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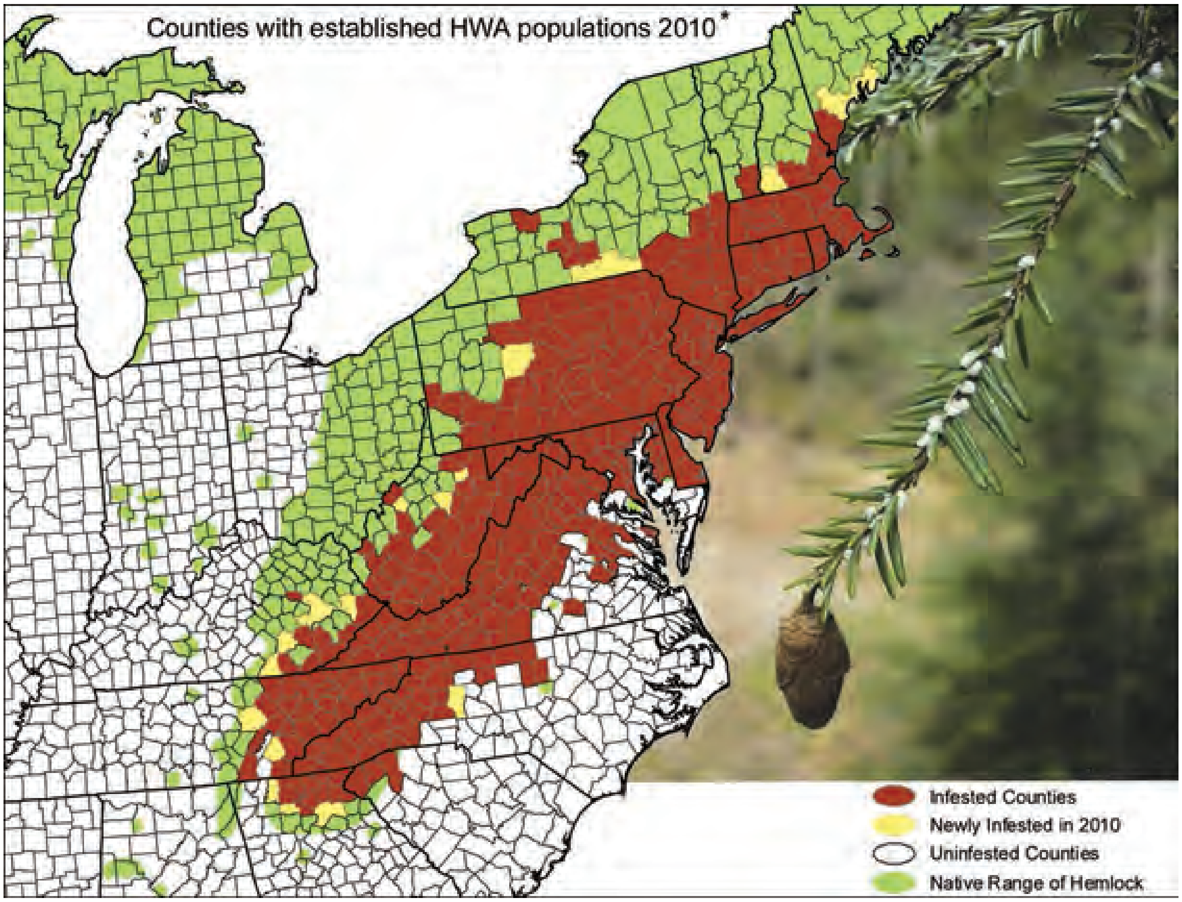
Hemlock Woolly Adelgid

Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States

Asheville, North Carolina
August 17-19, 2010



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Compiled by Brad Onken and Richard Reardon

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Compiled by Brad Onken[1] and Richard Reardon[2]

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FOREWARD

This is the 5th HWA symposium; the first meeting was held in Charlottesville, Virginia in 1995. Over the past fifteen years we have seen HWA spread like wildfire in the south, invade six additional states, and cause extensive tree mortality throughout the mid-Atlantic and southern regions. Sounds rather hopeless and yet we remain optimistic, and here's why.

During the first seven years (1995-2002), there were but a handful of scientists, forest health specialists and resource managers working on the HWA research and management issue. Investigations into biological control were limited and in its infancy, there were no chemical control options for protecting hemlocks in a forest environment, no standardized sampling methods, impacts to hemlock forests remained scattered regionally and until the late 1990s, some forest health specialists were even skeptical that HWA was really that big of a threat to hemlocks! Our basic knowledge of HWA biology was limited as was the consistency of funding to support the needed research and methods development. In 2001, a formalized plan, *An Exotic Pest Threat to Eastern Hemlock: An Initiative for Management of Hemlock Woolly Adelgid* was put forward and more stable levels of funding were made available annually by the USDA Forest Service beginning in 2003. This was the beginning of the HWA Initiative and the accelerated effort to research and develop management solutions. An effort that now involves 24 universities, seven institutions in China and Japan, 20 state agencies, four federal agencies and nine private institutions.

In the last eight years we have seen several effective systemic insecticides become labeled for forest applications resulting in tens of thousands of hemlocks treated and protected annually. Standardized survey methods have been developed, and thanks to investigative DNA technology, we now know that all HWA are NOT created equal and that the origin of HWA found in the East is southern Japan. We now have six institutions rearing HWA predators and we are seeing predators becoming established in many areas from Maine to North Carolina. The cold tolerant *Laricobius nigrinus* found in the interior west has been recently released is now becoming established in areas where the coastal strain of this beetle was difficult to establish. With all this, we also have approval to begin rearing and releasing the newly discovered beetle predator from Japan, *Laricobius osakensis* which has proven to be very promising.

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LONG-TERM EXPERIMENTAL ASSESSMENT OF *LARICOBIUS NIGRINUS* IMPACT IN THE NORTHEAST U.S.

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ABSTRACT

Laricobius nigrinus (Coleoptera: Derodontidae) was first collected near the coastal city of Victoria, British Columbia, Canada for release as a biological control agent to suppress tree-killing densities of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in the eastern United States. Beetles established in warm areas of the invaded range of *A. tsugae*, but had a low probability of establishment in cold areas. With the goal of locating beetles with greater cold-tolerance, we collected thousands of adults from its geographically isolated northern Rocky Mountain range.

To support planned releases of these inland *L. nigrinus*, the cold tolerance of field-collected coastal (Seattle, WA) and inland (Coeur d'Alene and Moscow, ID) adults and climate match index scores (CLIMEX v.2) of these collection areas and the eastern United States were compared. We found that inland *L. nigrinus* individuals were more cold tolerant than those of coastal *L. nigrinus*, based on higher survival in a winter field cage study in Massachusetts and a lower supercooling point in a laboratory bioassay. Inland *L. nigrinus* also had higher survival than coastal *L. nigrinus* after an 18 h exposure to -15 °C, which was 1.5 °C warmer than the mean supercooling point of coastal beetles.

Areas of the eastern United States infested with *A. tsugae* and receiving *L. nigrinus* releases matched the climate of Seattle and Coeur d'Alene reasonably well, but Coeur d'Alene had higher index values over a considerably larger area than Seattle.

Inland *L. nigrinus* appears preferable for release in the colder portions of *A. tsugae*'s invaded range in the eastern United States. To test this hypothesis, we have initiated paired coastal and inland beetle releases and control sites (n = 14) in hemlock dominated forests for long-term assessment of beetle establishment, population growth, and impact on *A. tsugae* and hemlock health. Modified USDA-Forest Service Forest Inventory and Analysis (Phase 3) plots were set up at each release and control site. Baseline forest structure, vegetation, and site data were collected and hemispherical photography was used to assess hemlock canopy health. Canopy sampling was used to document baseline densities of *A. tsugae* and elongate hemlock scale, *Fiorinia externa* (Hemiptera: Diaspididae). Initial beat sheet and canopy sampling for F_1 and F_2 *L. nigrinus* indicate that the beetle is establishing but results so far show no difference between coastal and inland beetle recovery rates. Re-measurement data collection will occur every other year for forest vegetation, pest densities, and hemlock health and yearly for *L. nigrinus* establishment and population growth.

DISPERSAL DYNAMICS AND PREY IMPACT OF *LARICOBIUS NIGRINUS* IN THE EASTERN UNITED STATES

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ABSTRACT

Laricobius nigrinus Fender is one of the introduced predators being used in the on-going biological control program against hemlock woolly adelgid, HWA (*Adelges tsugae* Annand). Between 2007 and 2010 observations of *L. nigrinus* dispersal within a hemlock stand and predatory impact on HWA were made at several release locations in the eastern United States. In fall and winter of 2007/2008, 300 beetles were released at two locations in Pennsylvania and two in Virginia to observe dispersal during site colonization. In the spring, for two years following beetle release, samples were cut from three crown stratum (< 7 m, 7 – 15 m, and > 15 m), of three release trees, at the four sites. Ninety-eight percent of F_1 offspring at the Virginia release sites were recovered above the first 7 m of the crown. At the Pennsylvania sites F_1 vertical dispersal sampling was compromised by a collapse of HWA populations on the release trees. Yet, there was a trend of the parent beetles moving upward in the crown for oviposition. There was a positive correlation between HWA and *L. nigrinus* density (in 2008 r = 0.49 (12), p = 0.0026 and in 2009 r = 0.23 (13), p = 0.0494). Dispersal between trees was compared from only the lowest crown strata of release trees and infested hemlocks at 10, 30, 50, and 100 meters from the release trees. At least one individual of the F_1 generation dispersed to a non-release tree at all four colonization study sites. The maximum dispersal distance was 50 meters observed one year after release at both of the PA release sites. Dispersal observations at four established *L. nigrinus* release sites (Bear Run, Bald Eagle S.F., PA; Rothrock, Rothrock S.F., PA; Middle Creek, Pisgah N.F., NC; and Laurel Creek, Great Smoky Mountains N.P., TN) were made from 2007 through 2009 at varying distances (50, 100, 150, 200, 300, and 600+ m). A similar population shift was observed at all four sites, with increasing *L. nigrinus* frequency at further distances from the release each generation. Offspring of the F_5 generation were recovered at a maximum distance of nearly 400 meters from the release area. *These results support sampling for predator establishment near the release trees and then shifting long-term monitoring to the surrounding stand. Sampling techniques limited to the lower crown underestimate predator populations.*

The impact of *L. nigrinus* at the Middle Creek, NC and Rothrock, PA paired release and control sites were evaluated from 2007 through 2010. In 2010, two additional paired release and control sites were evaluated; Laurel Creek, Great Smoky Mountain N.P., TN and North Fork, Jefferson N.F., VA. Either 300 or 600 beetles were released at each site. Also evaluated in 2010, was a unique 10,700 *L. nigrinus* beetle release made from 2008 through 2010 at the base of Grandfather Mountain Biosphere Reserve, NC. Several methods were used to assess predator impact on the prey and tree health. The first method compared before and after release measures of hemlock crown health (live crown ratio, transparency, and overall decline) and HWA density within a demarcated area of branches in the lower crown. The same measures were compared between the release and control sites with consideration for differences in initial HWA density and landscape factors. Results show tree health declined by varying degrees at both release and control sites. Similarities of initial parameters allowed comparison of two paired release and control sites. Both controls increased in number of dead tips and overall tree decline ratings were slightly greater than the release sites. A cage-exclusion experiment was attempted in 2009, yet was compromised by weather and human caused damage. The last method compared four years of observations in the eastern United States with the same parameters measured for two years at three locations in Seattle, WA, the predator's native environment. Measured impact parameters included predator density, prey density, prey survivorship, and HWA ovisac disturbance. Results show *L. nigrinus* densities reached the ranged observed in Seattle, WA at all release sites except North Fork, VA. HWA densities in the east fluctuated over several years. Most years HWA density and survival rates were greater than the range observed in WA. *Overall, the higher HWA densities and survival in the East indicate L. nigrinus release sizes of 600 or fewer adults, at initially low to moderately HWA infested hemlock stands, were not able to reduce HWA populations to a level tolerable by the hemlocks within seven years.*

Impact monitoring should continue. The described assessments were conducted at release sites intended to try to determine an optimal release strategy based on the number of predators available and the probability of establishment across many temperature gradients. Monitoring the long-term impact of large releases, such as at the Grandfather, NC site may lead to a more desirable reduction in HWA populations compared with smaller release sizes. Impact assessments were based upon measures of only release trees and primarily sampled from the lower crown. Results from dispersal observations indicate a better representation of *L. nigrinus* populations would be realized from samples taken from the upper crown and at increasing distances from the release area with increasing time. Parameter values varied greatly between years, demonstrating the need to sample for multiple years. Comparisons of parameters measured at releases made across the HWA infested range in the East with areas in the predators' native environment provide a meaningful comparison if the same sampling technique is applied with minimum subjectivity. *The results of this initial impact assessment provide data for discussion on how to design more effective HWA management strategies and to consider the complexity of the varied ecological systems hemlocks are part of.*

KEYWORDS

Laricobius nigrinus, dispersal, predator impact

POPULATION GENETICS OF *LARICOBIUS NIGRINUS* AND *L. RUBIDUS*, PREDATORS OF ADELGIDS IN NORTH AMERICA

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ABSTRACT

We report preliminary results using DNA-based methods to distinguish *Laricobius nigrinus* (Coleoptera: Derodontidae) from *L. rubidus* and characterize their population genetics. *Laricobius rubidus* is native to eastern North America where it is commonly associated with pine bark adelgid, *Pineus strobi* (Hemiptera: Adelgidae). It also feeds on the hemlock woolly adelgid, *Adelges tsugae*, but has not been shown to impact its populations. *Laricobius nigrinus* is native to western North America and has been released as a biological control agent of *A. tsugae* in multiple locations in eastern North America where *L. rubidus* occurs. The two species can be distinguished as adults by color and by differences in male genitalia. *Laricobius* larvae cannot be readily distinguished using morphology, and rearing them to the adult stage for identification is labor intensive, and many beetles die in the process. In addition, *L. nigrinus* and *L. rubidus* have been observed mating in the field (Mausel et al. 2008), and were found to be closely related sister species (Montgomery et al. submitted), which drew attention to the possibility of successful hybridization.

We used three molecular approaches to address these identification and hybridization issues. The first method we used to monitor post-release establishment and spread of *L. nigrinus* was polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP). This method uses restriction enzymes to cleave DNA at sites with specific short nucleotide sequences. Restriction enzymes were chosen that target four diagnostic nucleotide positions in the mitochondrial cytochrome oxidase I (COI) gene that reliably differ between the species. It should be noted that because COI is a mitochondrial gene, this assay only identifies an individual's maternal lineage, and therefore cannot detect hybrids.

The second method we developed is a real-time PCR assay that amplifies a 117 base pairs of COI and uses unique 20-nucleotide TaqMan® probes (Applied Biosystems). Separate probes were designed to bind specifically to *L. nigrinus* or *L. rubidus*, and were tagged with different colored dyes. During amplification, the dye associated with the beetle species' DNA that is present is released and detected. This method increases the cost per reaction compared to PCR-RFLP but it is less time consuming.

Finally, we developed microsatellite markers that could detect hybridization between *L. nigrinus* and *L. rubidus* have if it occurred in the eastern United States. Microsatellites are nuclear, co-dominant population genetic markers that, in contrast to mitochondrial DNA, provide information about both parental lines. Ten polymorphic dinucleotide microsatellite markers were isolated and characterized for both beetle species (Klein et al. 2010). Six of these were used to genotype *Laricobius* larvae collected from sites with known *L. nigrinus* release dates. To date, we have genotyped larvae from sites in Tennessee and Pennsylvania (*L. nigrinus* released in 2004), and North Carolina (*L. nigrinus* released in 2005). We found clear evidence of hybridization at all three sites, with backcrosses in both directions. The consequences of hybridization between *L. nigrinus* and *L. rubidus* for adelgid biological control are not known. Hybrid offspring could be more fit than their parents (hybrid vigor), which could enhance biological control but result in homogenization of the two species, or hybrids can be less fit (outbreeding depression or hybrid sterility), which could ultimately maintain species integrity but could impair control efforts. Introgression of genes between species could also change their host preferences. Future work will evaluate the impacts of hybridization on the efficacy of adelgid biological control.

KEYWORDS

biological control, hybridization, molecular diagnostics
DNA barcoding, microsatellites

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KEY CUES AND FACTORS FOR IMPROVING HWA PREDATOR RECOVERY EFFORTS

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ABSTRACT

Improved recovery methods for predators of hemlock woolly adelgid must take several ecological factors into account. Most of these factors were observed in the field in Seattle, Washington and applied in the field in the eastern US, in North Carolina. These principles could be applied wherever predator releases have been made to enhance recovery numbers.

For the native winter predator *L. nigrinus*:

1) Each fall, predator beetles emerge and must locate HWA;
2) Local HWA populations vary greatly from year to year;
3) Winter sun and HWA as food are essential to recovering beetles;
4) *L. nigrinus* larvae must drop to the ground to pupate; and
5) Adequate hemlock needle duff is necessary for *L. nigrinus* to pupate

For the native western summer predator *Scymnus coniferarum*:

1) It is present in low numbers during the winter on hemlocks;
2) It was found in high numbers on trees that did not have needle duff;
3) Life cycle is entirely within-tree;
4) Adults were also found on western white pine.

KEYWORDS

hemlock woolly adelgid, biological control, *Scymnus coniferarum*, *Laricobius nigrinus*

INTRODUCTION

Our HWA predator fieldwork in Northwestern North Carolina started in 2003, with the introduction of the winter HWA predator *Laricobius nigrinus*. The predator redistribution/recovery program has mainly been focused in the seven county High Country region of NC, and in the Puget Sound area of Washington State (for native HWA predator collections and pest/predator life history studies).

The High Country of North Carolina is one of the most biologically diverse areas in the world. This area includes Alleghany, Ashe, Avery, Mitchell, Watauga, Wilkes and Yancey Counties in North Carolina. The headwaters of five major river systems begin in this seven-county area, including the New (both North and South Forks), Watauga, Yadkin, Catawba, and French Broad Rivers. There are extensive native stands of Carolina and eastern hemlock, as well as eastern white pine (*Pinus strobus)*, Fraser fir (*Abies fraseri)*, and red spruce (*Picea rubens)* in this region. Abundant landscape plantings of blue spruce (*Picea pungens)*, and other landscape conifers like Mugo Pine (*Pinus mugo)* are also present. The diversity of conifers gives this area abundant alternate adelgid hosts for adelgid predators as well.

