitude greater than adjacent forests without barberry.

As noted in the section on gypsy moths, exotic insects can cause a cascade of changes across multiple trophic levels. In this case, mortality of eastern hemlock caused by the exotic hemlock woolly adelgid fostered development of a shrub layer dominated by thorny exotic species. The dense shrub thickets provided habitat favorable for high populations of white-footed mice (*Peromyscus leucopus*) that are the primary host of immature blacklegged ticks. While the short- and medium-term impacts of hemlock woolly adelgid are known, it is quite possible that additional effects as yet unforeseen will appear in the next several decades.

I am hopeful that the work being conducted by the scientists at this meeting and those unable to attend will develop the suite of biological controls that will allow hemlock to again flourish in the eastern forests. The persistence of hemlocks that have been repeatedly challenged by adelgids for nearly twenty years suggests that there is some inherent genetic resistance.

POTENTIAL THREATS

Unfortunately, it is likely that other exotic pests already established in the United States will soon be infesting Connecticut's forests. At this juncture, it appears that emerald ash borer (EAB) will cause the greatest damage. There are three ash species native to Connecticut: white ash (*Fraxinus americana*), green ash (*F. pennsylvanica*), and black ash (*F. nigra*), and the 10 million ashes in Connecticut's forest account for 3.5 percent of all trees (Miles 2008). Unless effective biological control measures for EAB are found in the very near future, ash will be largely extirpated from both forests and cities within twenty years. The ecological damage to our forests that will result from the loss of ash is difficult to judge but will include loss of thermal cover and small seeds consumed by wildlife (Poland and McCullough 2006).

The greatest impact of EAB may be experienced in our towns and cities. Street tree inventories indicate that ash comprises 3.4 percent of street trees in the suburbs and 2.9 percent of trees in New Haven. These percentages can be extended to estimate that along our streets there are 65,000 ash trees with diameters between 12-20 inches and 48,000 trees with diameters greater than 20 inches. The economic cost in lower real estate values, lost ecosystem services, and replanting is beyond this author to calculate, but in Connecticut, it will cost at least $20 to $50 million to remove ash trees along roads. The alternative is to have falling branches and trees disrupting power and phone lines, causing vehicle accidents and possibly injuring people with direct strikes.

Asian longhorned beetle (ALB) is just across the border in Queens, New York. It is fortunate that ALB infestations have been largely limited to towns and cities as maples—a favorite host—comprised 31 percent of all forest trees. Even a partial loss of maples and other species favored by ALB (e.g., *Salix*, *Ulmus*) will forever change our forests by lowering productivity, altering ecosystems, and muting fall colors. It is also fortunate that ALB infestations follow a pattern similar to southern pine beetles: if ALB escapes into our forests, management practices developed for southern pine beetle should provide a useful guide to controlling infestation foci. Maples comprise 30 percent or more of the street trees in Connecticut; as with EAB, ALB infestations will cause economic damage and disruption in developed areas.

CONCLUSIONS

I close by noting that both exotic insects and diseases have caused significant transformations in the composition of our forests. One hundred years ago, the Connecticut forest was dominated by stately American chestnut and had healthy populations of eastern hemlock, elm, and butternut. Gypsy moth was largely unknown. Now, chestnut has been reduced to a shrub, butternut and upland elms are nearly lost, and eastern hemlock is in decline. Restoration of these species and preservation of entire genera, including ash, will depend on your progress in selective resistance breeding and in developing biological, and chemical control. As a silviculturist and forest ecologist, I wish you good luck.

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Biological Control of Hemlock Woolly Adelgid: What Is it Going to Take to Make it Work?

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ABSTRACT

Biological control for hemlock woolly adelgid (HWA) has grown from a modest endeavor in the mid-1990s into the largest classical biological control program for a forest pest in North America today. There have been some notable advances in the past 15 years, but they often come with new challenges. Progress has not been linear. It is also realized that there is a finite period of time that we have to solve the HWA problem. Therefore, the approach we must take and communicate is to "hurry up and be patient." Establishment of some agents and several findings of potential new agents give hope to the prospects of a successful outcome from this effort. The expertise and infrastructure capacity that has been built over the past decade will be able to take advantage of continued investment. While no one can guarantee that biological control will eventually regulate HWA throughout its current and expanding range, it is possible that building a complex of natural enemies can contribute to the control of this pest particularly in areas where other biotic and abiotic factors come into play. Specific recommendations for areas to focus on in the future are given.

KEYWORDS

natural enemy complex, patience, teamwork, continued investment

THE PAST: BEGINNING IN 1992

The first decade of work in biological control of hemlock woolly adelgid is generally described in Cheah et al. (2004). The overall justification has been presented numerous times. There are 2.3 million acres of hemlock-dominated forests in the eastern U.S. No cultural or chemical control is known or likely to be developed to halt the continued spread and destruction of HWA over its expanding range. The early pioneers who identified potential agents in Japan were Mark McClure and Carole Cheah of the Connecticut Agricultural Experiment Station; in China, Mike Montgomery of the USDA Forest Service; and in western North America, Lee Humble of Forestry Canada. In 1996, the HWA Working Group (Kathleen Shields, Dick Reardon, Brad Onken, Rusty Rhea, and Dennis Souto) with outside input decided to invest a significant portion of the limited yet growing funding available for research and development and management of HWA toward biological control. This helped support the first major push of the study and release of *Sasajiscymnus* (= *Pseudoscymnus*) *tsugae* (*St*) (Coleoptera: Cocinellidae), from Japan, *Scymnus* spp. (Coleoptera: Cocinellidae) from China, and *Laricobius nigrinus* (Coleoptera: Derodontidae) from British Columbia and more recently Washington, Oregon, and Idaho. This was also followed by the screening and evaluation of potential pathogens by Bruce Parker and by Scott Costa and his associates at the University of Vermont.

Some highlighted accomplishments:

- Two surveys of native or established fauna associated with HWA in eastern North America were unable to detect any natural enemies with potential to help regulate populations of the target pest (Montgomery and Lyon 1996, Wallace and Hain 2000). This justified the effort toward finding natural enemies from western N. America and Asia.
- *Sasajiscymnus tsugae* was reared in high numbers first in Connecticut and subsequently in New Jersey at the New Jersey Department of Agriculture's (NJDA) Phillip Alampi Beneficial Insects Rearing Laboratory in Trenton. Eventually, labs at the University of Tennessee (UT), Clemson University, North Carolina Department of Agriculture in Raleigh, and a private company, Ecoscientific Solutions LLC, produced beetles in large numbers.
- Between 1995 and 2007, over 2 million *St* beetles were released in 16 states from Maine to Georgia. Although recovery has been limited, subsequent generations of this beetle have been found more than a mile from the nearest release site.
- Since 2004, 25,000 Chinese *Scymnus sinuanodulus* were released in 11 eastern states. Recovery of beetles has been confirmed at some sites. Climate analysis suggests that southern abiotic conditions are a better match for successful establishment of this predator and future releases will be limited to the southern range.
- Mass rearing techniques were developed for *L. nigrinus* at Virginia Tech. Beetles are now being reared at additional facilities at UT, Clemson University, University of Georgia, and the NJDA Phillip Alampi Beneficial Insects Rearing Laboratory. A field insectary at Virginia Tech was successfully established in 2001. Field collections from the Seattle area now provide consistent numbers of beetles for field releases and supplemental colonies at rearing facilities.
- Over 50,000 *Laricobius nigrinus* have been released at over 80 locations ranging from Georgia to Massachusetts since 2003. Beetles are established at nearly 60% of the monitored sites.
- Releases of all three beetle species have been carried out in a massive effort mostly on public land. This has been spearheaded by Brad Onken and Rusty Rhea, USDA Forest Service-Forest health Protection (FHP) in cooperation with multiple federal and state natural resource management agencies, universities, and one wild entomologist in the mountains of western North Carolina.
- Continued foreign exploration in western North America, China, and Japan has resulted in new natural enemies to study, adding to the biological control agent complex potential.
- Development of a whey-based fungal micro-factory technology to help grow *Lecanicillium muscarium* for use as a potential biopesticidal management option.

What were some of the challenges?

- Difficult to find very many *Sasajiscymnus tsugae* years after release; their establishment and efficacy are still in question in many areas.
- Finding a reliable source high quality food (HWA) to feed HWA predator colonies is an annual struggle for rearing labs.
- Mass rearing of *L. nigrinus* is difficult due to mortality during aestivation and the labor-intensive, time-consuming "hands on" effort required to produce large numbers of the predator.
- For foreign exploration, there have been several challenges:
  - trying to learn how to work through the bureaucracies of Japan and China, from permits for collections to approval to ship insects;
  - overcoming both the language and cultural differences between these countries and ours;
  - through 2006, there was limited long-term presence in these countries by a western scientist who can oversee the collection and transportation work.
- The scary notion that we are running out of time.

THE RECENT PAST AND THE PRESENT

Consistent support has been provided in recent years by the USDA Forest Service through the HWA Initiative. This has led to an unprecedented large-scale national and international effort toward the development of biological control for HWA. The fruits of this investment are described below.

New Agents Being Evaluated

- *Leucopis argenticollis* (Diptera: Chamaemyiidae) found by Glen Kohler (Oregon State University) has been observed to be a relatively common predator associated with HWA in western North America.
- A new *Laricobius* species has been discovered in Japan by Shigehiko Shiyake and Mike Montgomery, is currently being evaluated in Japan by Ashley Lamb, and is under quarantine in the U.S.
- A new effort is taking place to study *Tetraphleps galchanoides* (Hemiptera: Anthocoridae) in China, and it is under quarantine in the U.S.
- *Scymnus ningshanensis*, also from China, is close to being mass-reared for release on an operational basis.
- An interior Rocky Mountain strain of *L. nigrinus* is being studied at the Univeristy of Massachusetts (UMass) by Dave Mausel and Joe Elkinton to see if it can withstand the northern climate better than the coastal strain that has been reared and released since 2003.

Assessing Impacts

- Jerome Grant at UT, Joe Elkinton at UMass, and researchers at Virginia Tech are trying to quantify impacts that released predator species are having in the short and long term on HWA populations across the pest's range.
- Researchers at Virginia Tech have continued to assess the potential for competitive interaction among predator species and the compatibility of predators and insecticide use at the same locations.
- Ashley Lamb is carrying out a year-long assessment of natural enemy impacts on HWA in southern Japan.
- Virginia Tech is also developing a predator-release database to help us achieve long-term monitoring and assessment.

Rearing Natural Enemies

- The numerous labs mentioned earlier continue to refine their methodology for rearing predators. Virginia Tech produced over 15,294 *L. nigrinus* in fall 2007 and shipped most of them out for release to more than 27 new sites.
- Efforts with Lee Solter (University of Illinois) to ensure healthy predator colonies and that field-collected beetles are free from disease and contamination have become standard operating procedures.
- Development of artificial diets for predators is under investigation by Carole Cheah, Fred Hain, Alan Cohen, and colleagues.
- *Laricobius nigrinus* are now being harvested at the Virginia Tech field insectary.

DNA Diagnostic Testing

Differentiating among HWA and predator strains and closely related *Laricobius* species has become increasingly important. For example, *L. nigrinus* and *L. rubidus*, a native predator of pine bark adelgid that also feeds and reproduces on HWA, are often found together at release sites. Morphological differentiation of the larvae is not yet possible between these two species. Nathan Havill (Yale University) is developing the diagnostic techniques to help identify these and many other organisms we are finding in the field.

THE FUTURE: HURRY UP AND BE PATIENT

The learning curve for this system is steep. The tri-trophic interactions of the host plant and its ecosystem, the pest insect, and the growing potential natural enemy complex is not only very complicated, but also far from fully understood. Learning the biology of all these organisms and how they will interact within the hemlock ecosystem at landscape and regional levels takes time. We have now built a fairly large cadre of scientists and support staff who are able and eager to attack some of the more pressing questions that must be addressed. Additionally, resource managers throughout the eastern U.S. have also become familiar with this system. They provide the field support to implement biological control through release and monitoring of natural enemies. This enterprise in classical biological control is now the largest effort of its kind for any current forest pest in North America. The investment into building capacity in infrastructure and expertise has been remarkable. We are working in all areas with as much speed and effort as is possible without sacrificing the integrity of the effort. Nevertheless, after more than a decade, we cannot guarantee success.

As described above, progress continues to be made in foreign exploration, natural enemy evaluation, mass rearing of natural enemies, DNA diagnostics of the organisms in the system, development of release strategies, and the assessment of natural enemy establishment and impacts on the pest. But all this progress is not made easily or as quickly as we'd like. Every year, it seems as though we are making good progress in some areas, while at the same time, progress in other areas is stalled. Let's consider an example from our own lab.

Issues in Mass Rearing *Laricobius nigrinus*

Back in 2000, we started to spend a lot of time trying to develop a mass rearing technique for this insect faced with the following constraints: they feed only on HWA, are active in the winter (fall through spring), are dormant during the summer in soil, and only have one generation per year. The complexity of this effort is daunting. Just looking at the temperature we need to hold the different lifestages over the course of their life-cycle was a major challenge. In addition, the density of beetles for each type of container, the type of soil mixture, moisture, and depth all needed to be determined. Remarkably within three years, by 2002, we produced 21,000 adults. Unfortunately they emerged early and over 80% died. Lamb et al. (2007) solved this problem by maintaining the aestivating adults at higher temperatures (Table 1). This allowed us to control the date of emergence and time it to coincide with the onset of finding developing HWA in the field. So after this, it should have been smooth sailing.

Table 1. Temperature and photoperiod used for mass rearing *L. nigrinus* (after Lamb et al. 2005).

Life Stage of Predator	Temperature °C	Photoperiod (L:D)
Ovipositing adults (winter/spring)	4° day / 2° night	9.5 : 14.5
Larvae (winter/spring)	13°	12 : 12
Pupae (spring) for 6 weeks	13°	12 : 12
Aestivating adults (summer)	19°	12 : 12
Terminating aestivation	13°	12 : 12
Emerging adults (temps change at 2-week intervals)	10° day / 4° night 6° / 4° 4° / 2°	12 : 12 to 9.4 : 4.5

But we began doing two things that stopped our progress. First, we were sterilizing the soil mixture prior to use; second, we tried to streamline an extremely labor-intensive effort to save time and improve efficiency. Both brought about setbacks. Sterilization of the soil mixture appeared to allow saprophytic fungi to colonize the soil and make it unsuitable for aestivating beetles. Our streamlining effort involved putting more beetles into oviposition containers, more developing larvae into each modified Burlese funnel, using larger and therefore fewer aestivation storage containers, and less overall oversight in monitoring potential problems at each step of the way. As a result of these changes, we went from producing 8,000 beetles in 2004 to producing 3,430 beetles in 2005 and then down to 2,019 in 2006. We were going in reverse.

To address this slide, we went back to the fundamentals. Starting in 2006, we no longer sterilized the soil mixture. In 2007, we went back to the lower density of ovipositing beetles in containers and went back to the small soil containers for aestivating adults. We paid attention to every life stage and monitored for mortality, using sub-sampling at strategic points in their life cycle. The results were a complete turnaround. Virtually no contamination in the soil containers was observed, and we achieved emergence of over 15,000 adults, most of which were distributed to cooperators for release.

We have seen this same kind of progress with our foreign exploration efforts both in China and Japan. From both countries, we collected excellent potential biological control agents only to be delayed in their study and development by issues beyond our control or due to lack of understanding of the foreigh protocols (see Montgomery et al., Lamb et al., and McAvoy et al. in this issue). Even with the great news that *L. nigrinus* is establishing at many of the release sites that have been closely monitored (see Mausel et al. in this issue). Collecting and assessing *L. nigrinus* has been complicated by the widespread presence of *L. rubidus* (a native predator of pine bark adelgid), which is indistinguishable from *L. nigrinus* in the larval form and currently can only be identified through the use of molecular diagnostic tools or by rearing out the larvae to the adult stage. And we all now know how difficult the latter is.

The overall point here is that progress is not linear. All of our collective good faith effort takes time. And although we know we have a limited amount of time, we need to remain diligent in pushing this enterprise forward but also patient, because every new discovery seems to pose new problems.

So What Do We Need to Do to Maximize Our Chances for Success?

1. We need to maintain the expertise and infrastructure currently in place to allow for new discoveries and to move this process toward a solution to the HWA problem. This means: continue to invest in this program.
2. We need to continue to build and expand the complex of natural enemies. We are starting to learn that the geographic range of individual predator species may not cover the full range of HWA. This requires finding and studying HWA and its natural enemies in their native habitats. It also requires year-long investigations that quantify the impacts of different mortality agents.
3. We need to continue to evaluate the compatibility of the different agents with each other and with other developing pest management tactics, such as silviculture, chemical control, and breeding resistant trees.
4. If species are compatible, we should consider releasing more than one agent species at targeted locations.
5. As we continue to focus releases of biological control agents at the edge of the expanding HWA front, we should consider releasing agents in areas where a sufficient population of hemlocks have survived after the “first wave” of exposure to HWA. This would be contingent on having enough agents for release.

6. We need to consider employing additional field insectaries for natural rearing of biological control agents to help supplement and perhaps eventually replace production labs.
7. We need to be diligent in monitoring natural enemy releases and documenting their establishment and impact. This will help us focus on biological control agents and identify where they may be most effective. It is critical that everyone involved in releases send pre-treatment site data, beetle release data, and post-release evaluation data to Virginia Tech for entry into the online database.
8. This large-scale enterprise, involving universities, state and federal agencies, and private citizens, is working very effectively. Lines of communication need to be kept open. All participants need to be accessible and responsive to both established and new lines of collaboration. It must be recognized that, if you don't like the decisions made by the collective group, YOU SHOULD NOT NOT TAKE THINGS INTO YOUR OWN HANDS.
9. We should not oversell biological control as a "slam dunk, can't miss" success in waiting. We need to present our findings to resource managers and the public in reasoned tones backed up by science. Honesty and good faith efforts are most appreciated by all those affected by this devastating pest. Also, while all parties have some vested interest in the regions and locales that they work in, we need to think and act nationally. Those involved should consider national implications when forging ahead at the local level.

LAST WORD

Many of you could likely add to or revise the list of future efforts seen as necessary. Hopefully, this list will be a focal point for discussion among all of those involved and interested in using biological control to reduce the impact of HWA, and we hope that such efforts will help us keep hemlocks a meaningful and greatly valued component of our eastern forests.

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Laricobius nigrinus Establishment and Impact in Native and Introduced Habitats

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ABSTRACT

Different release locations, numbers of predators, and timing of release were evaluated for establishment of *Laricobius nigrinus* Fender for biological control of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), in the eastern U.S. In the Pacific Northwest, high predator-to-prey ratios and a density-dependent relationship between *L. nigrinus* and HWA indicate a potential strategy for pest suppression. Adults were released at 22 sites between 2003 and 2005 in eight eastern states. Release numbers were 75, 150, 300, 600, and 1,200 in fall, winter, or spring seasons, or in consecutive seasons. Monitoring of establishment consisted of beat-sheet sampling (for adults) and branch clipping (for immature stages) for three years post-release. Recoveries of F_3 generation individuals and increasing abundance over time indicate establishment at 59% of the sites.

A logistic regression with the three release variables was significant. Release location (i.e., USDA 1990 hardiness zone or recorded minimum temperature) was the only significant coefficient, and cold locations (i.e., zones 5a and 5b) were related with establishment failure. Multiple regression and commonality analysis were used to relate the release variables with larval abundance.

To detect impact, these analyses were conducted for changes in HWA sistentes density and eastern hemlock vigor index between 2004 and 2007. Release location was most related with larval abundance, HWA density, and hemlock vigor. Cold locations were related with low predator abundance, declines in HWA density, and increases in hemlock vigor. Paired release and control sites were tested for goodness of fit to compare distributions of HWA density and hemlock vigor over time. Predator impact on HWA density was detected, but densities remained above hemlock's physiological damage threshold and vigor declined. Investment in *L. nigrinus* appears worthwhile, and we propose the following strategy to optimize releases of a limited predator supply and increase establishment rates:

1. Do not release in zone 5a.
2. In zone 5b, release larger numbers, release in the spring, or release consecutively in the fall and following spring.
3. Observe whether any release numbers we tested by season results in high establishment rates in zones 6a and 6b.

KEYWORDS

classical biological control, predator, release, establishment, impact

An Overview of Lady Beetles in Relation to their Potential as Biological Controls for Hemlock Woolly Adelgid

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ABSTRACT

More than 63 species of lady beetles (Coleoptera: Coccinellidae) have been collected in China from hemlock infested with hemlock woolly adelgid, *Adelges tsugae*. The lady beetle species that seem most useful for biological control are in the genus/subgenus *Scymnus* (*Neopullus*), namely *S. camptodromus*, *S. sinuanodulus*, and *S. ningshanensis*. The geographic range of these lady beetles is limited to one or two provinces. Only five lady beetles were found in Japan; however, it has been surveyed there less intensively. Review of the life history of lady beetles and their prey indicates that lady beetles have been less effective for biological control of aphids than of scales. Because the generational period for adelgids is closer to that of scales than aphids, it is hypothesized that lady beetles that prey on adelgids will be more effective than those that prey on aphids. Introducing biological controls faces problems of climate-matching and often, time is required for agents to become adapted to the new environment.

KEYWORDS

Coccinellidae, biological control, China, Japan, climate

INTRODUCTION

The search in Asia for natural enemies suitable for biological control of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, began in 1992 and continues today. The first overseas explorer, Mark McClure, found five natural enemies in Japan during a six-week visit and imported two species, an orbatic mite and a previously unknown species of lady beetle (McClure 1995). In 1994, Sean Murphy of CABI in London went to Taiwan and China at the request of the USDA Forest Service to search for natural enemies. He found five families of predators: Cedidomyiidae, Coccinellidae, Derodontidae, Salpingidae, and Syrphidae (Murphy 1995). The most abundant predator in Taiwan was a derodontid beetle subsequently named *Laricobius taiwanensis* (Yu and Montgomery, in press). These pioneering efforts demonstrated that natural enemies of HWA in Asia are numerous and many are species not previously known to science.

This paper summarizes the surveys we and our colleagues made for natural enemies of hemlock woolly adelgids from 1995-2004. It quickly became apparent that lady beetles (Coleoptera: Coccinellidae) are both diverse and important predators of the hemlock woolly adelgid in China. Our report focuses on that family, although other families of natural enemies also play a major role in the region. We first provide an account of the species collected, then discuss the use of lady beetles to control other adelgids, and finally, comment on factors influencing successful use of lady beetles for biological control.

LADY BEETLES COLLECTED FROM HEMLOCKS IN CHINA AND JAPAN

Surveys were not done every year or systematically, and time spent actually collecting in the field was limited—usually only a few hours for each field site and only one to four days in each province. We investigated sites in Yunnan (1995-1998, 2002, and 2004), Sichuan (1995-1998 and 2003), Shaanxi (1998 and 2002), Guangxi (2003), and Hubei (2004).

Yu et al. (1997, 2000) recorded a total of 54 species of Coccinellidae collected from hemlocks in China. From 2002, we continued to search for natural enemies of the hemlock woolly adelgid in Shaanxi, Sichuan, Yunnan, Guangxi, and Hubei. In Hubei and Guangxi, we found hemlock but no adelgids. In these years, 27 species of lady beetles were found on Chinese hemlocks; three of these are new species (Yu and Montgomery, in press). As of 2005, the total number of species of ladybugs we have found on Chinese hemlocks is 63, with 28 species new to science and one new to China (Table 1). Of the 63 species, 48 are found in Yunnan, 13 in Sichuan, and 16 in Shaanxi. Six species are common to Yunnan and Shaanxi, four to Yunnan and Sichuan, and two to Sichuan and Shaanxi. There are no species common to all three provinces. Sichuan is probably under-represented because the first author (Yu) did not personally collect natural enemies in the province, and local partners targeted *Scymnus camptodromus* and paid little attention to other Scymninae or big-sized lady beetles.

Presently only 10 of the ladybug species in China are known to be predators of HWA. They are *Oenopia billieti* (Mulsant), *Adalia conglomerata* (L.), *Calvia championorum* Booth, *Sasajiscymnus* (=*Pseudoscymnus*) *ocellatus* (Yu), *S. camptodromus* Yu et Liu, *S. sinuanodulus* Yu et Yao, *S. ningshanensis*, *S. paralleus* Yu and Pang, *S. yunshanpingensis* Yu, and *S. geminus* Yu and Montgomery. The first three are in the subfamily Coccinellinae and are large species known to prey on aphids and other Homoptera. Because of their more general host range, they were not considered for importation. The remaining seven species are in the subfamily Scymninae, a subfamily of small (less than 3 mm long) pubescent lady beetles that are usually prey-specific (Hagen et al. 1999). Although seven of these species were imported, colonization was attempted for only the three species that were most abundant: *S. camptodromus*, *S. sinuanodulus*, and *S. ningshanensis*.

From 2002 to 2004, we also surveyed natural enemies of HWA in Japan. Previously, only one species, *Sasajiscymnus tsugae* (Sasaji and McClure 1997), was recorded from Japanese hemlocks. However, we found other ladybugs as well: *Scymnus* (*Pullus*) *posticalis* Sicard, *Adalia conglomerata* (Linneaus); *Harmonia axyridis* (Pallas), and *Scymnus* (*Pullus*) *giganteus* Kamiya. Of the five species, *S. tsugae*, *A. conglomerata*, and *H. axyridis* were collected as larvae in June 2004. The first two were reared into adults. Kamiya (1965) recorded *A. conglomerata* as a predator of *Adelges japonicus* Monzen. We observed that both its larvae and adults preyed on HWA. Other natural enemies (*Laricobius* spp., lacewings, Miridae etc.) were also found on hemlocks in China in addition to other ladybugs identified only to genus.

WORLD COCCINELLIDS PREDACEOUS ON ADELGIDS

Yu (2001) lists 48 species of lady beetles that prey on adelgids recorded from all over the world. Most of them were introduced into North America for biocontrol of the balsam woolly adelgid, *Adelges piceae* (Ratzenburg), in 1934-1969. Of these, five are Chilocorinae, 10 Scymninae, and 21 Coccinellinae. All but two of 16 lady beetles introduced into North America for control of *A. piceae* prey on other adelgids in their native region. Several of these species are known to be general predators on Aphididae, and one, *Chilocorus kuwanae* Silvestri, is well known as a predator of diaspine scale. The two species established in North America, *Aphidecta obliterata* (L.) and *Scymnus* (*Pullus*) *impexus* Mulsant, prey not only on *A. piceae* but on other adelgids and aphids as well. Another *Pullus* species, *Scymnus* (*Pullus*) *suturalis* Thunberg, a predator of adelgids on pines that was introduced from Europe to North America, also was found to attack HWA (Montgomery and Lyon 1996).

Successful biological control of balsam woolly adelgid was not achieved despite a 35-year effort that included 11 species of Coccinellidae (Schooley et al. 1984). What can we learn from this failure? Yu (2001) mentioned the following: 1) predators selected for introduction should prey and reproduce mainly or only on HWA in their native country; 2) higher latitude or colder areas of predator collection in China, such as Shaanxi Province, need to be explored for predators; 3) monophagous predators are to be preferred, their life cycle should be well synchronized with that of HWA, and they should be able to survive the summer aestivation period of HWA; and 4) introduction of multiple species may be necessary for effective predation.

COMMENTS ON BIOLOGICAL CONTROL OF HWA

Theoretically, can lady beetles control adelgids?

Although there have been hundreds of introductions of lady beetles for biological control, few have been successful. Generally, lady beetles are ineffective in the biological control of aphids (Dixon et al. 1995), although there are many examples of outstanding control of coccids by lady beetles (Clausen 1978; Dixon 2000). For a predator to effectively control a prey species, the "generation time ratio" of predator to prey should be less than 1.0 (Dixon et al. 1995 and 1997). Aphids with parthenogenetic, viviparous reproduction can have generation times of 1-2 weeks and their abundance is strongly responsive to host quality. On average, aphidophagous lady beetles develop slower than their prey aphids, with generation times often of more than a month, whereas the lady beetles that feed on coccids have generation times similar to their prey. Another hypothesis is that lady beetles feeding on coccids are more efficient, killing more prey because the coccids are immobile and the lady beetles feed selectively, consuming only the most nutritious parts of the prey (Dixon 2000). Surprisingly, the lady beetles that feed on coccids have lower fecundity than those that feed primarily on aphids, seem to eat prey at a slower rate, and grow more slowly (Dixon 2000). In sum, it seems that the greater prey

Table 1. Species collected in China from *Tsuga* spp. (1995 - 2004).

Species	Collection Provinces[1]	Relative Abundance[2]
Subfamily Coccidulinae		
Sumnius nigrofuseus Jing	Yun	2
Rodolia limbota	Sha	3
Subfamily Scymninae		
Stethorus (*Allostethorus*) descrip. pending	Yun	15
Clitostethus wenbishanus Yu	Yun	1
Scymnus (*Neopullus*) *camptodromus* Yu and Liu	Yun, Sic	119
Scymnus (*Neopullus*) *sinuanodulus* Yu and Yao	Yun	93
Scymnus (*Neopullus*) *ningshanensis* Yu and Yao	Sic, Sha	38
Scymnus (*Neopullus*) *lijiangensis* Yu	Yun	4
Scymnus (*Neopullus*) *thecacontus* Ren and Pang	Yun	1
Scymnus (*Neopullus*) *lycotropus* Yu	Yun	4
Scymnus (*Neopullus*) *nigromarginalis* Yu	Yun	1
Scymnus (*Neopullus*) *paralleus* Yu and Pang	Sha	3
Scymnus (*Parapullus*) *tsugae* Yu and Yao	Yun	2
Scymnus (*Parapullus*) descrip. pending	Sha	80
Scymnus (*Scymnus*) *najaformis* Yu	Yun	9
Scymnus (*Scymnus*) *unciformis* Yu	Yun	1
Scymnus (*Scymnus*) *paracrinitus* Yu	Yun	4
Scymnus (*Pullus*) sp. 1	Yun	1
Scymnus (*Pullus*) *nigrobasalis* Yu	Yun	2
Scymnus (*Pullus*) *baoxingensis* Yu	Sic	2
Scymnus (*Pullus*) *gucheng* Yu	Yun	2
Scymnus (*Pullus*) *yunshanpingensis* Yu	Yun	34
Scymnus (*Pullus*) *geminus* Yu and Montgomery	Yun	15
Scymnus (*Pullus*) *heyuanus* Yu	Yun	3
Scymnus (*Pullus*) *japonicus* Weise	Yun, Sha	4
Scymnus (*Pullus*) *ancontophyllus* Ren and Pang	Yun, Sic	5
Scymnus (*Pullus*) *robustibasalis* Yu	Yun	2
Scymnus (*Pullus*) *jaculatorius* Yu	Yun	1
Scymnus (*Pullus*) *toxosiphonius* Pang and Huang	Yun	7
Scymnus (*Pullus*) sp. 3	Yun	3
Pseudoscymnus heijia Yu and Montgomery	Yun, Sic	10
Pseudoscymnus ocellatus Yu	Sic, Sha	3
Pseudoscymnus truncatulus Yu	Yun	2
Pseudoscymnus sp. 1	Yun	1
Pseudoscymnus sp. 2	Yun	1
Cryptogonus *lijiangensis* Pang and Mao	Yun	1
Cryptogonus ocoguttatus Mader	Yun, Sic	3

Subfamily Chilocorinae		
Telsimia sp.	Yun	2
Subfamily Sticholotidinae		
Shirozuella quadrimacularis Yu	Yun	1
Shirozuella nibagou Yu	Sic	3
Subfamily Coccinellinae		
Hippodamia variegata (Goeze)	Yun	3
Propylea quatuordecimpunctata (L.)	Sic	1
Adalia bipunctata (L.)	Yun	11
Adalia conglomerata (L.)	Yun, Sha	6
Oenopia billieti (Mulsant)	Yun, Sha	4
Oenopia deqenensis Jing	Yun, Sic	21
Oenopia emmerichi Mader	Yun	3
Oenopia sexmaculata Jing	Sha	1
Oenopia signatella (Mulsant)	Sha	16
Oenopia zonatus Yu	Sic	3
Xanthadalia hiekei Iablokoff-Khnzorian	Yun	27
Coccinella septempunctata L.	Yun, Sha	3
Harmonia axyridis (Pallas)	Yun, Sha	9
Harmonia eucharis (Mulsant)	Yun	5
Harmonia quadripunctata (Pontoppidian)	Yun	1
Harmonia descrip. pending	Sic	1
Calvia quatuordecimguttata (L.)	Sha	20
Calvia championorum Booth	Sha	8
Calvia sp.	Yun, Sha	2
Halyzia sedecimguttata (L.)	Sic	1
Halyzia straminea (Hope)	Yun	1
Halyzia sanscrita Mulsant	Yun, Sha	2
Vibidia duodecimguttata (Poda)	Yun	4

[1] Yun = Yunnan, Sic = Sichuan, Sha = Shaanxi.
[2]Total numbers collected by the authors, Yao Defu, Nathan Havill or Tom McAvoy; does not include collections of mass numbers of *S. camptodromus* and *S. sinuanodulus* by others.

specificity of the coccidophagous lady beetles and the longer generation time of the coccids accounts for the success of these lady beetles as biological control agents.

When we consider the generational times of aphids and scales, adelgids seem closer to scales than to aphids. Both adelgids and scales have a mobile crawler stage but thereafter are sessile. Adelgids and most scales are oviparous. On the other hand, adelgids and aphids both have parthenogenic generations on secondary hosts. Cheah and McClure (2000) considered *A. tsugae* to be more similar to coccids than to aphids. They pointed out that it takes a couple of years for the adelgid to begin to cause decline in the host plant; thus, the adelgids would be less ephemeral than aphids. They also pointed out that the lady beetle *S. tsugae* has a generation time of five weeks, which is shorter than either the spring or overwintering generation of the adelgid. However, they mentioned that the aestivation of the adelgid, while the lady beetle is active, may be a 'bottleneck'. The development of *A. tsugae* during the winter months and initiation of egg laying in late winter may also make it difficult for lady beetles to control this prey.

Other families of predators have been used successfully for biological control of adelgids in the genus *Pineus* (Zilahi-Balogh et al. 2002). Chamaemyiid species (genus *Leucopis*) were successfully established in Chile, New Zealand, and Hawaii for biological control of *P. pini* (Macquart) (= laevis Maskill). An anthocorid predator introduced into Kenya, *Tetraphleps raoi*, has also been successful in reducing populations of *P. pini*. However, there are no examples of successful biological control of species in the genus *Adelges*.

Can better climate matching yield more successful predators?

An example of insufficient consideration of climate is the collection of predators from India and Pakistan for biological control of the balsam woolly adelgid in North America. In this case, none of the predators were recovered in the field in North Carolina (Amman and Speers, 1971) or in eastern Canada. The lady beetles imported for biological control of HWA generally come from latitudes further south than those where the adelgid occurs in the eastern U.S. *Sasajiscymnus tsugae* (Sasaji and McClure, 1997) was collected in the Osaka area on *Tsuga sieboldii*, and the holotype tree is located near Takatsuki City, Osaka Perfecture, Japan (N 34º 57', altitude 374 meters). The weather there is a little warmer and more rainy, but is a reasonable match with the climate where HWA now occurs in the eastern United States. The Chinese lady beetles *S. sinuanodulus* and *S. ningshanensis* were collected at more southern latitudes but higher altitudes, N 31.6º, 2,700 meters, and N 33.3º, 1,800 meters, respectively. The combination of low latitude and high altitude results in seasonal temperature averages that are similar to the high mountains of the southern Appalachians in the U. S. The main difference in where HWA occurs in east Asia and the eastern United States is the pattern of rainfall; dry winters and wet summers occur in Asia, whereas monthly rainfall is fairly even in eastern United States throughout the year (Wang et al. 1998; Montgomery et al. 2002).

Adaptation of natural enemies to new habits may take years or decades. For example, the mealybug destructor, *Cryptolaemus montrouzieri* Mulsant, was introduced to China from the Soviet Union in 1955 and was mass-reared and released in quantity in South China (Guangdong and Fujian Provinces) several times. Average maximum July temperature in Guanzhou, Guangdong, is 32.6°C. Establishment was not confirmed until 1978, more than 20 years later (Pang and Li, 1979). Comparison of survival at different temperatures shows that, after 30 years, the mealybug destructor adapted to the higher temperature in Guangdong (Table 2). Currently, home areas for the beetles introduced for biological control of HWA are warmer than the target's home areas and differ as well in varying degrees in rainfall patterns; thus, successful control of the hemlock woolly adelgid may be delayed while the beetles adapt to a new temperature regime.

Table 2. Percent hatch and survival at different temperatures of *Cryptolaemus montrouzieri* in 1962 prior to release in Guangdong Province, and three decades after release (1996).

	Year	Temperature (ºC)		
		32	**34**	**36**
Egg hatch	1962	4	0	0
	1996	51.3	36.2	7
Larval-pupal survival	1962	0	0	0
	1996	41.6	9.3	--

(Data from Li 1993, Chen et al. 2000)

What are the opportunities to introduce more lady beetles?

Considerable data on hemlocks, the adelgid, and natural enemies have been accumulated in the past decade. When considering the biological control of the adelgid, there still are many questions to ask: Where should we look? What should we look for? What may we find? In answering these questions, there is an inherent dilemma. For the safety of native species, natural enemies should be host-specific and narrow in distribution; unfortunately, the lady beetles meeting these requirements would, theoretically, be less successful in establishment in a new environment.

China has more potential as a source for biological control as it has a greater diversity of lady beetles on hemlock trees than Japan. However, the localized distribution of many of these beetles suggests limitations. Three provinces in China (Yunnan, Sichuan, and Shaanxi) have quite different lady beetle communities, and Scymnini species especially are different (Wang et al. 1998; Yu et al. 2000; Montgomery et al. 1996). The limited geographic range of the lady beetles in China, which are adelgid specialists, suggests that they may not adapt rapidly to a new environment. Although Japan appears to have a low diversity of specialist predators, a systematic survey needs to be done for both *Tsuga sieboldii* and *T. diversifolia*, which are associated with warmer and cooler climates, respectively.

Lady beetles considered to be less host-specific have not been evaluated for biological control of HWA, even though they may be important regulators of the adelgid in its native habitats. Examples of presumed generalists which may need further study are: *Adalia conglomerata* L., recorded previously to prey on *Adelges japonicus* (Kamiya 1965), *A. piceae* (Clausen 1978; Schooley et al. 1984), and the hemlock woolly adelgid (Yu et al. 2000); and *C. championorum*, distributed in many parts of China (Gansu, Shaanxi, Sichuan, and Yunnan provinces, and in Taiwan) (Yu and Wang 1999) from elevations of 1,580 m to 2,600 m and in northern India (Booth 1997). *Calvia quatuordecimguttata* (L.) is a species widely distributed in Asia and North America (Gordon 1985), and though there are no reports of its hosts in North America, a total of 20 specimens were collected from hemlock in fall of 2002 in Shaanxi Province, China (Yu and Montgomery, in press).

What use can be made of local natural enemies?

According to biological control theory, natural enemies should come from the native country or endemic area of the pest. However, there is increasing evidence that natural enemies present in the area where the pest has invaded could contribute to control by making a host shift to use the new resource. For example, the parasite *Chouioia cunea* Yang is used in China for control of the fall webworm, *Hyphantria cunea* (Drury), which is native to North America. It is easy to mass-rear, and five years after releasing the parasite, the parasitism rate is as high as 92% (Yang 2004).

For control of HWA in the eastern United States, several North American natural enemies have potential. *Laricobius rubidus* LeConte, native to the eastern U. S., has already been observed to feed and reproduce on HWA (Montgomery and Lyon 1996). *Laricobius nigrinus* Fender, which is native to western North America, has been successfully introduced to the other side of the continent and is a very promising potential control agent (Lamb et al. 2002). The role of native American lady beetles (such as *Mulsantina hudsonica* (Casey)) and established exotic species (such as *H. axyridis*) should not be overlooked.

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Evaluation of the Japanese *Laricobius* sp. n. and Other Natural Enemies of Hemlock Woolly Adelgid in Japan

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ABSTRACT

Since the initial importation of the Japanese *Laricobius* (Coleoptera: Derodontidae) to Virginia in 2006, we have studied the development of the predator at two different temperatures, measured feeding and oviposition rates, conducted preliminary host-range testing, and reared two generations. During this time, we have learned this predator differs from *Laricobius nigrinus* (Fender) in biology and life cycle. Host range tests have been relatively inconclusive, and laboratory survival has been low. These results prompted further investigation of this *Laricobius* beetle and hemlock woolly adelgid in Japan.

KEYWORDS

Laricobius sp. n., Japan, HWA predators, phenology, mortality factors

INTRODUCTION

As part of coordinated foreign exploration effort for hemlock woolly adelgid (HWA) predators in Asia, a new *Laricobius* species was discovered in Japan (Montgomery and Shiyake, unpublished report). The purpose of this project is to evaluate the newly imported biological control agent, *Laricobius* sp. n., in quarantine and in its native range in Japan. Preliminary basic biology and host-range studies have been conducted in the U.S. Results are summarized below. Phenology studies of *Laricobius* and HWA life cycles are underway in Japan. The presence of each life stage of HWA and its predators will be recorded over an entire year. The overall impact of natural enemies on the sistentes and progredientes generations will be estimated by examination of foliage under the microscope and by use of exclusion cages. Results from studies in Japan will support the effort to receive permission for removal of this species from quarantine in the U.S.

BIOLOGY AND LIFE CYCLE OF *LARICOBIUS* SP. N.

Field-collected adults had a high ovipositon rate (up to 11 eggs/day) at 9°C. Egg development rate is similar to *L. nigrinus*, but larval development rate is much faster at 9°C and 12°C (Table 1).

Table 1. The mean development rate (days±S.D.) of Japanese *Laricobius* eggs and larvae compared to the development rate of *L. nigrinus*.

Temperature	Stage	Japanese *Laricobius*	*L. nigrinus*
9° C	Egg	17.31±0.63	16.3±0.86
12° C	Egg	12.2±0.92	11.5±1.05
9° C	Larva	24.14±5.33	32.8±2.92
12° C	Larva	15.45±3.55	25.1±2.77

Pupae have similar survival when maintained at 12°C (65%) and 15°C (64%) after 30 days. Most individuals at 15°C had eclosed. If left undisturbed throughout the summer, adult survival is good at 15°C or at 18°C, if the temperature is higher than at pupation.

In 2006, over 9,000 larvae were reared to prepupae. Rearing conditions were suboptimal at ~18°C compared to the desired 12°C. Nevertheless, 1,530 adults emerged between August and December 2006 and held at 3°/5°C (night/day) (Figure 1b). A greater proportion of males emerged first, whereas ~80% of adults emerging in November and December were female. F_1 adults began laying eggs soon after emergence, first observed in mid-November, two weeks following emergence. Adults layed eggs at 0°C and were then cooled to as low as -7°C to get them to stop. Adults survived this temperature and still showed signs of feeding. In December, eggs began to hatch, and it was discovered that the larvae were feeding on HWA sistentes nymphs, something rarely observed in *L. nigrinus*. The Japanese predator larvae were surviving on this food source; however, they appeared dark in color and emergence from aestivation was much lower than the previous year (Figure 1c). In addition, many individuals experienced extreme temperatures as eggs and larvae (-7°C, and later, 4°C), which may also explain the poor summer survival.

A total of 9,000 larvae were reared, as in the previous year, but larval drop was slightly later; furthermore, adult emergence from aestivation was delayed compared to the previous year (Figure 1d). This may be due to the slow development at low temperatures, which caused larval drop to occur later in the spring, or it may be normal for individuals to emerge much later in the year and immediately begin laying eggs. These F_2 adults did begin to lay eggs in December; thus, the period between emergence and oviposition is very short (two to three weeks).

Between October and January 2007, 550 adults emerged and were held at 4°/6°C (night/day). Survival through aestivation was lower than the previous year and most adults to emerge were females. These F_2 adults did begin to lay eggs in December; thus, oviposition and funnel production is well underway as of January, and it is assumed individuals will develop under more normal conditions this year. The understanding of *Laricobius* life-cycle gained from field research in Japan will support this effort.

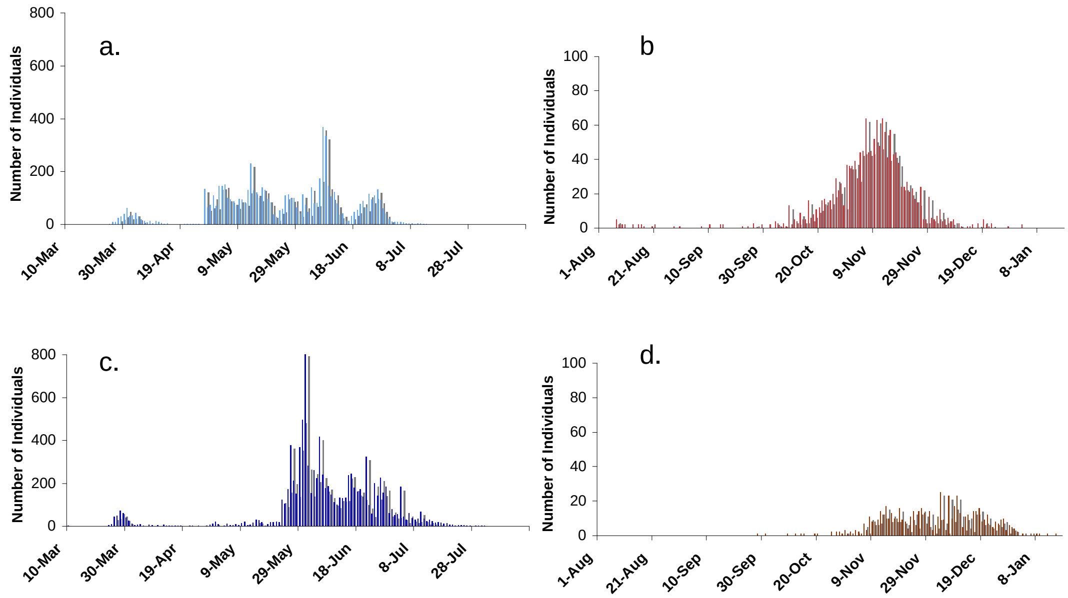

Figure 1. The number of *Laricobius* larvae to mature each day in spring 2006 (a) and 2007 (c) and the number of adults to emerge each day in fall 2006 (b) and 2007 (d).

HOST-RANGE TESTING

Host-range testing studies will follow the format and procedures used by Zialhi-Balogh et al. (2002) for *L. nigrinus*. The following non-target prey species are used in choice and no-choice feeding and oviposition bioassays: balsam woolly adelgid (BWA), pine bark adelgid (PBA), eastern spruce gall adelgid (ESGA). larch adelgid, woolly alder aphid, pine needle scale, elongate hemlock scale, and mealybugs. Development studies with some of these species have been conducted.

No-choice feeding and oviposition tests indicate adults prefer HWA over BWA and PBA but will feed and lay eggs on BWA and PBA when given no choice (Table 2).

Table 2. The mean number of adelgids eaten and eggs laid (±SE) when Japanese *Laricobius* is offered only one prey item: hemlock woolly adelgid (HWA), balsam woolly adelgid (BWA), and pine bark adelgid (PBA).

Host	N	Feeding Rate (eggs/day)	Oviposition Rate (eggs/day)
HWA	8	17.7±5.9	3.7±1.9
BWA	10	2.0±26	0.1±0.1
PBA	11	7.1±3.5	0.5±0.8

Development tests were inconclusive: fewer individuals developed to pre-pupae on BWA and PBA than on HWA, but no individuals successfully pupated. In a separate test, larch adelgid and woolly alder aphid do not appear to be suitable hosts (Table 3). The results of a third test were similarly inconclusive as no adults emerged from the soil. However, more individuals developed to pre-pupae on PBA than on HWA or ESGA (Table 4). BWA was not available for testing. It is possible that individuals are not being provided with a sufficient amount of food during these tests. When more food is provided, mold rapidly appears and larvae die. A different bioassay will be used in 2008.

Table 3. Number and percentage of *Laricobius* individuals surviving each life stage when offered only one prey item.

Stage	Hemlock Woolly Adelgid		Balsam Woolly Adelgid		Pine Bark Adelgid		Hemlock Woolly Adelgid		Larch Adelgid		Woolly Alder Aphid	
	n	%	n	%	n	%	n	%	n	%	n	%
Egg	26		22		20		10		20		20	
L1	11	100	14	100	18	100	9	100	0	0	0	0
L2	10	91	2	14	12	67	8	88	0	0	0	0
L3	9	82	1	7	12	67	7	78	0	0	0	0
L4	9	82	1	7	10	56	7	78	0	0	0	0
Pre-pupa	6	55	1	7	3	17	5	56	0	0	0	0
Pupa	0	0	0	0	0	0	?	?	0	0	0	0
Adults	0	0	0	0	0	0	4	44	0	0	0	0

Table 4. Number and percentage of individuals surviving each life stage when offered only one prey item.

Stage	Hemlock Woolly Adelgid		Pine Bark Adelgid		Eastern Spruce Gall Adelgid	
	n	%	n	%	n	%
Egg	30		30		14	
L1	28	93	29	97	14	100
L2	24	86	26	90	14	100
L3	11	46	21	80	12	86
L4	7	64	18	86	8	67
Pre-pupa	5	71	13	72	6	75
Pupa	0	0	0	0	0	0
Adults	0	0	0	0	0	0

Both behavioral activity and rearing experiences with this species have been different than with *L. nigrinus* and difficult to understand. We also had issues with understanding behavior and rearing of Chinese *Laricobius* spp. (Gatton 2005). As we began working with *L. nigrinus* back in 1997, we had access to field plots to study the insect's seasonal activity in its native habitat. We have not had that luxury with both the Chinese and the Japanese

Laricobius species. Without knowing the normal behavior and life cycle of *Laricobius* in the field, it is difficult to make appropriate adjustments to obtain optimal rearing conditions. A phenology study to determine the life history of this predator and its host in its native range will enhance our ability to research and rear this species in the U.S. The results of the host suitability tests are currently inconclusive as *Laricobius* sp. n. has been able to develop on other adelgids, sometimes with better survival than those on HWA.

Mausel (2007) determined that *L. nigrinus* has a significant impact on the populations of HWA in the Pacific Northwest. Since HWA is native to Japan (Havill et al. 2006), assessing the impact of natural enemies on HWA populations in its native range would help us predict what impact the biological control agents can potentially have in the U.S.

RESEARCH IN JAPAN

Phenology Study

The phenology of HWA and *Laricobius* sp. n. will be studied using an approach similar to that used by Zilahi-Balogh et al. (2003). The trees at Kobe Arboretum, Takatsuki, and Nara were ranked according to HWA infestation levels. Twelve trees with sufficient HWA were identified at three locations. There are nine trees at the Kobe Municipal Arboretum, one tree at the Myo-on-ji Temple in Takatsuki, and two trees on a mountaintop, Wakakusayama, east of Nara City. These locations are west, north, and east of Osaka, respectively. Beginning in early December 2007, each tree is being sampled for adult and immature predators and the number and life stage of HWA. Adult predators are sampled by beating three branches per tree; all predators are returned to the tree after counting. Immature predators and HWA are sampled by removing four 10-cm branch tips from each tree for examination under the microscope. The stage and number of adelgids are recorded, as well as dead adelgids and the cause of death (chewing insect, sucking insect, fungus, or mortality during aestivation). Immature numbers and stages are also recorded, and if unknown predators are found, they are reared out for identification or preserved in alcohol. These data will reveal which life stages of *Laricobius* and other natural enemies are present with respect to the stage of their host.

Predator Exclusion Study

The impact of natural enemies on HWA populations is being measured by comparing the number of adelgids on caged branches with no predators to branches open to predators. The four treatments include: caged branches from December to March, caged branches from January to May, open branches from October to March, and open branches from March to May (caged January to March). Two trees were selected at Wakakusayama, east of Nara City. HWA infestation levels were estimated on 40 branches and assigned 20 pairs. Each pair was randomly assigned a cage or open treatment. Branches assigned to cages were caged on December 26, 2007, after permission to use the trees was granted. Four 10-cm branch tips will be sampled from each of these 40 branches in March and April. The number of surviving sistentes will be counted, and treatment means will be compared using a standard t-test to determine the impact of predators from December to March and April.

PRELIMINARY RESULTS

HWA Phenology

Most adelgids were fourth instar sistentes on the first sample date (December 6), and by mid-January, most were adults, but very few eggs have been observed (Table 5). Interestingly, in the Takatsuki samples, very young adelgids (first and second instars) have been observed under the bud scales. In samples from December 19, two eggs were observed under the bud scales and hatched into crawlers a few days later. These eggs are likely the source of the young adelgids found there; however, the insects that laid these eggs are unknown. Samples have been sent to Nathan Havill for genetic analysis. They have been confirmed to be *Adelges tsugae*; however, their morphology and DNA differ from all other individuals collected in the past except one. They group with Japanese HWA but do not cluster with the two clades that were found in the mtDNA paper. It is out by itself with another sample collected at Unpenji Temple in Tukishima.

Table 5. Percentage of HWA at each life stage at three different sites during each sample period.

Sample Period	Site	Percentage of Individuals at each Life Stage						
		1st Instar	2nd Instar	3rd Instar	4th Instar	Adult	Adults with Eggs	Eggs/ Ovisac
	Kobe	0.9	0	7.6	88.7	1.4	0	
Dec 6 -11	Nara	2.9	0	2.9	88.1	6.0	0	
	Takatsuki	10.3	5.1	3.8	80.8	0	0	
	Kobe	0	0	0.6	89.1	10.2	0	
Dec 14 - 19	Nara	0	0.9	0.9	68.8	29.2	0	
	Takatsuki	18.4	0	5.2	73.7	2.6	0	
	Kobe	0	0.5	3.2	73.3	18.4	0	
Dec 22 - 27	Nara	0	1.3	0	56.3	42.5	2.9	1
	Takatsuki	26.2	5.6	4.4	59.8	3.8	0	
	Kobe	0.7	0	2.8	62.8	31.7	0	
Dec 31 - Jan 3	Nara	0	0	0	68.3	31.7	15.4	2
	Takatsuki	19.3	5.3	14.4	56.1	5.3	33.3	2
	Kobe	0	0.5	2.5	42.8	52.7	9.4	2
Jan 8 - 12	Nara	0	0	0	37.5	62.5	6.7	3
	Takatsuki	43.1	7.7	0	46.2	3.1	0	
Jan 14 - 18	Kobe	0	0	0	36.6	63.1	11.9	3.4
	Nara	0	0	0	11.8	88.2	20.0	5.3
	Takatsuki	12.6	14.3	19.8	46.2	13.1	0	
	Kobe	0	0.5	3.6	30.1	65.8	8.5	2.55
Jan 22 - 26	Nara	0	0	4.5	0	95.5	26.2	
	Takatsuki	5.8	2.5	15.0	75.8	0.8	0	3.45

Natural Enemies

The HWA at each site seem to experience different mortality factors. By early December, significant mortality could be observed, and the fall and winter predation are likely important contributors to HWA population regulation (Figure 2). There appears to be a chewing predator, particularly in Nara, that is responsible for some mortality (shown in grey); however, it has not been collected in the beatsheet samples yet. This predator could have been feeding throughout the fall and is now dormant.

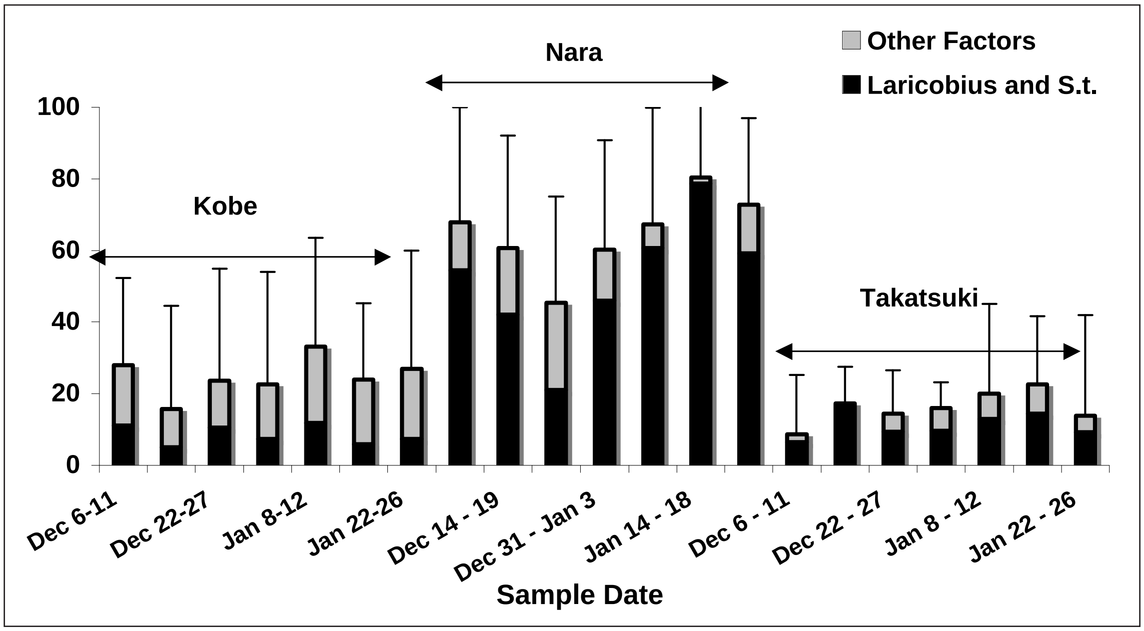

Figure 2. The average mortality observed in foliage samples during each sample period at each site. Proportions shown in black indicate the contribution of *Laricobius* and *St* adults to HWA mortality, and proportions shown in gray represent the impact of other natural enemies.

Laricobius is rather abundant at all three sites, particularly Nara, where nearly every beatsheet sample yields adult *Laricobius*, sometimes more than 25 individuals. At the other two sites, adults are less abundant but consistently present. Mortality caused by *Laricobius* "type" feeding (includes *St*) is indicated by black in Figure 2. It is very apparent that they are making an impact on adelgid populations at the Nara site but less so at the other two sites. *Laricobius* eggs were first observed in mid-December, when most adelgids were still fourth instars. Eggs seem to be laid in pairs on a single adelgid (often the adelgid has already been eaten); therefore, they appear to be less selective about their site of oviposition than *L. nigrinus*. Larvae were first observed in mid-January, and surprisingly, there are often no HWA eggs present. In effort to determine exactly what they are feeding on is currently underway.

Adult *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) have been observed in the beatsheet samples at the Nara and Takatsuki sites. It is the first record of *St* in Nara Prefecture. They are much less abundant than *Laricobius*; however, they have been observed during four of seven sample periods. (It is difficult to distinguish an adelgid consumed by *St* from one that has been consumed, for example, by *Laricobius*.)

Other predators consistently found at each site include several Dipteran larvae. Specimens have been difficult to collect as they are currently dormant within the budscales and are often damaged when the budscales are removed. They do not become active when placed on HWA, so further investigation of this predator will have to be done later in the year, when they become active in the field.

Perhaps the most exciting and unexpected find is one or more entomopathogenic fungi. Fortunately, there are two scientists in Japan (Drs. Degawa and Sakuma) that have taken an interest in them and are currently working on identification. Thus far, they have determined that it is within Entomophthoraceae, Entomophthorales. Dr. Degawa has been unable as yet to match the asexual and sexual stages. It has been found on four of the nine sample trees at the Kobe site, and where it is present, HWA mortality is high.

CONCLUSION

Based on field samples from December and January, it seems the behavior of *Laricobius* observed in the lab may be normal. Adults are active and ovipositing on fourth instar HWA in Japan in December. With increased knowledge of *Laricobius* and HWA in Japan, rearing and research should improve in the following years. Providing that all the alternative hosts can be attained for testing, host range studies will continue and may be completed in the near future.

The mortality agents operating at each of the three sites in Japan vary by site and by tree. Although *Laricobius* is making a measurable impact on HWA populations, it seems there are other factors contributing to the overall mortality. Field sampling at more sites will be required to understand the diversity of natural enemies and the roles they play in regulating HWA.

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EVALUATING CHAMAEMYIID PREDATORS OF HWA IN THE WESTERN UNITED STATES

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ABSTRACT

In a survey of predators associated with hemlock woolly adelgid (HWA) at 16 sites in western Oregon and Washington, three specialist predators were found to be the most common and abundant among the 55 species collected. *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), *Leucopis argenticollis* Zetterstedt (Diptera: Chamaemyiidae), and *Leucopis atrifacies* (Aldrich) (Chamaemyiidae) together comprised 59 percent of predator specimens recovered. Both *Leucopis* spp. are recorded as adelgid specialists in the literature. Furthermore, other chamaemyiid species have been used successfully in adelgid biological control programs in Chile and Hawaii. Collectively, this information suggests that *L. argenticollis* and *L. atrifacies* are good candidates for biological control of HWA in eastern North America.

Preliminary studies were conducted to determine the feasibility of using either *Leucopis* spp. for biological control of HWA in eastern North America. In the field, *Leucopis* spp. larvae were most abundant when progrediens and sistens eggs were present in the spring and early summer. *Leucopis* spp. larvae were also found in association with HWA nymphs in the fall. In laboratory assays, *Leucopis* larvae lived longer and were found in association with their prey more often when provided with HWA compared to two alternative adelgid prey species. In choice assays, *Leucopis* larvae chose HWA twice as often than the alternative prey species. *Leucopis argenticollis* were reared through one complete generation in an environmental chamber. Further studies will be needed to determine whether either *Leucopis* spp. should be released for biological control of HWA in eastern North America.

Linking Host Resistance Mechanisms and Hemlock Woolly Adelgid Populations

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ABSTRACT

In nature, host resistance and natural enemies are two factors that control herbivore populations. Within suitable hosts, resistance is a relative term and ranges from highly susceptible to highly tolerant or resistant. Host resistance includes characteristics that enable a plant to avoid, resist, or tolerate an attack of insects under environmental conditions that would otherwise result in host mortality. Host resistance also includes the collective heritable characteristics of a plant species, race, or individual that reduce the probability of successful utilization of that host by an insect species, race, or biotype. Understanding the variations in the geographical distribution and the prevalence of genetic and morphological variation in the resistance of hemlock species to hemlock woolly adelgid will aid in managing current and future populations of hemlock woolly adelgid (HWA).

We examined three mechanisms of defense: antixenosis, antibiosis, and tolerance. We examined morphological resistance factors of hemlocks that might interfere with mechanisms of HWA feeding and/or movement. To assess HWA survival, establishment, development, and fecundity, we used known hemlock species and western hemlock families to conduct a reciprocal transfer experiment. Finally, we documented the population dynamics of HWA on 2,185 hemlock trees of known genetic background.

Our results indicate the three mechanisms of host defense may, in part, explain why western hemlock are not severely damaged or killed by HWA. Our results indicate HWA survival, establishment, and population densities varied among hemlock species and families within western hemlock. Alternatively, HWA may be locally adapted to specific host types which may support the theories of local adaptation and/or deme formation.

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ESTABLISHING *SASAJISCYMNUS TSUGAE* IN THE SOUTH

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ABSTRACT

Since hemlock woolly adelgid was first found in the Great Smoky Mountains National Park in 2002, about 1.5 million *Sasajiscymnus tsugae* (*St*) adults have been released in hemlock-growing areas in the southeastern United States, including Georgia, North Carolina, South Carolina, and Tennessee. Recovery of *St* one or more years after release has been extremely limited in Georgia, South Carolina, and Tennessee. It has been recovered at a few sites in North Carolina. Most of the releases in the South, however, have occurred in the last three years. The subsequent health of hemlock trees in release areas is inconsistent; in some areas, trees are healthy, have good color and are producing new growth, while in other release areas, trees have died. Research using large tree cages is underway to further assess the survival, establishment, and impact of *St* on hemlock woolly adelgid on eastern hemlock. Monitoring of release sites should continue in southern states to better understand the role of *St* in population dynamics of hemlock woolly adelgid. This information, coupled with a more thorough understanding of the life cycle of the hemlock woolly adelgid in southern states, should assist forest land managers in the development and implementation of appropriate management strategies against hemlock woolly adelgid.

KEYWORDS

Sasajiscymnus, recovery, establishment, biological control, release

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, was first found in the southeastern United States in 1998, when it was documented in a few counties in North Carolina. It was later found in the Great Smoky Mountains National Park (GRSM) in 2002 and has since spread into Georgia, South Carolina, and Tennessee. Thus, HWA has spread throughout most of the hemlock-growing areas in the South, though it has not yet been found in Alabama.

Sasajiscymnus tsugae (*St*) (Sasaji and McClure), native to China, has been released throughout the eastern United States during the last decade (Cheah 2004). *Sasajiscymnus tsugae* is the most studied of any of the biological control agents considered for release against HWA (Cheah and McClure 1998, 2000, Sasaji and McClure 1997). Several laboratories in the South are rearing *St* for release on public lands in Georgia, North Carolina, South Carolina, and Tennessee. A rearing laboratory maintained by the North Carolina Department of Agriculture rears *St* beetles primarily for release in North Carolina, a laboratory at Clemson University produces *St* beetles primarily for release in Georgia, North Carolina, and South Carolina, and a laboratory at the University of Tennessee produces *St* beetles primarily for release in Tennessee, mainly in GRSM. Another laboratory at Harris Young College in Georgia also rears *St* for release in Georgia. In addition, some of the earlier released *St* beetles were provided by rearing laboratories at the New Jersey Department of Agriculture and EcoScientific in Pennsylvania.

The objectives of this paper are to: 1) discuss releases and recoveries of *St* in HWA-infested areas in southern states (Georgia, North Carolina, South Carolina, and Tennessee), 2) discuss ongoing research efforts at the University of Tennessee to assess establishment and performance of *St* in the South, and 3) discuss how these results can be applied by forest managers.

RELEASE OVERVIEW

Since 2002, when the first *St* releases were made in GRSM (Lambdin et al. 2006), about 1.5 million *St* beetles have been released in HWA-infested areas in these four southern states (Figure 1). Of these beetles, approximately 350,000 have been released at about 150 release sites in GRSM. About 73% (n=ca. 110) of these releases have occurred in the last three years.

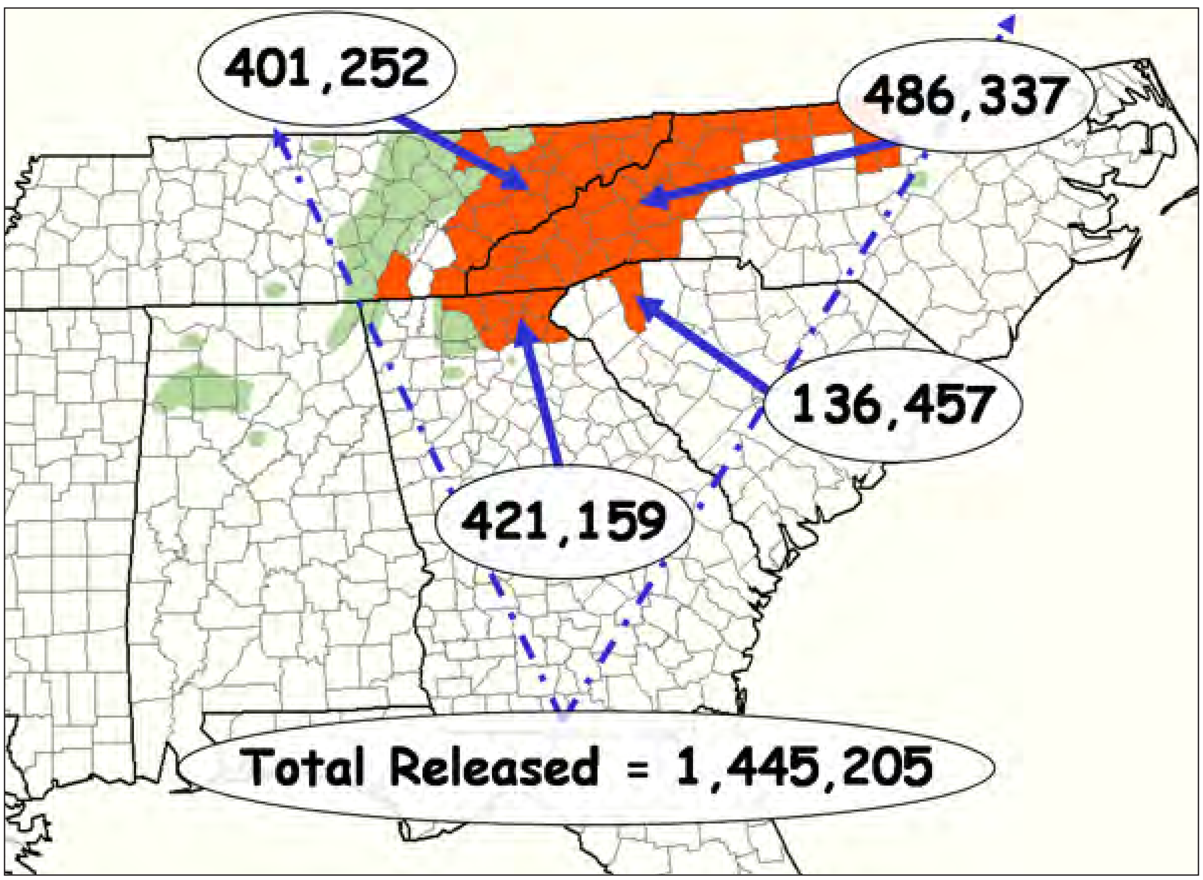

Figure 1. *Sasajiscymnus tsugae* releases in the southeastern United States, 2002-2007. The light colored (green) areas on the map illustrate distribution of eastern hemlock and the dark colored (orange) areas illustrate spread of hemlock woolly adelgid on eastern hemlock. The release numbers are based on data provided by various rearing laboratories and federal agencies; these are conservative numbers and may be slightly less than actual release numbers.

MONITORING AND RECOVERY

Large area-wide, comprehensive efforts to recover *St* in the South have not yet been implemented. In each state, however, researchers and cooperators have placed varying amounts of resources into documenting recovery and establishment of *St* in release areas. Recovery of adults has not been consistent. For example, *St* adults are found often within the first six months following release in all states (as late as early October in Tennessee). Lambdin and coworkers (2006) found three *St* adults four months after release in GRSM in June 2002.

It has been more difficult to document recovery the year following the initial release. In Georgia, no adults have been recovered at six release sites examined thoroughly with a bucket truck for two years (M. Dalusky, pers. comm.). In North Carolina, South Carolina, and Tennessee, only a few adults have been recovered at release sites one or more years after release (L. Burgess and K. Kidd, pers. comm.). Most of these release sites, however, were large areas with small numbers of beetles released. For example, a beetle release in GRSM usually consisted of 1,000 to 5,000 beetles released on several trees in a large area. In western North Carolina, where releases of large numbers of *St* beetles were concentrated on a few trees in a small area, adult *St* have been documented on eastern hemlock for several years (R. McDonald, pers. comm.).

Low recovery of *St* adults also has been observed in release areas in the northeastern United States. The low numbers of *St* beetles recovered at release sites may be attributed to several factors, including:

1. dispersal (following release, the beetles may move from release trees and inhabit other trees),
2. low survival (many of the earlier released *St* beetles were not acclimated to release conditions, which may have attributed to mortality),
3. inadequate timing of releases (adults may not have been released at the optimum time for the appropriate environmental conditions or for the appropriate HWA life stages),
4. mate location (how effectively are males and females locating each other after release and establishment in the field?),
5. predation (what effects do spiders and other established predators have on *St* eggs, larvae, pupae, and adults?),
6. low densities (are the beetles established but occurring at such low densities that their populations cannot be detected through normal sampling?),
7. large sampling area (is the release and sampling areas so large that beetles are easily overlooked?), and
8. insufficient time for populations to increase to easily detectable levels (is two to five years sufficient for populations of *St* to increase to detectable levels?).

Some anecdotal evidence suggests that populations of *St* are established in the southeastern United States. In some areas where *St* adults have been released, trees appear healthy, have good color, and are producing new growth. These release trees appear healthier than those where *St* has not been released. However, in other release areas, trees are in poor health or have died. Although the healthy appearance of hemlock trees in some release areas is encouraging, the results are inconsistent. In Tennessee and North Carolina, a few *St* have been recovered on HWA-infested plant material collected from the field and brought into the laboratory as food for predators (E. Bernard and K. Kidd, pers. comm.). It is difficult to confirm if this plant material contained field-produced *St* or if the material was contaminated with laboratory-produced *St*.

ONGOING RESEARCH AT THE UNIVERSITY OF TENNESSEE

Several research projects addressing biological control of HWA are underway or have been completed recently at the University of Tennessee. A few of these projects are outlined below.

Life Cycle. The life cycle of HWA in GRSM was documented (Deal 2007) and found similar to that reported in Connecticut (McClure 1989); however, there were a few important differences. HWA sistens began oviposition about one month earlier in Tennessee than in Connecticut, while the progrediens began oviposition about two months earlier in Tennessee. In both regions, sistens began aestivation in July and ended in October. These data suggest that HWA may feed one to three months longer in southern states, which may partially explain the rapid decline in tree health in this region.

Egg Releases. A protocol for the use of egg releases as a complementary means of establishing *St* has been developed and field-tested. The germinal idea of egg releases originated at the New Jersey Department of Agriculture. Temperature appeared to be the most important variable for survival of *St* eggs in the field; mortality of *St* eggs increased greatly as temperatures remained or dropped below freezing (Deal 2007, Grant et al. 2005).

Quantitative and Qualitative Assessment of *St*. A cooperative project with GRSM (Glenn Taylor) will further assess the establishment of *St* and its impact on tree health characteristics. As part of a large-scale study, 22 *St* release areas and 27 non-release/untreated areas have been established in GRSM. At each of these areas, hemlock trees will be intensively sampled for *St*. In addition, evaluation parameters (such as live crown ratio, crown density, crown transparency, twig dieback, HWA infestation level, new growth, etc.) will be assessed. These data will better define the establishment and impact of *St* on HWA in GRSM.

Large Tree Cage Assessments of *St*. The feasibility of using large tree cages (or screened enclosures) to assess establishment of *St* is underway. Cages have been developed and installed in the field. The tree inside each cage will be sampled every three months to assess survival and establishment of *St* as well as its impact on HWA.

DISCUSSION

Do we have unrealistic expectations of recovering *St* beetles at release sites in the southeastern United States, especially so soon following releases? If we consider the total number of beetles released (about 350,000) in GRSM and compare it with the area of land in the Park that has a hemlock component (from old growth to incidental hemlock), about 6.7 *St* adults have been released per hectare (2.7/acre). From that perspective, it is not surprising that *St* has not been recovered more often.

It is estimated that introduced biological control organisms generally require three to six years, depending upon prey and predator, to become established and begin exerting a noticeable effect on the targeted prey. Van Driesche and Bellows (2006) compared many biological control programs and concluded that, in most of these programs, declines in pest densities caused by introduced biological control organisms occurred over 6-10 generations of the prey. Applying this information to HWA, we should expect to find *St* in three to five years following release and also see declines of HWA. However, because of the high reproductive rate of the parthenogenic HWA females, *St* may require longer to exert pressure on HWA populations.

The vast majority of *St* releases in the South has occurred within the last one to three years. In fact, about 120 of the 150 release sites in GRSM were established since 2005, and two years is not sufficient time for establishment, population increase, and recovery. What is a sufficient time? Three years after release? Four years? Six years? Regardless of how long it takes to recover *St*, mortality of hemlock trees in the South is occurring at an alarming rate (about three years post-detection in most areas). The large tree cage assessments should enhance our understanding of the survival, establishment, and impact of *St* on HWA.

Release of *St* beetles has never been a primary short-term control strategy, but rather a long-term, sustainable and environmentally compatible population reduction strategy to lower HWA populations to non-threatening levels. Efforts to protect hemlocks using insecticides must continue while providing adequate time for establishment and population increases of *St*. This research has provided a better understanding of the life cycle of the HWA in the South and should enable us to better time pesticide applications as well as enhance our niche exploitation of HWA using introduced biological control agents. Egg releases also offer land managers an option to complement field releases of *St* adults when extra eggs are available from rearing laboratories. These egg releases can expand the areas where *St* are released and enhance management of HWA.