ECOLOGICAL FACTORS TO CONSIDER FOR INSECT SPECIES ABUNDANCE

Before we begin the discussion about specific pests and natural enemies, there are several concepts that should be introduced in order to better understand how insects colonize and establish in a particular area. These ecological concepts can help us to recover more predators in the hemlock and conifer ecosystem.

The ecological factors that determine species abundance (in this case, insects) in a particular area are: 1. plant species diversity; 2. plant structural diversity; 3. distance from colonizing source; 4. time (seasonal) available for colonization and 5. behavioral traits or additional requisites of the insect under consideration.

From these basic determinants we get the following:

1. **Plant species diversity** – not just hemlocks – other adelgid species present on other conifers provides additional food. More species and numbers of conifers are better, as they provide additional hosts and niches for predators. We meet these criteria of having high conifer biodiversity in the High Country.
2. **Plant structural diversity** – does the hemlock (in this case) have the requisites necessary for our natural enemies to reproduce, overwinter, pupate, etc? For example, *L. nigrinus* must have needle duff in order to pupate under its host tree. The selection of release sites for *L. nigrinus* must take into account the presence of food (HWA), winter sun, and needle duff necessary for pupation.
3. **Distance from colonizing source** – how far can these beetles disperse and in what direction in a certain period of time?
4. **Amount of time (seasonal) available for colonization**. Compare a predator with one generation per year, versus multiple generations that may have alternate hosts. A generalist may build up populations much quicker than a predator that only specializes in a certain prey at a certain time of year.
5. **Take advantage of behavioral traits of insects.** Some predators have additional requisites (pollen, flowers for nectar, specific egg laying or pupation sites).

Let's keep these concepts in mind as we consider improving the recovery and dispersal of our hemlock predators at release sites back East.

RELEASING *LARICOBIUS NIGRINUS* AT HEMLOCK HILL IN 2003

Working in conjunction with the USDA's Blue Ridge Resource Conservation and Development Council and the North Carolina Division of Forestry, we were fortunate to be involved with David Mausel's Ph.D. research at Virginia Tech that focused on the release methodology for the novel winter HWA predator, *Laricobius nigrinus* Fender. On December 31st, 2003, Hemlock Hill in Banner Elk, NC, became the 8th release of *L. nigrinus* adults made in the eastern US; as David Mausel released 300 beetles there at the rate of 30 beetles per ten trees.

At the time, we were unsure whether this predator of HWA would even be able to colonize against the Japanese strain of the HWA present in the eastern US, much less have an impact against HWA. Under guidance from Professors Loke Kok and Scott Salom at Virginia Tech and Brad Onken with the US Forest Service, we began our *L. nigrinus* release methodology and recovery project with David Mausel.

"SHEDDING LIGHT" ON RECOVERY AND DISPERSAL PATTERNS OF *L. NIGRINUS* FROM HEMLOCK HILL

We were initially fortunate in that we divided the release of 300 beetles into two halves. One hundred fifty beetles were released along the Elk River, a shady, cool, flood-prone area (5 trees with 30 beetles each), and the remaining 150 beetles were released near or at the top of the ridge on 5 trees, 30 per tree in an area which received winter sunshine (Figure 1). This gave us 2 release areas to compare – a shaded, cool, wet area, and a sunny drier, ridge area.

As we began to recover beetles over the first three years post-release, we noticed a pattern of distribution by the beetles during the late fall and winter months. We recovered more beetles in sunny areas with HWA (trees 8, 9 and 10), compared to cooler, shaded areas with HWA. For example, we recovered 101 beetles over the first three years in the upper ridge area, which received winter sun, versus only 7 beetles in the release trees 1 through 5 along the river, which did not receive winter sun (Table 1).

Next, from beat sheet samples taken during 2004 to 2008, we began to find that more beetles were dispersing in a southerly direction (Figure 2), following the presence of winter sun on hemlocks. This pattern of beetles dispersing into areas having winter sun continued for the next 2 years (Figure 3); with beetles now present more than a mile in every direction from the original release site.

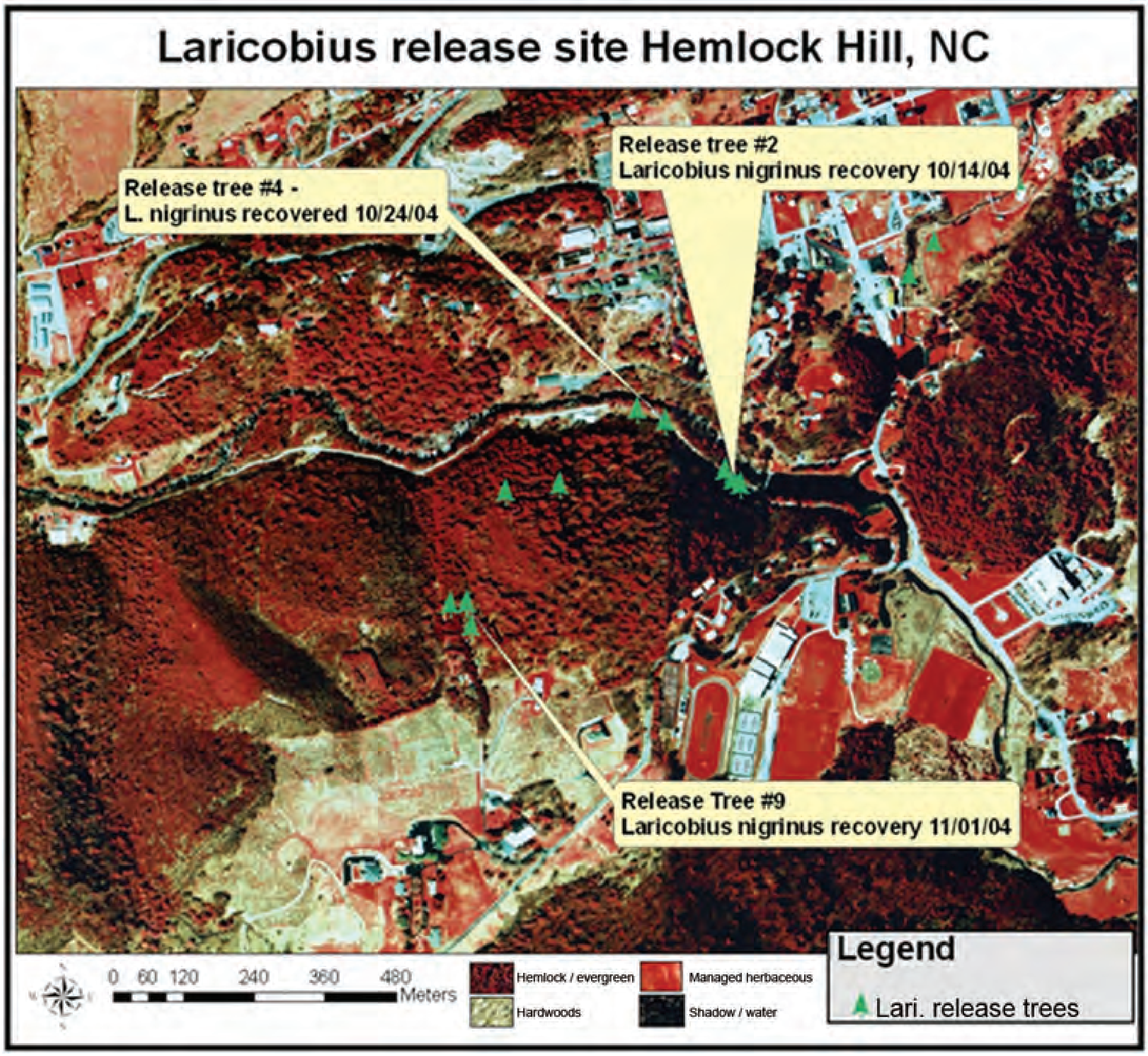

Figure 1. Location of *Laricobius nigrinus* release trees (in green - 10 trees, 30 beetles per tree) at Hemlock Hill in 2003. The yellow boxes are F1 recoveries during the 2004 season. Note that releases one through five were in a shaded area during winter, while releases six through ten were in areas that received abundant winter sun.

Table 1. Recovery of *Laricobius nigrinus* adults by season and place at Hemlock Hill 2004-2008. "River" is a shaded area during winter; "Ridge" is a sunny area at the top of the Hemlock Hill (see Figure 1). Larval numbers are from Mausel (2007).

Season or place	F1 Adults ('04/05)	F2 Larvae (April '05)	F2 Adults ('05/'06)	F3 Larvae (April '06)	F3 Adults ('06/'07)	F4 Adults ('07/'08)
Season						
Fall	3		12		93	80
Winter		10		314	109	23
Place (in Fall)						
River	2		1		4	41
Ridge	1		11		89	39

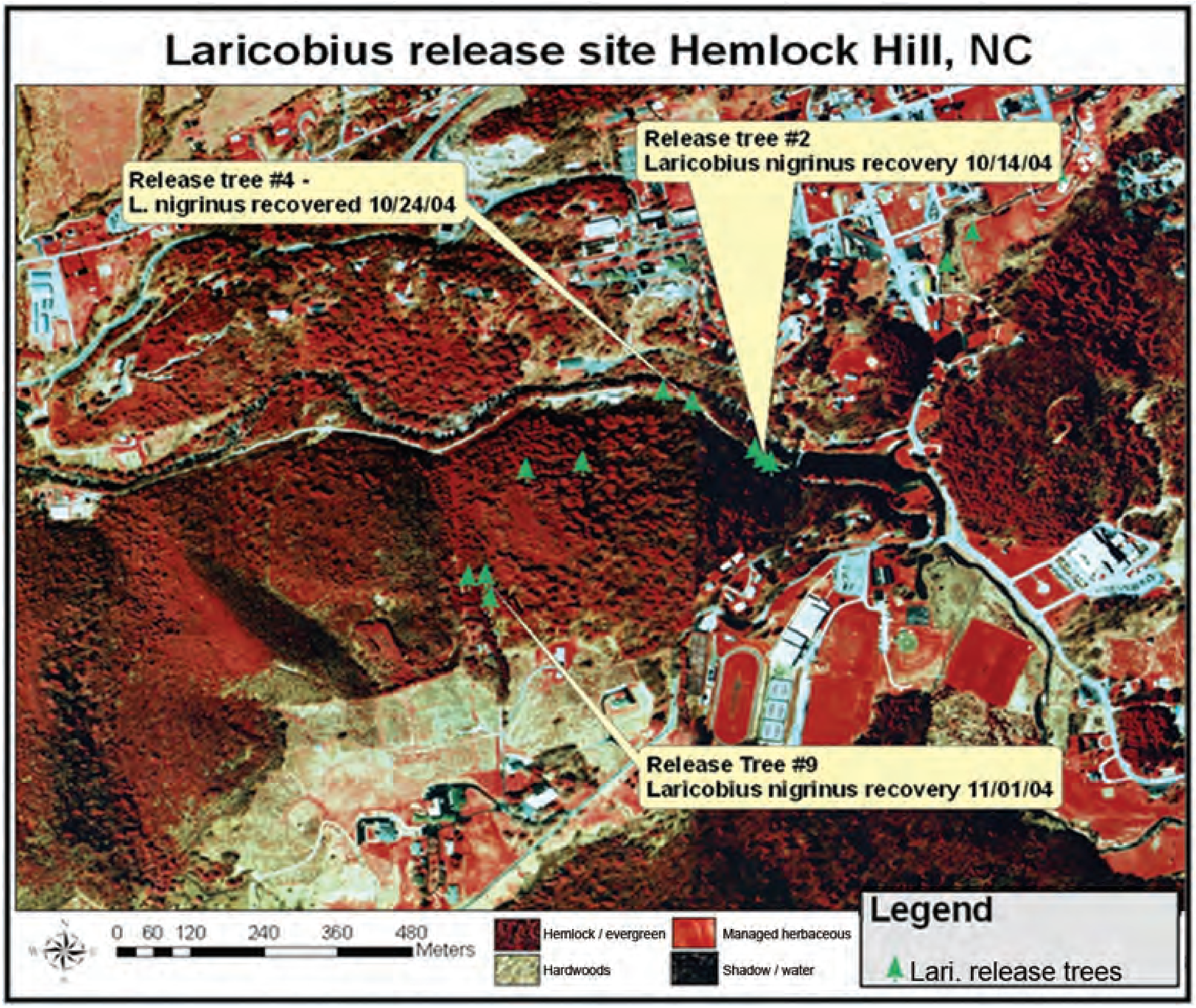

Figure 2. Pattern of dispersal of *Laricobius nigrinus* adults from 2004 through 2008 (light blue boxes). Adults appear to be more prevalent on south facing slopes during the coldest times of their season (Dec.-Feb.). Once warmer weather or sunlight is present, adult beetles move into north facing or shaded coves with HWA. Dispersal recoveries shown in 4 blue boxes: Mid-November of 2006: recovery of a single *L. nigrinus* adult on the main ridge of Hemlock Hill, 300+ meters from the nearest release tree. March/December 2007: recovery of a single *L. nigrinus* adult more than 3/8ths mile from the closest release site during March; December 2007 - Luker and McDonald found 2 adults in same area as above. January 2008 - Hamstead and McDonald recover 1 *L. nigrinus* adult below Tate Field scoreboard. This was 200 yards beyond prior known dispersal at that time, in a southerly direction.

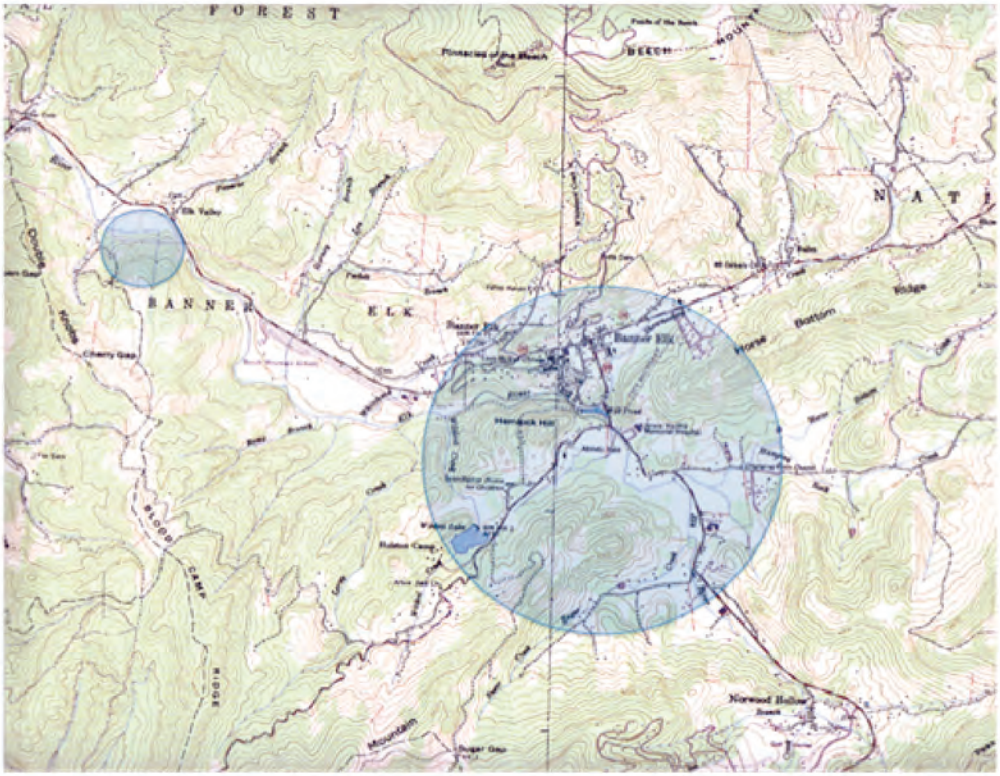

Figure 3. Current distribution of *Laricobius nigrinus* adults in the Hemlock Hill area; compare to Figure 2. Adults are now present in a 1-mile radius from the original release. Beetles appear to have moved mainly to the south. We collected 535 adults in November and April from the large blue shaded circle (original release area), and 46 adults from Lees-McRae Field Lab (smaller blue circle) in November 2009 and April 2010.

LESSONS FROM FIELD COLLECTIONS OF *L. NIGRINUS* IN SEATTLE

We began field studies of *L. nigrinus* with David Mausel in Seattle, starting in 2005. Our initial trips to Seattle were to see if we could capture enough beetles to start a colony at Virginia Tech. We had no idea how to best to collect beetles, nor any idea of how many beetles we might collect in a certain period of time. From those humble beginnings, now 5 years later, here's what we know in condensed form:

- Beetles emerge from October through December.
- Hemlocks must have adequate needle duff for *L. nigrinus* to pupate.
- Adults seek out warm, south facing coves with HWA and adequate winter sun.
- How do beetles locate HWA? Honeydew or a component in honeydew.
- Beetles stay in thermal pockets all winter until mean temperatures rise in spring.
- Beetles then disperse to cooler, shaded, north facing spots with HWA as temperatures warm and the sun's aspect rises.
- HWA exhibits bimodal patch dynamics; with peaks every 5+ years in Seattle.
- Scout where the HWA are present each fall.

- In Seattle, *L. nigrinus* populations cause HWA to crash locally
- We also find *L. nigrinus* on Douglas Fir and W. White Pine.
- 10% sugar water sprayed on limbs may act as attractant or an arrestant.
- We were able to collect 39,809 *L. nigrinus* beetles for redistribution and colony rearing since 200 7 in the Seattle area.
- The highest populations of *L. nigrinus* that we observed were in the urban community forest interface, not out in the forest. These park like areas with compacted soil and fertilization are the best places to collect beetles.

PREDATOR RECOVERIES IN NORTH CAROLINA

Using these ecological key factors initially outlined, along with observational cues, we were able to improve recovery and monitor colonization and establishment of Ln at our release sites in northwest NC. For example:

- Beat sampling collected 581 *L. nigrinus* adults from the Hemlock Hill area during 4 days of collecting (November 6 and 9, April 11 and 12, 2010). We focused on HWA in sunny areas.

- 816 *L. nigrinus* beetles were collected from the Hemlock Hill area during the past three years (2007-2009) and are increasing in numbers: 46(2007 – F4), 189(2008 – F5), 581 (2009 season – F5) (See Figure 4).

- 381 beetles collected during November 2009 were redistributed to an abandoned hemlock nursery to start a predator field insectary. (*We believe this is the first instance of locally collected L. nigrinus being used to start another field insectary*.)

- *L. nigrinus* adults are now common over a 1-mile radius from the original release site at Hemlock Hill (Figure 3).

- We are seeing new growth on hemlocks with predators in the Banner Elk and Elk River areas.

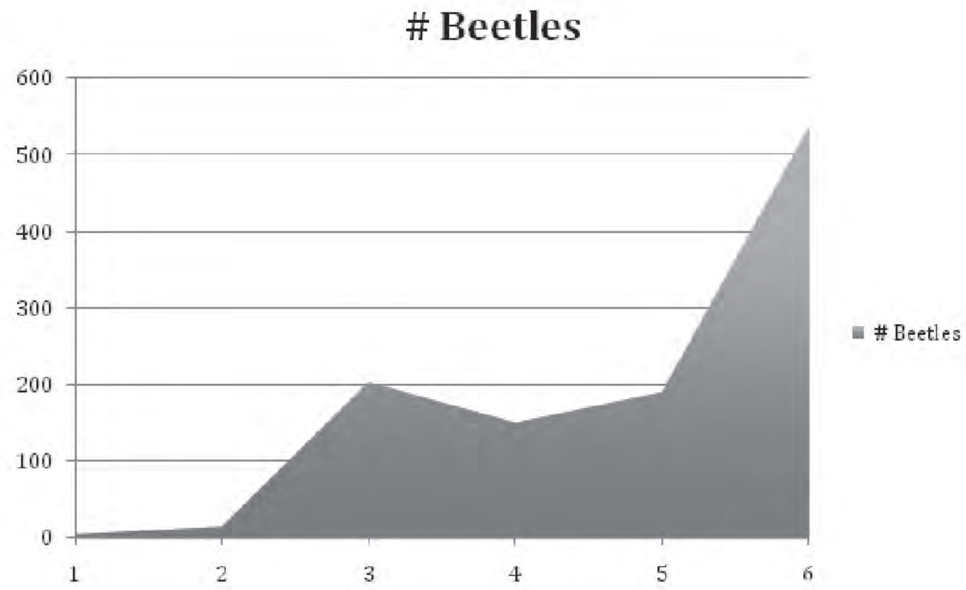

Figure 4. Number of *Laricobius nigrinus* beetles recovered by beat sheet method by generation at Hemlock Hill from 2004 to 2009 seasons. Note by the F6 generation (2009), beetle numbers had increased nearly 200-fold to 525 beetles – compared to the initial year's recoveries of 3 beetles (2004).

WHAT ABOUT SUMMER PREDATORS OF HWA IN SEATTLE?

Nathan Havill's DNA work from 2006 and 2007 (Havill, et al. 2006 and 2007) showed that the HWA in the Pacific Northwest is native. From a biological control standpoint, this could imply HWA in the Pacific Northwest may also have a complement of summer predators, similar to Japan and China. We began to look for summer predators of HWA in Seattle in late spring and early summer.

Starting in 2006, working with Dr. Michael Montgomery in northwestern North Carolina, we began to learn about the summer predators of HWA, mainly *Scymnus* spp. from China, but also *Sasajiscymnus tsugae*. We performed hemlock limb bag studies to show the efficacy of the Chinese and Japanese predators in controlling the progrediens (summer) generation of the HWA. Concurrently, during the early trips to Seattle, we observed several summer predator species - the Silverflies (Chamaemyiidae: *Leucopis* spp.) and what was later identified as *Scymnus coniferarum* by Dr. Montgomery (Figure 5).

Dr. Montgomery identified the ladybeetle *S. coniferarum* as a predator of HWA in the Puget Sound area and higher numbers of *S. coniferarum* could be collected from trees in places that did not have needle duff.

Since *S. coniferarum* reproduces within tree, this gives it an advantage in these areas – remember that *L. nigrinus* must leave the host tree to complete development in the needle duff at the base of the tree. Areas that show these kinds of characteristics are hemlock trees in parking lots, golf courses, cemeteries, parks, school grounds, etc. In natural areas, bluffs, rocky outcroppings, mountain ridges, along rocky creeks/rivers, and particularly windy areas.

Figure 5. The native summer HWA predator, *Scymnus (Pullus) coniferarum* (Crotch). This beetle is found on conifers throughout the Pacific Northwest and western US. Our field studies indicate that it is an important predator of HWA in the Pacific Northwest.

RECENT PROGRESS WITH *SCYMNUS CONIFERARUM* IN SEATTLE

- We collected 956 *S. coniferarum* from Oct. 2009 -June 2010; most (838) of these were collected in late April and late June (larvae and adults).
- *S. coniferarum* is widespread in the Seattle-Tacoma area.
- *S. coniferarum* is found in low numbers throughout the winter in certain areas.
- *S. coniferarum* appears to be a major summer HWA predator in this area.
- We were able to collect more beetle predators from trees that do not favor the development of *L. nigrinus* (trees that have the needle duff removed or covered by mulch).
- 24 *S. coniferarum* adults were collected on western white pine infested with adelgids (and aphids) from 3 infested pine trees.
- *S. coniferarum* has been recorded feeding on alternate adelgid hosts – in pines, firs, and spruces, as well as woolly apple aphid and citrus mealybug.

RECOVERING MORE SUMMER PREDATORS BACK EAST

From observation made in Seattle, we can predict that more summer predators would be prevalent in the same kinds of xeric hemlock environments found back East. We know that in the Pacific Northwest, there are larger populations of summer predators in areas that do not favor the development of *L. nigrinus*. Bluffs, rocky areas, outcroppings, and areas that are windy favor the within-tree developmental aspect of *S. coniferarum* – it does not have to leave the hemlock in order to complete development. We can also find small numbers of this predator on western white pine adelgids, so it has alternate hosts, and more than one generation a year, which may help in the functional predatory response against HWA.

We are attempting to achieve bracketing by having both summer and winter predators feeding on all life stages of HWA in the eastern US. This mimics the natural system we see in the Pacific Northwest, China and Japan where HWA is native. While there has been good success with establishing *L. nigrinus'*, we need more work on successfully introducing summer predators back in the East as bracketing of HWA by the correct predators will lead to sustainable control of the HWA.

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SUITABILITY OF *LARICOBIUS OSAKENSIS* AS A POTENTIAL BIOLOGICAL CONTROL AGENT OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

Laricobius osakensis Montgomery et Shiyake [Coleoptera: Derodontidae], a predator discovered on hemlock woolly adelgid (HWA) in Japan, was studied in its native range and in quarantine in the U.S. Its phenology in Japan, host range and interaction with other *Laricobius* spp. were determined. Its life cycle is synchronous with the HWA life cycle in Japan. In choice and no-choice tests, *L. osakensis* consumed more HWA and laid more eggs on HWA than any other insect tested and only completed development on HWA. In competitive interaction studies, adults and larvae did not appear to negatively affect the other *Laricobius* spp.

KEYWORDS

predator, HWA, biological control, Japan

INTRODUCTION

Hemlock woolly adelgid continues to expand its geographic range in the eastern U.S. and kill trees more quickly than foresters are able to manage. The biological control effort, considered the best option for saving hemlocks in the long term, began in the late 1990s. (Cheah et al. 2004). The goal has been to release multiple species of host-specific predators that can reduce HWA populations throughout the year and impede its expanding geographic range. A new host-specific predator was discovered in Japan on hemlock, *Tsuga sieboldii,* in 2005. It has been named *Laricobius osakensis* Montgomery et Shiyake. *Laricobius* spp. are known as adelgid predators (Leschen, 2000). *L. osakensis* comes from the same location as the strain of HWA from Japan that harms *Tsuga canadensis* in the eastern U.S. (Havill et al. 2006). A substantial effort was made to study the phenology of this predator in its native habitat. This was combined with laboratory experiments to assess host-specificity and compatibility with a native and introduced congeneric species.

METHODS AND MATERIALS

FIELD STUDY

Twelve HWA-infested hemlock trees, *T. sieboldii*, at three locations in the Kansai region of Japan were sampled each week over a 2-year period. On each sampling date, four branch samples (10-15cm long) were removed from the trees and examined for immature stages of *L. osakensis* using a microscope. In addition, a beat sheet ($1m^2$) was used to sample for adult predators on three branches of each tree.

HOST RANGE STUDIES

Choice and no-choice tests were conducted in petri dishes and the amount of host consumed and number of eggs laid on each host were determined. The alternate hosts used were: balsam woolly adelid (BWA), *Adelges piceae* (Ratz.), pine bark adelgid (PBA), *Pineus strobi* (Hartig), eastern spruce gall adelgid (ESGA), *Adelges abietis* (Linneaus), woolly alder aphid (WAA), *Paraprociphilus tessellates* (Fitch), elongate hemlock scale (EHS), *Fiorina externa* Farris, and pine needle scale (PNS), *Chionaspis pinifoliae* (Fitch). In choice tests, single adults were placed in petri dishes containing a known number of HWA and an alternate host. The amount of prey consumed and number of eggs laid on each were counted after 7 days. In the no-choice tests, single adults were placed in petri dishes with a known number of HWA or alternate host and the number of hosts consumed and eggs laid were determined after 5 days. Development tests were conducted by placing *L. osakensis* eggs on hemlock with HWA or on an alternate host. Larvae were observed for survival and developmental stage reached.

COMPETITIVE INTERACTION STUDIES

The four treatments consisted of three conspecific (same species) and one congeneric (different species) groups: three adult *Laricobius nigrinus*, three adult *L. rubidus*, three adult *L. osakensis* and one adult of each species. Each group was put in petri dishes containing 80 HWA. After 6 days, the number of HWA consumed, the number of eggs laid, and adult survival were determined. This was repeated using 3rd instar larvae of the same conspecific and congeneric groups. Larval survival and the number of HWA eggs consumed were calculated.

RESULTS

FIELD STUDY

In the Kansai region of Japan, adult *L. osakensis* are found on *T. sieboldii* from mid-November until late April. They lay eggs in HWA woolly masses from late December through April and larvae are present from January until May. The phenology of *L. osakensis* is synchronous with that of the winter generation of HWA on *T. sieboldii.*

HOST RANGE TESTS

Laricobius osakensis consumed more HWA and laid more eggs on HWA than on any other host in both the choice and no-choice tests. In addition, these predators survived for a short time as larvae on the adelgid alternate hosts, but were only able to complete development to adults on HWA. No development occurred on the non-adelgid alternate hosts.

COMPETITIVE INTERACTION STUDIES

In the adult group test, no differences were found in survival between the congeneric groups and the conspecific groups. The amount of prey consumed and number of eggs laid were same among the conspecific group and *L. nigrinus* and *L. osakensis* conspecific groups. The *L. rubidus* conspecfic group consumed less prey and laid fewer eggs than the congeneric and other conspecific groups. In the larval groups, survival among all four groups was the same. The conspecific *L. osakensis* group consumed more HWA eggs than the congeneric and other conspecific groups.

DISCUSSION

Given our knowledge of *L. nigrinus* in North America, the life cycle of *L. osakensis* in the Kansai region of Japan was not surprising. However, adults became active a little later (November) than expected. This may be because HWA on *T. sieboldii* in Japan breaks dormancy slightly later than HWA on *T. canadensis* in most of the eastern U.S.

The host range tests indicate that this predator is host-specific on HWA and cannot develop on other species. The competitive interaction tests show that the larvae consume more HWA than *L. nigrinus* and *L. rubidus* and may not negatively affect the other *Laricobius* species present on eastern hemlock.

These data suggest this predator poses no risk to native fauna within the eastern United States. This information was provided in the petition to release *L. osakensis* from quarantine. In May 2010, this predator was given FONSI (Finding Of No Significant Impact) status and is no longer required to be in quarantine. The next step will be to mass rear *L. osakensis* for release in the forest. Given what we know about its life history, we believe it will contribute to the biological control of HWA in the future.

ACKNOWLEDGEMENTS

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RECOVERY AND ESTABLISHMENT OF INTRODUCED PREDATORS OF HEMLOCK WOOLLY ADELGID IN THE SOUTHERN APPALACHIANS

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ABSTRACT

Since its introduction from Asia, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), has gradually spread into 17 states in the eastern United States (Trotter et al. 2008). A classical biological control program to introduce a complex of natural enemies has been implemented as part of an integrated management program against HWA. *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), which is native to Japan, was the first introduced species of natural enemy selected for mass rearing and release against HWA in the United States beginning in Connecticut in 1995 (Cheah et al. 2005). Since 2002, more than two million *S. tsugae* beetles have been reared in insectaries in several states and released throughout the eastern United States, with many of the releases made in the southeastern United States (Grant 2008; Salom et al. 2008). Of these, approximately 350,000 were released from 2002 to 2007 at ca. 150 sites in the Great Smoky Mountains National Park (GRSM), where HWA was first documented in 2002 (Lambdin et al. 2006). In addition, about 7,000 adults of another predatory species, *Laricobius nigrinus* Fender, native to the Pacific Northwest, has been released against HWA at 21 sites in the GRSM. However, limited information exists on the establishment of these introduced natural enemies in areas of the releases in the GRSM, as well as in the eastern or southeastern United States (Cheah and McClure 2000; Grant 2008; McClure and Cheah 1998; McDonald et al. 2008).

In 2008, a study was initiated to evaluate the establishment of *L. nigrinus* and *S. tsugae* released against HWA in GRSM. The objective of this study was to determine the presence and extent of established populations of these two introduced predatory species at multiple release sites in GRSM.

Sampling was conducted at 56 *S. tsugae* and 11 *L. nigrinus* release sites in 2008 (5 May to 7 July), 2009 (25 February to 16 June), and 2010 (19 February to 30 June) in GRSM. At each release site, four hours of sampling using beat-sheets was conducted. All release sites were identified using Park records which provided GPS coordinates; sites were located using a Garmin® GPS map 60 CSx GPS unit. Original release trees, which were identified by an aluminum tag, at each site were sampled where possible. On all hemlock

trees in the release site area, accessible branches from ground level to 2.5 m were sampled by tapping them five to eight times with a wooden rod while holding a white beat sheet (71 x 71 cm) beneath the branch to catch dislodged predators. Depending on the size of the tree, one to three beat-sheet samples were collected per tree. All *L. nigrinus* and *S. tsugae* observed during beat-sheet sampling were recorded, and representative specimens of suspected adults and larvae were taken to the laboratory, where larvae were reared in glass jars (2.64 L) on HWA-infested hemlock until pupation and emergence as adults. Field-collected and laboratory-reared adults were identified in the laboratory, and species identification was confirmed by James Parkman at the Lindsay Young Beneficial Insects Laboratory (University of Tennessee). Voucher specimens are housed in the Integrated Pest Management and Biological Control Laboratory at the University of Tennessee. Additionally, data describing general characteristics of sites at the time of release, as well as several years after release, was obtained from Park personnel.

Ten release sites (17.8%; 10 of 56 sampled sites) were positive for presence of *S. tsugae*, and 103 adults and 152 larvae were recorded. A significant inverse relationship between year of release and number of *S. tsugae* larvae (Pearson = -0.316; $P = 0.029$), adults (Pearson = -0.447; $P = 0.003$), and adults and larvae combined (Pearson = -0.440; $P = 0.003$) was documented. In other words, recovery of *S. tsugae* was greater at the older release sites. Trees in the *S. tsugae* release sites were rated healthier than those in the non-release sites during 2009. The attributes of sites positive for *S. tsugae* included: an average of 2,377 adults released per site, adults released from June 3 to July 10, average temperature at the time of release was ca. 27C, average elevation was 900 m, average new growth was about 72.5%, and the HWA infestation at time of release was estimated to be slight to high. On the other hand, the attributes of sites where *S. tsugae* were not collected were: an average of 3,452 adults released per site, adults released from June 2 to June 26, average temperature at the time of release was ca. 26C, average elevation was 719 m, average new growth was only about 55.0%, and the HWA infestation at time of release was estimated to be medium to high. In general, a comparison of trends suggests that temperature was slightly higher at the time of release at positive release sites, elevation was higher at positive release sites, new growth was considerably higher at the time of release at positive release sites, and HWA infestation was lower at the time of release at positive release sites.

Three sites (27.3%; 3 of 11 sampled sites) were positive for presence of *L. nigrinus*, and 23 adults were recorded; adults were recovered from 2004, 2006, and 2007 release sites, suggesting that length of time since release did not greatly influence recovery. In addition, 18 adult *Laricobius rubidus* LeConte (a native predator) were recovered from 18.2% (2 of 11) of the *L. nigrinus* release sites. Both *L. nigrinus* and *L. rubidus* were recovered from the same two sites. Research to assess hybridization of these two species in GRSM will be pursued.

This ongoing study documents the presence of *L. nigrinus* and *S. tsugae* persisting at numerous locations in GRSM. *S. tsugae* may take longer (as many as 6 to 7 years) than anticipated for populations to establish and attain readily measurable levels, as relatively greater numbers of *S. tsugae* were recovered from releases made in 2002 compared to releases made in subsequent years (Hakeem et al. 2010). Recovery of introduced predators provides essential information on species presence, establishment, distribution, dispersion, seasonality, density, and alternate hosts. However, recovery data do not provide insight into impact of these natural enemies on hemlock growth, tree health, tree survival, or adelgid populations. Further research is necessary to fully assess establishment of these introduced predators in GRSM and their impact on HWA.

KEYWORDS

Sasajiscymnus tsugae, *Laricobius nigrinus*, *Laricobius rubidus*
hemlock woolly adelgid, predators, biological control

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POPULATION DYNAMICS OF HEMLOCK WOOLLY ADELGID IN NEW ENGLAND

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ABSTRACT

Twenty years after the initial introduction of hemlock woolly adelgid (*Adelges tsugae*) in Massachusetts, most stands of eastern hemlock (*Tsuga canadensis*) remain intact with relatively low tree mortality. In contrast, eastern hemlock has experienced sharp declines and near eradication in parts of southern Connecticut and the southeastern U.S respectively; most trees die after just two to six years of infestation. This trend was expected to continue as the adelgid moved north; however, we have observed that adelgid populations in Massachusetts and elsewhere in northern New England have remained at relatively stable densities compared to previous studies. To document and understand this trend we collected life table data from six hemlock stands in Connecticut and Massachusetts from April 2004 through June 2007. On each sample occasion four 0.3m branches were haphazardly 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; 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 (Dec-Mar), progrediens mortality (Mar-June), sistens summer and fall mortality (June-Nov), and within the June–Nov period, aestivation mortality (Aug-Oct). We showed that adelgid mortality is consistently higher during the late spring and summer months compared to that occurring during the winter, the most extensively studied stage of mortality. Cold winter temperatures not only decrease survival, but also decrease fecundity of the overwintering generation. Likewise, cold spring temperatures negatively impact fecundity of the summer generation. In addition to temperature, winter and spring precipitation is also significantly associated with sistens survival and fecundity, respectively. Density dependent processes appear to be operating, and may be responsible for regulating populations of adelgid at the observed low densities. In particular it appears that high sistens density causes a reduction in adelgid fecundity in the following generation. In addition there is some evidence for density dependent mortality in various life stages. After seven years of observation, none of our study trees have died. These baseline density, survival and fecundity measures as well as their effects on tree health will hopefully shed light on the reasons for the differences in adelgid-hemlock interactions between the northern and southern U.S.