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Evaluation of Three Predators Released to Control the Hemlock Woolly Adelgid Using Whole-Tree Enclosures

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ABSTRACT

Predators released as biological controls of the hemlock woolly adelgid (HWA), *Adelges tsugae*, may have variable tendencies to disperse. Predators with high dispersal rates may fail to establish because they become too sparse to find mates after release. As a result, efforts to introduce them may be abandoned even though they might be effective at suppressing HWA if they did become established. Several coleopteran predators have been released against HWA with variable success. More information on their efficacy is needed so that decisions can be made as to which species should be emphasized in future efforts of mass rearing and release. Experiments using whole-tree enclosures allow us to evaluate the efficacy of predators in the absence of their dispersal. Here, we attempt to study the ability of three coleopteran predators, *Laricobius nigrinus* (*Ln*), *Sasajiscymnus tsugae* (*St*), and *Scymnus ningshanensis* (*Sn*), to decrease adelgid population density in whole-tree enclosures over the course of a year. Whole-tree enclosures give the beetles access to the forest floor, which may be important to their survival especially over the winter. This approach thus improves upon the many previous experiments with HWA predators inside mesh bags on branches, which are typically appropriate for only a few weeks or months.

Understory, 2-3 m tall hemlock trees at in a central Massachusetts forest were enclosed with screen cages in November 2006. There were eight replicates of five treatments: cages containing the predator *L. nigrinus*, cages containing *S. tsugae*, cages containing *S. ningshanensis*, cages without beetles (caged control), and uncaged trees (uncaged control). The density of adelgid was estimated by visual counts of ovisacs on 10 branches per tree in November. Predators were released inside cages on 29 November 2006. Branchlet samples collected in spring 2007 recovered a total of 12 *Ln* larvae from five cages and a total of five *St* larvae from four cages, indicating that adults had survived the winter and successfully reproduced. *Sn* was not observed since the release and likely has not survived (the adults were not fully mature and properly conditioned prior to the release). In order to determine how adelgid populations

were changing in size, the same 10 branches per tree sampled three times later in the season. In July 2007, branches were revisited to assess the density of progrediens; in August 2007, the density of newly settled sistens on new growth was recorded; and in December 2007, the density of sistens nymphs that produced woolly mass was measured.

In July, the density of HWA progrediens was significantly lower in *Ln* and *St* cages. This trend continued into August, when the density of newly settled sistens was also lower in *Ln* and *St* cages, indicating that these predators have been having an impact on HWA populations. By December, however, there were no differences in adelgid density among treatments, and trees were showing obvious signs of cage effects: many branches and, in some cases, large portions of trees were dead. This may have been a result of trees already in the understory receiving even less light inside cages or of the root systems of caged trees being damaged during the cage installation process. We were therefore unable to determine whether the lack of significantly lower HWA population sizes one year later were not observed because of cage effects or an inability of the predator species to regulate the adelgid.

LOW TEMPERATURE IN THE HEMLOCK WOOLLY ADELGID SYSTEM

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ABSTRACT

Hemlock woolly adelgid (HWA) vary in their susceptibility to temperatures considerably below freezing. Many individuals may die by -20°C (-4°F), but more cold-tolerant individuals will allow populations to persist. Managers wanting to gauge temperature impacts on future adelgid pressure to hemlock forests may benefit from using available daily records of minimum low temperatures. More robust data on adelgid temperature response will enhance development of models/tools to assist in their forecasts. Exotic predators being released to suppress adelgid populations (*Sasajiscymnus tsugae*, *Scymnus sinuanodulus*, *Scymnus ningshanensis,* and *Laricobius nigrinus*) may be slightly less cold-tolerant than the adelgid, but the cold-tolerance does not appear to be affected by feeding. These predators are able to feed at near 0ºC temperatures. In areas with relatively cold climates, the low winter temperature and the four predators tested could work together to regulate HWA populations.

KEYWORDS

cold tolerance, predator, microclimate, range

INTRODUCTION

Cold weather—or rather, low winter temperature—has a dynamic role in impacting populations of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, and defining the limits of their geographic expansion. The consequences of low temperature extends beyond mortality within HWA populations to influences on predators that might serve to reduce HWA numbers. A variety of predators are being released in the hemlock ecosystem in attempts to stabilize HWA populations at levels that will reduce their impacts to hemlock forests. We need not only a better understanding of predator survival at low temperatures but also of the behaviors they might have to protect themselves as temperatures decline and whether bouts of feeding during warming periods affect their cold tolerance.

Temperature within an ecosystem environment can be measured as ambient (i.e., the temperature without influences of solar radiation) or it can be measured as the microenvironment that a target organism might experience. Depending on where an insect is located at any given time in its lifecycle, its microhabitat might be on an hemlock needle, in the cracks of bark, or in the soil, among other places. Previous research (Costa et al. 2004) during the winter season in Massachusetts and Connecticut has compared the microclimatic extremes on hemlock foliage with ambient temperature records obtained in adjacent weather stations. Daily maximum winter temperatures on foliage were increased several degrees Celsius over ambient, with increases corresponding to recorded periods of sunlight. However, the difference between daily minimum temperature on hemlock foliage and ambient records fluctuated much less. The reason for this disparity between how maximum and minimum microenvironment temperatures reflect ambient conditions is that the lowest temperatures in the hemlock forest generally occurred at night when there was no sunlight to warm needles.

The similarity between ambient recordings of daily minimum temperatures and that found in the HWA microenvironment suggests the usefulness of ambient temperature records for assessing impacts of low temperature on HWA survival. Conveniently, this relationship makes the vast resources of meteorological data that is annually collected of great potential value in predicting areas where HWA pressure may be currently mitigated or expected to escalate in the coming year. There is a limited amount of information available on HWA cold tolerance to give a general indication of the range of HWA susceptibility to cold (Parker et al. 1999; Skinner et al. 2003). Data that exams mortality at narrower temperature increments is critical for more precise determinations of HWA response to cold.

The results presented in this paper provide updated data on HWA cold tolerance, the low temperature and feeding response of several HWA predators, and initial observations on predator low-temperature behavior.

Super-Cooling Point

For freeze-intolerant insects (as opposed to those that can tolerate freezing without dying), the temperature that freezing occurs can be considered the lowest possible temperature before mortality is expected to occur. The primary measurement used for determining the lethal temperature is the observation of super-cooling points (SCP) for individual insects. The basis of super-cooling point determination is the fact that, as water crystallizes or freezes, it releases a small amount of heat, which can be detected with a suitably sensitive temperature probe.

The term 'super cooling' stems form the observation that water can freeze at temperatures below 0ºC (32ºF) and thus allow organisms to survive normal freezing conditions. To assess the freezing point, insects are subject to rapidly decreasing temperatures (1-2ºC/minute) while their temperature is monitored at very short intervals (every second in our research). Later, the temperature records are examined and the lowest temperature achieved before the brief release of heat occurs is the super-cooling point for that individual.

Significance of HWA Super-Cooling Point Variation

The super-cooling point varies among individuals of a particular species and can also be influenced overall in a population by various factors such as time of year, preconditioning, and geographic sources of the insect, among other factors. Figure 1 depicts the SCP for HWA collected in the southern and northern extents of their range, and each individual point in the scatter plot represents an SCP determination. In general, many of the insects tested had a SCP around -18°C (0°F), and those from northern regions tended to tolerate slightly lower temperatures.

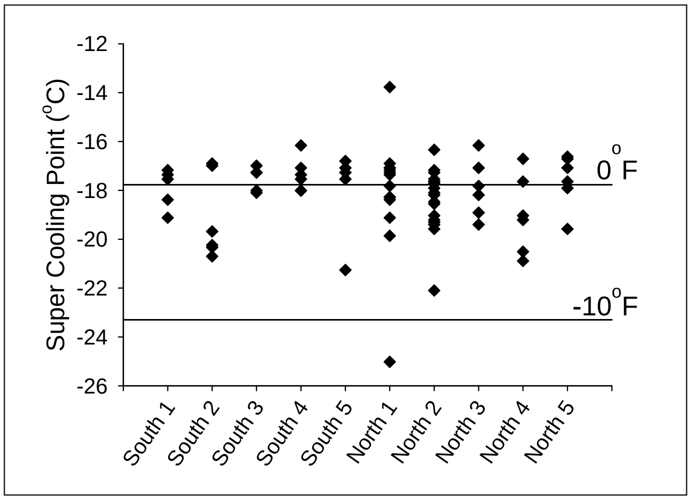

Figure 1. Super-cooling point of hemlock woolly adelgid collected during winter and early spring in the southern region (one site in Georgia, three in North Carolina, and one in Virginia) and northern region (one site in Pennsylvania, three in Connecticut, and two in Massachusetts). Each triangle represents a single determination. Horizontal lines provide reference between temperature scales.

Knowing the proportion of insects that will die at a particular temperature can have considerable value when coupled with temperature records for a given region. Importantly, Figure 1 shows that there are several insects that survive at very low temperatures. These few individuals can have profound affects on HWA populations in an area after what would appear to be a substantial winter cold event, especially with the high fecundity of HWA. However, for this kind of information to be of increased practical value for predicting temperature influence on upcoming HWA pressure in a forest, a more complete characterization of the frequency distribution of SCP values would be a benefit. Trotter is developing a comprehensive Hemlock Woolly Adelgid Simulation Model based on our research and a multitude of other available data. A subcomponent of this model involving HWA overwintering mortality could be adapted as a Forest Health HWA Impact Tool.

The practical impact that temperature can have on HWA populations and the synchrony between the SCP data in Figure 1 and what occurs in a forested setting can be deduced from results obtained under natural settings. Data collected in Massachusetts and Connecticut on the mortality of HWA over time in relation to microclimate low temperature shows that there was limited mortality during early January of those sistens of HWA that had survived summer aestivation. However, two low-temperature events occurred in mid-January in which microclimate temperatures fell to below -20°C. Subsequent to this, HWA mortality dramatically increased at both locations with more dying in Massachusetts, where temperatures were lower. The observation that 99.5% of the individuals died at Mount Tom, Massachusetts, and that damaging populations still persist there today highlights the significance of the few individuals with appreciable lower SCP values.

Predator Low-Temperature Feeding and Cold Tolerance

A data set is developing on four predators being released for management of HWA (*Sasajiscymnus tsugae* Sasaji and McClure, *Scymnus sinuanodulus* Yo and Yao, *Scymnus ningshanensis* Yo and Yao, and *Laricobius nigrinus* Fender) in relation to their ability to feed at temperatures ranging form 0-10°C. The adult beetles were placed individually in tubes, brought to 0°C in a low temperature bath, and then provided 15-25 HWA eggs, after which the temperature was raised to the target test temperature for 20 hours. Subsequent egg counts revealed that the beetles were able to feed down to 2.5°C (Figure 2; similar results were obtained with *L. nigrinus* but are not presented). This suggest that beetles active during the winter months are able to feed as temperature rise above freezing. Because sunlight can raise the microclimate temperature several degrees (Costa et al. 2004), it is likely that higher levels of feeding are possible or that beetles may feed even when ambient temperature conditions suggest otherwise.

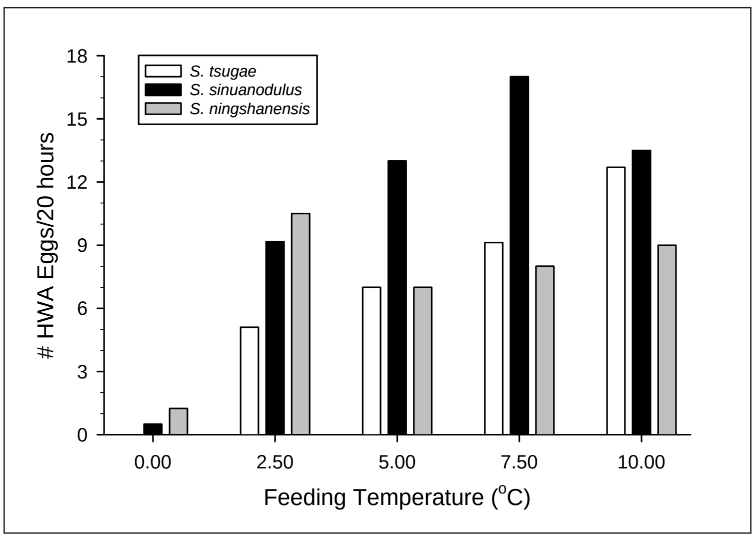

Figure 2. The feeding activity of *Sasajiscymnus tsugae*, *Scymnus sinuanodulus*, and *Scymnus ningshanensis* on hemlock woolly adelgid eggs at the temperatures indicated. Similar results were obtained with *Laricobius nigrinus,* although response at 2.5°C was not examined.

For insects feeding during the cold season, a question arises as to whether the consumption of food increases their susceptibility to cold. Initial observations made by determining the SCP of insects from the feeding trial above suggest that there is little difference in cold tolerance among fed and starved beetles (Figure 3; similar results were obtained with *L. nigrinus* but are not represented. The lack of influence on SCP favors the survival of insects feeding in an environment where temperatures can change rapidly and refuge from low temperatures is not readily available. These results coincide nicely with previous unpublished data for low temperature survival of *S. tsugae*. Note that the scatter of the SCP response (Figure 3) tends to be slightly above -18°C, whereas those for HWA (Figure 1) tended to be more around this point. Regardless, the differences are relatively minor, which is promising news for the geographic (environmental) range compatibility of HWA and the predators being released for its management.

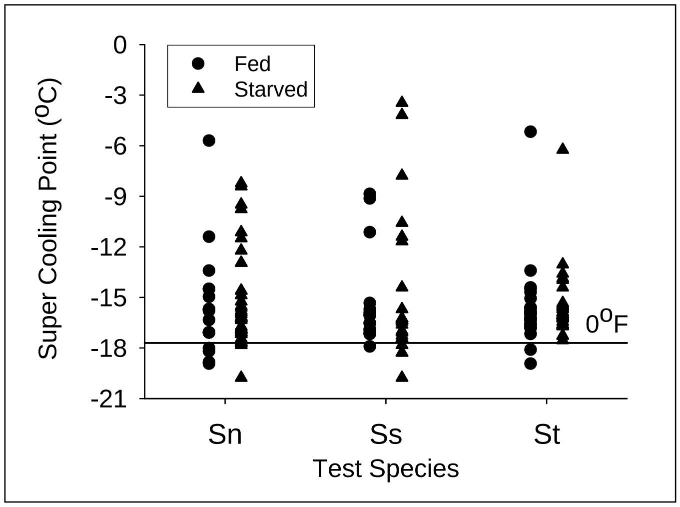

Figure 3. Super-cooling point of three hemlock woolly adelgid predators (*St* - *Sasajiscymnus tsugae*, *Ss* - *Scymnus sinuanodulus*, and *Sn* - *Scymnus ningshanensis*) that were either fed (●) or starved (▲) at low temperatures indicated in Figure 2. Each symbol represents a single determination. Horizontal lines provide reference between temperature scales. Similar results were obtained with *Laricobius nigrinus*.

Behavioral Response to Low Temperature

A critical question concerns the behavioral responses predators may have to protect themselves from freezing as temperatures fluctuate in the winter. For instance, do they remain on the foliage or do they seek refuge in bark or in the soil? Technology previously used to monitor the flight behavior of fruit flies under micro-gravity conditions (Miller et al. 2002) was adapted to monitor beetle behavioral responses to changing temperatures. The basic setup involves closed insect runways (10 lanes) etched into Plexiglas where the passage of beetles down the channel is detected at two points as they pass through infrared beams. To simulate a non-freezing refuge (e.g., soil), the temperature at the bottom of the channels can be regulated (5°C for this study) while the rest of the unit is cooled (i.e., via immersion in a low temperature bath). Temperatures were monitored at the top and bottom of the behavior chamber, which currently is placed vertically in the bath. This technology became available during early 2008 and we are just beginning its use as a research tool. The question being addressed is: Where do beetles go as the temperature drops?

Initial observations with *L. nigrinus* found considerable activity in the upper part of the chamber when temperature in the entire observation chamber was maintained at 15°C. But as temperatures began to decline beetle activity diminished. The temperature in the upper chamber dropped to near zero, while temperatures in the bottom chamber did not go under 5°C. As this occurred, the activity records indicated that the beetles moved downward toward the area of temperature refuge (i.e., where it was warmer). Our preliminary observations with *S. tsugae* were less convincing because of their reluctance to move; however, the activity profiles suggested that *S. tsugae* remained in the top of the chamber as temperatures declined. Conceivably, these preliminary results might suggest that, in the natural environment *L. nigrinus* either seeks refuge near warmth or by moving downward in response to declining temperatures, whereas *S. tsugae* may remain where they are located (upper chamber), presumably where they had been feeding on HWA.

HWA SYSTEM CONSIDERATIONS

The use of existing ambient low temperature records has excellent potential as a valuable resource for gauging impacts on HWA overwintering mortality. To increase the robustness of this approach for incorporation into a Forest Health HWA Impact Tool there is a need for expanded data on HWA SCP that captures the overall frequency distribution of SCP responses. The somewhat similar SCP profiles of HWA and its predators is a good sign and signals hope for wide compatibility within their geographic range. Additionally, there is indication that these predators may impact HWA populations when ambient and/or microenvironment temperatures rise modestly above freezing. These data suggest that in relatively cold climates the low winter temperature and the four predators tested could work together to regulate HWA populations.

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RECOVERY OF HEMLOCK WOOLLY ADELGID PREDATORS IN THE HIGH COUNTRY OF NORTHWESTERN NORTH CAROLINA, 2004-2008

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ABSTRACT

Three species of predatory beetles have been released to combat infestations of the hemlock woolly adelgid in the High Country region of northwestern North Carolina. They are the spring/summer predators *Sasajiscymnus tsugae* Sasaji (*St*), *Scymnus sinuanodulus* Yu and Yao (*Ss*), and the winter/fall predator *Laricobius nigrinus* Fender (*Ln*). A total of 890 adults of *St* have been released at three sites and adults were recovered from two of the three sites; one site was two years old. We also recovered *St* from a site where no releases were made by this group (Lees McRae Field Site). For *Ss*, 724 adults were released at three sites; within-season

recoveries were made at all three sites, but no F_1 adults have yet been found. For *L. nigrinus*, a total of 3,238 adults have been released at nine sites. Three of these sites are intensive study sites (Hemlock Hill, Holloway Mountain, and Lees-McRae Field). For Hemlock Hill, we have continuous adult recoveries in increasing numbers from 2004 to 2008 (establishment); adults have been found over 3/8th mile from the original release. The majority of beetle recoveries the first three years were in areas receiving winter light (southern facing coves or trees in the sun). HWA ovisacs with an *Ln* egg or larva at sampled sites ranges from 0 (River and Tate Field) to 31% (between release trees 9 and 10). Thirty one percent of HWA ovisacs with a *L. nigrinus* egg or larva translates to a predation rate of between 60 and 90 percent because a single larva consumes more than two ovisacs to complete development. The Holloway Mountain release site has establishment (F_3) and dispersal of *Ln* adults more than ¼ mile from the original release trees. Lees-McRae field release has F_2 beetles from the first wild-collected *Ln* (Oregon State University) release. We are achieving bracketing by having a spring/summer predator release with a fall/winter predator release at four sites and continuing strategic releases of beetles at ecologically sensitive areas, such as headwaters and other areas receiving adequate winter sunlight. We are cautiously optimistic that successful biocontrol of the HWA will occur in these release areas as we are seeing regrowth of hemlocks in the presence of predators at the three main release sites.

KEYWORDS

hemlock woolly adelgid, biological control, *Sasajiscymnus tsugae*, *Scymnus sinuanodulus*, *Laricobius nigrinus*

STUDY AREA

The High Country of northwestern North Carolina (Ashe. Avery, Alleghany, Mitchell, Yancy, Watauga, and Wilkes counties) is considered one of the most biologically diverse areas in the world. This area is rich in conifers and has native stands of both the Carolina and eastern hemlock. The High Country is also the headwaters for at least five major river systems—the New (both North and South forks), Watauga, Yadkin, Catawba, and French Broad rivers—making it an important source for trout and other riparian species. The hemlock woolly adelgid (HWA), *Adelges tsugae*, expanded its range into this area around year 2000; the High Country area is now considered generally infested.

In response to the threat to hemlock survival in this area, a cooperative effort between the USDA Forest Service, Virginia Tech University, Blue Ridge Resource Conservation and Development Council, Appalachian State University, and the author was implemented starting in 1999. Three species of adult predatory beetles of the HWA have been introduced during the course of these studies: the spring/summer predators *Sasajiscymnus tsugae* (*St*) (originally from Japan) and *Scymnus sinuanodulus* (*Ss*) (a Chinese HWA predator), and the fall/winter active native predator from the Pacific Northwest, *Laricobius nigrinus* (*Ln*) (Mausel 2007).

RELEASE OF *SASAJISCYMNUS TSUGAE*

Releases and recoveries of *St* adults are shown in Table 1. There have been at total of 890 adults released in three areas of the High Country. Recoveries of *St* beetles has occurred at three sites; one recovery is from a site where a release of beetles was made two years prior (Hattie Hill) and a second recovery of *St* beetles was made along the Elk River west of Banner Elk, though we have no record of releases made in this area. We are unsure whether this Elk River recovery is from dispersal or a release.

Table 1. Releases and recoveries of *St* beetles in the High Country of North Carolina, 2004-2007.

Release Date	Number/Whom	Release Site	No. Recovered	Date Recovered
April 2005	500/Graham	Hattie Hill (Bethel)	2	15 March 2007
April 2005	300/Graham	Grandfather Mountain	No sample	None
June 2006	90/RCM	Sugar Grove	2	April 2007
Unknown	0	Lees McRae Field (Elk River)	6	June 2006

RELEASE OF *SCYMNUS SINUANODULUS*

Releases of *Ss* adults are shown in Table 2. There have been a total of 724 adults of *Ss* released at three sites in the High Country. Within-season recoveries of *Ss* beetles have occurred at all three sites. Of note is the recovery of two *Ss* adults on the 25 November 2006 in Sugar Grove and a large number of adult *Ss* beetles found on both hemlocks and white pines at the Holloway Mountain release site. Monitoring has yet to find F_1 beetles.

Table 2. Release and recovery numbers for the Chinese HWA predator *Scymnus sinuanodulus*. All work with this predator has been cooperation with USDA Forest Service researcher Dr. Michael Montgomery.

Release Date	Number/Whom	Release Site	No. Recovered	Date Recovered
June-July 2006	228/RCM	Sugar Grove	10	July-November 2006
April 2005	400/MEM	Holloway Gap	63	April-October 2007
15 April 2007	96/RCM	Hemlock Hill	2	Late April 2007

RELEASE OF *LARICOBIUS NIGRINUS*

Cooperative work was performed with Virginia Tech on the three intensive study sites and six other release sites. Intensive study sites are: 1) Hemlock Hill (300 adults released 31 December 2003 and an augmentation of 300 beetles late March 2006); 2) Holloway Mountain (150 adults October 27, 2004, plus an augmentation of 240 adults late March 2006) and 3) Lees-McRae Field Research site (202 beetles from Oregon State University released December 2005/February 06). Adult beetles were recovered using beat-sheet sampling twice monthly from September to December and January to March of each year.

Larval sampling was by David Mausel and consisted of branch samples taken at mid- and upper-canopy of the 10 release trees

Release site #1: Hemlock Hill, Banner Elk

David Mausel, Ph.D., of Virginia Tech released 300 beetles (British Columbia strain) at Lees McRae College's Hemlock Hill, a significant area of old-growth hemlocks. Table 3 shows the recovery of *Ln* adults at the Hemlock Hill site post release. There was an exponential recovery by generation: three F_1 adults, 12 F_2 adults, 202 F_3 adults, and 103 F_4 adults (thus far: we will be sampling for winter 2008 numbers until March).

Table 3. Recovery of *Ln* adults by season and place at Hemlock Hill, 2004-2008. Larval numbers are from Mausel.

Season or Place	F_1 Adults ('04/'05)	F_2 Larvae (April '05)	F_2 Adults ('05/'06)	F_3 Larvae (April '06)	F_3 Adults ('06/'07)	F_4 Adults ('07/'08)
Fall	3		12		93	80
Winter	0	10	0	314	109	23
River	2		1		4	41
Ridge (Fall)	1		11		89	39

Release sites and dispersal patterns of *Laricobius nigrinus* at Hemlock Hill are shown in Figures 1 and 2. Once warmer weather or sunlight is present, dispersed adult beetles move into north-facing or shaded coves. In Figure 2, dispersal recoveries are shown in blue boxes: 1) mid-November of 2006 recovery of a single *L. nigrinus* adult on the main ridge of Hemlock Hill, nearly 1,000 feet from the nearest release tree; 2) March 2007 recovery of a single *L. nigrinus* adult more than 3/8ths mile from the closest release site; 3) December 2007, Luker and McDonald found two adults in same area as above; and 4) January 2008, Hamstead and McDonald recovered one *Ln* adult below Tate Field scoreboard, 200 yards beyond any prior known dispersal location.

The first two years post-release, we found beetles only on release trees; by the third year, we found abundant beetles in an areas that received abundant winter sunlight. Our survey data infer that beetles are more prevalent on south-facing aspects in winter months. This can help ensure success of beetle releases by strategically placing them in areas they prefer rather than northern aspects that receive no sun.

In order to determine predation rates of HWA ovisacs in the release area, HWA-infested branches were collected from February. to April. We focused on areas with high beetle recoveries (between release trees 9 and 10) as well as samples from the Elk River and Tate Field. Clipped branches were brought back to laboratory and ovisacs were dissected to determine presence of *Ln* egg/larva (Figure 3).

Release Site #2: Holloway Mountain

On 27 Oct 2004, 150 beetles were released on the north ridge of Grandfather Mountain at 3,6000 feet elevation; an additional 240 beetles were released on March 2006. In 2006, beetles were found 1/4 mile from the release site.

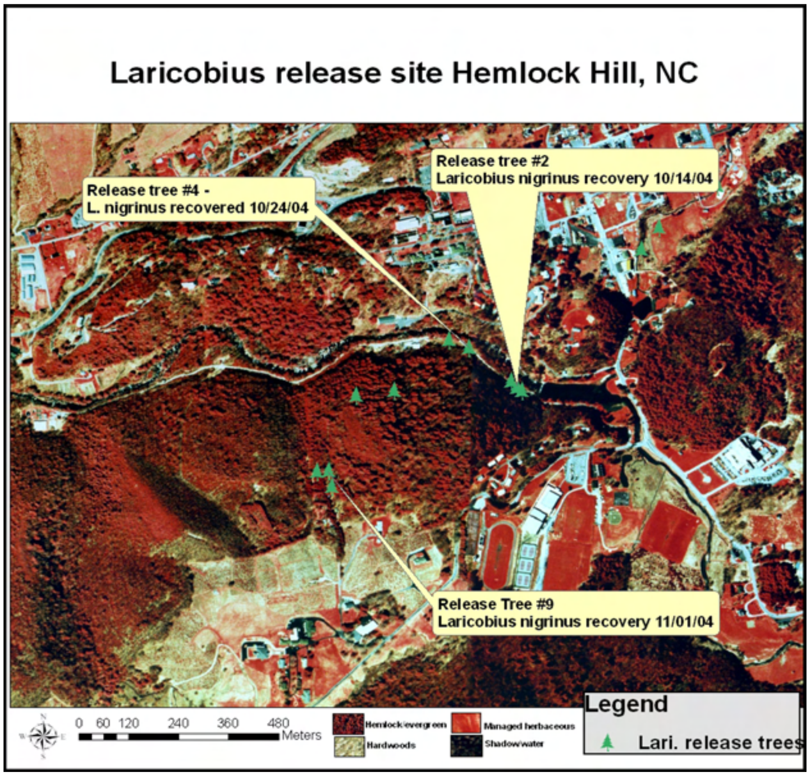

Figure 1. Release sites of *L. nigrinus* F_1 adults at Hemlock Hill. Compare this figure to the pattern of adult recoveries for F_2 to F_4 beetles in Figure 2. Infrared map courtesy of James Graham.

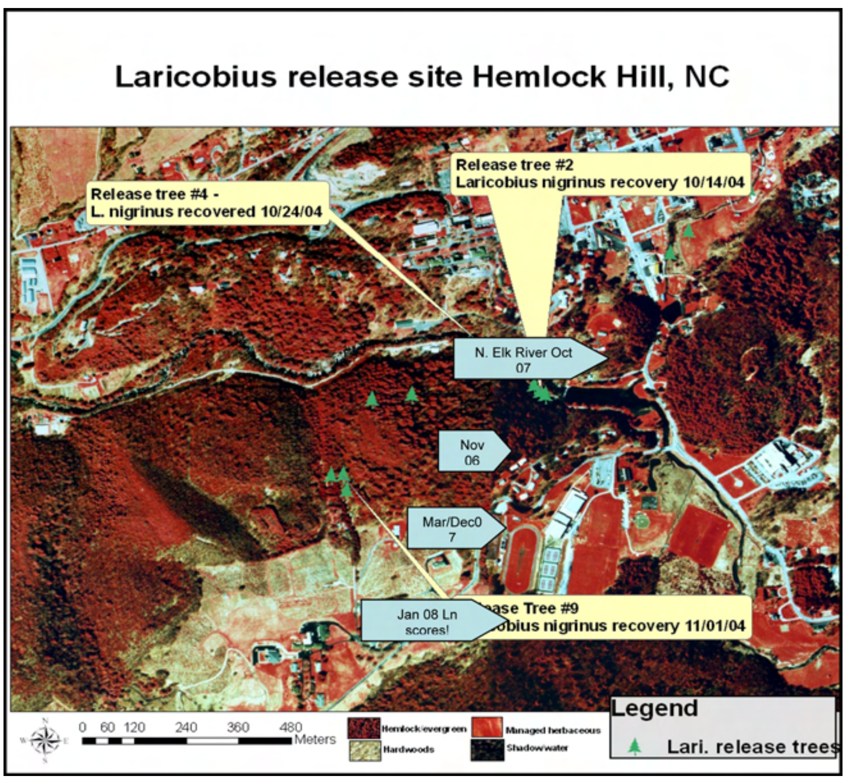

Figure 2. Pattern of dispersal of *Ln* adults from 2004 through 2008. Adults appear to be more prevalent on south-facing slopes during the coldest times of the year (December-February).

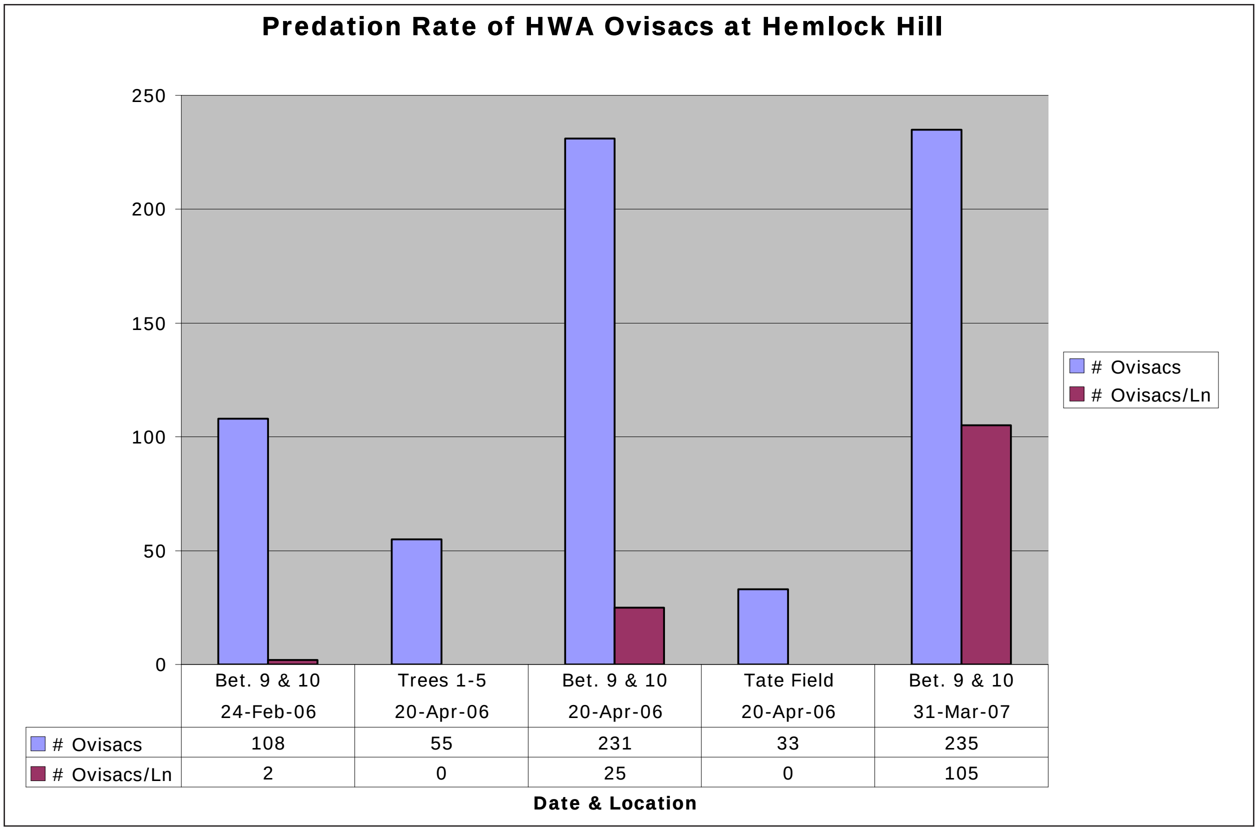

Figure 3. Predation rates of HWA by *L. nigrinus* through dissection of ovisacs. Ovisacs with a *Ln* egg or larva present were considered positive for predation. We found a 31% predation rate of ovisacs during late March 2007; HWA averages around 75 to 100 eggs per ovisac (smaller than western strain). It takes roughly 200-250 eggs for a *Ln* larvae to complete development: thus, 31% predation means that each larva eats an additional 2+ ovisacs, so the actual predation rate is between 60 to 90 percent after three years. Predation rates are approaching what is measured in the Pacific Northwest.

Table 4. Recovery of *Ln* adults at Holloway Mountain. Eight F_3 adults have been recovered during the '07/'08 season. This is the second site with establishment of *Ln* and dispersal of beetles more than ¼ mile from the original release trees.

F_1 Adults ('05/'06)	F_2 Adults ('06/'07)	F_3 Adults ('07/'08	F_1 Larvae (April '05)	F_3 Larvae (April '06)
1	9	8	2	24

Release site #3: Lees McRae Field Laboratory

Of 202 field adults received from Portland, Oregon, 100 were released 1 December 2005 (50 on each of two trees) and 102 adults were released on one tree 2 February 2006. Recoveries are presented in Table 5.

Table 5. Recovery of *Ln* adults at the Lees-McRae Field Laboratory site. Limited sampling at this site during fall of 2007 and winter of 2008.

F_1 Adults ('06/'07)	F_2 Adults ('07/'08)
6	1

Additional Release Sites

Additional releases of *Ln* adults in the High Country area are presented in Table 6.

Table 6. Additional releases of *L. nigrinus* adults in the High Country, 2005-2007. Additional resources to monitor these sites are needed.

Year of Release	Site	Release Date	*Ln* Total Number	*Ln* Source	F_1 Adults	F_2 Adults
2005	Sugar Grove	Dec.	146	WA Coll.	5	16
2006	Dugger Cr.	Nov. 06 +Jan. 07	1,100	WA Coll.	no sample	
2006	Simm's Cr.	March	200	WA Coll.	no sample	
2006	Hattie Hill	March	300	WA Coll.	2	
2006	Pensacola	March	100	WA Coll.	no sample	
2007	Beech Bog	Nov./Dec.	200	WA Coll.	no sample	
Totals			2,046		7	16

In addition to *Ln* adults, we also recovered adults of *L. rubidus* at most sites. We found *L. rubidus* on white pine saplings with pine bark adelgid, *Pineus strobi* (Hartig). The fewest *L. rubidus* were found at Hemlock Hill, and the most *L. rubidus* were found at Holloway Mountain. We are working with Virginia Tech and the USDA Forest Service to determine the impact, if any, of *L. rubidus* on HWA populations.

SUMMARY

Sasajiscymnus tsugae

- 890 *St* beetles were released at three sites (Hattie Hill, Grandfather Mountain, and Sugar Grove).
- Three sites showed positive for *St* (Hattie Hill, Sugar Grove, and Lees McRae Field); one site (Hattie Hill) two years old and another site (Lees McRae Field) has no record of *St* release.

Scymnus sinuanodulus

- 724 adults of the Chinese predator *Ss* were released at three sites (Sugar Grove, Holloway Mountain and Hemlock Hill).
- *Ss* adults were found during the season of release at all three sites.
- *Ss* adults also were found on pines, suggesting *Ss* feeding on pine bark adelgid.

Laricobius nigrinus

- Releases of adult *Ln* beetles starting in 2003 resulted in recovery of next-generation adults at five release sites: Hemlock Hill (F_4), Holloway Mountain (F_3), Lees McRae Field (F_2), Sugar Grove (F_2) and Hattie Hill (F_1). Four other release sites were not sampled.
- Both lab-reared beetles from Virginia Tech and field-collected adults from the West Coast have established or colonized hemlocks.
- Hemlock Hill (2003 release) results:
  - Exponential increase in adults each generation: F_1=3, F_2=12, F_3=202, F_4=103 (still sampling).
  - *Ln* adults dispersal over a ½-square-mile area.
  - More beetles present on sunny, south-facing aspects.
  - 31% of HWA ovisacs infested with *Ln* at the site between release trees 9 and 10; this translates to a 60 to 90 percent predation rate.
  - The site has the potential to be used as a nursery to provide beetles for distribution to other nearby areas.
  - We are seeing regrowth at Hemlock Hill, Holloway Mountain, and Lees-McRae field sites in the presence of predators.
  - Resource managers should make continued releases of all three predators a priority.

ACKNOWLEDGEMENTS

The author wishes to thank all persons associated with biological control of the HWA for their efforts, especially Virginia Tech, USDA Forest Service, North Carolina Division of Forestry's Urban and Community Forestry Grant Program, Lees McRae College, and the Blue Ridge Resource Conservation and Development Council.

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Arthropods Associated with Eastern Hemlock

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KEYWORDS

arthropods, guilds, canopy community, detritivores, oribatids

ABSTRACT

We conducted a literature review of arthropods associated with eastern hemlock, *Tsuga canadensis* (L.) Carriere, to interpret the results of several field surveys. To date, 95 insect species and three mites have been found to be associated with eastern hemlock. Many of these species are generalists, though a few are specialists that feed on conifers. None of the arthropods cited in the literature were found to feed exclusively on eastern hemlock.

We compared the results of field surveys conducted at Shenandoah National Park (Virginia) and Great Smoky Mountains National Park (Tennessee) with our surveys at the West Virginia University Forest and Botanic Garden (West Virginia). Arthropods in these studies were collected using a variety of methods: branch beating, branch clipping, Malaise traps, trunk traps, light traps, and pitfall traps. The project objectives were to survey eastern hemlocks and catalog the insect (arthropod) fauna associated with them. 180 to 215 species of insects and 33 species of mites were identified. In addition, we also collected two undescribed oribatid mite species. A comparison of arboreal guild compositions among studies determined that the majority of species in these studies were detritivores. The broad sweeping branches of eastern hemlock appear to function as collectors of leaf litter and debris, forming the basis for a complex food web. This canopy community of arthropod fauna appears to be dependent on the crown structure of eastern hemlock and will be impacted by the foliar reduction and eventual loss of eastern hemlock caused by the hemlock woolly adelgid.

We presented a discussion of detritivores, oribatid mite biology, and our future analysis plan for these and other data.

Managing Hemlock Woolly Adelgid at Great Smoky Mountains National Park: Situation and Response

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ABSTRACT

National Park Service mandates and policy require prompt action when invasive species threaten native biodiversity. Great Smoky Mountains National Park manages extensive hemlock forests and has worked aggressively to preserve these resources and associated species dependent upon them. Developed areas (trail corridors, picnic areas, and campgrounds) have been treated using a combination of insecticidal soap and systemic insecticides, while landscape-level control measures include two species of biological control beetles and systemic insecticides.

KEYWORDS

hemlock woolly adelgid, Great Smoky Mountains National Park

INTRODUCTION

Great Smoky Mountains National Park in Tennessee and North Carolina contains the most extensive old-growth forests in the eastern United States. Approximately 20 percent of the Park's 800-square-mile territory was never harvested for timber, and most of the Park is currently managed as wilderness. The National Park Service (NPS) mandate requires managers to "preserve and protect" these resources "unimpaired for future generations." NPS policy and federal Executive Orders also emphasize managing invasive exotic species threatening native ecosystems and biodiversity. Great Smoky Mountains National Park (GRSM) began removing exotic plants such as kudzu as early as the 1940s and continues to systematically document, treat, and monitor over 50 exotic plant species at over 900 sites. The Park began control of balsam woolly adelgid on Fraser fir using insecticidal soap in the mid 1980s and has a lengthy history of research and monitoring on other non-native forest health threats, including dogwood anthracnose, butternut canker, beech scale/beech bark disease, and mountain ash sawfly. Park staff anticipated the arrival of hemlock woolly adelgid (HWA) in the southern Appalachians by conducting surveys of old-growth hemlock forests and associated species of arthropods, plants, and birds and responded promptly when a trail crew worker made the first report of HWA infestation.

PROGRAM OVERVIEW

The hemlock woolly adelgid control project has greatly expanded since the non-native insect was first discovered in GRSM in 2002. Financial support comes from the Friends of the Smokies, Great Smoky Mountains Association, USDA Forest Service (USFS), and NPS. A full-time coordinator and six subject-to-furlough forestry technicians were hired in 2004. HWA surveys were conducted throughout the Park, concentrating on the nearly 1,500 acres of old growth, 35,000 acres dominated by hemlock, heavily visited developed areas, and roadsides.

GRSM has coordinated and cooperated with several important research projects related to HWA, including studies of avian and arthropod diversity in hemlock forests, mychorrhizal associates, efficacy of biological and chemical controls, and hemlock structure and ecology. Mycologists from Mississippi State University are conducting an etcomycorrhizal study (2006-2009) to support potential future reforestation efforts for hemlock. One hundred and seventy macrofungi were collected in 2006-2007 and identified from two plot areas of different elevations in GRSM. Species richness at both locations included 58 fungal genera (111 species). Also, 41 genera (87 species) from Copeland Creek and 33 genera (39 species) from Gabes Mountain plots were identified. A total of 18 of the genera were the same across locations, while 36 genera were unique to Copeland Creek and 23 genera were unique to the Gabe's Mountain plots. The five most common genera were *Lactarius* (16 species), *Amanita* (13 species), *Russula* (six species), *Tricholoma* (four species), and *Cortinarius* (four species). All of the fungi listed above were previously reported to be ectomycorrhizal associates with forest trees. In addition, environmental samples (soil from the rhizosphere) were taken to compare field collections with molecular sequence data. Preliminary molecular data indicate an average of 21 unique species associated with the roots of non-treated control trees. Trees receiving a low-dose treatment of imidacloprid have an average of 19.5 unique species (M. Alexander and R. Baird, Mississippi State University, pers. comm., Jan. 2008).

In 2006, GRSM began cooperating with Will Blozan and Jess Riddle of the Eastern Native Tree Society in the Tsuga Search project to document and treat eastern hemlock trees of exceptional size. This research contributed significantly to our knowledge of the resource (particularly of more remote old-growth stands) and hemlock tree physiology and structure. Trees over 170 feet tall were measured, among the largest specimens known for hemlock.

By 2006, infestations were identified in all major watersheds of the Park, although there are still areas where HWA has not been seen and/or where infestations are in the early stages and hemlock stands appear healthy. Hemlock mortality was very visible from main roads for the first time in 2006 and increased in 2007. Severe drought contributed to physiological stress in many tree species, especially on drier sites, and hemlocks were no exception. Hemlock forests in Cataloochee, Cades Cove, and along US 441 were showing widespread decline and mortality. Water quality impacts are not yet known, but some hemlock-dominated streams were tinted amber by dissolving hemlock bark following heavy rains in 2007. As more hemlocks die, lose tannin-laden bark, and collapse into streams, water quality and stream dynamics will be dramatically altered throughout the Park and will affect downstream communities.

Planning for HWA control followed NPS Integrated Pest Management policy and was coordinated with USFS and other agencies (USDA's Animal and Plant Health Inspection Service—APHIS, and theTennessee and North Carolina Departments of Forestry and Agriculture). An Environmental Assessment was completed in 2005 that included public input and inter-agency review prior to expanding treatments from developed areas to natural areas. Integrated Pest Management for HWA includes surveys, pre- and post-treatment assessments, and chemical and biological controls.

Biological control organisms are used under the guidance of the U.S. Department of Agriculture, which has approved three predatory beetles specific to adelgids for HWA (Figure 1). Releases of predatory beetles (*Sasajiscymnus* sp.) as a biocontrol began in 2002. The University of Tennessee started rearing beetles and supplying them to the Park in 2004 and reached full capacity in 2006. In 2007, the Park received 67% of the beetles produced by the lab, with the remainder going to USDA Cherokee National Forest and the Tennessee Department of Agriculture. In 2006, the lab started large-scale production of a new predator beetle (*Laricobius* sp.), providing 2,486 to the Park. Although it is too early assess the overall success of these biocontrols, preliminary monitoring results are encouraging.

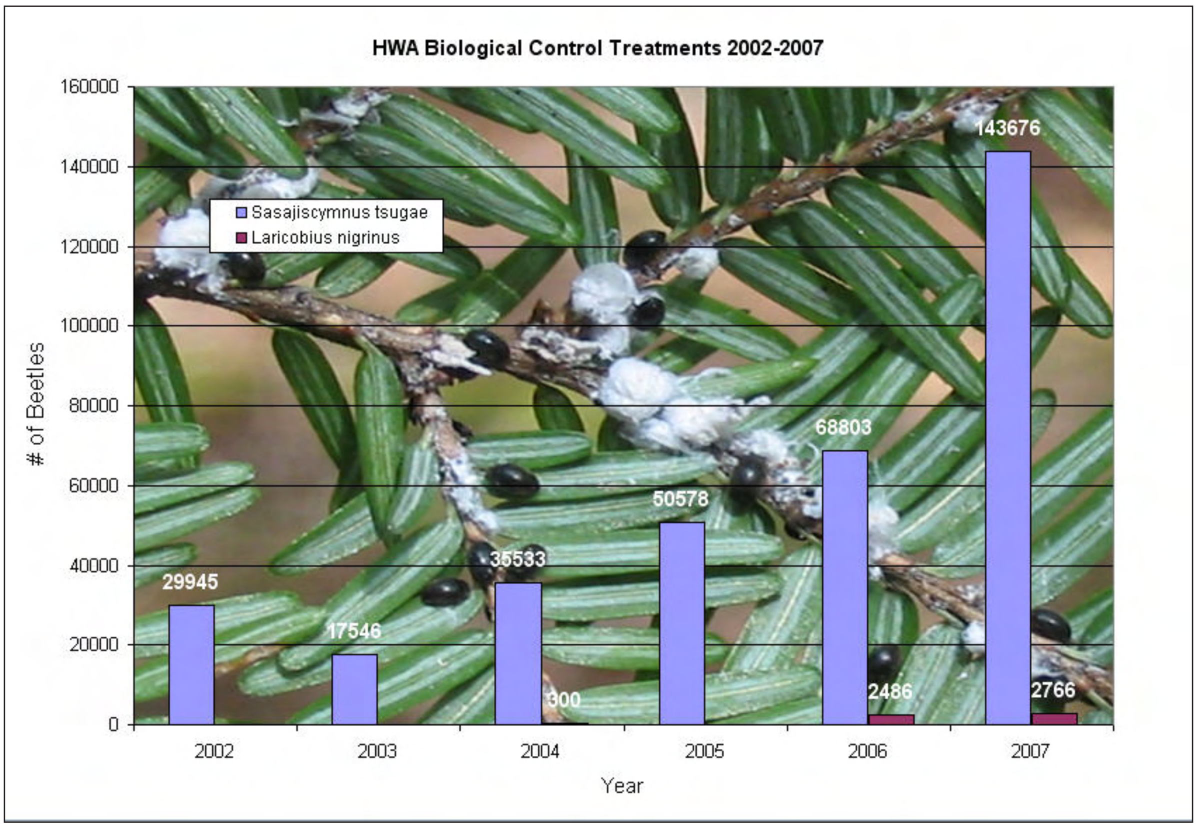

Figure 1. Predator beetles released into the Great Smoky Mountains National Park.

Chemical control activities include foliar treatments with insecticidal soap and systemic insecticides applied through the soil or trunk of individual trees. Through January 2008, over 75,000 hemlocks covering approximately 2,200 acres have been systemically treated. Foliar treatments done on an annual or semi-annual basis have covered 480 acres. All of the developed areas (campgrounds, picnic areas, roadsides, visitor centers, and backcountry campsites) in the Park have received an initial treatment. The Park's backcountry campsites receive over 350,000 camper-nights each year, and over 500,000 hikers on 850 miles of maintained trails. Total annual Park visitation is up to 10 million annually.

MONITORING

In 2005, permanent hemlock health monitoring plots consisting of three mature overstory hemlocks, and three generally smaller hemlocks with branches reachable from the ground at four cardinal directions (north, east, south, and west) were established at 22 *S. tsugae* release areas, 36 systemic imidacloprid application areas, 15 foliar treatment areas, and 27 untreated (control) areas.

Tree health was evaluated using the crown condition rating system of the USDA Forest Service, Forest Inventory and Analysis/Forest Health Monitoring programs, as well as a new growth and HWA infestation count on individual branches developed by Rich Evans at Delaware Water Gap National Recreation Area.

Analysis of crown ratings (live crown ratio, density, transparency, dieback, and vigor) did not conclusively show a treatment effect. This may be due to the possibility that, by treatment type, only a few sites had ratings far outside the mean values for plots of the same treatment type.

Evaluation of branch tips (number of terminals, number of new terminals, and number of terminals with HWA) showed a treatment effect. The greatest difference (lowest mean) was shown for biocontrol treated plots for two of the three parameters: number of new terminals and number of new terminals with HWA—see Figures 2 and 3.

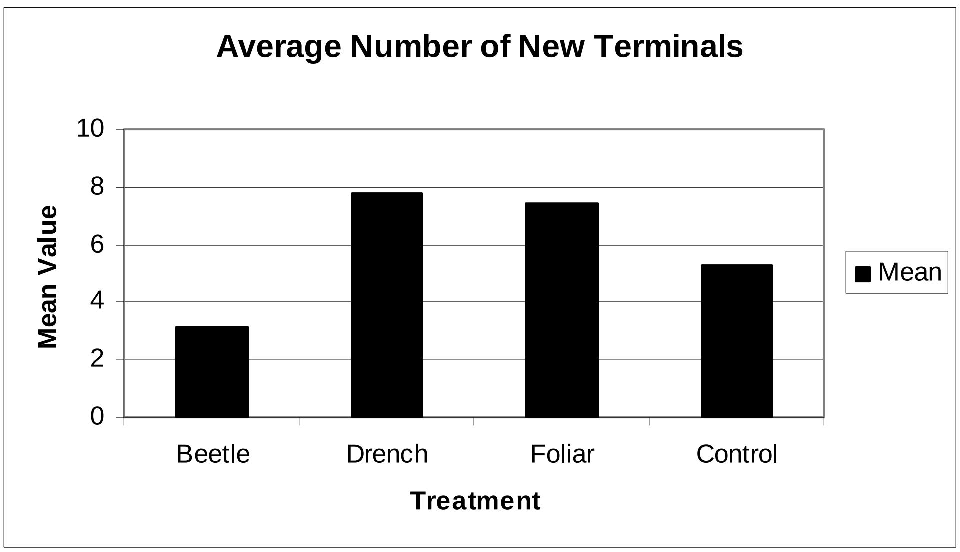

Figure 2. Average (mean) number of new terminals (branch tips) by treatment.

Biocontrol-treated plots had the lowest mean number of new terminals and the lowest mean number of terminals with HWA. Control plots had the highest mean values for number of terminals with HWA.

The biocontrol plot results may have two explanations. At release, beetles are placed on accessible branches that could be the same branches that were evaluated in this study. The beetles feed on many HWA on those branches before moving off to higher branches. The evaluated branches could contain fewer HWA than other branches due to this 'cleaning effect'.

These previously moderately to heavily infested branches would still exhibit dieback and have little new growth even though they had few adelgids at evaluation time.

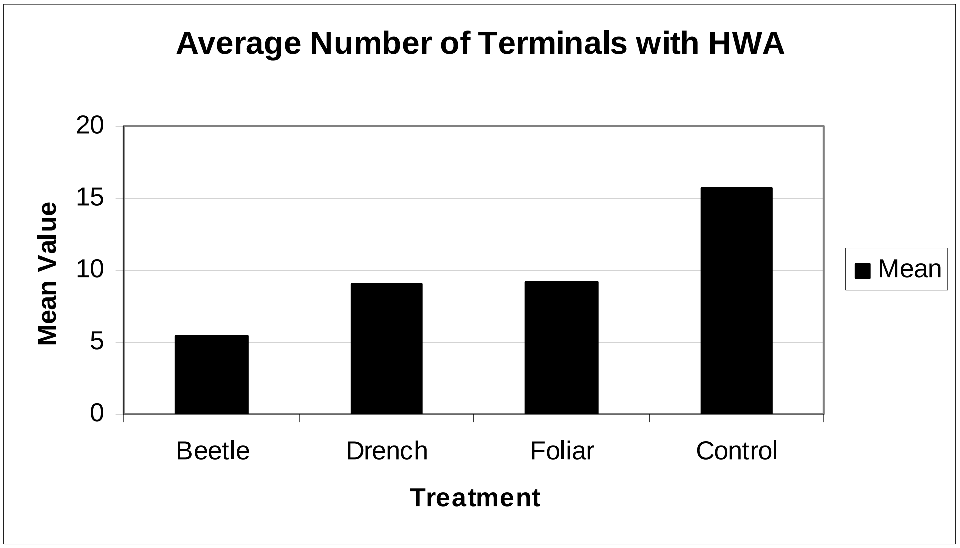

Figure 3. Average (mean) number of terminals (branch tips) infested with HWA.

Other treatments showed fewer 'mean terminals with HWA' than controls suggesting an observable treatment effect. The mean number of new terminals by treatment test was not significant but did show that chemical treatment had a higher mean value than biocontrol or no treatment.

TREATMENT SUMMARIES

In 2006, 33 Eastern Hemlock Conservation Areas were established in the Park. These areas range from 5 to 184 acres and total 1,249 acres. All of the areas selected are old-growth hemlock forests and are distributed throughout the Park. Conservations Areas are patterned after those in the USDA National Forests, representing diverse geographic, geologic, elevational, and hydrologic situations to best preserve fragments of the hemlock ecosystem. Most of these areas can be seen be the visiting public by means of the Park's trail system. These areas receive a combination of systemic and biocontrol treatments. To date, thirty of these sites have received initial systemic treatments and biocontrol releases.

Additional highlights include:

- **Cades Cove.** Loop road and developed areas receive foliar treatment on an annual basis. A total of 943 hemlock trees have been treated systemically at the campground, ranger station, and historic areas.
- **Tremont.** Entrance road and administrative portion of Middle Prong trail have been treated. A total of 359 hemlock trees have been treated at developed areas, including the Institute.
- **Elkmont.** A total of 1,373 hemlock trees have been treated at the campground and historic district.

- **Cosby.** A total of 806 hemlock trees were treated along roads and accessible trails and in campgrounds and developed areas.
- **Oconaluftee.** A total of 207 hemlock trees were treated along roads and accessible trails and in developed areas.
- All of the major Park roads have been or are presently undergoing evaluation and treatment as part of hazard tree management. So far, 4,500 roadside hemlocks have been treated.
- All of the backcountry campsites have been scouted and treated systemically if deemed necessary. A total of 2,082 hemlocks were treated among all backcountry campsites.
- Along the Boogerman Loop trail in Cataloochee, North Carolina, 1,482 trees were treated.
- Along the Rainbow Falls Trail in Tennessee, a total of 1,013 trees were treated.
- A total of 1,189 trees were treated along the Trillium Gap Trail in Tennessee to Grotto Falls.
- A total of 2,411 potential hazard hemlock trees along 34 miles of Newfound Gap Road have been treated.
- Trees around the Park headquarters area were treated: a total of 422 trees.
- A total of 1,009 trees have been treated along the Ramsey Cascades Trail.

Treating trees in developed areas helps ensure visitor safety, protect aesthetics, and reduce maintenance costs. For example, removal of a 15-inch-diameter hazard tree costs the Facility Management Division approximately $150. Treating a 15-inch-diameter hemlock costs an estimated $16.00. Treatments completed at Elkmont saved the Park over $61,000 in potential hazard tree removal costs. Systemically treated trees are protected for three to five years, but the most promising long-term landscape level solution is establishment of biological controls.

PROJECT OBJECTIVES

The objectives of HWA management in the Park are to:

- Preserve old-growth hemlock forests in differing elevations, soil types, aspects, and watersheds. This strategy has the greatest potential to preserve genetic diversity and habitat variations represented in the Park.
- Preserve hemlocks in heavily visited areas of the Park for visitor enjoyment and safety as well as to reduce hazard tree removal costs.
- Preserve hemlocks along road-sides for aesthetics and to reduce maintenance costs.

The success of this project is contingent on meeting these objectives well into the future. Trees already treated must be maintained, while many valuable old-growth stands - potential conservation areas—in Cosby and Greenbrier are still in good condition and could be saved. By fall 2008, this choice may not be available as the trees will likely be heavily infested and in decline.

CONCLUSION

The initial outbreak of an exotic forest insect infestation is usually the most devastating phase; in time, pest populations can be reduced through a combination of natural and introduced predators and parasites, decrease in host in population, and genetic host resistance. Large-scale insecticidal treatment is, and will continue to be, necessary during this phase with HWA. Over time, these treatments will be greatly reduced as HWA populations decline. At present, the Park can still make decisions about whether or not to save large tracts of old-growth hemlock. By this time next year, we will most likely be forced to shift our focus to maintaining previously treated trees and monitoring the devastating effects of HWA on watersheds, wildlife, understory plants, and visitor safety.

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Research, Monitoring, and Management of Eastern Hemlock Forests at Delaware Water Gap National Recreation Area

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ABSTRACT

Beginning in 1993, the Delaware Water Gap National Recreation Area (DEWA or "the park") has conducted a program to address the threats that forest decline associated with hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, on eastern hemlock, *Tsuga canadensis* (L.) Carriere, poses to valued park resources and visitor experiences. This program includes annual monitoring of HWA populations and hemlock tree health in permanent hemlock forest plots in the park, studies of ecosystems and biodiversity associated with hemlock dominated forests in the park, and efforts to manage HWA and maintain hemlock-dominated ecosystems and visitor use areas in the park.

HWA infestation levels and hemlock tree crown conditions and new growth have been monitored annually in permanent plots in the park since 1993. HWA infestation levels have varied greatly over this period, but the annual variation is consistent among sites, with peak HWA infestation levels occurring in 1999-2000 and 2007. The amount of new growth during the pre- and initial-infestation periods from 1995 to 1998 showed little variability, being fairly consistent annually within each site and among sites. In contrast, new growth following HWA infestation was highly variable annually.

Our management approach has been to 1) identify and prioritize management concerns related to hemlock decline and mortality, 2) identify resource values of individual hemlock stands, 3) prioritize stands for specific management actions, and 4) complete management actions in order of priority. Management concerns for the park include: increased hazardous trees, negative effects on esthetics of visitor use areas and recreational activities, invasions of alien plants, impacts of white-tailed deer (*Odocoileus virginianus*), alteration of micro-climates and ecosystem functions, loss of native biodiversity, and increased fire risk. Management actions we have taken include avoiding soil compaction from visitor use in hemlock forests, releasing biological control agents for HWA, chemical suppression of HWA, hazardous tree mitigation, construction of deer exclosure fences, planting native trees, and suppression of invasive alien plants in declining hemlock stands.

We have released a total of 75,700 *Sasajiscymnus tsugae*, 4,500 *Scymnus sinuanodulus*, and 2,700 *Laricobius nigrinus* beetles. These biocontrol beetles do not appear to have been effective at suppressing HWA infestations to date. Twenty-eight percent (28%) of hemlock plot trees had died as of 2006. A model we developed predicts that, by 2014 (within seven years), half (50%) the hemlock trees in the park will be dead, and that by 2022 (within 15 years), 80% will be dead.

A total of 975 hemlock trees have been treated by soil injection of Merit 75WSP® (Bayer Corporation) and 157 hemlock trees by stem injection of IMA-jet® (Arborjet Inc.) to date. Approximately 150 acres in seven different declining hemlock forest areas have been treated to eliminate or suppress invasive alien plants. A Reforestation Project was initiated in 2006 to inform and demonstrate to visitors the effects of HWA and hemlock decline and current park management concerns and responses.

KEYWORDS

hemlock, adelgid, alien, monitoring, management

INTRODUCTION

Delaware Water Gap National Recreation Area (DEWA or "the park") covers approximately 70,000 acres (28,300 hectares) along the Delaware River in northeastern Pennsylvania and northwestern New Jersey. Eastern hemlock, *Tsuga canadensis* (L.) Carriere, forests account for 5% of the forested area of the park and occur on spatially patchy, isolated ravines, on steep slopes, and on flat, moist benches (Young et al. 2002). Hemlock often accounts for 50% to 80% of the basal area in these stands (Sullivan et al. 1998). Many hemlock stands in DEWA are recognized as "Outstanding Natural Features" having "high intrinsic or unique values" (National Park Service 1987). Trout streams and scenic waterfalls are associated with many park hemlock stands, and recreational activities like hiking, fishing, bird watching, picnicking, and general "sight-seeing" are very popular in these areas.

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, was first detected at DEWA in 1989. Since 1993, we have conducted a program to address the threats that HWA and decline of eastern hemlock poses to valued park resources and visitor experiences. This program includes annual monitoring of HWA populations and hemlock tree health in permanent hemlock forest plots in the park, studies of ecosystems and biodiversity associated with hemlock-dominated forests in the park, and efforts to manage HWA and maintain hemlock dominated ecosystems and visitor use areas in the park. The efforts and support of many agencies and cooperators, especially the USDA Forest Service, have contributed greatly to this program. Table 1 provides a time-table of 25 hemlock and HWA-related research, monitoring, and management activities at DEWA through 2007. The following is a summary of monitoring and management activities.

MONITORING METHODS

Annual Monitoring

A total of 78 permanent hemlock plots in the park were established using the methods described by Onken et al. (1994). Plots were located randomly within each of six separate hemlock stands. The six hemlock stands were not located randomly but were chosen to represent a range of geographic and physiographic areas of the park. Each plot includes 10 hemlock trees permanently marked with individually numbered aluminum tags. The crown conditions of

Table 1. Summary time-table of 25 hemlock and HWA-related research, monitoring, and management activities at DEWA.

	Year														
Program Activity	**93**	**94**	**95**	**96**	**97**	**98**	**99**	**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**
Monitoring															
Establish Permanent Plots	[+]	[+]				[+]									
Monitor Plot Tree Health	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]
Monitor HWA & New Growth			[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]	[+]
Park-wide HWA Survey			[+]				[+]								
Park-wide Hemlock Health (LandSat)															
Research															
Small Mammals, Adams & VanCampens	[+]	[+]													
Amphibians, Adams & VanCampens	[+]	[+]													
Terrestrial Arthropods, Adams & VanCampens	[+]	[+]													
HWA Population Estimation Method		[+]	[+]	[+]	[+]										
Vegetation & Light, Adams & VanCampens		[+]	[+]								[+]				
Stream Fish, 14 pairs Hemlock v Hardwood					[+]										
Stream Arthropods, 14 pairs Hemlock v Hardwood					[+]										
Stream Chemistry, 14 pairs Hemlock v Hardwood							[+]	[+]	[+]						
Breeding Birds, 14 pairs Hemlock v Hardwood						[+]		[+]							
Stream Amphibians, 14 pairs Hemlock v Hardwood								[+]							
Vegetation in 10 Hemlock Stands											[+]	[+]	[+]	[+]	
Hemlock Dendrochronology											[+]			[+]	
Imidacloprid Insecticide Efficacy												[+]	[+]	[+]	[+]
Management															
Release *Sasajiscymnus tsugae*								[+]	[+]	[+]	[+]	[+]			
Cut Hazardous Trees										[+]		[+]	[+]		
Release *Scymnus sinuanodulus*														[+]	[+]
Release *Laricobius nigrinus*														[+]	[+]
Apply Imidacloprid Treatments														[+]	[+]
Suppress Invasive Alien Plants							[+]					[+]	[+]		
Install Deer Fences													[+]		

plot trees have been assessed annually using the "Visual Crown Rating Methods" developed and used by the USDA Forest Service. In addition, the overall condition of individual trees has been categorized annually as either healthy, in slight decline, moderate decline, severe decline, or dead.

HWA infestation levels and the amount of new twig growth have also been measured annually between the last week in May and the first week in July on a subset of hemlock trees in or adjacent to the plots. At each plot sampled, one to four branches on each of one to four trees were examined. On each sampled branch, the number of twigs on the distal 25 cm of the branch was counted, and the proportion of those twigs having HWA and the proportion having new growth was determined (Evans 1996). An index of HWA infestation level for each stand was calculated as the average proportion of twigs infested with HWA for all branches sampled in each stand. An index of new twig growth for each stand was calculated as the average proportion of twigs having new growth for all branches sampled in each stand.

Predicting Hemlock Decline and Mortality

A simple mathematical model was developed to describe the spread of HWA infestations throughout the park and forecast the resulting hemlock decline and mortality. In the first part of the model, the "logistic" equation was used to model the cumulative percentage of hemlock trees in the park that become infested over time:

Cumulative percent hemlocks infested up to the year "y" = $\frac{100}{1 + e^{-r\,y}}$

where e = 2.71828 (the natural logarithm),
and r = the maximum rate of spread of HWA

Data from DEWA presented in Eschtruth et al. (2006) was used to estimate r:

$$r = 0.2608$$

In the second part of the model, hemlock trees decline and die at assigned rates after being infested with HWA. The initial conditions of the model were set to fit the fact that, in 1994, 93% of hemlocks in the permanent plots were either "healthy" or in "slight decline," and none were dead. For modeling purposes, trees in "slight decline" were included in the "healthy" category. In the model, hemlocks were removed from the "healthy" category four years after HWA infestation. For hemlock mortality, the model assumes that, of existing hemlocks, 15% die five years after initial HWA infestation, 35% die after 10 years, 25% die after 15 years, 15% die after 20 years, 5% die after 25 years, and 5% survive indefinitely. The average time to hemlock tree mortality after HWA infestation in this model is just over 12 years (ignoring the trees that survive indefinitely).

RESULTS

HWA Populations and Hemlock Tree New Growth

Figure 1 shows annual average HWA infestation levels and amount of new twig growth at four stands during the past 13 years. HWA infestation levels have varied greatly among years at each site over this period, ranging from 80% to 90% at Mount Minsi and Donkeys Corner in 1999 and 2007 to nearly zero at Mount Minsi and VanCampens in 2001 and less than 10% at all four sites in 2004. A consistent pattern of annual variation is apparent, with peak HWA infestation levels occurring in 1999-2000 (except at Adams Creek) and again in 2007, and minimal infestation levels occurring at all four sites in 2001 and 2004. The amount of new growth during the pre- and initial-infestation periods from 1995 to 1998 showed little variability, being fairly consistent annually within each site and even among sites. New growth during this period ranged from 45% to 80% at Mount Minsi, and from 35% to 50% at Adams Creek. In contrast, new growth following HWA infestation was highly variable annually. New growth ranged from near zero in 2000 at all the sites except Adams Creek (which had not yet been highly infested) to between 50% and 80% at all four sites in 2005 (following low HWA infestation levels in 2004). A fairly consistent pattern of annual variation in new growth is apparent, though less pronounced than it was for HWA infestation levels. Given the very high levels of HWA infestations at these sites in 2007, it does not appear that the biological controls released in previous years (see below) have been effective at suppressing HWA infestations to date.

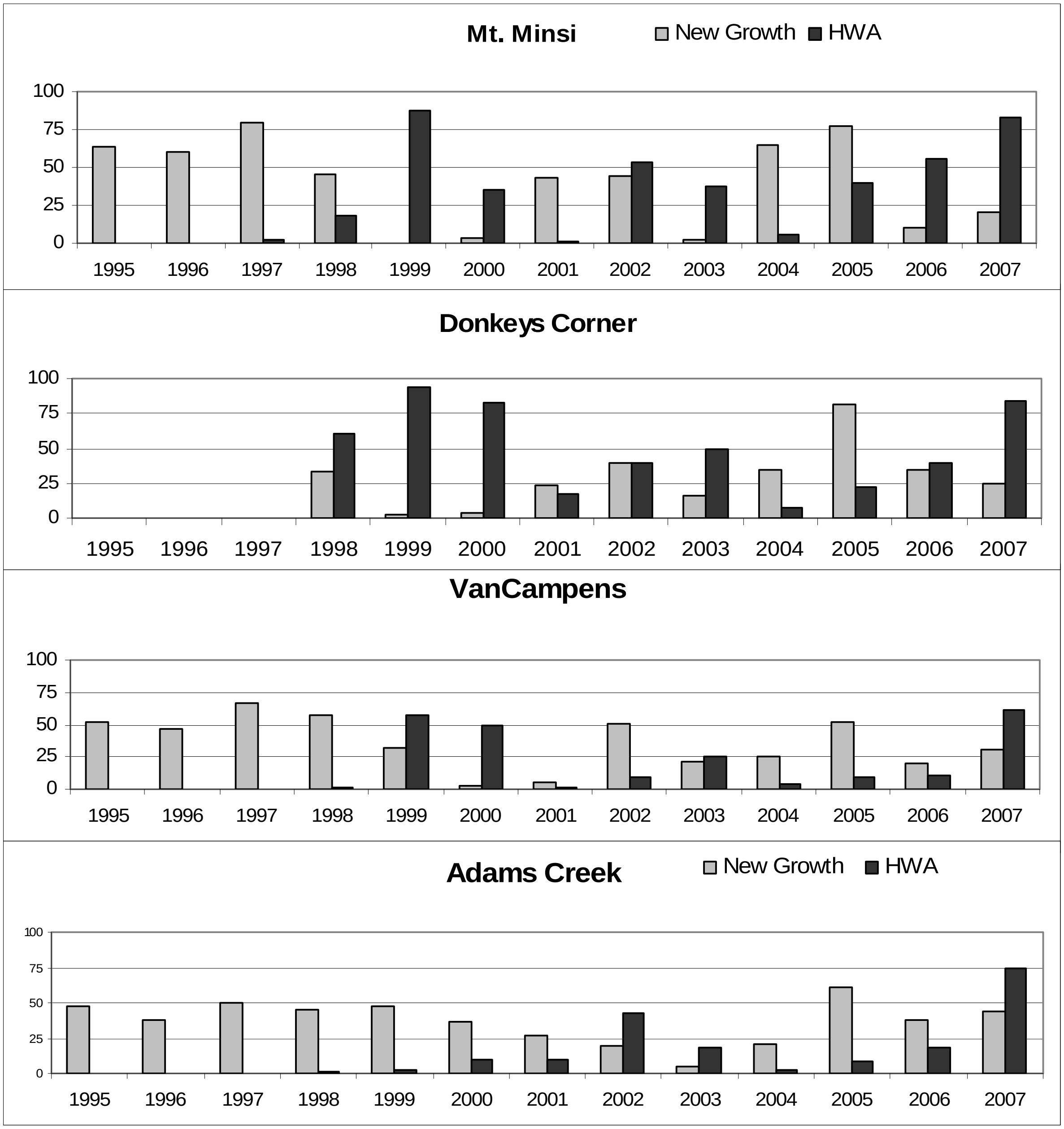

Figure 1. Annual average hemlock new growth (percent of twigs producing new growth) and HWA infestation levels (percent of twigs infested with HWA) at each of four monitoring sites from 1995 through 2007. Data were not collected at Donkeys Corner before 1998.

Hemlock Decline and Mortality

As of 2006, 28% of DEWA hemlock plot trees had died, and all the remaining plot trees were in moderate or severe decline; no healthy or slight decline trees remained. The predictive model of HWA spread and consequent hemlock decline and mortality has underestimated the rate of hemlock decline and mortality in our monitoring plots to date (Figure 2). As of 2006, the model predicted only 20% hemlock tree mortality, and 37% of trees remaining healthy. Even so, the model predicts that 50% of park hemlocks will have died by 2014 and 80% by 2022. The high HWA infestation levels of 2007 are likely to accelerate hemlock decline and mortality in coming years.

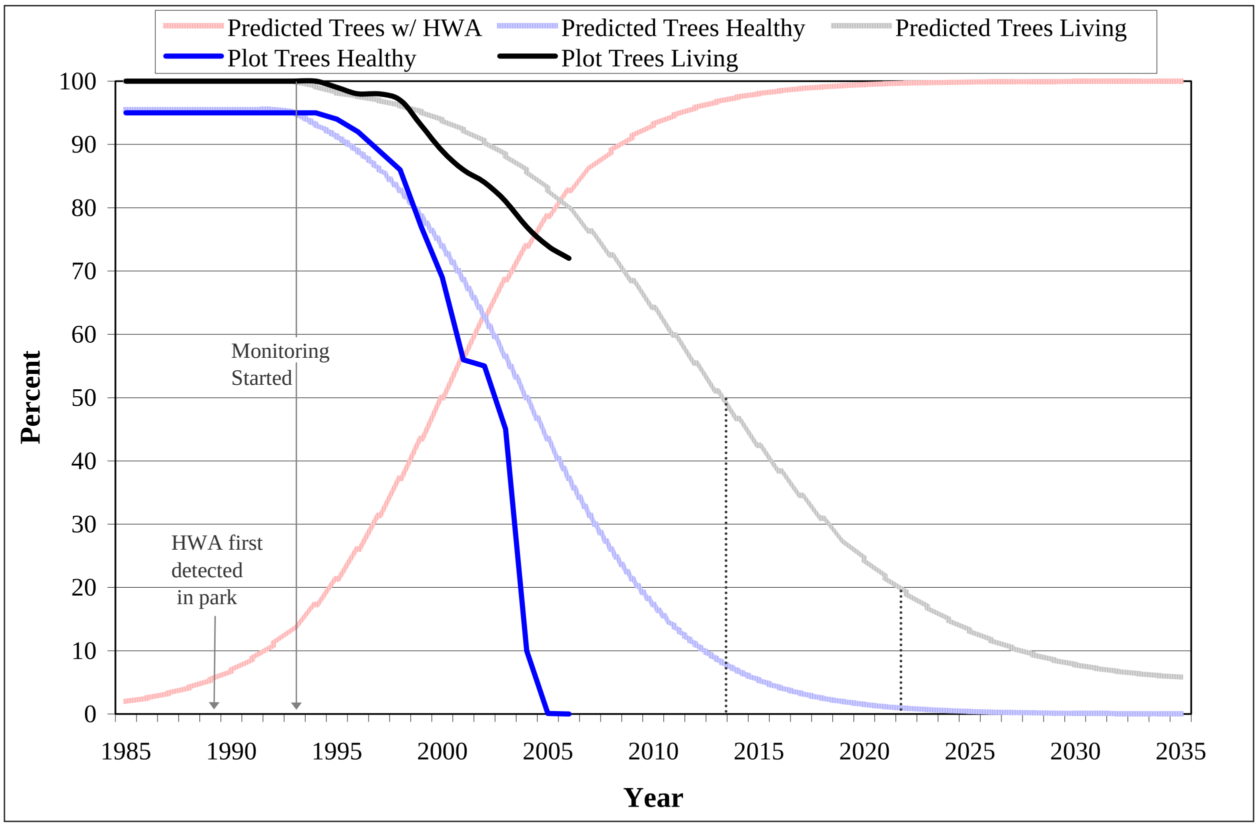

Figure 2. Hemlock health monitoring data compared to the predictive model of HWA spread and hemlock health decline (see text). The dotted lines indicate the predicted dates when 50% of the hemlocks will be dead and when 80% will be dead.

MANAGEMENT OF HWA AND HEMLOCK

Our approach to management of HWA and hemlock can be summarized by the following steps:

1. Identify and prioritize hemlock and HWA management concerns and goals.
2. Identify visitor use and ecological values of individual hemlock stands.
3. Prioritize individual hemlock stands for management actions based on their visitor use and ecological values.
4. Apply best available management practices to address specific concerns at prioritized hemlock stands.

Our primary hemlock management concerns include the following (the management goals would be to avoid these):

1. Increases in hazardous trees.
2. Negative effects on esthetics of visitor use areas.
3. Negative effects on recreational activities.
4. Invasions of alien plants.
5. Impacts of white-tailed deer (*Odocoileus virginianus*) herbivory.
6. Alteration and disruption of micro-climates and ecosystem functions.
7. Loss of native biodiversity, particularly brook trout (*Salvelinus fontinalis*).
8. Increased fire risk.

Management actions we have taken in response to HWA infestations and hemlock decline include avoiding soil compaction from visitor use in hemlock forests, releasing biological control agents for HWA, chemical suppression of HWA, hazardous tree mitigation, construction of deer exclosure fences, planting native trees, and suppression of invasive alien plants in declining hemlock stands. To avoid soil compaction and damage to hemlock tree roots, elevated boardwalks were installed at Dingmans Falls visitor use area in 1995. Management actions have been matched with the specific ecological and visitor use values of each area (Table 2). Thus, for example, we have cut hazardous trees and applied imidacloprid insecticide to suppress HWA and keep hemlock trees alive at highly developed visitor use areas like Childs Park, Dingmans Falls, and Raymondskill Falls. In contrast, our only management action at an undeveloped area with low visitation like Conashaugh has been to suppress invasive alien plants to protect rare and native species.

Table 2. Management actions at priority hemlock areas in relation to primary values of the areas.

	Primary Area Values			Management Actions			
Area	Development & Recreation Level	Water & Wetland Values	Native Plant Values	Hazardous Tree Mitigation	HWA Chemical Suppression	HWA Biocontrol Releases	Invasive Plant Suppression
Childs Park	High & High	Wild Trout	Rare Species	Yes	Yes	--	Yes
Dingmans Falls	High & High	Wild Trout	Rare Species	Yes	Yes	--	Yes
Raymondskill Falls	High & High	Wild Trout	Rare Species	Yes	Yes	--	Yes
Adams Creek	Low & Medium	Wild Trout	Rare Species	--	--	Yes	Yes
Hornbecks Creek	Low & Medium	Wild Trout	Diverse Species	--	--	Yes	Yes
Toms Creek	Low & Medium	Class A Trout	--	--	--	planned	Yes
Conashaugh	Low & Low	Wild Trout	Rare Species	--	--	--	Yes
Hogback Ridge	Low & Low	Palustrine	Rare Species	--	--	Yes	Yes
Spackmans Creek	Medium & Medium	Wild Trout	Rare Species	planned	planned	--	planned
Tumbling Waters	Medium & Medium	Wild Trout	--	planned	planned	--	--
Buttermilk Falls	Medium & High	--	--	planned	Yes	Yes	--
Upper VanCampens	Low & Low	Wild Trout	--	--	--	Yes	--
Lower VanCampens	Medium & Medium	Wild Trout	--	Yes	Yes	Yes	--
Hemlock Pond	Low & Medium	High Elevation	Rare Species	--	--	Yes	planned

Biological Control of HWA

Biological control agents provide the only hope in the foreseeable future of limiting the damaging effects of HWA in large or remote hemlock forests over the long term. Beginning in the year 2000, we have released a total of 75,700 black "Japanese lady beetles" (*Sasajiscymnus tsugae*) at 14 locations in the park (including VanCampens and Adams Creek), 4,500 *Scymnus sinuanodulus* beetles at two locations, and 2,700 *Laricobius nigrinus* beetles at four locations in the park (Table 3). Yet, the high HWA infestation levels observed in 2007 (Figure 1) indicate that, at least to date, these beetles have had little or no meaningful effects on HWA infestation levels.

Table 3. Numbers of HWA biological control agents released within DEWA.

	Biological Control Agent		
Year	***Sasajiscymnus tsugae***	***Laricobius nigrinus***	***Scymnus sinuanodulus***
2000	15,000	—	—
2001	10,000	—	—
2002	25,700	—	—
2003	17,500	—	—
2004	7,500	—	—
2005	—	—	—
2006	—	310	1,000
2007	—	2,390	3,500
Total:	**75,700**	**2,700**	**4,500**

Chemical Suppression of HWA

Judicious use of insecticides is the only practical method of suppressing HWA and keeping individual trees alive in the immediate future. With support from the USDA Forest Service, imidacloprid was applied to suppress HWA at three priority visitor use areas in 2006 and 2007 (Childs Park, Dingmans Falls, and Raymondskill Falls). To date, a total of 975 hemlock trees have been treated by soil injection of Merit 75WSP® and 157 hemlock trees by stem injection of IMA-jet®. Each treated tree was tagged to document the year and season of treatment (spring of 2006 or fall of 2007). Soil injection was the preferred and "default" method of application because stem injection is more costly, requires more time, and is most effectively applied during limited hours in the morning. However, use of stem injection is required or advised in wet areas and where trees are growing on bedrock and within 50 ft. to 100 ft. of surface waters (depending on soil characteristics). Other factors limiting soil applications are the maximum annual dose per acre (currently 182 grams, or 0.4 lbs, of active ingredient per acre per year), distance from water supply, and the ability to transport water to the application site. In practice, the annual dose per acre limitation means that typically we can only treat about 20% to 25% of the hemlock trees on a square acre in DEWA.

Invasive Alien Plant Suppression

We selected fourteen hemlock stands as priorities for invasive plant suppression, based on stand size, location, and landscape setting; park management zoning; botanical resources (including rare species); and recreational use. All but one of these stands are within the "Outstanding Natural Feature" zone in the park. Targeted invasive alien plants include Japanese barberry (*Berberis thunbergii*), tree-of-heaven (*Ailanthus altissima*), multiflora rose (*Rosa multiflora*), autumn olive (*Elaeagnus umbellata*), shrub honeysuckles (*Lonicera* spp.), Asiatic bittersweet (*Celastrus orbiculatus*), wineberry (*Rubus phoenicolasius*), garlic mustard (*Alliaria petiolata*), and Japanese stiltgrass (*Microstegium viminium*).

Prior to treatment, we conducted surveys to assess invasive plant populations and to identify treatment areas. We rated invasive plant patch levels as heavy, medium, or light, and mapped their locations. Invasive plant patches adjacent to these hemlock stands were also mapped, especially if they were near streams or drainage ditches, because these can serve as propagule sources for invasive alien plants.

Invasive plants have been suppressed in nearly 150 acres in eight hemlock stands in the park to date. Park staff, National Park Service Exotic Plant Management Team staff, and private contractors have been actively involved in this effort. A combination of mechanical removal and herbicide (glyphosate and imazapyr) treatments have been used.

Reforestation "Demonstration" Project

In 2005, nearly 100 hazardous hemlock trees were cut within a 5-acre area of the Raymondskill Falls visitor use area in the park. A Reforestation Project was initiated at this area in 2006 to inform and demonstrate to visitors the effects of HWA and hemlock decline, and park management concerns and responses. The objectives of this project are to:

1. Eliminate or minimize populations of invasive plants.
2. Maintain existing hemlock trees.
3. Foster regeneration of eastern hemlock and other native trees.
4. Minimize erosion and maintain good soil conditions for reforestation.
5. Inform and educate the public.

It is worth noting that the trees cut in 2005 were left on site to help minimize erosion, minimize invasion of alien plants, maintain good soil conditions, and foster regeneration of hemlock and other native trees. Although the downed trees may be perceived as un-attractive by some people, this is a temporary condition, and the aesthetic concern is far outweighed by the ecological benefits of leaving the trees on-site.

Several actions were taken in 2006 and 2007 to help maintain hemlocks and foster native tree regeneration. Soil injections of Merit 75WSP® were applied to 200 hemlock trees, and stem injections of IMA-jet® were applied to 13 hemlock trees. Thirty-five sapling white pines (*Pinus strobus*), 17 eastern red cedars (*Juniperus virginiana*), and two sugar maples (*Acer saccharum*) were planted in this area. To ensure protection of native plants from deer browsing (see Eschtruth and Battles, in press), high-tensile, 8-foot-high woven wire fencing was installed around 2.3 acres at the site in fall 2006. Japanese barberry, multiflora rose, and "tree-of-heaven" (or "tree-from-hell") have been eliminated (or nearly so) from this site. However, garlic mustard and Japanese stiltgrass persist at this site.

CONCLUSION

Hemlock woolly adelgid is only one example of problems caused by introductions of invasive alien species. The consequences of the introduction and spread of HWA are complicated and exacerbated by other environmental changes, such as the presence of other alien insects like elongate hemlock scale (*Fiorinia externa*), invasive alien plants, elevated deer populations, and changes in climate. Efforts to control HWA infestations and mitigate the numerous effects of widespread hemlock decline and mortality on ecosystems, native biodiversity, and immediate human concerns (safety, aesthetics, and recreation) are extremely difficult and costly, and have only had limited success to date. Given such circumstances, what are we to make of the National Park Service congressional mandate (set forth in the "National Park Service Organic Act" of 1916) to conserve the parks "in such manner and by such means as will leave them unimpaired for the enjoyment of future generations"?

> Nature in the 21st century will be a nature we make; the question is the degree to which this molding will be intentional or unintentional, desirable or undesirable.
>
> Dr. Daniel Botkin (1990)

ACKNOWLEDGMENTS

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Status of *Ex Situ* Conservation Efforts for Carolina and Eastern Hemlock in the Southeastern United States

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ABSTRACT

Carolina hemlock (*Tsuga caroliniana* Engelmann) and eastern hemlock (*T. canadensis* Carriére) are threatened across their natural range in the southeastern United States by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. Chemical and biological controls have much promise for limiting the spread and damage done by this exotic pest but are not yet effective on a widespread basis. Alternate conservation strategies are necessary to protect the dwindling gene pools of these import conifer species until such a time that adelgid management tools are available. Since 2003, Camcore (at North Carolina State University) and the USDA Forest Service have been collaborating to collect seeds from populations of both Carolina and eastern hemlock throughout the southern U.S. These seeds have been placed in cold storage or have been germinated to establish *ex situ* conservation plantings in Latin America and the Ozark Mountains of Arkansas. The goal is to conserve the genetic resource of each species in perpetuity until hemlock restoration is possible. To date, seeds have been sampled from 13 populations and 84 mother trees of Carolina hemlock and 18 populations and 110 mother trees of eastern hemlock. Here we discuss results of genetic diversity studies with both species, our conservation strategy, and progress on seed collections and *ex situ* conservation planting establishment.

KEYWORDS

gene conservation, *Tsuga caroliniana*, *Tsuga canadensis*, *Adelges tsugae*

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an exotic insect pest of Carolina hemlock (*T. caroliniana* Engelmann) and eastern hemlock (*Tsuga canadensis* Carriére) that threatens to eliminate these important tree species from their geographic ranges in the eastern United States (McClure et al. 2001). Efforts to manage HWA populations silviculturally, chemically, and via the introduction of natural enemies from the adelgid's native range (biological control) are ongoing and show much promise (Cheah et al. 2004; Ward et al. 2004, Cowles et al. 2006). However, considering the speed with which the adelgid spreads and kills trees, it is likely that some hemlock populations will be severely depleted or completely lost before widespread adelgid control becomes a reality. *Ex situ* conservation, the movement of germplasm (seeds) from its place of origin to other more protected areas, is one viable option for conserving these populations that face possible extinction.

Carolina hemlock is a rare conifer endemic to southern Appalachian Mountains. It is found in a relatively small number of isolated populations in Virginia, Tennessee, Georgia, and North and South Carolina, where it is typically found growing on mountain bluffs or exposed ridges between 600 and 1500 meters elevation (Farjon 1990). This species is most often described as occupying dry, coarse, nutrient-poor soils, although field research by Camcore has revealed that the species is much more broadly adapted to a greater variety of site types than originally thought (Jetton et al. 2008). Eastern hemlock has a much larger range, extending from Nova Scotia south to northern Georgia and as far west as northern Minnesota, with isolated populations occurring in Indiana, central Kentucky, and northern Alabama (Farjon 1990). It is typically a riparian species occupying moist, acidic soils and grows from sea level to about 730 meters elevation (Godman and Lancaster 1990).

The rapid progression of HWA makes urgent the need to protect dwindling genetic resources of both hemlock species. To that end, in 2003 Camcore (International Tree Conservation and Domestication, North Carolina State University) and the USDA Forest Service embarked on a joint, three-phase project to move representative seed samples of both Carolina and eastern hemlock to *ex situ* conservation banks in South America and the Ozark Mountains, where HWA is not present. The first phase, conducted from 2003 to 2006 with seed collections and the establishment of field *ex situ* conservation banks for Carolina hemlock, has been previously documented (Tighe et al. 2005; Jetton et al. 2008). The second phase began in 2005 and is a four-year project to conduct seed collections from eastern hemlock throughout its range in the southern U.S. and establish *ex situ* conservation banks. Phase 2 is currently ongoing. The third phase will sample seed from eastern hemlock populations in the northern and midwestern portions of the species' range and is tentatively set to begin in 2009. This effort will ensure that the gene pools of these important conifer species live on in perpetuity and that seeds will be available to repopulate lost hemlock stands once effective HWA management is available. These plantings will also provide a breeding population for adelgid resistant hybrids should HWA control remain elusive. This article reports on our progress with Phases 1 and 2 of the conservation effort.

HEMLOCK GENETIC DIVERSITY AND CONSERVATION STRATEGY

The goal of any *ex situ* conservation program is, by collecteding seed from an appropriate number of trees and populations, to capture a representative number of alleles to protect dwindling gene pools (FAO et al. 2004). To do this, one must understand the population genetic structure and the environmental factors that influence genetic diversity (Eriksson et al. 1993). Researchers at Camcore and the USDA Forest Service are addressing these population genetics issues for Carolina and eastern hemlocks with several recent and ongoing molecular marker studies.

An amplified fragment length polymorphism (AFLP) molecular marker study of Carolina hemlock indicates that this species has a moderate amount of overall genetic variation and a fairly high amount of genetic differentiation among populations. Additionally, the results suggest that a recent *ex situ* seed collection of nine populations has more than adequately conserved the genetic variation present throughout the range of the species (Camcore 2006).

Population genetic studies of eastern hemlock have focused on the southern portion of the species' range. An allozyme investigation of genetic variation across 20 eastern hemlock populations in the southeastern U.S. suggests that the species has a low level of diversity in the region compared to most other conifers but greater population differentiation (Potter et al., in press). Populations along the eastern periphery and in the Appalachian interior exhibited greater diversity than those to the west of the mountain chain, indicating that the glacial refuge of the species was located east of the southern Appalachian Mountains, the area in which *ex situ* conservation seed collections should be concentrated to capture greater genetic variation.

An ongoing microsatellite marker study using primer combinations recently isolated from both hemlock species should further elucidate the relationships among eastern hemlock populations in the Southeast. Microsatellite molecular markers, also known as simple sequence repeats (SSRs), generally exhibit much higher variability than enzyme markers such as allozymes, and should therefore allow for improved testing of hypotheses about how post-Pleistocene migration and isolation have affected the genetic composition of eastern hemlock populations.

Previous conservation efforts by Camcore with Central American and Mexican pines indicate that, for species with low to moderate levels of genetic diversity, a sample size of six to eight populations throughout a species' geographic range and 10 to 20 trees per population will conserve most alleles with frequencies of 5% or greater (Dvorak et al. 1999). Thus far, our molecular studies with Carolina and eastern hemlocks have revealed that these species have low to moderate genetic diversity in the southern U.S. Based on these results, our conservation strategy for hemlocks is to sample 10 mother trees in 1) as many populations of Carolina hemlock as we can identify and 2) 60 populations of eastern hemlock distributed throughout the seven states in the southern region in which it occurs.

PROVENANCE SEED COLLECTIONS

Carolina Hemlock

Since 2003, Camcore has explored 21 Carolina hemlock populations distributed across the species' range (Figure 1). Due to its rarity and small geographic range, our conservation goal for this species is to sample 10 trees per population in as many populations as we can locate. We have collected seed from 13 provenances yielding a total of 84 mother trees that are represented in the hemlock seed bank at North Carolina State University (Table 1). Collections at three sites in North Carolina (Linville Falls, Carolina Hemlocks Campground, and Wildcat) provided the full complement of 10 mother trees sampled. The Tallulah Gorge collection is also complete as this sample contains three of the four known trees at this site. Despite yearly evaluations at the remaining nine sites where we have made partial seed collections and eight others we continue to explore, we have been unable to locate the additional cone bearing trees necessary to complete the sampling. We surmise this could be due to a number of factors, including but not limited to variation in cone production cycles among trees within populations, lack of proper exposure to sunlight for less dominant trees, or a combination of stress from drought and HWA infestation.

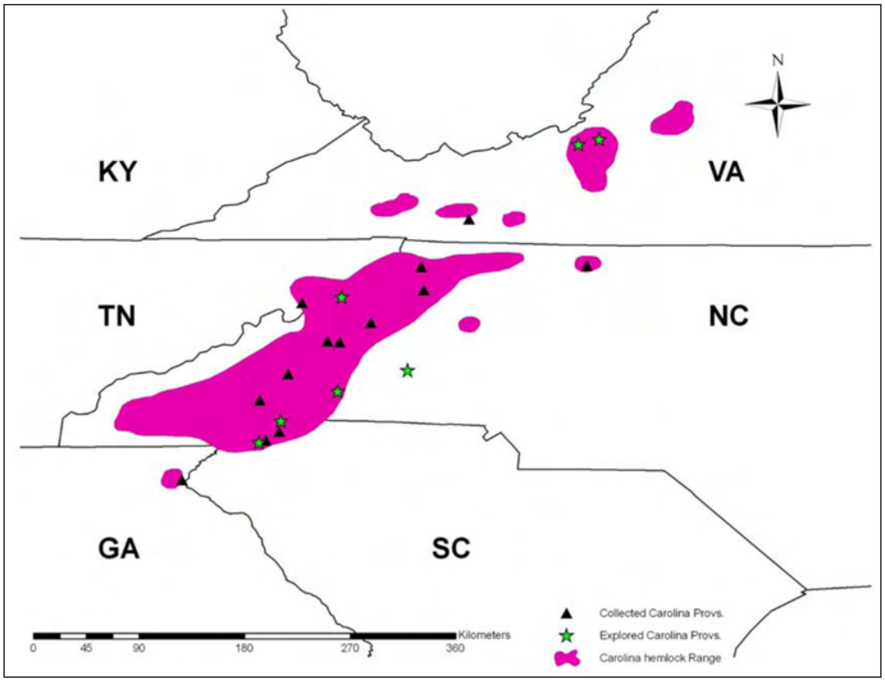

Figure 1. Camcore Carolina hemlock site explorations (stars) and seed collections (triangles), 2003-2007.

Eastern Hemlock

We have had greater success locating cone producing populations of eastern hemlock. Between 2005 and 2007, Camcore explored 56 eastern hemlock provenances across the southeastern U.S. (Figure 2). Seed collections were made in 18 of these, yielding a total of 110 mother trees currently represented in the Camcore seed bank (Table 1). This is a little more than one-sixth our conservation goal of 600 mother trees (60 populations, 10 trees per population) for this species. However, similar to our experience with Carolina hemlock, most eastern hemlock populations have yielded small amounts of seed or no seed at all during collection efforts. Again, we feel this could be due to a number of factors, including within-population cone-cycle variation and lack of exposure to sunlight. In the case of eastern hemlock, we feel that much of the difficulty in finding cone bearing trees is due to severe range-wide drought and HWA-related decline. Field observations suggest that Eastern hemlocks decline much more rapidly and succumb more quickly to adelgid infestation than do Carolina hemlocks, a pattern likely to be magnified under current drought conditions in the southeastern U.S. Among the many symptoms of adelgid infestation that may be more acute in eastern hemlock is the abortion of vegetative and reproductive buds leading to reduced cone production (McClure et al. 2001). Furthermore, we have explored several sites in Kentucky, Tennessee, and Alabama in the western portion of the eastern hemlock range. These sites are experiencing severe drought but are far removed from the adelgid infestation, suggesting that low water availability may also be a significant limiting factor to successful hemlock reproduction.

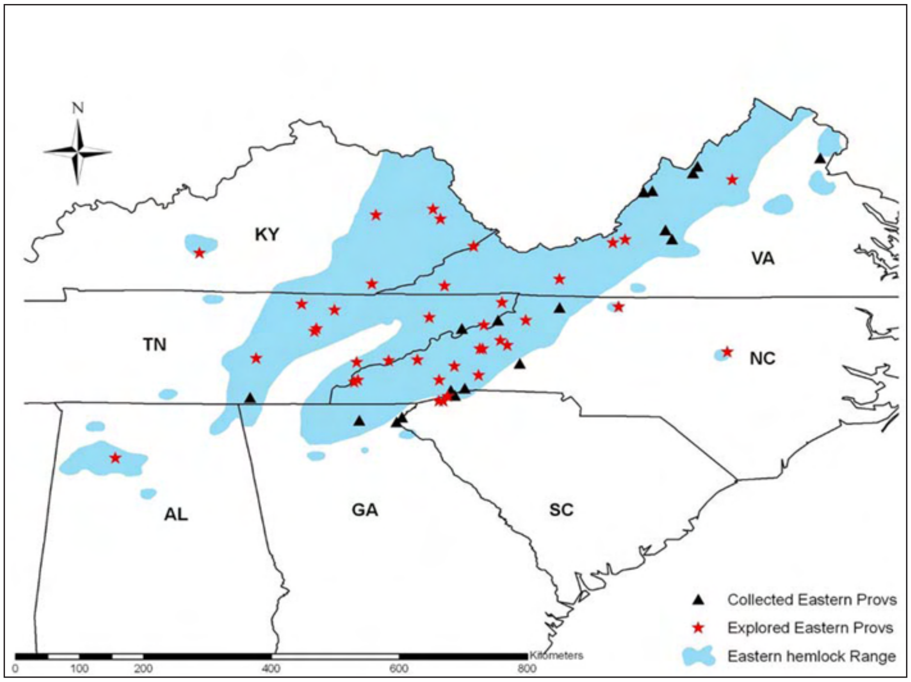

Figure 2. Camcore eastern hemlock site explorations (stars) and seed collections (triangles) 2005–2007.

Table 1. Seed Collections of Carolina and eastern hemlock in the southeastern U.S. by Camcore, 2003–2007.

Prov. #	Provenance	County/State	Latitude & Longitude (Dec. Deg.)	Elevation (m)	Collection Year(s)	# Mother Trees
Carolina Hemlock						
1	Linville Falls	McDowell, NC	35.94 N 81.92 W	995	2003	10
2	Table Rock	Pickens, SC	35.04 N 82.73 W	956	2003	3
3	C. Hemlocks Campground	Yancey, NC	35.80 N 82.20 W	823	2003	10
4	Caesar's Head	Greenville, SC	35.11 N 82.63 W	933	2003, 2006	5
5	Cradle of Forestry	Transylvania, NC	35.35 N 82.78 W	1017	2003	8
6	Wildcat	Watauga, NC	36.20 N 81.52 W	297	2003	10
7	Hanging, Rock	Stokes, NC	36.39 N 80.27 W	146	2003	5
8	Bluff Mtn.	Ashe, NC	36.38 N 81.54 W	1375	2003	8
9	Crabtree	Yancey, NC	35.80 N 82.20 W	1132	2003	6
10	Cripple Creek	Wythe, VA	36.75 N 81.17 W	766	2006	4
13	Tallulah Gorge	Rabun, GA	34.73 N 83.38 W	576	2005	3
14	Cliff Ridge	Unicoi, TN	36.10 N 82.45 W	671	2006	6
21	Biltmore Estate	Buncombe, NC	35.33 N 82.32 W	650	2007	6
Eastern Hemlock						
13	Tallulah Gorge	Rabun, GA	34.73 N 83.38 W	576	2005	13
14	Cliff Ridge	Unicoi, TN	36.10 N 82.45 W	671	2006	2
16	Stone Mtn.	Alleghany, NC	36.38 N 81.02 W	470	2006, 2007	7
17	South Mtn.	Burke, NC	35.59 N 81.60 W	400	2007	10
20	Quantico	Prince Wm., VA	38.53 N 77.38 W	53	2006	7
25	North Creek	Botetourt, VA	37.54 N 79.58 W	345	2006, 2007	10
26	Hone Quarry	Rockingham, VA	38.46 N 79.13 W	600	2006, 2007	7
27	Beech Mtn.	Avery, NC	36.22 N 81.94 W	959	2006	5
33	Helton Creek	Union, GA	34.75 N 83.89 W	727	2007	4
37	Todd Lake	Augusta, GA	38.36 N 79.20 W	612	2006, 2007	5
38	DuPont State Forest	Transylvania, NC	35.18 N 82.60 W	766	2006, 2007	10
39	Blowing Springs	Bath, VA	38.06 N 79.89 W	526	2007	3
40	Hidden Valley	Bath, VA	38.15 N 79.76 W	580	2007	4
41	Cave Mtn. Lake	Rockbridge, VA	37.57 N 79.53 W	371	2007	3
43	Jones Gap	Greenville, SC	35.12 N 82.58 W	470	2007	2
46	Prentice Cooper	Marion, TN	35.13 N 85.42 W	536	2007	3
50	Carl Sandburg Home	Henderson, NC	35.27 N 82.44 W	745	2007	5
51	Chattooga River	Oconee, SC	34.79 N 83.31 W	377	2007	10

FLORAMAP™ CLIMATIC MODEL AND SOIL SAMPLING

Two important factors to consider when selecting sites for the establishment of *ex situ* conservation stands are that 1) areas must have suitable climate and weather for the species of concern, and 2) soil conditions must be suitable to meet the species' resource needs (FAO et al. 2004). To address these issues for hemlock, Camcore is utilizing a computer climate model and range-wide analyses of soil cores from hemlock stands.

We used the FloraMap™ climate model (Jones and Gladkov 1999) to select areas of the world with climates suitable for growing hemlocks. Based on the geographic coordinates and elevations of the hemlock populations in Figure 1, FloraMap™ predicted with high probability (>90%) that Carolina hemlock will survive when planted along the coastal areas of Washington and Oregon, in isolated areas of the Himalayan Mountains, and at similar southern hemisphere latitudes in Chile, from the city of Concepción south towards Valdivia. Predictions of survival in the Pacific Northwest and Asia are not surprising since other hemlock species occur naturally in these regions. However, because adelgids also occur on the hemlocks in these regions (Havill et al. 2006), they are not suitable for hemlock conservation plantings. FloraMap™ also predicted that Carolina hemlock can be grown in the Ozark Mountains of Arkansas and small areas of southern Brazil near the city of Lages, although with a lower probability (40%) of suitable climatic matches. Similar results were found for a model based on eastern hemlock populations in North Carolina and Virginia (Figure 2). Based on this climatic modeling, Camcore has targeted Chile, Brazil, and the Ozark Mountains for *ex situ* conservation of Carolina hemlock. We are cooperating with forest industries in Chile and Brazil, the University of Arkansas, and USDA Forest Service in the Ozarks to grow seedlings and acquire land for planting. Our progress here is outlined in the following section. The methodology and analyses conducted with the FloraMap™ model have been previously documented by Tighe et al. (2005) and Jetton et al. (2008), and readers are referred to these for specific details on model parameters and outputs.

Unfortunately, the FloraMap™ program does not contain a soils component. Therefore, to further refine our site selections within the regions selected by the climate model for hemlock planting, we have conducted a range-wide analysis of soil conditions in eastern and Carolina hemlock stands in the southern U.S. (Jetton et al., in press). We are using this data to pin-point the best possible sites in Chile, Brazil, and the Ozarks for *ex situ* conservation bank establishment.

PROGRESS ON *EX SITU* CONSERVATION BANK ESTABLISHMENT

Carolina Hemlock

Camcore currently has Carolina hemlock seedlings growing at forest nurseries in Chile, Brazil, and at the University of Arkansas. In Chile, Camcore cooperative member Bioforest-Arauco is coordinating the nursery production, field establishment, and subsequent management of the trees for seed production. This planting will represent all Carolina hemlock populations and open-pollinated families (64 families total) collected in 2003 (Provenances 1 – 9; Table 1). Seedlings will be established on a plantation site in the Los Alamos Zone as 3-0 stock in September 2008. In Brazil, Camcore members Klabin SA and Rigesa-MeadWestvaco are coordinating seedling production, establishment, and management. At their Tres Barras nursery facility, Rigesa is growing seedlings for all Carolina hemlock populations and families listed in Table 1 with the exception of the Biltmore Estate provenance. Rigesa will grow the seedlings up to 3-0 stock plants, at which time half of the Carolina hemlocks will be given to Klabin. Both companies will establish *ex situ* conservation banks on plantation sites in Santa Catarina State in 2010.

Dr. Brad Murphy in the Department of Horticulture at the University of Arkansas (Fayetteville) is growing Carolina hemlock seedlings for the Ozarks-based *ex situ* conservation plantings. He has recently germinated seedlings for all populations and families listed in Table 1 (excluding the Biltmore Estate) and should have 3-0 seedlings available for planting in late 2010 or early 2011. These seedlings will be established and managed by the USDA Forest Service at seed orchard sites on the Ozark and Ouachita National Forests.

Eastern Hemlock

Rigesa-MeadWestvaco and Dr. Murphy have agreed to also coordinate *ex situ* conservation of eastern hemlock in Brazil and Arkansas, respectively. Rigesa received seed for all eastern hemlock provenances and families collected in 2005 and 2006 (Table 2) with its Carolina hemlock seed shipment. Seedlings have been germinated and should be ready for field establishment in 2010. Dr. Murphy will receive eastern hemlock seeds from all collections listed in Table 1 in 2008. As with Carolina hemlock, the USDA Forest Service will coordinate the establishment and management of eastern hemlocks in Arkansas.

Camcore member CMPC Forestal Mininco has agreed to coordinate the *ex situ* conservation of eastern hemlock in Chile. Unfortunately, since our initial shipment of Carolina hemlock seeds to Bioforest-Arauco, the Chilean Servicio Agrícola Ganadero – SAG (Ministry of Agriculture) has changed its regulations regarding the shipment of exotic plant seeds into the country. Therefore, we cannot ship seeds to CMPC until they have completed construction and received SAG certification of a new quarantine greenhouse at their Centro Experimental Escuadrón nursery facility. The company has made excellent progress with the greenhouse and it should be ready to receive its their first shipments of eastern hemlock seed in late 2008.

CLOSING REMARKS

Camcore and the USDA Forest Service continue to make good progress with the conservation of Carolina and Eastern hemlocks in the southern U.S. Although we continue explorations and seed collections for Carolina hemlock, our conservation effort with this species is largely complete ,and AFLP analysis indicates that our work has adequately conserved its genetic diversity across the range (Camcore 2006). Our efforts with Carolina hemlock are now focused on conservation bank establishment, breeding, and seed production for eventual reintroduction purposes. With respect to breeding, we have established a small seedling seed orchard of Carolina hemlock in the mountains of North Carolina and plan to establish more of these in the future.

Gene conservation collections for eastern hemlock are still in progress. Through two years of field work we have catalogued approximately one-sixth of our seed goal for the species. Hemlock decline associated with HWA infestation and the severe range-wide drought is having a significant impact on the availability of seeds across the southeast. This has certainly slowed our progress and may effectively eliminate some populations before trees are able to recover and produce seed. One option to increase seed availability may be to apply soil or trunk injected imidacloprid treatments to mother trees we have targeted for seed collection in eastern hemlock populations identified as critical to the conservation effort. Of course, the success of such an effort will depend on the duration of current drought and its effect on tree uptake and mobilization of the insecticide. In the meantime, we will continue to collect what seed is available each year and send that seed to our cooperators for inclusion in *ex situ* conservation plantings.

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WATER USE BY EASTERN HEMLOCK: FROM IMPLICATIONS FOR USING SYSTEMIC INSECTICIDES TO ECOSYSTEM FUNCTION

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ABSTRACT

We present data from two water-use studies for eastern hemlock and co-occurring species. In the first study, we quantified the distribution of water flux within hemlock stems and the seasonal variability of daily water use in New England and the southern Appalachians. We found that the maximum water flux in the stem occurs in the outer 2 cm of sapwood. Further, eastern hemlock is physiologically active for eight months in New England, in contrast to its year-round activity in the southern Appalachians. The timing of maximum water use in the southern Appalachians occurs during April, while in New England peak water use occurs in July and August. We also found an exponential relationship between tree diameter and water use. This relationship has implications for the current dosage recommendations for chemical insecticides, which are based on a linear relationship between tree diameter and dosage. Simple mathematical and graphical models derived from these data can be used by land owners, natural resource managers, and tree care specialists to estimate the amount and timing of water use by eastern hemlock based on tree size and climatic conditions.

In the second study, modeled estimates of transpiration of hemlock stands, and within the stand, we compare transpiration rates among hemlock trees with the three woody species that will likely replace hemlock in the southern Appalachians: black birch, red maple, and rosebay rhododendron. At the stand scale, we estimate that the loss of hemlock will result in approximately 10% reduction in annual transpiration, with spring and winter reductions reaching approximately 30%. Among tree species, under similar climatic conditions, leaf-level transpiration by black birch was six times greater than hemlock while red maple and rhododendron was only three times greater than hemlock. Integrating among all leaves, red maple and hemlock water use were similar, while black birch water use was twice that of hemlock in the growing season. Water use by rhododendron was only a small fraction of that of hemlock due to the small size of the shrub and low leaf area supported. We conclude that if hemlock is replaced by 1) black birch, then annual transpiration will increase, 2) red maple, then annual transpiration will be similar, and 3) rhododendron, then annual transpiration will decrease markedly. Replacement by either red maple or black birch will also change the seasonality of transpiration.

Best Management Practices for Systemic Chemical Control of Hemlock Woolly Adelgid in Forests

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ABSTRACT

Hemlock woolly adelgid (*Adelges tsugae* Annand) threatens native hemlock (*Tsuga canadensis* and *T. caroliniana*) in the eastern United States. Imidacloprid used as a soil-applied systemic insecticide is effective against *A. tsugae* in natural forests and in landscapes. Non-target impacts are a special concern because hemlock is ecologically important, often growing next to streams that contain aquatic species sensitive to imidacloprid, some of which are protected or endangered fauna. Environmental risk can be mitigated by applying the minimum effective dosage in forests as determined by a field dose-response experiment. Adelgid populations responded to imidacloprid dosage with approximately a linear relationship between the percent population reduction (probability scale) vs. log of dosage; 50% reduction in populations could be achieved with 0.15 g per 2.5 cm of trunk diameter at breast-height (DBH), or 10% of the maximum labeled dosage.

However, effectiveness was found to vary with DBH: the dosage predicted to giveapproximately 90% reductions in adelgid populations is given by the equation $\log(\text{dosage}) = 0.0153 \cdot \text{DBH} - 1.074$, where the dosage is grams of imidacloprid per 2.5 cm of trunk DBH and DBH is measured in centimeters. The most convenient method to apply this information will be to calibrate DBH tapes in dosage units using this logarithmic equation. For trees less than 82 cm DBH, these dosages are less than the maximum labeled dosage of 1.5 g imidacloprid per 2.5 cm DBH. Trees larger than 82 cm DBH may require treatment in two successive years.

A combination of optimum dosing of trees and adoption of the tablet formulation when treating trees in sensitive habitats should minimize the risk of contaminating aquatic resources with imidacloprid. This approach will provide satisfactory multiple-year suppression of adelgids where an immediate reduction in adelgid populations is not needed. For quarantine purposes or where rapid and short-term suppression of adelgids is needed, adelgid populations can be suppressed with a foliar spray of horticultural oil and/or bifenthrin (not appropriate near aquatic resources) or a trunk application of dinotefuran. The multiple-year benefits obtainable with soil application of imidacloprid or a similar long-lasting neonicotinoid could be combined with these shorter-term options to realize the benefits of quick and long-lasting treatments.

KEYWORDS

Imidacloprid, *Adelges tsugae*, optimum dosage, DBH

Analytical Approaches to Imidacloprid and Metabolite Analysis

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ABSTRACT

This presentation is an overview of the four analytical techniques that have been used to analyze imidacloprid in treated hemlock. The four techniques and the matrices through which imidacloprid has been analyzed in hemlock are:

1. Enzyme-linked immunosorbent assay (ELISA) for xylem fluid,
2. Gas chromatography with positive chemical ionization mass spectrometry (GC-PCI/MS) for xylem fluid,
3. Flow injection analysis with nitric oxide chemiluminescence detection (FIA NOCL) for xylem fluid and needles (Lagalante and Greenbacker 2007), and
4. Liquid chromatography tandem mass spectrometry, (LC/MS/MS) for xylem fluid and needles.

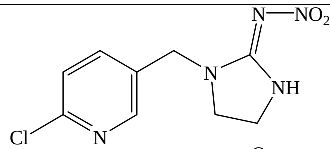
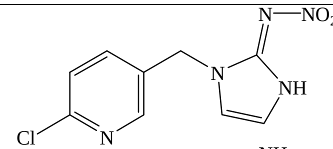
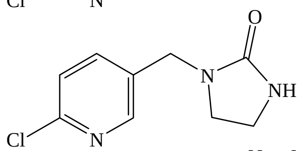
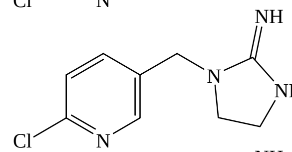
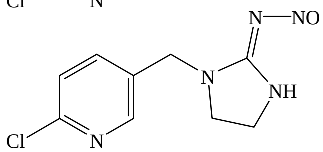
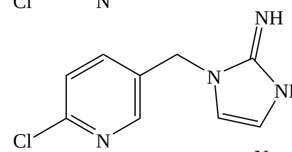
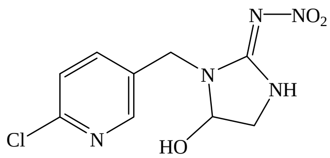
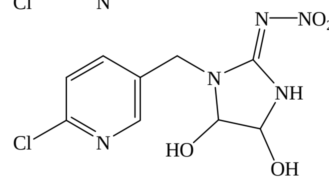
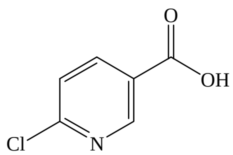
ELISA is a relatively low cost method that does not require sophisticated instruments. However, significant, varying cross-reactivity is found for the urea, N-nitroso, olefin, des nitro, and des nitro olefin metabolites that can lead to falsely elevated concentrations of imidacloprid in hemlock xylem fluid. See Table 1 for chemical structures of imidacloprid and its metabolites.

To overcome the non-specificity of ELISA, a GC-PCI/MS method was developed to quantify selectively imidacloprid in hemlock needles (Jones 2007). Comparative results between the two techniques indicated that indeed the ELISA results were elevated in comparison to the GC-PCI/MS determinations, potentially due to the presence of metabolites.

FIA NOCL is a method based upon photochemical degradation of imidacloprid to nitrite, chemical reduction of nitrite to nitric oxide, and then chemiluminescent detection of nitric oxide with ozone. Although we were successful in developing the FIA NOCL method, it also suffers from cross reactivity for the metabolites containing N-nitro or N-nitroso functionality.

Lastly, a LC/MS/MS technique was developed using specific multiple reaction monitoring (MRM) ion transitions that has metabolite specificity. An initial application of the method was to hemlocks treated with imidacloprid-treated trees in 2005 in the West Virginia Botanical Gardens. Analysis of hemlock needles indicates the presence of imidacloprid hydrolysis

Table 1. Imidacloprid and metabolites studied.

imidacloprid 1-(6-chloro-3-pyridylmethyl)-*N*-nitro-imidazolidin-2-ylideneamine		**olefin** 1-(6-chloro-3-pyridylmethyl)-*N*-nitro-1,3-dihydro-imidazol-2-ylideneamine	
urea 1-(6-chloro-3-pyridylmethyl)-imidazolidin-2-one		**des nitro** 1-(6-chloro-3-pyridylmethyl)-imidazolidin-2-ylideneamine	
***N*-nitroso** 1-(6-chloro-3-pyridylmethyl)-*N*-nitroso-imidazolidin-2-ylideneamine		**des nitro-olefin** 1-(6-chloro-3-pyridylmethyl)-1,3-dihydro-imidazol-2-ylideneamine	
5-hydroxy 1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol		**dihydroxy** 1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-4,5-diol	
6-chloro-nicotinic acid			

and dehydrolysis pathways as well as oxidation pathways, while reductive pathways are not present (Figure 1). At two and five months post-treatment, imidacloprid and seven of its metabolites were observed in the stem injected trees. At five months after treatment, residues in the soil treated trees were still below detection limits. The high levels of the olefin metabolite in stem-injected trees are of particular interest due to its 10-fold increase in insecticidal efficacy over the parent compound. Control samples were not taken for analysis until seven months post-treatment.

KEYWORDS

imidacloprid, metabolites, ELISA, GC-PCI/MS, chromatography, flow injection analysis.

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LABORATORY STUDIES OF IMIDACLOPRID IMPACTS ON HEMLOCK WOOLLY ADELGID, *LARICOBIUS NIGRINUS*, AND *SASAJISCYMNUS TSUGAE*

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ABSTRACT

Eastern hemlock branches infested with hemlock woolly adelgid were treated with systemic doses of imidacloprid in the laboratory. In choice and no-choice tests, *Laricobius nigrinus* and *Sasajiscymnus tsugae* were impacted from feeding on adelgids from treated branches.

KEYWORDS

imidacloprid, *Laricobius nigrinus*, *Sasajiscymnus tsugae*

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an exotic invasive pest from Japan (Havill et al. 2006) that infests and kills eastern hemlock trees, *Tsuga canadensis* (L.) Carrière, throughout much of their native range in the eastern United States. A classical biological control program is underway, and *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) are two biological control agents that have been released in the eastern United States to control HWA. Imidacloprid, a neonicotinoid insecticide, is commonly used against HWA in forest environments. Trunk and soil injections of imidacloprid are the primary methods of control in forest and urban landscapes and can provide protection against infestation for several years after application (Cowles and Cheah 1999, Doccola et al. 2003, Webb et al. 2003). There continues to be applications of imidacloprid in public and private forests and parks, often geographically close to releases of adelgid predators in a coordinated biological control program. The purpose of this study was to investigate if imidacloprid treatments could potentially exhibit nontarget impacts on beneficial predators of HWA.

SPIKE TESTS AND DETERMINATION OF LC_{50} FOR HWA

HWA-infested hemlock branch sections were placed in vials containing 20 mLs of 0, 1, 10, or 100 ppm imidacloprid concentrations prepared in water. Branch sections were removed and

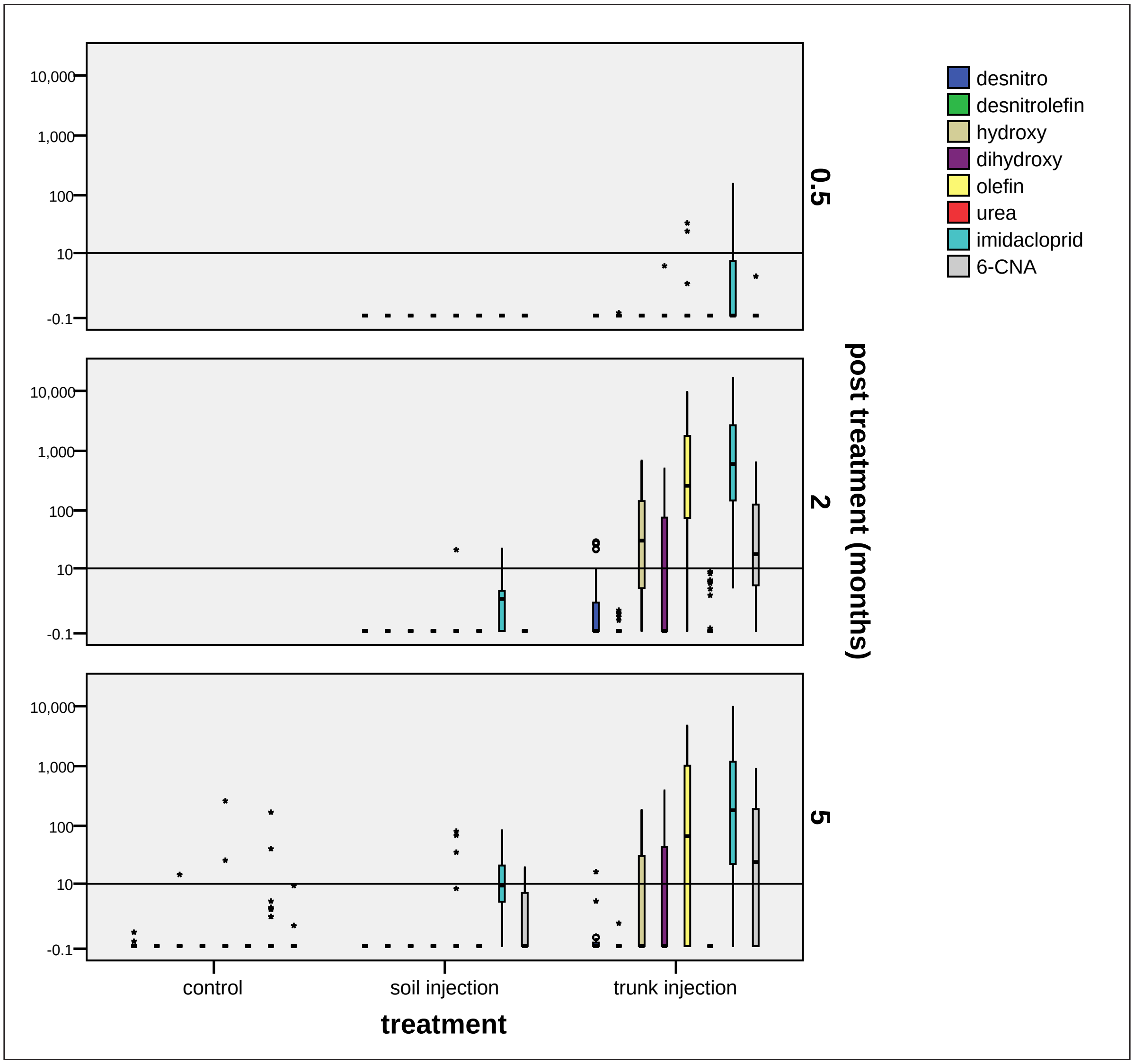

Figure 1. Effect of treatment method on imidacloprid and metabolite levels in treated and control trees. The solid line at 10 ppb indicates the limit of detection by LC/MS/MS. (Control samples were not taken for analysis until seven months post-treatment although they are shown in the five-month portion of the figure).

HWA were observed under a microscope to determine if they were alive or dead after 10, 20, and 30 days. The amount of new growth was measured to the nearest centimeter, and the number of live adelgids per centimeter was recorded for each branch. HWA mortality was highly correlated with the amount of imidacloprid recovered from the branches. Mortality was higher as imidacloprid concentrations in the branches increased. Mortality increased over time and the highest adelgid mortality observed was 30 days after treatment. Imidacloprid was extracted from hemlock wood tissue using liquid chromatography dual-mass spectrometry (LC/MS/MS). Probit analysis of HWA mortality and imidacloprid concentrations recovered from branch wood tissues determined the LC_{50} and its 95% confidence limit (CL) to be 242 and 105-411 ppb, respectively. HWA exhibited high mortality from imidacloprid in the 30-day trial, and it can be inferred that in the field where HWA will be exposed to imidacloprid for periods of time much longer than 30 days, a biologically efficacious dose of imidacloprid in hemlock branches would be less than 242 ppb. These results suggest that HWA is highly susceptible to imidacloprid, and that even very low concentrations (<242 ppb) are efficient in causing substantial HWA mortality.

LABORATORY NO-CHOICE TESTS FOR PREDATORS

In no-choice tests for predators in the lab, hemlock branches were placed in 20 mL of 0, 1, or 100 ppm imidacloprid in water. One beetle was placed on each branch and was observed every five days for a total of 20 days. Beetles were observed for signs of poisoning as well as whether or not they were still alive. The number of adelgids consumed on the branch was counted at each observation period. For *L. nigrinus*, beginning 20 days after treatment, mortality was significantly higher on the 100 ppm branches than on controls. For *S. tsugae*, mortality on treated branches was higher than controls but was not significantly different. *Sasajiscymnus tsugae* consumed the same number of adelgids on treated branches as in controls, while *L. nigrinus* beetles consumed significantly fewer adelgids from the 100 ppm branches than the number of adelgids consumed on controls. LC/MS/MS analysis of branches determined that wood from the 1 ppm branches contained 67-200 ppb imidacloprid, while concentrations of branches with 100 ppm treatment ranged from 4.5 to 15.2 ppm. *Laricobius nigrinus* and *S. tsugae* mortality was highest from feeding on the 100 ppm branches. The imidacloprid concentrations were high enough to kill more than 90% of HWA after 30 days. Mortality could be through starvation or poor prey quality rather than direct mortality from the insecticide. When given no choice in prey, both beetle species will feed on HWA residing on treated branches.

LABORATORY CHOICE TESTS FOR PREDATORS

In predator choice experiments in the lab, HWA infested hemlock branches were placed into 20 mLs of 0, 1, 10, or 100 ppm imidacloprid in water. One predator beetle was placed into an arena containing two branches, one branch cut from a treated branch, the other from an untreated branch. Beetles feeding on the two branches were observed every five days for 20 days. Beetles consumed significantly fewer adelgids on the 100 ppm branches than those on the untreated branches probably because, on the 100 ppm branches, over 90 % of the adelgids were dead by the end of the trial and the beetles prefer to feed on live adelgids over dead ones. Beetles were observed feeding more on control branches than treated branches, suggesting a feeding preference on healthier, untreated adelgids. Beetle mortality generally increased in the higher treatments; however, means were not significantly different from control mortality. It is unclear if beetles died from natural causes associated with feeding on poor quality adelgids or from ingesting imidacloprid in the adelgids.

IMPACTS OF TOPICAL APPLICATION OF IMIDACLOPRID ON PREDATORS

Laricobius nigrinus and *S. tsugae* beetles were individually treated with 0, 0.005, 0.05, 0.5, 5, or 50 ng of imidacloprid in acetone. Imidacloprid solutions were applied to the ventral abdomen, after which beetles were observed every 24 h for 6 days. The LD_{50} value six days after exposure was 1.8 ng and 0.71 ng per beetle for *L. nigrinus* and *S. tsugae*, respectively. Both beetles displayed tremors and paralysis after treatment, with increasing intensity of poisoning symptoms and mortality over time and with increasing treatment concentration. Both beetles are susceptible to imidacloprid from topical applications, although in practice, the systemic treatment of imidacloprid into hemlocks makes it unlikely that predators would be exposed to topical doses of imidacloprid within the hemlock and HWA system. *Sasajiscymnus tsugae* was more than twice as susceptible to imidacloprid, probably in part because their smaller size and volume would result in a higher concentration of imidacloprid per milligrams of body weight. This experiment provides a reference point for susceptibility to imidacloprid concentrations that the beetles may be exposed to when feeding on HWA on treated trees.

SUMMARY

Data reported here are from laboratory studies only. In these studies, imidacloprid displayed biological efficacy against HWA at very low concentrations (<242 ppb). The two predator species displayed sensitivity to imidacloprid from topical applications in the nanogram range, although *S. tsugae* was twice as susceptible as *L. nigrinus*. Both predators displayed a preference for feeding on untreated branches over treated ones, suggesting that beetles may prefer to feed and lay eggs on branches where imidacloprid is not present and HWA populations are healthier and denser. The two predators may be negatively affected by feeding on adelgids from treated trees; however, mortality and fitness seem to be affected as a result of reduced prey quality and density rather than direct mortality associated with directly ingesting the insecticide. Some individuals did display poisoning symptoms after feeding on treated adelgids, suggesting that imidacloprid could potentially be passed from an adelgid to a predator under specific conditions. *Laricobius nigrinus* was more sensitive to feeding on adelgids from treated branches than *S. tsugae*. This could be because *L. nigrinus* is more intimately linked to HWA: for instance, this predator lays eggs within HWA ovisacs while *S. tsugae* lay eggs on the bark. Also, *L. nigrinus* seemed to carefully consume whole adelgid adults, while *S. tsugae* was more often observed feeding on eggs or partially consuming adelgids. Adelgid eggs might not have imidacloprid within them and could be a safer food source for both predators and their larvae, although further experiments are required to test this hypothesis. In the field, very low concentrations of imidacloprid are capable of controlling HWA, and any negative effects that imidacloprid would have on HWA predators would probably be due to reduced prey quality and density. Imidacloprid exposure through feeding of adelgids on treated trees is possible, but predator preference for healthier food stock could drive them away from treated stands towards denser, healthier adelgid populations. Both chemical and biological control of HWA are important in the effort to save hemlocks in the eastern forests, and both methods should be employed for maximum efficacy; however, predator releases should not be made near hemlocks treated with imidacloprid until HWA populations have recovered.

ACKNOWLEDGMENTS

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Environmental Fate of Imidacloprid

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ABSTRACT

Two methods are being employed to control hemlock woolly adelgid (*Adelges tsugae* Annand) on hemlocks: biological control and chemical control. Currently, the most effective chemical control appears to be the injection of imidacloprid, a systemic insecticide that acts on the adelgid's nervous system. Imidacloprid may be applied as a trunk injection directly into the tree or applied to the soil via soil drenches or injections.

While injecting imidacloprid directly into the soil minimizes the risk to humans and other animals, there is concern about the pesticide reaching nearby streams. Some research has focused on the lethal concentrations of imidacloprid to various aquatic organisms, but little work has been done on field experiments involving imidacloprid treatments. The overall objective of this study is to determine if soil injection with the systemic insecticide imidacloprid for hemlock woolly adelgid control near streams can adversely impact aquatic invertebrates, particularly insects. Specific objectives include: 1) determine if there is a detrimental effect on aquatic invertebrates immediately following soil injection with imidacloprid, 2) determine if there is a long-term impact on aquatic invertebrates by periodic sampling over a two-year period, and 3) if there is an impact, determine the length of recovery time needed for aquatic invertebrate assemblages.

Four streams were selected in the Blue Ridge province of the southern Appalachian region for treatment of the surrounding watershed with imidacloprid. The study sites in the Chattahoochee National Forest in Georgia included an unnamed tributary of Holcomb Creek, Addie Branch, and Billingsley Creek. Sixty trees around these streams were treated with soil-injected imidacloprid in November 2005. The fourth treatment site was Dryman Fork at the Coweeta Hydrologic Laboratory in North Carolina. In May 2006, 88 trees bordering the stream were treated with tree injections of imidacloprid and 109 trees were treated with soil injections. The adjacent watershed was used as the reference condition.

Streams were sampled biweekly for three months following treatment and monthly thereafter for two years. A Surber sampler was used to sample four riffles in each stream, and water samples were taken for chemical analysis.

We compared the number of taxa, number of EPT (Ephemeroptera + Plecoptera + Trichoptera) taxa, average abundance, and North Carolina Biotic Index (NCBI) for each season in the treatment streams to the reference stream to determine the impact of the imidacloprid treatments. If the indices in the treatment stream were significantly lower than in the reference stream, we then analyzed the within-stream seasonal variation.

The October 2007 water sample from Holcomb Tributary was found to contain less than 1.0 ppb of imidacloprid. No other samples contained imidacloprid.

The average number of taxa in Addie Branch in winter 2006/07 was significantly lower than in the reference stream, but not lower than the previous season in that stream. Average abundance in Addie Branch for fall 2006 and winter 2006/07, as well as in Dryman Fork for fall of 2007, were significantly lower than in the reference stream but again not significantly lower than the previous season in the same stream. The average number of EPT taxa in Addie Branch in summer 2006 was significantly lower than in the reference stream and significantly lower than the spring 2006 sample. The data for this metric followed the same pattern as the other treatment streams, but with a more pronounced decrease in taxa due to emergence of adults. NCBI scores for the treatment streams were never significantly lower than in the reference stream. All scores were less than 4.18, falling in the water quality class of excellent.

Only a trace amount of imidacloprid entered one of the streams over a period of two years, and no effect was observed on the aquatic macroinvertebrates in that stream. Our results indicate that soil injections of imidacloprid can safely be used in the southern Appalachian area to control hemlock woolly adelgid. However, extreme caution should be used when applying these results to other areas with different soil types that may not bind imidacloprid as tightly.

KEYWORDS

imidacloprid, streams, nontarget, aquatic macroinvertebrates

A New Strategy to Control Hemlock Woolly Adelgid Using Imidacloprid and an Arboricultural Method to Gauge Hemlock Health

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ABSTRACT

Imidacloprid, a systemic insecticide is labeled for use against hemlock woolly adelgid (HWA) and armored scales. In this study, we applied imidacloprid by soil and/or tree injection, neither of which is, in itself, unique. What is new is combining both as a strategy for insect management and tree protection. Designed as a two-year study, applications to trees were made in 2007. Evaluations include HWA and elongate hemlock scale (EHS) densities, tree response, and imidacloprid residues. One desired outcome would be a method for the forest manager to gauge hemlock health. In 2007 results, 94.2% of hemlocks were categorized as being in poor to very poor health (little or no growth or dieback); 52.2% of hemlocks had symptoms of dieback. Low HWA densities (mean of 0.41 HWA/cm of twig) and poor tree condition are typical of very late-stage infestation. HWA survival is most closely associated with new growth (17.5% of trees); the potential of re-emergence therefore exists. EHS was present in the study trees as well (mean of 1.15 EHS/needle), with highest numbers observed in three-year-old needles. Protecting the extant canopy is critical to tree recovery; therefore, management of EHS is a management priority. Successful treatment methods are those that aid hemlock recovery and provide protection over time. Combining tree and soil injection is a new strategy that may provide the forest manager with key advantages, such as quick and simple applications for near- and long-term tree protection.

KEYWORDS

imidacloprid, hemlock woolly adelgid, tree microinjection, soil injection, hemlock health

INTRODUCTION

Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine), a neo-nicotinoid insecticide, is labeled for use in management of hemlock woolly adelgid (HWA), *Adelges tsugae* (Homoptera: Adelgidae), and suppression of armored scales. It may be applied as a canopy spray, to the soil, or injected directly into the tree. For forest and woodland applications, the latter two methods offer the most versatility. For example, the Kioritz soil injector and the Arborjet QUIK-jet tree injection equipment are light-weight, easy to carry, use low volumes of concentrate, and are simple to use. Typically, the forest manager will choose one application method, but there may be significant advantages to combining soil and tree injection as a strategy for near-term therapeutic treatment and longer-term tree protection.

As part of this study, we developed a simple method to help quantify hemlock (*Tsuga canadensis* (L) Carriere) response that may be used by the forest manager. This method is based on the impact HWA has on twig growth. McClure suggested an inverse relationship between HWA pressures and hemlock growth (1991); this growth loss occurs with increasing HWA pressure and may take three or more years. Ward (1991) suggested an HWA threshold of 25-30 HWA/100 needles as negatively impacting twig growth; we use 2.0 HWA/cm twig growth (Doccola et al. 2007). In hemlock, we counted needles per cm and calculated a mean of 7.2, or 2.8 HWA/10 needles, which is comparable to HWA densities reported by Ward. HWA feed in the xylem ray parenchyma cells (McClure 1987) on twigs at the needle base, symplastic tissue rich in carbohydrates, and other essential nutrients; as carbohydrates are siphoned off by insect feeding, less is available to the tree for basic life functions (i.e., growth, metabolism, reproduction, carbohydrate storage, and defense) (Shigo 1989). As infestation progresses, twig lengths decrease, followed by loss of new growth altogether. Photosynthetic capacity, and therefore carbohydrate manufacture, is reduced. The capacity of the tree to meet existing and new mass requirements becomes limited. Unchecked, HWA can compromise photosynthetic capacity further; below a critical level, twigs die back and shed needles. As the resource becomes depleted, the HWA numbers also drops. These hemlock responses to HWA infestation may be used by the forest manager as indication of treatment efficacy and hemlock health.

Elongate hemlock scale (*Fiorinia externa*) (Hemiptera: Diaspididae) infestation exacerbates hemlock decline by increasing needle shedding[(1)]. Alleviating pest pressures by therapeutic treatments makes tree recovery possible. Of additional concern in woodlands are inconsistent patterns or low levels of precipitation. New shoot growth and root uptake of soil-applied imidacloprid are dependent upon adequate soil moisture.

This study compares three treatment methods and three formulations for tree protection to answer the questions: 1) Does method of application and formulation of imidacloprid make a difference to outcome? 2) Can methods and formulations be combined to advantage? 3) Is there sufficient activity maintained and protection provided to hemlocks?

[(1)]http://www.na.fs.fed.us/spfo/pubs/pest_al/ehscale/ehscale.htm

METHODS

Sixty-four hemlocks were treated with the insecticide formulations containing the active ingredient (a.i.), imidacloprid for HWA and EHS infestations. Eight treatments were assigned in a complete randomized block design. Study trees ranged from 10.6 to 34.7" (26.5 to 86.8 cm) tree diameter at breast-height (DBH) with a mean DBH of 19.6" (49 cm). The eight treatments were: 1) controls, 2) IMA-jet microinjection (0.15 g a.i./DBH") (0.06 g a.i./cm DBH), 3) IMA-jet Micro-Infusion™ (0.3 g a.i./DBH") (0.12 g a.i./cm DBH), 4) MERIT Tree Injection, low rate (0.15 g a.i./DBH") (0.06 g a.i./cm DBH), 5) MERIT Tree Injection, high rate (0.3 g a.i./DBH") (0.12 g a.i./cm DBH), 6) IMA-jet microinjection + MERIT soil injection (1.45 g a.i./DBH") (0.6 g a.i./cm DBH), 7) IMA-jet Micro-Infusion™ + MERIT soil injection, and 8) MERIT soil injection. Treatments were applied on August 29-30, 2007, at the Biltmore Estate, Asheville, North Carolina.

The Tree I.V. and the QUIK-jet injector are methods of Micro-InfusionTM and tree microinjection, respectively. The QUIK-jet injector delivers a fixed, measured dose per injection site. The number of application sites into the sapwood varied depending upon the method used. The configuration of the injection site was standardized using a 0.375" (9 mm) bradpoint bit. We drilled 1.5" (3.75 cm) into the sapwood to create a 2.5 cm^3 capacity site. The Tree I.V. is equipped with four injector tips, and sites were located every 8" of stem circumference. The Tree I.V. delivered the higher volume dosages by trunk injection. Sixteen trees were treated using this method.

The QUIK-jet injector applied the lower volume dosages. Sites were located every 6" of stem circumference for the QUIK-jet injector. Thirty-two trees were treated using this method. Tree injection formulations of imidacloprid were applied in concentrate as formulated. Only IMA-jet was applied by Tree I.V. at the 6 mL/DBH"(2.4 mL/cm DBH) rate. IMA-jet at the 3 mL/DBH" (1.2 mL/cm DBH), MERIT Tree Injection at 0.75 or 1.5 mL/DBH" (0.3 – 0.6 mL/cm DBH) were applied by QUIK-jet injector.

Soil applications were applied using the Kioritz soil injector. Soil applications were made 12-36" (30-90 cm) of the tree bole. Each MERIT 75 WSP 1.6 oz (45.4 g) packet was mixed in 32 oz (960 mL) water. Thus prepared, the mixture supplies 1.2 oz of a.i. (34.1 g), sufficient to treat a 23.5" DBH (58.8 cm) tree at the 1.45 g a.i./DBH" (0.58 g a.i./cm DBH) rate. Twenty-four (24) trees were treated by soil injection.

In November, 2007, 70 days after treatment (DAT) twig samples were taken from the study trees. Four branches were cut from the mid- to upper tree canopy by aerial lift truck, each between 40 to 45 cm in length, and shipped to Arborjet, Inc, Woburn, Massachusetts, for evaluation. Thirty-two (32) additional branch samples from eight non-infested, healthy hemlocks were cut for comparison. Samples were held at 40°F (4.5°C) for the extent of the bioassay assessments. Branches were cut to 30 cm length. Each sample was scanned using a Canon Color Image Scanner (CanoScan 8800F) and identified with the tree identification number and year. The digital images were inserted in a Microsoft PowerPoint file and tip growth was determined on a percentage basis, adapting the Webb et al. (2003) method. In infested twig samples, dieback, no growth, or new growth may be observed. Here, dieback is defined as the loss of current year twig growth and needle drop in one-year-old or older twigs.

We quantified tree response by assigning a numeric value, such as '-1', '0', or '+1'. The positive numeric values are further assigned a value that corresponds to percent tip growth observed. Percent was determined by number of tips with growth divided by the total counted x 100. We assigned a '-1' value to samples with dieback, '0' value to samples with no growth and '1' to samples with <10% tip growth, '2' to 10-25% tip growth, '3' to 26-50% tip growth, '4' to 51-75% tip growth, and '5' to >75% tip growth. The mean of the four samples generated a tree rating. The number of trees with each rating was tallied, and percentages of trees in each health category were calculated. Scans will be made of twigs sampled annually for three years to record tree response. Of four branches taken, one was selected at random for bioassay assessments; three were forwarded to the USDA Forest Service/Villa Nova for imidacloprid residue analyses. One terminal and two laterals (three samples) x three years of growth x 64 treatments generated 192 analyses.

Annual twig growth (ATG) was measured in cm and recorded for the most current three increments (e.g., 2007, 2006, and 2005 growth). ATG is the internodal length measured from terminal bud or bud scar to bud scar. Current season twigs are light orange-brown, whereas three-year-old twigs are dark brown or gray[(2)]. In non-infested hemlock, we cut to three bud scars for our sample. However, in infested twigs with no growth or dieback, we count back from the terminal bud, two bud scars. Dendrochronological checks were made to assist in dating infested twigs. A transverse section of the xylem was made by scalpel and the number of growth rings counted. In healthy samples, one-year-old xylem was formed in the current year (e.g., 2007), whereas one-year-old xylem in twigs with no growth was formed the year prior (e.g., 2006). In samples with dieback (needle loss), we assumed that terminal growth was formed the previous year and dated that wood to the previous year. In this case, twig samples to two-year-old wood (i.e., formed in 2005 or earlier) were evaluated. Dendrochronological checks were cross-referenced to twig color: light orange-brown twigs with one annual growth ring were formed in the current year; darker brown twigs with one-year growth ring were formed the previous year, and those with two annual growth rings were formed three years previously.

HWA and EHS scale infestations were assessed by microscopic examination. Each branch generated five branchlets, each of which were assessed from the most current twig. Three growth increments were assessed when current growth was present. When samples had no current year growth, the last two growth increments were assessed. For HWA, the number of live sistens was counted. Dead HWA on twigs were ignored. The number of HWA/cm was calculated. All EHS life stages present on needles were counted on 1 cm of needles of twig. The number of needles per centimeter were also tallied. The number of EHS was multiplied by the increment length with intact needles. EHS per year was determined by multiplying EHS by length of growth increment. EHS by number of needles was also calculated.

[(2)]http://hort.ufl.edu/trees/TSUCANA.pdf

RESULTS

Tree Treatments and Application Efficiency

Two applicators completed the treatments (56 treated and eight controls) in two days. Application time varied with the method used. In terms of speed of application, the QUIK-jet injector was fastest, the Kioritz injector was intermediate, and the Tree I.V., the slowest. Mean application times for the methods were 2, 5, and 10 minutes, respectively. The difference in time is a function of volume and tree (high) or soil (low) resistance to formulation absorption. For example, the Tree I.V. delivered 18 mLs/ injection site, or 7.2 times the volumetric capacity of the injection site. The uptake time is dependent upon tree transpiration and absorption into restrictive sapwood tracheids. The dosages applied by QUIK-jet injector, on the other hand, were 0.6, 1.2, or 2.4 times that of the volumetric capacity of the injection site. Therefore, QUIK-jet applications may be expected to be three to 12 times faster than Tree I.V. Soil injections met with little or no resistance to application. The Kioritz injector, however, is limited to 5 mL per stroke, and therefore requires numerous pump strokes to complete an injection—for example, to deliver 960 mLs requires 192 pump strokes, which accounts for the intermediate time required for application.

HWA Densities and Annual Twig Growth

Because of the high percent of dieback we observed in hemlock branches, only a sub-sample of seven controls and six randomized treatments were assessed for HWA and ATG. Eight (8) non-infested trees are included for comparison of annual twig growth. This data is presented in the Table 1.

Table 1. Comparison of twig growth according to treatments.

	1-Year Twigs			2-Year Twigs			3-Year Twigs		
	Twig length (cm)	HWA (n)	HWA/cm	Twig length (cm)	HWA (n)	HWA/cm	Twig length (cm)	HWA (n)	HWA/cm
Means UTC (n=35)	0.92	0.29	0.32	3.74	1.17 0.31	4.73	0.67	0.14	
Means Treatments (n=30)	1.88	2.57	1.37	4.51	0.73	0.16	4.53	0.57	0.13
Means Non-infested (n=31)	8.84	---	---	12.10	---	---	11.58	---	---

Statistical analyses were conducted in Minitab, version 15. Statistical significance was observed for the number of HWA/linear cm for 2007 at 95% CI (p=0.034), but no significance was found in 2006 or 2005 twigs between treatments and controls. The number of HWA/linear cm ranged from a low of 0.13 to 1.37. Interestingly, the HWA in treatment trees were higher than in the controls, probably due to limited sample size. The highest numbers of HWA/cm were in current year twigs with the greatest growth. Low densities were observed on older twigs of either treated trees or controls. Twig measurements were made at the end of the growing season and treatments were in late August; therefore, this observation of increased growth is not a treatment effect.

One-way ANOVA of 2007 twig length compared treatments, controls, and non-infested (healthy) hemlock indicates strong significance (p=0.000 at a 95% CI) between healthy and infested mean twig growths. Mean HWA/100 needles was calculated at 4.05, well below the threshold described by Ward. The low HWA numbers and reduced twig growth are indicative of a remnant HWA population and late infestation. HWA infestation is likely older than the three years of growth analyzed. Subsequent twig checks to five-year-old twigs suggest that the trees were infested originally in 2003.

EHS Scale on Hemlock Needles

Mean numbers of EHS on untreated controls increase with twig age (p=0.002) at 95% CI. There were no significant differences in treatments (n=168) or control trees (n=24), suggesting that EHS pressures are the same across all treatments. Dieback from scale in hemlock has been reported when density reaches 10 EHS/needle. Mean scale density was low (0.09/needle) in the 2007 sample, but growth was absent in 57.97% of the samples evaluated due to HWA infestation. Mean EHS densities were higher in 2006 and 2005 needles, at 0.93 and 2.43/needle, respectively. Second-year (2005) needles represent the highest percentage of intact biomass; protecting extant needles is therefore critical to tree survival.

Needle Density

The number of needles per centimeter was also determined. Infested trees had mean needle density of 2.16, 7.37, and 7.86/linear cm for 2007, 2006, and 2005 twig increments, respectively (n=69). Needle density was significantly lower in 2007 compared to the two years previous growth (p=0.000) due to HWA limiting growth. Non-infested trees had needle densities per year of 9.97, 6.74, and 4.87/linear cm, respectively (n=31). Mean three-year needle density for non-infested trees was 7.19/linear cm.

Precipitation in Asheville, North Carolina

Hemlock growth is sensitive to available moisture. Onken (1994) found that there was a direct relationship between the amount of rainfall that trees receive in a given year and the amount of new growth in the next year. Table 2 summarizes monthly rainfall data in 2006 for Asheville, North Carolina[3]. The mean annual precipitation was 48.29", 103% of normal. The fall months in particular were above normal. 2007 growth in non-infested trees was 8.8 cm, in contrast to 1.4 cm in HWA-infested trees. Based on this data, the trees received adequate moisture for growth in 2007, and infestation rather than moisture limited twig growth in

[3]http://www.weather.gov/climate/index.php?wfo=gsp

Table 2. 2006 Monthly precipitation data for Asheville, North Carolina.

Rainfall Month	Rainfall (inches)	Normal (inches)	Departure from Normal	% of Normal
January	3.58	4.06	-0.48	88
February	2.55	3.83	-1.28	67
March	0.91	4.59	-3.68	20
April	4.58	3.50	1.08	131
May	1.69	4.42	-2.73	38
June	5.16	4.38	0.78	118
July	2.81	3.87	-1.06	73
August	7.12	4.30	2.82	166
September	7.80	3.72	4.68	210
October	2.93	3.18	-0.25	92
November	4.52	3.82	0.70	118
December	4.64	3.40	1.24	136
Yearly totals	**48.29**	**47.07**	**1.22**	**103**

the study trees. Monthly totals were also tallied for 2007; the mean annual precipitation was 34.39", 74.06% of normal. In non-infested hemlock, 2007 twig growth was less (though not statistically significant) than the previous two years (means of 8.8, 12.1, 11.6 cm, respectively). These drier conditions are not encouraging for hemlock recovery in 2008.

Hemlock Health Scale

Three basic conditions were observed in infested trees and compared to non-infested trees: dieback, no growth, or growth. Hemlock growth varies with tree age, environmental conditions and pest pressures. For this reason, we grouped tree response into broad, descriptive categories (see Table 3).

Table 3. Hemlock health scale.

Tree Rating Numeric Scale	Observation of Terminal Growth (% annual twig growth)	Tree Health Descriptive
-1	no growth and needle loss	very poor, dieback
0	no growth	very poor, no growth
1	<10% tip growth	poor
2	10-25%	poor to below normal growth
3	26-50%	below normal to normal growth
4	51-75%	normal to healthy growth
5	>75%	healthy, normal growth

The 2007 results appear in Table 4. Of the trees studied, 94.2% are in poor to very poor condition, 52.2% of infested hemlock rated very poor, with dieback, 30.4% rated very poor, with no growth, and 11.6% rated poor. Healthy, normal growth was observed in non-infested trees only.

Table 4. Hemlock condition in 2007.

	Dieback	No Growth	Tip Growth				
			<10%	10-25%	26-50%	51-75%	>75%
Tree Rating	-1	0	1	2	3	4	5
Totals (n)	36	21	8	3	1	0	0
%	52.2	30.4	11.6	4.4	1.5	0.00	0.00

DISCUSSION

This is the first of three evaluations. This paper includes preliminary evaluation of method, tree condition, and a hemlock health scale that may be used by the forest manager to assess tree condition. Three application methods and three formulations were used either alone or in combination for therapeutic and protective activity in hemlock.

In application, the QUIK-jet microinjections were simple and fast (2 minutes/tree) but designed for low volume applications. This is an efficient method because the injection site is configured to accept the low volume applied and therefore independent of tree uptake (i.e., transpiration) rate. The dosages applied are designed for insecticidal but not necessarily for long-term activity.

The Tree I.V. applications took more time (10 minutes/tree) to apply because uptake of large volumes depends on tree transpiration rate. Larger dosages were designed to deliver a higher concentration of active ingredient for increased insecticidal activity, especially in larger diameter (>19” DBH) (>47.5 cm DBH) trees. The larger dosage exceeds the volumetric capacity of the injection site; therefore, uptake rate is dependent upon absorption into the axial tracheary elements. However, uptake of large volumes in restrictive tracheid trees can be slow (Cruziat et al. 2002). The advantage of applying a systemic insecticide directly into the sapwood is that activity against the pest is quicker (at least in theory) than for soil-applied insecticide.

The Kioritz soil injector applied MERIT 75WSP simply and relatively quickly (5 minutes/tree). Soil application of imidacloprid has been demonstrated to show insecticidal activity over time (McClure 1987), even over years (Cowles et al. 2006). However, time to activity is generally slow. Imidacloprid moves slowly in soils because of its high octanol-water partition coefficient (0.57) and relatively low water solubility (0.5g/L)[4]. Though less likely to leach in soils; it may be only slowly taken up into trees. Combining tree and soil injection may provide the forest manager with advantages, such as quick and simple applications for immediate and long-term titration of imidacloprid into the sapwood tissues. At the time of writing, no imidacloprid residue data was available for the samples taken at 70 DAT.