EFFECTS OF TEMPERATURE ON THE EGGS AND LARVAE OF *SCYMNUS CAMPTODROMUS*

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ABSTRACT

Classical biocontrol of hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand) will require the establishment of a complex of natural enemies from the native range of HWA. *Scymnus camptodromus* Yu and Liu has a broad geographic range in China (Yunnan and Sichuan Provinces), is one of the most abundant predators of HWA in Yunnan and Sichuan provinces, is found across a wide range of habitats and HWA densities. These characteristics, in combination with authorization from the Animal and Plant Health Inspection Service (APHIS) to release it from quarantine make it a candidate for release as a biological control. The biology of this species is similar to that of the two *Scymnus* species already released for the control of the hemlock woolly adelgid, with the exception that its eggs go through a summer diapause, coincident with the time during which HWA aestivates. To break the summer diapause the eggs need to be exposed to temperatures ≤ 10 °C. Eggs will proceed to hatch once the diapause is broken after they accumulate enough heat, though eggs can hatch when held at a constant 10°C. Larvae will develop at 10-25 °C but survive to pupation better at 15-20°C. The average winter temperatures in the eastern United States where HWA is present are similar to these for the regions were *S. camptodromus* is collected in China, though the summer climate where the beetle is endemic in China is cooler and wetter. All these factors taken together suggest that *S. camptodromus* phenology would make it a good candidate for potential release against HWA since the timing of its oviposition and development so closely mirror that of HWA.

KEYWORDS

egg diapause, temperature, hatch, development

INTRODUCTION

The tools being developed to manage hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand) include classical biological control, silviculture, host resistance, chemical control, regulatory efforts, and increasing public awareness. Biological control has the potential for providing sustainable long-term control through establishment of a complex of natural enemies from the native range, and so efforts to develop biological control agents for HWA began in the mid-1990s (Cheah et al. 2004). Several HWA predators from the adelgids native range in Asia and the Pacific Northwest have been released into eastern North America including two closely related beetle species from China, *Scymnus* *sinuanodulus* Yu and Yao imported from Yunnan province and first released in the eastern U.S. in 2004 (Yu et al. 2000) and *Scymnus ningshanensis* Yu and Yao imported from Shaanxi and Sichuan provinces and released for the first time in 2007. Another lady beetle native to China (*Scymnus* (*Neopullus*) *camptodromus* Yu and Liu) is still being evaluated to determine its potential for release against HWA in eastern North America. This species has a broad geographic range in China (Yunnan and Sichuan Provinces), is one of the most abundant predators of HWA in Yunnan and Sichuan provinces, is consistently present over a wide range of habitats and HWA densities (Li et al 2007), and permission to remove it from quarantine was confirmed in 2005 by the Animal Plant Health Inspection Service, Plant Protection and Quarantine.

The biology of this species is similar to that of the two previously released Scymnus species except that, instead of hatching in about 10 days, the eggs of *S. camptodromus* enter a summer diapause and do not hatch until the following spring (Cheah et al. 2004). This summer egg diapause may be an adaptation this beetle has developed to allow it to survive the summer when the stages of HWA it feeds on are not available (Figure 1). The only other record of an egg diapause in the Coccinellidae is for *Scymnus* (*Pullus*) *impexus* (Mulsant) which feeds on *Adelges piceae* (Ratzeburg) in Europe (Delucchi 1954).

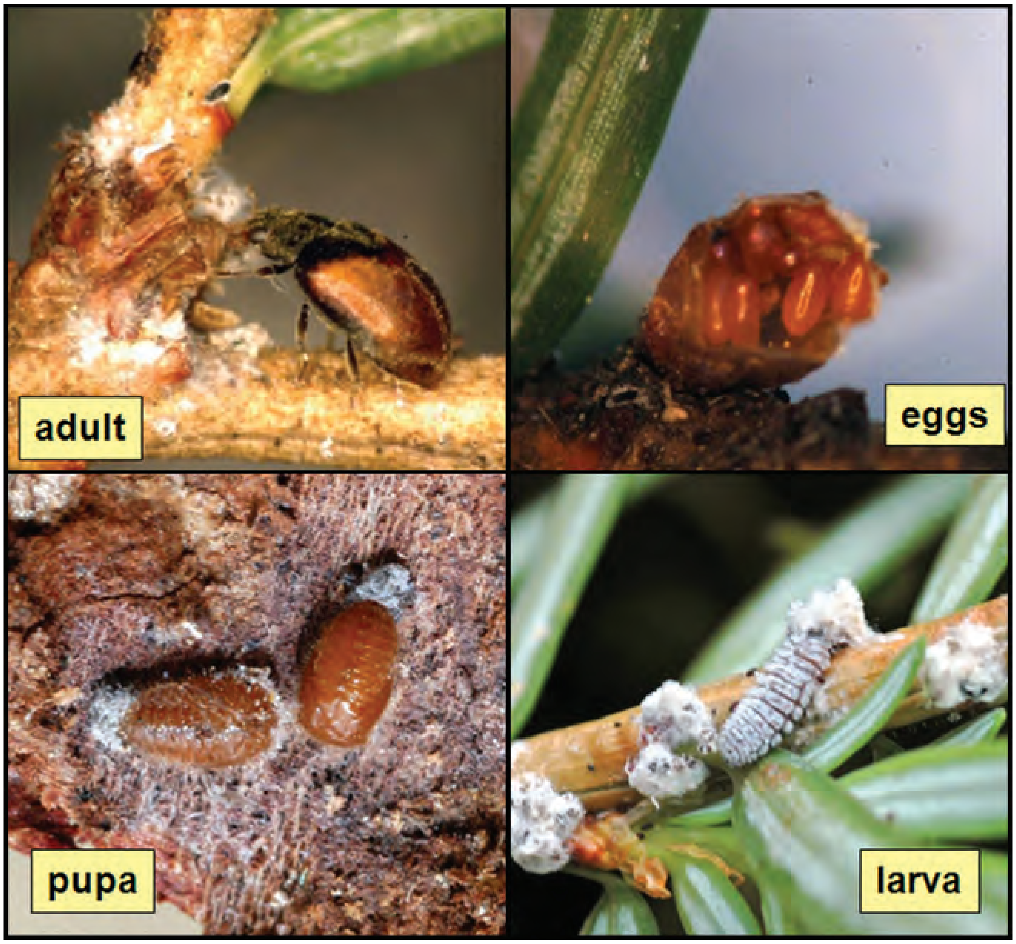

Figure 1. Life stages of *Scymnus camptodromus*. Adult feeding on hemlock woolly adelgid (HWA), eggs laid in an old hemlock male cone base, larva feeding on HWA eggs, and pupae under a hemlock bark flap.

The egg diapause of *S. camptodromus* has limited the rearing of this species for use for conducting research; therefore a series of experiments was conducted to evaluate the effects of temperature on the egg diapause to determine temperatures that will allow eggs to hatch. The impact of temperature on larval development was also evaluated to see which east coast climates would be good matches for this predator. In addition, extensive studies of development, phenology, and oviposition are generally conducted for every predator before its release to develop a thorough understanding of their biology.

EFFECTS OF TEMPERATURE ON *S. CAMPTODROMUS* EGGS

The eggs of *S. camptodromus* will not hatch if held at constant temperatures at or above 15 °C, except for the rare egg that does not go in to diapause. At these higher temperatures there is no sign of development within the egg; development does not begin until diapause is broken. At a constant 10 °C, the eggs will hatch after an average of 227 ± 32 days. Eggs will hatch after exposure to 5 °C and the percentage hatch increases with increasing time at 5 °C. If eggs are moved to 10 °C for development and hatch, the optimum time at 5 °C is about 56 days, but if they are moved to 15 °C it takes longer than 84 days. Eggs held at 15 or 20 °C after exposure to less than an optimum period of time at lower temperatures often desiccate or do not complete development. This may be due to the chill requirement being only partially met at the lower temperatures or because the embryo runs out of energy to complete the process at the warmer temperatures. With increasing time at lower temperatures the eggs hatch faster when moved to temperatures above 10 °C which may allow more to hatch successfully. *S. camptodromus* eggs are clearly adapted to break diapause in the fall or winter and complete their development and hatch in late winter to early summer when HWA begins laying eggs.

EFFECTS OF TEMPERATURE ON *S. CAMPTODROMUS* LARVAL DEVELOPMENT

The larvae of *S. camptodromus* will develop at constant temperatures between 10 and 25 °C if sufficient HWA eggs and nymphs are available. At 10 °C the larvae take an average of 90 ± 3 days to pupate and at 25 °C they take 20 ± 1 days. Survival to pupation is only about 50% at 10 °C, >70% for 15-20 °C, and 25% for 25 °C. The poor success at 25 °C in the laboratory is probably partly due to problems with maintaining the necessary quantities of the appropriate HWA stages in the rearing containers at this temperature, but there were also signs that the larvae that pupated at this temperature were not as healthy (e. g. deformed adults and the developmental rate was not much faster than at 20 °C). Time as a pupa decreased with increasing temperatures between 10 and 25 °C. *S. camptodromus* larvae appear to be well adapted to completing their development during the spring when HWA is active and the highest quantities of HWA eggs would be available.

IMPLICATIONS FOR RELEASE OF *S. CAMPTODROMUS* AS A PREDATOR FOR HWA

The average winter temperatures in the eastern United States where HWA is present are similar to those in regions were *S. camptodromus* was collected in China, though the average summer temperatures are higher on the east coast of the U.S.. *S. camptodromus* should be able to hatch and develop in the eastern United States and align its developmental period with that of the HWA. The *S. camptodromus* eggs should maintain their diapause through the summer on the east coast and not break diapause until HWA breaks aestivation. *S. camptodromus* adults that during the summer would have to find suitable prey until HWA is again available. Host range studies that are underway should provide clues to what alternate prey they may utilize during the summer and indicate that *S. camptodromus* may only oviposit on hemlock when HWA eggs are present. All these factors taken together suggest that *S. camptodromus* phenology would make it a good candidate for potential release against HWA since the timing of its oviposition and development so closely mirror that of HWA.

ACKNOWLEDGEMENTS

We thank Jason Biroscak, Rebecca Lomanchinsky, Paul Moore, Vicente Sanchez, and Alice Vandel who provided technical assistance. We also thank Li Li, Defu Yao, and Jianhua Zhou for assistance in obtaining adults to start the colony.

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REARING AND HOST-RANGE STUDIES OF *SCYMNUS CAMPTODROMUS*

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ABSTRACT

Scymnus camptodromus has several attributes that make it promising as a natural enemy of hemlock woolly adelgid (HWA) in the eastern United States. It has a phenology closely matched with that of HWA, and it is found throughout HWA's range in China from warm to cold regions. It has an earlier larval feeding period than other *Scymnus* spp. being investigated, and thus may have greater impacts on HWA populations. Ability to rear large numbers of *S. camptodromus* has been limited because of an egg diapause, but now methods to break the diapause and rear the beetles have been developed. Given the potential of this species for control of HWA and its ability to thrive in colder climates, this species is being evaluated for its impact on non-target species as well as the development of mass rearing procedures. This work will be followed by investigation of the effectiveness of this predator against HWA on infested hemlocks.

Of the 6 geographic populations of *S. camptodromus* brought to the U.S. from China from Sichuan and Yunnan Provinces, we propose to concentrate on one strain from each province. So far we have used one strain from Yunnan province (MNP) in our studies since this strain has produced the largest number of viable offspring in rearing. In the spring of 2010, when adults of *S. camptodromus* became available, choice tests of 19 beetle adults were performed using eggs of 3 non-target adelgid species: Cooley spruce gall adelgid *(Adelges cooleyi)* on Douglas-fir, larch adelgid *(Adelges laricis)* on Japanese larch, and pine bark adelgid *(Pineus strobi)* on eastern white pine in comparison with HWA on Eastern hemlock. The choice test arena for each predator contained branch tips of hemlock infested with HWA and 2 alternate prey items on their respective hosts at a time (total of 3 branch tips of different adelgid species at one time). The adelgid adults had been removed and equal numbers of eggs of each adelgid were left for *S. camptodromus* to feed on. Beetles were allowed to feed for 72 hours.

Predators consumed significantly more HWA eggs than of the other 3 prey items and there were significant differences in the number of HWA consumed depending upon which combination of other prey items was present. Predators were 6.6-fold more likely to eat HWA eggs over larch adelgid, 4-fold more likely to eat eggs of the combined group of Cooley spruce gall adelgid and pine bark adelgid over larch adelgid, and 1.6-fold more likely to eat HWA over the combined group of Cooley spruce gall adelgid and pine bark adelgid. Female beetles only laid eggs on hemlock infested with HWA during these choice tests, which is consistent with findings at the Forest Service Quarantine Facility.

Preliminary no-choice larval development tests of using two first instar *S. camptodromus* larvae were conducted in an arena containing pine bark adelgid eggs on host plant material at 15 °C and foliage containing prey were replaced as needed. The time to pupation and time to adult eclosion were recorded. Only one of the two beetle larvae reared on pine bark adelgid pupated and eclosed to adult, but it was abnormal and died within 24 hours. It was missing a leg and the wings were deformed. Further no-choice larval rearing will be done in the future, as will experiments to determine which hosts the beeltes will oviposit near.

Our goal is to develop a mass rearing procedure for *S. camptodromus* in the field. Mass rearing methods for *S. camptodromus* are under development. A hoop house was constructed at Rock Springs at Penn State in the fall of 2009 and contains about 20 hemlocks that were planted in the early spring of 2010. An irrigation system was installed, soil pH optimized, and a fertilizer regime implemented. Infestation of these trees by HWA will be started this fall. Once an adequate infestation level is established, we will introduce beetles to the rearing facility. The lab at Pennsylvania State University has reared about 100 *S. camptodromus* of the MNP strain from egg to adult so far, but this remains labor intensive due to the need to break egg diapause.

KEYWORDS

host preferences, choice test, non-choice test

ACKNOWLEDGEMENTS

We thank Jason Biroscak who provided technical assistance. Funding for this project was provided by the Northeastern Area State and Private Forestry (09-DG-1142004-330).

USING BEHAVIORAL ASSAYS TO DEVELOP FITNESS-BASED QUALITY CONTROL FOR THE HWA PREDATOR, *SCYMNUS CONIFERARUM*

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ABSTRACT

Insect predators employ the sequence of host selection behaviors to locate their prey. The behaviors include habitat location, host location within the habitat, host acceptance and host use. In efforts to use predators to control non-native invasive insects, practitioners of biological control often focus on host suitability, which includes host acceptance and host use. Following confirmation that the organism of interest accepts and uses a single host, scientists rear and release the organism into selected habitats. The overall goal of this research is to determine factors that may influence the ability of *Scymnus coniferarum*, a potential predator of HWA, to locate its host. Implementation of quality control procedures at the onset of assessing this biological control agent of HWA will increase the overall efficiency and efficacy of the effort. Specifically, we quantified responses of the predator based on factors that alter behavior in other predator-prey systems. Objective one quantified if/how rearing parameters of the predator affects its chemosensory response to its prey and prey host material (hemlocks); this provided timely information regarding behavioral shifts during mass rearing and informs practitioners that adjustments in rearing may be needed to ensure the efficacy of predators. Objective two linked variability of beetles physiological condition (age, satiation, mating status) with its behavioral responses to its prey and prey host material. All behavioral responses were tested using the four-arm olfactometer (Analytical Research Systems, #OLFM-4-C-2440PE. Gainesville, FL USA). Two criteria quantified behavior: 1- time spent in each field; 2- if the insects chose an odorized field at the beginning of the test and remained in that field (final position). Host location: *S.coniferanum* located hemlock in 4-way olfactometer. Level of satiation: Twelve hours without food increased the percent and speed of locating E. hemlock, however, 24 hrs w/o food decreased orientation to any plant material. Age of insect: Insects 6-10 days old are most likely to walk towards plant material; 7 day old beetles walk toward E. hemlock more often than toward other plant material. Maternal host: field caught vs. lab reared experiments are in progress. Behavioral trials for the remaining objectives, including prior experience, mating status, and sex, will be conducted during 2010-2011 life cycle of *S. coniferarum*.

EVALUATION OF *LEUCOPIS* SPECIES (DIPTERA: CHAMAEMYIIDAE) FROM THE PNW AS POTENTIAL BIOLOGICAL CONTROLS FOR THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

In a survey of predators associated with 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% of predator specimens recovered. From more recent collections in the same region, Dr. Stephen Gaimari, California Department of Food and Agriculture, verified that there is a third species of *Leucopis*, *L. piniperda* (Malloch), also associated with HWA. These *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, L. atrifacies,* and *L. piniperda* are good candidates for biological control of HWA in eastern North America.

We have focused on two objectives, (1) determine the synchrony of *Leucopis* spp. and HWA at field sites in Oregon and Washington, and (2) determine the suitability of HWA and alternative adelgid species as prey for *Leucopis* spp. in laboratory feeding trials. In the field, *Leucopis* spp. larvae were found throughout the year, but were most abundant when progrediens and sistens eggs were present in the spring and early summer. Several feeding trials have been completed using HWA and several alternative prey including, balsam woolly adelgid, Cooley spruce gall adelgid and pine bark adelgid. Although some *Leucopis* spp. larvae have fed and developed to the pupal stage on the alternative prey, they have demonstrated a preference for HWA.

Currently, the greatest challenge in studying the biology and ecology of these *Leucopis* spp. is that we do not have a way to rear the individual species. Consequently, we must work with field collected larvae and there are no characters to identify species in the larval stage. We do not know what species were included in a feeding trial until the individuals develop to the adult stage. Since many individuals die before reaching the adult stage, we do not know the species for all larvae used in the trials. Dr. Nathan Havill, USDA Forest Service, has been working on developing bar codes to identify the *Leucopis* species. We have also established a raised bed containing about 50 hemlock trees that are about 1 meter tall. We plan to infest these trees with HWA and build enclosures around groups of trees in an effort to establish colonies of the individual *Leucopis* species for further studies.