The low HWA numbers and greatly reduced twig growth are indicative of a remnant HWA population and late infestation. HWA infestation is likely older than the three years of growth analyzed. Although a low (17.5%) percentage of trees had growth, the HWA reservoir has the potential to re-infest trees. Of the trees evaluated, 87.5% were treated with imidacloprid; of these, 47.9% of hemlocks had little or no growth while 52.1% had dieback

[4]http://extoxnet.orst.edu/pips/imidaclo.htm

symptoms. Webb et al. (2003) reported that trees with little growth but no dieback recovered quickly, producing dense foliage following imidacloprid treatment, compared to trees with dieback, which recovered, but at a slower rate.

Of concern is EHS in the compromised hemlock. Although the numbers were less than 10 EHS/needle, EHS pressures were evident across treatments. The highest EHS pressures were in the three-year-old needles (mean of 2.43 EHS/needle) and the lowest in current-year needles (0.09 EHS/needle). The low EHS density in current growth is a function of tip dieback. EHS in the two-year-old growth was 0.93/needle. Second-year (2005) needles represent the highest percentage of intact biomass; protecting extant needles is therefore critical to tree survival.

Also of concern is soil moisture. Although 2006 precipitation was 103% of normal, 2007 was drier (74.06% of normal). In non-infested hemlock, 2007 twig growth was less (though not statistically significant) than the previous two years (means of 8.8, 12.1, and 11.6 cm, respectively). Hemlock recovery is at least in part dependent on adequate soil moisture, especially in treatments of soil injected imidacloprid.

A possible source of error may be in the determination of the age of wood evaluated in samples with dieback. Although we counted growth rings (dendrochronology), cross referenced with twig color and presence or absence of axillary buds (i.e., origin of current year growth), dieback may have progressed more slowly than assumed in the analysis. Although we standardized counting back two bud scars in these samples, the tips may have been older. Incidental checks of HWA pressure on samples date to five years; therefore, it is possible that the twigs cross-checked to two growth rings may have dated back to four years, rather than to three years.

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Optimizing Fungal Production for Hemlock Woolly Adelgid Suppression

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ABSTRACT

The entomopathogenic fungus *Lecanicillium muscarium* ((Petch) Zares and Gams) is active against aphids and scales, and isolates have been recovered from hemlock woolly adelgid (HWA). *Lecanicillium muscarium* (Mycotal™, Koppert Biological Systems) plus the nutritive base sweet whey, an inexpensive byproduct of cheese, is a promising formulation for suppression of HWA populations. The formulation goals are to reduce the initial number of spores applied to save application costs, increase post-application abundance of spores, and overcome physical constraints such as temperature and humidity. Sweet whey and *L. muscarium* formulations generate spore production on an inert surface without direct contact of an insect host; we call the production of spores in this manner a whey-based fungal "microfactory."

Microfactory production was characterized in different combinations of sweet whey (0%, 5%, 10%, and 15%) and spore concentration (1 x 10^6, 1 x 10^7, and 1 x 10^8 spore/ml) applied to lids of Petri dishes. Dramatic 42-fold and 29-fold increases in spore production occurred with the addition of 10% sweet whey to 1x 10^6 and 1x 10^7 spore/ml, respectively. Increasing whey concentration increased the number of spores that were recovered. Spore production was also obtained on hemlock foliage, with similar trends in influence of spore and whey concentration. The hemlock branches also contained HWA, and their mortality was evaluated. Adelgid mortality was highest in formulations containing sweet whey, but whey had an independent effect on mortality.

Antimicrobials and humectants may have value as a formulation constituent. Antimicrobials may decrease competition between the fungus and microbes present in the sweet whey, spray units, and on foliage for the whey nutrient resource. Humectants may enhance the microclimate and extend wetting of the microfactory on foliage. Whey-based fungal microfactory technology has promise for enhancing the potential of *L. muscarium* for suppression of HWA, and a pilot study is planned to evaluate their effectiveness in hemlock forests.

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ECOLOGICAL AND MANAGEMENT IMPLICATIONS OF HEMLOCK LOGGING IN MASSACHUSETTS

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ABSTRACT

The recent unimpeded infestation of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, across the northeastern U.S. has created a situation in which large-scale hemlock decline and mortality is occurring. HWA has already infested over 40% of the towns in Massachusetts, and as a result, many landowners are choosing to pre-emptively harvest their eastern hemlock (*Tsuga canadensis* (L.) Carr.) stands. Information from timber harvesters, state agencies, and studies of landscape patterns of hemlock decline in southern New England indicate that the recent broad-scale increase in logging associated with HWA is occurring with little ecological assessment and in the absence of scientific background for conservationists, land managers, or policy makers. We are continuing our efforts to compare the impacts of hemlock logging with the impacts of HWA infestation on the magnitude and trajectory of community and ecosystem dynamics.

Ten sites were selected for intensive study throughout central Massachusetts on public and private lands where hemlocks were harvested between one and 12 years ago. Forty-two to 97 percent of hemlock stems and basal area were removed from these sites. Sapling densities of 10,500 to 24,000 stems ha^{-1} dominated the vegetation at older cuts and consisted primarily of black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.), and white pine (*Pinus strobus* L.). Seedling densities averaged 5 m^{-2} across sites, peaked at 20 m^{-2} at six-year-old to nine-year-old sites, and consisted of hemlock, black birch, and red maple. Additional understory species that were common in cuts sites included various raspberry (*Rubus* L.) species, often averaging 30 percent cover, hay-scented fern (*Dennstaedtia punctilobula* (Michx.) Moore), bristly sarsaparilla (*Aralia hispida* Vent.), and sedge species (*Carex* L.). Harvesting resulted in soils that were 3°C to 5°C warmer and tended to be drier in recent vs. older cuts. Net nitrogen mineralization rates in organic soils averaged just over 10 kg nitrogen (N) $ha^{-1}yr^{-1}$ in recent cuts and approximately 5 kg N $ha^{-1}yr^{-1}$ in older cuts. Mineral soil net mineralization rates were lower among all cuts, ranging from 2.1 to 3.5 $ha^{-1}yr^{-1}$. Nitrification rates were low in both soil horizons at all harvest ages. Recent cuts had much higher nitrogen capture ($NH_4 + NO_3$) on resin bags averaging around 1200 μg N g $resin^{-1}$ vs. 300 to 600 μg N g $resin^{-1}$ captured in older cuts.

Findings from this study corroborate past work (Kizlinski et al. 2002), as cutting resulted in high birch and maple establishment and higher N availability in recent cuts. In contrast to prior studies, cutting in Massachusetts sites also led to abundant conifer regeneration of white pine and hemlock, suggesting that seed tree availability, seedbed characteristics, and lack of

HWA at several of the sites cut in Massachusetts contributed to the higher conifer seedling densities found in this study. In addition, nitrification rates and nitrate capture on resin bags were lower in Massachusetts sites than those reported on sites with a long history of HWA that were then subsequently logged.

KEYWORDS

Adelges tsugae, hemlock logging, vegetation dynamics, nitrogen cycling

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Incorporating Hemlock Woolly Adelgid Impacts into the Forest Vegetation Simulator Model

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KEYWORDS

Forest Vegetation Simulator, disturbance modeling, impacts

BACKGROUND

The hemlock woolly adelgid (HWA), *Adelges tsugae*, is a small aphid-like insect that feeds on the xylem ray parenchyma cells near the base of hemlock needles. This insect, a native of Asia and western North America, was first noted in eastern North America in 1951 and has now spread to infest hemlock in at least 17 states. The increased hemlock mortality driven by this insect requires land-managers and silviculturalists to develop stand management plans that account for the potential impact this invasive species. The Forest Vegetation Simulator (FVS) was developed to simulate stand development through time under a variety of land-management actions and has the flexibility to include impacts by fire, pathogens, insects, and other disturbances. Using available data that describe the timing, intensity, and trajectory of infestations, we have developed two simple event monitor addfiles (*.kcp files) for the northeastern and southeastern FVS variants.

EVENT MONITOR STRUCTURE

The event monitor adfiles for both FVS variants are based on three key assumptions: the assumed time of infestation, the assumed intensity of the infestation (population growth rate), and the assumed population behavior (cyclical vs. saturated). First, the timing of infestation is determined by the user for a given stand based on the distance from known populations of the hemlock woolly adelgid. Multiple publications, including Souto et al. (1996), Orwig and Foster (1998), Yorks et al. (1999), and Evans and Gregoire (2007), document rates of HWA spread across the landscape. These primary sources should be used as tools for producing an estimate of the anticipated date of arrival—or more specifically, used to bracket the estimated time or arrival. This date or variation around the anticipated date can then be modified by the user by editing the FVS HWA event monitor in any text editing program. Use of bracketing (i.e., running the simulation assuming infestation in the near future and comparing that with simulations based on infestation occurring in the more distant future) can provide examples of "worst" and "best" case scenarios of infestation timing.

The second assumption used to drive the FVS HWA event monitor is the population growth rate expressed by the adelgid in a newly infested stand. At the present, quantitative time-series data for stands growing in varying conditions and in variable geographic locations are not available. However, data collected over the last 15 years at the Delaware Water Gap National Recreation Area (Evans 2004) provide an estimate of the time period between population detection (i.e., date of infestation) and deterioration of host condition. Within the Delaware Water Gap, trees "generally" began to deteriorate in about five years; however, significant variation occurred among stands in the park and even between trees in a stand. Bracketing this variable by running the stand development simulation with varying rates of impact should be done to assess the impact this variation may have and to provide "best" and "worst" case scenarios, though the default in the FVS HWA event-monitor is five years (assuming the user calculates stand state in five-year intervals). Although these data provide a starting point for our understanding of long-term HWA dynamics, it should be noted that these data were collected from a single region in a northern location and may be appropriate only for the northeastern variant or portions of it. In the southeastern United States, HWA populations have been observed to grow much more rapidly, causing the deterioration and mortality of hemlocks in as little as two years. Unfortunately, little quantitative time-series data is available for the South due to its recent infestation, and so estimates of the time between infestation and tree impact are based on qualitative observations. As additional observations and data become available, they will be incorporated into the southeastern variant by including a lag between an infestation event and impact or by shifting the infestation date (i.e., a longer lag-time can be simulated by simply shifting the infestation to a later date).

The third assumption incorporated into the FVS HWA event monitor is that northern and southern populations of the hemlock woolly adelgid have different population dynamics: specifically, that populations in the northeastern variant may cycle through time with gradually reduced population peaks (McClure 1991). In this system, population densities of the adelgid grow to a point that degrades the condition of the hemlock, and in so doing, reduces its ability of the hemlock to support large populations of the hemlock woolly adelgid. As adelgid populations crash due to poor host condition, trees begin to recover, triggering a resurgence in adelgid populations. Populations may not grow to the density of the first wave, however, due to a reduced carrying capacity by the hemlock (i.e. hemlocks may not recover to pre-infestation conditions). Over time, the decrease in hemlock condition produces the gradually reduced peaks in adelgid abundance. In the FVS HWA event monitor, this is effected by allowing only "moderate" to "low" infestations after a "catastrophic" infestation. Population cycles are randomized within the FVS to simulate some of the stochasticity associated with external factors such as climate, but ratings of "zero" adelgid are not allowed, as it is assumed that once a stand is infested, it will remain infested. These cycles may result in tree death in as little as four years or may continue for 15 years or longer. The net affect in northern stands is that hemlocks may be lost from the system over the course of several decades, with tree mortality happening in waves.

In the southeastern United States, however, populations of adelgids may grow so quickly that they reduce the quality of the host hemlock beyond a point at which recovery is possible. In this system, trees may die quickly without repeated cycles of impact and recovery. The current version of the FVS HWA event monitor can be adjusted to reflect these differences in impact, and as additional data becomes available, rates can be adjusted and bracketed to improve estimates of stand development.

SUMMARY

The ecology of stand development is highly complex and is ultimately determined by local, proximate factors. However, the general impacts of an invasive species can often be estimated using existing knowledge, and the resulting estimates of stand impact can be used to forecast stand conditions using known "rules" in the system. Here we have incorporated some basic observations or rules regarding the population dynamics of the hemlock woolly adelgid, and that the resulting changes in tree condition in northern and southern hemlock stands to produce an even monitor that reflects the potential impacts of the HWA.

The resulting benefit for the management of this invasive adelgid is two-fold. First, this simulation can be useful for land managers seeking to understand the potential development trajectories forested systems that include hemlock make take as they are infested and to play "what if" games to evaluate multiple management scenarios. Second, this event monitor highlights areas needing additional research on hemlock woolly adelgid biology and ecology: for example, more data is needed on the relationship between the timing of infestation and the deterioration and mortality of hemlock trees in the Northeast.

In the southeast where infestations are young but often severe, the importance of gathering both pre- and post-infestation data and quantifying the interaction between adelgid arrival and density with tree condition is even more important. As this data becomes available and is integrated into the event monitor, the quality of the simulation will improve. The FVS HWA event monitor should be viewed as a dynamic tool that will evolve in step with our understanding of the biology and ecology of the hemlock woolly adelgid. Only through the continued integration of more specific, site-appropriate data will land managers be able to reduce the size of brackets used for estimating the relevant adelgid and hemlock parameters and refine the scope of potential stand development trajectories.

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The Role of Volatile Terpenoids in the Relationship of the Hemlock Woolly Adelgid and Its Host-Plants

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ABSTRACT

The terpenoid profiles in the needles of the hemlock species were found to be related to geographic distribution of the species and their presumed ancestry. Although a definitive association of individual terpenoids with resistance to the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, was not identified, isobornyl acetate and α-humulene seem to be linked with resistance. Variability in space and time of several components may be as important in determining resistance as the level of a specific terpenoid. Seasonal variability is higher in the leaf cushion, where HWA feeds, than in the needle where the terpenoids are stored. High levels of isobornyl acetate (or its isomer bornyl acetate) are also a characteristic of the foliage of many other North American conifers. We hypothesize that selective pressure from native defoliators such as the hemlock looper, in the absence of native adelgids and scale insects, may have resulted in the phytochemistry of the *Tsuga* species in eastern North America drifting away from protection against sucking insects. Studies of the nutrients and semiochemicals in the leaf cushion xylem ray parenchyma, where the adelgid feeds, are needed to further define the basis of hemlock resistance to HWA.

KEYWORDS

Adelges tsugae, terpenes, chemical ecology, host-plant resistance

PRESENTATION

This talk presented an overview of our three peer-reviewed publications and offered new insight into the previously published data. Over the past several years, we have endeavored to link the volatile semiochemicals of hemlock (*Tsuga*) species from throughout the world to the biological responses of the hemlock woolly adelgid (HWA). We have examined volatile terpenoids in different species (Lagalante and Montgomery 2003), in cultivars of eastern hemlock (Lagalante et al. 2007), and the temporal and spatial variation of terpenoids in eastern hemlock

(Lagalante et al. 2006). We began by developing an analytical method: headspace solid-phase microextraction/gas chromatography/mass spectrometry (SPME/GC/MS). The technique identified 51 semiochemicals (terpenoids) in hemlock needles. Quantitative analyses of the relative abundance of the terpenoids were used to address the following three questions:

1. How does the relative abundance of terpenoids in each species of hemlock relate to the biogeography and resistance of hemlock species?
2. Are terpenoids different in eastern hemlock selected for horticultural characteristics?
3. How do terpenoids in eastern hemlock vary seasonally in the needles and leaf cushion?

For the first question, the volatile chemical signatures were obtained for seven hemlock species, and principal component analysis (PCA) was used to elucidate similarities and differences in the terpenoids of the species (Lagalante and Montgomery 2003). The PCA extracted two principal components, and the plot of the coefficients corresponded to the geographical location of the species from east to west, except *T. mertensiana*, which is in a separate taxonomic section. (This species behaves as an outlier; hence, all analyses were rerun with it excluded, and the comments below exclude it.) For the first component, which accounted for 75% of the total variance, isobornyl acetate had a much higher loading than any other terpenoid. This terpenoid is most abundant terpenoid in *T. canadensis* and *T. caroliniana* (Figure 1). There are several terpenoids that are lower in the eastern North American species than the other hemlock species: α-humulene, β-caryophyllene, γ-cadinene, δ-cadinene, and γ-muurolene. Of these, α-humulene is most strongly associated with resistance; it is present in 3-4% in the eastern North American species, 6% in *T. sieboldii*, and 11-12% in *T. chinensis*, *T. diversifolia,* and *T. heterophylla* (Figure 1).

A subsequent PCA, using data that were log-transformed to reduce the influence of major components such as isobornyl acetate and α-pinene, grouped *T. caroliniana* closer to the Asian species (see Figure 2 in Lagalante et al. 2007). This arrangement corresponds to the molecular phylogeny of the genus. The separation of *T. canadensis* from the other species appears to be a consequence of high concentrations of piperitone and borneol: both measured approximately 3% in *T. canadensis* and <0.08% in the other species.

The second question was addressed by quantifying volatiles from 13 cultivars of *Tsuga canadensis* using SPME/GC/MS. Multi-dimensional scaling of the dissimilarity of terpenoid profiles for six species and 13 cultivars produces an arrangement (Figure 2) which is similar to that produced by the PCA in Lagalante et al. (2007). Although most of the cultivars of *T. canadensis* have a terpenoid profile that is similar to wild-type *T. canadensis*, four of the cultivars stand apart from the other cultivars: 'Barry's dwarf', 'Callicoon', 'Snowflake', and 'Albospica'. The latter two cultivars belong to the white-tip group; otherwise, the variation in terpenoid chemistry generally did not correspond with the considerable differences in morphological characters observed. 'Albospica' and 'Snowflake' resemble the Asian species in having relatively high levels of α-humulene and germacrene D.

The third question was addressed by measuring the terpenoid content of eastern hemlock in a forest setting over an annual cycle of shoot development. This was related to the temporal feeding pattern of the adelgid that has two generations on hemlock each year. The

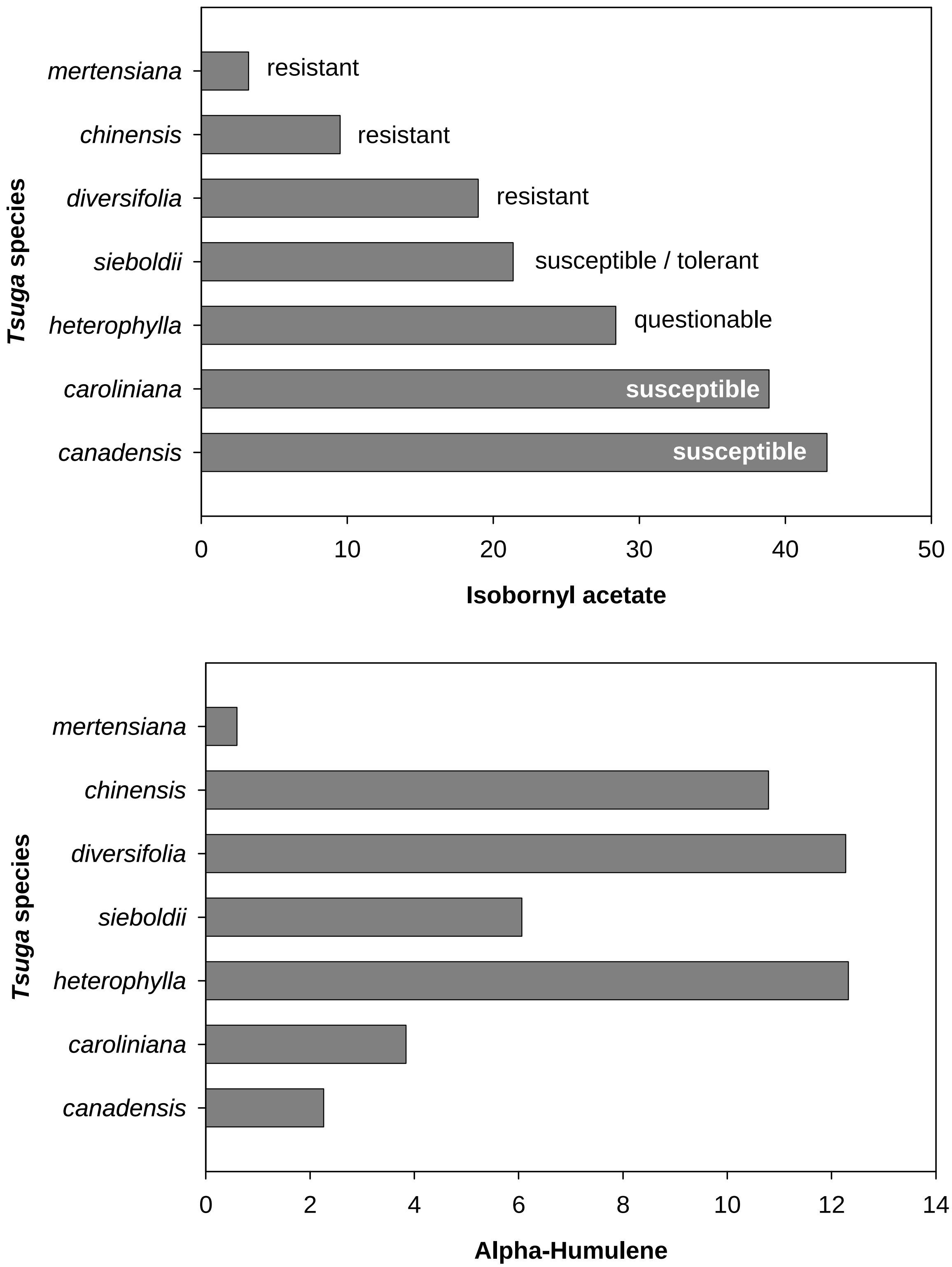

Figure 1. Relative percentage for two of the 51 terpenoids recovered from needles of seven species of hemlock. The resistance of each species to *Adelges tsugae* in the eastern United States is based on Del Tredici and Kitajima (2004) and the authors' unpublished evaluations.

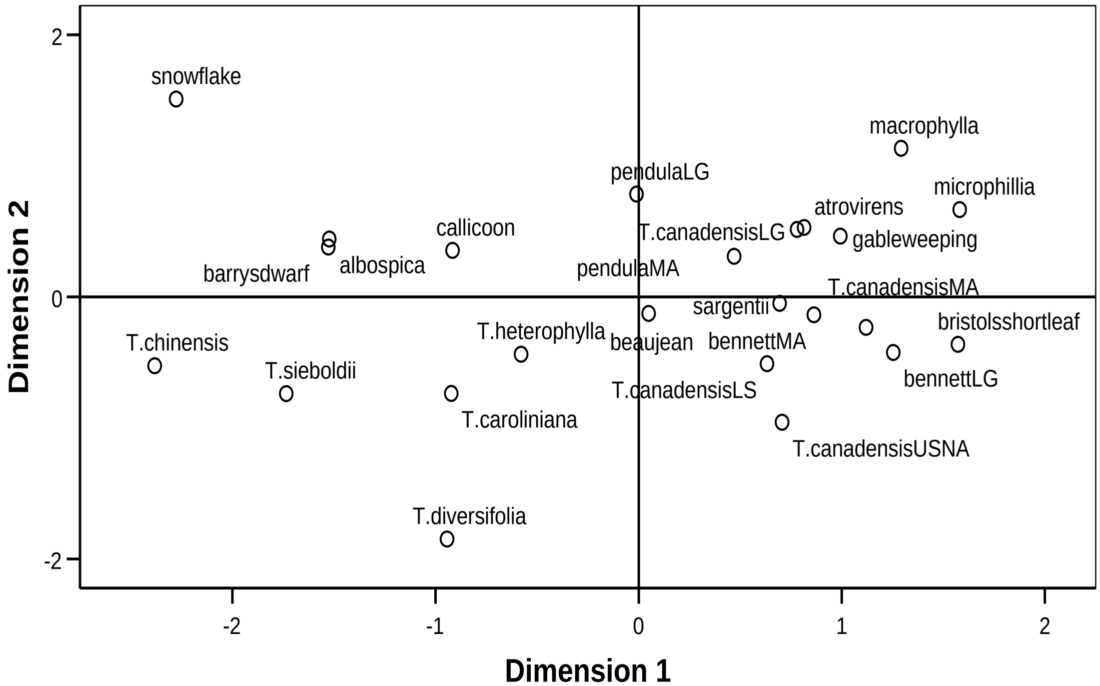

Figure 2. Derived configuration based on multidimensional scaling using Euclidian distances calculated for 23 terpenoids in six hemlock species and 13 named cultivars of *T. canadensis* (lower case names). The non-cultivar *T. canadensis* are from four locations (LG, MA, USNA, and LS) and the cultivars 'Bennett' and 'Pendula' were replicated in the two arboreta (LG and MA).

spring (progrediens) generation is shorter, lasting from May to July, with the adelgid feeding on the previous year's growth. This is also the same part of the hemlock twig where its mother fed. The following generation (sistens) appears in July and settles on the new growth, but it enters diapause and does not begin growing until the fall. The adelgid develops during winter when temperatures are above freezing, becoming an egg-laying adult in late winter or early spring. In additional to seasonal changes, this study took a closer look at the micro-site where the adelgid feeds. Although the terpenoids are stored in the resin canal located in the hemlock needle, the adelgid feeds in xylem ray parenchyma in the leaf cushion. To examine the variation of terpenoid composition in space and time, the needle and leaf cushion of the current and previous years' growth were analyzed over a one-year period. Comparison of these four fractions found significant variation for all of the 23 terpenoids present above 0.1%, including complex interactions between the temporal or spatial factors. Isobornyl acetate and α-humulene increased in the leaf cushion as it matured, whereas α-pinene, myrcene, and germacrene D decreased. Ordination and factor analysis revealed that the terpenoids vary more over time in the leaf cushions than in the needles, with the overall terpenoid composition in the leaf cushion becoming more similar to that in the needle as the tissues mature. The influence of age of the leaf cushion on terpenoid levels was greatest for myrcene and germacrene D, which measured >16% in immature leaf cushions and <4% in mature leaf cushions and immature and mature needles (Figure 3). By entering a non-feeding diapause during the late spring and summer, the sistens HWA would avoid the unstable, variable levels of terpenoids in the immature leaf cushion.

Alpha-humulene, which was associated with resistance in the study comparing species, was relatively more abundant in the leaf cushion than in the needle, especially during the periods when the adelgid is feeding (see Figure 3). Higher levels at the specific feeding site during the actual time period of feeding is what one would expect in a resistant host if the terpenoid were toxic. However, *T. canadensis* is susceptible. An explanation may be that the total terpenoid level in the leaf cushion is only about one-tenth the level in the needle; thus, the absolute level of α-humulene in the leaf cushion may not be toxic. It should also be noted that all of these studies were made using lightly infested or uninfested, healthy hemlocks in order to avoid herbivore induced effects.

In order to determine the extent that terpenoids are a basis of resistance, we, like the adelgid, must probe the leaf cushion. The species comparison, which looked at terpenoids in the needles, needs to redone with the leaf cushion. A key to understanding hemlock resistance to HWA is in-depth knowledge of both the primary and secondary chemistry of the xylem ray parenchyma cells on which the adelgid feeds. These cells store starches and lipophilic compounds, including steryl esters and waxes. Thus, the parenchyma cells may not only provide the adelgid with nutrients but also the precursors of the woolly wax it uses as a protective covering. The parenchyma cells are produced by the cambium, and the radial parenchyma is a living link from the bark to the inner wood. Since disturbance of this link could lead to whole-tree trauma, the *de novo* synthesis of terpenoids in response to feeding stress may be more critical than static reserves of terpenoids. Thus, an examination of the induced changes in the chemistry of the ray parenchyma is also critical to understanding the basis of hemlock resistance to HWA.

The major conclusions from these works can be summarized as follows:

- Terpenoid profiles in hemlock species relate strongly to geographic distribution and phylogeny.
- By feeding in mature leaf cushion, HWA avoids the high variability of terpenoids found in the developing needles.
- High levels of isobornyl acetate in the two eastern North American species of hemlock may reflect their co-evolution in the presence of defoliators and the absence of sucking insects.

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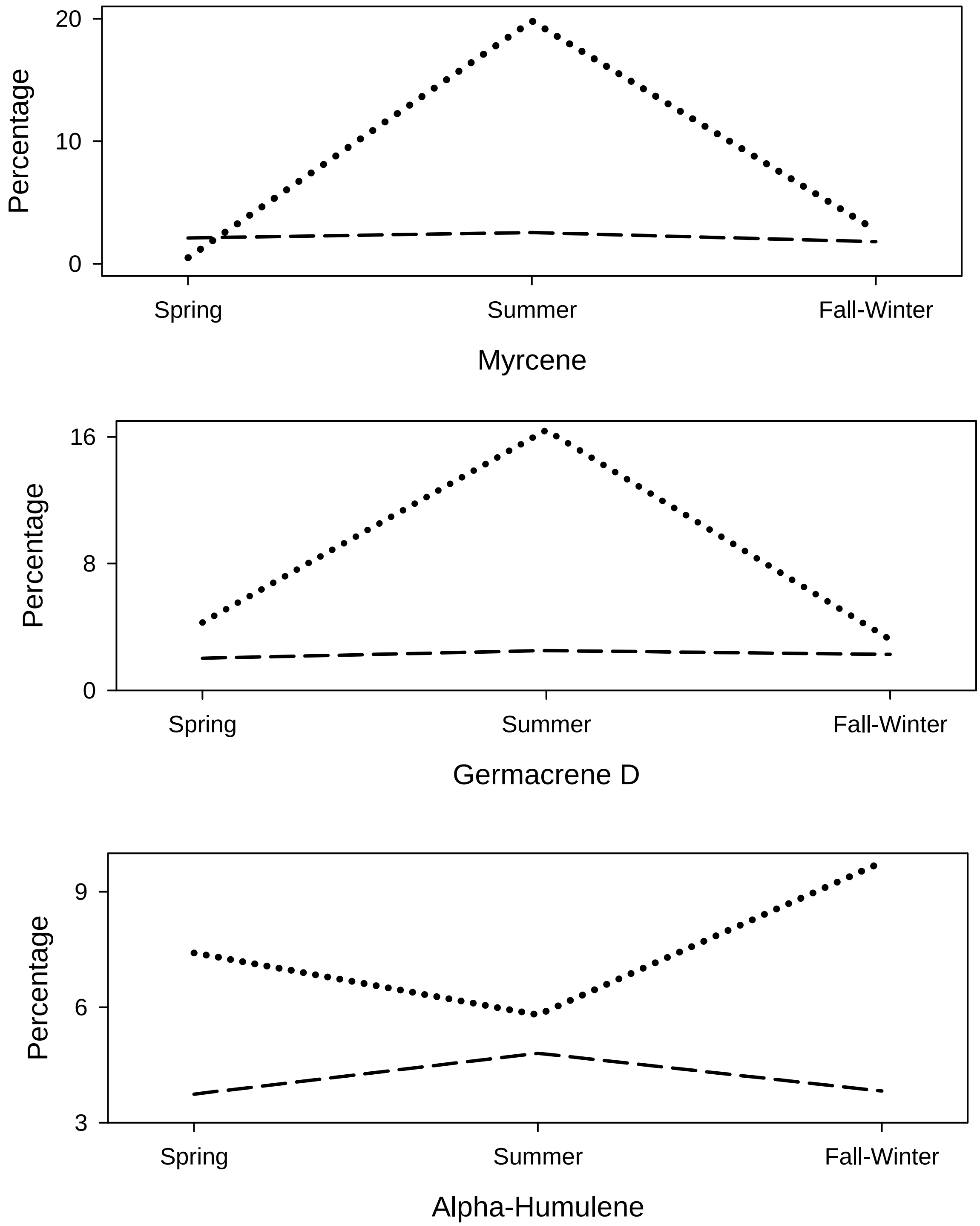

Figure 3. Seasonal comparison of selected terpenoids as the percentage of total terpenoids in needles (dashed line) and leaf cushions (dotted line). Spring is measured on the previous year's growth (penultimate internode) on which HWA both settles and feeds; Summer is the new growth (terminal internode) on which HWA settles and enters diapause, and Fall-Winter is the now-mature terminal internode on which HWA is now feeding.

Production and Evaluation of Eastern Hemlocks Potentially Resistant to the Hemlock Woolly Adelgid

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ABSTRACT

As the hemlock woolly adelgid (HWA) has spread throughout the forests of the Northeast, it has killed countless eastern hemlocks while possibly sparing a small minority of trees with some degree of innate resistance. There are, as yet, no published records of HWA resistance in *T. canadensis*, but on rare occasions, a relatively healthy tree (referred to as 'putatively resistant') is found amidst a devastated stand. As HWA susceptibility is influenced by many factors, including plant nutritional status and prior attack by HWA and other insects, we chose to vegetatively propagate cuttings from putatively resistant forest trees in order to grow and evaluate these plants for HWA resistance under standardized greenhouse conditions. We found that a combination of IBA and NAA rooting hormones gave the best rooting results of cuttings taken in mid-winter. When six-month-old rooted plants were inoculated with adelgids, there was much lower settlement on putatively resistant plants than on control plants (collected from *T. canadensis* growing in northern Massachusetts).

KEYWORDS

hemlock woolly adelgid, *Adelges tsugae*, *Tsuga canadensis*,
vegetative propagation, pest resistance

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, has become a serious pest of the native North American eastern hemlock species *Tsuga canadensis* (L.) Carriere and *T. caroliniana* Engelm.. Several *Tsuga* species resist HWA, including western hemlock, *T. heterophylla* (Raf.) Sarg.; mountain hemlock, *T. mertensia* (Bong.); Chinese hemlock *T. chinensis* (Franch.) E. Pritz.; and Japanese hemlocks, (*T. diversifolia* (Maxim.) Mast. and *T. sieboldii* Carriere) (McClure 1992). Although these resistant species can be quite useful in managed landscapes, they are not suitable for replacing eastern hemlocks in forested settings. If HWA-resistant eastern hemlocks could be identified and propagated, they would be useful for both managed landscapes and stand-level reforestation of HWA-devastated areas. To date, no one has demonstrated HWA resistance in eastern hemlocks. Although a few relatively healthy trees can be found in otherwise devastated areas, it is unclear whether their health is linked to innate resistance or to growing conditions (McClure and Cheah 1992, Pontius et al. 2006, Preisser et al. 2008). HWA resistance should ideally be evaluated under standardized conditions in order to isolate the effect of innate plant resistance. Although it is possible to collect and grow seed from putatively resistant plants, this approach is problematic as the resulting seedlings may have at least one susceptible parent. For documenting resistance levels among selected clones, vegetative propagation quickly yields a population of progeny that are genetically identical to parent plants. Del Tridici (1985) and Jetton et al. (2005) successfully propagated hemlocks from branch cuttings, and Butin et al. (2007) experimentally inoculated hemlocks with adelgids—two techniques that are central to our research. In this paper, we discuss the selection, propagation, maintenance, and evaluation of hemlocks that may be resistant to the HWA.

MATERIALS AND METHODS

Rooting Trial One

As part of a larger research effort (Preisser et al. 2008), we surveyed 142 hemlock stands in Connecticut and Massachusetts in 2005 for potentially HWA-resistant eastern hemlock trees. In forest stands where the mortality rate of mature hemlocks exceeded 95%, we sought mature (>10 m height) trees that appeared healthy and largely free of HWA and the elongate hemlock scale, *Fiorinia externa* (Marlatt). We report data on six putatively resistant hemlocks identified in our 2005 survey: two trees at each of three sites near the towns of East Haddam, Madison, and Old Lyme in Connecticut. We also sampled a tree from the Arnold Arboretum (Jamaica Plain, Massachusetts) that was identified as potentially resistant by Peter Del Tredici.

Table 1. Trees from which cuttings were taken in 2005 and 2006.

Location	Site Code	Coordinates
Jamaica Plain, MA	AA	42.29° N 71.12° W
East Haddam, CT	BB1	41.46° N 72.33° W
East Haddam, CT	BB2	41.46° N 72.33° W
Madison, CT	M1	41.38° N 72.63° W
Madison, CT	M2	41.38° N 72.63° W
Old Lyme, CT	OL1	41.37° N 72.37° W
Old Lyme, CT	OL2	41.37° N 72.37° W

Cuttings were taken in late July 2005 and in late January 2006 using a 4 m pole-pruner to clip healthy terminal growth from branches at varying heights. Cuttings were placed in plastic bags with wet paper towels and kept refrigerated at 4.4°C for a few days until they could be treated. Cuttings were trimmed to a uniform length of new growth: ~8 cm. for July cuttings and ~25 cm for January cuttings. The bottom 1/3 of each cutting was stripped of foliage, wounded on one side, and the end cut at a 45° angle with a grafting knife. Each cutting was then dipped in one of five rooting hormone treatments for five seconds. The rooting treatments consisted of the following combinations of IBA (indole-3-butyric acid) and NAA

(1-napthaleneacetic acid): 1) Hormex© 45 (4.5% IBA) powder; 2) 1:1 aqueous dilution of Dip 'N Grow® (1% IBA) and 0.5% NAA with water; 3) 1% aqueous solution of KIBA (potassium salt of IBA); 4) Hormodin® #3 (0.3% IBA) powder; and 5) no hormones (control). The number of cuttings receiving each treatment varied according to the amount of usable plant material collected from each tree (Table 2).

Table 2. Cuttings taken in 2005 and 2006.

	Summer cuttings			Winter cuttings		
Site Code	**Taken**	**Treated**	**# of cuttings**	**Taken**	**Treated**	**# of cuttings**
AA	NA	NA	NA	2/7/2006	2/8/2006	82
BB1	8/5/2005	8/6/2005	22	1/28/2006	1/30/2006	85
BB2	8/5/2005	8/6/2005	20	1/28/2006	1/30/2006	73
M1	7/25/2005	7/26/2005	106	1/17/2006	1/20/2006	59
M2	7/25/2005	7/26/2005	73	1/17/2006	1/20/2006	28
OL1	7/25/2005	7/26/2005	67	1/17/2006	1/20/2006	25
OL2	7/25/2005	7/26/2005	58	1/17/2006	1/20/2006	67

These cuttings were then stuck in 12 cm-deep square flats (36 cm by 36 cm) filled with rooting media (2:1 horticultural perlite:milled peat moss by volume) and placed in a propagation bed in a greenhouse at the University of Rhode Island's East Farm (Kingston, Rhode Island). The propagation bed measured 1.5 m wide by 30 m long and had bottom heat and plastic-sheeting sides. There was also a misting system controlled by a leaf wetness gauge that averaged ~10 seconds of mist every 10 minutes. The greenhouse maintained a minimum temperature of 12°C while the cuttings received a constant bottom heat of 21°C. After three months we began weekly fertilization with 200ppm of 20-20-20 Peter's soluble fertilizer. The cuttings were kept under mist for another two months before potting.

Five months after taking cuttings, the plants were removed individually from the rooting media and the number of roots counted. Rooted plants were transplanted into 1.95L pots (15.2 cm tall, 13.3 cm square), filled with Sun GroMetro Mix® 510 growing media and fertilized with a low level of Osmocote® fertilizer (1.0mg/m2 of standard release 19-6-12) in preparation for HWA resistance trials (described below).

Rooting Trial Two

Based upon the results of Trial One, we repeated the rooting experiment using branches from the same Connecticut trees. We also used samples of new plant material provided by cooperators in Pennsylvania and New Jersey (Table 3).

One hundred cuttings per tree were treated with Dip 'N Grow®, the best rooting treatment identified in the previous trial. The plants used in this trial were propagated in the same greenhouse and maintained as in Rooting Trial One. Cuttings were trimmed to 25cm and treated as in the previous season with one exception: because some of the cuttings were infested with elongate hemlock scale, all cuttings were dipped into a 1% mixture of horticultural oil insecticide (Sunspray™ Ultra-fine) and allowed to dry before the rooting treatment.

Table 3. Trees from which cuttings were taken in 2007.

Location	Site Code	Coordinates	Taken	Treated
East Haddam, CT	BB1	41.46° N 72.33° W	1/30/2007	2/1/2007
East Haddam, CT	BB2	41.46° N 72.33° W	1/30/2007	2/1/2007
Pelham, MA	C1	42.36°N 72.43° W	2/3/2007	2/5/2007
Pelham, MA	C2	42.36° N 72.43° W	2/3/2007	2/5/2007
Madison, CT	M1	41.38° N 72.63° W	2/1/2007	2/1/2007
Madison, CT	M2	41.38° N 72.63° W	2/1/2007	2/1/2007
Walpack, NJ	NJ1	41.13° N 74.91° W	1/26/2007	1/29/2007
Walpack, NJ	NJ2	41.13° N 74.91° W	1/26/2007	1/29/2007
Walpack, NJ	NJ3	41.13° N 74.91° W	1/26/2007	1/29/2007
Walpack, NJ	NJ4	41.13° N 74.91° W	1/26/2007	1/29/2007
Walpack, NJ	NJ5	41.13° N 74.90° W	1/26/2007	1/29/2007
Old Lyme, CT	OL1	41.37° N 72.37° W	1/30/2007	2/1/2007
Old Lyme, CT	OL2	41.37° N 72.37° W	1/30/2007	2/1/2007
Drums, PA	PA1	41.07° N 75.92° W	2/12/2007	2/19/2007

Rooting treatments for Trial Two included: 1) a 1:1 ratio of Dip 'N Grow® and water; 2) a 1:2 ratio of Dip 'N Grow® and water, and 3) a no-hormone tap water control. The 100 cuttings per tree were divided into eight five-cutting groups for each of the two concentrations of hormone (= 80 total cuttings) and four five-cutting groups for the control (= 20 total cuttings). Each group was placed randomly within a flat. All of the cuttings were inserted into identical flats of the same rooting media and maintained in the same location under the same conditions of the first trial. Six replicates were used in each flat to maintain equal spacing within the flat.

The cuttings were allowed five months to develop roots before being removed from the media for examination and transplantation in late June 2007. Prior to being transplanted into individual pots, each surviving cutting was rinsed and given a rating of 0-3 as a combined measure of root number, root length, and overall size of the root system. A '0' rating indicates that the cutting was still alive at the end of five months but did not produce any roots. A rating of ‘1’ indicates the cutting developed few short roots in fair condition. A ‘2’ rating indicates a denser root system of 5-10 main roots with many root hairs. Finally, a ‘3’ rating indicates a dense root system of greater that 10 highly branched main roots densely covered with root hairs. See Figure 1 for examples of rated seedlings.

Figure 1. Root ratings 0 (left) through 3 (right).

HWA Exposure

In May 2006, the successfully-rooted cuttings from the Rooting Trial One were individually potted in 15.3 cm tall x 14 cm square pots with Sun Gro® Metro Mix 510 growing media. Plants were maintained in the greenhouse under ambient light and temperature and fertilized weekly through a proportioner set to deliver 200 ppm of nitrogen using a liquid 20-20-20 fertilizer. At the same time, we also potted HWA-resistant western hemlocks and HWA-susceptible *T. canadensis*. The western hemlocks used were two-year-old bare rooted seedlings shipped from Western Maine Nurseries in Fryeburg, Maine. The control eastern hemlocks used were randomly-selected seedlings collected from the Cadwell Memorial Forest in Pelham, Massachusetts.

In April 2006, HWA-infested branches were cut from eastern hemlock trees on the University of Rhode Island (URI) Kingston campus and from Saint Patrick Cemetery in Fall River, Massachusetts. The branches were placed in 20L buckets of water to keep the cut ends submerged and held at 4.4°C to delay crawler emergence. In early June, these infested branches were used to inoculate five plants from each plant source (only two western hemlocks) using the protocol described in Butin et al. (2007). Inoculated plants included the six putatively-resistant Connecticut trees, one tree from the Arnold Arboretum, and the western hemlock and eastern hemlock controls. In these inoculations, an 8-cm twig of heavily HWA-infested hemlock foliage was inserted into a florist’s water pic and twist-tied to the stem of each potted hemlock. HWA eggs hatched within a few days and all plants were initially exposed to immense numbers of crawlers. Inoculated plants were held in the greenhouse until mid-August 2006, when they were examined with a hand lens and settled adelgids were counted.

RESULTS

Rooting Trial One

By mid-May 2006 the successfully-rooted cuttings had developed a root mass adequate for transfer into individual pots. January cuttings were more successful than the smaller July cuttings. Most cuttings from the July collection failed to develop roots even after eight months in the mist bed, and the few that did root died several weeks after being potted. The best rooting results were obtained from January cuttings with 1:1 solution of Dip 'N Grow® and Hormex© 45 powder treatments, both of which gave over 60% rooting success for some trees. However, the Dip 'N Grow® treatment yielded the best rooting overall (see Table 4).

Table 4. Results of 2006 winter cuttings (number rooted/number treated).

Site Code	Hormex© 45	1:1 Dip 'N Grow®	1% KIBA	Hormodin® 3	Control
AA	0/18	12/18	2/18	0/10	0/18
BB1	11/17	14/17	6/17	8/17	1/17
BB2	9/15	8/15	5/15	10/16	2/12
M1	2/14	6/15	0/15	0/15	NA
M2	5/7	4/7	5/7	0/7	NA
OL1	3/7	1/6	2/6	0/6	NA
OL2	0/17	3/17	0/17	0/16	NA
Totals	30/95	48/95	20/95	18/87	3/47
% Rooted	31.58%	50.53%	21.05%	20.69%	6.38%

Rooting Trial Two

The second trial had a slightly lower overall rooting success rate than Rooting Trial One. The 1:1 concentration of Dip 'N Grow® in 2007 resulted in 35.9% rooting (Table 5) compared to 50.5% in the previous year. Only one of six Connecticut trees (M2) had rooting success exceeding the 2006 average. Although the difference between the two hormone concentrations was negligible, both hormone treatments significantly increased percent rooting compared to the control group (Figure 2). The same relationship among treatments can be seen in root ratings (Figure 3).

Hemlock Adelgid Exposures

By August 2006 there were substantial differences in numbers of settled adelgids among the plant groups (Figure 4). There were far fewer adelgids on the putatively resistant eastern hemlocks from Connecticut than on the field-collected controls. However, the plant from Arnold Arboretum appeared quite susceptible to HWA. The western hemlock had the fewest settled adelgids—slightly lower, on average, than the Connecticut plants (Figure 4). Due to mortality of some of the plants between the time of inoculation and examination, numbers of replicates among treatments are not equal, and one of the trees could not be evaluated.

Table 5. Results of 2007 winter cuttings. Treatment 0 = control, Treatment 1 = 1:1 ratio of Dip 'N Grow® to water, Treatment 2 = 1:2 ratio of Dip 'N Grow® to water. Root ratings (described in text) range from 0 – no roots through 3 – extensive roots.

	Plant Source													
Percent Rooting														
Treatment	**BB1**	**BB2**	**C1**	**C2**	**M1**	**M2**	**NJ1**	**NJ2**	**NJ3**	**NJ4**	**NJ5**	**OL1**	**OL2**	**PA1**	**TRT. MEAN**
0	0.00	25.00	15.00	10.00	5.00	10.00	0.00	10.00	30.00	20.00	10.00	25.00	20.00	15.00	13.93
1	20.00	40.00	7.50	12.50	47.50	55.00	30.00	37.50	40.00	35.00	40.00	42.50	27.50	67.50	35.89
2	35.00	57.50	25.00	10.00	46.67	45.00	17.14	15.00	20.00	20.00	27.50	57.50	35.56	35.00	31.92
Source Mean	18.33	40.83	15.83	10.83	33.06	36.67	15.71	20.83	30.00	25.00	25.83	41.67	27.69	39.17	27.25
Root Rating															
Treatment	BB1	BB2	C1	C2	M1	M2	NJ1	NJ2	NJ3	NJ4	NJ5	OL1	OL2	PA1	TRT. MEAN
0	0.00	0.83	0.63	0.50	0.25	0.38	0.00	0.25	0.60	1.00	0.38	0.75	0.31	0.63	0.47
1	0.67	1.15	0.50	1.00	1.20	1.57	1.06	1.13	1.93	1.31	1.96	1.30	1.28	1.67	1.27
2	0.88	1.25	0.91	0.56	1.42	1.07	1.14	0.96	1.33	1.13	1.29	1.38	0.97	1.22	1.11
Source Mean	0.52	1.08	0.68	0.69	0.96	1.01	0.73	0.78	1.29	1.15	1.21	1.14	0.85	1.17	0.95

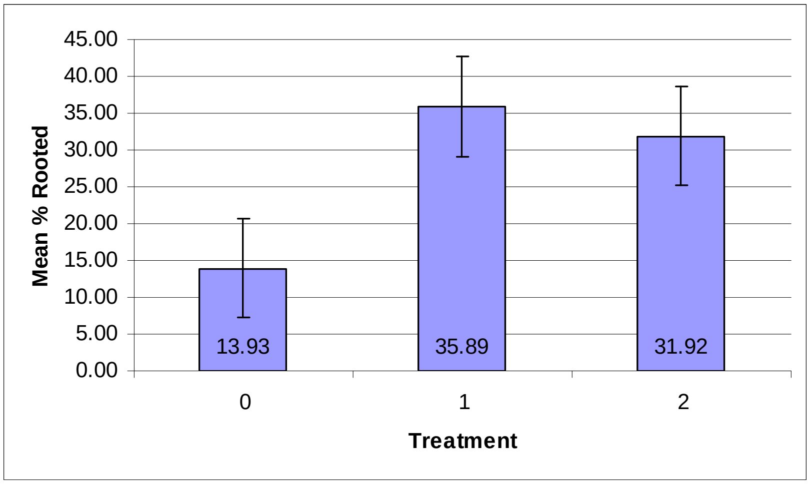

Figure 2. Mean number of cuttings rooted. Treatment 0 = control, Treatment 1 = 1:1 ratio of Dip 'N Grow® to water, Treatment 2 = 1:2 ratio of Dip 'N Grow® to water.

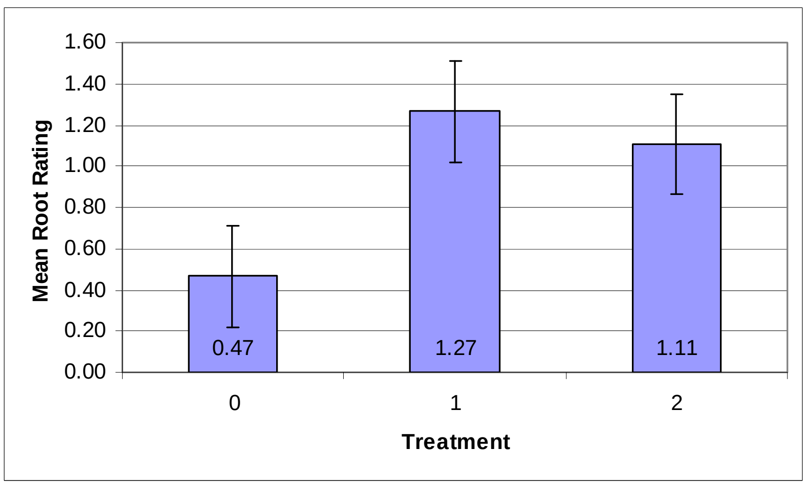

Figure 3. Mean root rating attained for each treatment for rooted cuttings. Treatment 0 = control, Treatment 1 = 1:1 ratio of Dip 'N Grow® to water, Treatment 2 = 1:2 ratio of Dip 'N Grow® to water.

DISCUSSION

Rooting

Our research shows that winter cuttings are an efficient way to propagate *T. canadensis*, with average rooting success ranging from about 30% to 50% when using the most effective rooting hormone. All hormone treatments increased rooting success relative to untreated controls, with Dip 'N Grow® (IBA + NAA) being the most effective. It remained equally effective when diluted 1:1 and 1:2 with water. Del Tredici (1985) also had good success using IBA for rooting *T. canadensis*. Jetton et al. (2005) found no benefit from using NAA alone as a rooting treatment. Thus, we are confident that IBA is important for rooting *T. canadensis*, and it appears to be more effective when combined with NAA, as in the commercial Dip 'N Grow® product.

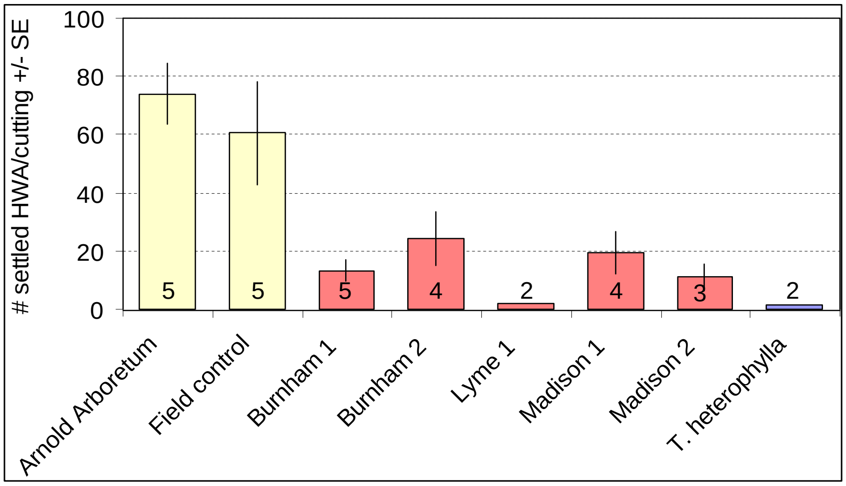

Figure 4. Average number of settled adelgids per plant. Numbers in the columns refer to the number of rooted cuttings used in the evaluation of each tree. Error bars represent standard errors.

We had no success with summer cuttings: the few that rooted quickly died. However, Jetton et al. (2005) had relatively good success (41% rooting of *T. canadensis*) with relatively small (3-6 cm) June cuttings. Although Del Tredici (1985) obtained better rooting success with January cuttings than with those taken in June, he pointed out in the same paper that summer cuttings of *T. canadensis* "might well prove to be the most economical way to produce healthy plants." Summer cuttings develop a good flush of growth in the spring following an overwintering chill period. Winter cuttings generally don't develop a good growth flush for about 18 months. Summer cuttings also do not require the use of bottom heat. Thus, summer cuttings may warrant additional investigation—perhaps with more shade and better protection from summer heat than we provided in 2005.

Several factors other than timing and hormone treatment also contribute to the success of rooting hemlock cuttings. Larger cuttings did considerably better than the small summer cuttings, likely because of a larger reserve of carbohydrates, greater surface area for root development, and more leaf surface area for photosynthesis (Hoad and Leakey 1996). The presence of elongate scale on some of the experimental trees at the time of collection may have also played a role in reducing energy reserves to produce roots. Recent summer droughts in Connecticut may have also affected rooting success (Hartmann et al. 2002). Furthermore, the trees used in this experiment were chosen for their potential resistance to HWA rather than their overall vigor, a factor which could have reduced rooting success.

Nutrient management is crucial for plants used in adelgid experiments. Although a minimal amount of fertilizer is needed to maintain plant growth and vigor in pots of soil-less media, overfertilizing promotes HWA attack (McClure 1991) and can mask innate plant resistance. Pontius et al. (2006) hypothesize that high nitrogen (N) and phosphorus (P) favor HWA attack while calcium (Ca) and potassium (K) inhibit these insects. Thus, we have opted for a moderate rate of fertilizer. After three months in the mist bed, cuttings are fertilized weekly with 200 ppm of 20-20-20 soluble fertilizer until potting. Subsequently, each plant receives 1.5 teaspoons of Osmocote® Plus 15-9-12 controlled-release fertilizer. This dose provides five to six months of feeding at the lowest dosage listed on the label.

Adelgid Exposures

The adelgid transfer method of Butin et al. (2007) was quite effective in establishing HWA on our test plants and all plants were quickly exposed to large numbers of crawlers. By the time we counted these adelgids six weeks after inoculation, there were substantial differences in adelgid populations among the various trees. Our results suggest that the putatively resistant trees from Connecticut may be HWA-resistant and that these resistance levels may approach those of western hemlocks. This interpretation is supported by the fact that rooted *T. canadensis* cuttings from the Arnold Arboretum tree received identical treatment but became heavily infested. While exciting, these are only preliminary results from trials that were intended more to develop the rooting process than to evaluate the resistance of trees. We started with only five plants from each clone, and there was plant mortality during the trial. The relatively high mortality in Connecticut plants (7/25) compared to the field controls and Arnold Arboretum plants (0/10) may reflect a greater stress on these plants, which may have reduced adelgid survival. Other factors, such as past infestation history on those sites, may have also played a role in adelgid settlement in this trial. The real significance of this work is that we have a process to clonally propagate trees in order to evaluate them for HWA resistance.

In continuing tests, we are maintaining rooted plants for an additional year before testing them for adelgid resistance. Following rooting, these plants are potted and maintained outdoors under partial shade and provided a moderate level of fertilizer. They are overwintered in an unheated greenhouse under a protective blanket in preparation for adelgid inoculation in the following spring. In addition to propagating putatively resistant trees, we are similarly propagating and maintaining several *T. canadensis* clones started from plants on the northern edge of the current HWA distribution. These plants, which have never been exposed to HWA, will serve as controls in future tests. This additional time should allow plants to recover from the trauma of rooting and transplanting and minimize any carryover effects from growing conditions prior to our taking cuttings. Our evaluation of clonally-produced plants maintained under identical conditions eliminates the environmentally induced variability in HWA/host plant interactions, allowing a very rapid and accurate assessment of resistance. If useful levels of resistance are found, we may refer back to our repository of potted clonal material for further experimentation and distribution.

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EVALUATING HEMLOCKS FOR HEMLOCK WOOLLY ADELGID RESISTANCE

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In 2006, a procedure was developed to directly challenge hemlock with hemlock woolly adelgid (HWA) ovisacs. *Tsuga canadensis* and *T. chinensis* were both challenged that season. Infestation occurred on *T. canadensis*, confirming that the challenge procedure was effective, yet the *T. chinensis* had no infestation. In 2007, seven hemlock species (*T. canadensis*, *T. chinensis*, *T. diversifolia*, *T. heterophylla*, *T. mertensiana*, *T. sieboldii*, and *T. yunnanensis*) were challenged with HWA and elongate hemlock scale (EHS). The hemlock species can be classified into three groups: highly HWA-susceptible (*T. canadensis* and *T. heterophylla*), moderately HWA-susceptible (*T. diversifolia*, *T. sieboldii* and *T. yunnanensis*), and highly HWA-resistant (*T. chinensis*). All hemlock species were highly susceptible to EHS. *Tsuga mertensiana* suffered heavy losses early in the challenge before its susceptibility to HWA and EHS could be evaluated. Resistance or susceptibility to HWA in hemlocks did not correlate to EHS resistance or susceptibility on either a species or individual level.

EVALUATION OF GROWTH CHARACTERISTICS OF *TSUGA* SPECIES FROM NORTH AMERICA AND ASIA AND SUSCEPTIBLITY TO HEMLOCK WOOLLY ADELGID

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ABSTRACT

In an effort to identify possible replacement species for *Tsuga canadensis* lost to hemlock woolly adelgid in the Northeast, field plots were established in fall of 2003 to assess the growth characteristics of species of *Tsuga* from western North America and Asia and to gauge their susceptibility to hemlock woolly adelgid (HWA), *Adelges tsugae*. In addition, we sought to assess the impact of nitrogen fertilization on adelgid susceptibility. Species evaluated include two species from western North America (*T. heterophyllya* and *T. mertensiana*), two species from Japan (*T. diversifolia* and *T. sieboldii*), and one species from China (*T. chinensis*); *T. canadensis* and *T. caroliniana* were included in the plots for comparison.

Plots were established in an area infested for at least 10 years by hemlock woolly adelgid and were arranged in a split-plot design, with fertilization as the whole plot factor and hemlock species as the subplot factor. To facilitate establishment of adelgids, shoots of *T. canadensis* infested with egg masses of *A. tsugae* were attached to branches of test trees during spring of 2005 and 2006. Fertilizer was applied to half-plots every spring and fall at 1.5 lb N/1000 ft^2, and trees were assessed for growth and adelgid susceptibility at the same times.

Dramatic differences were observed in survivorship and growth of the test species: *T. canadensis*, *T. heterophylla*, and *T. chinensis* had the highest survivorship (>85%) and also achieved the greatest heights (among these three species, *T. canadensis* grew tallest, followed by *T. chinensis*, then *T. heterophylla*). The growth form of *T. chinensis* and *T. heterophylla* were fairly similar to *T. canadensis*, with the exception that branches of *T. chinensis* tends to "weep" more than *T. canadensis*, and *T. heterophylla* often exhibited signs of winter damage in the form of browned needles in spring. The remaining species had markedly lower survivorship, ranging from 45% to 55%. Adelgid susceptibility also varied greatly among test species, ranging from approximately 95% for *T. canadensis* down to 0% for *T. chinensis* (the other species were intermediate to these two extremes). Fertilization had no apparent effect on tree growth or susceptibility to adelgids, perhaps because the rate used was chosen to maintain tree health without unnecessarily stimulating tree growth; higher rates of fertilizer might have had more noticeable effects on tree growth and adelgid susceptibility. In sum, judging from favorable survivorship, growth habit, and adelgid susceptibility, *T. chinensis* appears to be a highly acceptable replacement for *T. canadensis* in areas infested by *A. tsugae*.

Resistance of Hemlock Species and Hybrids to Hemlock Woolly Adelgid

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ABSTRACT

In this study, *T. canadensis*, *T. caroliniana*, and *T. sieboldii* showed high levels of susceptibility and *T. chinensis* exhibited high levels of resistance to hemlock woolly adelgid; the hybrids of the resistant *T. chinensis* and *T. caroliniana* or *T. chinensis* and *T. sieboldii* showed intermediate resistance to HWA. After six years in a field setting, many of the hybrid plants are attractive as replacement species and appear suitable for landscape use

KEYWORDS

Tsuga interspecific hybrids, insect resistance

INTRODUCTION

The eastern North American native hemlock species, *T. canadensis* [L.] Carriere and *T. caroliniana* Engelm., are highly susceptible to injury from the hemlock woolly adelgid (HWA), *Adelges tsugae*, while the Asian species, *T. chinensis* (Franch.) E. Pritz, *T. diversifolia* (Maxim.) Mast., and *T. sieboldii* Carriere are reported to show more resistance (Del Tredici and Kitajima 2004, McClure 1992 and 1995, and Montgomery 1999). The U.S. National Arboretum initiated a hemlock breeding program in the 1990s to assess the potential for controlled hybridization among different hemlock species utilizing both the HWA-susceptible eastern species and the HWA-resistant Asian species. In 2002, we reported on the extent of self-compatibility and hybridization from controlled pollination of several hemlock species (Bentz et al. 2002, Pooler et al. 2002). Attempts to hybridize *T. canadensis* with three Asian species were unsuccessful. However, more than 50 authentic hybrids from crosses between *T. caroliniana* and *T. chinensis* were identified by DNA fingerprinting. Crosses between the Asiatic species also were also successful. The hybrids, seedlings of the parent species, and self-pollinated progeny were planted in a randomized block design at the USDA's South Farm, Beltsville, Maryland, in the fall of 2002. Hybrids between *T. chinensis* and *T. caroliniana* and between *T. chinensis* and *T. sieboldii* are showing good survival and vigorous growth at this site, while survival of *T. caroliniana* has been poor. In 2006, a study was begun to assess the resistance of these hemlock species and their hybrids to HWA and to evaluate their horticultural and landscape qualities.

In April of 2006 and 2007, trees of *T. canadensis*, *T. caroliniana*, *T. chinensis,* and *T. sieboldii* and interspecific hybrids between *T. caroliniana* and *T. chinensis* and between *T. chinensis* and *T. sieboldii* were inoculated with crawlers of the progrediens generation of the HWA. Due to variability in size and health of trees, unequal numbers of trees were used (Table 1). Small infested branches, collected from heavily infested trees located at the U.S. National Arboretum, were divided into bundles of two to four branches. The average number of egg masses per bundle, 554 and 626 in 2006 and 2007, respectively, was determined by counting all egg masses on 10 randomly selected bundles. These bundles were placed mid-branch on an interior branch of each tree and enclosed in mesh fabric bags in one to two days during the first week of April. In mid-June when approximately 50% of the progrediens were adults, establishment and development of HWA was monitored by randomly removing three twigs (2006) and five twigs (2007) from each bagged branch and taken to the laboratory for examination under the microscope. Bags remained on trees until July of each year to contain dispersal to adjacent branches or trees. The entire branch was removed from the test trees in November or December to sample HWA sistens populations on new growth, and HWA sistens on 10 twigs per tree were counted in the laboratory.

Table 1. 2007 census (number per centimeter) of progrediens generation 10-weeks after inoculation with HWA crawlers on plants from controlled pollinations of *Tsuga* species.

Hosts	N	Total Settled	Developing	Aptera Adults
Tsuga chinensis	10	0.18±1.05	0.01±0.71	0.00±0.15
T. chinensis × caroliniana	14	1.96±0.89	0.52±0.60	0.02±0.13
T. chinensis × sieboldii	15	2.16±0.86	0.69±0.58	0.08±0.12
T. sieboldii × chinensis	12	2.91±0.96	1.06±0.65	0.09±0.14
T. caroliniana × chinensis	21	3.75±0.73	1.41±0.49	0.04±0.10
T. sieboldii	10	8.93±1.05	5.81±0.71	0.95±0.15
T. caroliniana	7	9.77±1.26	6.17±0.85	1.11±0.18
T. canadensis	11	9.96±1.00	6.99±0.68	1.83±0.14

Values are means ± SE per cm of previous year's twig growth.

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The June 2006 and June 2007 data (Table 1) show high levels of infestation on *T. canadensis*, *T. sieboldii,* and *T. caroliniana*; intermediate infestation of the hybrids; and poor to no infestation of *T. chinensis* by the progrediens generation. Evaluation of the wintering, sistens generation on the new growth is shown in Table 2.

Table 2. Number sistens settled on new growth in December 2007 following inoculation the previous April.

Hosts	N	Settled/cm
Tsuga chinensis	10	0.03
T. chinensis × caroliniana	14	0.08
T. chinensis × sieboldii	15	0.44
T. sieboldii × chinensis	12	0.62
T. caroliniana × chinensis	21	0.30
T. sieboldii	10	3.87
T. caroliniana	7	2.97
T. canadensis	11	5.53

These results reflect the progrediens generation, except that the number on the hybrids is closer to the number on *T. chinensis*. The sistens were still in the first instar and appeared to be dead on all samples. We are not sure if this is an effect of leaving on the bags during the hot weather, summer drought, or the juvenile character of the trees.

In this study, *T. canadensis*, *T. caroliniana*, and *T. sieboldii* showed high levels of susceptibility and *T. chinensis* exhibited high levels of resistance; the hybrids between the resistant *T. chinensis* and *T. caroliniana* or *T. sieboldii* showed intermediate resistance to HWA. Further testing under natural infestation conditions is planned to evaluate whether the hybrids will demonstrate similar and/or adequate field resistance on a long-term basis. It will also provide opportunity to evaluate the flowering phenology of the hybrids to determine whether the potential for interbreeding with native populations exists. Lastly, after six years establishment in the field planting, it is apparent that many of the hybrid plants are attractive and suitable for horticultural and landscape use. Propagation for HWA testing and landscape evaluation is proceeding.

ACKNOWLEDGMENTS

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Endosymbionts of Hemlock Woolly Adelgid

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ABSTRACT

Bacterial endosymbionts are an essential feature of the biology of insects that feed on nutrient-poor diets, such as plant sap. Where studied, these bacteria perform essential fitness functions, such as providing (through gene overexpression) essential amino acids missing or in low concentrations in the diet, increased thermal tolerance, defense against endoparasitoids, and factors related to host-plant specificity. In Aphididae, the closest relatives of Adelgidae, the primary endosymbiont, *Buchnera aphidicola*, is the main provider of supplementary nutrition, while up to three other secondary endosymbionts have been implicated in other fitness effects. Endosymbionts of Adelgidae and hemlock woolly adelgid (HWA), *Adelges tsugae*, have not been investigated with modern molecular genetic techniques. Our study employed these methods to identify and characterize symbionts of *A. tsugae*.

HWA samples were obtained from Japan, western North America (WNA), eastern North America (ENA), Taiwan, and China. We used polymerase chain reaction, subcloning, and automated sequencing to amplify and sequence bacterial 16S rRNA genes from whole insects. Sequences were submitted in GenBank queries and included in phylogenetic analyses to determine their bacterial origin and relationships to known insect endosymbiont and free-living species. Fluorescent *in situ* hybridizations with bacterial-specific probes were performed on cryosectioned insects to determine the location of bacteria in the insect body.

Up to five different bacteria were recovered, depending on the sample. All populations contained a *Pseudomonas* sp. (gamma-Proteobacteria) symbiont, which resided in the hemolymph. Sequences of this bacterium from Japan on *Tsuga sieboldii* and ENA were identical. All populations also harbored a gamma-proteobacterial symbiont most closely related to *Buchnera aphidicola* and two other insect endosymbionts and was the major occupant of the bacteriome. Samples from Japan on *T. sieboldii* and ENA, only, contained a third gamma-proteobacterial species most closely related to *Serratia symbiotica*, a secondary endosymbiont of aphids. This symbiont resided in central cells of the bacteriome. Limited samples from Japan (from *T. sieboldii*) and ENA and WNA contained one or two additional symbionts belonging to the beta-Proteobacteria subdivision. One symbiont was nested within *Burkholderia* and the other was closest to Janthinobacterium. These were detected only in salivary glands and only in first instars/crawlers. Our data suggest that the HWA infestation in ENA originated from a source population in Japan on *T. sieboldii*, most likely from the Osaka region. The *Serratia* endosymbiont could be investigated in future studies for possible causal effects related to virulence of the infestation in ENA. The apparently facultative association of beta-Proteobacteria with early insect stages might be investigated for effects on early establishment and feeding success.

Climate Matching: Implications for the Biological Control of Hemlock Woolly Adelgid

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ABSTRACT

Classical biological control programs are faced with a daunting challenge: inserting a new species into an existing ecological system. In order for the newly introduced biological control species to survive and reproduce, the recipient ecosystem must provide the required biotic and abiotic requirements. The Adelgid Biological Control simulator (ABCs), a simulation program built to help evaluate the suitability of biological control species for the control of the hemlock woolly adelgid (*Adelges tsugae*), indicates that the Asian predatory beetle *Scymnus sinuanodulus*, imported to control the adelgid, can complete one generation annually, and that the production of a second generation is limited, not by the availability of warmth or prey, but by the need for adults to experience low temperatures to trigger oviposition. These findings support the use of a pre-conditioning regime in which adult beetles are first exposed to low temperatures prior to release to ensure oviposition begins in the year of release.

INTRODUCTION

Invasive species are a global environmental problem. The arrival, establishment, and spread of invasives bring both ecological and economic costs (Pimentel et al. 2000). Managing invasives often requires a multifaceted approach, including mechanical, chemical, and biological treatments. Efforts to control the hemlock woolly adelgid (*Adelges tsugae*) have included each of these with the pre-infestation removal of hemlock, the use of insecticides such as imidacloprid, and the release of non-native natural enemies for classical biological control. While pre-emptive salvage of hemlock eliminates the potential for infestation, it also eliminates this species from forested ecosystems. Insecticides can maintain hemlocks in a forested setting; however, the application of insecticides is costly and risks harming non-target organisms. Classical biological control may therefore play a key role in the maintenance of hemlock in eastern forests.

Although biological control is conceptually appealing, putting it into practice is a major challenge. Successful biological control programs are contingent on identifying natural enemies from the native range of the invasive, ensuring the biological control agent is host-specific, mass rearing the agent, and releasing it into an environment in which it can survive and reproduce. The population dynamics of the agent must then track that of the invasive species, reducing its numbers and/or its impact. In the case of *A. tsugae*, a number of predatory species of beetles have been identified, tested to examine their host range, mass reared, and released. However, establishment has been difficult to confirm, and questions regarding the potential for each species to survive and reproduce have been raised.

The interactions among a biological control agent, an invasive species, and the abiotic environment are complex, and although much is known about the biology of both *A. tsugae* and the biological controls identified and released, identifying key factors in the life history and the abiotic environment that may facilitate or prevent the establishment of predators is a major challenge. Simulation offers a tool to address these challenges by synthesizing information in a way that allows multiple factors to interact simultaneously. In this paper, we describe a new simulation linking the life history of *A. tsugae*, *Scymnus sinuanodulus*, and temperature.

The Adelgid Biological Control simulation (ABCs) was built using Stella™ (ISEE Systems™, Version 9.0). The structure of the simulation was based on the life histories of the adelgid and its predator, with each life history being broken into segments as organized in Figure 1. Imbedded within each segment are the rules that govern the behavior of the individuals within it, including: 1) the number of individuals entering that segment, 2) the percentage of individuals that survive that segment, 3) limiting factors that may arrest the development of individuals within that segment (for example, some life stages of *S. sinuanodulus* require adelgid eggs to complete development), 4) the time required to complete the segment, 5) the reproductive rate for individuals within that segment, if appropriate, and 6) the number of individuals leaving that segment. The code used to build the simulation is available from the author and provides the most up-to-date list of the rules used to define inter-variable interactions. This simulation was used to address three key questions regarding the suitability of *S. sinuanodulus* for release in the eastern United States:

1. Using exemplar southern and northern locations (Asheville, North Carolina, and Amherst, Massachusetts), do seasonal temperatures allow for *S. sinuanodulus* to complete development?
2. Does the timing of seasonal temperatures coincide with the availability of adelgid eggs, a requirement necessary for the completion of the *S. sinuanodulus* life-cycle?
3. Does the timing of temperature and adelgid egg availability allow for the completion of more than one generation of the beetle?

PARAMETER ESTIMATES

The parameters used in the simulation were based on information available in the published literature. Temperature values for Asheville, North Carolina, and Amherst, Massachusetts, were obtained through the online databases available at http://www.ncdc.noaa.gov (see Figure 2). Temperature requirements for the development of *S. sinuanodulus* were obtained from Lu and Montgomery (2001). The timing of the life-history of the adelgid was estimated using Figure 4 in McClure (1987).

SIMULATION RESULTS

The simulation suggests that seasonal temperatures in Asheville, North Carolina, allow the beetle *S. sinuanodulus* to complete development; however, the beetle produces only one generation per year. Closer examination of the simulation results indicate that the lack of a

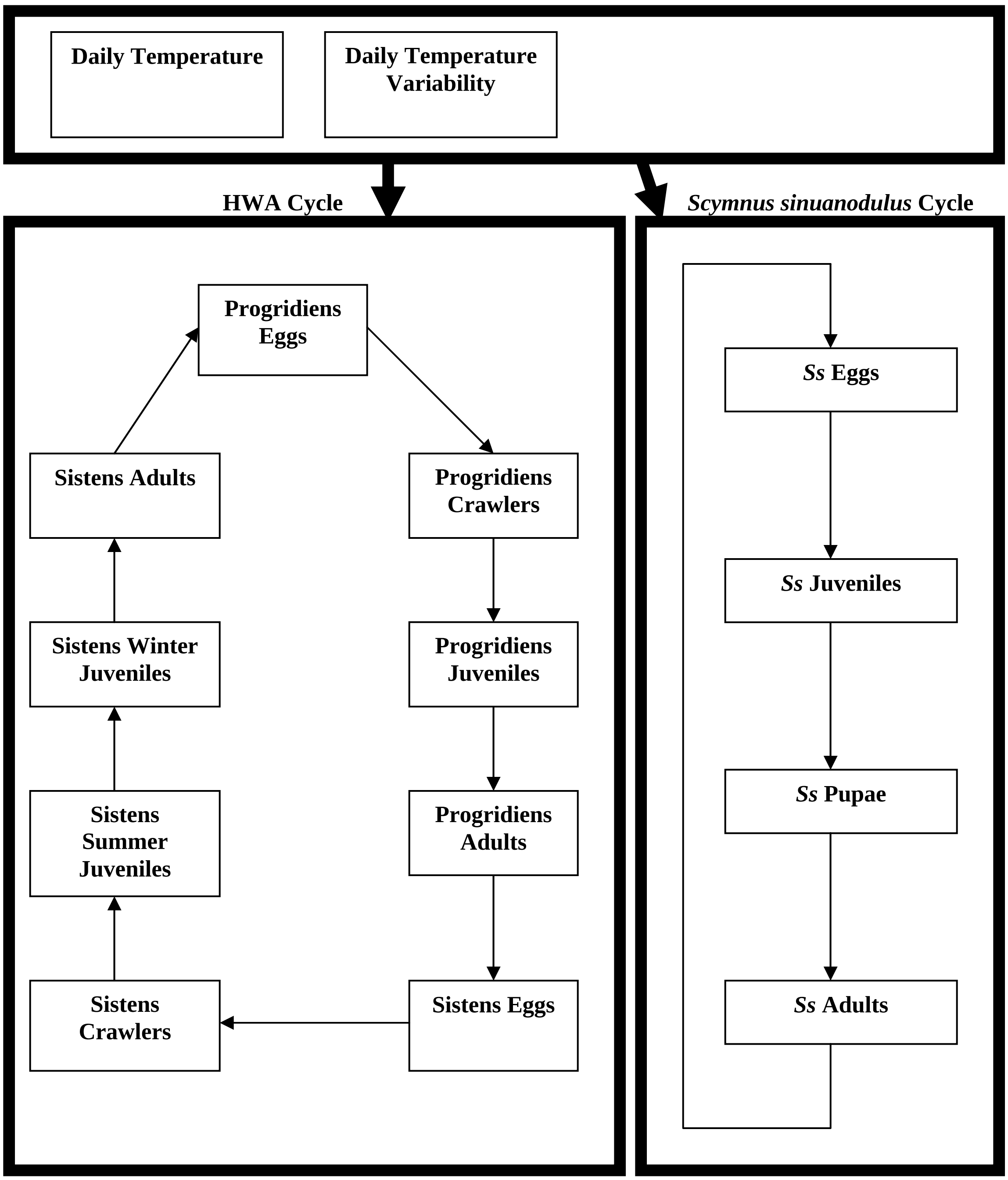

Figure 1. Adelgid Biological Control simulation structure.

second generation is the result of inadequate low, rather than high temperatures. As Lu and Montgomery (2001) discuss, *S. sinuanodulus* requires exposure to temperatures below 5°C to initiate oviposition. As Figure 3 shows, beetles were "released" in the simulation in the spring of the second year but did not produce eggs until the following spring. The lag between release and oviposition highlights the importance of pre-treating the beetles prior to releasing them (Montgomery, pers. comm.).

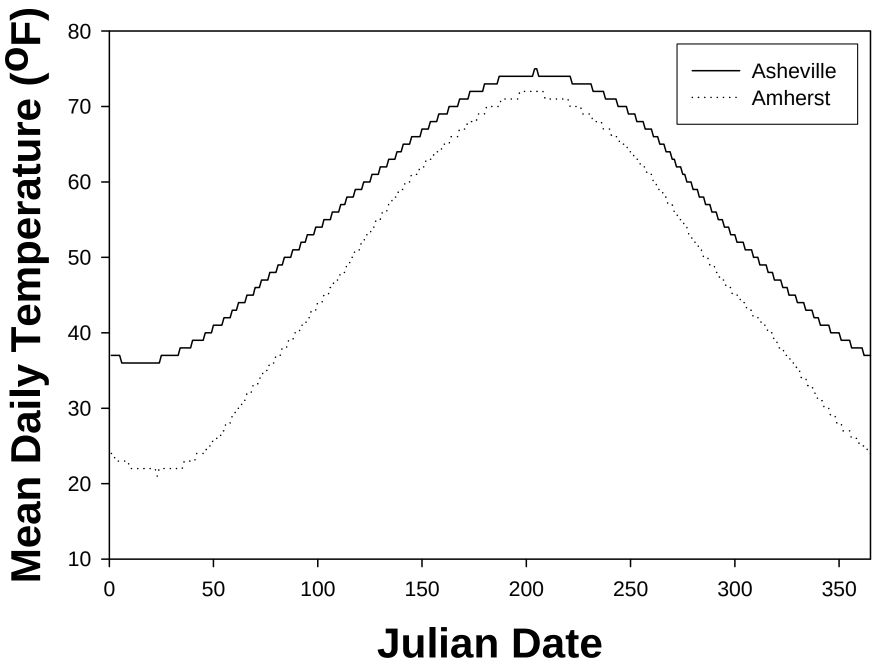

Figure 2. Temperature profiles for Ashevill, NC, and Amherst, MA.

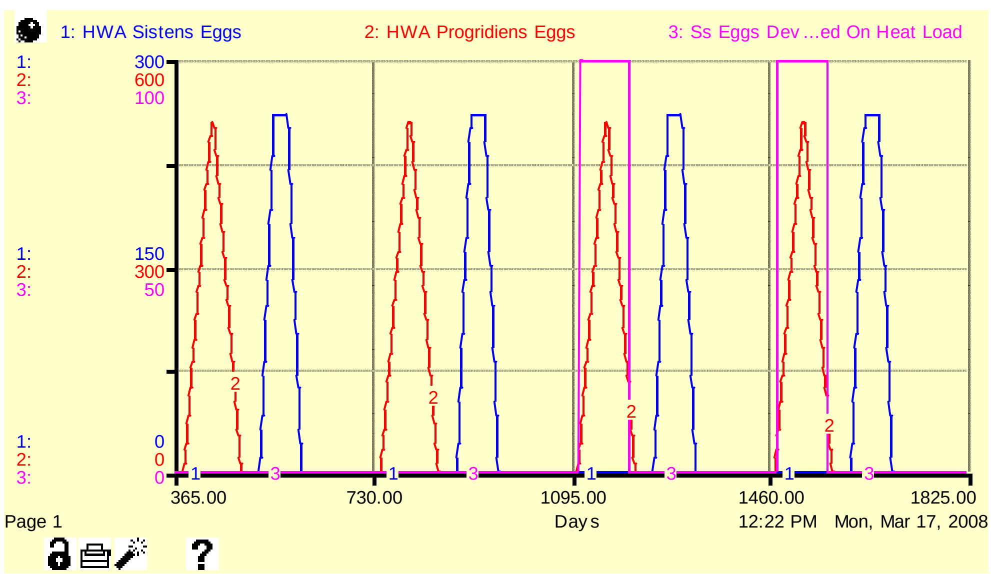

Figure 3. Simulation results for Asheville, NC.

Using temperatures from the northern site (Amherst, Massachusetts), the simulation yields similar results (Figure 4). Again, the production of a second generation is limited, not by available heat, but by the need for adult beetles to be exposed to low temperatures before they will initiate oviposition. However, the simulation does show that the overall lower temperatures in the north results in an lengthening of the time it takes for the beetle to complete each of its life stages.

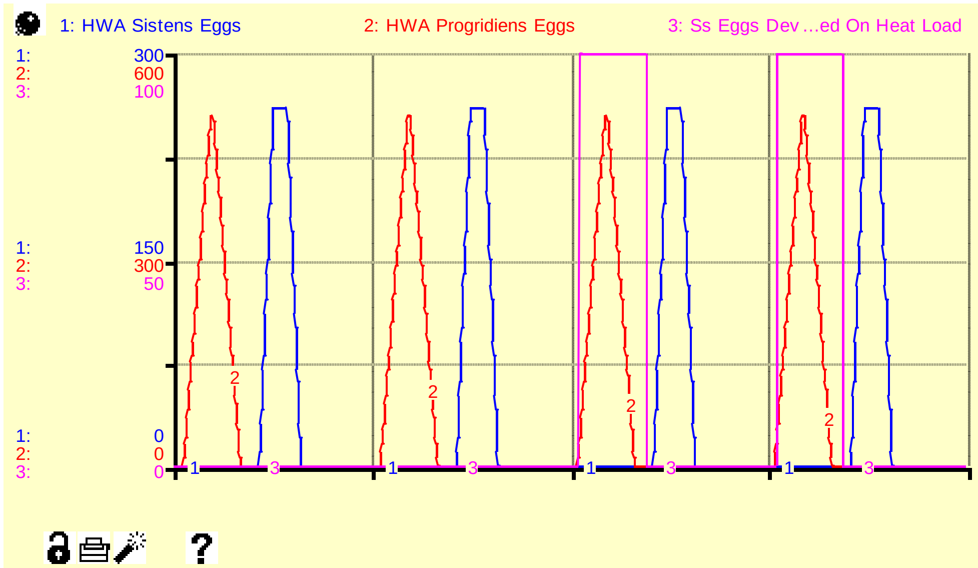

Figure 4. Simulation results for Amherst, MA.

SUMMARY

These two simulations suggest three patterns in the population dynamics of the predatory beetle *Scymnus sinuanodulus*. First, the simulations from both the northern and southern locations indicate that seasonal warmth is not likely to be a limiting factor in the completion of the beetle's life-cycle. To the contrary, the need for adults to experience low temperatures to initiate oviposition is a limiting factor, as the spring release of the beetles in the simulation shows that eggs were not produced until the following spring. In management applications, beetles released in the spring should be first exposed to low temperatures if eggs are to be laid in the current year. Failure to pre-condition *S. sinuanodulus* adults before field release may result in a delay in the appearance of the F_1 generation. Compounding the problem, not all adults may survive the year between release and oviposition, which may reduce establishment success.

The second pattern shown by both simulations is that *S. sinuanodulus* will likely lay its eggs in synchrony with *A. tsugae* sistens. As the beetle eggs hatch and the juveniles begin to feed, they may be able to impact the eggs laid by both sistens and progridiens. The potential for this impact further highlights the importance of pre-conditioning beetles before release to encourage oviposition in the first spring.

Finally, these simulations specifically (and the Adelgid Biological Control simulation environment more generally) highlight the need for additional information on the biology and ecology of the hemlock woolly adelgid and its natural enemies. For example, these simulations assume that the beetles survive for one year and so have one reproductive event. Yet, laboratory studies (Montgomery, pers. comm.) have shown that the beetles can survive two years and perhaps even longer, potentially producing more than one generation of eggs. This generational overlap may result in increased population growth rates and cause the population dynamics of the beetle to behave like that of a bivoltine species. The simulation also highlights the need for a better understanding of the environmental mechanisms that drive the development of the hemlock woolly adelgid. The timing of the availability of eggs is likely to change from one year to the next as winter and spring conditions vary. These changes in timing may have an impact on the beetles that depend on the adelgid populations; yet, little is known about how the timing of adelgid development varies or how this may impact natural enemies. As research on these species continues and as additional information on the biology and ecology of the hemlock woolly adelgid and its natural enemies becomes available, it can be integrated into the simulation to improve our understanding of this complex system, and to explore new management options.

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Molecular Ecology of Hemlock Woolly Adelgid, Its Hosts, and Its Natural Enemies

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ABSTRACT

Molecular analyses show that the hemlock woolly adelgid (HWA) has distinct native lineages in western North America, Japan, China, and Taiwan, while in eastern North America, HWA is not native and was introduced from Japan some time before 1951 (Havill et al. 2006 and 2007). The typical holocyclic lifecycle in the family Adelgidae involves primary hosts in the genus *Picea* and secondary hosts in other conifer genera (Havill and Foottit 2007). It is therefore feasible that in the regions where it is native, HWA alternates between *Tsuga* and *Picea* species. A database of DNA barcodes (approximately 650 base pairs from the COI gene) being compiled at the Canadian Centre for DNA Barcodes (Guelph, Ontario) includes HWA as well as adelgid samples collected from various Asian *Picea* species. We found an exact match between Chinese HWA and adelgids from galls on *Picea likiangensis* (Franch.) Pritzel and between Japanese HWA and adelgids from galls on *Picea torano* (K. Koch) Koehne. This confirms that HWA host alternates in at least part of its ranges in China and Japan. We are currently working to further understand HWA lifecycles and the relationships between HWA and its hosts with population genetic methods using microsatellites.

The beetle *Laricobius nigrinus* Fender (Derodontidae) is native to western North America and has been released throughout the range of introduced HWA in eastern North America. There are three additional *Laricobius* species reported to be present in North America: *L. laticollis* Fall is native to western North America, *L. rubidus* LeConte is native to eastern North America and is routinely collected from HWA-infested eastern hemlock, and *L. erichsonii* Rosenhauer was introduced from Europe in the 1950s and 1960s as a biological control of balsam woolly adelgid, but to our knowledge it has not been collected in North America in recent decades. A molecular phylogeny using mitochondrial and nuclear DNA sequence data shows that *L. nigrinus* and *L. rubidus* are closely related. A restriction fragment length polymorphism (RFLP) assay was developed based on nucleotide differences in the COI gene that are fixed for each species. This assay provides rapid and inexpensive identification of *Laricobius* larvae to aid post-release monitoring of establishment and spread of *L. nigrinus*. It should be noted, however, that since mitochondria are strictly maternally inherited, that

this assay only identifies the maternal genealogy of the beetles and is not useful for detecting potential hybrids. While there is currently no evidence that *L. nigrinus* and *L. rubidus* can successfully produce hybrid offspring, this possibility should be addressed given the close relationship of the two species.

KEYWORDS

DNA barcoding, lifecycle, *Laricobius*, *Tsuga*, *Picea*

ACKNOWLEDGEMENTS

Many people have graciously contributed to these projects both intellectually and by providing samples. We wish to especially thank Gisella Caccone, Steve Cook, Gina Davis, David Mausel, Shigehiko Shiyake, Carol von Dohlen, Guoye Yu, and Gabriella Zilahi-Balogh for their contributions.

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Mortality Factors and Population Rate of Increase for the Hemlock Woolly Adelgid

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ABSTRACT

Using survival and fecundity data, we construct a lifetable for the hemlock woolly adelgid in order to understand year-to-year fluctuations in adelgid population density. The major questions we address are: 1) what are the sources and relative amounts of mortality occurring at each stage of the adelgid lifecycle? and 2) how can we use this knowledge to understand and manipulate adelgid population growth?

Six hemlock stands in Connecticut and Massachusetts were repeatedly visited from April 2004 through June 2007. On each occasion, four 0.3m branches were randomly sampled from each of four hemlock trees and brought back to the lab for examination. Each year, we measured sistens density and overwintering mortality in April; sistens fecundity in June; and progrediens density and fecundity, amount of new growth, and density of newly settled sistens on new growth in August. With this information we were able to calculate the rate of population increase for the adelgid from one year to the next at all sites. Mortality was assessed throughout four parts of the adelgid lifecycle: sistens overwintering mortality (December-March), progrediens mortality (March-June), sistens summer and fall mortality (June-November), and aestivation mortality within the June-November period (August-October).

At all sites, adelgid populations decreased during 2004, increased slightly in 2005, and increased about 20-fold in 2006. Among the parts of the lifecycle considered, the highest and least variable amount of mortality occurred during the progrediens generation (March-June). Possible causes of mortality during this stage include predation, production of sexupare, and failure of nymphs to settle. Mortality during the other stages was much more variable among years. Overwintering mortality was significantly associated with winter temperature. In 2004 and 2005, total mortality occurring during the sistens generation was greater than that occurring in the progrediens generation. In 2006, total sistens mortality was about equal to progrediens mortality.

In the northern part of the range, where adelgid populations sometimes decline due to overwintering mortality and in other years increase very little, a small amount additional mortality from a biological control agent may be able to prevent widespread mortality of hemlock trees. Using adelgid survival and fecundity data, we may be able to predict how much mortality from these agents is necessary each year. Lifetable data may help us identify other important sources of adelgid mortality that we do not now understand. They may also help us determine where viable adelgid populations would be able to establish both now and in the future as global climate change continues.

Diet Development for Hemlock Woolly Adelgids and Their Predators

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ABSTRACT

We report here on progress in developing artificial diet-based rearing systems for hemlock woolly adelgids and one of their key predators, *Sasajiscymnus tsugae*. This report is divided into two parts: 1) summary of efforts to develop an artificial diet and diet-based rearing system for hemlock woolly adelgid (HWA), *Adelges tsugae* (Homoptera: Adelgidae), and 2) summary of efforts to develop a diet for adults and larvae of *S. tsugae* (Coleoptera: Coccinellidae). Efforts to develop a successful diet for HWA are complicated by complex and poorly understood feeding mechanisms and feeding targets. We offer evidence here for HWA feeding on particulate foods that are inherently more nutrient-dense than simple liquid solutions. Diet development for *S. tsugae* is somewhat complicated by the specific nature of adaptation to completing its life cycle on HWA. However, despite these complexities, we have succeeded in developing a diet that supports robust feeding by adults and larvae and which sustains high adult survival for over two months, allowing them to oviposit viable eggs with high levels of hatch (greater than 90%).

KEYWORDS

hemlock woolly adelgid, *Sasajiscymnus tsugae*, artificial diet, hemlock, biological control

HWA DIETS

Our earlier efforts to provide diets for HWA resulted in difficulties in eliciting robust feeding. We realized that much of the failure resulted from a lack of understanding of the fundamentals of feeding by these complex insects. Therefore, our recent research has been focused on developing a clear understanding of HWA feeding.

The first objective was to establish whether HWA uses particulate or liquid foods. We began our research on diet development under the assumption that HWA ingests liquids from parenchyma tissues of hemlock. However, failure to sustain HWA individuals on several liquid diets with complete nutrient profiles suggested that these insects may ingest foods that are not liquids. Observations of these facts confirmed our suspicions: 1) HWA and their close relatives balsam woolly adelgids (BWA) do not produce a copious excretory fluid as do aphids, whiteflies, leafhoppers, and other Homoptera that produce honeydew or a similar clear fluid that exceeds the insects biomass on a daily basis; 2) HWA guts (Figure 1a and b) are not suited to concentration of dilute foods such as xylem sap, phloem sap, or other "plant juices"; and 3) extensive investigation of stylet placement in plant tissues by Young et al. (1995) and Cohen et al. (unpublished data) has led to unclear conclusions about the nature of feeding. Young et al. (1995) found that HWA stylets frequently terminated in parenchyma storage cells in xylem rays of hemlock needles (Figure 2a and b). These authors contrasted HWA feeding with that of other adelgids and aphids that utilize as nutrients solutes in phloem. However, it is not clear from the study by Young et al. (1995) exactly which materials from xylem ray-parenchyma storage cells are ingested by HWA. We feel that, in light of our observations stated as 1) and 2) above, it is unlikely that a simple solution of parenchyma cell sap would be adequately concentrated to meet the growth needs of HWA. Unlike aphids (which feed generally on phloem sap) and leafhoppers (which feed generally on xylem fluid), HWA does not have the anatomical complexity of gut structures to accommodate the filtration and concentration processes evident in the two former groups of insects. Furthermore, the lack of copious excretory liquid suggests that HWA ingests a fairly, if not highly, concentrated plant material.

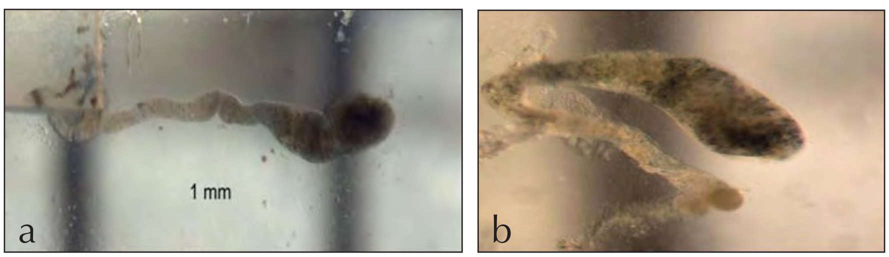

Figure 1. Guts from adelgids: a) from a balsam woolly adelgid adult and b) from a hemlock woolly adelgid adult. Note the simple structure of these guts—characteristic of insects that feed on concentrated nutrients rather than dilute nutrients such as plant saps.

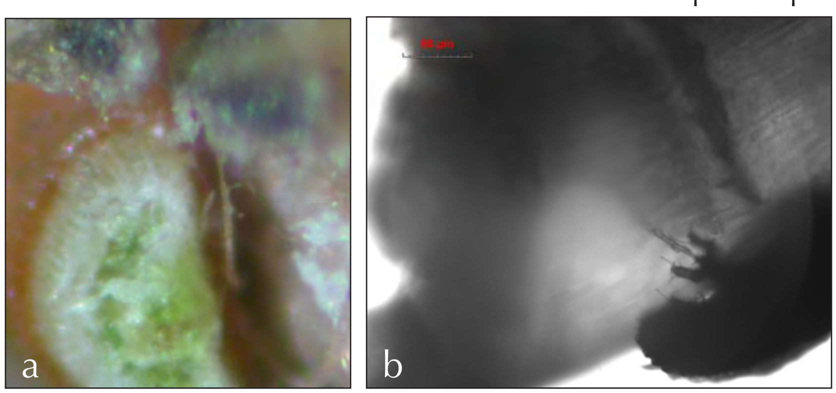

Figure 2. a) Adelgid stylet sheath in gap between hemlock needle and stem. This sheath (the T-shaped structure near the middle of the photograph) was started from the upper part of this image and moving toward the bottom. It can be seen to branch to the left and right. The green color of the needle is evident to the left of the branching stylet. b) Adelgid feeding on hemlock, forming a stylet sheath that is inserted into the needle base.

Thus far, we have had the best success in our diet trials when we provided HWA crawlers with a gelled diet composed of particulate materials such as Spirulina, *Arthrospira platensis*, and *Arthrospira maxima* (Phylum: Cyanobacteria). The Spirulina-based diets contain about 1-2% sucrose, 1-2% Gelcarin 812, 0.5% Vanderzant vitamins, 1-2% yeast extract, 0.1% Wesson salts, 0.2% sodium propionate, and 0.2% potassium sorbate. We have been testing these diets under the assumption that, for diets to be successful, they must meet the following four criteria (described in Cohen 2004): 1) palatability, 2) nutritional value, 3) bioavailability, and 4) stability. Simply put, the diet must elicit robust feeding and contain all growth and development requirements of the target species. The diet components must be absorbed by the insect's digestive system in appropriate amounts, and the diet must not spoil due to microbial growth, spontaneous oxidation, or enzymatic degradation.

SASAJISCYMNUS TSUGAE DIETS

Several introduced predator species from Japan, China and the Pacific Northwest are currently being reared for deployment in the HWA-infested states as part of the national biological control program supported by the USDA Forest Service. The mass rearing of *Sasajiscymnus tsugae* and other imported predators are completely dependent on extensive collections of healthy, heavily infested hemlock foliage. The availability of infested foliage is seasonal and can be unpredictable in quality. This dependence on natural prey collections and the necessity for labor-intensive techniques has placed numerical limitations on the large-scale production of specialist predators for adelgid control. Development of a predator artificial diet or dietary supplement for insectaries would greatly improve biological control efforts.

After testing numerous formulations (60 diets), a diet based on chicken eggs has shown excellent nutritive potential, and this, combined with a diet presentation system composed of uninfested hemlock foliage, has elicited good feeding response by adults and enabled survival of adults on diet alone for 8-10 weeks in 2006 while retaining reproductive capability. Research in 2006 has focused on new techniques for the production of a diet gel texture acceptable to *S. tsugae* that reduces congealment on mouthparts and allows the insect to traverse easily over the diet surface to feed. New cold-gelling techniques with sodium alginate and various calcium treatments were tested. The gelling of the base diet when dipped in a calcium solution forms a thin gel membrane around the liquid diet core, and beetles appeared to accept this formulation and were able to feed on it. This method of preparation has a preparatory advantage over the previously tested hot gel method using Gelcarin 812 as it does not require a heat source.

In 2006, *S. tsugae* readily fed on calcium-treated cold-gelled egg diet presented without hemlock foliage in Petri-dishes, but problems of desiccation resulted in diet globules drying up overnight. In early 2007, *S. tsugae* adult response to the egg-based artificial diet with calcium treatments on uninfested hemlock foliage was surprisingly much reduced. This was eventually found to be due to the inferior quality of the more recent freeze-dried formulations, which resulted in uneven dispersion of the diet particles in the hydrocolloid matrix. Equipment malfunction of the freeze drier resulted in an inferior base diet, which proved less attractive to *S. tsugae*. This behavioral observation also attested to the very specialized feeding preferences of this predator and the challenges of consistent diet formulation. However, cold-gelling techniques with sodium alginate appeared to provide an adequate protective skin around the diet that could be contacted without adhesion and penetrated by both larval and adult mouthparts.

In 2007, superior diets were produced for testing with *S. tsugae* adults. Extended and improved behavioral assays involved monitoring the adult feeding response and other behaviors in large 14 cm Petri-dish arenas (n = 5-10 adults/arena), which allowed for more natural, uncrowded behavior on 10-12cm hemlock tips. Adults are able to fly off the hemlock tips and move around the arena freely to exhibit real choice behavior. Ambient temperatures were also maintained between 23-25°C as adult *S. tsugae* show more activity and feeding behavior at these temperatures. Results showed that a non-gelled formulation of egg diet and honey preferentially attracted prolonged adult feeding when presented together with adelgid first instars just breaking dormancy and developing second instar nymphs (Figure 3).

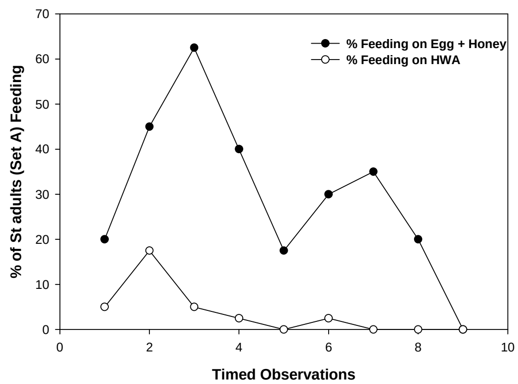

Figure 3. Preferential feeding by adult *S. tsugae* on egg diet and honey, given a choice of diet and HWA first and second instar nymphs.

Adults also were attracted over time (2 hours) to feed on dry egg diet powder mixed with honey and presented on filter paper in the absence of hemlock (Figure 4). This method was then tested as a supplement for maintaining long-term survival of adults on infested hemlock in holding cages kept at 14°C.

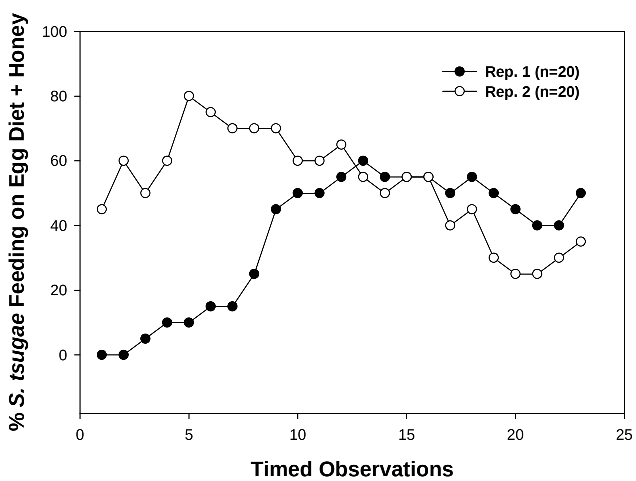

Figure 4. Mature adult *S. tsugae* response to egg diet and honey presented on filter paper.

Results showed that, at 14°C, survival of mature adults in holding cages (n = 35-50/cage) with egg and honey supplement was superior to that in holding cages which had a supplement of Bug Pro© Gardens Alive beneficial insect supplement and honey (Figure 5). Survival on the latter supplement was unpredictable, while survival in cages with egg and honey supplement was consistently high even when holding hemlock material was desiccated and highly deteriorated with no live adelgids available for feeding. Mean percentage adult survival with egg and honey supplement was 85.1%, while mean percentage survival with Bug Pro© and honey supplement was 43.9%.

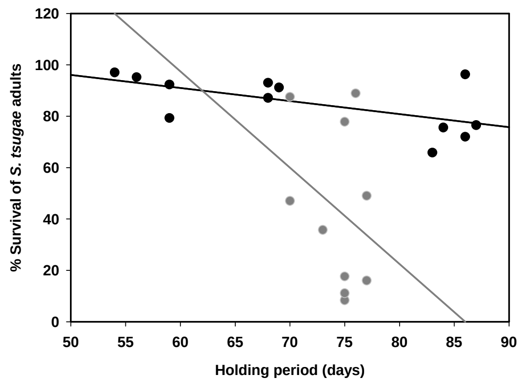

Figure 5. Comparative *S. tsugae* colony cage survival on adelgid-infested hemlock and supplements: 1) Cohen egg diet and honey (•) and 2) Bug Pro© Gardens Alive and honey (•).

Adults also significantly preferred gelled egg diet formulations over a pollen derivative diet in choice tests. Cold gelling of either diets produced an acceptable texture for both adult and larval feeding, but larval survival depended on having fresh moist diet available every two days as larvae are not attracted to dried diet and easily starve or desiccate to death. Mature larvae (third and forth instars) could survive for weeks with 50% survival at 17 days on diet alone but were unable to molt. This will be investigated further. Adults did not prefer gelled egg diets over second instar adelgid nymphs but fed equally on either HWA or diet by the second day. With larger HWA stages (third and fourth instars and adults), adults would start feeding preferentially on HWA, but by the third and fourth days, would switch to preferential feeding on the artificial diets—and on the egg diet in particular (Figure 6)—probably influenced by the depletion of adelgids with increased consumption over time. However, this behavior shows that *S. tsugae* will accept artificial diets for feeding when HWA is less available or of inferior quality.

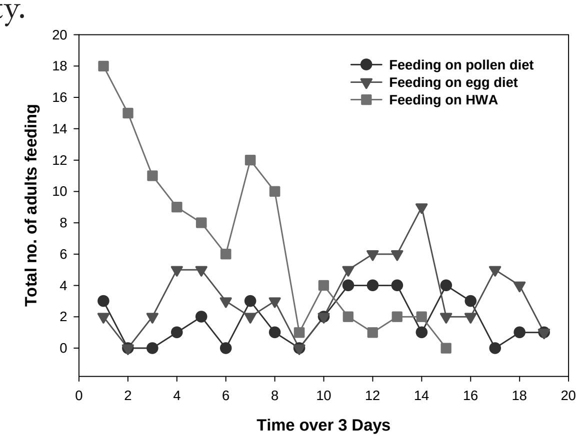

Figure 6. Change in adult *S. tsugae* feeding preferences with time when presented simultaneously with artificial diets and HWA.

Adults were then tested for their ability to survive on egg diet alone. Over 80% survival has been recorded at 8 weeks when adults have been maintained on gelled egg diet alone presented on uninfested hemlock, but frequent diet changes are necessary (every 2-3 days). Oviposition was also elicited when no live adelgids were present, but current observations indicate that an adelgid stimulant component is necessary, and this is being further investigated. Eggs that were laid on diet twigs were viable, with over 90% hatch.

RHEOLOGICAL PROPERTIES OF DIETS

One of our most novel discoveries in this work and related research is the importance of texture as a suitability factor of the artificial diets for both HWA and their predators. We have used a combination of cold-gelling agents to give a firm texture to the outside of the *S. tsugae* diet and gelling agents (or hydrocolloids) to give suitable viscoelastic properties to the diets. Besides viscoelasticity (G' and G" in Figure 7), there are several other features of texture that are proving to be important to the palatability of diets by insects, including *S. tsugae*. Figure 7 shows measurements made with rheological equipment testing four insect diets. Properties such as gumminess, hardness, fracture force, chewiness, and adhesiveness all seem to impact the "decision" of beetles to feed on or reject a given diet. We are currently trying to understand the range of values measured objectively with rheological techniques to determine the complex of textural characteristics that result in a highly palatable diet versus a diet that the insects reject.

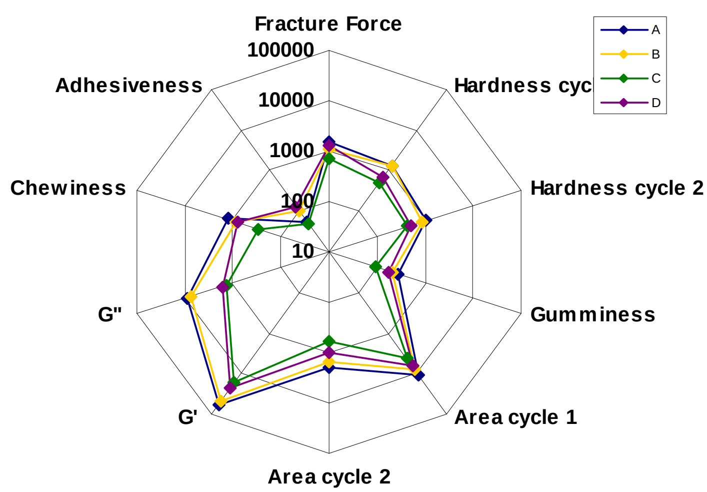

Figure 7. Diagram of rheological properties of four insect diets made with different gelling agents. The diagram illustrates the various texture qualities of diets, such as hardness, adhesiveness, chewiness, gumminess, and viscoelastic proportions (G′ and G″). We have found these qualities to be greatly significant in determining the suitability of insect diets for rearing *S. tsugae*.

DISCUSSION

HWA

The exact nature of HWA feeding is not clear, but our work provides evidence that, like many Hemiptera, these insects may use extra-oral digestion where solid components of cells are pre-digested by the insects' salivary enzymes, then ingested a concentrated nutrient "broth." Our preliminary work with particulate diets based on algae and yeast provide some promise that particulates will be more useful than the strictly dissolved solute-based liquid diets that we have tested in the past. We have also made considerable progress in using flow-through systems to overcome the problem of diets becoming stale over the long feeding periods displayed by HWA. Our current efforts are intensely centered on more complete understanding of the nature of feeding by HWA and the exact feeding targets so that we can use these as physical and biochemical models for artificial diet.

Sasajiscymnus tsugae

We have succeeded in showing that *S. tsugae* adults will readily accept and feed on a gelled egg diet in the absence of HWA when the diet is presented on hemlock, with long-term survival on diet alone if diets are replaced regularly and frequently—every 2-3 days—to remove mold and maintain palatability and freshness. Shelf life of prepared diets is also very important as diets need to be formulated at a minimum of every 3 weeks and refrigerated. The texture provided by the gelling agents is very important as it allows adults and larvae to insert mouthparts to feed without interference and obstruction. Mature larvae will now readily feed for extended periods of time on fresh gelled diets. Premature desiccation of diets remains a challenge, especially for larvae, and will be the focus of future research.

ACKNOWLEDGMENTS

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Changes in Decomposition Dynamics in Hemlock Forests Impacted by Hemlock Woolly Adelgid: Restoration and Conservation of Hemlock Ecosystem Function

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ABSTRACT

This study examines changes in decomposition, forest floor mass, and eastern hemlock (*Tsuga canadensis* (L.) Carr.) foliar chemistry associated with infestation by hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. HWA populations were associated with higher levels of foliar and fine-branch percentage of nitrogen (%N), but the relative cause and effects of this relationship remain unknown. Fine branch %N, which includes the tissue that these insects feed upon, was more strongly associated with HWA population density than foliar %N, suggesting that studies of HWA population dynamics could benefit from more detailed study of eastern hemlock fine branch chemistry. Decomposition rates of a common substrate were increased by HWA infestation and associated stand decline. However, hemlock replacement by hardwood species results in an even greater effect on decay rates. We summarize published studies of litter decay from sites representative of eastern hemlocks range and recommend experimental plantings of slow-decomposition rate species (such as pine) for restoration and retention of ecosystem function associated with eastern hemlock.

KEYWORDS

foliar chemistry, decomposition, hemlock restoration, hemlock ecosystem function

INTRODUCTION

Eastern hemlock (*Tsuga canadensis* (L.) Carr.) is a regionally important tree and ecosystem type in the eastern United States and Canada. High shade-tolerance of eastern hemlock and relatively slow growth rates often leads to a multi-layered canopy that supports unique avian and invertebrate communities in New England and likely elsewhere in its native range (Tingely et al. 2002; Ellison et al. 2005b). Eastern hemlock often dominates the canopy in historically uncleared sites with relatively low soil quality (Foster 1992) and greatly adds to landscape-level vegetation heterogeneity within southern New England. The ecosystem characteristics associated with eastern hemlock are greatly altered by infestation by hemlock woolly adelgid

(HWA), *Adelges tsugae* Annand, and associated stand decline (Cobb and Orwig 2002; Orwig et al. 2002). Eastern hemlock stands are notable for low overstory diversity and deep, slowly decomposing forest floors (Rogers 1978, McClaugherty et al. 1985). These slow rates of litter decay are a consequence of relatively cool microclimate conditions in the forest floor, high litter pH levels, and low foliar litter quality, which also slow rates of nitrogen (N) turnover (Daubenmire 1930; McClaugherty et al. 1985). HWA infestation radically alters these characteristics by causing high tree mortality levels; increasing understory light levels, temperature, and N turnover rate; and lowering forest floor moisture (Orwig and Foster 1998; Jenkins et al. 1999; Kizlinski et al. 2002; Yorks et al. 2003; Cobb et al. 2006; Orwig et al. 2008). Extensive hemlock mortality has occurred in southern New England, the mid Atlantic, and southern Appalachian mountains (cf. Orwig et al. 2002; Eschtruth et al. 2006; Preisser et al. 2008). At present, these functional characteristics of eastern hemlock are at risk of loss from the New England landscape, and little effort has been allocated to retention or restoration of these processes within the HWA infestation zone. No effective control measures for HWA at landscape to regional scales have emerged, necessitating a greater understanding of hemlock ecosystem function, habitat uses, and dependencies of species associated with hemlock ecosystems.

Comprehensive studies of ecosystem response to HWA infestation are a key step in developing management centered on the function of impacted forests. We lack comprehensive understanding of insect herbivory impacts on ecosystem processes, which are likely to vary from one plant-herbivore complex to another (Schowalter et al. 1986; Hunter 2001; Chapman et al. 2003). In a recent study, Cobb et al. (2006) found no differences in mass loss rates between green foliage from HWA infested vs. uninfested stands. However, over the course of the eighteen-month study, litter nitrogen percentage (%N) was significantly higher in infested foliage despite a lack of clear initial differences in foliar chemistry between the two litter types. Overall, these changes are likely to increase N concentrations in the forest floor and increase N release at later infestation stages or following salvage harvest (Jenkins et al. 1999; Kizlinski et al. 2002; Orwig et al. 2008). However, direct effects of HWA herbivory are far less dramatic than those resulting from changes in dominant species composition. Replacement of relatively low-quality hemlock foliage with high N, low lignin black birch foliage (*Betula lenta* L.) has been shown to greatly increase rates of litter decay, increase nitrification rates, and reduce forest floor mass (Kizlinski et al. 2002; Cobb, unpublished data). These changes result from greatly altered canopy structure, microclimate, and dominant species phenology. Studies of HWA impacts and the ecology of eastern hemlock forests provide knowledge necessary to develop restoration techniques needed in regions impacted by HWA. However, a rigorous definition and testing of such techniques has not been undertaken, and therefore, the effectiveness of restoration efforts is highly uncertain.

We designed a series of field studies to evaluate the impacts of HWA infestation, stand decline, and hemlock loss on foliar litter decomposition. This paper will summarize these studies and synthesize additional knowledge from ecological studies of eastern hemlock forests not impacted by HWA. The objectives of our field studies were to: 1) determine the impacts of HWA herbivory on litter mass loss and chemical quality, 2) quantify the effects of microclimate change on conditions for litter decay, and 3) evaluate the effects of hemlock loss and replacement with black birch on litter decomposition. These objectives span a continuum of ecosystem change following infestation by HWA to include uninfested stands, intact hemlock stands with high HWA populations, declining stands, and stands where hemlock has been functionally eliminated. We discuss these studies in the context of eastern hemlock functional processes and offer general recommendations for restoration.

METHODS

Building on previous studies (Orwig and Foster 1998; Kizlinski et al. 2002; Cobb et al. 2006), we combined *in situ* measures of HWA population dynamics, foliar chemistry, green foliage decomposition, common substrate mass loss, and forest floor mass dynamics in 11 hemlock stands ranging from those with no HWA infestation to stands with near-complete mortality in order to determine the magnitude of decomposition and forest floor change in response to infestation. Soil temperature, moisture, and understory light levels were monitored for three years in the majority of these stands. Overstory mortality was monitored in all stands on an annual basis.

Effects of HWA on Twig and Foliar Chemistry

We monitored HWA population levels and foliar chemistry in three stands by sampling fifteen trees in 2002. Sample dates were early May before full extension of current-year foliage and emergence of summer sistens (spring), late July (summer), and early September (fall). Three to five hemlock branches, including current year, one-year-old, and two-year-old foliage, were removed from study trees and returned to the laboratory on ice. Five branch tips were selected randomly, and total HWA population number, number of needles, and length of shoots were recorded across shoot sections of different ages. Infestation density was calculated on a relative scale (#HWA $\#needles^{-1}$ $shoot^{-1}$). Not all trees were sampled at each time point: six of 15 HWA-infested trees were added during the July sampling, and sampling of all age classes was not possible at each collection time. Sampled branches were then removed, dried at 45°C for 24 hours, and subsequently analyzed for total N and carbon (C) by dry-combustion autoanalysis. Foliage and stem material were processed and analyzed separately. Additionally, in the spring and summer sampling, all HWA egg sacs were removed and analyzed for total C and N with the same technique.

Decomposition Environmental Conditions Associated with HWA

Effects of overstory mortality and changes in dominant canopy species on decomposition were quantified with a cellulose paper common substrate study. Approximately 7.5 grams of cellulose paper (Whatman #1) were enclosed in 1 mm^2 mesh bags. Five bags were pinned to the forest floor surface and five bags were buried at the forest floor-mineral soil interface in three locations within nine study stands. Common substrate bags were deployed in May 2001 and collected monthly until a site reached 100% mass loss (after the fourth collection). Collected bags were cleaned of debris, returned to the laboratory on ice, and immediately dried at 105°C for 24 hours and weighed. Forest floor depth was measured at four locations at each common substrate incubation site.

Long-Term Impacts of HWA and Hemlock Replacement

Long-term effects of HWA infestation and subsequent species change were evaluated with a four-year study of litter decay. Multiple native tree species commonly observed in the understory of declining stands (Orwig and Foster 1998), and previously infested and uninfested green hemlock foliage (c.f. Cobb et al. 2006) were incubated adjacently. Senescent litter of hemlock, black birch, an equal mix of hemlock and black birch, red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.), and fine branches of hemlock were decomposed adjacently at the Prospect Hill hemlock stand within the Harvard Forest. Green hemlock litter (Cobb et al. 2006) and senescent litter were deployed in fall 2001 and collected after 6, 12, 24, and 48 months. Decay constants (*k*) were calculated annually according to Olsen (1963):

Eq. 1: $\ln (M_{t2} / M_{t1}) = -kt$

where M_{t1} is the mass remaining at an initial time t_1 and M_{t2} is the mass remaining at the time step of the next collection (t_2).

Published values for litter decay rates, initial chemistry, and chemical dynamics during decay of common eastern species were gathered from a literature search. Data were compiled into a database and standardized to values reported in this study via Equation 1. Additional metadata such as overstory dominance in study stands, duration of study, and total study length were recorded.

Data were analyzed with one-way analysis of variance (ANOVA) using HWA as the independent variable and time as a continuous covariate. Assumptions of normal distribution of error and homogeneity of variance were evaluated by examining plots of residual values vs. predicted values and formal tests for goodness of fit of data to a normal distribution. Total HWA were square-root transformed prior to analysis. Analyses with continuous predictor variables were analyzed by regression analysis, with HWA population levels as the independent variable. Reported error ranges are one standard deviation. All analysis was conducted with the JMP statistical analysis software package (Version 7).

RESULTS

Effects of HWA on Twig and Foliar Chemistry

HWA population levels were highly variable among foliar age classes and across the growing seasons during the 2001 (Figure 1). During the spring sampling, HWA populations were almost entirely composed of overwintering sistens distributed exclusively on one- and two-year-old foliage. By the summer sampling, HWA populations (progrediens and new sistens) had increased substantially, and individual HWA were found at high population densities on new foliage. This sampling corresponded with a precipitous decrease in HWA numbers on two-year-old foliage. By the fall sampling, total HWA densities had decreased and were similar to initial population densities in the spring except that densities in one- and two-year-old foliage had decreased.

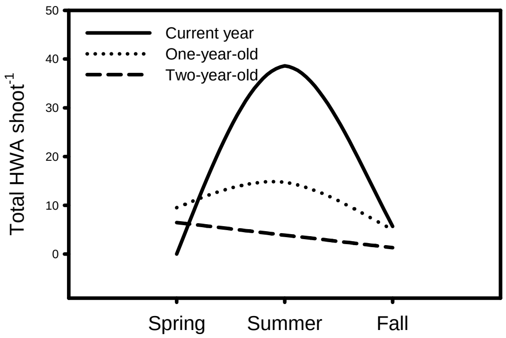

Figure 1. HWA densities on nine eastern hemlock trees over the course of the 2002 growing season. Total number of HWA were standardized for the number of needles on the shoot sampled.

Fine branch %N was 41% lower compared to foliar %N (0.95 ±0.54 vs. 1.59 ±0.51) and significantly decreased over the growing season and across foliar age class (Table 1). Percentage N in stem material from infested trees was higher compared to uninfested trees (1.11% ±0.52 vs. 0.62% ±0.42; p<0.001) and was affected by HWA density. Foliar %N levels were higher in infested trees compared to uninfested trees (reported in Stadler et al. 2005) and were affected by HWA population density and foliar age but not sample date. Standardized beta values from these separate analyses suggest the association of stem %N and HWA density was greater than that of foliar %N (41% greater effect). Foliar %N levels were correlated with stem %N. Percentage C and N in egg masses were significantly different between the spring (3.49%N ±0.26; 62.7%C ±1.26) and summer (3.09%N ±0.24; 63.9%C ±1.10) samples. Percent N in egg masses decreased between spring and summer while the %C increased (11% p=0.0003 and 2% p=0.0165, respectively). Recovery of egg masses during the fall sampling was inconsistent due to egg sac breakdown between measurements.

Table 1. Regression analysis of foliar and fine branch eastern hemlock chemistry on total HWA number. Standardized regression coefficients (β), a measure of effect size for each independent variable, are presented for statistically significant variables. Age represents the age of sampled material (i.e., current, one-, or two-year-old foliage). Adjusted r^2 values and degrees of freedom for error for the full model are included.

Parameter	Variable	P>|T|	B	R^2_{ADJ}	N
Stem %N	Total HWA	<0.001	0.427	0.515	64
	Sample date	0.0019	-0.32		
	Substrate age	0.0248	-0.306		
Foliar %N	Total HWA	0.023	0.293	0.537	74
	Sample date	0.19	NS		
	Substrate age	<0.001	-0.71		
Foliar %N	Stem %N	<0.001	0.605	0.357	64

Decomposition Environmental Conditions Associated with HWA

Our cellulose common substrate decayed rapidly below ground but slowly on the forest floor surface (Figure 2-A). HWA infestation and associated stand decline did not result in significant differences in common substrate mass loss at the forest floor surface compared to uninfested stands. Hemlock stands replaced with birch had the greatest overall mass loss of cellulose at the forest floor surface, but this trend was not significant ($p>0.05$). However, mass loss of cellulose paper buried at 5cm was significantly more rapid in black birch dominated and HWA-infested stands compared to uninfested stands (Figure 2-B). Forest floor depth and mass was significantly lower in stands with black birch canopies compared to those with hemlock overstories, regardless of infestation (Figure 3; depth not shown). Forest floor depth was linearly related to percentage of cellulose paper mass remaining at 5cm depth (depth = 0.10 * %mass remaining – 2.99; $r^2=0.627$; $p<0.01$).

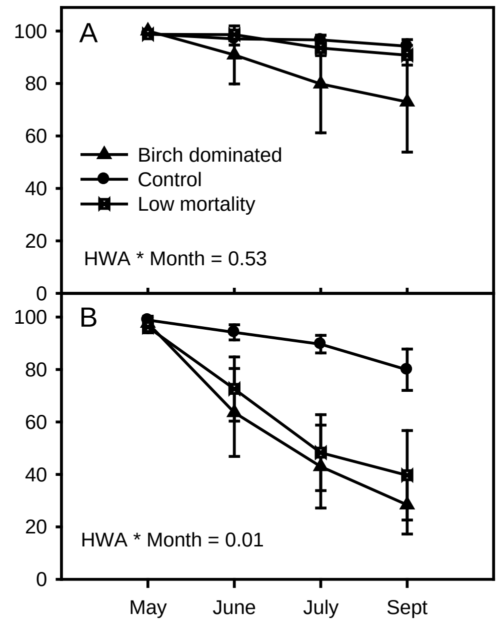

Figure 2. Cellulose paper mass loss at the forest floor surface (A) and buried 5cm below the surface (B) during the 2001 growing season in three hemlock stand types (see text for description). Significance values for time and HWA interactions are given.

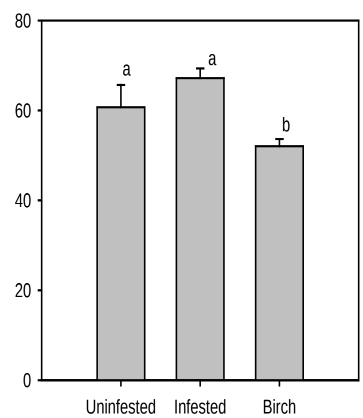

Figure 3. Forest floor mass from stands at different stages of HWA decline compared to uninfested control stands. Infested stands are hemlock dominated but in decline, while birch stands are former hemlock stands that now contain a developing canopy of black birch. Three sites of each stand type are included in this analysis.

Long-Term Impacts of HWA and Hemlock Replacement

Long-term incubation of green hemlock litter showed no differences in mass loss dynamics between infested and uninfested foliage (Figure 4). Foliage decay rates differed by site of origin, but these effects were not a function of previous impacts by HWA. Green litter material decayed more rapidly (50% faster) than senescent litter. Red oak, black birch, and red maple placed at the Prospect Hill stand decayed at approximately the same rate and magnitude over the study period (Table 2). Red oak litter reached the greatest overall mass loss over three years, followed by black birch and red maple, although *k* rates were highly variable over time. Measured rates of litter decay were similar to those obtained from our literature search of studies conducted on these species (Table 2) except that the relative rank of most to least rapid decay was different for the various hardwood species. Red maple and birch had consistently more rapid decay rates in the published studies we examined; however, many of these measurements were made in hardwood stands, whereas our measurements were made in an intact eastern hemlock forest.

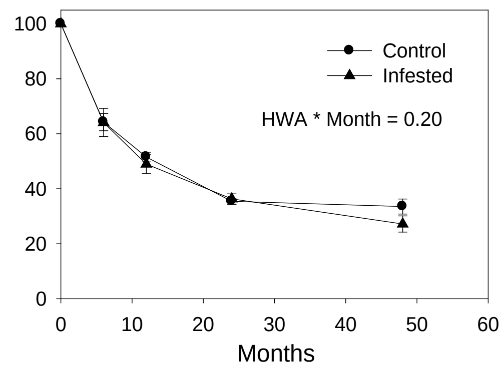

Figure 4. Long-term patterns of infested and uninfested (control) green hemlock foliage. Green hemlock litter is the same material used by Cobb et al. (2006).

Table 2. Decay constants and average mass remaining after two years for several senescent litter types decomposed in a hemlock-dominated forest at the Prospect Hill site and data from published foliar decay studies conducted at sites representative of New England hemlock forests.

	Measured Values		Literature Data		
Litter Type	***k* yr[1]**	**% Mass Remaining**	***k* yr[1]**	**Rank**	**# of Sites**
Red oak	0.43	42.1 (±6.4)	0.51	3	5
Black birch	0.39	45.4 (±7.5)	0.72	2	3
Red maple	0.32	52.8 (±0.5)	0.83	1	4
Mix[1]	0.27	57.3 (±5.3)	NA		
Hemlock	0.25	60.5 (±1.0)	0.29	6	9
Hemlock wood[2]	0.25	60.2 (±4.8)	NA		
Pine	NA	NA	0.34	4	2
Norway spruce	NA	NA	0.29	5	15

[1]Equal mix of senescent eastern hemlock and black birch.
[2]Senescent fine hemlock branches.
NA - Species not included in field study or data not available in the literature.

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In Table 2, *k* values were calculated as the average of the first two years of decay, the time period typically associated with the first phase of decomposition. Data are averages arranged from most rapid to slowest rates of decay observed. Values in parenthesizes are one standard deviation. Not all species for which data were gathered from the literature were included in the litter decay study and vice versa. The relative rank (fastest to slowest decay) and number of sites where foliage was incubated are listed in the left-most columns.

DISCUSSION

Induced changes in foliar chemistry are a common response to herbivory (Schowalter et al. 1986; Hunter 2001), but the lack of effective resistance in eastern hemlock indicates that either induced responses do not occur or that they are ineffective to HWA. Our measurements of seasonal foliar and twig chemistry, combined with population dynamics, illustrate the temporal dynamics of foliar chemistry and population densities, but these studies cannot address the relative drivers this association. Are plant N levels higher because HWA populations are more dense on these trees? Or are HWA populations more dense on these trees because plant N levels are higher? Unlocking this riddle requires experimental manipulations and further monitoring of HWA population dynamics and foliar N levels. This is the first study to document a relationship between HWA densities and hemlock fine branch %N. Furthermore, the effect of fine branch %N was greater than the effect of foliar %N on HWA population levels. If plant chemistry is driving HWA population levels, these patterns suggest that more detailed study of fine branch chemistry may be useful for understanding tree susceptibility to HWA.

Our common substrate decay study and associated measures of forest floor mass indicate that the greatest changes in decay rate occur after overstory mortality has led to the development of a black birch canopy. These data indicate that changes in overstory canopy structure have substantially greater effects on decomposition dynamics than herbivory alone. A central problem with common substrate decay studies is that only relative differences in decay rates are extendable to other substrate types such as litter or fine woody debris. This occurs because cellulose is highly degradable compared to litter that` is rich in lignin and secondary chemicals. However, follow-up measurements of senescent hemlock, black birch, and equal hemlock-black birch mixes indicate that the relative differences in decay rates are similar to those that actually occur for litter (Cobb and Orwig 2002; Cobb, unpublished data). These data demonstrate the importance of hemlock overstories in maintenance of functional and structural features associated with eastern hemlock. Across our study sites, slow decay rates and thick forest floors remained components of forests where the overstory remains dominated by eastern hemlock. These patterns are useful when planning to conserve similar functional processes in declining stands by selecting and planting other shade-tolerant conifers. Silvicultural techniques that regulate light levels over time, such as shelterwood cutting, are effective methods for maintaining hemlock advance regeneration (Goerlich and Nyland 2000) and suggest that planting of shade-tolerant conifers during the early stages of HWA infestation could reduce dominance of black birch in future stands.

Our previous study of HWA herbivory impacts on litter decay found no changes in mass loss but significant chemical changes in decomposing litter from HWA infested trees (Cobb et al. 2006). This same pattern has since been documented following eastern tent caterpillar (*Malacosoma americanum*; Lepidoptera, Fabricius) herbivory on red oak (*Quercus rubra* L.; Frost and Hunter 2008). This study (Figure 3) confirms our originally reported pattern that HWA herbivory does not result in changes in green foliage mass loss even over a much longer time period. Overall, herbivory-related changes in foliar chemistry over the course of decomposition are likely to contribute to increased N availability in infested stands (Orwig et al. 2008). These changes may be an inevitable consequence of HWA invasion of eastern hemlock stands, pushing these systems into novel states (Seastedt et al., in press). Impacts of HWA herbivory may be impossible to ameliorate with restoration efforts and should be considered as changed characteristics of these forests.

Discrepancies of hardwood litter decay rates between our study and those reported in the literature may be related to differences in decay environment among different forest types (Elliot et al. 1993). Further work is needed to understand the relative dynamics of hardwood litter decay in eastern hemlock stands as they decline in order to better understand how overall patterns reported in the literature correspond to the actual rates that would result from restoration or succession in HWA-impacted stands. However, conservation efforts focused on retaining functional processes will be better served by selecting species with lower decay rates, such as any of the conifer species in Table 2. Native pine (*Pinus* L. sp.) and Norway spruce (*Picea abies* (L.) Karsten) have litter decay rates comparable to those of eastern hemlock (Table 2). Additional research examining ecosystem function characteristics associated with various species commonly replacing hemlock is needed. In the absence of effective biological and chemical control of HWA at the landscape scale, such efforts are the sole potential for maintenance of functional characteristics associated with hemlock for future forests of southern New England.

CONCLUSIONS

Understanding ecosystem level impacts resulting from HWA outbreaks and associated stand decline is important when selecting appropriate management actions in infested stands. Hemlock mortality results in ecosystem impacts with much greater magnitude compared to those from HWA herbivory alone. Loss of hemlock-specific ecosystem functions such as slow nutrient cycling, long-term storage of soil carbon, and ecosystem specific floral and faunal communities are often cited as deleterious impacts of the regional HWA outbreak (Tingley et al. 2002; Yorks et al. 2003; Ellison 2005a). However, management efforts have not focused on maintaining or restoring particular ecosystem processes in declining stands. A management focus solely on chemical or biological control ignores the most heavily impacted stands, which further exacerbates loss of biodiversity and ecosystem function in declining stands.

Our decomposition studies suggest several potential techniques that may be useful in restoring ecosystem function provided by hemlock forests in the New England landscape. Further, these restoration recommendations are likely applicable in other impacted hemlock stands, such as those in the mid-Atlantic and southern states in the United States:

1. Select shade-tolerant conifers with slow litter decay and nutrient cycling rates in restoration treatments.
2. Plant restoration species in HWA-infested stands before high mortality levels occur.
3. Consider and continue to explore the functional processes associated with restoration species in declining stands for use in adaptive management of hemlock woolly adelgid.

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Patterns of Spread of Hemlock Woolly Adelgid in Carolina Hemlock Populations

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ABSTRACT

Census and mapping of individuals was carried out in four populations of Carolina hemlock to uncover patterns of spread of hemlock woolly adelgid. Spatial cluster analysis was used to distinguish among three patterns of adelgid transmission: random spread, spread in a wavelike pattern, and spread from multiple foci. The finding of clusters in all populations refuted the random spread hypothesis. In the two recently infested populations, trees in all size classes were in good condition, but infestation clusters were evident. In the population with a more advanced infestation but under chemical treatment, tree condition was poorer but infestations were light and seedlings were abundant and in excellent condition. Close monitoring of adelgid infestations and treatment of tree clusters with high densities of adelgids is recommended.

KEYWORDS

Carolina hemlock, cluster analysis, hemlock woolly adelgid, infestation, transmission

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, has shown a remarkable ability to extend its range in the eastern United States and to spread within populations of both the wide-ranging Canada hemlock (*Tsuga canadensis* (L.) Carr.) and the southern Appalachian-endemic Carolina hemlock (*T. caroliniana* Engelm.). Over the past two years, HWA infestations have become common in the mountains of east Tennessee and western North Carolina. Demographic studies have documented the spread of HWA at the level of states and counties (Evans and Gregoire 2007; Pontius et al. 20005; Orwig et al. 2002), but there is scant information on the local, fine-scale patterns of infestation. The goal of this study was to determine whether it was possible to discern patterns of pathogen transmission within populations. With limited biological and chemical resources, knowledge of spread patterns could guide potential treatment strategies, which may include whether or not to focus on infested trees or areas adjacent to infestations and whether or not to target particular age classes. We focus on the Carolina hemlock because the limited distribution subjects this species to risk of decimation or perhaps extinction in the wild.

Three alternative but not mutually exclusive patterns of transmission can be envisioned: random spread in which infestations arise and spread with no discernable pattern, spread in a wavelike pattern from a point of introduction, and spread in all directions from multiple foci. For example, the multiple foci alternative may arise as a combination of random dispersals followed by wavelike spread from each new focus.

METHODS

Census and mapping was conducted in four populations of Carolina hemlocks. All populations were located on National Park Service lands associated with either the Blue Ridge Parkway (Linville Falls and Doughton Park) or the Appalachian Trail (Nolichucky and Laurel Fork) (Table 1). The Nolichucky and Doughton Park populations were in the very early stages of HWA infestation; Laurel Fork was more advanced; and the infestation at Linville Falls was advanced, and chemical (imidacloprid) treatments had been conducted.

Table 1. Characteristics of Carolina hemlock populations censused.

Population	Location	Owner	Elev. (ft)	Stage 1	Treatment
Nolichucky	Unicoi Co., TN	AT/USFS	1750	early (fall 2006)	none
Laurel Fork	Carter Co., TN	AT/USFS	2400	mid (2004-5)	none
Linville Falls	Burke Co., NC	BRP/NPS	3350	late (2002-3)	chemical
Doughton Park	Allegheny Co., NC	BRP/NPS	3600	early (2006?)	none

[1]Infestation stage and approximate date of HWA introduction.

In each population, a start point for a line transect was delineated with GIS coordinates. From this point, a line was extended for 100-200 meters, from which all Carolina and Canada hemlocks, including seedlings, were assigned x-y coordinates corresponding to distance on the line and distance perpendicular to the line on either side. Coordinates will allow us to re-census these populations and field-test the mode-of-spread hypothesis derived from the cluster analyses presented here.

For each hemlock, we measured two demographic characters (plant height and diameter at breast-height) and three epidemiologic characters (number of cardinal quadrants showing presence of adelgids [0-4], degree of infestation [absent-low-medium-high], and tree condition [excellent=no impact; good=light and spatially restricted impact; fair=conspicuous loss of foliage; poor=dying tree; dead]). All epidemiologic characters were assessed visually.

DATA ANALYSIS

Demographic characteristics were visualized using size pyramids. The spread hypotheses were evaluated using spatial cluster analysis. Hypothesis testing was possible because each pattern of spread gives rise to a different clustering expectation. Tree locations on the x-y coordinate system were used to conduct spatial cluster analyses using a Poisson model as implemented by SaTScan software (Kulldorff 1997). Monte Carlo replicate randomizations were used to assess significance of the scan statistic. Separate analyses were conducted for each of the four populations and for each of the three epidemiologic characters for a total of 12 analyses.

RESULTS

1. Demographics

The Nolichucky population of Carolina hemlock was relatively small and intermixed with Canada hemlocks. There was a notable absence of seedlings and large trees (>20" DBH) in this population (Figure 1). This size class distribution, if reflective of ages, suggests a recently established population colonized over a short time period. In contrast, there were abundant seedlings at Linville Falls, which led to an age pyramid with an extended base, but Linville Falls also had a relatively high number of large trees. Laurel Fork had a more uniform size pyramid with a relatively small number of seedlings, while Doughton Park had a more classic pyramid-shaped size structure.

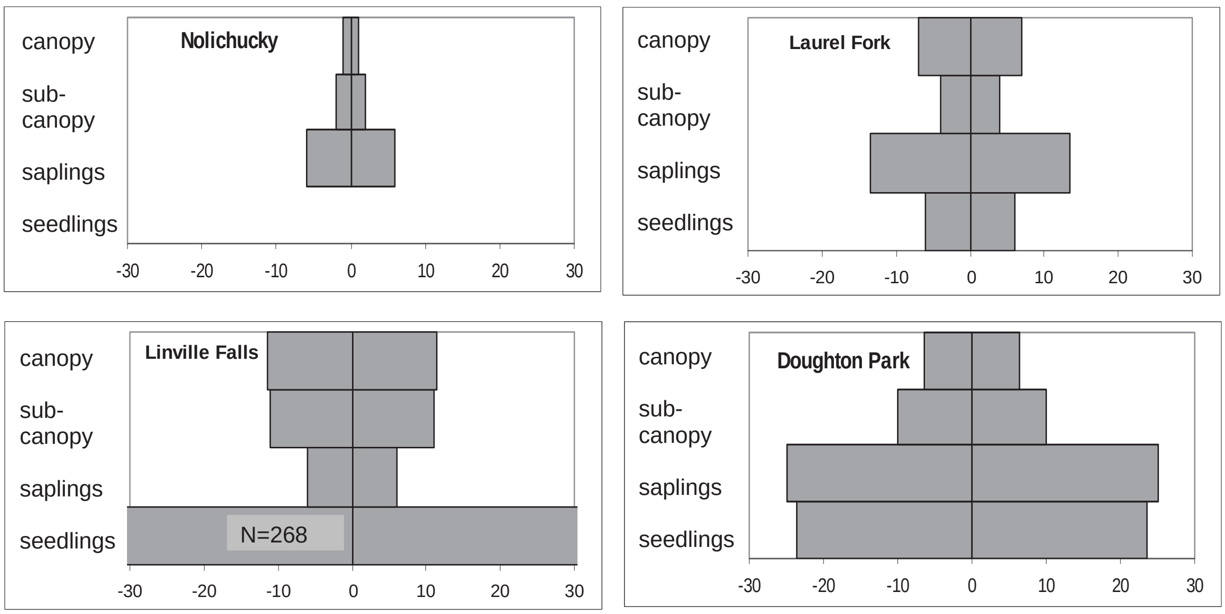


Figure 1. Size pyramids for four Carolina hemlock populations. Size class totals are the combined negative and positive values of the x-axis.

2. Epidemiology

Seedlings were analyzed separately from other height classes because: 1) their numbers would bias results from some populations, and 2) they tended to have different degrees of infestation or their condition was not typical of larger specimens. In all populations, seedlings tended to be in relatively good condition despite infestations in some populations (Figure 2). The notable exception was at Linville Falls, where seedlings were abundant and all were in excellent condition and uninfested.

Regarding non-seedlings, Nolichucky was in a very early stage of infestation, and although adelgid infestation was widespread, virtually all trees were in healthy condition (Figure 2). Similarly, Doughton Park was at an early stage of infestation, with the leading indicators (quadrants infested and degree of infestation) higher than the lagging indicator (tree condition). In contrast, Linville Falls had been infested for several years, and the area we censused was under chemical treatment, with the initial trees treated four years prior and more trees treated in subsequent years. Consequently, few adelgids were observed, but the trees showed extensive signs of impaired health (loss of foliage and dead branches).

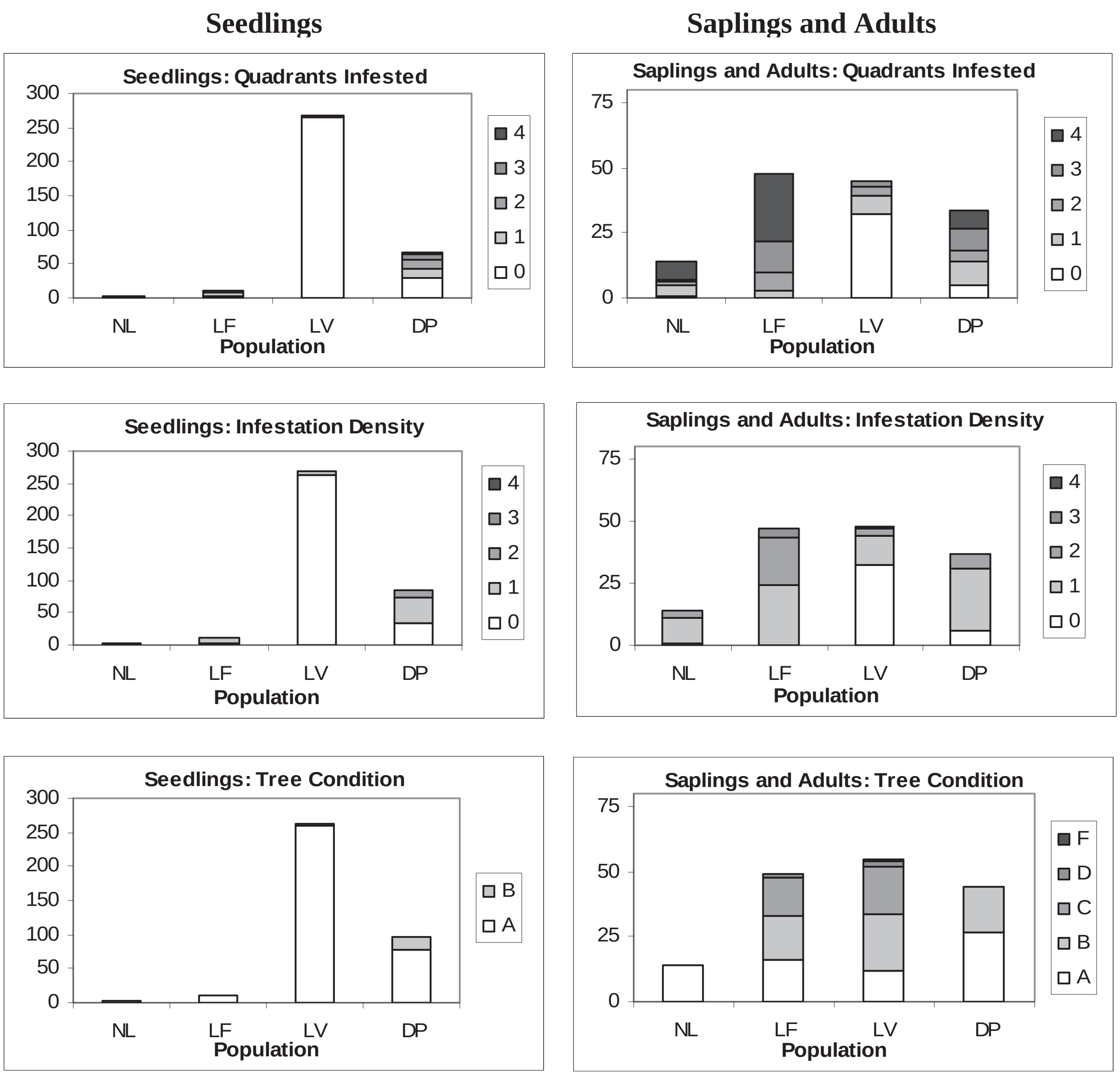


Figure 2. Epidemiologic characteristics of Carolina hemlocks in four populations. For “Infestation” characters, ‘0’ signifies absence and ‘4’ is highest level of infestation. For “Tree Condition”, ‘A’ is excellent and ‘F’ is dead. NL=Nolichucky; LF=Laurel Fork; LV=Linville Falls; DP=Doughton Park.

3. Cluster Analysis

Significant clusters were observed in all four populations (Table 2). The presence of clusters refutes the random spread hypothesis. There were infestation clusters at Nolichucky (Figure 3-A), Doughton Park (Figure 3-B), and Linville Falls, and there were clusters of trees in declining condition at Laurel Fork (Figure 3-C), Doughton Park and Linville Falls (Figure 3-D). In these figures, each plot is a scaled representation of the locations of trees within a population. The size of the circle is scaled to the size of the tree, but many seedlings may be represented by a single small circle (e.g., the cluster of two small green dots enclosed by a green circle near the bottom of Figure 3-D). For “Quadrants Infested,” the more filled the circle, the more quadrants infested; for "Infestation Density," the higher the red bar from the circle, the denser the infestation, and for "Tree Condition," red indicates poor condition ('F') and green indicates excellent condition ('A'). Significant clusters are enclosed in circles. A red circle indicates a cluster of individuals with either more severe infestation or in poorer condition; a green circle indicates a cluster of individuals with lesser infestation or in better condition.

Table 2. Summary of significant clusters.

	Significant Clusters					
	Quadrants Infested		Infestation Density		Tree Condition	
	High	Low	High	Low	Poor	Good
A. Untreated Populations						
Nolichucky	1					
Laurel Fork		1		1	1	1
Doughton Park	1	1	1	1	1	
B. Treated Population						
Linville Falls	2		2		4	2

There were no adelgids noted at Nolichucky in an informal survey in the fall of 2006. When this census was conducted in the spring 2007, Nolichucky had become colonized by HWA but all trees were relatively undamaged, thus accounting for the absence of tree condition clusters. Similarly, the infestation at Doughton Park appears relatively recent, as evidenced by almost all trees in excellent to good condition. However, there are clear areas of infestation foci, and we can expect tree condition to decline if no adelgid control measures are taken.

Linville Falls was unique as a census site because this area has a longer and more serious infestation history and the site censused had been chemically treated for several years. Thus, this site provided the opportunity to observe the combined effects of a long-standing infestation and several years of chemical treatment. There were multiple clusters at Linville Falls. Clusters of trees in poorer condition tended to be larger trees, while the clusters of individuals in excellent condition were mainly seedlings (Figure 3-D). Infestations were relatively light at Linville Falls, and infestation clusters tended to be small in size. Thus, Linville Falls appears to have responded to chemical treatment by a marked reduction in the infestation, but trees and saplings showed the negative effects of infestation, while seedlings were generally adelgid-free and in excellent condition. It is unknown whether the excellent condition of seedlings can be attributed to protection from uptake of residual chemical in the soil (Cowles et al. 2006) or if their condition is a reflection of the benefit of a currently light local infestation of HWA.

Management Implications

Chemical treatment of larger trees led to the unanticipated benefit of a healthy seedling crop, as seen clearly at Linville Falls. This could argue for selective protection of some infested trees to maintain a seed source and to create seedling beds in a protection zone. Protection of seedlings may be particularly important due to the limited time of seed viability in hemlocks (Sullivan and Ellison 2006). Although very preliminary, the finding of infestation clusters suggests treatment should focus on infestation foci and adjacent areas.

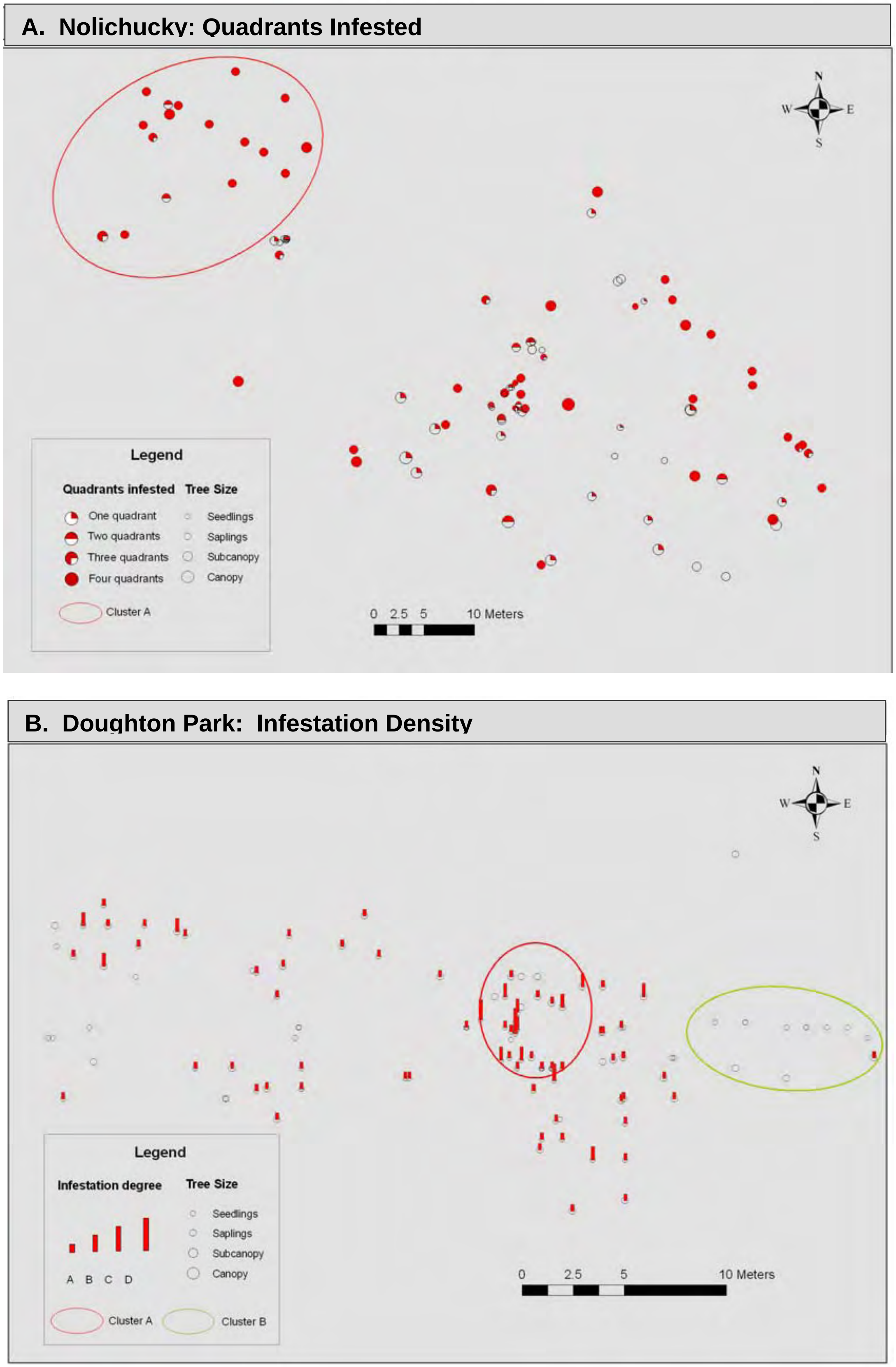
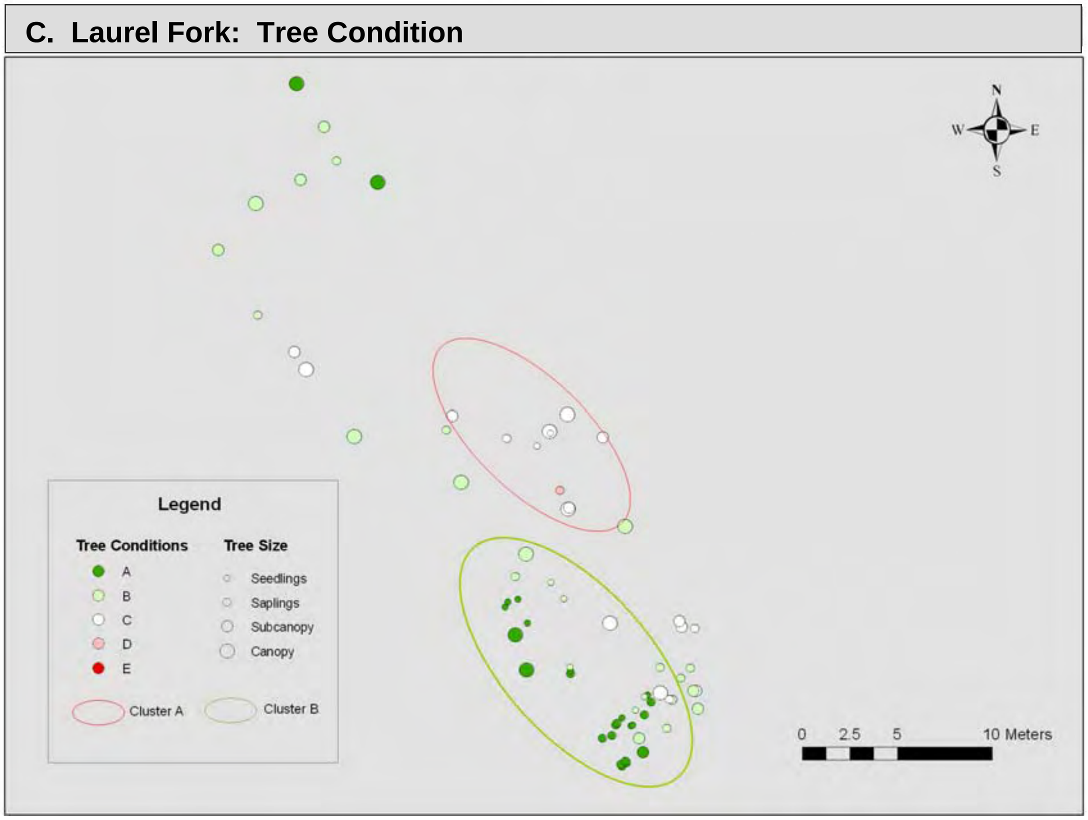


Figure 3, A-B. Spatial clusters in four Carolina hemlock populations. (See description in text.)