PACKAGING AND PRESENTATION OF ARTIFICIAL DIETS FOR HEMLOCK WOOLLY ADELGID PREDATORS

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ABSTRACT

We have determined that artificial diets that we developed for *Sasajiscymnus tsugae* were prone to deterioration from microbes, desiccation, and free-radical damage. Therefore, we undertook studies of diet packaging techniques that would help protect the diets from forces of deterioration. We used as controls our FDFE3 and F100 Diets (both based on chicken egg yolks and functional diet components such as fructose, preservatives, vitamins, and texturing agents). The control diets were hydrated either with water or honey. Both diets were attractive to both adult and larval *S. tsugae*, but the honey-diets lasted longer than the water-based diet in terms of mold and complete desiccation to crisp dryness. We used packaging techniques that included stretching Parafilm around diet (by hand and with the Cohen, Harsh, Smith 2002 technique). We also used encapsulation with molten wax droplet technique (Cohen, 1983), and we also created films from coatings of alginate (activated with calcium) and chitosan (activated with acetic acid). All the film-coatings were attractive to the *S. tsugae*, but the Parafilm and wax droplets were not attractive and elicited almost no feeding. We are continuing to work on improvements of the packaging techniques, using improved wax mixtures that are softer and thinner, and we are undertaking tests with spray encapsulation with alginate, chitosan, and wax mixtures. We are also making further exploration of making the diet itself more attractive and nutritive by using fermentation technology coupled with our existing diet technology and volatile attractants to formulate field manipulation (attraction, retention, and nutrition).

INTRODUCTION

Development of artificial diets for predators of hemlock woolly adelgids (HWA) would be a very important aid in management of HWA because mass rearing programs for adelgid predators are often impeded by the lack of fresh, high quality food sources for their predators. Therefore, an artificial rearing system for the predators, including a suitable artificial diet would help offset costs and losses of predator stock. We have been engaged in development of artificial diets, and we have succeeded in developing two relatively successful diets based on chicken eggs and supplementary materials. Though we have not been able to successfully rear the predators through their entire life cycle, we have been able to sustain adult predators for several months free of natural host materials (HWA). This was considered a breakthrough, but our diet delivery system where we used gelling materials such as alginate did not have a suitable shelf-life. Therefore, we undertook a research project on a diet packaging/presentation system. We used several existing systems and some novel ones that have not been used previously with arthropods.

MATERIALS AND METHODS

We used several film/membrane systems to coat proprietary formulations known as FDFE3 and F100 as follows: 1. sodium alginate (1% added to the diets and activated to form a skin or artificial cuticle on its surface) 2. chitosan (1% added to diets and activated to form a film or skin with 20% acetic acid), 3. Parafilm stretched by hand and with various pointed implements (as described in a US patent by Cohen et al. 2003), 4. semi-liquid cells coated with various wax mixtures, including soft dental wax, beeswax, paraffin, and petroleum jelly mixtures heated in a Reacti-therm hot plate and dipped onto Parafilm sheets (Cohen 1983). We also presented the insects (*Sasajiscymnus tsugae* and *Laricobius nigrinus*) with diets that were not packaged with coatings or films. We also used diets suspended in honey with no film after freeze-drying the diets then combining them with honey.

RESULTS

All diets were accepted by both species of predators, with the highest acceptance (> 90%) for the uncovered diets and for those with films of alginate or chitosan. The lowest acceptance was with the wax-encapsulated diets (< 5%) and Parafilm-covered diets (< 20%). The diet coatings that gave the best protection from desiccation and oxidation of diets were the wax encapsulations (which lasted more than 10 days without drying out or discoloring) and the Parafilm, which gave equal protection to the diets. All of the chemical film diets (alginate and chitosan) and uncovered diets quickly dried out and became discolored within 24 hours. We did find that the diet coatings without protection against desiccation could be rehydrated with addition of fresh water. However, addition of water caused acceleration of the onset of mold. One of the best behavioral responses was with diets suspended in undiluted honey.

DISCUSSION

We found such extensive acceptance of the diets that were uncoated, suspended in honey, or coated with thin alginate or chitosan films that we are optimistic about our progress with artificial diets. The lesser acceptance of the diets coated with artificial membranes or wax encapsulation media was less encouraging in terms of our ability to maintain colonies with minimal labor which would be involved with changing the diet every two days. However, the most encouraging aspects of this work are the prospects of our being able to use the diet formulations in situations when suitable field-collected adelgids are not available, but when our colonies of predators are still active and need some source of nutrition. This success has also suggested the possibility of using the diets under field conditions to sustain predator populations in off seasons so that when the adelgids again become active, we can have healthy, robust predators that are in adequately healthy condition to begin control of HWA populations. This work suggests that the artificial diets along with appropriate packaging systems and delivery systems for the diets can help make predators more available to forest managers who rely on robust, active predators of HWA.

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HEMLOCK WOOLLY ADELGID SUPPRESSION WITH AERIAL APPLICATION OF MYCOTAL FUNGUS IN A MICROFACTORY FORMULATION: CAN IT WORK?

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ABSTRACT

The question is 'can we impact forest populations of hemlock woolly adelgid by aerially applying a commercial insect-killing fungus that is enhanced with a microfactory formulation additive'. Results from the 2009 Pilot Study, where 1.25 acre plots of hemlock forest in TN were aerially treated with *Lecanicillium muscarium* in Mycotal, a commercial biopesticide from The Netherlands, found HWA population growth reduced by >50%; but the reduction occurred only when the tank mix was augmented with a fungal enhancer based on whey microfactory technology. The ultimate answer to the "can it work" question will depend on a variety of factors, some biological and ecological, while others are practical, economic and regulatory. Notwithstanding, evidence from the achieved impact on HWA populations combined with an understanding of biotic and abiotic influences on HWA population dynamics suggests that *L. muscarium* can help bring HWA under management.

KEYWORDS

Biopesticide, fungal enhancer, entomopathogen, *Lecanicillium muscarium*

INTRODUCTION

In developing a biopesticide to protect hemlock trees from insect pests certain other questions will also arise, such as: will the fungus integrate without harming beneficial predators, will there be a commercial product available, will it be cost-effective, are State and Federal regulatory hurdles surmountable, and who will fund management implementation. These ancillary factors must be kept in mind throughout the process of selecting and testing insect-killing fungi for implementation. In previous research, the insect-killing fungus *Lecanicillium muscarium* contained in the commercial product 'Mycotal' emerged as the best candidate for development (Reardon *et al.* 2004). We report on a pilot study to examine the efficacy of Mycotal for suppression of HWA when applied aerially to hemlock stands.

Lecanicillium muscarium (=*Verticillium lecanii*) is an insect-killing fungus that has been isolated from most terrestrial zones. Its host range is relatively narrow, infecting such insects as adelgids, thrips, aphids and whiteflies (Humber and Hansen 2005). In fact, HWA in the Eastern US were found to be infected by a fungus identified as *V. lecanii,* among other insect-killing fungi (Reid 2003). Fungi kill insects by growing profusely within their body cavity, after they germinate and penetrate from the outside, which requires suitable temperature and relative humidity. When several species of insect-killing fungi were tested in small-scale field trials, *L. muscarium*, then called *V. lecanii*, proved to be most consistently effective against HWA sistens (Reardon *et al*. 2004). Additionally, no negative impact of *V. lecanii* to *Sasajiscymnus tsugae*, an introduced predatory beetle, was found in a bagged foliage study (Reardon *et al*. 2004).

For an insect-killing fungus to be successfully deployed for forest pest management it must first be registered by the US EPA as a biopesticide, which can be time consuming and costly for fungi recently isolated and with no commercial history. Mycotal is a commercial biopesticide produced in The Netherlands that contains the insect-killing fungus *Lecanicillium muscarium*. Although not yet registered in the US, this biopesticide has a completed registration package for its European registration that will accelerate the timeline for its availability in the US. With the support of the manufacturer, Koppert Biological Systems, Inc., permits were obtained from USDA-APHIS-PPQ to allow Mycotal's importation and release into the environment for field testing. This allowed us to treat up to 10 acres/yr of hemlock forest with the fungus.

Fungal microfactory technology was developed to improve the effectiveness and economic feasibility of fungi applied over large acreage for pest management. Microfactory technology relies on the nutritive value of cheese whey for fungal growth and reproduction, which occurs after the fungus is applied in the field (Grassano 2008). MycoMax™ fungal enhancer is a formulation adjuvant based on this technology that was optimized for use with Mycotal against HWA. In laboratory experiments with the enhanced fungal formations, the number of fungal spores increased 10-100 fold and extensive fungal growth was observed on foliage collected from the field.

SUMMARY 2009 PILOT STUDY TENNESSEE WILDLIFE AND RECREATION AREA - TITUS CREEK

On the evening of May 28 and again on the morning of May 29, 2009 replicate 1.25 acre plots of hemlocks infested with adelgid crawlers were treated *via* helicopter (10 liters/acre). To account for density-dependent population effects a total of 12 plots were grouped into 4 blocks on the basis of pre-treatment HWA population counts. Four plots, one from each block, served as 'No Spray' control, 4 were treated with *L. muscarium* alone (1 x 10^8 spores/ml: Mycotal, Koppert Biological Systems) and 4 received *L. muscarium* enhanced with the microfactory formulation (5% w/v MycoMax); the oil adjuvant Addit (0.25% v/v : Koppert Biological Systems) and the sticker Hyperactive (0.05% v/v : Helena Chemical) were also added to both fungal treatments. Treatments were applied aerially using a Bell Jet-Ranger helicopter with mounted AU6539 Micronair atomizers. Targeting of treatments was accomplished with an on-board navigational system and ground-truthed plot locations. Temperature, rainfall, relative humidity and leaf wetness were collected within the study area. Weather conditions immediately post- treatment were generally favorable for the germination and growth of *L. muscarium*, with RH from 85-100% and temperatures nearing 20°C.

A total of 3000 foliage samples were collected to assess HWA population status pretreatment in spring 2009, in summer 5 weeks post-treatment and again in March 2010.

Both lower and upper (~ 15 meters) canopy foliage was collected and examined during the 2009 and 2010 spring evaluations, but only the lower canopy was sampled during summer 2009. At each tree elevation, 5 sample branches (~25 cm) were collected from each of 10 pre-selected and tagged trees. HWA were quantified on the spring (2009 and 2010) samples using a modification of the 'Rich Cowles' method where only up to 20 HWA woolly masses are counted/branch (Cowles and Montgomery 2006). The difference between post and pre-treatment counts were considered to assess treatment impact. Minute HWA sistens are present during summer sampling period and each of these must be counted microscopically and punctured to determine its survival status; alive insects are indicated by body turgor and burgundy colored blood; the HWA on five branchletes/ branch were examined (~12,000 individuals). Data were log transformed when necessary and analyzed using GLM is SAS (SAS Institute 2008) (Alpha=0.05); P-diff was used for pre-planned means separation (SAS Institute 2008).

When foliage was collected 5-weeks post-treatment to assess populations of aestivating sistens the number of alive HWA tended to be lower in fungal treated plots, and more so when the fungal enhancer was included, 73% and 43% of control populations, respectively (Figure 1), although the differences were not significant (P>0.05). The latter post-treatment sample, taken in spring 2010, revealed an anticipated density-dependent crash of HWA populations in the lower elevation of block (4) where the HWA density was extremely high the previous year. The change in HWA population from the preceding spring reflected this crash in a significant interaction between block (based on initial population) and fungal treatment, which masked the overall affect of the fungus in the analysis (Figure 2). The crash was most prominent in the block 4 control plot, whereas the dramatic reduction was not as evident in the plot of the corresponding block receiving enhanced fungus. It is unclear whether this failure to completely crash was the result of the enhanced fungal treatment or that initial HWA populations were somewhat lower.

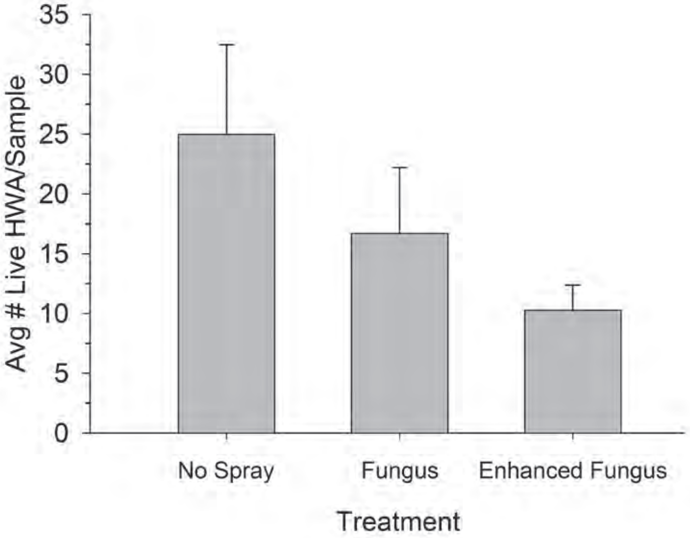

Figure 1. The number of live HWA sistens found in the lower hemlock canopy 5 weeks post-treatment for plots receiving no spray or aerial treatment of *L. muscarium* fungus (Mycotal) with or without fungal enhancer (MycoMax). Vertical lines indicate the standard error of means. There were no significant difference (P>0.05) among treatments.

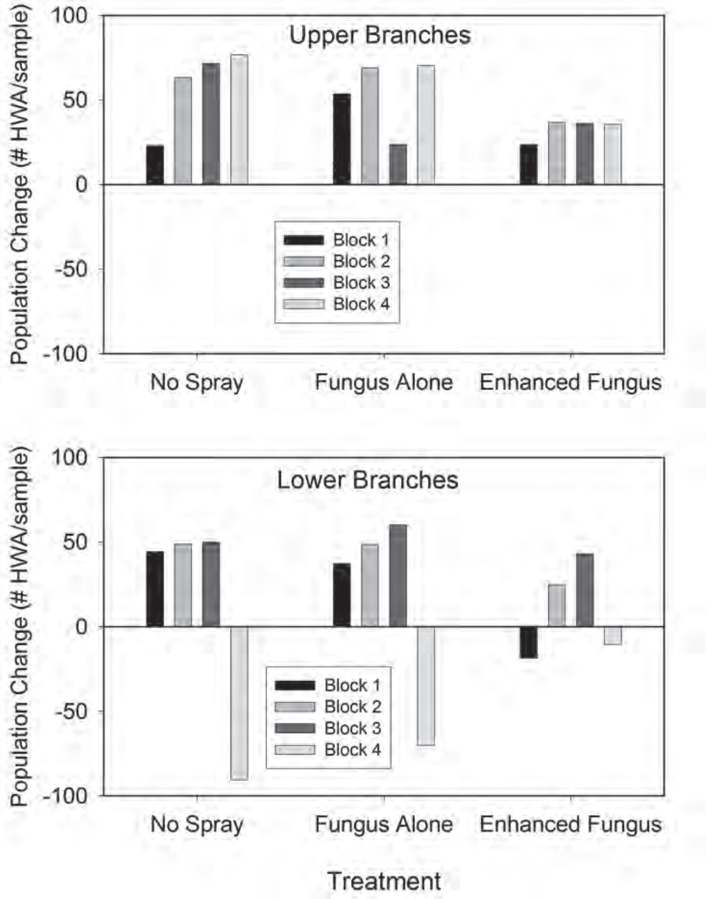

Figure 2. The change in HWA populations between pre- and post-treatment samples taken from the lower and upper branches (~ 15 meters). The sum of 5 samples/elevation/tree was used to calculate population change. In most plots HWA populations were continuing to expand, except that a density dependent crash in HWA populations occurred in the lower canopy of block 4, which is characterized by a significant ($P<0.05$) Block (density) X Treatment interaction.

Regardless of the cause, the presence of a residual HWA population may be of some benefit by providing food for continued survival of HWA predators, instead of the dearth caused by the density-dependent tumult in unregulated HWA populations.

The population change data was re-analyzed without block 4 to obtain a more precise evaluation of treatment effects. In this case, there was a significant ($P<0.05$) reduction in HWA population growth when hemlocks were treated with fungus enhanced in the whey microfactory formulation (Figure 3). Although HWA population in the enhanced fungus plots still continued to grow, they expanded at a rate less than half that found in control plots. HWA population expansion in plots treated with fungi, but without the fungal enhancer, tended to be lower than the untreated control although the difference was not significant. These results reflect that observed in summer (Figure 1) and suggest HWA populations were impacted by aerial application of the insect-killing fungus when formulated with the fungal enhancer.

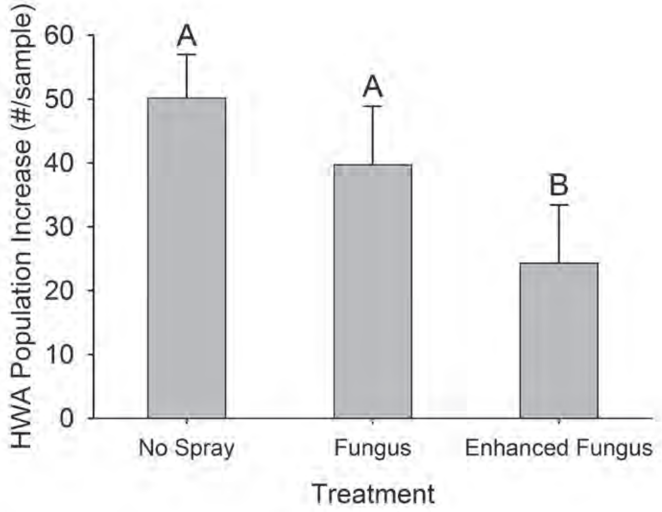

Figure 3. The change in HWA populations between pre- and post-treatment samples taken from the lower upper branches (~ 15 meters), combined over elevation. Results from block 4 were excluded because of an anticipated density dependant crash in HWA population. Plots were either not treated (No Spray) or treated with *L. muscarium* fungus (Mycotal) with or without fungal enhancer (MycoMax). The growth of HWA populations was significantly (P<0.05) reduced in plots receiving the enhanced fungal treatment, as indicated by different capital letters over standard error bars.

The influence of reducing population growth on longer-term HWA population dynamics are uncertain. To explore possible outcomes, a preliminary assessment was made using a HWA Population Simulator developed by Trotter (2010) to model, among other factors, impacts of cold induced, over-wintering mortality; mortality to the summer and/or overwintering sisten population is analogous to the fungal impact reported herein. Nominal settings were made in the model to reflect potential impacts of other biotic and abiotic factors on the progridiens and sistens population: egg production=50% of maximum, egg mortality=50%, crawler mortality=90%. The mortality of juvenile and adults progridiens was set to 50% to suggest predator influences and overwintering mortality of sistens was held at 0%; sisten summer mortality was adjusted to capture different levels of fungal impact, i.e., 0, 25%, 75% yearly mortality.