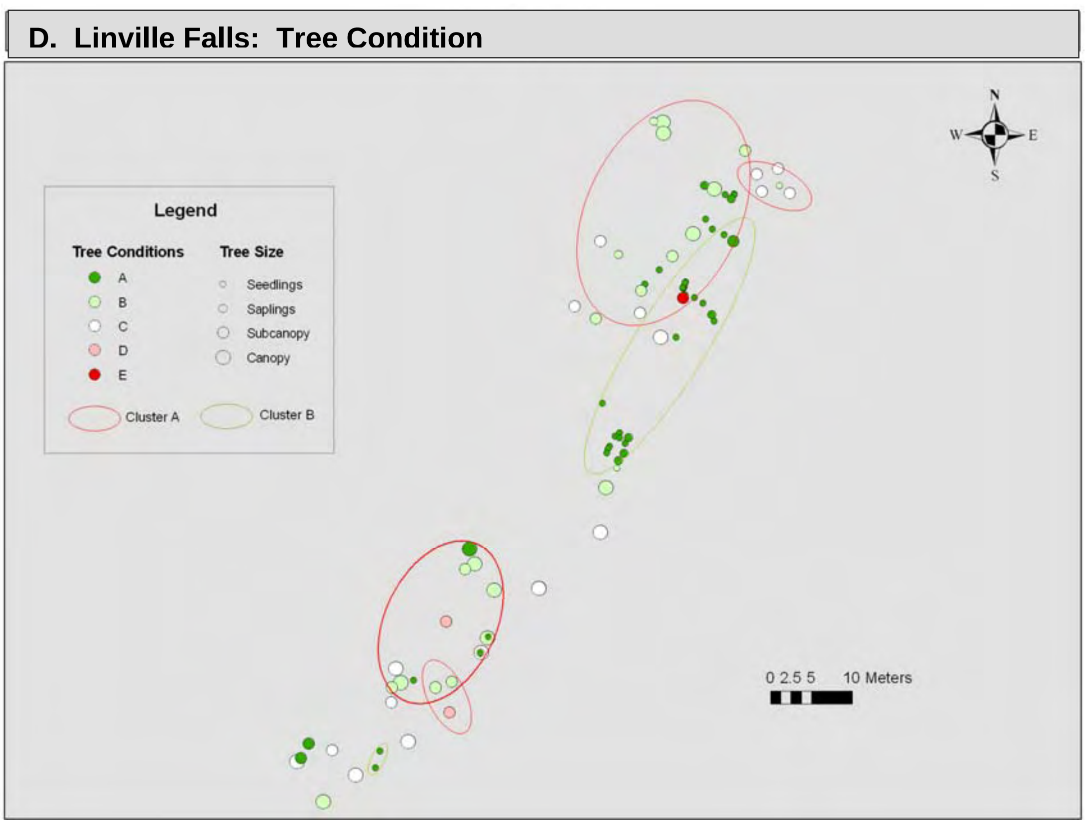


Figure 3, C-D. Spatial clusters in four Carolina hemlock populations. (See description in text.)

Unanswered Questions

Our first-year results provide baseline data on infestation patterns within Carolina hemlock populations. Subsequent surveys are needed to uncover patterns of spread over time and to address the following questions:

- Are clusters of high infestation simply expanding from one year to the next?
- Does infestation in untreated populations progress from high infestation to poor tree condition?
- Are seedlings, small trees, and larger trees equally vulnerable to HWA colonization?
- Does transmission of HWA follow human trails to expand infestations?
- Do clusters of low-infestation and good-condition trees persist over succeeding years?

SUMMARY

Cluster analysis was successful in identifying population sectors of high and low infestation and trees in poorer and better health. The clusters that indicate negative impacts of adelgid attack (more quadrants infested, higher infestation density, and trees in poorer condition) clearly reflect regions within populations where the adelgids have established. An unexpected outcome is that, with the exception of one small area within Linville Falls, there were no locations with significant clusters for all three negative impact indicators. This is probably a reflection of our choice of two populations in very early stages of infestation (Nolichucky and Doughton Park) where tree health is still relatively good. In contrast, at Linville Falls, the combination of a longer infestation and chemical treatment has resulted in the mix of larger trees in poorer condition, but subsequent clearing of the infestation by chemical treatment accounts for the generally light infestation. Many trees were adelgid-free, including a seedling population that is large and in excellent condition.

ACKNOWLEDGMENTS

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Using Dendrochronology to Model Hemlock Woolly Adelgid Effects on Eastern Hemlock Growth and Vulnerability

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ABSTRACT

This study examined the relationship between eastern hemlock (*Tsuga canadensis* (L.) Carr.) crown condition and changes in radial growth associated with infestation by hemlock woolly adelgid (HWA), *Adelges tsugae* (Hemiptera: Adelgidae). Tree-ring chronologies of eastern hemlock were used to develop a binomial decline index based on three consecutive years of below-average growth. Radial growth decline was modeled using logistic regression as a function of an extensive array of tree, crown, and site variables that were collected over an 11-year period in Delaware Water Gap National Recreation Area. Some site-related variables, such as site-location and aspect, were significantly related to decline probabilities when considered individually. However, the total proportion of response variance accounted for was low, and the only site variable included in the final model was mean plot-level HWA infestation level. For every 1% increase in mean percent HWA infestation per plot, there was an 8% increase in the likelihood that a tree would be classified as being in decline. Tree crown variables such as live crown ratio, crown density, and the modified ZBadj index, a combination of foliage transparency and branch dieback, had the most explanatory power, both individually and in the final model. These crown variables were relatively accurate predictors of the degree of hemlock growth decline during HWA infestation.

Effects of Eastern Hemlock Mortality on Riparian Ecosystems in the Southern Appalachians

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Eastern hemlock (*Tsuga canadensis* L.) is an important evergreen tree species in the southern Appalachians. Although it occurs on a wide range of mesic sites, hemlock is most prevalent in riparian areas. The introduced hemlock woolly adelgid (HWA), *Adelges tsugae*, is causing widespread hemlock mortality throughout its range. In 2003, we began estimating the extent of HWA infestation and degree of crown loss on hemlock trees within the Coweeta Basin. While estimates of both were low in 2003, by 2005, infestation had reached 100%, resulting in 80% crown loss.

In 2004, we initiated a study to monitor and predict changes in ecosystem processes due to loss of eastern hemlock in riparian areas. We established eight plots in riparian areas with more than 50% hemlock basal area. Hemlock trees were in the initial stages of HWA infestation on all plots. On one-half of the plots, we girdled the hemlock trees to accelerate mortality. We allowed HWA to progress on the remaining four plots (HWA-infested plots). We also established four plots in riparian areas with no significant hemlock component. We monitored changes in microclimate, carbon pools and cycling rates, soil nutrient pools and cycling rates, species composition, biomass increment, and transpiration. Our results show that, by 2005, girdling had resulted in higher soil moisture than HWA-infested plots.

By 2007, soil moisture was similar presumably due to near 100% mortality in both treatments. Both treatments were higher in soil moisture than hardwood plots. Differences in soil temperature were minor across treatments, with the highest occurring in hardwood plots. In 2004, fine root (<0.5 mm) biomass accounted for 45% of all root biomass, of which 31% was made up by hemlock. By 2006, fine root biomass had declined by one-third across both girdled and HWA-infested plots, presumably due to hemlock fine root mortality, as a similar decline was not seen in the hardwood plots. Soil CO_2 efflux rates were similar in girdled and HWA-infested plots in 2004; however, rates in 2005 and 2006 declined significantly (~20%) from 2004 in both treatments. Above-ground woody biomass increment was substantially reduced three years after infestation in HWA-infested and girdled plots but not in hardwood plots.

Observed small changes in microclimate are not likely to be biologically significant. Observed biological responses (e.g., soil CO_2 efflux, fine root biomass, and biomass increment) were directly related to hemlock mortality. In general, girdled and HWA-infested plots exhibited similar responses in magnitude and timing for most variables. In the long term, we predict that increased inputs of hemlock woody debris and changes in species composition will significantly alter structure and function in these ecosystems.

Controlling Hemlock Woolly Adelgid with New Forms of Mycoinsecticides

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ABSTRACT

New formulations of *Beauveria bassiana* and *Lecanicillium muscarium* were used for management of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. The formulations were prepared with whey, a waste product from the production of cheese, and vegetative oil. Codominant hemlock trees (2.0-2.5 m tall) with high populations of HWA were randomly selected for these field trials. Treatments were applied with fungal suspensions containing 5 x 10^9 conidia per milliliter from the ground using a modified ultra-low volume (ULV) sprayer. Two applications of 20 ml/tree were made seven weeks apart. The first application was made in late spring as the crawlers started to hatch and the second in mid-summer as hemlock trees completed expansion of their new growth (2 to about 10 cm length). The two applications were formulated to optimize on contact of the crawlers and settles with the fungal propagules. HWA mortality was determined at 0, 3, 7, 13, and 18 weeks post-spray. Mortality ranged from 85% to 90%. Fungal formulations based on whey and vegetable oil were more efficacious than those based on whey alone. Mycopesticides did not show any negative impact on several non-target components of the forest community.

KEYWORDS

hemlock woolly adelgid, entomopathogenic fungi, mycopesticide, ULV spray, *Beauveria bassiana*, *Lecanicillium muscarium*, whey, oil

INTRODUCTION

Morphological and biological characteristics of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, together with their high reproductive potential and cold-resistance, provide this forest pest advantages for success in colonization of hemlock forests in North America (McClure et al. 2003). HWA is an exotic invasive species and has a relatively limited number of natural invertebrate enemies. Mortality from native predators is generally inconsistent and low (McClure and Cheah 2002; Zilahi-Balogh et al. 2002). There is limited detailed information on infection pathology of HWA from different geographical locations. First reports of HWA individuals with visible signs of pathological infection were from Massachusetts in 1996. Fungi were found associated with approximately 37% of the 6,000 HWA examined. Fungi from 17 different fungal genera were isolated, including species from *Lecanicillium*, *Beauveria*, *Paecilomyces,* and *Acremonium* (S. Gouli et al. 1997). Isolates from the first three genera were identified as *Lecanicillium lecanii*, *Beauveria bassiana*, and *Paecilomyces farinosus*. Subsequently, similar species of entomopathogenic fungi were isolated from HWA collected in Sichuan Province, Peoples Republic of China. After confirmation of identification, these fungi were deposited in the USDA-ARS Collection of Entomopathogenic Fungal Cultures (Humber and Hansen 2005). Besides data on entomopathogenic fungi, there is information about symbiotic bacteria associated with HWA (Shields and Hirth 2005). This work created the opportunity to conduct research focused on the use of insect-killing fungi as a component of a total integrated pest management (IPM) strategy: entomopathogenic fungi can be used as mycopesticides, and the discovery of endosymbiotic bacteria provides an opportunity to search for ways to study an aposymbiotic effect.

Of numerous entomopathogenic microorganisms, the fungi are the only ones that are able to penetrate through the host cuticle. Personnel at the Entomology Research Laboratory (ERL) have been involved with HWA management using insect-killing fungi through numerous phases, from search, isolation, cultivation, and identification to mass production (Gouli and Gouli 2004 and 2006); formulation and pilot testing in the forest (S. Gouli et al. 1997; Reid 2002 and 2003; Parker et al. 2006). Two strains of *Beauveria bassiana* and a one strain of *Verticillium lecanii* (=*Lecanicillium muscarium*) proved to be efficacious during pilot testing. The fungi were formulated based on oil and whey carriers with maximal concentration of conidia suitable for application using an ultra-low volume (ULV) sprayer. The new fungal formulations were used in a small-scale forest trial and delivered suspensions onto selected hemlock branches strongly infested with HWA. Previous studies were conducted in late fall of 2005 and then late spring of 2006 and 2007. All field trials indicated significant reductions of HWA populations, but both spring and fall HWA treatments had certain shortcomings. In the case of the spring treatment, part of the HWA population survived as they had colonized new growth, which does not have fungal propagules. As a result, these insects had a normal rate of development and reestablished populations at their initial pre-spray levels. The fall treatment produced similar results as the survivors successfully overwintered and colonized new growth in the spring. The field trials reported herein were designed to solve this problem by including a two applications of entomopathogenic fungi seven weeks apart targeting new growth of hemlock where the majority of HWA crawlers and settles were located.

MATERIALS AND METHODS

Field experiments were conducted in Purgatory Chasm Reservation (Sutton, Massachusetts) in late spring and mid-summer to examine the efficacy of new formulations based on whey and whey with oil. Two entomopathogenic fungal isolates, *Beauveria bassiana* (*Bb*) and *Lecanicillium muscarium* (*Lm*) were used. *Beauveria bassiana* strain CA-603 was initially isolated from soil, and *L. lecanii* strain EHS-132 was isolated from from elongate hemlock scale, *Fiorinia externa*. Both pathogens were repeatedly used for inoculation of HWA and then reisolated from infected insects. Fungal biomass for field experiments was mass-produced on millet. Pure conidia were formulated as stable suspensions based on whey concentrate and/or whey with 10% oil. Conidial suspensions for treatment of trees contained 5 x 109 conidia/ml. Forty-two codominant hemlock trees (2.0 – 2.5m tall) were randomly selected for treatment.

Eighteen of these trees were reserved for controls, of which six received no treatment, six were sprayed with whey, and six were sprayed with whey plus oil. Each of the four treatments was replicated six times (24 trees total) and included *Bb* plus whey, *Bb* plus whey and oil, *Lm* plus whey, and *Lm* plus whey and oil. The first application, using a modified ULV sprayer, was carried out on June 6, 2007. For the second application, made on July 25, 2007, we randomly selected three trees from each of the controls and treatments previously sprayed; these, we used for a second application (Figure 1).

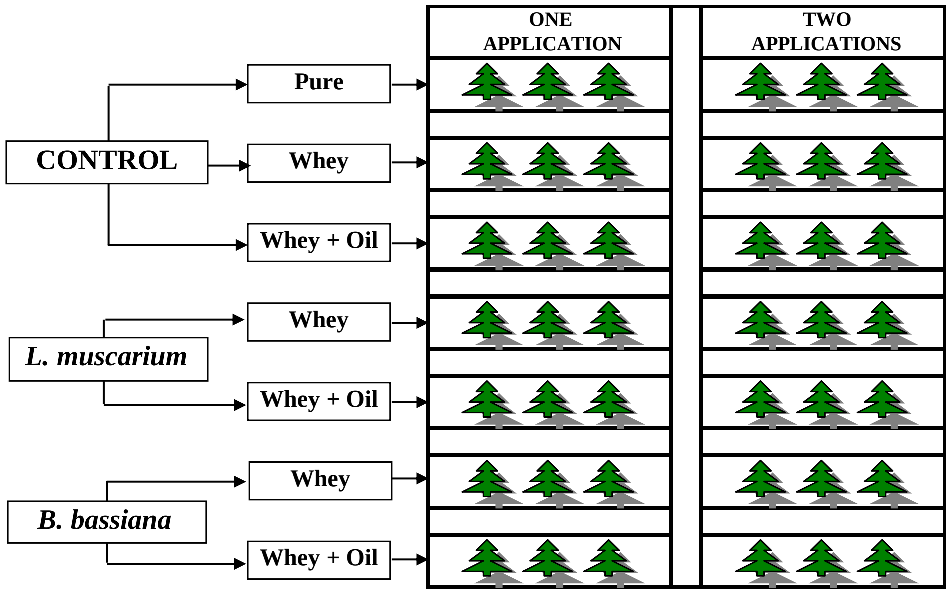

Figure 1. Schematic of the procedures followed for a single and double application of entomopathogenic fungi for management of HWA in Purgatory Chasm, Massachusetts. First application: June 6, 2007; second application: July 25, 2007.

Samples for determining HWA population levels and mortality of settles were collected pre-spray and 0, 3, 7, 13, and 18 weeks post-spray. From each tree, we clipped four twigs approximately 5 cm long containing new hemlock growth. These were taken from the middle and upper canopy. Each sample was placed individually in sterile graduated 50 ml conical plastic tubes containing 16 g of sterilized sand and 7 ml sterile distilled water. The sand held the twigs upright and held water to prevent desiccation and maintain a humid environment. Each tube was covered loosely with a cap that allowed ventilation to the tube, and the tubes were then transported to the laboratory. The tubes were placed in plastic bags to prevent any desiccation and held at 22°C with 16:8 LD. Numbers of adult HWA with eggs were counted on 2 cm of old growth immediately below the area of new growth. The new generation of settles was counted on 5 cm of new growth.

Mortality of insects was determined under a binocular microscope at 40x power within one day of collection. Pre-spray samples were examined one day following collection; 0-day samples (taken immediately following the spray application) were held in the laboratory in conditions as mentioned above for one week and were then examined for mortality. Settles were deemed dead if they showed signs of mycoses (i.e., they were off-color or had obvious mycelia growing from their bodies), failed to respond following very slight probing with a blunt mineutin, or failed to demonstrate a positive haemolymph response. Adult HWA mortality is difficult to determine visually because of the cottony masses that cover the adult female, her cadaver, and her eggs. If there was uncertainty whether the individual was dead or alive, we prepared squash slides, stained them with cotton blue stain, and further examined it under a light microscope. The condition of cells in the haemolymph was a clear indication of mortality (V. Gouli et al. 2000). Invertebrates (nontargets) from the hemlock samples were examined in a similar manner as above for manifestation of mycoses.

STATISTICAL ANALYSES

Data were analyzed using two-way analysis of variance (for laboratory experiment) and three-way ANOVA (for field collected data) following the general linear model procedure (PROC GLM). Count and percentage data were transformed into logarithmic (log10) or arcsine square-root scale, respectively, before the analysis. In the event of significant differences, a Student-Newman-Keuls (SNK) test was used to separate means (SAS 2003).

RESULTS

Pre-spray samples of 5 cm of new growth had between 13.5 and 25 crawlers and 7.7 to 14.4 settles. The natural mortality of crawlers ranged from 0 to 13.1%. No mortality of settles was observed. The number of adults with eggs per 2 cm twigs ranged from none to five, with a mean of 0.2. The total number of adults, although not counted beyond 2 cm, was much higher.

Hemlock control samples taken at time 0 (immediately following spraying) after one week in the laboratory had 1.5% to 5.7% mortality of settles. Settles on hemlock branches sprayed with *Bb* showed definite signs of mycoses, and 64.7% to 69.1% were dead. Those sprayed with *Lm* also showed mycoses, and 62.9% to 70.7% were dead. This was a clear indication that the ULV application was successfully applied to the hemlock trees and hit the target HWA (Figure 2).

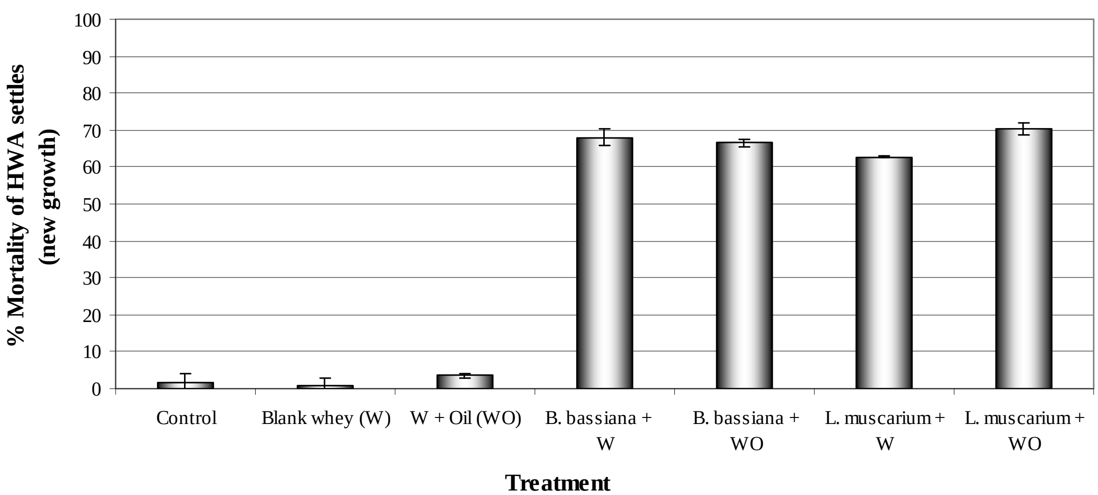

Figure 2. Mortality of HWA settles sprayed in the field and held in the laboratory for one week post-application. W= formulation with whey; WO= formulation with whey and oil.

Control mortality of settles after a single application ranged from 0.4% to 6.2% after 3 weeks, and then the number had a tendency to increase gradually after 7, 13, and 18 weeks: 2.2%-4.9%, 4.5%-8.7%, and 6.9%-10.2%, respectively. Mortality of settles on hemlock trees sprayed with the fungal formulations based on *Bb* ranged from 50.3% to 60.0% at 3 weeks; 51.8% to 61.6% at 7 weeks; 63.2% to 64.2% at 13 weeks, and 61.3% to 65.0% at 18 weeks.

The efficacy of *Lm* formulations was approximately at the same level (Figure 3). The mortality after 7 weeks increased slightly and then tended to level off to about 65%, indicating a significant number of settles survived. It is believed that two factors led to this result: HWA eggs do not all hatch at the same time and, therefore, there was increase in those hatching following the application of June 6th; and these new crawlers had new growth to settle onto, which did not have spray on it.

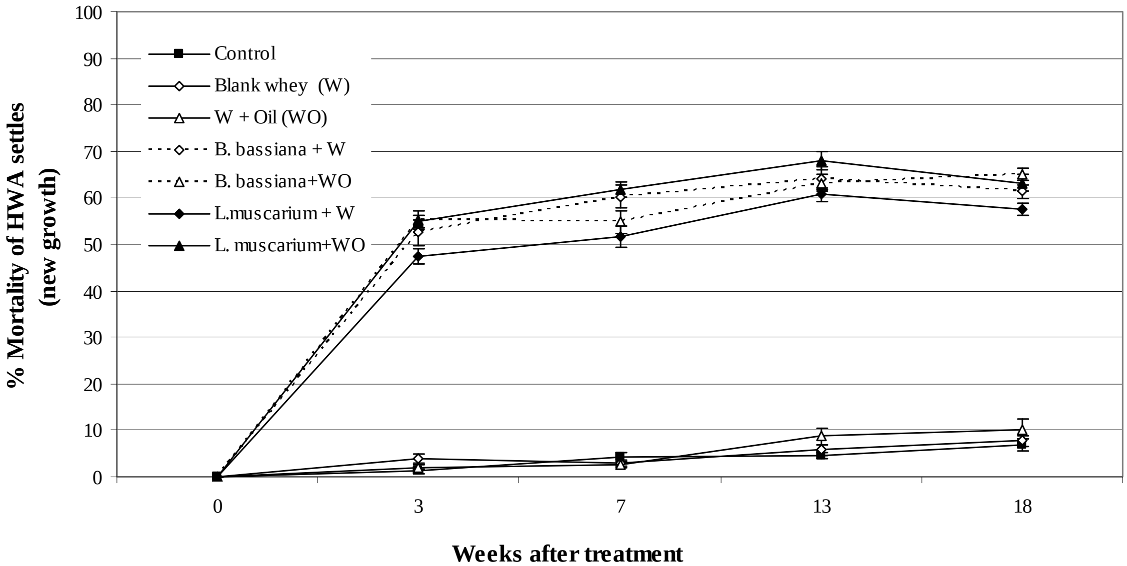

Figure 3. The mortality of HWA settles following a single application of whey formulations of *Beauveria bassiana* and *Lecanicillium muscarium*.

The mortality of HWA settles after the second application of mycopesticides is shown in Figure 4. It was significantly higher than after the first application. In case of *Bb*, the difference was from 14.8% to 22.1% higher after 13 weeks and from 19.4% to 26.4% after 18 weeks. For formulations with *Lm*, the difference was 13.1% to 17.8% higher after 13 weeks and from 25.9% to 28.4% higher after 18 weeks (Figure 5).

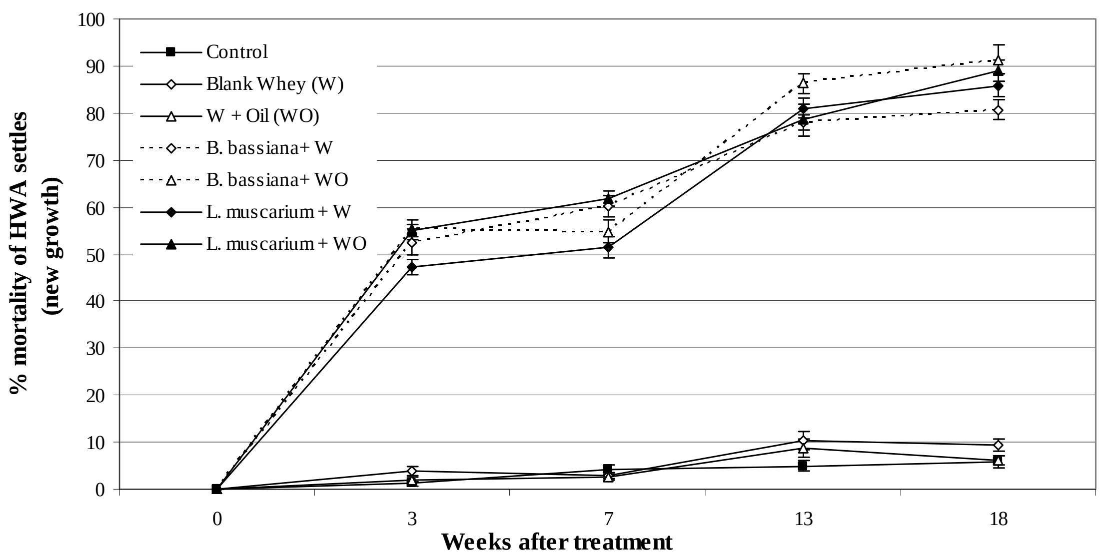

Figure 4. The mortality of HWA settles following two spray applications of mycopesticides. W= whey formulation; WO= whey + oil formulation.

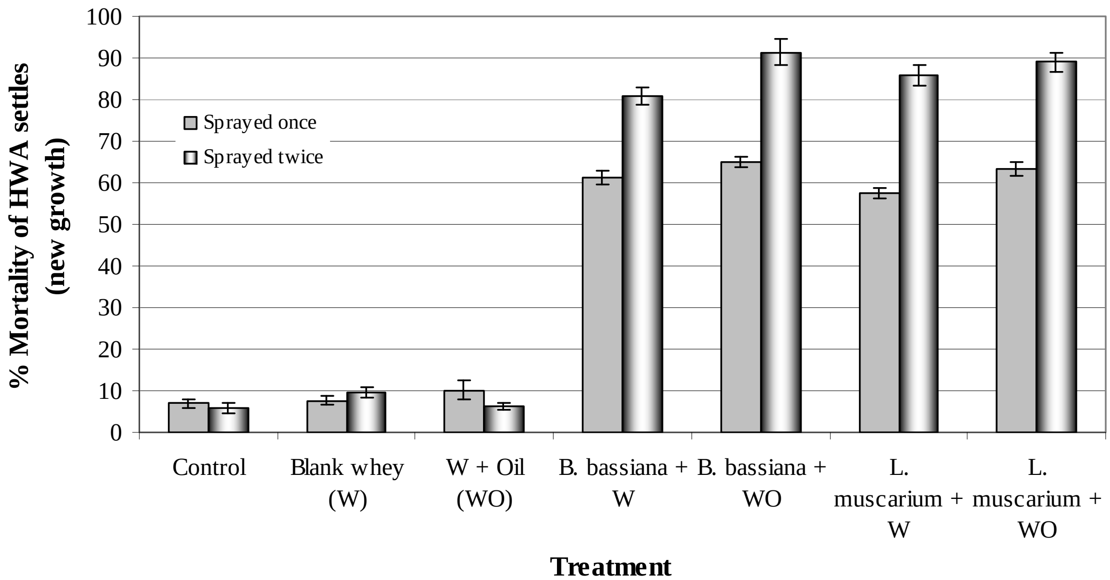

Figure 5. A comparison of mortality of HWA settles after one and two applications of *Bb* and *Lm* 18 weeks post application. W= whey formulation; WO= whey + oil formulation.

HWA mortality between the two fungal isolates and the two different types of formulation did not show significant differences (Figure 6 and Figure 7). These field experiments in 2007 confirmed the need for further development of entomopathogenic fungi for the integrated pest management of HWA on hemlock. The next logical step would be to carefully conduct a small-scale pilot aerial application.

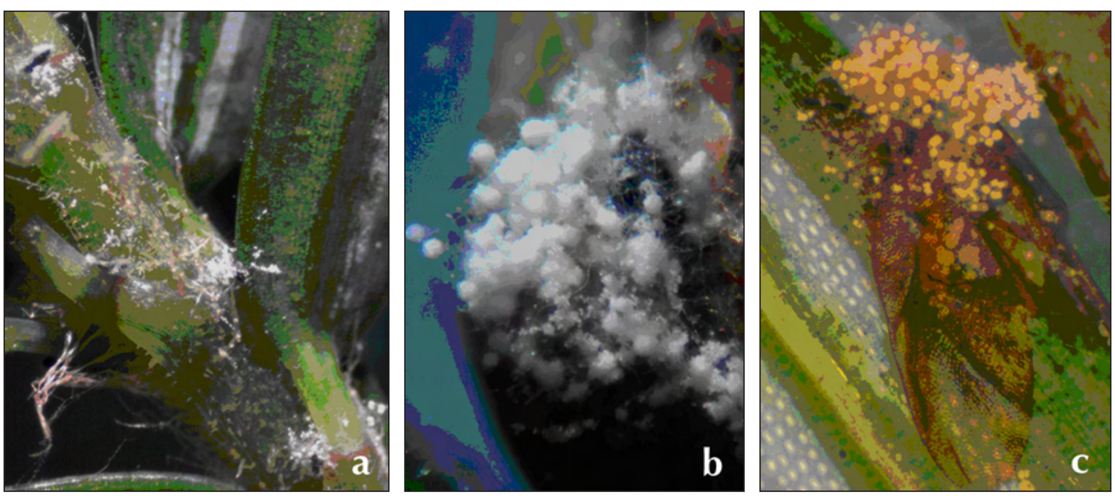

Figure 6. HWA with signs of *B. bassiana* mycosis (a, b - settles; c - winged adult)

Pre- and post-application samples were used for estimation of both HWA and non-target arthropod mortality on experimental trees. The hemlock samples yielded the following arthropods: *Dicyrtoma* sp. (Collembola, Dicyrtomidae), spider mites of conifers (non-identified), and non-identified thrips. *Dicyrtoma* sp. did not show any signs of mycoses during the experimental period. Strong fungal epizootics were registered inside mite and thrips populations. Specific azygospores from the genus *Massospora* (Zygomycetes, Entomophthorales) were discovered during microscopic analysis of cadavers (Figure 8a, b, and c).

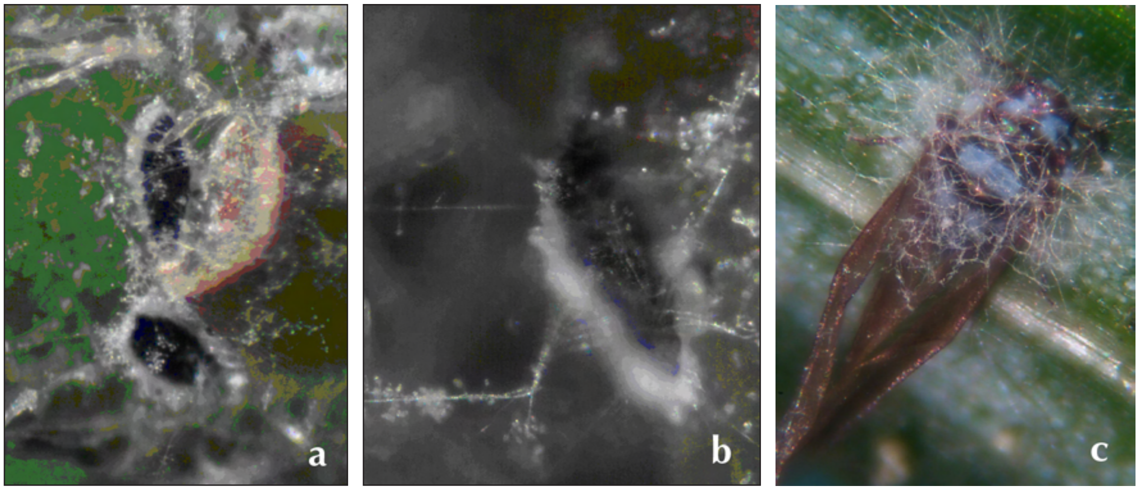

Figure 7. HWA with signs of *L. muscarium* mycosis (a, b - settlers; c - winged adult).

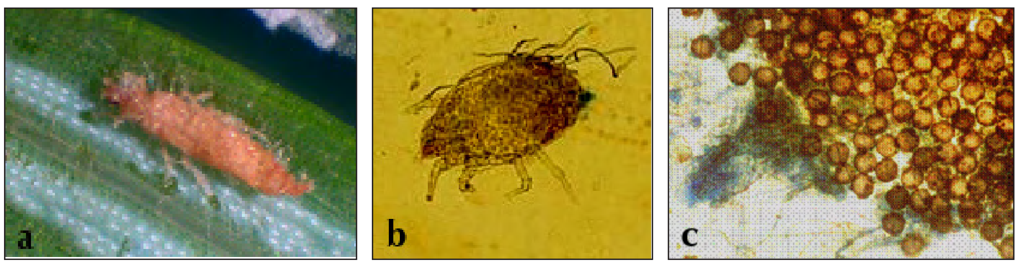

Figure 8. Entomophthoroses of arthropods taken from hemlock samples sprayed with *Bb* and *Lm* for management of HWA: thrips (a) and mite (b) with signs of entomophthoroses; resting spores of fungus *Massospora* sp. (c).

The density of the non-target invertebrates was relatively low, and we did not see any samples with visible signs of mycoses provoked by *B. bassiana* or *L. muscarium*.

SUMMARY

Four different formulations of insect killing fungi based on *Beauveria bassiana* (strain CA603) and *Lecanicillium muscarium* (strain EHS-132) were compared for their efficacy against *Adelges tsugae*. For each isolate, two prototype formulations containing 5 x 10^9 conidia ml were developed and compared with the control (no treatment) and with two blank formulations. Pre- and post-application mortality of HWA settles populations was used to evaluate the efficacy of the formulations. Laboratory incubation of sprayed twigs shows 63% to 70 % mortality of settles in all fungal-based formulations within seven days of application. Mortality in all control treatments remained lower than 5%. Results after a single spray application were compared to those of a double application. Under field conditions, a single application of fungal formulation resulted in 60% to 68% mortality 13-18 weeks post-spray. In contrast, two applications of fungal formulations caused 78% to 91% mortality 13 – 18 weeks post-spray, which is about 23% to 25% increase in HWA control activity. Throughout the study period, mortality in all the control treatments remained lower than 10% in both single and double applications. Both tested isolates were equally effective against HWA; however, their efficacy was affected by the type of formulation carriers used.

CONCLUSIONS AND RECOMMENDATIONS

The current and previous laboratory and field experiments we have conducted showed that indigenous strains of the entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* (=*Verticillium lecanii*) formulated with whey and whey plus oil can be effective for HWA management in the Northeast. Our formulated fungi do not appear to be a threat to several non-target species. It is necessary to make a double application of fungi for maximum control of HWA. The first treatment should be made when crawlers and new growth of hemlock starts (an elongation of ~ 2 cm). The second treatment should be made when new growth of hemlock has ceased (mid-summer). This strategy provides the maximum contact of the insect body and plant surface with fungal propagules. The use of entomopathogenic fungi for HWA management is not a "silver bullet," and consideration should be given to combining this strategy with introduction of other natural enemies in a total integrated management approach.

ACKNOWLEDGMENTS

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Incidence of Elongate Hemlock Scale and Its Parasitoid *Encarsia citrina* in the Eastern United States

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ABSTRACT

Elongate hemlock scale (*Fiorinia externa*) is an invasive species from Japan that was first detected in the U.S. in 1908. In the U.S., elongate hemlock scale attacks eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) and has established in most states where these trees occur. Scale density is much higher in the U.S. (from 21 to over 400 scales per 100 needles) compared to Japan (less than one scale per 100 needles) despite the presence in both regions of its primary parasitoid, *Encarsia citrina*, which is believed responsible for low densities in Japan. Previous work has shown that elongate hemlock scale and *E. citrina* have two synchronous generations in southern Japan (Kyoto), whereas there are one-and-a-half generations of elongate hemlock scale in the northeastern U.S. (Connecticut), which are thus asynchronous with *E. citrina*. In this study, we tested the hypothesis that if there are two generations of elongate hemlock scale in the southern U.S., this should produce a seasonal pattern similar to that in Kyoto, allowing for two generations of *E. citrina* and resulting in better host-parasitoid synchrony. Such improved synchrony would then predict increased parasitism and decreased scale density in the southern U.S. (North Carolina) as compared to the northern U.S. (Connecticut).

To test this hypothesis, four eastern hemlock forest stands in Connecticut, four in Pennsylvania, and three in North Carolina were sampled weekly in 2006 to assess elongate hemlock scale density and life stage phenology, parasitism rates, and *E. citrina* flight phenology. These data confirmed the presence of a full second generation of elongate hemlock scale in North Carolina; however, phenology was still asynchronous with *E. citrina* flight, and parasitism rates were lower than in either Pennsylvania or Connecticut.

INTRODUCTION

Elongate hemlock scale, indigenous to Japan, was first reported in the United States on Long Island, New York, in 1908 (Ferris 1942). Since then, elongate hemlock scale has spread along the Appalachian Mountains from Massachusetts to northern Georgia and South Carolina, and west to Ohio, Michigan, and Minnesota. Since 1998, elongate hemlock scale has spread further north in New England and increased in density (Preisser et al. 2008). This spread, together with experimental evidence (Preisser et al., in press), demonstrates that elongate hemlock scale is evolving greater cold tolerance and will likely continue to move northward into areas not yet invaded. This represents a serious threat to eastern hemlock forests that, up until now, have been protected by cold winter temperatures.

Elongate hemlock scale settles permanently on the undersides of hemlock needles where they insert long thread-like mouthparts to feed on fluids within the needle. Several scales feeding on the same needle can cause needle death, and on trees with heavy infestations, this can result in the loss of a large proportion of a tree's foliage. Sustained heavy infestations of elongate hemlock scale can cause tree mortality. In Connecticut, elongate hemlock scale density varies widely, from 21 to 420 scales per 100 needles (McClure and Fergione 1977; McClure 1978). In its native Japan, density ranges from 0.0 to 0.15 scales per 100 needles (McClure 1986; Lyons, unpublished). Low densities of elongate hemlock scale in Japan are associated with parasitism by *Encarsia citrina* (Hymenoptera: Aphelinidae). Parasitism rates by this wasp of greater than 90% were reported by McClure (1986) for elongate hemlock scale in Japan, although other sources report much lower rates (10-40%) (Lyons, unpublished). This aphelinid is a widespread polyphagous species that also occurs in the United States, attacking various armored scales (Diaspididae). Parasitism rates by *E. citrina* on elongate hemlock scale in Connecticut vary widely, from near zero to greater than 90% (McClure 1981). Parasitism rates in other states are not known.

McClure hypothesized that this parasitoid and the susceptible life stage of elongate hemlock scale were not well synchronized in Connecticut, so that, when the second flight of parasitoids in August search for hosts, few individuals of the susceptible stage (second instar) of the scale are present. In Japan, two complete generations of elongate hemlock scale occurred in the areas studied by McClure (Kyoto, 34°55'-35°05' latitude, 80-150m elevation.) There, parasitoids emerging from the first generation of scale had young of the second generation of scale to serve as hosts in which to overwinter. In Connecticut, elongate hemlock scale has only one generation (McClure 1978). As a result, parasitoids emerging in late summer did not have a second generation of scale to serve as hosts (as reported by McClure). If this asynchrony is the reason for poor control of elongate hemlock scale by the parasitoid *E. citrina* in Connecticut, then by extension, this hypothesis would predict that better control would occur in southern states, where shorter winters would allow two complete generations of elongate hemlock scale, resembling the pattern in Kyoto, Japan. Our research tested the McClure hypothesis by testing this prediction.

Simultaneously we are interested in other possible explanations for the high elongate hemlock scale density observed in southern New England. Among these alternatives are that the parasitoid *E. citrina* in the United States, although morphologically indistinguishable from its counterpart in Japan, may be a genetically differentiated population, with a different level of efficacy. Work on this issue is underway but not reported on here.

METHODS

Field Sites

Forest hemlock stands were scouted and research sites located in Connecticut, Pennsylvania, and North Carolina. These three states represent the approximate northern, middle, and southern parts of the range of elongate hemlock scale. Sites were used to collect data from which we constructed elongate hemlock scale life tables at locations with short, moderate, and long growing seasons. The longer growing season of North Carolina was expected to allow for two complete generations of elongate hemlock scale, thus enabling us to examine the consequences of improved host-parasitoid synchrony. Four research sites were located in North Carolina, in the Bent Creek Experimental Forest; four sites were selected in the Mont Alto State Forest in Pennsylvania, and four sites were selected in the Tunxis and Nathan Hale state forests in Connecticut. Permits were obtained to collect samples at all study sites. Collectors were hired to send samples from Pennsylvania and North Carolina to the University of Massachusetts. Hemlock stands in all locations were chosen based on a thorough visual survey of hemlock foliage for the presence of elongate hemlock scale. A survey of elongate hemlock scale density in random hemlock forest stands within a 25-mile radius of the selected study sites was conducted to determine how representative scale density and parasitism rates in the study sites were to the regional average.

Elongate Hemlock Scale Phenology in the U.S.

Work at the above mentioned research sites was done in 2006 and 2007. Here, we discuss results from 2006 only. Weekly samples from sites in each of three states (Connecticut, Pennsylvania, and North Carolina) were collected from April to December, 2006. At each site, ten hemlock trees were flagged and numbered. One 15-20 cm branch was collected weekly from each of the ten flagged trees for the duration of the study. Needles were examined on one randomly selected newest-growth tip from each of the ten branches collected. When necessary, additional new-growth tips were examined until a minimum of 20 needles and 10 living scales had been examined from each sample branch (sample size: 100 live scales per site per week for all 11 sites). All scales were classified as live or dead and male or female. The life stage, presence of eggs, and whether or not parasitoid larvae, pupa, or an exit hole was present were also noted for each scale. These data described the phenology of scale life stages at each site as well as the phenology of parasitoid larvae and pupae. In addition, collection of these data allowed calculation of the scale density, parasitism rate, generational mortality, and sex ratio for elongate hemlock scale in the three distinct geographic locations.

Flight Phenology of *Encarsia citrina*

To measure timing of flight of adult *E. citrina*, weekly samples of foliage were collected from 11 sites in three states (Connecticut, Pennsylvania, and North Carolina) from April to December. Ten hemlock trees (different from those used to collect scale) were flagged at each field site and given a number. One 15-20 cm branch was collected weekly from each of these trees for the duration of the study. Resampling the same trees lowered the impact of tree-to-tree scale density variation on our estimates of wasp phenology. Each branch was trimmed from the base until it weighed 4 grams for a total of 40 grams of hemlock foliage from each site per week. This foliage was placed in two emergence containers (20 g each) and held in growth chambers for parasitoid emergence. Growth chambers were programmed weekly with the previous week's mean day and night temperature, approximating the number of degree days experienced at the field sites. Mean daytime and nighttime temperatures were calculated from hourly temperature data obtained from the nearest available weather station. Each foliage sample was held for two weeks and the number of emerged parasitoids counted weekly. Counts were taken weekly to coincide with weekly elongate hemlock scale data. Parasitoid phenology was then compared to phenology of second instars of elongate hemlock scale to measure the degree of host-parasitoid synchrony.

RESULTS

Elongate Hemlock Scale Phenology in the U.S.

In all three states, regional elongate hemlock scale density (number per 100 needles) tended to be lower compared to the specific study sites (Connecticut: 73 vs. 153; Pennsylvania: 84 vs. 185; North Carolina: 9 vs. 36). This was likely due to the fact that the regional survey included all hemlock stands, including those with no elongate hemlock scale, which could not be considered for use as study sites. Similarly, regional parasitism rates tended to be lower compared to that at study sites (Connecticut: 56% vs. 70%; Pennsylvania: 53% vs. 77%; North Carolina: 34% vs. 47%).

Elongate hemlock scale phenology differed markedly with increasing latitude (from North Carolina to Pennsylvania to Connecticut). First instars abundance peaked in mid-July in Connecticut, peaked twice in Pennsylvania (early June and July), and in North Carolina, first instar populations had several small spikes in May and June, with a larger spike in late July. Second instars first appeared in Connecticut in early July and remained at a relatively constant level for the remainder of the year. In Pennsylvania, second instars appeared in early May and then steadily decreased until a peak in early July and dropped to a relatively constant level for the rest of the year. In North Carolina, second instars had a series of peaks throughout the year at approximate five-week intervals starting in early May. In Connecticut, third instars were present at the beginning of the study and represented the previous year's generation that successfully overwintered. New generation third instars begin to appear in mid-July and peaked in late August and early September. A similar pattern was seen in Pennsylvania and North Carolina, except that in North Carolina, a large spike in abundance was seen in early October at nearly twice that seen in July. This likely represents a second generation of elongate hemlock scale reaching maturity. Overall, these phenology data suggested that elongate hemlock scale does not have distinct generations with clear even development of life stages in the northern, middle, or southern portions of its invaded range, but that two generation of the scale do occur in North Carolina.

Synchrony of Flight of *Encarsia citrina* with Second Instars of Elongate Hemlock Scale

In 2006 in Connecticut, *E. citrina* flight was asynchronous with the presence of elongate hemlock scale second instars throughout the year, consistent with McClure's findings (1986). This same relationship was found in Pennsylvania as well. In North Carolina, better synchrony of wasp flight and scale second instars was seen in the spring and summer, but synchrony deteriorated in the fall. *Encarsia citrina* abundance decreased steadily in September and was zero by mid-October, while second instar elongate hemlock scale remained abundant through November.

DISCUSSION

This study did not support the hypothesis that, at more southern latitudes, a complete second generation of elongate hemlock scale would occur and lead to better synchrony with the parasitoid *E. citrina*. Synchrony in North Carolina, relative to Connecticut, was improved in the spring and early summer, but not in late-summer and fall. *Encarsia citrina* was absent in the fall even though second instar elongate hemlock scales were present in substantial numbers. Despite improved spring-early summer synchrony and lower scale density seen in North Carolina, percent parasitism was substantially lower in North Carolina than in Pennsylvania or Connecticut. This was unexpected, as improved synchrony was predicted to increase parasitism rates (McClure 1986).

Two components of this study that are still in progress may help to more clearly explain the relationship between elongate hemlock scale and *E. citrina* in the U.S.: 1) a bagged cohort study to directly assess the impact of *E. citrina* impact on elongate hemlock scale in each study region, and 2) a molecular analysis to determine if cryptic species of *E. citrina* might exist between Japan and the eastern U.S. Results of these studies are being analyzed.

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Entomopathogenic Fungi for Management of Invasive Armored Scales in Northeastern Forests

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ABSTRACT

Armored scales are major pests of plants world-wide. Of importance is that invasive species arrive with limited natural enemies. As a rule, local entomophilous microorganisms are the first factor limiting numbers of invasive species. Entomopathogenic fungi are an important resource for armored scale management. Three invasive species of armored scales, elongate hemlock scale (*Fiorinia externa*), shortneedle conifer scale (*Nuculaspis tsugae*), and European fruit lecanium scale (*Parthenolecanium corni*), were examined as hosts of pathogenic fungi. Three groups of fungi, including real entomopathogens, facultative entomopathogens, and concomitants, were isolated and identified. Epizootic processes provoked by fungi were observed in all sampled insect locations. Manifestation of mycoses in scale populations fluctuated among very light (≤1%), light (1-20%), moderate (21-50%), and heavy (51-85%).

An explosive epizootic was observed in *F. externa* populations, and the following fungi were isolated from this epizootic: *Myriangium* sp., *Colletotrichum acutatum* subsp. *fiorinia*, *Cordyceps* sp., *Beauveria bassiana*, *Lecanicillium muscarium*, *Botrytis* sp., *Phialophora* sp., *Nectria* sp., *Rhinocladiella* sp., and *Trichoderma viridae*. The shortneedle conifer scale was contaminated with *C. acutatum* subsp. *fiorinia*, *B. bassiana*, *L. muscarium*, and *T. viridae*. The fungi *Hirsutella* sp., *B. bassiana*, *L. muscarium*, *Paecilomyces farinosus,* and *Fusarium* sp. were isolated from *P. corni*. The activity of the fungi isolated was tested under laboratory condition using *F. externa* as the host. Entomopathogenic fungi are valuable for development of epizootiological direction of pest control in forests.

KEYWORDS

armored scales, *Fiorinia externa*, *Nuculaspis tsugae*, *Parthenolecanium corni*, entomopathogenic fungi, *Myriangium* sp., *Colletotrichum acutatum* subsp. *fiorinia* *Hirsutella* sp., *Nectria* sp., *Cordyceps* sp., *Beauveria bassiana*, *Lecanicillium muscarium* *Paecilomyces farinosus*, *Phialophora* sp., *Rhinocladiella* sp., *Fusarium* sp., epizooty, insect mortality

INTRODUCTION

Many countries have problems with new invasive pests, including noxious microorganisms, plants, and different groups of animals. One major reason for this influx is the transportation opportunities that result from the globalization of trade. As a rule, exotic invasive organisms create the most serious problems for agriculture and forestry around the world. The hemlock woolly adelgid (HWA), *Adelges tsugae*, the elongate hemlock scale (EHS), *Fiorinia externa* Ferris, and the shortneedle conifer scale (SCS), *Nuculaspis tsugae*, are causing a significant decline of hemlock trees in the U.S. and Canada. Sugar maple and other deciduous trees are damaged by the European fruit lecanium scale (EFL), *Parthenolecanium corni* Bouche. Management of these dangerous exotic invaders should be based on effective biological resources. Among entomopathogenic microorganisms, only fungi are known as mortality agents of scales that provoke insect disease (Weiser 1966; Lipa 1975; Gouli et al. 1981; Parker et al. 2005). This factor is associated with specific morphological and biological properties of the hosts and the fungi.

The importance of fungi in epizootics has been documented, and numerous fungal epizootics in forest insects unknown as pests have been reported in the last ten years (S. Gouli et al. 1999; Gouli and Gouli 2001). For example, the entomophthoralean fungus, *Entomophaga maimaiga*, has been observed reducing gypsy moth populations in the U.S. and Canada (Hajek et al. 2004 and 2006). So, too, the epizootic of EHS has been documented in New England (McClure 2002; Parker et al. 2005).

Scales have a protective covering and feed by piercing tissue and sucking nutrients. This feeding mechanism actually protects the midgut from microbial contamination. Yet, the suppression of gypsy moth populations by a fungus, for instance, has stimulated research activity of microbials for forest pest management of other species. Thus, the objectives of this research were to isolate and identify the entomopathogenic fungi responsible for the epizootic of EHS, determine their mass-production potential, and evaluate fungal efficacy in the laboratory and field.

MATERIALS AND METHODS

Samples of the *F. externa* and *N. tsugae* were collected from 2003 to 2007 from 35 different locations in New York, North Carolina, Pennsylvania, Connecticut, and Vermont, while *P. corni* was found on trees at the Jericho Research Forest, University of Vermont. Pathological material was examined using two methods: direct microscopic analyses for exposure of propagules relating to entomophthoralean fungi and inoculation of artificial media. The microscopic analyses were done by preparing squash slides and examining them under a phase contrast microscope. Smears were prepared from dead and diseased insects and stained with methyl cotton blue (Goettel and Inglis 1997). Isolation and culturing was done on potato dextrose agar (PDA), Sabouraud dextrose agar and yeast (SDAY), and our experimental nutrient media based on TC-100 medium for cultivation of insect cells. Identification of fungal isolates was based on traditional morphological methods and rDNA analysis (Marcelino 2007).

Insecticidal activity of fungal strains was tested on natural populations of EHS. They were field-collected from eastern hemlock trees at the Mount Tom Forest Preserve (Holyoke, Massachusetts) located outside the known area of the *F. externa* epizootic. Hemlock tree branches 5 cm long with new growth and infested with a healthy populations of EHS were used for these experiments. Conidial suspensions for inoculation of insects contained of 10^6 and 10^7 conidia/ml sterile distilled water (SDW) with 0.02% Silwet. Control EHS were sprayed with SDW with 0.02% Silwet. Bioassays were repeated three times with four replicates/treatment. For bioassays of EFL, selected branches of maple trees with heavy infestation of the scales were treated in the forest.

Statistical analysis included a post-hoc Turkey-Kramer test for comparison between fungal isolates within a test insect species. The effect of suspension concentration was determined with an adjusted least square means. $P \leq 0.05$ was considered statistically significant. All statistical analyses were performed using SAS (SAS 1990) and plotted SPSS (SPSS 2005).

RESULTS

Each of the populations of the three species of scales in the field contained individuals with typical signs of mycoses. Manifestation of this mycoses fluctuated and was ranked according to the scale: very light (≤1% of the population with mycoses), light (1-20%), moderate (21-50%), and heavy (51-100%) (Table 1).

Direct microscopically analyses of the scales did not expose any morphological structures related to entomophthoralean fungi. The pathological material from the three scale species contained mycelial masses and conidia typical of hyphomycetous fungi (Table 2; photographs in Figures 1 through 9). Sometimes, the traditional approach for identification of these fungi did not provide positive results. In particular, identification of *C. acutatum* subsp. *fiorinia* was difficult. Initially, this fungus was identified in China as *Aschersonia marginata*. The final identification was based on rDNA analyses. Unfortunately, the modern molecular-genetic information devoted to Hyphomycetes fungi does not contain a wide variety of species, and sometimes it is difficult to identify uncommon species.

For fungal isolates responsible for the epizootic in EHS populations, a phylogenetic analyses using six of the most commonly studied nuclear genes in molecular phylogenetics (D1/D2 domain of the 28 rDNA gene, ITS region, β-Tubulin 2, GPDH gene, GS gene and HMG box at the MATI-2 mating-type gene) and RAPDs was made. These analyses showed the fungal strains were closely related to phytopathogenic strains of *C. acutatum* and that they may represent a single population lineage of this species (i.e., *Colletotrichum acutatum formae specialis fiorinia*). This new subspecies displays a propensity to induce rapid disease and mortality in *F. externa* populations. Though a large body of information exists regarding the phytopathogenic genus *Colletotrichum*, the *C. acutatum* subsp. *fiorinia* (*Caf*) is only the second reported entomopathogenic strain of this genus. Periodically, this strain is isolated together with the entomopathogenic fungi *Myriangium* sp. (*Ms*), and *Cordyceps* sp. (*Cs*).

All fungi isolated from scales can be divided into three groups: 1) real entomopathogens, 2) facultative entomopathogens, and 3) concomitants with phytopathogens and antagonists. Epizootic processes provoked by fungi were observed in all sampled locations. Manifestation of mycoses in scale populations fluctuated from being very light (≤1% of the population), light (1-20%), moderate (21-50%), and heavy (51-85%). It is noteworthy that an explosive epizootic was observed in *F. externa* populations in some locations.

Table 1. Forest sites where epizootics were found impacting scale populations, 2002-2006. EHS = elongate hemlock scale; SCS = shortneedle conifer scale; EFL = European fruit lecanium scale.

State	Year	Site Found	Scale species	Natural scale mortality, %	Collector
NY	2002	Bedford	EHS SCS	15.1 50.5	McClure
	2003 2005 2006	South Salem	EHS	61.8 67.3 83.2	L. Schwarzberg S. Gouli J. Marcelino
	2003	Ward Pound Ridge Reservation	EHS	10.9	L. Schwarzberg
	2004	Esopus	EHS	2.0	L. Schwarzberg
	2003	Mohonk	EHS	42.0	L. Schwarzberg
	2004	Mianus River	EHS	36.8	L. Schwarzberg
	2005	Gorge	EHS	0.5	L. Schwarzberg
PA	2004	Bowmans Hill	EHS	5.5	M. Blumenthal
	2004	Valley Forte	EHS	4.8	M. Blumenthal
	2004	Zoedosburg	EHS	10.8	M. Blumenthal
	2004	Barph Storm	EHS	27.6	M. Blumenthal
NJ	2005	Ringwood St. Park	EHS	21-50	J. Marcelino
	2005	Wanaque Reservoir	EHS	21-50	J. Marcelino
CT	2006	Macedonia State Park	EHS	21-50	J. Marcelino
VT	2006	Jericho Research Forest Fairfax	EFL EFL	1.5-3.2 3.5-4.2	M. Skinner M. Skinner

Table 2. Fungi isolated from locations where epizootics were found associated with scales on trees.

Scale Species	Fungi	Figure Reference
Elongate hemlock scale	*Myriangium* sp. *Colletotrichum acutatum* subsp. *fiorinia* *Cordyceps* sp. *Beauveria bassiana* *Lecanicillium muscarium* *Botrytis* sp. *Phyalophora* sp. *Nectria* sp. *Rhinocladiella* sp. *Trichoderma viridae*	1, 2 1, 2 3 4, 6 5, 7
Shortneedle conifer scale	*Colletotrichum acutatum* subsp. *fiorinia* *Beauveria bassiana* *Lecanicillium muscarium* *Trichoderma viridae*	1, 2 4, 6 5, 7
European fruit lecanium scale	*Hirsutella* sp. *Beauveria bassiana* *Lecanicillium muscarium* *Paecilomyces farinosus* *Fusarium* sp.	9 4, 6 5, 7 8

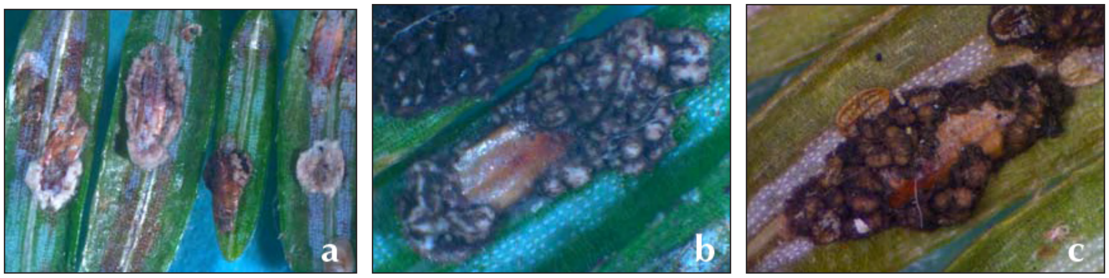

Figure 1. Mycosis of elongate hemlock scale caused by *Myriangium* sp. and *Colletotrichum acutatum* subsp. *fiorinia*. (Sclerotic masses on scale bodies initially have white color (a) and than have darkened (b, c).

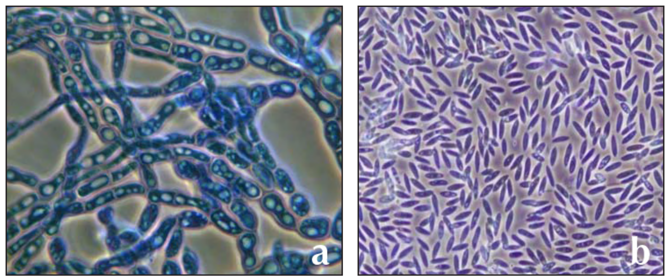

Figure 2. *Myriangium* sp. (a) at 100x magnification, and *Colletotrichum* (b) at 40x magnification (b). Cotton blue stain.

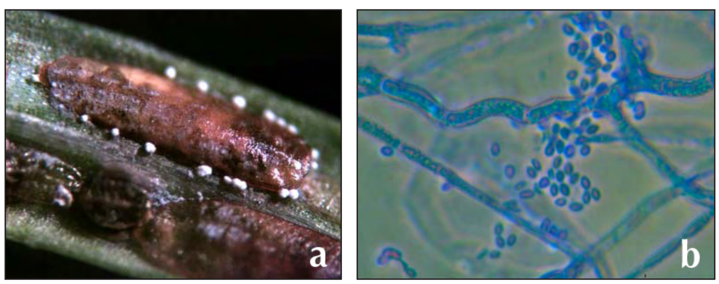

Figure 3. Mycosis of elongate hemlock scale caused by *Cordyceps* sp. (a) and *Cordyceps* sp. under light microscope (b), cotton blue stain, objective x 100.

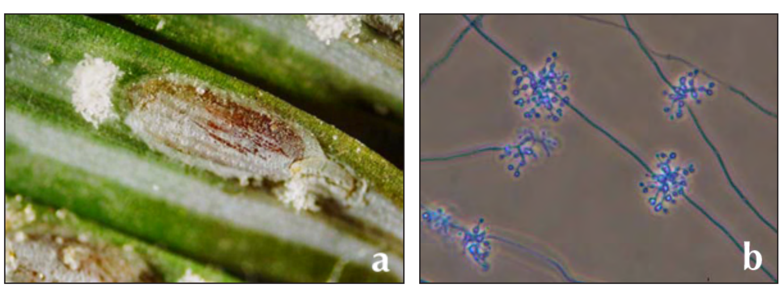

Figure 4. Mycosis of elongate hemlock scale caused by *Beauveria bassiana* (a) and *B. bassiana* under light microscope (b), cotton blue stain, objective x 100.

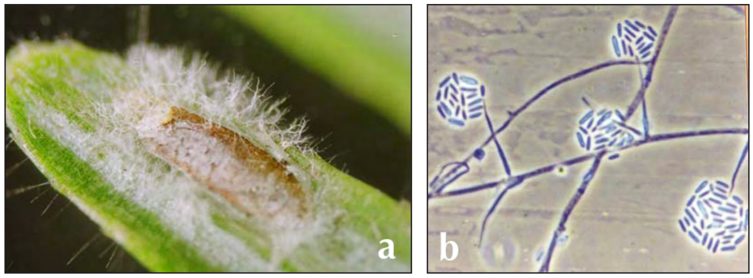

Figure 5. Mycosis of elongate hemlock scale caused by *Lecanicillium muscarium* (a) and *L. muscarium* under light microscope (b) cotton blue stain, objective x 100.

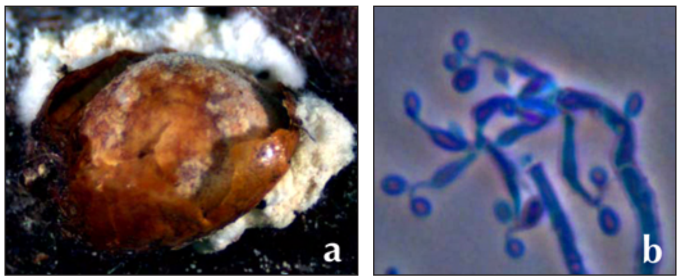

Figure 6. Mycosis of European fruit lecanium scale caused by fungus *Beauveria bassiana* (a) and *B. bassiana* under light microscope (b), cotton blue stain, objective x 100.

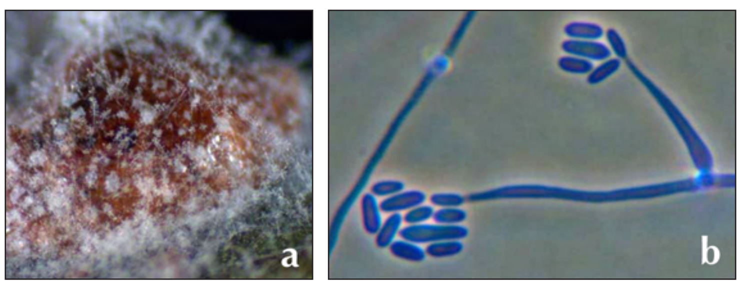

Figure 7. Mycosis of European fruit lecanium scale caused by *Lecanicillium muscarium* (a) and *L. muscarium* under light microscope (b), cotton blue stain, objective x 100.

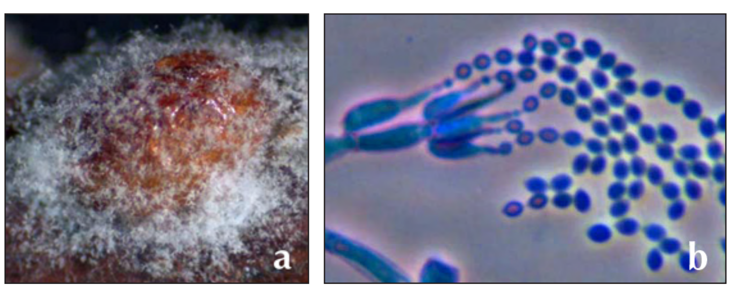

Figure 8. Mycosis of European fruit lecanium scale caused by *Paecilomyces farinosus* (a) and *P. farinosus* under light microscope (b), cotton blue stain, objective x 100.

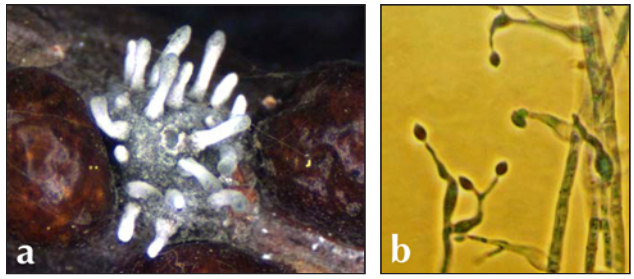

Figure 9. European fruit lecanium scale with signs of *Hirsutella* sp. mycosis (a) and *Hirsutella* sp. under light microscope (b), cotton blue stain, objective x 100.

The specific character of disease manifestation (Figure 1) and results of mycological analyses of EHS bodies and sclerotic masses led us to conclude that the two fungal species, *Myriangium* sp. and *Colletotrichum acutatum* subsp. *fiorinia*, were responsible for the natural epizootic in *F. externa* and *N. tsugae* populations. Only these fungi can form specific sclerotic masses in culture. Unfortunately, the sclerotic masses contain only mycelia without any other morphological structures that are suitable for preliminary identification of fungi using direct microscopic analyses.

Colletotrichum is a well-known genus containing phytopathogenic species; there is only one report that we found of an entomopathogenic species in the *Colletotrichum*. This was *C. gloeosporioides*, infecting the citrus scale in Brazil (Cesnik and Ferraz 2000). To confirm that our isolate of *Colletotrichum* was an entomopathogenic species, bioassays were conducted using field-collected scales. A series of fungal inoculations of EHS were done under laboratory conditions. Initially, eight isolates of *Colletotrichum* were tested to obtain the most active strains. Definite symptoms and signs of infection were observed among the insects treated (Figures 10 and 11).

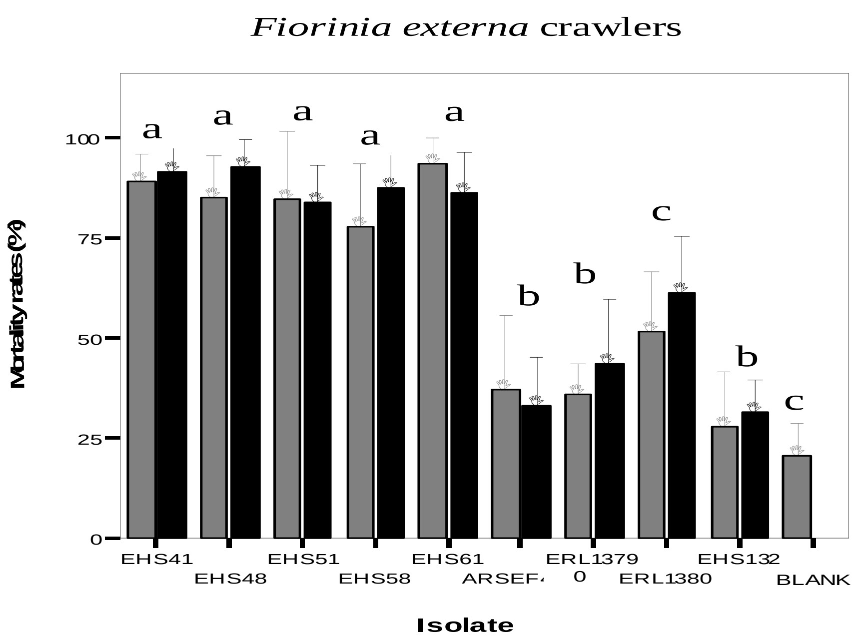

Figure 10. Mortality of elongate hemlock scale crawlers treated with isolates of *C. acutatum* subsp. *fiorinia*.

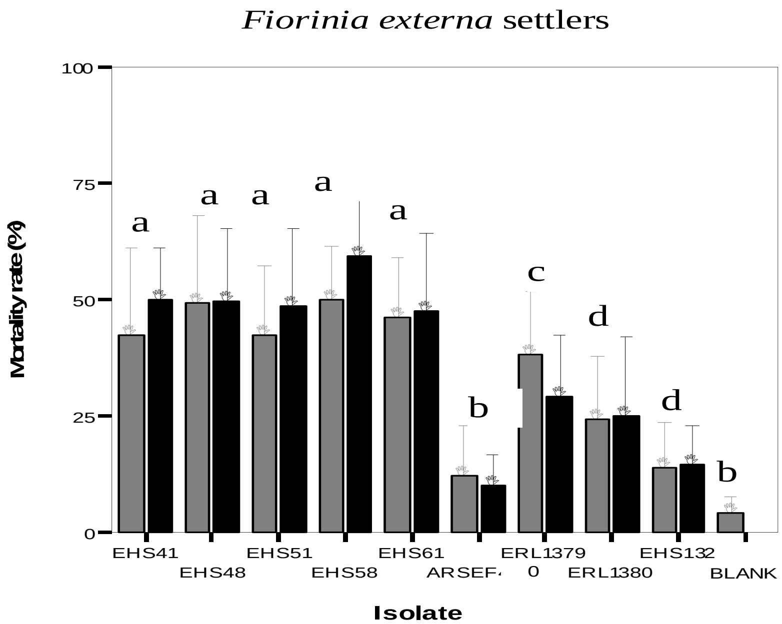

Figure 11. Mortality of elongate hemlock scale settlers treated with isolates of *Colletotrichum acutatum* subsp. *fiorinia*.

The high level of EHS mortality in several northeastern states is caused by several fungi that are not well known. For example, *Myriangium* sp. is an obligatory scale pathogen that is very difficult to culture on artificial media. It grows slowly, and development does not accompany the formation of typical conidia. At the present time, the information about cultural peculiarities of *Myriangium* and pathogenesis of mycoses is limited. It is possible that the natural epizooty in *F. externa* populations is linked with a fungal complex, but at the present time, it is not known what importance each species in this natural epizootic has.

Several combinations of fungi, including *Colletotrichum* with *Myriangium* and *Colletotrichum* with *Cordyceps* sp., were tested under laboratory conditions against *F. externa*. Hemlock twigs with new growth (10 cm) naturally infested with visually healthy EHS adults and immatures were kept individually in tubes containing 5 g sterilized sand and 2.5 ml SDW. The twigs were sprayed with conidial suspensions with a total concentration of 1 x 10^7 conidia/ml; each fungus in the combination contained 0.5 x 10^7 conidia/ml. Two concentrations of conidia (0.5 x 10^7 and 1.0 x 10^7) for single species of fungi were used as controls. EHS crawler mortality after one and two weeks, respectively, was: for *Colletotrichum* strain EHS-41, 62%-83% and 79%-91%; for *Myriangium* strain ERL-1400, 35%-48% and 47%-68%; for *Cordyceps* strain EHS-132, 40%-54% and 65%-72%; for *Colletotrichum* mix with *Myriangium*, 76%-82% and 78%-88%; for *Colletotrichum* mix with *Cordyceps,* 79%-87% and 81%-91%; and for the control, 11%-16% and 15%-19%. EHS settlers mortality after one and two weeks, respectively, was: for *Colletotrichum* strain EHS-41, 43%-57% and 50%-67%; for *Myriangium* strain ERL-1400, 28%-35% and 33%-48%; for *Cordyceps* strain EHS-132, 29%-38% and 39%-55%; for *Colletotrichum* mix with *Myriangium*, 61%-68% and 57%-70%; for *Colletotrichum* mix with *Cordyceps,* 59%-68% and 65%-81%; and for the control, 3%-6% and 5%-8%. *Myriangium* and *Cordyceps* caused a relatively low level of mortality because they grow slowly. Mixed fungal infections demonstrated a synergistic effect, but insect cadavers contained only mycelial masses, and identification of fungal species responsible for the mortality was impossible. *Colletotrichum* and an accompanied fungus were isolated on artificial media. This allowed us to conclude that both species developed simultaneously in the body of scales.

A separate experiment was conducted in a deciduous forest with European fruit lecanium scale and *Hirsutella*. Selected branches of scale-infested maple trees were treated using a suspension of *Hirsutella* having a concentration of 5 x 10^7 conidia/ml SDW. External signs of mycosis on the scales were subsequently observed among 35% of the population. But development of epizootic process must have been in progress because the fungus *Hirsutella,* like *Myriangium*, has specific peculiarities of growth and sporulation.

CONCLUSION

Entomophilous fungi are an important factor affecting the density and harmfulness of scale populations. The dynamics of the natural epizooty expansion in populations of *F. externa* and *N. tsugae* and the study of pathogeneses of mycoses in the laboratory helped us conclude that *Myriangium* sp. and a new subspecies, *Colletotrichum acutatum*, are the principal causative agents of EHS mortality. *Colletotrichum acutatum* is a ubiquitous species, and this pathogen is an important part of epizootiological processes.

Microscopic analyses of cadavers and diseased insects from nature did not reveal any morphological spore-forming structures of *C. acutatum*. It is possible that this fungus is a stress factor accelerating the epizootic process. Fungi from the genus *Myriangium* have a very limited possibility of distribution because this group of fungi is characterized by slow growth and limited spore production. The possibility of using fungi from genus *Colletotrichum* for scale control is problematic because the genus contains mainly phytopathogenic species. As a stress factor, *Colletotrichum* is distributed everywhere, but the presence of *Myriangium* is limited. *Myriangium* is an ideal pathogen for causing and expanding an epizootiological event for control of forest pests. The primary problem is with the mass-production and formulation of this insect pathogen. It is critical to receive additional information related to pathogenesis of *Myriangium* mycosis at the organismal and population levels.

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Comparing Hemlock Woolly Adelgid and Elongate Hemlock Scale Control in Ornamental and Forest Hemlocks

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ABSTRACT

Eastern hemlock, *Tsuga canadensis*, and Carolina hemlock, *T. caroliniana*, are important members of native forest communities and urban landscapes in the eastern United States. The hemlock woolly adelgid (HWA), *Adelges tsugae*, is the most important sternorrynchan pest of these two species in both natural and managed settings. However, three additional exotic armored scales (Diaspididae) infest *T. canadensis* and other conifers in the eastern United States. These include elongate hemlock scale (EHS), *Fiorinia externa* Ferris, *Nuculaspis tsugae* (Marlatt), and cryptomeria scale, *Aspidiotus cryptomeriae* Kuwana. The elongate hemlock scale and *N. tsugae* are serious pests of hemlocks and several conifers in the Northeast and mid-Atlantic regions of the United States (McClure and Fergione 1977; Stimmel 1986). McClure (2002) stressed that, while control of these pests is difficult in native forest stands, good progress has been made in managing these pests in managed urban landscapes. This report summarizes our recent work and the work of several others who have evaluated insecticides to manage *A. tsugae* and *F. externa* in managed landscapes and forests.

KEY WORDS

hemlock woolly adelgid, elongate hemlock scale, systemic insecticides, growth regulators, imidacloprid

HEMLOCK WOOLLY ADELGID AND INSECTICIDAL CONTROL

Early attempts to control the hemlock woolly adelgid revealed good efficacy of foliar insecticides, including insecticidal soap and oil as well as numerous petrochemical insecticides (McClure 1987 and 1988). Thorough coverage was the key to effective control with contact insecticides applied to foliage. In 1992, McClure presented convincing evidence that several systemic insecticides, including oxydemetonmethyl, bidrin, and acephate, provided excellent levels of control when injected or implanted through the bark of the tree.

In the early 1990s, a new class of insecticides became available to the green industries and foresters, the neonicotinoids. Imidacloprid was the first of its class to become widely used against a wide variety of sucking insects, and its systemic activity allowed it to be applied through the trunk of the tree or through the soil, from which it was absorbed by roots and distributed through the canopy. McClure et al. (2001) noted that soil applications had advantages over trunk injections or implants in that they do not wound the tree. McClure et al. (2001) also noted that healthy sap flow was vital to transporting and distributing systemic insecticides from the soil throughout the canopy of the tree. Severe damage by the adelgid reduced the ability of hemlocks to transport and distribute imidacloprid. Steward and Horner (1994) demonstrated that imidacloprid applied as a soil injection provided excellent control of hemlock woolly adelgid on established Canadian hemlocks in a formal public garden. Rhea (1996) investigated the use of insecticidal soap, horticultural oil, and imidacloprid on HWA populations on mature hemlocks in recreation areas in national forests in Virginia. All three materials provided very good levels of control greater within the first year of application. Doccola et al. (2003) was the first to demonstrate satisfactory levels of control with injections of imidacloprid (Imicide) through the bark of hemlocks. Mortality of adelgids on imidacloprid-treated trees was more than twice that of adelgids on untreated trees. In a recent study, Cowles et al. (2006) found that several types of soil applications of imidacloprid provided good to excellent levels of control of HWA, ranging from 50% to 100% in the first year and from 83% to 100% in the second year following application. Conversely, none of the trunk injections attempted with Mauget systems, Wedgle systems, or Arborjet systems provide control.

We have conducted several studies evaluating the efficacies of conventional and neonicotinoid insecticides for control of HWA and EHS in managed landscapes and forests. The following vignettes summarize the results of these studies and their implications for managing these pests.

HWA Control on Hemlocks in Residential Landscapes with Soil Drenches

The first study was conducted in a residential landscape in Frederick County, Maryland. Hemlocks in this study were specimen trees ranging in size from 13 to 58 cm diameter at breast-height (DBH). All had been infested with hemlock woolly adelgid for several years. Prior to the application of imidacloprid, the condition of all trees was evaluated by examining their canopies for new growth, needle loss, and dieback. Trees were rated and placed into one of three categories based on the condition of their canopies: healthy, poor, or with dieback. We found that hemlocks recovered dramatically with new growth once the pressure of the adelgids was reduced following an application of imidacloprid.

Interestingly, the response of trees to imidacloprid therapy differed in relation to their condition at the onset of the experiment. Trees with the healthiest, most foliated canopy improved the least following the reduction in adelgid populations. Trees with little new growth but no dieback recovered the quickest and most dramatically, and trees in the poorest condition at the onset recovered impressively but more slowly. Trees left untreated remained sparsely foliated, with dieback. Despite being surrounded by untreated trees with adelgids, trees treated with imidacloprid remained free of HWA for more than 816 days after treatment, when the study was terminated (Webb et al. 2003).

HWA Control on Hemlocks in Native Stands with Trunk Injections of Imidacloprid

Our second study examined the efficacy of the Ima-jet formulation of imidacloprid (5% active ingredient) injected through the trunk of the tree with the Arborjet Tree I.V. injection system in reducing populations of HWA in a forest setting. Trees used in this study were part of a native stand of eastern hemlocks in the Green Ridge State Forest in Allegheny County, Maryland. Eighteen eastern hemlocks were assigned to one of two treatments: half received trunk injections of imidacloprid and the other half served as untreated controls. Trees were treated on May 27, 2005. The DBH of each tree was measured and imidacloprid was applied using the label recommended rates of 2 ml for trees less than 11 inches DBH and 4 ml for trees between 12-23 inches DBH. Diameters of all trees ranged from 8-20 inches of DBH. At the time of application two branches infested with adelgids were marked with plastic tags on each tree. On the distal 30 cm of each branch, the number of adelgids was counted. These counts were combined to give an adelgid density expressed in number of adelgids per 60 cm of branch. Counts were repeated on October 20, 2005, and June 13, 2006, on each tree. Data were examined to see if assumptions for analysis of variance were met. For pre-counts made in May 2005 and post-counts made in June 2006, assumptions were met and data were analyzed using the general linear models procedure (Zar 1999, Statistix Version 8). Data collected in October 2005 did not meet the assumptions for an analysis of variance and were analyzed with a Kruskal-Wallice non-parametric analysis of variance test (Zar 1999, Statistix Version 8).

The results of the analysis of pre-counts indicated that HWA densities did not differ between treated and untreated hemlocks ($F_{1,16} = 0.24$, $P < 0.63$) (Figure 1). The non-parametric analysis of counts taken five months after the application of imidacloprid detected marginally significant differences in the densities of HWA on treated and untreated trees ($\chi^2 = 3.17$, $P < 0.07$) (Figure 1). Counts taken in June 2006 revealed a significant reduction in the number of adelgids on trees injected with imidacloprid ($F_{1,15} = 8.16$, $P < 0.01$) (Figure 1). This study provides evidence that hemlocks can be protected from HWA in forest sites inaccessible to vehicles using the Arborjet Tree I.V. system. This system is easily transported and can be used by one or two individuals.

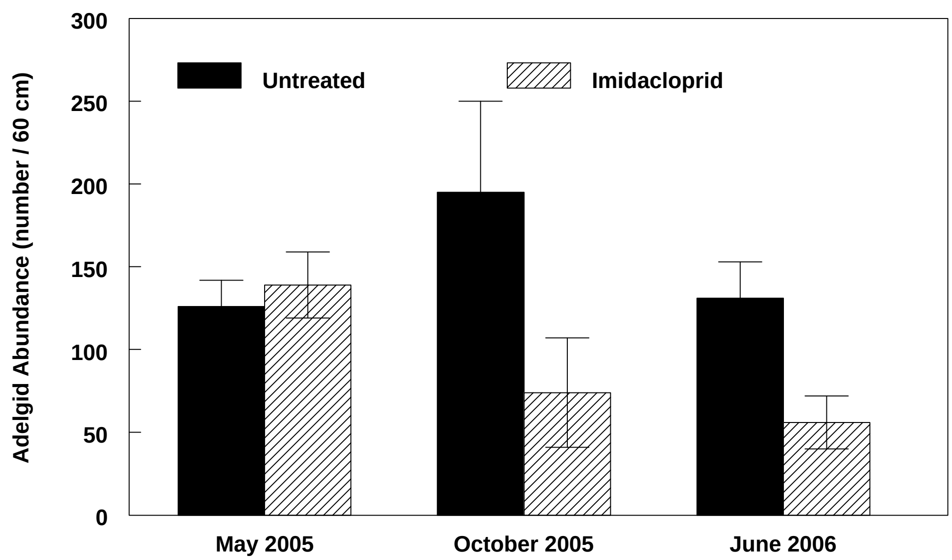

Figure 1. Density of hemlock woolly adelgid on untreated hemlocks and those treated with Arborjet Tree I.V. injections of imidacloprid. May 27 represents the density at the time of the pesticide applications and October 2005 and June 2006 represent the density of the same branches approximately five and 13 months later, respectively. Bars represent means and vertical lines are standard errors.

HWA Control on Hemlocks in Native Stands with Soil Drenches of Imidacloprid and Dinotefuran

Our third study evaluated the efficacies of two neonicotinoid insecticides, dinotefuran (Safari), and imidacloprid (Merit), in a forest setting. Hemlocks used in this study were part of a large population of eastern hemlocks growing under natural conditions in a forest in Harford County, Maryland. We visited the site on June 6, 2006, and active adelgid infestations were confirmed on 16 small trees distributed over approximately one acre of forest. All trees in the study were less than 10 inches DBH.

We performed pre-treatment counts of adelgid populations on these trees by counting the adelgids on two small branches on each tree. Trees were then randomly assigned to four treatment categories. Adelgid densities did not differ among treatments on June 6 for the pre-treatment counts ($F_{4,11}$ = 0.85, P = 0.52). At the time of treatment, densities were fairly light, only about two to three adelgids per centimeter of branch (Figure 2). We applied dinotefuran at the rates of 3 grams, 6 grams, and 12 grams of insecticide per inch DBH as a soil drench. We also treated trees with a soil drench of imidacloprid at the rate of 2 grams per inch DBH. Four trees were left untreated. We returned to the site on October 23, 2006, to see how well our insecticides performed. Once again, we counted all adelgids on previously marked branches. By October, adelgid densities differed significantly among the trees in different treatments by a Kruskal-Wallice non-parametric analysis of variance (χ^2 = 11.4, P = 0.02).

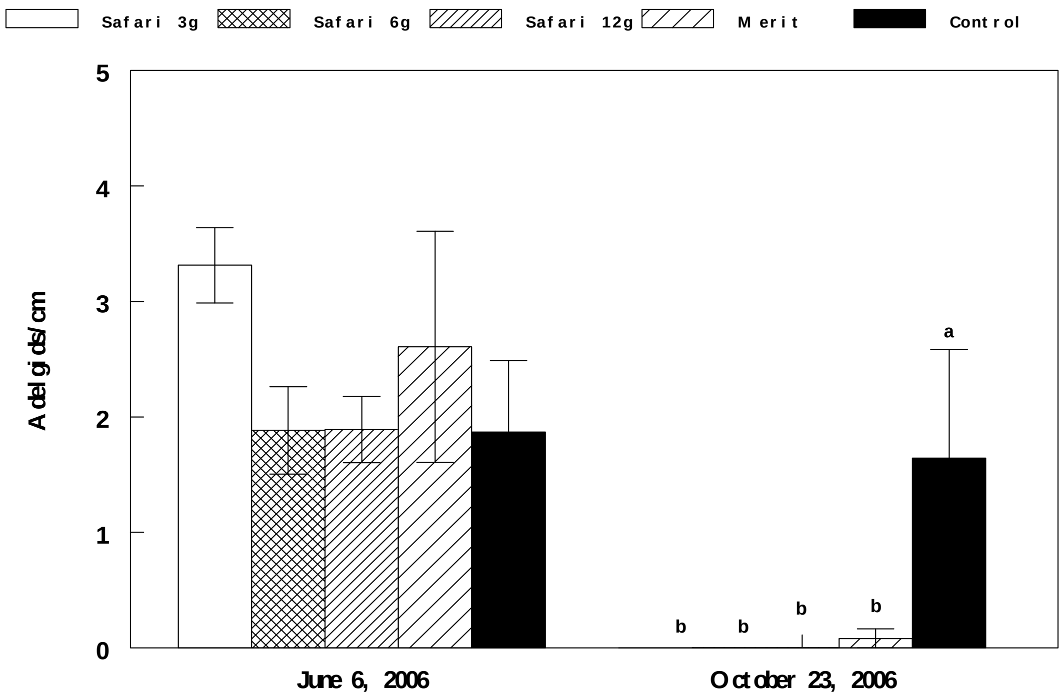

Figure 2. Effects of soil drenches of Safari (dinotefuran) and Merit (imidacloprid) on hemlock woolly adelgid on eastern hemlocks in a native forest. Pre-counts were made on November 6, 2006, and post-counts were made on April 18, 2007. Bars represent means and vertical lines are standard errors. Means that share a letter do not differ by a multiple comparison test (P< 0.05).

All rates of dinotefuran annihilated the adelgid, and no living adelgids could be found on the treated trees (Figure 2). Imidacloprid also provided very high levels of control, and only a few living adelgids were seen. There was no indication of phytotoxicity in any treatment. One interesting facet of this study was that all equipment needed to apply the pesticides was transported more than a half mile to the application site in a deep ravine by three people. A stream approximately 100 feet from the application site was used as the source of water, which was transported to the trees in a five-gallon pail. This study clearly demonstrates the feasibility of using soil drenches of systemic insecticides to control HWA in remote sites inaccessible to vehicles.

HWA Control on Hemlocks in a Residential Landscape with Soil Drenches of Imidacloprid and Dinotefuran

Our fourth study investigated eastern hemlocks that were part of a managed planting of growing in a lawn at an estate near Charlottesville, Virginia. These were very large trees ranging in size from 15 to 55 inches DBH. Counts of the HWA were made prior to the application of insecticides on November 6, 2006, and active adelgid infestations were confirmed on 28 hemlock trees distributed over approximately five acres of lawn. We applied Safari (dinotefuran) at the rates of 3 grams and 6 grams per inch DBH and a soil drench of Merit (imidacloprid) at the rate of 2 grams per inch DBH. Trees were treated on November 6, 2006. We performed pre-treatment counts of adelgids on these trees by marking several heavily infested twigs, removing two twigs about 20 cm long, and returning these to the laboratory where the number of adelgids was determined microscopically. Post-counts of adelgids were made on April 18, 2007, at 163 days after treatment. On this sample date, we removed two additional twigs and counted the number of adelgids as in the pre-counts. Data were examined for normality and homogeneity. Adelgid densities at the pre-treatment counts were not normal and were normalized with a square-root transformation. A Bartlett test indicated homogeneity of variance. Treatments were compared with an analysis of variance on the transformed data (Zar 1999, Statistix 8.1). Post-treatment counts of adelgid densities were not normal and could not be normalized through transformations. Treatments were compared with a Kruskal-Wallice non-parametric analysis of variance (Zar 1999, Statistix 8.1).

Adelgid densities did not differ among treatments on November 6, 2006, for the pre-treatment counts ($F_{3,24} = 0.45$, $P < 0.72$) (Figure 3). On April 18, 2007, 163 days after insecticides were applied, adelgid densities were significantly reduced on trees that received dinotefuran but not on trees treated with imidacloprid (Kruskal-Wallice non-parametric analysis of variance ($\chi^2 = 17.49$, $P < 0.0001$) (Figure 3). This report calls into question whether the dose of 2 grams per inch DBH of imidacloprid is sufficient to adequately control HWA on very large trees in landscape settings, or at least in the timeframe of this study.

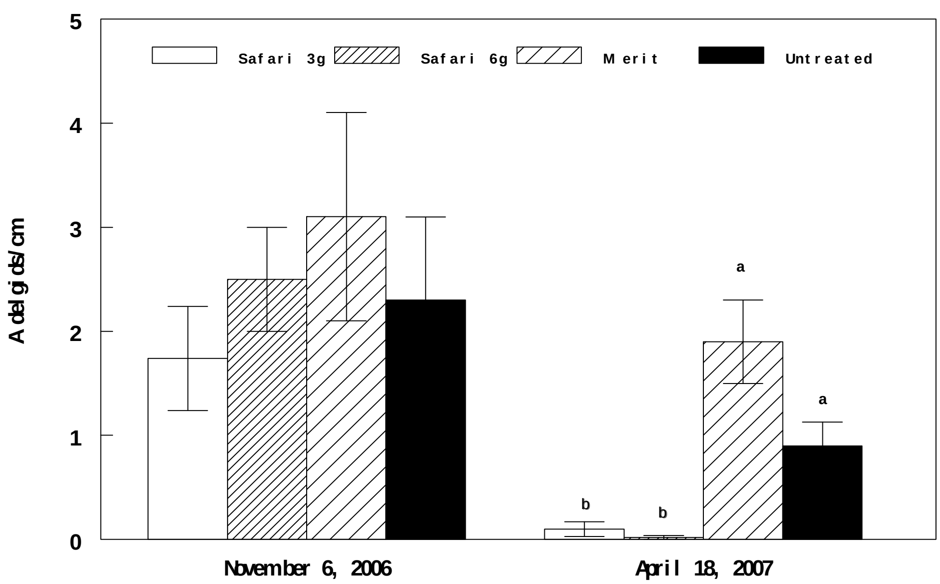

Figure 3. Effects of soil drenches of Safari (dinotefuran) and Merit (imidacloprid) on hemlock woolly adelgid on eastern hemlocks in a residential landscape. Pre-counts were made on November 6, 2006, and post-counts were made on April 18, 2007. Bars represent means and vertical lines are standard errors. Means that share a letter do not differ by a multiple comparison test (P< 0.05).

ELONGATE HEMLOCK SCALE AND INSECTICIDAL CONTROL

Prior to advent of systemic neonicotinoid insecticidal control of EHS relied on the use single or multiple applications of contact insecticides, such as dimethoate or horticultural oil. Well-timed applications targeted vulnerable stages such as crawlers (Davidson and McComb 1958; Wallner 1962; McClure 1977; McClure 2002). Dimethoate and horticultural oil continue to be widely used to control EHS in nurseries and Christmas tree plantations with varying levels of success. For example, Heller and Kline (2005) produced high levels of control of elongate hemlock scale on Frazer fir with multiple applications of dimethoate. However, control of EHS scale is complicated due to overlapping generations over much of the growing season (Davidson and McComb 1958). In landscape settings, contact insecticides are most useful where trees can be completely covered and are not obstructed or near environmentally sensitive areas such as rivers or streams.

As with HWA, hydraulic applications to large trees are often complicated or impossible in many urban forest situations due to proximity of buildings, presence of vehicular and pedestrian traffic, and restrictions related to pesticide movement and exposure to non-target organisms. Similarly, in remote locations in natural forest stands such as at the headwaters of small, mountain streams, it may be impossible to access infested trees by vehicles with hydraulic applicators. In these situations, systemic insecticides may provide the only means of administering insecticides.

Presently, we are conducting a study to examine the efficacy of two approaches for controlling elongate hemlock scale: 1) hydraulic applications of the relatively new insect growth regulator pyriproxyfen (Distance) and horticultural spray oil; and 2) soil and trunk injections of the systemic insecticides imidacloprid (Merit), dinotefuran, (Arborjet), and acephate (Acecap). The objectives of our study are threefold: first, we are interested in determining if single applications of insecticides listed above provide significant control of elongate hemlock scale; second, we are interested to how these materials perform within a single growing season and for one and two years after the application; and finally, we are examining the effects of these applications on the community of natural enemies found in the canopies of treated and untreated trees.

The study is being conducted at the United States National Arboretum in Washington, DC. Sixty-three eastern hemlocks infested with EHS are being used in the study. Hemlocks are mature trees and parts of managed landscape plantings. They range in size from 6 to 37 inches DBH. Trees were randomly assigned to one of seven experimental regimes: foliar application of pyriproxyfen, foliar application of horticultural spray oil, soil drench of imidacloprid, trunk injections of imidacloprid, implantations of acephate, and trunk injections of dinotefuran. Branches were removed with hand or pole pruners from trees and the number of living scales counted on four samples of young foliage from each tree. Trees were sampled on four dates in 2004 and three dates in both 2005 and 2006.

Results of the efficacy trail are encouraging and have been submitted to a refereed journal for consideration. This submission precludes a more detailed report of our findings here. However, reductions in scale abundance were observed with foliar sprays of pyriproxyfen and horticultural oil and soil drenches of imidacloprid on several dates, both in the year of application and in subsequent years. The abundance of natural enemies—notably, the parasitoid *Encarsia citrina* was also affected by applications of insecticides.

ACKNOWLEDGMENTS

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Interactions Between Hemlock Woolly Adelgid and Elongate Hemlock Scale in New England Hemlock Forests

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ABSTRACT

The rapid population growth rates and high population densities of invasive species often leads to strong competition between invasive herbivores (Denno et al. 1995). On the east coast of the U.S., eastern hemlock (*Tsuga canadensis*) forests are threatened by two invasive herbivores, the elongate hemlock scale (EHS), *Fiorinia externa*, and the hemlock woolly adelgid (HWA), *Adelges tsugae*. Despite predictions that HWA would largely extirpate hemlocks in southern New England by the late 1990s, healthy hemlock stands still exist in areas where HWA has been present for over 15 years (Preisser et al. 2008). One possible explanation of the slower rate of hemlock mortality in these areas is the range expansion of EHS into southern New England in the mid-1970s. Although recent surveys have shown hemlock mortality to be more strongly related to HWA density than to EHS density (Preisser et al. 2008), the interactions between these two species remain largely unexplored (McClure 2002). In summer 2005 and 2007, we re-surveyed 141 hemlock stands (first surveyed in 1997-98; see Orwig et al. 2002 for details) in a 7,500 km^2 transect from southern Connecticut to northern Massachusetts. While HWA has declined in population density over this period, EHS continues spreading north and east into New England and has sharply increased in both density and abundance between 1997 and 2005 and between 2005 and 2007.

One explanation for the spread of EHS into New England involves its local adaptation to colder northern winters. In order to assess whether northern populations of EHS show greater cold-tolerance than do southern populations, we collected EHS-infested hemlock foliage from four Maryland sites (the southern population) and four Connecticut sites (the northern population) in March 2004. We then used a freezer to expose EHS-infested foliage to one of seven treatments: 0, 6, 12, 18, 24, 30, or 36 hours at -15°C. One week after the treatments were applied, we counted the percent of surviving scales (up to 100 scales/branch) per treatment per site per location (Maryland or Connecticut). In the absence of cold shock, southern EHS populations had higher survival than did northern populations. Survival of southern EHS decreased significantly as the length of the cold shock treatment increased; in

contrast, survival of northern EHS populations was unaffected by up to 36 hours at -15ºC. Our finding that northern EHS populations are more cold-tolerant than southern populations suggests that local adaptation has facilitated this species' invasion.

Another explanation for EHS expansion into New England involves the possibility that HWA may somehow facilitate EHS spread into hemlock forests. We performed a multi-year experiment in which uninfested hemlock foliage was inoculated with HWA only, EHS only, both, or neither species. We assessed the experimental foliage every six months for 2.5 years for insect density and foliage growth. The population densities of both HWA and EHS were ~30% lower in the HWA+EHS treatment than when each species was grown alone, suggesting strong interspecific competition rather than facilitation. Foliage growth in the HWA-only treatment was lower than in the other three treatments; there was no difference in foliage growth between the EHS-only, HWA+EHS, and control treatments. Our results suggest the need for larger-scale experimental manipulations of both invasive herbivore species in order to understand the potential impact of mixed HWA and EHS infestations on forest health.

KEYWORDS

Adelges tsugae, *Fiorinia externa*, *Tsuga canadensis*, competition, range expansion

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Hemlock Woolly Adelgid Initiative: Progress and Future Direction

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ABSTRACT

In 2001, the USDA Forest Service in cooperation with the National Association of State Foresters and the National Plant Board proposed a five-year program that would accelerate development and implementation of management options to reduce the spread and impact of hemlock woolly adelgid. From 2003-2007, this "Hemlock Woolly Adelgid Initiative" has involved researchers and resource managers from four federal agencies, 20 state agencies, 24 universities, seven institutions in China and Japan, and more than nine private companies. Accelerated research and technology development during the five-year initiative resulted in a significant increase in our knowledge of hemlock woolly adelgid and its impact on hemlock resources. New management strategies have improved short-term control and provided opportunities for a potential long-term solution for this devastating pest. Although the Initiative has been highly successful, the job is not done. Recommendations for future program direction and priorities are discussed.

KEYWORDS

HWA Management Initiative, priorities, accomplishments, management tools, research, technology development

KEY ISSUES

Reducing the rate of artificial spread and overall impact of hemlock woolly adelgid (HWA) have become primary management goals. During the first five years of the Initiative (2003-2007), state officials reported newly found HWA infestations in 74 new counties (for a total of 303 counties) and two additional states (South Carolina and Kentucky). HWA currently infests nearly one-half of the native range of hemlock in the East. It can be found from southeastern Maine to northeastern Georgia and as far west as eastern Tennessee and Kentucky (Figure 1). HWA continues to spread at an average rate of 12.5 km per year, primarily by wind and birds. Slowing the natural spread of HWA is not likely to occur given the relative randomness of movement and difficulties in early detection of initial infestation in forest settings. Controlling artificial spread (movement of infested nursery stock or other hemlock products from infested to uninfested areas), however, can reduce the occurrence of isolated infestations. Regulating movement and quick response to newly discovered infestations is necessary.

Isolated infestations have occurred in Vermont, Ohio, and Michigan as the result of infested nursery stock being transported from the generally infested area. Forestry and agriculture officials have undertaken eradication efforts in Maine, New Hampshire, Vermont, Ohio, Wisconsin, and Michigan and have implemented quarantines. See Figure 1 for a map of the currently infested area.

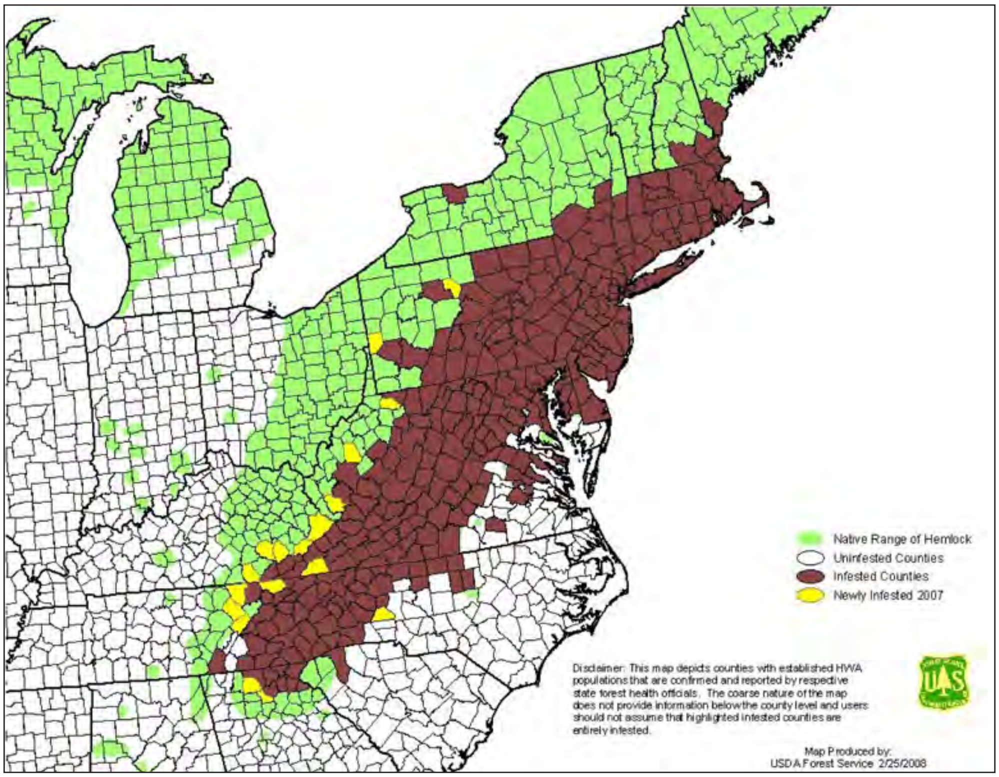

Figure 1. Hemlock woolly adelgid infestation, 2007.

KEY ACCOMPLISHMENTS

Over the last five years:

- Two new predatory beetles (*Laricobius nigrinus* and *Scymnus sinuanodulus*) for HWA biological control have been established and the range of *Sasajiscymnus tsugae* has been expanded to 15 states.
- Six laboratories (four at universities and two at state agencies) are now actively rearing three predator species.
- A systemic insecticide (imidacloprid) has been registered for use in the forest environment, and methods that increase its effectiveness have been developed.
- Resistant hemlock genotypes identified from devastated landscapes or through breeding with resistant species are under evaluation and development.
- Molecular genetics has been used to pinpoint the origin of HWA in the eastern U.S. and suggest that several distinct populations of HWA occur worldwide.

- Improved assessments of impact have been developed through classical economics and remote spectral sensing.
- Several survey methods have been developed that can be tailored to specific objectives.
- Silvicultural studies were initiated on public lands to determine whether thinning hemlock-hardwood stands to improve tree vigor in advance of HWA will minimize hemlock mortality once infested.

Information transfer has greatly improved access to available information: a website dedicated to HWA is in place that provides both the public and researchers up-to-date information, three symposia were held that provided a forum for managers and researchers to find out what is being done over the full range of topics, and proceedings from the symposia were published.

FUTURE PROGRAM DIRECTION

The knowledge base needed to develop and implement an effective HWA management program was increased significantly during the 2003-2007 period of this initiative. Progress in managing HWA has been made, but additional investments in research and technology development are needed to advance long-term management strategies, particularly in the areas of biological control and insecticide management. The recommended future program activities that follow provide general program direction regarding needed research and methods development over the course of the next five years and is not intended be a comprehensive list. Specific program activities will often change as new information becomes available.

Biological Control

Although three predatory beetles have been established for biological control of HWA, their ranges are spreading slowly. Additional species and better methods are needed to accelerate spread and to increase populations to levels that can maintain HWA below damaging levels. Two additional beetles have been approved for release, and better methods of mass rearing them need to be developed. Several candidates in quarantine or in the western U.S. need further evaluation and development of rearing methods. Priorities include:

1. Conduct extended-length (at least one-year) studies to assess the impact of natural enemies on HWA populations in their native habitat, such as in Japan, the source of HWA in the eastern U.S.
2. Continue quarantine screening of newly discovered predators and begin field evaluations of promising biological control candidates.
3. Continue development of artificial diets suitable for mass rearing of predators, and transfer developed technology to rearing facilities.
4. Phase-out mass rearing and release of *S. tsugae* in the southern range as other predators become available for rearing and release.
5. Phase-out mass rearing of *L. nigrinus* in the laboratory, and rely more on field collections from western North America for establishing healthy beetles and field insectaries.

This phase-out should be concomitant with the build-up of mass-rearing capacity for the Japanese *Laricobius* predator beetle when it becomes available for release.

6. Assess the cold-hardiness of *L. nigrinus* collected in Idaho, and determine whether it will improve establishment success in colder climatic regions in the East.
7. Continue rearing and establishment of *S. sinuanodulus* in southern range.
8. Begin mass rearing, release, and evaluation of *S. ningshanensis* in northern regions and higher elevations in the South.
9. Continue studies of biology and develop rearing methods for *Leucopis argenticollis.*
10. Continue to monitor all natural enemy species released, and assess their ability to establish, disperse, and impact HWA across the pest's geographic range. Part of this effort includes requiring all cooperators to input release data (e.g., site and predators) into an online predator-release database currently being developed.
11. Develop genetic identification tools (as there are no morphological methods for species identification for the larvae and pupae) and more information on the population structure of the natural enemies being released and evaluated.

Insecticidal Management

Soil application to manage HWA on individual, high-value trees or groups of trees has been shown to be effective, but the cost is high and alternatives for environmentally sensitive areas are needed. More efficacious methods for stem application are needed. Aerial application options are needed to treat large or otherwise non-accessible hemlock stands. Protection of high value hemlock trees and groups of trees should continue until natural enemies become established and provide control of HWA. Priorities include:

1. Continue evaluating new insecticide formulations and application methods for efficiently and effectively treating hemlock stands
2. Continue ground-based field trials of fungal pathogens, including *Lecanicillium lecanni* (Verticillium) using newly developed formulation technology.
3. Conduct pilot project to evaluate treatment efficacy of aerially applied insecticides including fungal pathogen formulations.
4. Evaluate the efficacy and application of the newly developed tablet formulation of imidacloprid.
5. Continue studies on optimal dosage and uptake of systemic insecticides.
6. Continue studies on movement and persistence of imidacloprid in soils.
7. Continue studies on methods to measure low levels of imidacloprid and its metabolites.

Silvicultural Management

In the face of changing forests, land managers need better analytical tools and experimentally assessed protocols for developing and exploring silvicultural options. To acquire them, we should:

1. Continue efforts to evaluate the effect of pre-infestation thinning of mixed-hardwood stands on vulnerability of hemlock to HWA infestation.
2. Continue modification of the Forest Vegetation Simulator (FVS) to include a HWA extension in cooperation with FHTET and Forest Service Research. Using existing and newly acquired knowledge about the biology of the HWA and its impact on stand structure, this tool will allow forest managers to visualize forested ecosystems after impact by HWA and under various silvicultural treatments.

Host Resistance

There are three approaches to increasing the resistance of hemlocks in the eastern U.S. to HWA. One approach is to incorporate the natural resistance of species that have co-evolved with HWA into the native eastern U.S. species through breeding programs. Another approach is to identify hemlocks with higher resistance among existing growing stocks and propagate them. A third approach would be to identify whether and how the environment may induce resistance.

1. Continue development and evaluation of hybrid *T. caroliniana* and *T. chinensis*, including generation of F_2 and backcross populations.
2. Verify if the above hybrids will naturally hybridize with *T. caroliniana.*
3. Determine nature of incompatibility between *T. canadensis* and other *Tsuga* species and test the means to overcome this incompatibility so that hybrids can be created with *T. canadensis*.
4. Make outplantings of the most promising hybrid crosses over the natural range of eastern hemlocks in order to verify their growth characteristics in a variety of environments.
5. Select specimens of *T. canadensis* that have survived in generally infested areas and verify whether this resistance is genetic.
6. Identify the role of environmental factors on the tolerance of hemlock to HWA.
7. Conserve the gene pool of eastern and Carolina hemlock until biological control and/or other means are available to reduce HWA below damaging levels.

Biology and Ecology

Recent breakthroughs in elucidating the genetics of HWA has helped identify the source of HWA in eastern North America. Important questions of the genetics of HWA populations, its hosts and its natural enemies remain. Additional information is needed on how climate—not only cold winter temperatures but also hot summer temperatures—affect HWA population dynamics. Knowledge of the feeding behavior and nutritional requirements of HWA would help in resistance studies and insecticidal management.

With the identification of the source population, the climatic preferences of the hemlock woolly adelgid can be estimated. Once identified, the key climatic factors that facilitate or limit HWA growth can be used to identify areas with similar conditions, from which biological control agents may be identified. Once identified, analysis of the similarities of requirements between the adelgid and the biological control can help managers tailor the use of specific biological controls to regions of the landscape. These analyses are being based on global climate data sets in combination with custom-built population models. Used in combination with other systems such as the FVS, these tools may assist land managers with selecting appropriate species, timing, conditioning, and distribution of released biological control agents. This work is being integrated with a landscape-wide study of the relationships between the HWA and environment to assist with identifying those areas most at risk for infestation. Information requirements include the need to:

1. Complete a global database on environmental parameters for HWA.
2. Elucidate the population genetic structure of HWA at a sufficiently fine level and link this to life history and host interactions to assist development of management options.
3. Develop models of climate space and HWA development.
4. Define the feeding behavior and nutritional requirements of HWA.
5. Develop DNA barcodes to identify interceptions of adelgids, thus promoting biosecurity.

Host-Pest Interactions

The elongate hemlock scale does not appear to be a significant pest in the southern U.S. or in its native habitat in Japan, but for unknown reasons, does appear to be spreading and impacting hemlock health in the mid-Atlantic and New England regions. Further investigations are needed to determine the cause and if intervention is possible. We still need to:

1. Determine the significance of biological control agents of EHS in southern U.S. and Japan and evaluate potential candidates for establishment in affected regions in eastern U.S.
2. Determine whether the scale parasitoid *Encarsia citrina* found in Japan is genetically different from *E. citrina* found in eastern North America.

Impact Assessments

Methods to evaluate impacts to hemlock resources are currently available using Landsat imagery and hyperspectral remote sensing technologies. The economic damage to property values in residential communities have been assessed in the northern range of HWA but may not be applicable to the loss of hemlocks in communities in the southern range. Furthermore, the socio-economic valuation of the forest resources at risk due to HWA and the value of treatment options in terms of ecosystem response, public acceptance and economic cost/benefit have not been assessed. We need to:

1. Conduct comparable community-based economic assessment in the southern Appalachians.
2. Assess the socio-economic impacts of existing management and no-management options for HWA on a regional scale utilizing current management strategies and available impact information.
3. Assess the socio-economic impacts to primary recreational areas such as the Great Smoky Mountains National Park and Delaware Water Gap National Recreation Area, where significant impacts have occurred and management actions are underway.

Survey and Monitoring

Sufficient methods are now available for survey, monitoring, and research purposes. No further methods development is needed. We need to:

1. Continue annual regional assessments of newly infested areas by using current survey methods and supporting county level surveys conducted by states.

Information Transfer

Maintaining an updated USDA Forest Service HWA website and publishing new information related to management of HWA has been an effective way of reaching both resource managers and private land owners. Publishing HWA newsletters and conducting periodic HWA symposia with published proceedings has also proven effective at distributing new information and developing collaboration among researchers and resource managers.

1. Continued support in updating the HWA website with new information as it becomes available.
2. Continued publishing periodic newsletters and conducting HWA symposia in alternating years.

Posters

Preliminary Assessment for Presence of a Native and Introduced *Laricobius* Species Within Four Hemlock Woolly Adelgid-Infested Hemlock Stands

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ABSTRACT

Introduced from the Pacific Northwest, *Laricobius nigrinus* Fender continues to show promise as a biological control agent for hemlock woolly agelgid (HWA), *Adelges tsugae* Annand, in the eastern United States. Understanding the dispersal potential of this introduced predator in its newly established range may facilitate strategic planning of future release locations based on hemlock density, HWA density, or a combination of these factors. A native predator of pine bark adelgid, *Laricobius rubidus* (LeConte), can also complete its life cycle on HWA and is commonly recovered on infested hemlocks.

Between March 30 and April 29, 2007, up to sixteen points were sampled at four forested sites of known *L. nigrinus* establishment. The morphologically indistinguishable larvae of both species were recovered from the samples and identified using molecular diagnostics. *Laricobius nigrinus* was recovered up to 100 meters from the original release area at two sites, representing movement of the F_2 and F_3 generations. Even in the absence of its traditional host, *L. rubidus* was confirmed at 56% of the total points sampled. Continued sampling of *L. nigrinus* dispersal and *Laricobius* spp. impact on HWA is planned for 2008 and 2009.

KEYWORDS

Laricobius, dispersal

Assessment of Imidacloprid and Horticultural Oil on Non-Target Phytophagous and Transient Canopy Insects Associated with Eastern Hemlock in the Southern Appalachians

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ABSTRACT

Systemic imidacloprid and horticultural oil are the primary chemicals used to control infestations of hemlock woolly adelgid, *Adelges tsugae* Annand. The impact of imidacloprid and horticultural oil on non-target canopy insects is unknown. A study was initiated in November 2005 to assess the effects of imidacloprid soil drench, soil injection, and tree injection applications, and of horticultural oil applications on aspects of the phytophagous and transient insect community (overall species richness and abundance, guild species richness and abundance, and individual species).

Mean species richness and abundance were significantly reduced by one or more application methods. Species richness was significantly reduced among detritivore and phytophaga guilds by soil drench applications. Species abundance was significantly lower across all guilds, with the exception of the haematophaga guild, and differed significantly in the number of specimens on the control trees. From 293 species evaluated, 35 were found to be directly effected by one or more of the chemical treatments. Of the 35 species, 27 were lepidopterans that are hemlock feeders and pupate in the soil. The additional eight species were psocopterans that feed on decaying micro-fungi on the tree.

Soil drenching had the most significant effect, while soil injection and horticultural oil had a moderate effect. Tree injection and horticultural oil applications were most similar to the control; however, it is important to note that tree injections were found not to translocate imidacloprid uniformly throughout the tree. This research will provide land managers and owners information on the effect of treatment methods and their potential impact on non-target species.

KEYWORDS

non-target insects, imidacloprid, horticultural oil

ACKNOWLEDGEMENTS

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Predator Beetles at Work: Evidence-based Assessments of Private *Sasajiscymnus tsugae* Release Sites in Western North Carolina

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ABSTRACT

Private site releases of *Sasajiscymnus tsugae* predator beetles offer an important opportunity to expand our knowledge about biological control of the hemlock woolly adelgid (HWA), *Adelges tsugae*. Because of cost considerations, private site beetle releases may utilize much lower release densities (100-300 per acre) than reported for USDA releases on public lands (1,000-5,000 per acre). Evidence-based assessments can be facilitated by the accessibility of private sites. This report focuses on the development and application of measurement strategies for a two-year assessment of a set of riparian woodland sites and a one-year assessment of a set of woodland and neighborhood sites. A digital measurement strategy for quantifying changes in hemlock crown density is proposed, and observations on beetle behavior at release sites are suggested. Low-density releases proved effective in both neighborhood and woodland settings. Tests of low-density waterway releases suggest a strategy for initiating biological control coverage in riparian woodland areas. Need for evidence-based USDA policies and practices concerning biological control of HWA is noted.

RELEASES

2006

The research reported here was initiated in 2006 as an environmental intervention to save a large number of severely HWA-defoliated eastern hemlocks (*Tsuga canadensis*) on a 150-acre headwaters tract on the Blue Ridge escarpment in Western North Carolina. The waterways and hemlocks on the property were mapped by the landowner/author in 2005. In April 2006, 3,000 HWA predator beetles, *Sasajiscymnus tsugae* ("Sassie"), purchased from a local private insectary lab (Conservation Concepts) were released in hemlock areas across the property.

Cost considerations motivated the author to draw upon published results from prior field work in Connecticut (McClure 2001; McClure and Cheah 2003) to design a low-density, colony-based release design intended to establish self-sustaining predator beetle colonies in the field. This release effort included several dose-response components to assess different release densities, ranging from 30 to 100 beetles per release site and 100 to 300 beetles per multi-site colony.

The larger hemlock trees on the property were located primarily along approximately 9000' of small waterways on the property and ranged in size from 20-35" DBH and up to over 100' in height. All were heavily defoliated from an HWA infestation of unknown duration first noted in 2003. Some dead trees were present in the worst infestation area and several large hemlocks had been "topped" by wind gusts in 2003. Ten large trees around a pond had been treated in July 2004 with imidacloprid trunk injections, with no visible subsequent effects on tree health or new foliage production. Most of the larger trees on the property had produced little or no new foliage in the 2005 growth season.

Because there were no expectations for short-term results, no baseline observations of the trees were recorded prior to predator beetle releases. Careful observations of hemlock foliage changes were begun in mid-June 2005 in response to dramatic production of new foliage on large trees around the pond. By mid July, the same kinds of new crown foliage production were visible on crowns of larger trees in the vicinity of beetle release areas. After leaf-fall in mid-October, most of the larger trees along the waterways were observed to have produced visible amounts of new crown foliage. (This new-crown-growth phenomenon was not observed in comparable size trees along the waterways on other properties in the immediate area.) This production of new crown growth did not appear to be limited to the large trees in the immediate area of predator beetle releases but extending hundreds of feet—in one instance, over 1000' along a waterway onto an adjacent property.

Some release site trees produced new foliage on lower branches as well; the amount of new foliage produced appeared to be greater on trees with substantial sunlight exposure. Prior chemical treatment may have also have contributed to the quantity of growth buds and, hence, the amount of new foliage produced. However, this was confounded with the effect of sunlight: all previously treated trees were also exposed to considerable sunlight because of their location around the pond. Nonetheless, the positive association between sunlight exposure and amount of new foliage production was indisputable.

Smaller trees and the middle and lower branches of most of the larger trees (with the exception of some release area trees with full sunlight exposure) typically produced little or no new foliage in this first year, while crowns of larger trees in the same area showed substantial new growth. It was hypothesized that this new growth was made possible by reductions in adelgid density due to predation by adult beetles and larvae (McClure and Cheah 2003; Evans 2004). To test this hypothesis, sample twigs collected from these new growth areas in October/November 2006 were examined for HWA crawlers, and very low crawler densities were observed. (New growth twigs collected from adjoining areas had very high crawler densities.)

If we presume that observed foliage changes are a result of predator beetle behavior, then we can use these foliage changes as indicators to indirectly observe and track the movement of the beetles during the first season after release. Use of this method suggests that, after release, the beetles tend to move to the tops of the largest hemlocks in the area to feed and lay eggs. Then, they move to adjacent tall trees, repeating the process over and over again, sometimes over considerable distances. Note that the new growth used as an indicator here is produced during the spring hemlock growth cycle (May/July in this area). Therefore, this indicator only represents the first approximately three months behavior of released and F_2 beetles.

It is important to remember that the larger trees in this area were severely defoliated - the process observed here might differ for healthier trees with heavier adelgid densities. The released beetles were approximately 4 weeks of age and hence reproductively active—the process might differ for younger beetles that are not yet reproductively active, but in these situations, the released beetles appeared to move considerable distances during the first several months.

Why were the beetles' early activities apparently concentrated at the crowns of the larger hemlocks? Several hypotheses have been suggested. Perhaps on heavily defoliated trees, the crown holds the largest adelgid population (food supply). Perhaps the beetles were attracted to higher locations or to the light that was present at such locations. Or perhaps those light conditions facilitated growth buds that could be released due to the beetles predation.

2007

The largely unanticipated short-term results from the 2006 releases prompted a number of new efforts for the 2007 release season. These included 1) a quest for a reliable measurement strategy for documenting changes in hemlock crown density, 2) the refinement of a low-density release strategy that would be cost-effective in private applications, 3) grass-roots efforts to involve community groups in planning and implementing beetle releases, and 4) continued (second year) observation of the 2006 release sites noted above.

1) Measuring Changes in Hemlock Crown Density

The magnitude of short-term changes observed in hemlock foliage in 2006 (changes that could not be quantified in the absence of baseline measurements) motivated a search for an objective measurement strategy for hemlock foliage change. The standard USDA crown density measurement strategy, using multiple trained raters with crown density-foliage transparency cards (Cheah et al. 2005), was not readily applicable to private sites where no professional raters were available. An alternative digital strategy involving no human judgement component seemed preferable for such private applications. The foliage changes that were so obvious to the human eye should also be detectable in photographic images. But how could information on hemlock crown density (HCD) be extracted from such images?

The quest for a digital measurement strategy built on the inputs and knowledge of several individuals with professional graphic and photographic backgrounds. Christine von Lersner (2007) first suggested ideas for using Adobe Photoshop software to extract pixel density information from photographs. Rita and Steve Buchanan (pers. comm.) followed up with suggested refinements in the procedure using the Histogram display from Photoshop Elements to quantify pixel density information.

My contribution to defining the problem was to focus on a special type of photograph: one that silhouettes the hemlock crown against an open sky. (While such trees are not representative of trees in hemlock areas; the hope was that they could be useful for comparisons over time and across different areas.) In such photographs, the hemlock trunk, branches and foliage (density) are represented by darker pixels, while the background (transparency) is represented by lighter pixels. The first operation utilizes the Histogram in Photoshop Elements to chart the percentage dark/light content of the pixels in a digital photograph from darkest to lightest. The second operation uses the histogram to calculate the cumulative density at a darkness level representing hemlock foliage. This density level can be compared with corresponding measures for other photos to measure change in one tree or to make numerical comparisons between different trees.

The photos and data below (Figure 1) illustrate the application of this measurement strategy to a 2007 *Sasjiscymnus tsugae* release site on hemlocks near the Horsepasture River in Sapphire, North Carolina. Two sets of photos were taken in mid-April and mid-July, approximately three months apart. Below are the 'before' and 'after' photos for a site containing a pair of defoliated hemlocks. At the top are the photos, cropped in order to focus the measurement procedure on the same crown areas for comparison. At the bottom are the histogram outputs for the two photos.

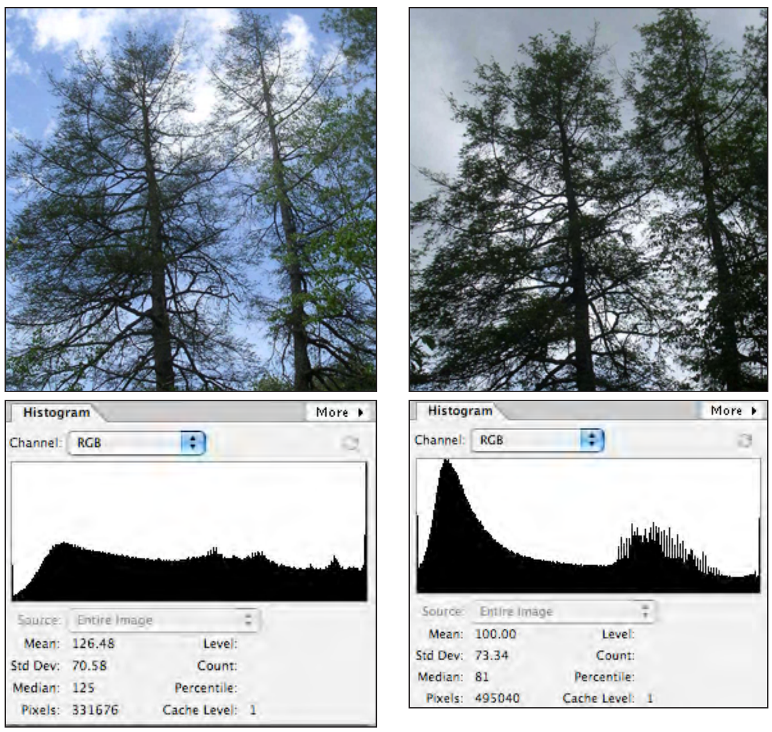

Figure 1. Digital measurement strategy for hemlock crown density: mid-April (left) photograph and histogram and mid-July (right) photograph and histogram.

These photographs indicate that there appears to be more foliage on the trees in mid-July than mid-April. The histogram allows us to quantify these density differences in terms of the percentage of pixels in the darker color range. The histogram is a plot representing the distribution of pixel values across the spectrum on the horizontal axis and pixel frequency on the vertical axis. It also provides cumulative percentile information—from dark to light—at any specified level that can be used to obtain quantitative measures of pixels representing hemlock crown density.

The corresponding histograms for these two photos illustrate even more clearly the extent of foliage growth on the hemlocks in the two pictures. The histogram for the April photo shows a relatively uniform pixel value distribution moving horizontally from dark (left) to light (right), with a spike at the far right for the white clouds. The histogram for the July photo shows a definite peak for darker pixels in the lower part of the distribution, attributable to new foliage.

We can use these histograms to measure hemlock crown densities by calculating the percentile of darker pixels in a photograph. The proposal here is to use cumulative percentile numbers derived from a photograph's histogram to represent the crown density in the pictured tree(s) as well as to measure changes in that density over time. However, this will require selecting a darkness-level benchmark to capture hemlock foliage growth.

Table 1. Comparisons of hemlock crown density measurement benchmarks.

	Darkest 1/2	Darkest 1/3	Darkest 1/4
mid-April	51.12%	34.75%	25.35%
mid-July	61.99%	51.30%	44.39%
3-month change	10.87%	16.55%	19.04%

The histograms illustrate that changes due to new foliage growth appear well down into the darker end of the spectrum. This will help us to identify the "best" point in the spectrum for measuring these foliage changes. The table above contains readings for three different darkness levels: darkest half, darkest third, and darkest fourth. A review of histograms for these and other photographs suggests that differences due to foliage changes are concentrated in the darkest 20% of the spectrum. The results in the table indicate that either the 25% or 33% benchmarks would have considerable sensitivity to the three-month foliage changes represented here. Specifically, the 25% benchmark, representing the darkest 25% of the pixel color spectrum, indicates a 19.04% increase (due to new foliage growth), while the 33% benchmark, representing the darkest 33% of the pixel color levels, indicates a 16.55% increase (due to new foliage growth).

Variability in light conditions is always a concern in photographic comparisons, and field comparisons will often exhibit such variability. However, it appears that the proposed use of the histogram adequately separates the variability in backlight levels from that of hemlock foliage. Background light is represented on the right side of the distribution—note the spike for white clouds for the April photograph. But the hemlock crown density is derived from the darkest portion of the histogram spectrum and does not appear to be unduly influenced by the relative lightness or darkness of the background. A more serious concern may be the exposure of subject trees to illumination from the front, biasing the foliage measurement. This issue warrants further investigation, but overcast, dusk, and dawn appear to offer the best conditions for photos to be used in this measurement technique.

2) Low-Density Beetle Release Strategies

My 2006 releases utilized much lower beetle release densities than typically noted in the literature. While this was primarily driven by cost considerations, a careful effort was made to include a dose-response component in the release design, utilizing systematic variations in the number of beetles released at different sites as well as the number of beetles per hemlock acre. Asaro et al. (2005) previously reported that per tree releases of 300 beetles were as effective in reducing adelgid densities as releases of 3,000, but lower release densities were apparently not considered and per acre densities were not reported. The per-tree densities implemented here ranged from 30 to 100 per site, and the per-acre (of hemlocks) release densities ranged from 100 to 300 per acre. (It should also be noted that all the trees in these release areas were well above the 50% defoliation level that is often cited as the upper limit for effective biological or chemical treatment.)

One bit of good news from these trials was that even small releases of 50 beetles per tree and 100 per hemlock acre appeared to be effective at initiating the biological control process over a substantial area. While some positive correlation was noted between release density and quantity of new foliage production, the relationship was not strong. Other situational issues such as tree health, sunlight, and stage of infestation appear to be more important determinants of quantity of foliage production after beetle release. This should not be too surprising, as the first several months of beetle activities could not affect the production of hemlock growth buds. It could only "release" the buds that had already been produced but were being suppressed by the ongoing adelgid infestation (Evans 2004).

The colony-based aspect of the release design utilized multiple sites within 100' of one another, with 30-50 beetles per site (depending on tree size) to define a self-sufficient colony. Such colonies could then be spaced as local conditions and resources dictate. Extension of these low-density release procedures to a wider range of private release sites in 2007 allows further assessment of a variety of issues and procedures.

3) Expanding Private Beetle Release Efforts

News about the surprising results of the 2006 releases was circulated through local media in Brevard and Cashiers and through presentations to local property owner and other public interest groups. This led to a grass-roots movement in the Brevard area involving public officials, private landowners, and residential developers that led to the 2007 release of about 25,000 "Sassie" beetles purchased from Conservation Concepts and EcoScientific Solutions. Efforts in the Sapphire/Cashiers area were a bit more limited, resulting in the release of about 12,000 purchased beetles.

While these release efforts were limited by the supply of beetles available, several obstacles to the involvement of private individuals in biological control of HWA were noted. Foremost among these were active USDA Forest Service media efforts (by personnel in Asheville, Pisgah National Forest and Nantahala National Forest) to discourage landowner involvement, both for larger woodland tracks and for neighborhood-level applications. This was reinforced by numerous statements by local Forest Service and other USDA officials stating that there was "no information" about the effectiveness of HWA predator beetles and by an active media campaign by chemical interests (led by a private arborist) (Slade 2007; Preston 2007) dismissing the effectiveness of HWA biological control efforts. While the latter efforts are understandable in light of economic incentives, the former suggest the need for more careful attention to evidence-based HWA policies on the part of USDA/Forest Service policy-makers.

Not all the private releases noted above were known to the author, and not all of those known have yet been carefully assessed. The discussion here will employ a case-study format to present some sites where assessment efforts have been completed and where ample evidence on first-year results is available.

Most hemlocks in the Brevard area appeared to be in an earlier stage of HWA infestation than trees at the 2006 sites and were in a less-defoliated condition. In contrast, many hemlocks in the Cashiers and Sapphire area were just as or possibly even more defoliated than 2006 sites ,with a significant number of dead trees present in some locations.

Generally speaking, the results at these 2007 private release sites paralleled those observed in 2006 and reported above. The dramatic renewals of new top growth in defoliated larger trees were apparent here as well, but the large hemlock tree-hedges (15-30' in height) that were common in Brevard neighborhoods also proved to be very responsive to beetle-induced re-foliation. Several wild 2007 "test releases," designed to obtain more accurate distance measures for predator beetle releases on waterways, will also be reported.

Case 1: In-town neighborhood area. A condominium 4-plex in downtown Brevard had a 15' hemlock hedge along the front and one side, totaling about 400'. Trees were in mid-level infestation, with adelgids present at all levels, foliage "graying," and bare tips present on many branches. A release was made of 200 beetles at 25 beetles per site, distributed at equal intervals along the hedge. By mid-July, all areas of the hedge were covered with extensive new growth. Adjoining properties benefited as well. Two larger (30') and more severely defoliated tree hedges—one 150' in length, running parallel to the complex across a two-lane highway, and the other perpendicular to this and running 300' feet away from the property—were also covered with new foliage.

Case 2: Residential development area. Sherwood Forest, a large environmentally-oriented development (1,000 acres) in the Brevard area, initiated biological control efforts to replace chemical control efforts. About 6,000 beetles were released on "green" and trail areas as well as on privately-owned tracts of residents and of adjoining property owners. Assessments after leaf-fall indicated substantial re-foliation of larger hemlocks in release areas and along waterways. Plans are in place to extend biological control coverage to additional acreage.

Case 3: Municipal efforts for biological control of HWA. Proposals were made in fall 2007 to both the City of Brevard and Transylvania County for purchase of predator beetles for release at municipal sites in the Brevard area (see Acknowledgements). City officials readily agreed to a 1,000 beetle purchase for release in several small parks and a 400-acre watershed area adjacent to the city. County deliberations included USDA extension officials (see Acknowledgements) in a series of meetings that lead to a positive vote by County Commissioners for a similar purchase. The County release was directed to an 8-acre mansion property (Silvermont) in downtown Brevard. This property contained approximately 300 medium-size to large hemlocks and was surrounded on all sides by private properties containing large hemlocks.

Brevard City park and watershed releases involved younger trees in relatively early infestation areas, where hemlock health was not yet significantly impaired and defoliation was at an early stage. Spot checks at these release sites indicate reductions in adelgid densities and continued production of new growth but none of the dramatic foliage changes observed on more severely defoliated trees.

The County release was done by ecology students at Brevard College. These trees were larger and more heavily infested than those in the city. Heavy HWA infestations had moved from the ground level to higher-level branches, and some "graying" of the hemlock foliage was apparent. While a formal assessment will be conducted by the release group, significant new growth is apparent on the crowns of many hemlocks in the release area.

Case 4: Wild waterway test areas. Observations from 2006 releases along waterways suggested an unexpectedly long range of influence by newly released "Sassie" predator beetles. Several tests were conducted to further examine this issue. The first was a relatively isolated waterway (Democrat Creek) in a new Nature Conservancy tract: Silver Run Preserve in the Nantahala National Forest.

This site was located in the vicinity of the 2006 release area, and the medium to large trees along the waterways were severely defoliated with little new foliage being produced. In March 2007, two sets of 50 *Sasajiscymnus tsugae* beetles were released at locations near the confluence where two tributaries come together to form the creek, which then runs several miles south before emptying into the Whitewater River. (The Whitewater River is benefiting from numerous USDA-sponsored beetle releases by the Clemson insectary beginning in 2005, creating large areas of re-foliating hemlocks along this waterway.)

Dramatic new crown growth was observed at these two release sites by mid-July, but a more careful assessment was delayed until after the fall of deciduous leaves in mid-November. Because of the defoliated state of hemlocks in this area, new crown growth offered a clear indicator of developments. New growth was observed at least 1,500 feet down the creek to the south, about 800 feet east up one tributary, and 600 feet to the north up the other tributary.

At a second waterway release site utilizing 100 beetles, new crown growth was observed for an area extending over 2,000 feet downstream and about 500 feet upstream. This suggests that a series of low-density waterway releases might be an effective strategy for extending biological control into riparian areas.

4) Second Year Observations at Beetle Release Sites

In 2007, continued observations of the 2006 beetle release sites described above have suggested further inferences concerning the behavior of beetles, hemlocks, and adelgids during the process of establishing biological control. During the winter of 2006, observations of wind-break hemlock twigs distributed across forest floors indicated that a majority of the upper-level new growth showed no adelgid re-infestation (from HWA crawlers emerging in fall 2006). Twigs with HWA present showed a range of infestation densities from light (less than 5 per twig) to heavy (most new needles affected). The adelgids were not gone, but neither were they overwhelming the recently-produced new hemlock growth.

Over the course of the 2007 growing season, the emergence of new growth continued in the 2006 new growth areas (mostly in the crown). However, in this new second-season growth also extended to grey and defoliated branches on middle to lower sections of the larger trees, as well as to smaller trees in the area. This "trickle-down" effect continued the re-foliation of the largest trees and initiated this process on middle to smaller trees in the release areas.

By fall 2007, the density of HWA crawlers on lower branches of both larger and smaller hemlocks in release areas was lower than noted in the previous year. In addition, individual beetles were regularly found on small trees in the area, although never in large quantities. During winter 2007, hemlock twigs and branches that had been cleaned of emergent crawlers were commonly found on trees of all sizes.

These observations suggest several interesting hypotheses for further investigation at these sites and others. First, it suggests that the extensive movement and egg-laying of newly released, reproductive-age beetles beyond immediate release areas (as noted in the first season) did not hinder the growth of beetle populations in the release areas to biologically effective levels. Second, it suggests that the low-density release strategies utilized (and discussed above) were effective in initiating biological control of HWA. Third, it suggests that it would be better to think of biological control of HWA as a process than as an event. Because of the extended hemlock growth cycle, the extension of biological control benefits to recovery of normal foliage on all trees in an area will be a multi-year process. However, two years into the process, trees that show foliage recovery have shown no indication of adelgid population resurgences or of damage to recovering new foliage areas.

DISCUSSION

Forest Managers

Evidence from the use of low-density releases of *Sasajiscymnus tsugae* predator beetles offers new options for extending biological control of HWA across a broader range of woodland areas. Unlike conventional, high-density beetle releases that require relatively healthy trees with heavy adelgid infestations, low-density releases can be utilized in hemlock areas with trees experiencing substantial defoliation and relatively low adelgid densities. While not all trees may be saved in such areas, a release of 100 beetles in a severely defoliated hemlock cove or waterway area can establish a self-sustaining beetle colony that will cover a substantial area and provide support for recovery of surviving hemlocks in the area, resulting in long-run protection to these ecologically critical zones. Waterway releases (e.g., at roadway crossings of creeks or rivers) offer an effective way of dispersing beetles into riparian woodlands to protect important hemlock habitats.

USFS

Evidence from low-density *Sasajiscymnus tsugae* releases in both private woodlands and neighborhood areas suggests the need for a more careful evaluation of USFS policy statements in light of evidence-based criteria. Several public statements made by USFS representatives in western North Carolina—that predator beetles cannot help trees that are more than 50% defoliated (Slade 2007) and that predator beetles are not effective for use in neighborhoods (Ellison 2007)—appear to be contrary to the evidence presented above.

More generally, the negative orientation of local USFS statements about predator beetle effectiveness seems to be based more on “opinion” than evidence, and efforts to discourage private landowners from utilizing biological control strategies appear to be misguided, at the very least. Our native hemlock habitats exist on both private and public lands. And just as predator beetle releases in state and national forest areas can benefit hemlocks on adjoining private properties (Connor 2006), private releases can benefit adjoining public lands.

So long as increasing beetle supplies to private landowners does not reduce supplies to public lands, maximizing private as well as public releases of HWA predator beetles should be encouraged. The USFS could even attempt to guide, rather than discourage, private participation in biological control of HWA. For example, there are many nature-oriented groups in our communities (e.g., hikers, anglers, and birders) that would be capable of purchasing beetles in 1000-unit lots and conducting low-density releases in environmentally sensitive public areas —areas that are not being addressed by current public release efforts.

It has taken four decades to recognize the HWA problem and another decade to find a solution. There should no delay in getting this solution deployed as widely as possible on both private and public lands. There is still time to save many of our native eastern and Carolina hemlock trees and habitat areas.

ACKNOWLEDGEMENTS

I would like to extend thanks to Diana Zerby for her enthusiastic efforts to promote biological control efforts in Brevard and Transylvania County, to Eric Caldwell and Jill Sidebottom from USDA Extension for participating in the county review process, and to Tommye Scanlin and Jack Anthony for their help with photographic issues. And special thanks to my wife, Noel A. Thurner, for sharing my despair at seeing the hemlocks dying, my joy at seeing their recovery, and my urgency in sharing this information with others.

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Naturally Occurring Adelgid Resistance in Eastern Hemlocks

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ABSTRACT

As hemlock woolly adelgid (HWA), *Adelges tsugae*, has spread throughout the eastern United States, there has been massive mortality within stands of eastern hemlock, *Tsuga canadensis*, following HWA infestation. Occasionally, however, a few healthy-looking eastern hemlock trees persist. The University of Rhode has begun an initiative to locate, propagate, and experimentally evaluate the level of resistance in these rare individuals. Rooted cuttings from 18 individuals fitting our criteria for potential resistance are currently being grown in a greenhouse at East Farm on the University of Rhode Island-Kingston campus in preparation for testing their level of HWA resistance (following procedures in Caswell, in prep.). Eastern hemlocks that show increased resistance to HWA will be used in landscape management and reforestation.

Recent examination of HWA-resistant *Tsuga* spp. reveal a number of characteristics that may underlie their resistance. First, Lagalante and Montgomery (2003) analyzed seven different *Tsuga* spp. and identified significant between-species differences in their terpenoid profiles. Second, McClure (1991) recognized that increases in foliar nitrogen are capable of stimulating HWA population growth. Finally, Pontius et al. (2006) examined foliar chemistry among susceptible and resistant *Tsuga* spp. and identified calcium (Ca), phosphorus (P), potassium (K), and nitrogen (N) as key cations whose concentrations differed significantly between the two groups. We are examining carbon-to-nitrogen (C:N) ratios, Ca, P, and K concentrations as well as the terpenoid profiles of eight of the 18 trees in addition to testing for HWA resistance. Should these trees prove resistant to HWA attack, our analyses should help reveal the basis for this highly-desirable trait.

KEYWORDS

Adelges tsugae, *Tsuga canadensis*, host-plant resistance

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GENOMIC MARKERS FOR CAROLINA HEMLOCK, EASTERN HEMLOCK, AND FIVE OTHER *TSUGA* SPECIES

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ABSTRACT

Populations of Carolina hemlock (*Tsuga caroliniana*), a native to the southern Appalachian Mountains, and of eastern hemlock (*Tsuga canadensis*), a species with a widespread range in eastern North America, have been devastated by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. Two sets of polymorphic microsatellite markers (TcSI and Tcn) were developed to aid the genetic studies of these threatened species. These molecular markers were independently developed from the genomic DNA of two hemlock trees located at Linville Falls, North Carolina. A set of 34 TcSI markers was derived from the *T. caroliniana* tree and a set of 15 Tcn markers was derived from the *T. canadensis* tree. Amplification of both sets of markers was tested in samples representing seven hemlock species: *T. caroliniana*, *T. canadensis*, *T. chinensis*, *T. mertensiana*, *T. diversifolia*, *T. sieboldii*, and *T. heterophylla*. Thirty-one TcSI and no Tcn markers were polymorphic in *T. caroliniana*. Six TcSI and 15 Tcn markers were polymorphic in *T. canadensis*. Both TcSI and Tcn markers were polymorphic in at least one of the other five hemlock species. These markers will be useful for characterizing the genetic diversity of hemlocks for conservation, breeding, and hybrid verification efforts.

Maine's Slow-the-Spread Program for Hemlock Woolly Adelgid

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ABSTRACT

The Maine Forest Service and Maine Department of Agriculture manage hemlock woolly adelgid (HWA) with an integrated slow-the-spread approach. Four tiered intervention principles guide HWA management activities: exclusion, eradication, containment, and mitigation. Management is a joint effort of the State's Forest Health and Monitoring (Department of Conservation, Forest Service) and Plant Industry (Department of Agriculture) divisons. The divisions' management tools include biological, chemical, and physical control; public outreach and regulation; and research, survey, and monitoring. The approach delays the artificial movement of HWA into the state's uninfested hemlock resource and allows time for the development new management approaches and new understanding of the pest, its host, and its environment.

KEYWORDS

hemlock woolly adelgid, citizen science, integrated pest management, Maine

MAINE'S HEMLOCK WOOLLY ADELGID MANAGEMENT TOOLBOX

Maine's Slow-the-Spread management approach for the hemlock woolly adelgid (HWA) is a joint effort between the Department of Conservation's Forest Health and Monitoring Division and the Department of Agriculture's Division of Plant Industry. Four hierarchical principles guide the management efforts:

- Exclusion,
- Eradication,
- Containment, and
- Mitigation.

Regulation, outreach and education, control, survey and monitoring, and research are among the categories of tools the divisions use.

Maine first established an external quarantine on potentially infested hemlock materials in 1988, shortly after HWA was detected in southern New England. The quarantine has been strengthened several times since it was first enacted. Maine's current internal and external quarantine limits movement of hemlock products from six towns in southern York County Maine, infested counties and towns in eastern states, and the entire states of Alaska, California, Oregon, and Washington. Maine's quarantines work towards the principles of exclusion and containment of hemlock woolly adelgid. Other important regulatory tools include Emergency Orders to respond to a changing situation, authority to condemn and destroy infested material, and compliance agreements to allowing regulated movement of lower-risk forest products.

Maine's outreach efforts address all four principles of the slow-the-spread approach. Activities include presentations for landowners, public, and industry about HWA and quarantines, displays at garden shows, posters for trailheads and other public places, a series of Web-pages dedicated to hemlock woolly adelgid, door-to-door campaigns associated with other management activities, public service announcements, press releases, and articles for newsletters. Maine Forest Service pioneered a "Take a Stand" volunteer initiative in which citizen scientists are trained to survey for hemlock woolly adelgid using Costa and Onken (2006) survey methods. Over 75 volunteers have gone through an intense day-long training session about HWA biology, management, and survey and monitoring. The volunteers "Take a Stand" to survey annually. Several new detections of HWA have been reported by trained Take a Stand volunteers. Volunteer surveys augment what the Maine Forest Service can survey on the ground; more importantly, volunteers are partners in spreading the word about hemlock woolly adelgid.

The Maine Forest Service employs several control tactics, including biological, chemical, and physical control. Two species of predator beetles have been released in southern York County. As of December 2007, 20,500 *Sasajiscymnus tsugae* and 1,700 *Laricobius nigrinus* had been released across nine sites. Sampling at release sites has yielded *S. tsugae* adults and larvae on infested hemlocks adjacent to the release sites. More releases across a broader area are planned for the future. Chemical control is targeted to sites where there is a high risk of human-adelgid interaction and high potential for artificial spread. Physical control is important in outlying areas, where trees are removed and destroyed, and at high risk sites where chemical control is not feasible or desired. Control methods address the principles of eradication, containment, and mitigation.

Annual surveys for hemlock woolly adelgid help define the management problem. Detection surveys are conducted in all York County towns to determine quarantine boundaries, along major travel corridors in the southern third of the state, at and near facilities with compliance agreements to monitor effectiveness of agreements, and at sites where outplanted infested hemocks were detected to monitor for new infestations. Survey and monitoring help define the problem, assess management effectiveness, and guide future actions.

The Maine Forest Service cooperates in research activities as resources allow. Partners in research efforts include the USDA Forest Service, The Connecticut Agricultural Experiment Station, and The University of Maine. Among Maine Forest Service research interests are: HWA biology, biological control, risk mapping, chemical control, and hemlock ecology.

Maine's Slow-the-Spread management approach, guided by the principles of exclusion, eradication, containment, and mitigation, has delayed the artificial advance of HWA into the as-yet uninfested hemlock resource. Outreach and education efforts have garnered strong public support for the hemlock woolly adelgid management and forest health and monitoring programs. The management tools used allow responsiveness to changing knowledge and conditions.

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Epicuticular Wax and Infestation Success: Possible Cause and Effect for Differential Susceptibility to Hemlock Woolly Adelgid Among Carolina Hemlock Provenances

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ABSTRACT

Hemlock (*Tsuga*) species native to Asia and western North America are believed to be at least partially resistant to infestation by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. In the eastern United States, where it is an exotic invader, HWA is considered a serious threat to both eastern hemlock (*T. canadensis* Carriére) and Carolina hemlock (*T. caroliniana* Engelmann). It has caused significant hemlock mortality from southern New England to northern Georgia (McClure et al. 2001).

It is generally accepted that both of these hemlock species are highly susceptible to adelgid attack, and little hope exists for finding natural HWA resistance in either species (Cheah et al. 2004); however, anecdotal evidence gathered from foresters throughout the southern Appalachian region and field reconnaissance by researchers at North Carolina State University indicates that Carolina hemlock may harbor some tolerance to HWA infestation. Adelgid populations on this species appear to grow more slowly, and significant tree decline and mortality has not occurred with the same severity as with eastern hemlock. This is particularly evident in areas where the two hemlocks occur sympatrically. Increased levels of HWA tolerance in Carolina hemlock are intuitive given this species' close genetic relationship with hemlocks of Asian origin (Vining 1999), and recent research suggests the possible existence of adelgid resistance mechanisms in this species.

Following artificial inoculation in a climate-controlled greenhouse, HWA infestation levels on eastern hemlock seedlings were nearly four-fold greater than on seedlings of either Carolina or putatively resistant western hemlock (*T. heterophylla* Sargent), and adelgid densities did not differ significantly between the two latter species. Similar greenhouse studies indicated significant differences in HWA infestation rate and fecundity among Carolina hemlock seed sources. Although overall infestation levels were low, adelgid densities were consistently higher on seedlings from the Carolina Hemlocks Campground (North Carolina) and Caesar's Head (South Carolina) populations, while those from the Linville Falls, Cradle of Forestry, and

Bluff Mountain populations (all North Carolina seed sources) remained uninfested. Results for HWA fecundity mirrored the results for infestation rate.

Gas chromatography–mass spectrometry analysis of the surface wax layer of needles collected from seedlings from these same Carolina hemlock seed sources indicate a potential role for epicuticular wax components in this differential susceptibility to HWA among Carolina hemlock provenances. The leaf surface wax layer of seedlings from the Carolina Hemlocks Campground and Caesar's Head populations, which supported high adelgid infestation and fecundity, contained high concentrations (0.5 - 0.6 ng/ml) of the high molecular weight alcohol n-hexacosanol, a known feeding stimulant of silkworm moth larvae (*Bombyx mori* L.) on mulberry (*Morus alba* L.) leaves (Mori 1982). Much lower concentrations (0.1 - 0.15 ng/ml) of n-hexacosanol were found in epicuticular wax extractions from those Carolina hemlock seed sources that did not support adeglid infestation. However, high n-hexacosanol concentration was not correlated with increased susceptibility to initial HWA infestation for all Carolina hemlock seed sources that supported high adelgid densities. Additionally, this analysis identified other components of hemlock epicuticular wax that may play a role in determining HWA feeding preference but have yet to be quantified by seed source.