The outputs from the modeling exercise when the initial population is set to 500 individuals are depicted in Figure 4. Note that models are designed to explore possible outcomes and results should be considered with that in mind. When no (0%) fungal impact was incorporated the HWA population spiked regularly twice each year as crawlers emerged, escalated in between and went off the chart after 2 years, much like what occurs naturally. At 25% yearly impact, the severe escalation was delayed for 1 year. Incorporating the fungal impact obtained in the pilot study (~50% suppression in expansion), the spikes in crawler numbers were still evident but the general escalation of the HWA population is appreciably inhibited. Surprisingly, when the fungal impact was increased to 75%, crawler spikes began to subside and overall populations decreased over time! However, it must be reiterated that results from this modeling exercise can only be used to help understand interactions of potential mortality factors and cannot be substituted for real world evaluation of longer-term impacts.

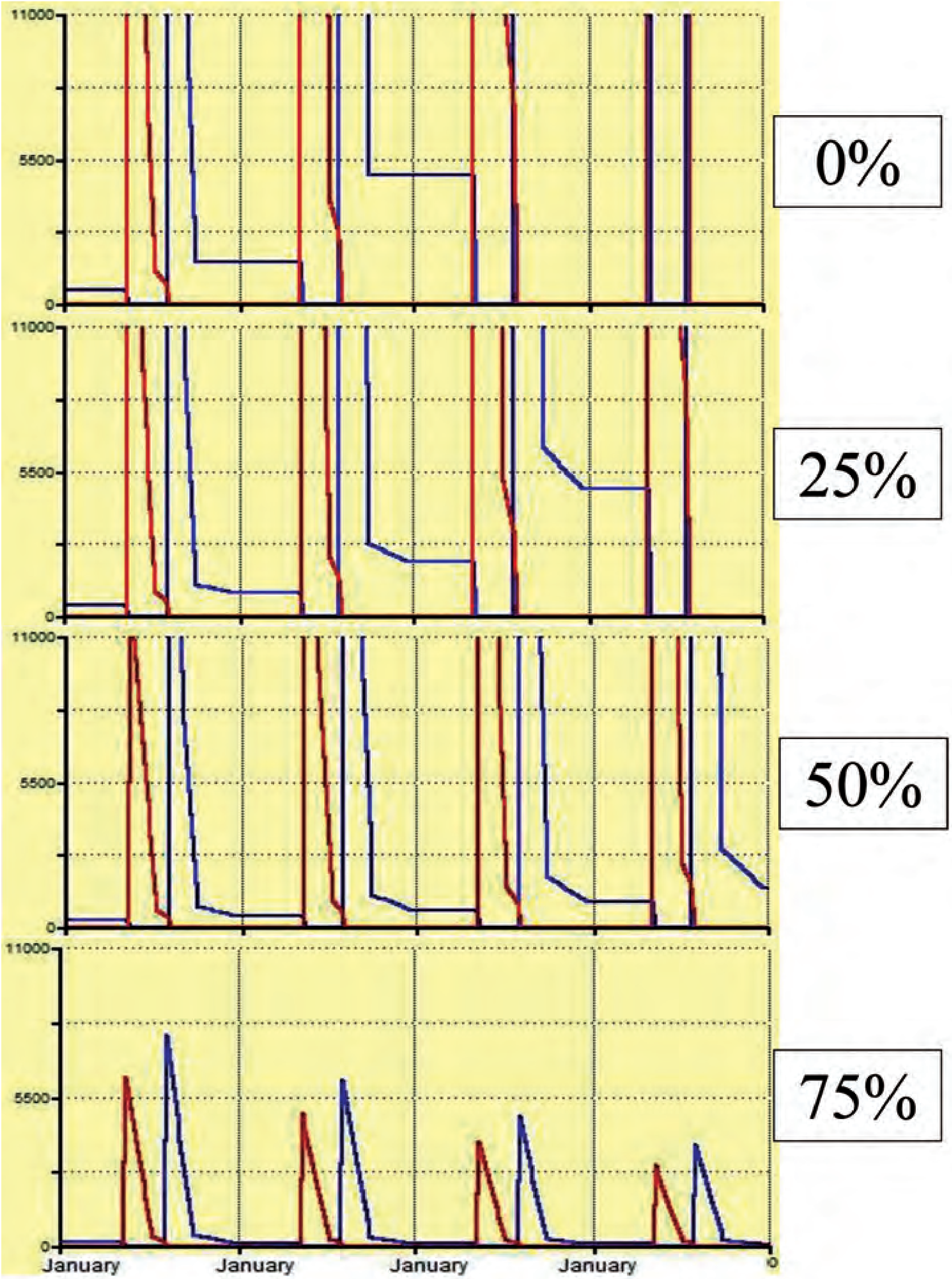

Figure 4. Results from the hemlock woolly adelgid population simulation model (Trotter 2010) at different levels of projected yearly impact by insect-killing fungus, i.e., 0, 25, 50, and 75% reduction in summer sisten populations. Repeating pairs of large spikes represent yearly populations of progridien and sisten crawlers, respectively, followed by a trough indicating the sisten population that would summer aestivate and overwinter. The Y-axis ranges from 0-11,000 individuals and crawler spikes go off-scale. The model is started with 500 individuals and other parameters are defined in the text.

The question remains, ‘can it work’, can we use aerial applications of insect-killing fungi as biopesticides to broadly suppress HWA populations and protect hemlock trees. A second aerial application at a higher fungal rate was made in 2010, for which the results were unavailable, and a larger trial is planned for 2011 in two climatic zones (TN-southern and PA-northern) to investigate interaction with low winter temperatures -- from these a clearer picture of fungal impacts is expected to emerge. However, the results obtained in the 2009 Pilot Study are a positive harbinger compared to other unsuccessful attempts at broad scale application of insect-killing fungi for pest suppression, and suggests the utility of using the fungal enhancer in the whey-based microfactory formulation. There is an understandably strong desire to observe rapid knockdown of HWA populations. In reality, massive disease outbreaks with insect-killing fungi are poorly understood and not usually captured through spray and count experimental approaches. While rapid knockdown is hoped for through making ‘inundative’ applications of fungal spores done in the 2009 and 2010 Pilot Studies, the benefits of broadly inoculating HWA populations to enhance prospects for future disease outbreaks should not be ignored.

ACKNOWLEDGMENTS

The author is only the presenter and hopeful catalyst of this work, which would never have been accomplished without the steadfast intellectual and practical support of at least the following individuals: Brad Onken, Karen Felton. Dick Reardon, Rusty Rhea, Dennis Souto, Michael Bohne, Bob Tichenor, Brant Miller, Jerome Grant, Joe Elkins, Wes Pilley Justin Walden, Ashley Lamb, Bud Mayfield, Brad Register, and the Costa Boys. The author is also indebted to Helicopter Applicators Inc. and Koppert Biological Systems, Inc. for supply of Mycotal. The 2009 Pilot Study was funded under USFS Cooperative Agreement Number: 07-CA-11420004-117, Whey-Based, Fungal Micro-Factory Technology for Enhancement Mycopathogen Deployment in Forest Pest Management. COI Statement - Dr. Scott Costa is also President of Costa Enterprises, Ltd, which holds exclusive rights to whey-based fungal microfactory technology.

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THE HWA PREDATOR RELEASE AND RECOVERY DATABASE

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INTRODUCTION

For the past several years, state and federal agencies have released lab reared or wild predatory beetles of the hemlock woolly adelgid (*Adelges tsugae*, or HWA), in an effort to control this pest. Until recently, data pertaining to the release, monitoring, and recovery of *Laricobius nigrinus* (Coleoptera: Derodontidae), *Scymnus sinuanodulus* (Coleoptera: Coccinellidae), and *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) have been maintained on paper data forms or in small local data bases and, as a result, have been inaccessible to HWA researchers at regional and national levels. In 2007, we received funding to develop and implement the HWA Predator Release and Recovery Database (PDB) which would be housed in the Department of Entomology at Virginia Tech in Blacksburg, VA. The PDB will impose an organizational structure on the data and serve as a central repository for information collected on release and recovery efforts. Implementing the PDB will facilitate improved access to and use of the data; provide project-wide reporting, mapping, and analysis; ensure that data from all cooperators are maintained, archived and available; and improve decision-making for future actions.

DATABASE STRUCTURE

The database is developed in Oracle® database management system and is installed on a Windows 2003 Server platform in the Department of Entomology at Virginia Tech. Each release site record contains approximately 85 data fields distributed among four predator related database tables plus an additional table containing information related to database users (Figure 1).

In addition to information on HWA predator release sites, the database contains data on the individual and organization performing the activity, site and stand conditions, weather, origin of beetles released, release tree information, post-release survey, and predators recovered.

The database is organized around release sites. Each site is geographically referenced and contains from one to several release trees. Data for post-release monitoring, sometimes referred to as recoveries, are in almost all cases associated with release sites, as are subsequent beetle releases called augmentations.

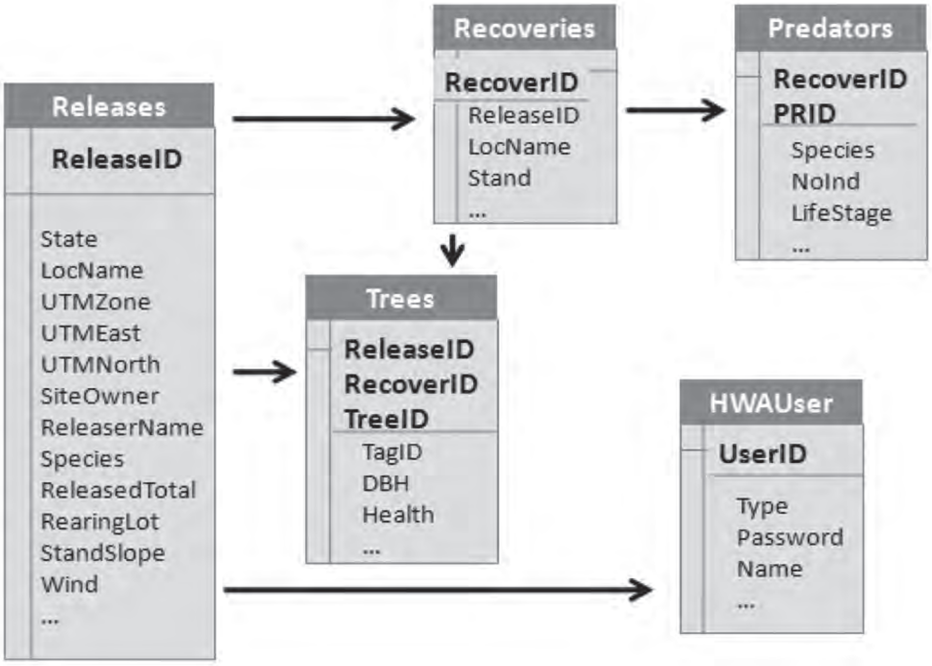


Figure 1. Database table structure and relationships in the HWA PDB.

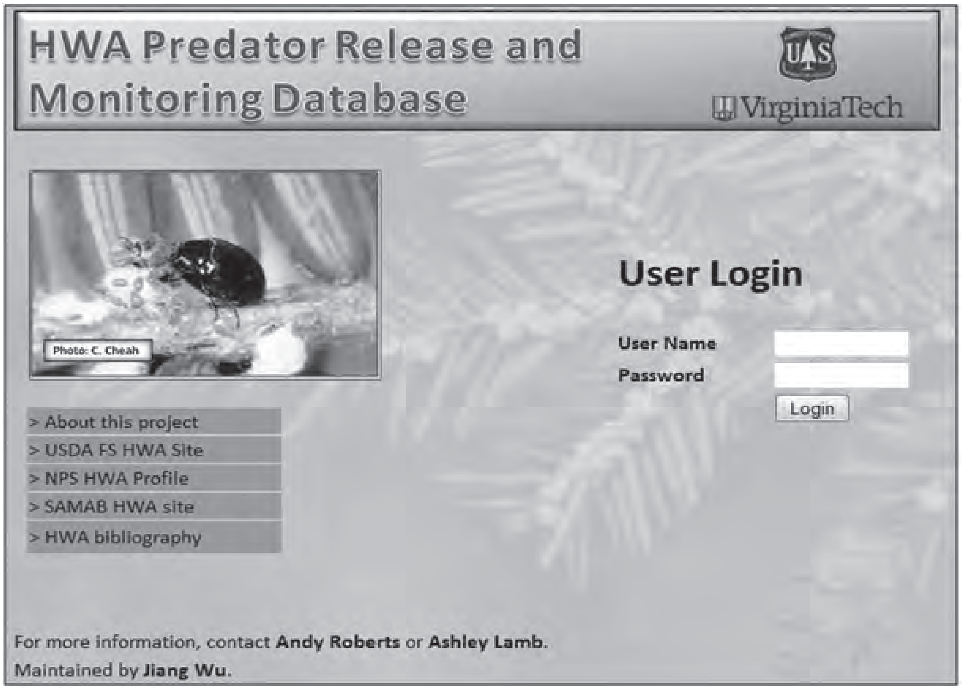
DATABASE FUNCTIONALITY

The PDB is accessed through a web portal (http://hwa.ento.vt.edu)(Figure 2).

Two types of user accounts are available. The «VT User» account is reserved for persons who require both read and write privileges for the entire database. This includes database managers and technicians whose responsibility is to enter or correct data from multiple agencies. A «Remote User» account is assigned to non-VT personnel whose responsibility is to enter and maintain data for a specific agency. Currently, the site is publicly available and anyone can log in as «guest» without a password. Guest logins may view all site content but have no editing capability. Presently, there are approximately 40 registered users, but only a portion of those are actively involved with data entry and management.

The functionality of the database and associated web site are fairly standard and consist of data entry and editing for both initial release data, post-release monitoring and predator recovery, database query and reporting, map query and display, and data download. In addition, there is access to documents addressing field protocols, data entry protocols, and user account administration. The main page for the remote user interface is shown in Figure 3.

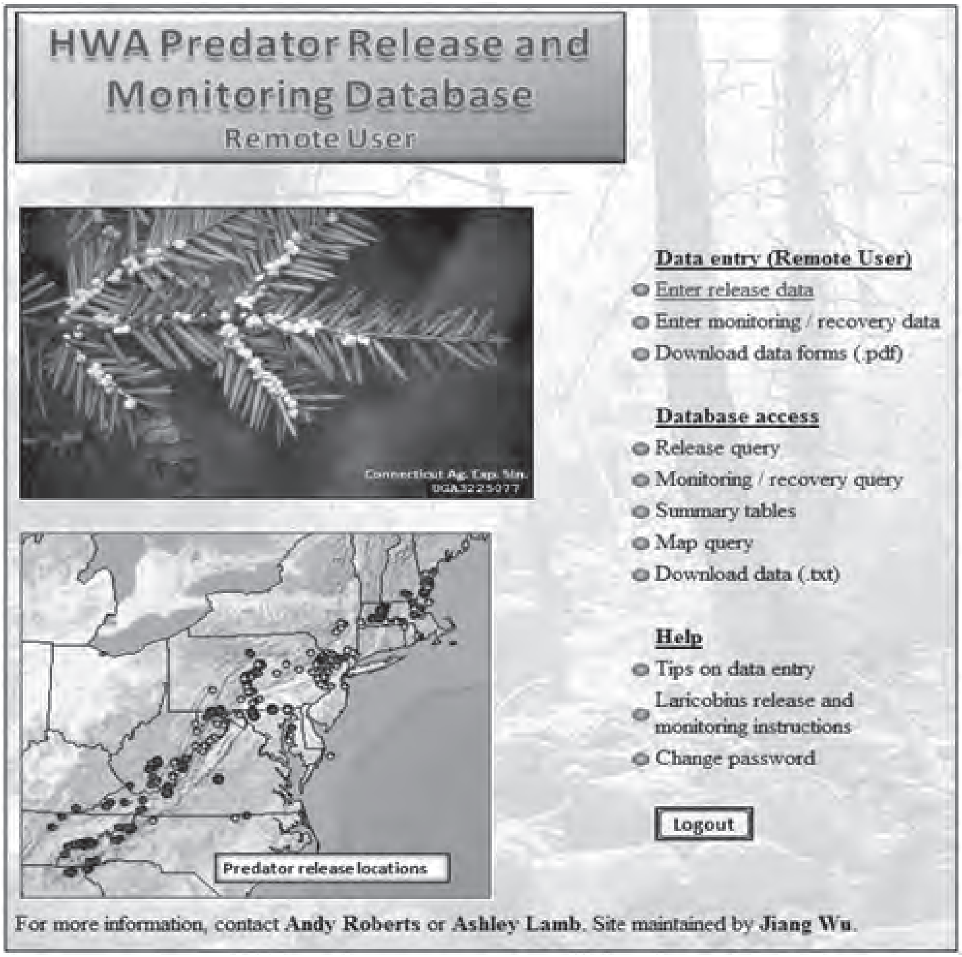
The VT User main page is similar to the remote user page except it includes access to data entry screens for earlier versions of the release data forms and access to database user statistics. It also allows someone with VT User privileges to review and approve data submitted by a remote user.



HWA Predator Release and Monitoring Database

For more information, contact **Andy Roberts** or **Ashley Lamb.**
Maintained by **Jiang Wu.**

Figure 2. The login screen to the PDB web portal.



HWA Predator Release and Monitoring Database
Remote User

For more information, contact **Andy Roberts** or **Ashley Lamb**. Site maintained by **Jiang Wu**.

Figure 3. The primary page on the Remote User database site.

Data entry is straightforward. Because predator-release field protocols and data collection have varied greatly over time and have been conducted by several different agencies, we intentionally made the PDB relatively unconstrained. There are few required fields, and there are few constraints on the values and formats of most fields. This results in a somewhat messy database, but the decision was to capture as much data as possible, including historical records, rather than expend resources on cleaning up data, which is up to a decade old. It is hoped that, as the database matures and begins to consist primarily of current records, consistency and clarity in the data will emerge. One area where we have imposed quality control at the data entry stage is in geographic coordinates (Figure 4).