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Interactions Between Invasive Herbivores: *Adelges tsugae*, *Fiorinia externa*, and Their Impact on Eastern Hemlock Growth and Foliar Chemistry

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ABSTRACT

The rapid population growth rates and high population densities of invasive species often leads to strong competition between invasive herbivores (Denno et al. 1995). Eastern hemlock (*Tsuga canadensis*) forests are threatened by two invasive herbivores, the elongate hemlock scale (EHS), *Fiorinia externa*, and the hemlock woolly adelgid (HWA), *Adelges tsugae*. Despite predictions that HWA would largely extirpate hemlocks in southern New England by the late 1990s, healthy hemlock stands still exist in areas where HWA has been present for more than 15 years (Preisser et al. 2008). One possible explanation of the slower rate of hemlock mortality in these areas is the range expansion of EHS into southern New England in the mid-1970s. Although recent surveys have shown hemlock mortality to be more strongly related to HWA density than to EHS density (Preisser et al. 2008), the interactions between these two species remain largely unexplored (McClure 2002).

In spring 2007, we inoculated previously-uninfested hemlock foliage with one, both, or neither herbivore species. After four months, we measured the impact of each herbivore on the population density of the other species as well as their individual and combined effects on foliar chemistry. EHS densities were significantly lower in the presence of HWA; however, EHS had no impact on HWA density. In terms of foliar chemistry, we found HWA-infested foliage (in both the HWA and HWA+EHS treatments) was lower in percent nitrogen (%N) and had a higher carbon-to-nitrogen (C:N) ratio than uninfested foliage. In contrast, the EHS and control treatments did not differ in %N and C:N ratio. These findings represent the first part of a three-year study to determine the impact of these species' interactions at a landscape level over time.

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A Proposed Method to Calculate the Active Ingredient per Acre of Imidacloprid Resulting from Soil Applications

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ABSTRACT

According to the U.S. Environmental Protection Agency (EPA), the maximum amount of imidacloprid active ingredient (a.i.) that can be applied into the soil is 0.4 lb a.i. per acre (Bayer, imidacloprid label) for non-tablet formulations. However, there is no common method in use to determine the a.i. per acre of soil applied imidacloprid. A proposed method was developed using Merit® (75 WP) soil injected trees (0.56 g a.i./cm DBH) at Mt. Lake, Virginia. Coordinates using GPS were recorded for 76 treated trees. Trees were mapped using GIS software. Three maps were generated, with 50-, 75-, or 100-ft-radius circles drawn around each tree location. A polygon was drawn around all contiguous tree circles. The area of each polygon was recorded. The total amount of active ingredient (a.i.) per acre applied in each polygon was calculated. The amount of a.i. per acre could then be found. The 50-ft buffer was found to be too small an area, and the 100-ft buffer was too large. Based on the grouping of the hemlock trees, the 75-ft radius circle should provide a more than adequate buffer zone to prevent imidacloprid contamination of the water system. The amount of a.i. per acre applied in this study ranged from 0.20 to 0.67 lbs per acre using the 75-ft-radius buffer. Calculating a.i. per acre can also be done without GPS using graph paper by mapping the trees by hand, circling each tree with a 75-ft-radius circle, connecting the overlapping circles, and counting the squares within each polygon created.

KEYWORDS

active ingredient per acre, buffer zone, hemlock woolly adelgid, imidacloprid, chemical control

INTRODUCTION

Imidacloprid (Merit®) is an effective systemic insecticide for the control of hemlock woolly adelgid (HWA) (Cowles et al. 2006; Webb et al. 2003). The use of this insecticide has increased, especially in forest settings where hemlock stands are frequently found along open water and trees can attain large diameters, thus increasing the imidacloprid dose required per tree. A limit of 0.4 lb of active ingredient (a.i.) per acre for soil applications of Merit® is the maximum amount allowed by the EPA. With the increased use of soil injections of imidacloprid in forests the possibility of contamination of water increases. Although migration of imidacloprid is usually very limited, rockiness of soil or applying in soil with low organic matter can increase migration (McAvoy et al. 2005). Calculation of active ingredient (a.i.) of insecticide per acre in uniformly spaced plantings of trees such as fruit or nut tree plantings is straightforward. Making this calculation using traditional methods would not be appropriate as hemlocks are unevenly distributed in the forest. Currently, no method exists to determine the amount of a.i. per acre of soil treatments for large stands of hemlocks in forest or urban settings.

Control of HWA was begun at Mountain Lake Resort, Virginia, in 2001. Due to the high number of trees in close proximity and the number of trees with large diameters, there was a concern that the limit of 0.4 pounds a.i. per acre would be exceeded. Consequently, a method to determine the a.i. per acre was developed. Standard units are used here because these units are used on the product labels and are more useful to forest managers.

METHODS

Hemlock trees were treated in 2001. All trees were soil injected with imidacloprid (Merit® 75 WP) at a rate of 0.07 oz of product per inch of DBH (0.56 g a.i./cm DBH, 0.00314 lbs a.i./inch DBH) using a Kioritz® soil injector. To determine the amount of a.i. of imidacloprid per acre, coordinates for each tree were determined using GPS (Garmin, GPS II Plus®). Trees were mapped using GIS software (ArcView 3.0). Three maps were generated with a 50-, 75-, or 100-ft-radius circle drawn around each tree location (Figures 1 through 3). A polygon was drawn around all contiguous tree circles, and the area of each polygon was calculated. The total amount of a.i. applied within each polygon was based on the sum of the imidacloprid soil injected per tree. The total amount a.i. per acre applied in each polygon was then calculated.

A mapping method not using GPS or GIS was also investigated. A map was made by plotting trees on graph paper. A 75-ft radius circle was drawn around each tree and the contiguous tree circles were connected (Figure 4). The number of squares within each polygon was counted to obtain the total area. The a.i. per acre was then calculated as described above.

RESULTS

Trees with 50-ft-radius circles had more polygons or groups (19) (Table 1) of trees than the 100-ft-radius polygons (8) (Table 2). The 50-ft-radius polygons also contained a smaller number of acres than the 100-ft-radius polygons: 0.4 and 2.8 acres, respectively. As a result, the 50-ft-radius polygons also had more trees per acre than the 100-ft radius-polygons: 7.9 and 3.0 trees per acre, respectively. Consequently, the mean a.i. per acre of imidacloprid applied in each polygon was higher in the 50-ft-radius polygons than the 100-ft-radius polygons: 0.64 and 0.24 lbs a.i./acre, respectively. As the radius of the circle around each tree increased, the a.i. per acre decreased. The 75-ft-radius polygons had a mean a.i. of 0.38 lbs per acre and 4.6 trees/acre (Table 3). In four groups of 75-ft-radius polygons, the 0.4 lbs a.i. per acre level was exceeded.

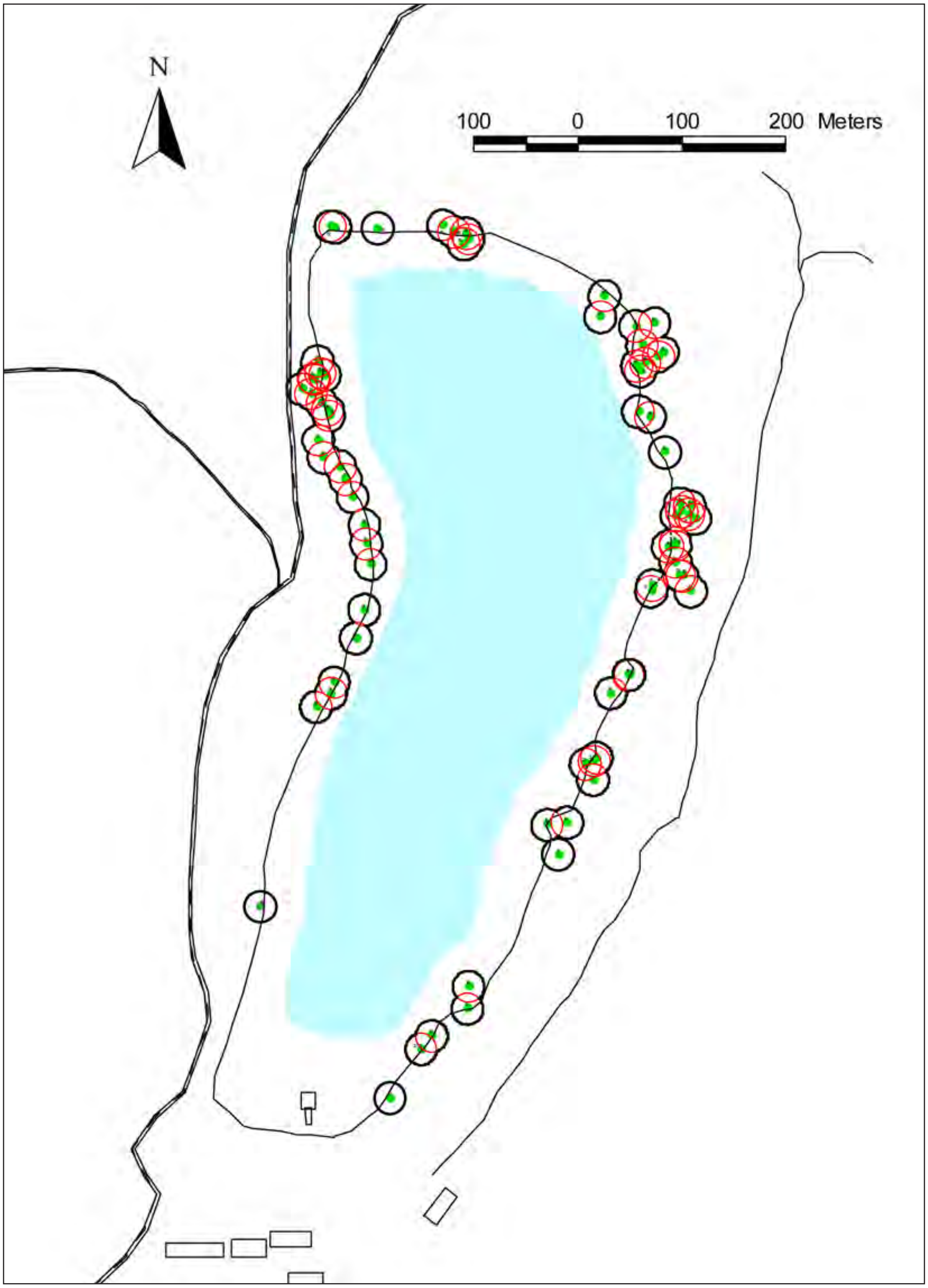

Figure 1. Groups (1-19) of hemlock trees at Mt. Lake with 50-ft-radius circles.

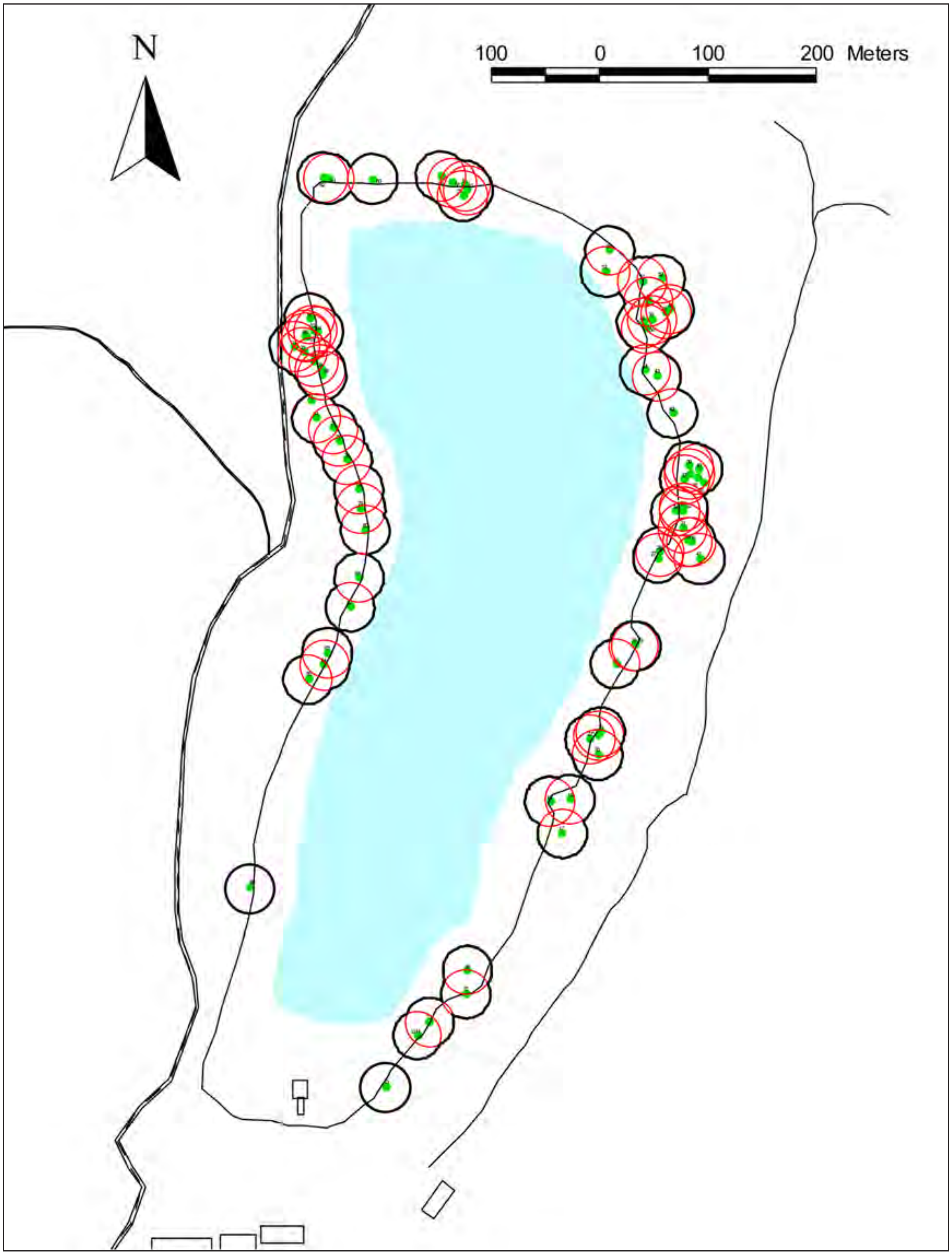

Figure 2. Groups (1-12) of hemlock trees at Mt. Lake with 75-ft-radius circles.

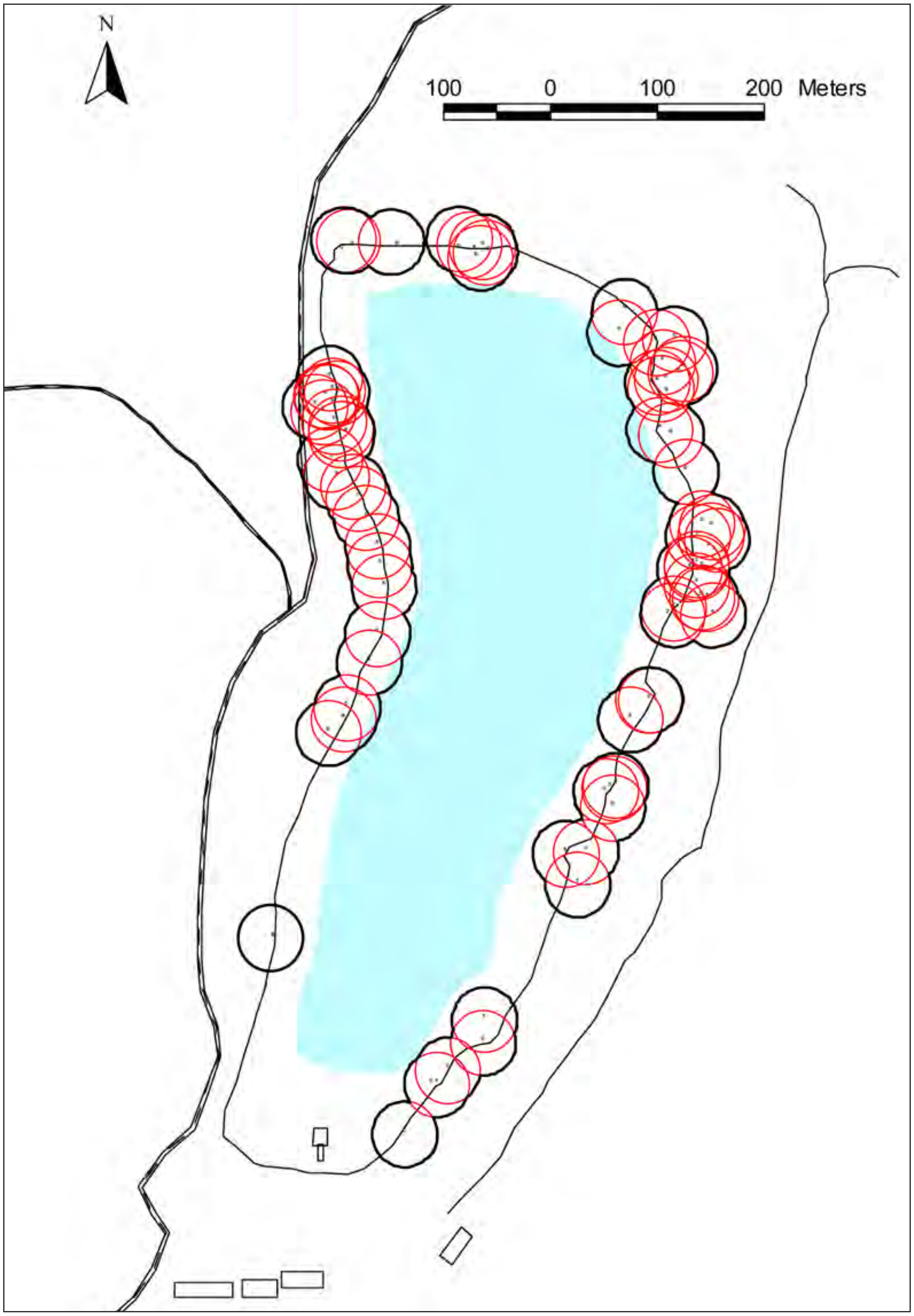

Figure 3. Groups (1-8) of hemlock trees Mt. Lake with 100-ft-radius circles.

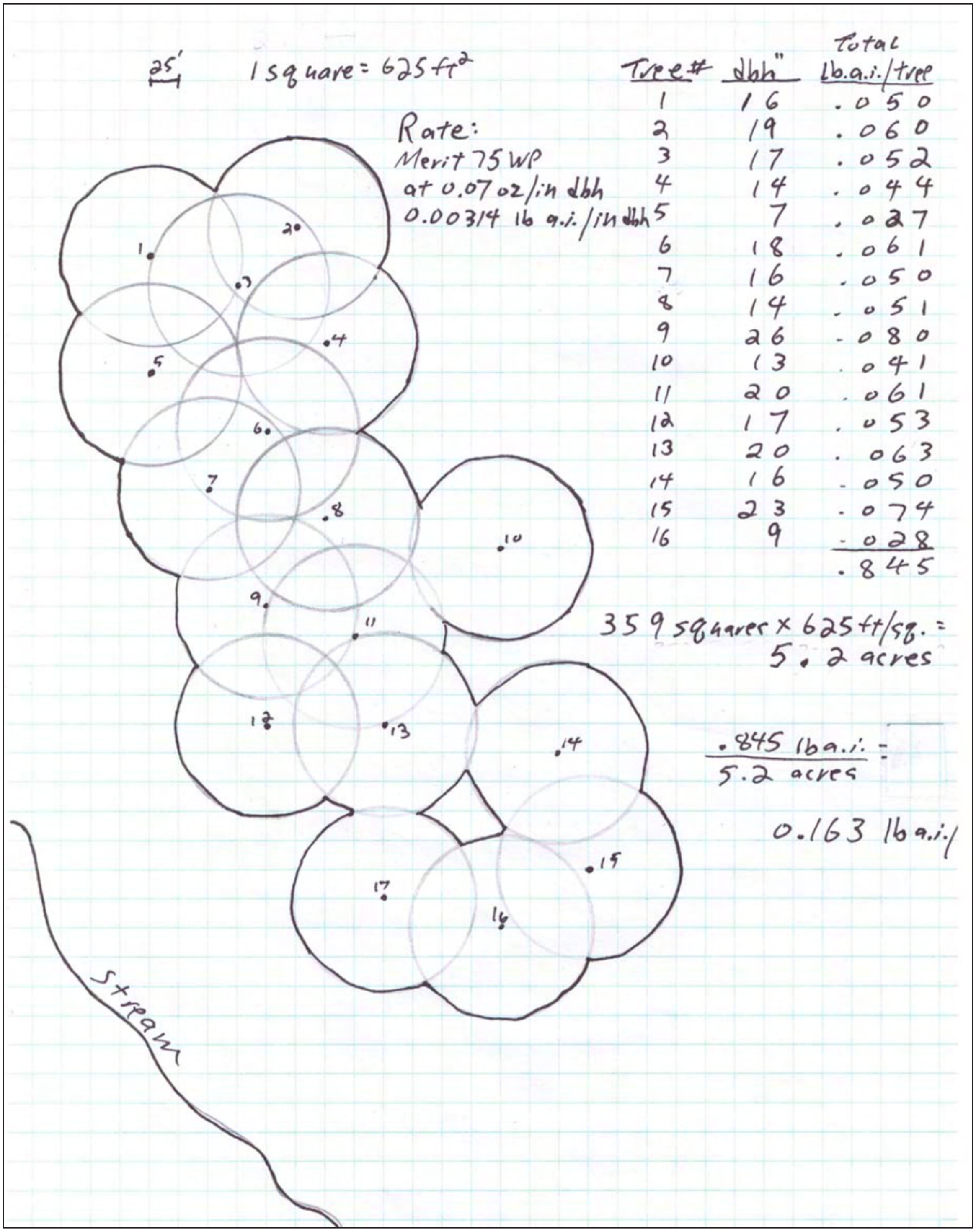

Figure 4. Graph paper method of determining a.i. per acre using 75-ft-radius circles.

Table 1. Number of trees, diameter at breast height (DBH), number of acres, trees per acre, and lb a.i. per acre using 50-ft-radius circles for groups of hemlocks at Mt. Lake, Va., treated with imidacloprid.

Group #	# Trees	Mean DBH (inches)	# Acres	Trees/Acre	Lb a.i./Acre
1	2	18	0.2	9.3	0.53
2	1	20	0.2	5.6	0.34
3	5	21	0.4	11.5	0.73
4	2	20	0.3	6.2	0.38
5	8	24	0.8	10.3	0.76
6	2	32	0.3	7.3	0.72
7	1	26	0.2	5.6	0.44
8	15	27	1.2	12.4	1.03
9	3	33	0.3	8.7	0.89
10	4	28	0.4	10.4	0.88
11	2	32	0.3	6.3	0.63
12	1	26	0.2	5.6	0.45
13	2	30	0.3	6.2	0.57
14	3	18	0.3	10.2	0.55
15	1	37	0.2	5.6	0.64
16	1	28	0.2	5.6	0.47
17	3	32	0.4	7.7	0.76
18	2	26	0.4	5.6	0.45
19	18	29	1.8	9.9	0.90
Sum/Means	76	27	0.4	7.9	0.64

Table 2. Number of trees, diameter at breast height (DBH), number of acres, trees per acre, and lb a.i. per acre using 100-ft radius-circles for groups of hemlocks at Mt. Lake, Va., treated with imidacloprid.

Group #	# Trees	Mean DBH (inches)	# Acres	Trees/Acre	Lb a.i./ Acre
1	3	19	1.4	2.2	0.13
2	5	21	1.2	4.1	0.26
3	28	26	6.5	4.3	0.34
4	3	33	1.1	2.7	0.28
5	7	29	2.4	2.9	0.26
6	6	25	2.6	2.3	0.18
7	1	27	0.7	1.4	0.12
8	23	29	6.2	3.7	0.34
Sum/Means	76	27.0	2.8	3.0	0.24

Table 3. Number of trees, diameter at breast height (DBH), number of acres, trees per acre, and lb a.i. per acre using 75-ft-radius circles for groups of hemlocks at Mt. Lake, Va., treated with imidacloprid.

Group #	# Trees	Mean DBH (inches)	# Acres	Trees/Acre	Lb a.i./Acre
1	3	19	0.9	3.5	0.20
2	5	21	0.8	6.4	0.40
3	13	25	2.6	4.9	0.37
4	15	27	1.8	8.1	0.67
5	3	33	0.7	4.5	0.46
6	4	28	0.7	5.8	0.49
7	3	30	0.9	3.3	0.31
8	5	23	1.2	4.2	0.29
9	1	37	0.4	2.5	0.28
10	1	28	0.4	2.5	0.21
11	3	32	0.7	4.0	0.40
12	20	29	3.5	5.7	0.51
Sum/Means	76	27	1.2	4.6	0.38

Based on the distribution of hemlock at Mt. Lake, which is typical of old growth stands, and our experience with treating hemlocks, the 75-ft-radius circle appeared to be the more practical and efficient to use and encompassed a reasonable number of trees. Imidacloprid is very stable when applied to high-organic-content soils and movement is very limited. The possibility of imidacloprid migrating beyond 75 feet is very unlikely but does increase when applied in rocky or low-organic-content soils. Also, the possibility of springs below the surface, especially in mountainous areas, may cause chemical migration. The 50-ft-radius circle may not be large enough an area to avoid over-treating, while the 100-ft-radius circle may be too large an area for effective treatment. Therefore, the 75-ft radius appears to be the most appropriate buffer radius for determining the a.i. per acre of imidacloprid. Using graph paper allowed the use of this method if land managers do not have easy access to a GPS device or GIS software and appeared to be a practical alternative (Figure 4).

Before treating a large hemlock stand, the trees should be mapped as described above using the 75-ft-radius buffer. After mapping, if the total amount of a.i. per acre is greater than 0.4 lb per acre, then trees within the polygon should be excluded until the total a.i. per acre is below the 0.4 lb per acre limit. Hemlock trees can then be treated.

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Phenology, Biology, and Collections of *Tetraphleps galchanoides*, a Predator of Hemlock Woolly Adelgid in China

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ABSTRACT

Tetraphleps galchanoides Ghauri (Hemiptera: Anthocoridae) was tested as potential agents for biological control of hemlock woolly adelgid (HWA). Adults, nymphs, and eggs were collected from 2005 to 2007 in Yunnan and Sichuan provinces on HWA-infested hemlock. Adults were found throughout the year, with peak adult collections occurring from early May to late-June. Eggs were found from early June through July and in November. Nymphs were found from April to May. In no-choice feeding tests, nymphs fed only HWA or pine bark adelgid, *Pineus strobi* (Hartig), and developed to the adult stage. Nymphs developed only through the first instar on balsam woolly adelgid, *Adelges picea* (Ratz.). Nymphs fed and developed on the bean aphid, *Aphis craccivora* Koch, in the laboratory in Beijing. Oviposition did occur in *Tsuga canadensis* (L.) Carr. needles. A total of 53 eggs were oviposited by one female in *T. canadensis* needles. More research is needed to determine the host specificity of *T. galchanoides*.

KEYWORDS

Tetraphleps galchanoides, hemlock woolly adelgid, biological control, Anthocoridae

INTRODUCTION

The holotype of *Tetraphleps galchanoides* Ghauri (Hemiptera: Anthocoridae) (Figure 1) was reported feeding on *Adelges* sp. on hemlock near Lachung, Sikkim, India (Ghauri 1972). Zheng and Bu (1990) and Bu and Zheng (1991 and 2001) reported this species in Lixian and Baoxing, Sichuan Province, Peoples Republic of China, on hemlock woolly adelgid (HWA) on *Tsuga chinensis* (Franchet) Pritzel. In was first evaluated as a biological control agent for HWA in 1996 by workers in a Sino-American cooperative program on biological control of loblolly pine mealy bug and HWA. This initial work found that *T. galchanoides* nymphs and adults ate 2.75±1.4 and 5.64±1.58 HWA eggs or first instars, respectively, daily (Yao and Wang 1998). In October 2002, early instar nymphs were discovered on foliage collected in Sichuan Province, China, and shipped to the quarantine laboratory at Virginia Tech University (VTU). In choice tests, *T. galchanoides ate* more HWA eggs than eggs of pine bark adelgid (PBA), *Pineus strobi* (Hartig) (Hemiptera: Adelgidae), and completed development feeding on HWA (McAvoy et al. 2007). Another three-year Sino-American program on biological control of HWA conducted from 2005-2007 provided additional information on *T. galchanoides*, which is summarized in this report.

Figure 1. Adult *Tetraphleps galchanoides*.

METHODS

Collections in China for *T. galchanoides* began in 2005 in Yunnan and Sichuan provinces, China. Collections of *T. galchanoides* were kept in China for study or shipped to the quarantine facility at VTU. Collections were made by beating hemlock branches over a beat sheet and collecting adults or nymphs that fell. Foliage was collected and examined under a dissecting microscope for eggs in the needles and adults or nymphs on the foliage.

In a shipment received at the VTU quarantine facility on Dec. 13, 2007, 63 eggs and one live female were found. Forty-eight eggs (76%) hatched. Nymphs and adult were reared at 15°C with a 12:12 (L:D) photoperiod in no-choice feeding studies using HWA on *Tsuga canadensis* (L.) Carr., PBA, and balsam woolly adelgid (BWA), *Adelges piceae* (Ratz.) (Hemiptera: Adelgidae). Both nymphs and adult were fed *ad libitum*.

RESULTS

Based on collections from 2005 to 2007 primarily in Yunnan Province, adults were found generally throughout the entire year (Figure 2). The greatest number of adults was collected from mid-May to late June. This may be the peak period of adult emergence. The greatest number of adults collected was 252 on one collecting trip from May 23 to 28, 2007. As adults were found consistently throughout the year, it is difficult to determine when generations of this species occur based on adult collections. A higher proportion of males than females were found in all collections, ranging from 67% to 92% males.

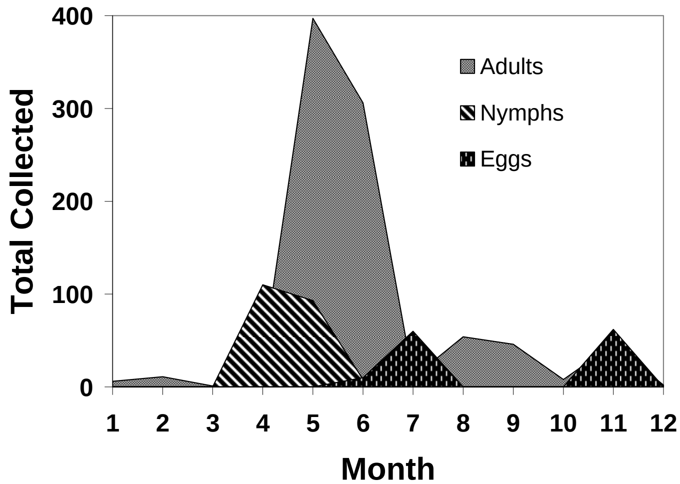

Figure 2. Phenology of *Tetraphleps galchanoides* based on collections made in China.

Eggs were found from early June through July following peak adult occurrence. Eggs were also collected in November: this may indicate that there are two generations per year. Although no nymphs were found after eggs were observed, they were observed from April to May. Perhaps eggs aestivate during the summer and hatch in the fall. Eggs recovered in the December 2007 shipment did hatch and may indicate that hatch occurs in December in the field, but this needs to be confirmed by field observation. More field collections are needed to determine accurately the phenology of this species.

Forty-eight of the 62 eggs began hatching (77% hatch rate) two days after the shipment arrived. In the BWA no-choice tests, nymphs completed only the N-I stage (Table 1). Nymphs feeding exclusively on HWA or PBA (Figure 3) successfully completed development to the adult stage. Nymphs fed and developed on the bean aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae), in the laboratory in Beijing. When five aphids were left overnight in a Petri dish with an adult bug, an average of four aphids were killed. The aphids were not killed immediately, and the entire aphids were not eaten. Chacko (1973) reported that adults of *Tetraphleps raoi* Ghauri rarely eat the entire host when food is abundant.

Table 1. Development and survival of *Tetraphleps galchanoides* nymphs feeding on hemlock woolly adelgid, pine bark adelgid, and balsam woolly adelgid in no-choice tests at 15°C.

	Hosts								
	Hemlock Woolly Adelgid			Pine Bark Adelgid			Balsam Woolly Adelgid		
Stage	n	# Days ±SD	% Survival	n	# Days ±SD	% Survival	n	# Days ±SD	% Survival
Hatched eggs	18			14			16		
N-I	13	7.7±1.1	72	6	6.9±0.7	43	5	5.2±1.1	31
N-II	6	3.9±1.8	33	5	4.7±1.0	36	0	-	0
N-III	3	6.2±1.5	17	5	6.9±1.2	36			
N-IV	3	8.3±0.6	17	5	7.9±0.6	36			
N-V	2	14.4±0.7	11	3	14.2±0.9	21			

Figure 3. *Tetraphleps galchanoides* N-III nymph feeding on pine bark adelgid.

Tetraphleps galchanoides eggs are normally inserted into the tissue on the underside of the needle with only the operculum visible (Figures 4-6). The female began ovipositing in *T. canadensis* needles seven days after the shipment arrived and continued ovipositing for 21 days. This is the first confirmation of *T. galchanoides* ovipositing in *T. canadensis*. A total of 53 eggs were oviposited, with a mean of 2.3 eggs per day. Egg development took 18.4 days at 15°C. Half of the eggs were oviposited in the plant tissue while half were oviposited on the lower needle surface. This has been observed in other *Tetraphleps* species (Chacko 1973).

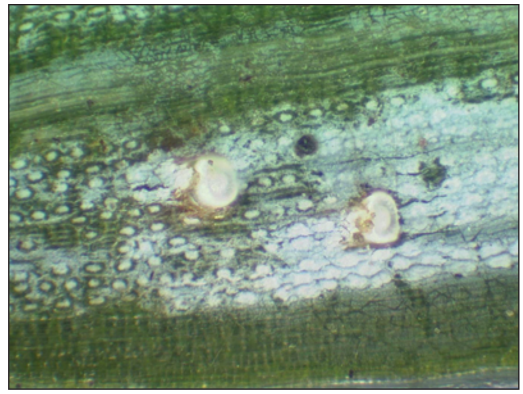

Figure 4. *Tetraphleps galchanoides* egg operculum on underside of needle.

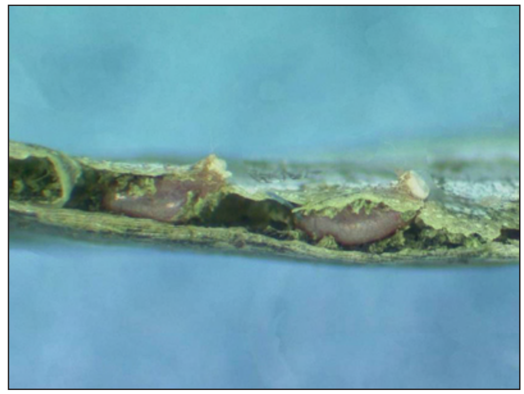

Figure 5. *Tetraphleps galchanoides* eggs in cross section of hemlock needle.

Figure 6. *Tetraphleps galchanoides* hatched egg and first instar nymph.

Although, nymphs may be able to complete development on more species than HWA, oviposition may be restricted to hemlock needles, possibly making this species facultatively host-specific to hemlock. More research is needed to determine its host specificity.

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Hemlock Woolly Adelgid Phenology and Predacious Beetle Community on Japanese Hemlocks

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ABSTRACT

Monthly samples of the hemlock woolly adelgid (HWA), *Adelges tsugae*, and predatory beetles were taken from *Tsuga sieboldii* near the border of Osaka and Kyoto prefectures. The beetles were collected by sweeping the canopy up to 5 meters height with nets. The phenology of HWA life stages were monitored by collecting branches and determining, under the microscope, the number of each stage of HWA present per cm twig length. The phenology of HWA on the hemlock appears to be similar to that observed in the eastern United States. Predacious beetles present included many generalist species, especially in the spring months. *Sasajiscymnus tsugae* was present in every month except January and February. A new species of *Laricobius* (Derodontidae) was present from November to May. The new *Laricobius* beetle would be a good compliment to *S. tsugae*, which was exported to the U.S. in 1994 from these study trees.

KEYWORDS

Adelges tsugae, Japan, *Laricobius* new species, *Sasajiscymnus tsugae*, seasonal history

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an introduced pest in the eastern United States that can be lethal to the native hemlocks, *Tsuga canadensis* (L.) Carriere and *T. caroliniana* Engelman, growing there. HWA is innocuous in Japan, China, and western North America, where it is a native insect (Havill et al. 2006). Because HWA populations are not effectively regulated by natural enemies in the eastern U.S., classical biological control is considered the most promising option for controlling this pest in a forest setting (Cheah et al. 2004). Predators are the only natural enemies known to attack the family Adelgidae. Recently, Havill (2006), using DNA, pinpointed the origin of HWA in the eastern U.S. to a population in the central part (Kansai region) of Honshu Island, Japan. Because the Osaka Museum is interested in natural history information on insect fauna in the Kansai district and the USDA Forest Service desires information on HWA and its natural enemies in its native habitat, the two groups initiated a cooperative study in 2005.

METHODS

Tsuga sieboldii Carriere trees in a landscape setting at Nakahata, Takatsuki, Osaka Prefecture, were sampled every month for a year beginning in October. *Sasajiscymnus tsugae* (Sasaji and McClure) was collected and exported to the United States from one of these trees in 1994 (Sasaji and McClure 1997). The number and stage of HWA on the twig samples were determined using a microscope. During each visit, HWA-infested hemlock were collected and branches were swept using nets that could reach up to 5 meters into the canopy. Adult predacious beetles and insects in the net were sorted to type and counted. Occasional samples for predacious beetles on hemlock trees were taken at the Kobe Arboretum, Hyogo Prefecture; Koyasan, Wakayama Prefecture; Mt. Tsurugi, Tokushima Prefecture; Maji Village, Kochi Prefecture; and the lower slopes of Mt. Fuji.

RESULTS AND DISCUSSION

HWA Life Cycle

In July, almost all HWA were diapausing first instars on the new twigs. They began developing in September, and the adults, covered with wool, appeared in December. Most individuals were adults by February, and many eggs were found underneath the wool beginning in February. In spring, many crawlers hatched, dispersed, settled on twigs near their mothers, and developed rapidly. Eggs of the winter and spring generations were present from March to July. HWA phenology at Takatsuki seems to be similar to that in the eastern U.S., except that a few HWA may break diapause earlier in the fall and a few crawling stages were found during the winter (Figure 1).

Predators

Twenty-seven predacious species in five families of Coleoptera were collected in sweep nets from Japanese hemlocks (Table 1). Although some immature insects were collected, the table includes only adults that could be identified. In addition to beetles, larvae of hoverflies such as *Heringia familiaris* Matsumura (Syrphidae) and green-lace wings (Chrysopidae) were observed from spring to summer. The months with the greatest diversity of predacious beetles were April-June, when HWA eggs were plentiful. Elaterids and cantharids were abundant

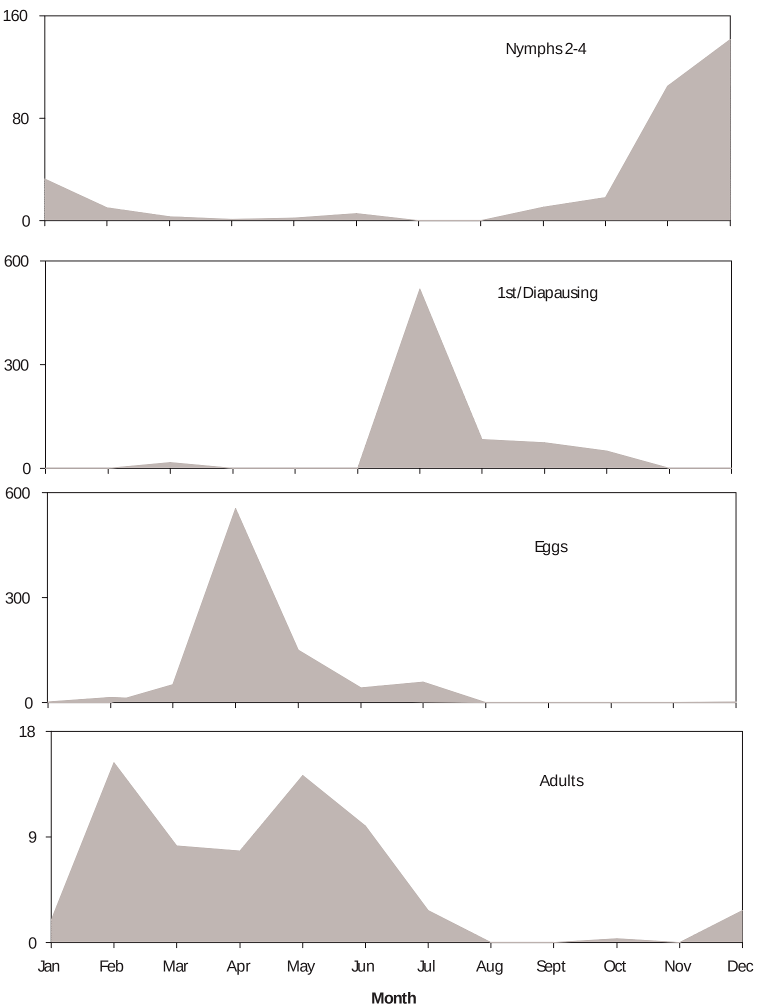

Figure 1. Phenology of *Adelges tsugae* life stages at Takatsuki, Japan

during this season The cantharids are large and voracious, and one *Asiopodabrus* sp. individual consumed four sacs of eggs and adults and 17 settled nymphs overnight in the laboratory. The coccinellid, S. *tsugae*, was observed on the hemlocks for more months than any other beetle; the only months it was not collected were January and February It seems to be the predator most likely responsible for the decline in number of the diapausing HWA nymphs during the summer. *Sasajiscymnus tsugae* was not found on other conifers near Takatsuki, and HWA is the only prey on hemlock on which we have seen it feeding. We have found *S. tsugae* only in the Kansai area (Osaka, Hyogo, Wakakusayama, and Wakayama prefectures) on *T. sieboldii* growing in landscape settings, but other scientists have collected it in other areas of Honshu Island on pine and in marsh grasses far from any hemlock. Adults of a derodontid beetle, *Laricobius* sp. nov., were present from November to May (the peak, in March). In Japan, the derodontid beetle is abundant and fairly widespread and seems to greatly reduce the over-wintering HWA adults and the eggs laid by this generation.

Table 1. Predacious beetles collected in sweep nets from hemlock trees.

Family/Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Elateridae												
Ampedus carbunculus (Lewis)					●							
Cardiophorus niponicus				●								
Dalopius bizen Kishii					●							
Displatynychus adjutor (Candeze)						●						
Dolerosomus gracilis (Candeze)				●	●							
Kibunea eximia (Lewis)					●							
Melanotus annosus Candeze						●						
Melanotus correctus Candeze					●							
Spheniscosmomus cete cete (Candeze)					●							
Spheniscosmomus koikei (Kishii et al)					●							
Cantharidae												
Athemus vitellinus (Kiesenwetter)					●							
Athemus suturelles (Motchulsky)					●							
Micropodabbrus longipes longipes					●							
Hatchiana heydeni (Kiesenwetter)					●							
Hatchiana sanoi Imasaka					●							
Asiopodabrus lictorius (Lewis)					●							
Asiopodabrus malthinoides				●	●	●						
Derodontidae												
Laricobius sp. nov	●	●	●	●	●						●	●
Melyridae												
Dasytes japonicus Kiesenwetter					●							
Laius hisstrio Kiesenwetter						●	●					

Table 1 (cont.). Predacious beetles collected in sweep nets from hemlock trees.

Family/Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Coccinellidae												
Chilocorus kuwanae Silvestri		●		●		●						
Oenopia hirayamai (Yuasa)	●											
Phymatosternus lewisii (Mulsant)		●										
Sasajiscymnus tsugae (Sasaji et al)			●	●	●	●	●	●	●	●	●	●
Scymnus giganteus H. Kamiya				●	●			●				
Scymnus hoffmanii Weise									●			
Harmonia axyridis (Pallas)			●	●					●	●	●	●

CONCLUSIONS

The Japanese *Laricobius* species appears to be an important natural enemy in the region that is the source of the HWA that is in the eastern United States. It seems to be a very promising candidate for biological control. More study of its life cycle and host-range in its endemic area coupled with host-range testing on potential non-target species in the U.S. is needed prior to its release. In addition to the *Laricobius* beetle and *S. tsugae*, many generalist species were collected from the hemlocks, and some of these are voracious on HWA. Based on initial observations, it appears that *Laricobius* is an important predator during the winter and early spring months, with generalists active in late spring and early summer, and only *S. tsugae* remains active on the tree during the summer months when HWA is in diapause. Thus, the Japanese *Laricobius* should be a good compliment to the Japanese *S. tsugae* already released for biological control of HWA in the eastern U.S.

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IMPROVING THE ACCURACY OF CROWN VOLUME ESTIMATES IN EASTERN HEMLOCK

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KEYWORDS

impulse laser, crown volume

ABSTRACT

Soil and trunk injection treatments for the control of the hemlock woolly adelgid use the diameter of the tree at 1.3 m height as the basis for determining dosage. It is our hope that additional information such as crown volume, in addition to DBH, can be used to better adjust the dosage needed for treatment.

In order to investigate this relationship between dosage and tree size, an accurate estimate of crown volume was needed. Traditionally, crown volume has been approximated by assuming some geometric solid. In many cases, a conoid or paraboloid formula has been employed due to the ease of calculation, but this may not provide the accuracy needed to develop specific treatment guidelines based on tree diameter and crown size.

Thirteen hemlock trees (*Tsuga canadensis* (L.) Carr.) were selected for this study. Ten trees were selected from four counties in northern West Virginia, two from western Maryland, and one from southwestern Pennsylvania. An Impulse 200 LR laser hypsometer was used to collect crown measurements. For each sample tree, crown volume was estimated by assuming a conoid and paraboloid geometric form and using the estimated crown diameter at the base of the crown and the measured crown length. We felt an improvement in accuracy could be obtained by fitting a polynomial to the average crown radius data and using the solid of revolution to obtain volume. Initial work indicated that a third order polynomial of the form:

$$Y = a_0 + a_1 x + a_2 x^2 + a_3 x^3$$

worked well and could be applied to the variable shaped crowns that were found for this species. 267

In general, the third degree polynomial fit the crown radii data well, with r^2 values ranging from 0.72 to 0.98, with most (77%) of the trees having r^2 values greater than 0.90. In this particular study, no actual true crown diameter was known, but based on the visual fit of the model through the measured crown radii, this flexible polynomial fit the data points well. Crown volume estimates based on the assumption of a conoidal form resulted in a root mean squared difference of 201.6 m^3. When assuming a paraboloidal form, the root mean squared difference was 362.3 m^3. In both instances, using a geometric form to approximate tree form generally resulted in large over-estimations of crown volume in 77% of the trees. Visual examination of these differences indicated that this volume error increased proportionally with tree size. The ability to more accurately estimate tree crown volume should improve pesticide dosage estimates for this species.

Spatial Distribution of Fine Roots and Soil Carbon Beneath Eastern Hemlock

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KEYWORDS

eastern hemlock, fine root biomass, soil carbon, topographic position, bioavailibility

ABSTRACT

Soil-related parameters (such as moisture, texture, temperature, and rooting depth) and vegetation-related parameters (such as tree density and species complex) impact the longevity, sorption, desorption, and bioavialibility of soil-injected chemicals, but little is known about these parameters in eastern hemlock forests. As a first step to elucidating some of these effects, we explored the variability in soil- and vegetation-related parameters. Specifically, as fine roots (smaller than 2 mm in diameter) are important in the uptake of nutrients, water, and soil-injected chemicals, soil carbon is an important soil property. We explored the relationship between these parameters and topographic position.

The distribution of fine roots (less than or equal to 2 mm diameter) and soil carbon in an eastern hemlock (*Tsuga canadensis* Carriere) stand was investigated. Three equally sized trees (22 to 24 cm diameter at 1.3 m height) at three topographic positions (footslope, backslope, and shoulder slope) were sampled in the summer of 2007. At each tree, we sampled the soil and roots using a 15.2-cm soil corer. Six cores to a depth of 30 cm were taken at randomly selected cardinal directions at bole (30.4 cm), mid-crown, and drip-line from opposite sides of the tree. The cores were separated into 7.6-cm-depth increments. Live and dead roots were separated from the soil, washed, and sorted by size. Soil carbon was determined on a LECO TruSpec CHN Analyzer.

Fine root biomass and soil carbon did not differ significantly between the three sample locations beneath the crown of the eastern hemlock trees sampled. Both fine root and soil carbon decreased with increasing depth at all slope positions, with 43% to 57% of the biomass and 40% to 63% of carbon in the top 7.5 cm of soil. Due to the lack of replication, any findings are tentative, but these data do suggest that parameters such as root distribution and soil carbon may change with landscape position. If so, landscape position may need to be considered when using soil-injected chemicals.

Hemlock Ecosystem Monitoring in Southern West Virginia

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ABSTRACT

We initiated a long-term hemlock ecosystem monitoring study in 1998 on the New River Gorge National River (NERI) and Gauley River National Recreation Area (GARI), in Nicholas, Fayette, and Raleigh counties, West Virginia, to quantify ecosystem response to invasion by the hemlock woolly adelgid (HWA). Hemlock vigor and degree of adelgid infestation were quantified in 1998-2007, vegetation structure and composition were sampled in 1999 and 2007, and avian populations were sampled 1999-2007. The HWA was detected on eight of 36 sampling plots in 2004 and 22 of 36 by 2007. Now that most of the monitoring plots are infested with HWA and the literature suggests that tree mortality can occur within four to six years (McClure 1991), we expect hemlock mortality to ensue. In this paper, we summarize data collected pre-infestation and during the initial stages of infestation.

KEYWORDS

adelgids, vegetation structure composition, songbirds

INTRODUCTION

Introduced forest pests such as the hemlock woolly adelgid (HWA), *Adelges tsugae*, can substantially change native forest ecosystems and the wildlife communities they support. The HWA, introduced in Virginia in the early 1950s (Stoetzel 2002), is a serious pest of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*), causing extensive hemlock mortality (Souto et al. 1996). The HWA was first detected near our study areas in West Virginia on the Bluestone River in April 2000 and the New River Gorge National River in March 2002 (J. Perez, pers. comm.).

Prior to adelgid infestation, we established a long-term hemlock ecosystem monitoring project on the New River Gorge National River (NERI) and Gauley River National Recreation Area (GARI). The study was initiated in 1998 with baseline surveys of adelgid presence and hemlock vigor in fall/winter 1998. In summer 1999, baseline vegetation composition and structure were quantified (Wood 1999a, 1999b), and baseline songbird surveys were initiated (Wood 2004). Our study, designed to detect change on a landscape scale, covers an extensive area spanning approximately 97 km from north to south and ranging in elevation from 427–853 m (1400–2800 ft). Our pre-infestation data on the NERI/GARI hemlock forests provides a rare dataset of the extant Appalachian hemlock ecosystem prior to the arrival of the HWA.

Hemlock ecosystems harbor avian species dependent on conifer habitats (Tingley et al. 2002). After infestation by adelgids and as the plant community changes from hemlock dominated stands to hardwood-dominated stands or perhaps early successional stands, the avian community also will change. To document these changes, it is necessary to identify species richness and abundance of the avian community before the adelgid arrives and as changes in the hemlock community ensue. Local species demographics are often tied to aspects of the plant community such as vertical structure, density, and relative abundance of species (Franzreb 1983); changes in this structure can result in changes in the avian community (Litwin and Smith 1992).

The overall objectives of our long-term study are to 1) document HWA invasion and the resulting hemlock decline and mortality, 2) quantify the changes in plant species composition, structure, abundance, and richness over time, and 3) quantify avian richness and abundance to relate these songbird metrics to changes in vegetation characteristics. The vegetation sampling was specifically designed to detect changes in all layers of the plant community by collecting vegetation data in the following categories: 1) density and species richness of trees, saplings, shrubs, and vines, 2) cover and vertical structure of vascular plants, 3) frequency of occurrence of understory vascular plants, 4) hemlock diameter, live crown ratio, vigor, and straightness, and 5) density and diameter of all other tree species. As HWA invasion is just beginning to occur on our study plots, this paper summarizes data from pre-invasion and during initial outbreaks of HWA.

STUDY AREA AND METHODS

We established 36 long-term hemlock ecosystem monitoring plots in the NERI and GARI, in Nicholas, Fayette, and Raleigh counties, West Virginia, during November 1998 (Wood 1999a and 1999b). Three moisture levels were each replicated 12 times: hydric, mesic, and xerix. Study plots ranged from the northern-most sites at Carnifex Ferry State Park along the Gauley and Meadow rivers to the southern-most sites at Kate's Branch and Grandview State Park near the New River, a distance of approximately 97 km. The 36 plots were intentionally placed in hemlock stands representing a variety of habitats with varying degrees of soil moisture, elevation, slope, and aspect as directed by the National Park Service (NPS) with the intention of gathering the maximum amount of data on biodiversity.

Each plot was 400 m^2 (0.04 ha) in size, with dimensions similar to other HWA stand-level monitoring studies (Mahan et al. 1998; Orwig and Foster 1998). Depending on site conditions, plots were placed either within a hemlock stand or within an isolated patch of hemlock trees and were square (20 m x 20 m) or rectangular (10 m x 40 m). On sites where hemlock was a co-dominant rather than a dominant tree species, plot-centers were deliberately placed where there was a visible amount of hemlock canopy cover. Centers and corners of each plot were permanently marked. Plots were placed at least 250 m apart when possible to accommodate the avian study. Eight pairs of points were less than 250 m apart; none were less than 100 m apart. Generally, 250 m between point count stations is considered sufficient for independence between avian sampling stations (Ralph et al. 1993), although some studies have used distances of 100 m (Pendleton 1995).

Hemlock Stand Age

A total of 108 hemlock trees, three from each plot, were cored with a tree increment borer in April 1999. Whenever possible, one core was taken from a tree randomly selected from each of the three largest diameter classes in each plot. Tree rings were counted, without staining, using a dissecting stereo-microscope. If the exact center of the tree was missed in the coring process or if the radius of the tree was greater than the length of the borer, the total number of rings was estimated. This estimate took into account both the measured radius of the tree and the growth rate of any older trees cored at the same geographical location in the study area. Mean age of the three cored trees was used to represent stand age. Stand ages were compared among moisture-gradient classes using a fixed-model, nested analysis of variance (ANOVA) that included geographic location, moisture-gradient class, and the replicated plot effect.

Hemlock Diameter, Vigor, Live Crown, and HWA Infestation

We initiated baseline surveys of adelgid presence and hemlock tree vigor, live crown ratio, and diameter in fall/winter 1998. These data were collected each fall/winter except 1999 and 2005. Data were collected to coincide with the November-April period in which the current season's HWA population typically exhibits woolly characteristics (Onken et al. 1994).

All hemlock trees that were rooted within or intersecting the outside perimeter of each 400 m^2 plot were tallied and diameter at breast-height (DBH) was measured. A tree was defined as any stem 8 cm DBH or bigger. Each tree was given a crown-vigor class rating that was an index of the health of the live crown based on Onken et al. (1994). The entire crown was inspected using binoculars and ranked as '1' = >95% healthy crown, '2' = >75-95%, '3' = >50-75%, '4' = >25-50%, and '5' = >0-25%. Additionally, a live crown ratio was visually estimated as the percentage of the total tree height with live foliage (Miller et al. 1998). The extent of HWA infestation was rated for each hemlock. Degree of HWA infestation was ranked from 1–4, with '1' = heavily speckled and visible from 30 m, '2' = moderately speckled, '3' = lightly speckled with only a few scattered specks, and '4' = none.

The distribution of hemlock trees assigned to 10 DBH classes was compared between 1998 and 2006 with a Cochran-Mantel-Haenszel (CMH) chi-square statistic, which allowed us to simultaneously test for differences among the three moisture classes. The CMH test also was used to compare the distribution of hemlock trees assigned to the four HWA ranks and to the five categories of live crown ratios in 1998 vs. 2006 and among the three moisture classes.

Vegetation Sampling

Vegetation structure and composition initially was sampled in summer 1999 and then re-sampled in summer 2007 using methods summarized in a final report (Wood 1999b) and detailed in field sampling protocols (Wood 1999a). Personnel were trained in vegetation sampling protocols.

Shrub and sapling stems (those greater than 8 cm DBH and less than 1.4 m tall) were counted and identified to species on four belt transects (each was 2 x 10 m, or 20 m^2) that typically began 1 m from the plot center and extended towards each plot corner. All tree stems (those greater than 8 cm DBH and greater than 1.4 m tall) on the 400 m^2 plot were tallied, identified to species, and their DBH measured. Stem density of hemlock saplings, hemlock trees, and hardwood trees and overall species richness were compared between the two years with a fixed-model, nested ANOVA. For species richness and tree density, the ANOVA model accounted for geographic location and moisture gradient class. For sapling density, the ANOVA model accounted for geographic location, moisture gradient class, replicate, and transect.

Percent cover of vegetative forms (trees, shrubs, forbs, grasses/sedges/rushes, ferns, mosses, snags, dead saplings and shrub stems, fallen logs, woody debris, and leaf litter), bare ground, and bare rock was estimated on the four 10 m-long line-transects on each plot. Cover was vertically stratified based on the layers of vegetation present in each plot (i.e., understory, shrubs, low midstory, high midstory, subcanopy, and canopy), and presence/absence of each pertinent structural component was recorded at every decimeter (100 points per transect). The average maximum and minimum heights of each vertical stratum were estimated at the time of sampling and varied from transect to transect (Wood 1999a). This method of estimating the height of vegetative strata has been used effectively by The Nature Conservancy in community-classification sampling and captured the structural diversity measures important to avian species. In this paper, we present only results of hemlock cover in the six strata due to space limitations. We used ANOVA to compare cover for each strata between years and accounted for geographic location, moisture gradient class, replicate, and transect.

Avian Sampling

The center of each vegetation sampling plot served as the center of the bird point count station. During 1999-2007, songbird richness and abundance were quantified using standardized sampling protocols on circular point count plots (Ralph et al. 1993) within a 50-m radius and at an unlimited distance. All birds seen or heard from one-half hour after sunrise to 1030 hours during appropriate weather conditions were recorded. Each count lasted 10 minutes and was conducted twice during the breeding season to detect both early and late migrants. All points were sampled once during the first or second week of June; they were re-sampled the third or fourth week of June. We attempted to have at least one week between the two samples at each point. All point-count personnel were skilled in bird identification by sight and sound.

We summarized avian richness and abundance on each plot in each year using count data from an unlimited radius to increase sample size of detections and to avoid variability among observers in distance estimation. Species richness was the total number of different species detected, while relative abundance was the maximum number of individuals of a given species detected over the two sampling periods (Wood 2004). Species also were categorized into habitat (forest interior, interior-edge, or edge) guilds to examine abundance and species richness for each guild. Yearly trends in avian species richness and abundance were examined with a general linear ANOVA model in which our primary variable of interest—year—was treated as a continuous variable, and we accounted for variability in the data due to geographic location and moisture gradient class.

RESULTS AND DISCUSSION

Hemlock Health and Cover

At the beginning of the study in 1998, hemlock tree ages ranged from 38-328 years. Trees in the hydric plots (n = 36, mean = 156.3 years, range = 66-328) were older than mesic (n = 36, mean = 74.3, range = 44-131) and xeric (n = 36, mean = 69.2, range = 38-136) plots (F = 59.69, P = 0.0001). The hydric plots sampled in this study tended to be less accessible (rockier and further from existing roads) than the other plots which likely contributed to their older stand age.

Average (+SE) diameter of live hemlock trees was similar in 1998 and 2006 (1998: n = 482, mean = 25.1+0.7; 2006: n = 485, mean = 26.2+0.7) and the distribution across diameter classes did not change (Cochran-Mantel-Haenszel statistic = 1.2, P = 0.27). About 1/3 of the live hemlock trees in both years were in the smallest (8-14 cm) DBH class. Largest trees occurred in the hydric plots where maximum DBH was 99.1 cm, while the maximum DBH in mesic and xeric plots was 68.6.

In November 1998, no plots had evidence of HWA infestation. We obtained five years of pre-infestation data on the hemlock trees as HWA was first detected on eight of the study plots in 2004. These eight plots had individual trees with an HWA index of '3', indicating low severity of infestation. In fall/winter 2006-07, HWA was detected on 154 trees ranging in size from 8-74 cm DBH. Mean HWA rank was 3.8 in hydric plots, 3.7 in mesic plots, and 3.2 in xeric plots, indicating that xeric plots were infested more often and more heavily (Cochran-Mantel-Haenszel statistic = 63.15, P = <0.0001) (Figure 1).

Of the 518 hemlock trees tallied and measured during November 1998, 36 were dead. In fall/winter 2006-07, 41 of 526 hemlock trees tallied were dead. Stem density of dead hemlock trees did not change across years (F = 0.05, P = 0.82). In 1998, most live hemlocks appeared to be very healthy: 69.3% of the 482 live trees had >95% healthy crown, while only seven trees (1.5%) had vigor = '3' (50-75% healthy crown). By 2006, 22.7% of the 485 live trees had crown vigor = '3'. The majority of low vigor trees were in the smallest DBH classes (Figure 2).

Stem density of live hemlock trees and saplings did not change across the two sampling years (Table 1). Although HWA infestation is beginning to affect hemlock crown vigor, it has not resulted in significant hemlock mortality or changes in live stem density. Stem density of hardwood trees declined slightly and approached significance. Species richness (excluding hemlocks) was not different across the two years but did vary among the three moisture gradients (F = 3.99, P = 0.02). The hydric moisture class had a mean richness of 4.4, while mesic was 5.7 and xeric was 5.6.

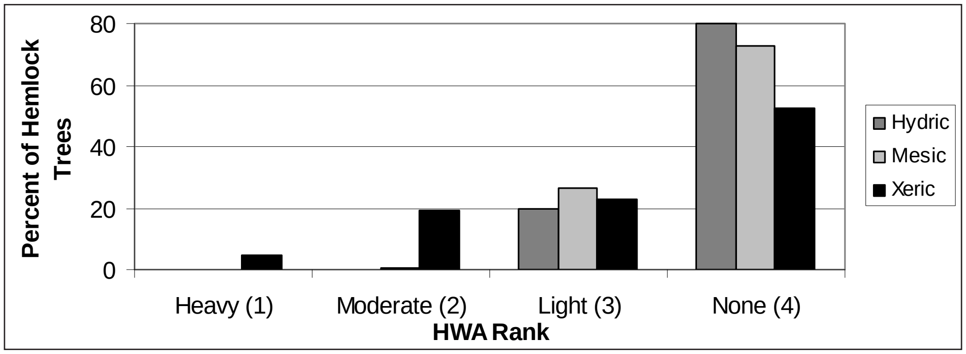

Figure 1. Percent of hemlock trees in each HWA infestation rank by moisture class, 2006.

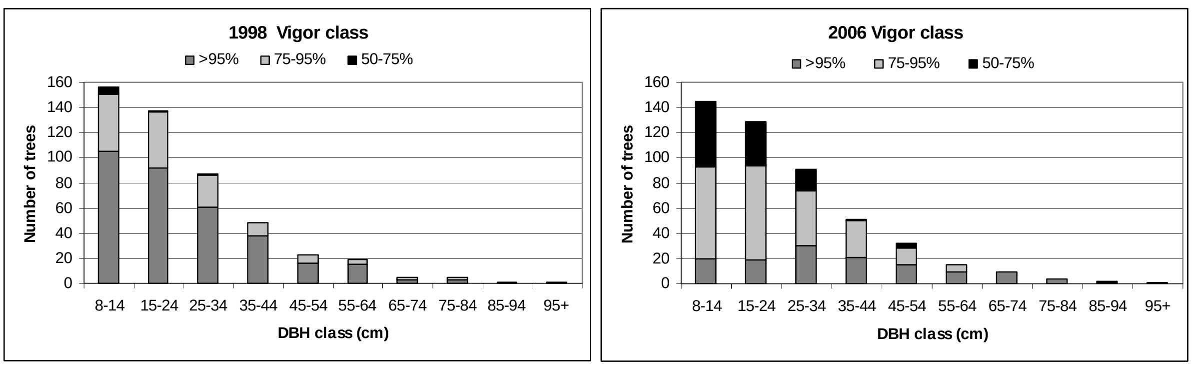

Figure 2. Number of hemlock trees assigned to each vigor and DBH class in 1998 vs 2006.

Table 1. Hemlock and hardwood stem density (mean and standard error) and overall species richness. Tree stem density values are number per 400 m^2 plot; sapling density and species richness values are number per 20 m^2 transect.

	1999		2007		F	P
	Mean	SE	Mean	SE		
Density						
Hemlock trees	13.4	1.3	13.5	1.3	0.00	0.95
Hemlock saplings	1.1	0.2	0.9	0.2	1.54	0.22
Hardwood trees	13.2	1.0	11.2	1.0	3.20	0.08
Species richness	5.6	0.4	4.7	0.4	3.34	0.07

Hemlock cover in some of the vegetative strata changed between 1999 and 2007 (Table 2). Cover in the canopy strata increased but decreased in the subcanopy, which might reflect growth of some trees into the canopy strata over the past eight years. The large increase in high midstory cover suggests that hemlock saplings also have grown substantially since 1998. In general, hemlock canopy and midstory cover appear to be increasing at this stage of HWA infestation and did not change enough to currently affect plant or wildlife communities.

Table 2. Mean percent hemlock cover in six vertical strata during 1999 and 2007.

	1999		2007		F	P
Hemlock Strata	Mean	SE	Mean	SE		
Canopy	30.3	2.8	41.3	2.8	7.5	0.007
Subcanopy	37.2	2.8	27.8	2.8	5.7	0.02
High midstory	2.8	1.9	31.5	1.9	113.9	<0.0001
Low midstory	14.5	2.0	17.8	2.0	1.3	0.25
Shrub layer	8.3	1.1	7.6	1.1	0.2	0.64
Understory	5.0	0.8	2.7	0.8	4.7	0.03

Avian Richness and Abundance

During 1999-2007, 75 different avian species were detected on and adjacent to the sampling plots. The majority were songbird species. Overall species richness of songbirds declined over time ($R^2 = 0.57$, F = 10.66, P = <0.0001), as did richness of each habitat guild (forest interior: $R^2 = 0.39$, F = 3.31, P = <0.0001; interior-edge: $R^2 = 0.47$, F = 8.89, P = <0.0001; edge: $R^2 = 0.47$, F = 3.95, P = <0.001) (Figure 3). Declines in all guilds and the limited change detected in vegetation structure on the plots suggest that songbird declines were not likely related to changes in vegetation structure but were due to other factors.

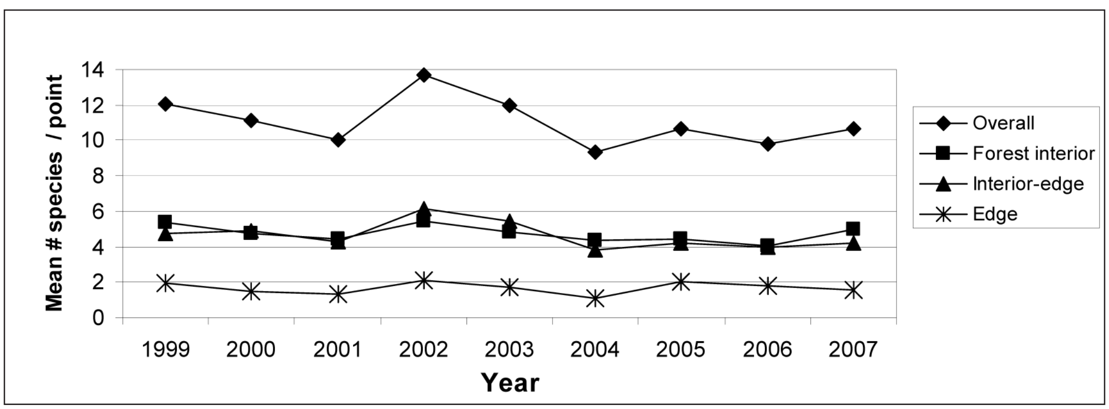

Figure 3. Annual trends in songbird species richness, overall and by guild.

Becker et al. (in press) found that only two bird species in their study, the Acadian flycatcher (ACFL), *Empidonax virescens*, and the black-throated green warbler (BTGW), *Dendroica virens*, were positively associated with living hemlocks. These two species are considered to be strong hemlock associates (Tingley et al. 2002). In our study, both of these species had declining trends as well (ACFL: $R^2 = 0.57$, F = 8.70, P = <0.0001; BTGW: $R^2 = 0.59$, F = 5.91, P = <0.0001) (Figure 4). In contrast, Becker et al. (2007) found that wood thrush (WOTH), *Hylocichla mustelina*, were negatively associated with the amount of living hemlocks and suggested that this species was benefiting from the increased number of dead trees and canopy gaps that resulted from HWA infestation on their study areas. In our study, wood thrush abundance had increased slightly ($R2=0.53$, $F=3.62$, $P= <0.001$) from the early years of our study (Figure 4). Statewide in West Virginia, these three species have had varying trends based on Breeding Bird Survey data; ACFL and WOTH have declining trends, while BTGW have been increasing (Sauer et al. 2007).

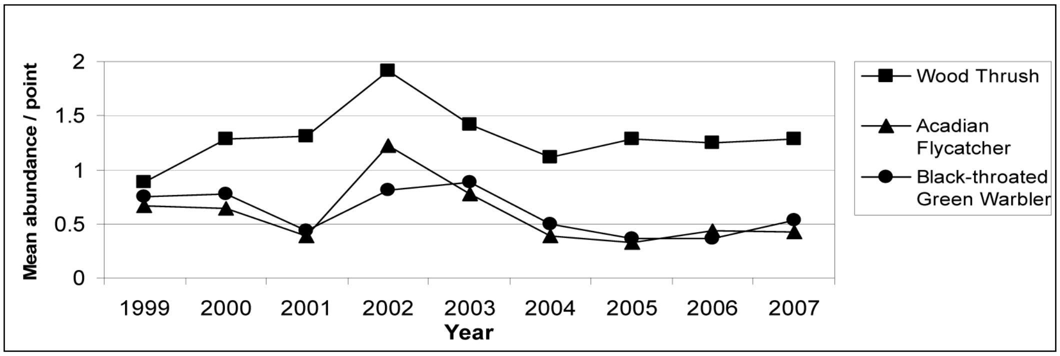

Figure 4. Annual trends in songbird species abundance.

CONCLUSIONS

In 2004, eight plots were infested with HWA. In 2007, 22 of the 36 hemlock monitoring plots were infested. Hemlock tree mortality can occur within four to six years of infestation (McClure 1991); thus, we expect hemlock mortality to begin occurring within the plots in the near future. Continued monitoring is critical for documenting the response of the hemlock ecosystem to HWA and to improve our understanding of the impacts of the decline or elimination of this habitat type.

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Symposium Agenda

Agenda: Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States

Welcoming address	Donald Smith
The historical and future impacts of exotic insects and diseases on connecticut's forests	Jeffrey Ward
Biological Control of Hemlock Woolly Adelgid	
Biological control of hemlock woolly adelgid: what is it going to take to make it work?	Scott M. Salom
Rearing labs summary	Rusty Rhea
Laricobius nigrinus establishment and impact in native and introduced habitats	David Mausel
An overview of lady beetles in relation to their potential as biological controls for the hemlock woolly adelgid	Michael E. Montgomery
Evaluation of the Japanese *Laricobius* sp. n. and other natural enemies of hemlock woolly adelgid in japan	Ashley Lamb
Evaluating Chamaemyiid predators of hemlock woolly adelgid in the western united states	Glenn R. Kohler
Linking host resistance mechanisms with hemlock woolly adelgid populations	Kimberly Wallin
Establishing *sasajiscymnus tsugae* in the South	Jerome F. Grant
Evaluation of three predators released to control the hemlock woolly adelgid using whole-tree enclosures	Joseph Elkinton
Low temperature in the hemlock woolly adelgid system	Scott D. Costa
Recovery of hemlock woolly adelgid predators in the high country of northwestern North Carolina, 2004-2008	Richard McDonald
Arthropods associated with eastern hemlock	Richard M. Turcotte
Managing hemlock woolly adelgid at Great Smoky Mountains National Park: situation and response	Kristine Johnson
Research, monitoring, and management of eastern hemlock forests at Delaware Water Gap National Recreation Area	Richard Evans
Status of *ex situ* conservation efforts for Carolina and eastern hemlock in the southeastern United States	W. Andrew Whittier

Water use by eastern hemlock: from implications for using systemic insecticides to ecosystem function	Chelcy R. Ford
Best management practices for systemic chemical control of hemlock woolly adelgid in forests	Richard S. Cowles
Analytical approaches to imidacloprid and metabolite analysis	Anthony F. Lagalante
Laboratory studies of imidacloprid impacts on hemlock woolly adelgid, *Laricobius nigrinus*, and *Sasajiscymnus tsugae*	Brian M. Eisenback
Environmental fate of imidacloprid	James Hanula
Eeonicotinoid insectices for hemlock woolly adelgid control	Kris Braman
A new strategy to control hemlock woolly adelgid (*Adelges tsugae* Annand) using imidacloprid and an arboricultural method to gauge hemlock health	Joseph J. Doccola, Brenda I. Cruz, Peter M. Wild, John Joseph Aiken, Reed N. Royalty, and William Hascher
Core tect: developing and evaluating a controlled-release imidacloprid formulation to manage hemlock woolly adelgid	Nate Royalty
Optimizing fungal production for hemlock woolly adelgid suppression	Stacie Grassano
Ecological and management implications of hemlock logging in Massachusetts	David A. Orwig
Incorporating hemlock woolly adelgid impacts into the forest vegetation simulator model	R. Talbot Trotter III
The role of volatile terpenoids in the relationship of the hemlock woolly adelgid and its host-plants	Anthony F. Lagalante
Production and evaluation of eastern hemlocks potentially resistant to the hemlock woolly adelgid	Richard Casagrande
Evaluating hemlocks for hemlock woolly adelgid resistance	Benjamin K. Hoover
Evaluation of growth characteristics of *Tsuga* spp. from North America and Asia and susceptiblity to hemlock woolly adelgid	Paul A. Weston
Resistance of hemlock species and hybrids to hemlock woolly adelgid	S.E. Bentz
DNA analysis of resistance at the cellular level	Alison Morse

Helmlock Woolly Adelgid Biology	
Endosymbionts of hemlock woolly adelgid	Carol D. von Dohlen
Climate-matching: implications for the biological control of hemlock woolly adelgid	R. Talbot Trotter III
Molecular ecology of hemlock woolly adelgid, its hosts, and its natural enemies	Nathan P. Havill
Mortality factors and population rate of increase for the hemlock woolly adelgid	Annie Paradis
Diet development for hemlock woolly adelgids and their predators	Allen C. Cohen
Changes in decomposition dynamics in hemlock forests impacted by hemlock woolly adelgid: restoration and conservation of hemlock ecosystem function	Richard C. Cobb
Patterns of spread of hemlock woolly adelgid in Carolina hemlock populations	Foster Levy
Using dendrochronology to model hemlock woolly adelgid effects on eastern hemlock growth and vulnerability	Mary Ann Fajvan
Effects of eastern hemlock mortality on riparian ecosystems in the southern Appalachians	Chelcy R. Ford
Controlling hemlock woolly adelgid with new forms of mycoinsecticides	Svetlana Gouli
Hemlock Woolly Adelgid and Elongate Hemlock Scale	
Incidence of elongate hemlock scale and its parasitoid *Encarsia citrina* in the eastern United States	Kristopher Abell
Entomopathogenic fungi for management of invasive armored scales in northeastern forests	Vladimir Gouli
Comparing hemlock woolly adelgid and EHS control in ornamental and forest hemlocks	Robert Ahern
Interactions between woolly adelgid and hemlock scale in new england hemlock forests	Evan Preisser
Closing	
Hemlock woolly adelgid initiative: progress and future direction	Brad Onken
National perspective on hemlock woolly adelgid	Rob Mangold