The database accepts coordinates in either Universal Transverse Mercator (UTM) or in latitude / longitude, which may be in any of the common formats: decimal degrees, degrees decimal-minutes, or degrees minutes seconds. Constraints have been placed on these coordinates so that values out of range for the respective coordinates are not allowed.

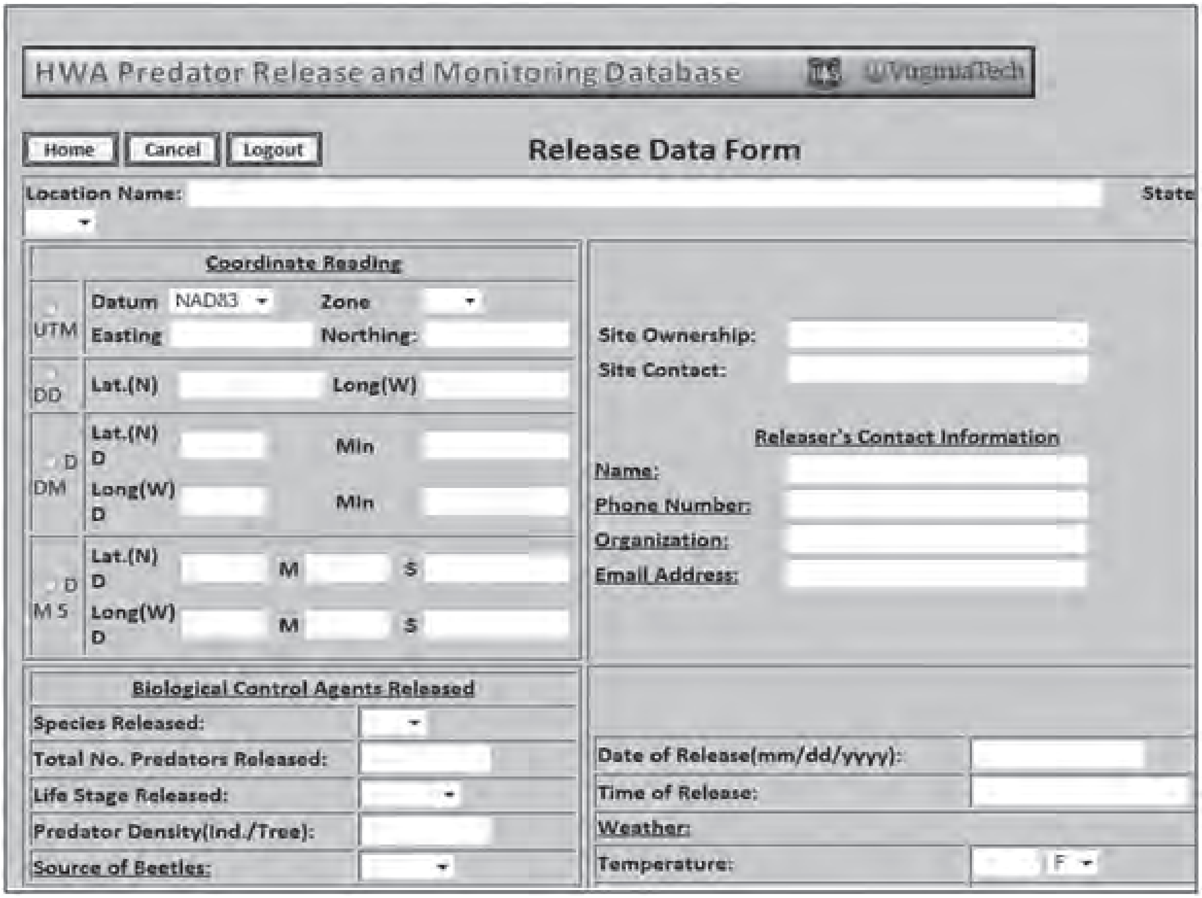
Actions which occur at a site after release data have been entered include updating release information, adding post-release monitoring data, or adding augmentative release data. Data entry in these cases is accomplished by using a database query to access the initial release data and then selecting the appropriate action (Figure 5). When post-release data are entered in this way, the database populates the online data forms with pertinent information from the release records and thus expedites data entry. In addition, this reduces possible omissions or data entry errors by the user. An example is shown in Figure 6 where all relevant release information for the Gauley River site has been pre-loaded into the post-release monitoring data form.

DATABASE CONTENT

The number of predator releases and the number of beetles released by species and state is presented in Table 1. These data were derived from text files downloaded on August 3, 2010, and include both initial and augmentative releases.

Some readers may notice that the database contains far fewer records than what has been collected over the years by the various agencies involved. This is because there still is a great deal of data that has yet to be entered into the system. The reasons for this are varied and efforts are ongoing to remedy the discrepancy between the contents of the database and existing information.

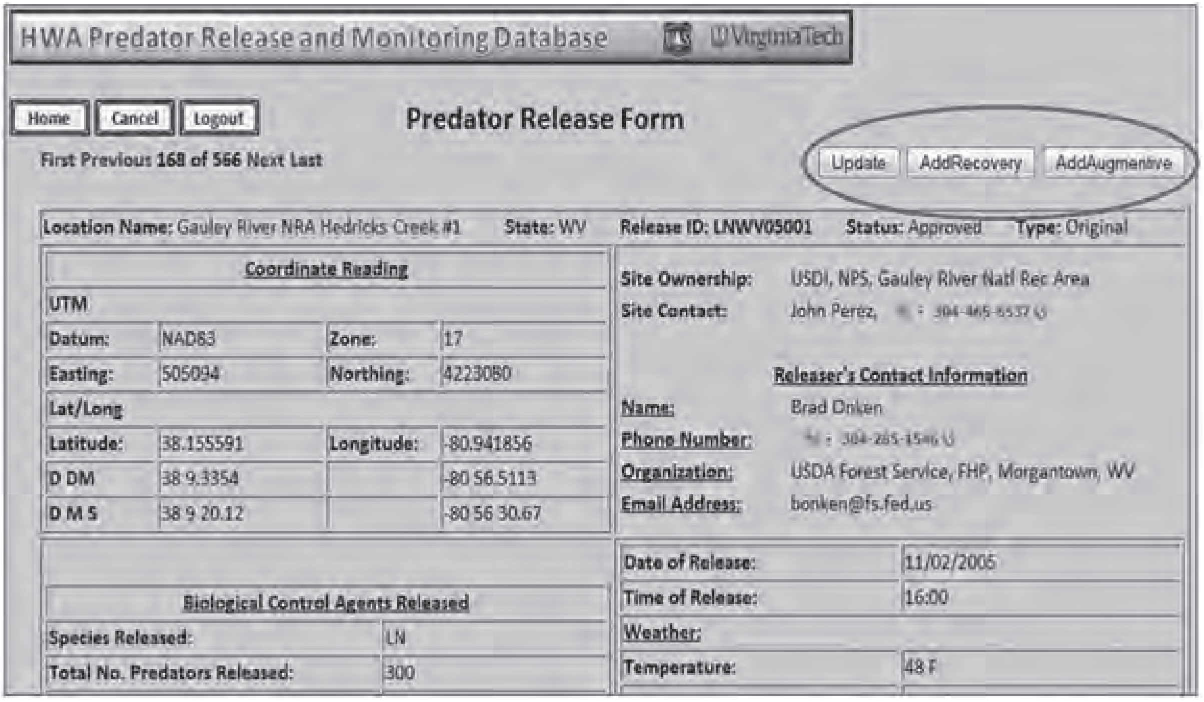
Accessing data through the web portal allows more refined searches than a simple export from the database. It can perform queries based on the values of specific variables, such as Location Name, State, Species, Year. In addition, the default Summary Report generates a table by species of the number of locations and beetles released to date and a yearly time series of post-release monitoring results (Figure 7). For example, of the 61,376 *L. nigrinus* released at 183 locations, there were 36 post-release surveys in the following year, 18 of which were positive for beetle recoveries (Figure 7). To date, there were a total of 42 post-release surveys at these sites and beetles were recovered at 21 of those. It must be remembered that the reported numbers are for unique locations, not



Datum NAD83

Temperature: F

Figure 4. The predator release data entry screen.



Predator Release Form

First Previous **168 of 566** Next Last

Location Name: Gauley River NRA Hedricks Creek #1 **State:** WV **Release ID: LNWV05001** **Status:** Approved **Type:** Original

Coordinate Reading

UTM			
Datum:	NAD83	Zone:	17
Easting:	505094	Northing:	4223080
Lat/Long			
Latitude:	38.155591	Longitude:	-80.941856
D DM	38 9.3354		-80 56.5113
D M S	38 9 20.12		-80 56 30.67

Site Ownership: USDI, NPS, Gauley River Natl Rec Area
Site Contact: John Perez, ☎ : 304-465-6537

Releaser's Contact Information

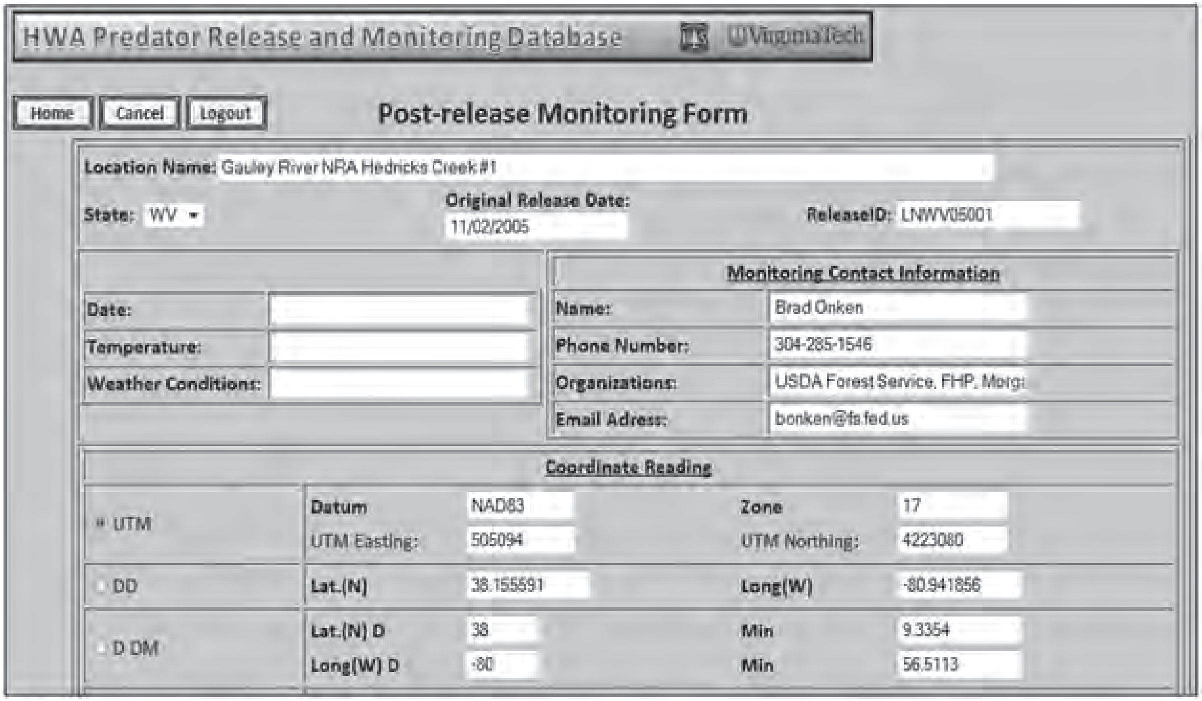
Name: Brad Onken
Phone Number: ☎ : 304-285-1546
Organization: USDA Forest Service, FHP, Morgantown, WV
Email Address: bonken@fs.fed.us

Biological Control Agents Released

Species Released:	LN
Total No. Predators Released:	300

Date of Release:	11/02/2005
Time of Release:	16:00
Weather:	
Temperature:	48 F

Figure 5. The results returned by a query of the release data. This template is used to access data entry for subsequent actions at the site.



Post-release Monitoring Form

Location Name: Gauley River NRA Hedricks Creek #1

State: WV

Original Release Date: 11/02/2005

ReleaseID: LNWV05001

Date:	
Temperature:	
Weather Conditions:	

Monitoring Contact Information

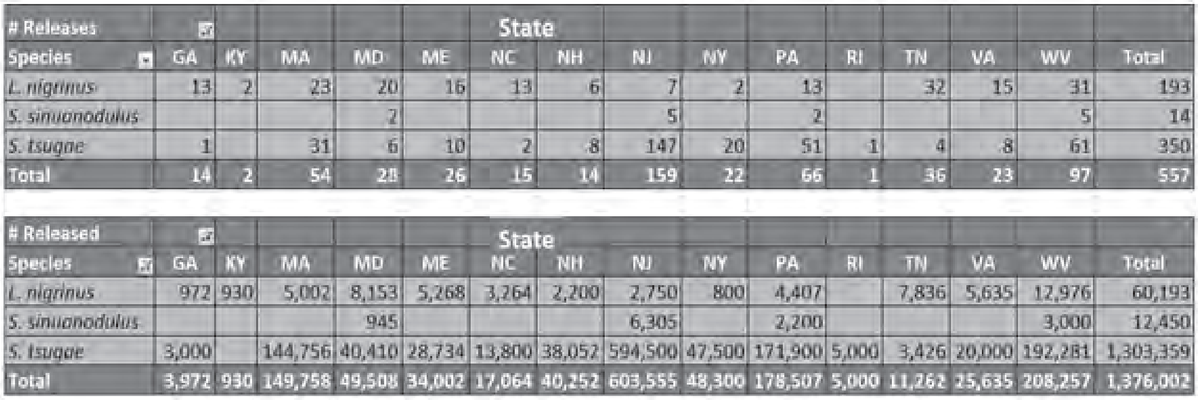
Name:	Brad Onken
Phone Number:	304-285-1546
Organizations:	USDA Forest Service, FHP, Morg
Email Adress:	bonken@fs.fed.us

Coordinate Reading

● UTM	**Datum**	NAD83	**Zone**	17
	UTM Easting:	505094	UTM Northing:	4223080
○ DD	**Lat.(N)**	38.155591	**Long(W)**	-80.941856
○ D DM	**Lat.(N) D**	38	**Min**	9.3354
	Long(W) D	-80	**Min**	56.5113

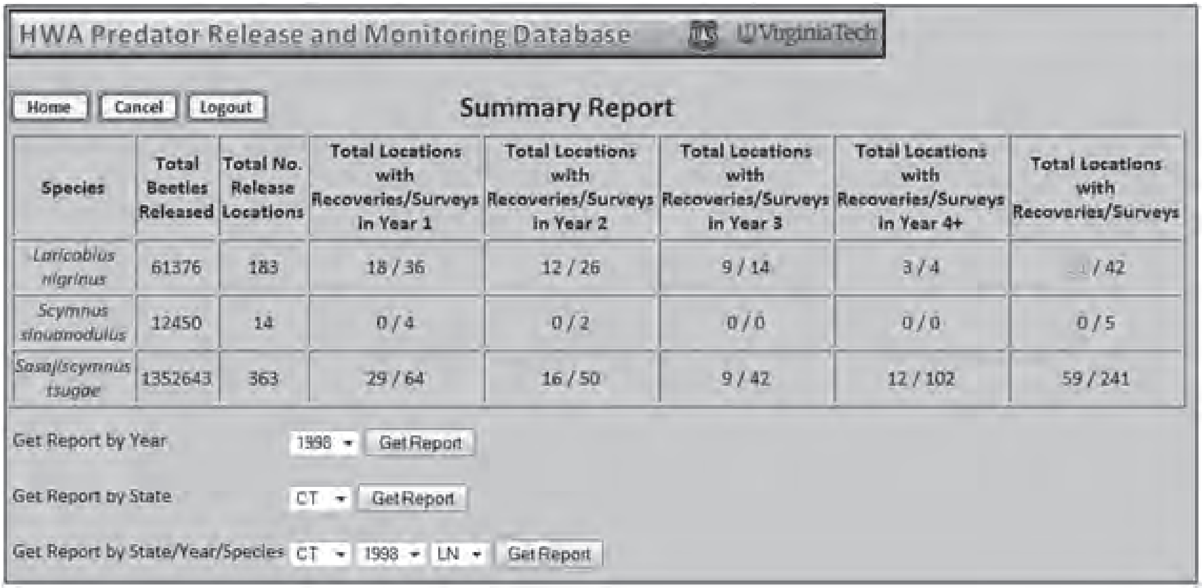
Figure 6. The post-release monitoring and predator recovery data entry form.

Table 1. Numbers of predator release sites and beetles released in the database by species and state.



# Releases	State													
Species	GA	KY	MA	MD	ME	NC	NH	NJ	NY	PA	RI	TN	VA	WV	Total
L. nigrinus	13	2	23	20	16	13	6	7	2	13		32	15	31	193
S. sinuanodulus				2				5		2				5	14
S. tsugae	1		31	6	10	2	8	147	20	51	1	4	8	61	350
Total	14	2	54	28	26	15	14	159	22	66	1	36	23	97	557

# Released	State														
Species	GA	KY	MA	MD	ME	NC	NH	NJ	NY	PA	RI	TN	VA	WV	Total
L. nigrinus	972	930	5,002	8,153	5,268	3,264	2,200	2,750	800	4,407		7,836	5,635	12,976	60,193
S. sinuanodulus				945				6,305		2,200				3,000	12,450
S. tsugae	3,000		144,756	40,410	28,734	13,800	38,052	594,500	47,500	171,900	5,000	3,426	20,000	192,281	1,303,359
Total	3,972	930	149,758	49,508	34,002	17,064	40,252	603,555	48,300	178,507	5,000	11,262	25,635	208,257	1,376,002



Summary Report

Species	Total Beetles Released	Total No. Release Locations	Total Locations with Recoveries/Surveys in Year 1	Total Locations with Recoveries/Surveys in Year 2	Total Locations with Recoveries/Surveys in Year 3	Total Locations with Recoveries/Surveys in Year 4+	Total Locations with Recoveries/Surveys
Laricobius nigrinus	61376	183	18 / 36	12 / 26	9 / 14	3 / 4	[illegible] / 42
Scymnus sinuanodulus	12450	14	0 / 4	0 / 2	0 / 0	0 / 0	0 / 5
Sasajiscymnus tsugae	1352643	363	29 / 64	16 / 50	9 / 42	12 / 102	59 / 241

Get Report by Year 1998 ▾ Get Report

Get Report by State CT ▾ Get Report

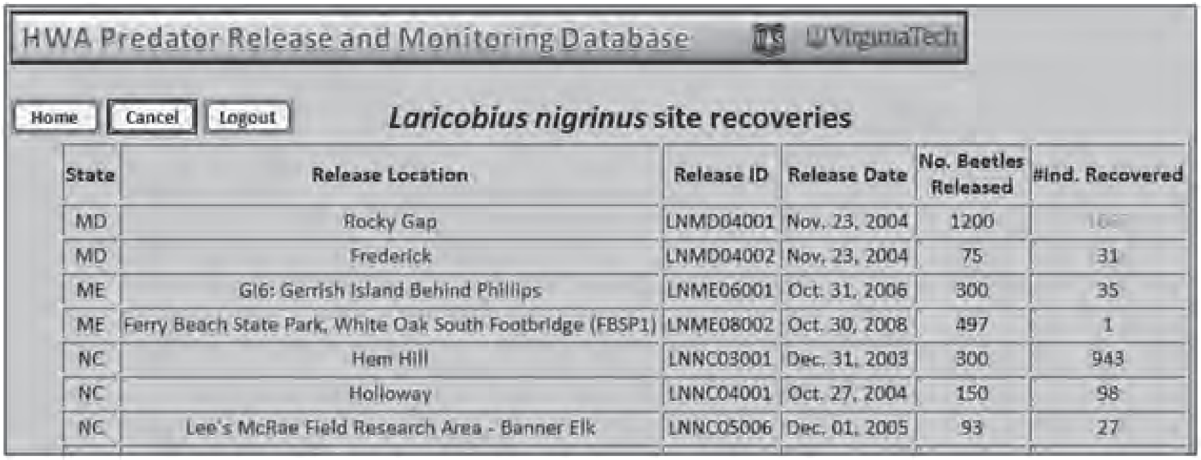
Get Report by State/Year/Species CT ▾ 1998 ▾ LN ▾ Get Report

Figure 7. The default Summary Report from the database.

total number of surveys. Because some locations have been surveyed multiple times over the years the grand total does not equal the sum of each yearly total.

One useful function of the PDB is the ability to "drill down" through the data to reveal more detail. For example, clicking on the 21 sites with positive recoveries will bring up a list of those sites with associated data as well as the number of beetles recovered at each (Figure 8).

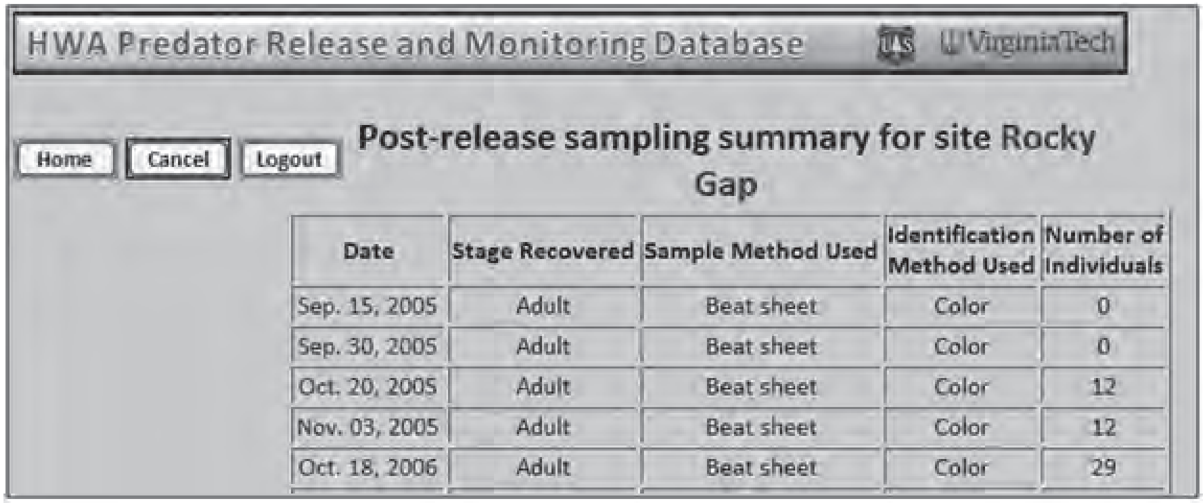
Selecting the 1067 individuals recovered for a release site will return a list of the results for each individual survey (Figure 9). In the returned data table, selecting the value in the Date field will return the original survey record for that activity.



***Laricobius nigrinus* site recoveries**

State	Release Location	Release ID	Release Date	No. Beetles Released	#Ind. Recovered
MD	Rocky Gap	LNMD04001	Nov. 23, 2004	1200	[illegible]
MD	Frederick	LNMD04002	Nov. 23, 2004	75	31
ME	GI6: Gerrish Island Behind Phillips	LNME06001	Oct. 31, 2006	300	35
ME	Ferry Beach State Park, White Oak South Footbridge (FBSP1)	LNME08002	Oct. 30, 2008	497	1
NC	Hem Hill	LNNC03001	Dec. 31, 2003	300	943
NC	Holloway	LNNC04001	Oct. 27, 2004	150	98
NC	Lee's McRae Field Research Area - Banner Elk	LNNC05006	Dec. 01, 2005	93	27

Figure 8. Example of records returned through the site query.



Post-release sampling summary for site Rocky Gap

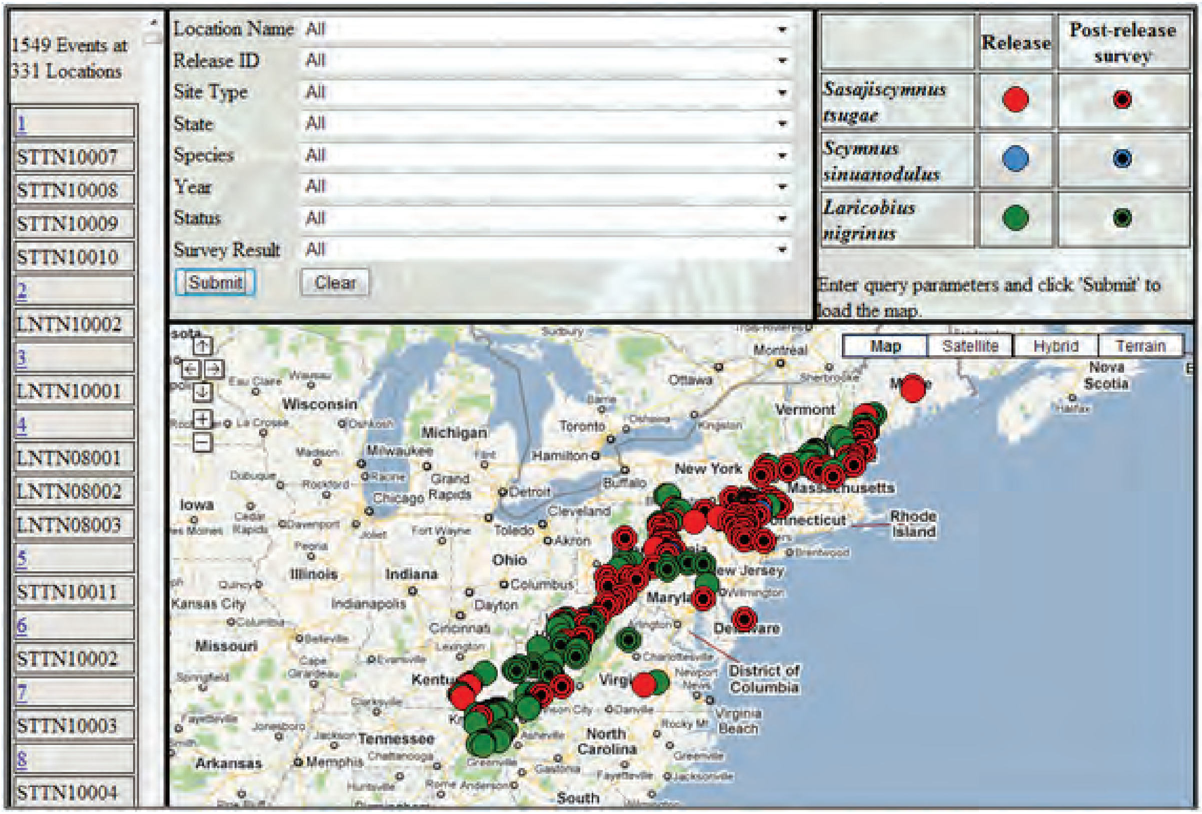
Date	Stage Recovered	Sample Method Used	Identification Method Used	Number of Individuals
Sep. 15, 2005	Adult	Beat sheet	Color	0
Sep. 30, 2005	Adult	Beat sheet	Color	0
Oct. 20, 2005	Adult	Beat sheet	Color	12
Nov. 03, 2005	Adult	Beat sheet	Color	12
Oct. 18, 2006	Adult	Beat sheet	Color	29

Figure 9. Example of monitoring records returned from the database for a specific site.

MAP QUERY

The PDB incorporates a simple map display developed in a Google Earth® application. Only records with geographic coordinates are displayed on the maps. As with the reporting section of the web portal, the mapping function supports querying the database using several data fields (Fig 10). Symbology is simplified to differentiate among species and between release and post-release monitoring sites. The map display groups data by unique geographic coordinates, which are numbered and listed in the panel to the left of the map. Release sites as well as post-release monitoring sites are grouped under each unique location. For example, site 1 in Figure 10 references four release records that may or may not occur on the same date.

As with all Google maps, the base layer can be either a street layer, a terrain layer (Figure 11) or remotely sensed imagery. Each location has a hyperlinked reference to all activities at that site. For example, in Figure 11 data for an initial release of 1000 *L. nigrinus* (ID=LNWV08005) and a subsequent survey (ID=WV090002) are referenced by the point in the center of the map.



1549 Events at
331 Locations

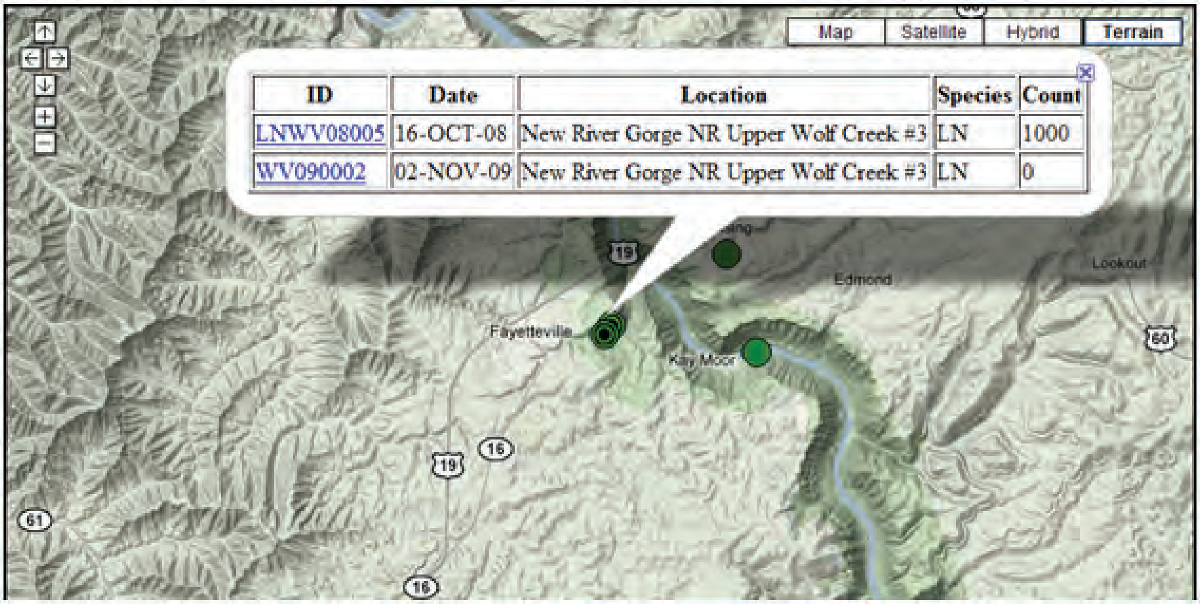
1
STTN10007
STTN10008
STTN10009
STTN10010
2
LNTN10002
3
LNTN10001
4
LNTN08001
LNTN08002
LNTN08003
5
STTN10011
6
STTN10002
7
STTN10003
8
STTN10004

Location Name: All
Release ID: All
Site Type: All
State: All
Species: All
Year: All
Status: All
Survey Result: All

	Release	Post-release survey
Sasajiscymnus tsugae	●	●
Scymnus sinuanodulus	●	●
Laricobius nigrinus	●	●

Enter query parameters and click 'Submit' to load the map.

Figure 10. Results of map query displaying all georeferenced records in the database.



ID	Date	Location	Species	Count
LNWV08005	16-OCT-08	New River Gorge NR Upper Wolf Creek #3	LN	1000
WV090002	02-NOV-09	New River Gorge NR Upper Wolf Creek #3	LN	0

Figure 11. Map display with linked data records and terrain background.

DATA EXPORT

The entire PDB can be exported as a text file. Information on releases, release trees, recoveries, recovery trees, and predators are downloaded in separate files which will easily load into spreadsheets such as Microsoft's Excel® and GIS software such as ESRI's ArcGIS®.

DATABASE ISSUES

There are a number of important issues to address in the continued development of the PDB. One of the most important of these is the necessity to incorporate past survey data which exists on paper and/or local data files. Equally important is to obtain buy-in from groups that are releasing and monitoring beetles. Another task is to correct data that has been submitted, primarily by editing or adding coordinates and including missing data. This is most important for geographic coordinates. Since inception, we have added or corrected coordinates for over 200 release sites, but there still remain dozens of records with no geographic information. It will help to standardize the naming procedures of release sites such that location names provide more consistent location information. We hope that the user community will recognize the advantages of the centralized, standardized, online database and eagerly contribute to the data housed in it.

ACKNOWLEDGMENTS

In addition to those listed above as contributors, Jiang Wu and Ian Firkin, both at Virginia Tech, have devoted much time to the database and mapping applications. Karen Felton with the USDA in Morgantown greatly assisted in developing the database structure and schema. Funding for this project is supplied by the USDA Forest Service HWA Initiative through the Northeastern Area.

ASSESSMENT OF INTRODUCED PREDATOR SPECIES AGAINST HEMLOCK WOOLLY ADELGID ON EASTERN HEMLOCK USING WHOLE-TREE CANOPY ENCLOSURES

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ABSTRACT

Numerous predatory species have been evaluated against hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), since its introduction into North America where it has caused extensive mortality of eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Englemann (Cheah et al. 2004, Lambdin et al. 2006, Ward et al. 2004). Most of these predatory evaluations have been conducted in the laboratory, incubators, quarantine facilities, or sleeve cages in the field. A pilot study was conducted to assess the feasibility of using whole-tree canopy enclosures (i.e., cages) to assess survival, colonization, interactions, and establishment of introduced predators on HWA on eastern hemlock, as well as to assess their impact on population densities of this serious invasive pest and on tree health. This project focused on the use of large (ca. 8.3 m tall) cages to qualitatively and quantitatively assess the successful field application (colonization and impact) of three introduced biological control agents [*Laricobius nigrinus* (Fender), *Sasajiscymnus tsugae* Sasaji and McClure, and *Scymnus sinuanodulus* Yu and Yao] on populations of HWA in the southeastern United States (Tennessee).

This study was conducted at Blackberry Farm near Walland, Tennessee, near the boundary of the Great Smoky Mountains National Park. Nylon-screened cages (ca. 8.26 m tall and 6.35 m basal diameter) were constructed by Camel Manufacturing, Pioneer, TN, and erected in the field from 30 October to 19 December 2007. Fifteen HWA-infested eastern hemlock trees (ca. 8 m tall) were selected and used in this study. Twelve cages were erected in November and December 2007 over HWA-infested hemlock trees and the following treatments (three replications per treatment) were applied beginning in December 2007: a) releases of *L. nigrinus* (190 adults/caged tree), b) releases of *S. tsugae* (300 adults/caged tree), c) releases of *S. sinuanodulus* (90 adults/tree), and d) control (no releases of predatory beetles). In addition, three non-caged, HWA-infested trees also were included in the study design as control trees. Adults of *L. nigrinus* and *S. tsugae* were obtained from Lindsay Young Beneficial Insects Laboratory, University of Tennessee, and adults of *S. sinuanodulus* were obtained from the University of Georgia and the USDA Forest Service Northern Research Station, Connecticut. Cages were removed from trees in July 2009.

Following their releases, all three species of introduced beetles survived, colonized, and reproduced inside the cages, as larvae of each species were recovered in 2008. Adult *L. nigrinus* were found inside the cages in March and November 2008 (about one year after initial placement in cages), adult *S. sinuanodulus* were found in April, June, and July 2008, and adult *S. tsugae* were found in April, May, June, July, and November 2008 (about eight months after initial placement in cages). No *S. sinuanodulus* was found on foliage in the cages during the November 2008 sampling period. In 2009, prior to cage removal, adult and larval *L. nigrinus* and *S. tsugae* were recovered from trees inside the cages, but no *S. sinuanodulus* was recovered. In July 2009, HWA densities had declined about 62% on trees caged with *S. tsugae* and about 85% on trees caged with *L. nigrinus*, suggesting that both of these predatory species reduced HWA populations during this period.

In 2010, on previously caged trees, adult and larval *S. tsugae* (F3 to F5 generations) were recovered from February to November (for more information, see the related Abstract 'Seasonality of Established Populations of *Sasajiscymnus tsugae* on Eastern Hemlock in the Southern Appalachians' by Wiggins et al. in this Proceedings). In 2010, adult and larval *L. nigrinus* (F2 to F3 generations) also were recovered. Following removal of the cages, *L. nigrinus* and *S. tsugae* dispersed throughout the site (for more information, see the related Abstract 'Coexistence of Two Introduced Predatory Beetle Species of Hemlock Woolly Adelgid on Eastern Hemlock' by Hakeem et al. in this Proceedings). The lack of recovery of *S. sinuanodulus* may be due to the low numbers of adults released inside each cage and/or the low vigor of many of the released adults. These results also suggested that predation of HWA by *L. nigrinus* and *S. tsugae* benefited tree health (for more information, see the related Abstract 'Assessment of Tree Health Characteristics following Releases of Predatory Beetles on Eastern Hemlock' by Wiggins et al. in this Proceedings).

The use of whole-tree canopy enclosures provides a viable technique to assess single species (and possibly species complexes) of introduced predators against HWA on eastern hemlock. Populations of *L. nigrinus* and *S. tsugae* were successfully established using whole-tree canopy enclosures and their impact on HWA was documented. These enclosures allow the use of whole trees in natural, field situations and provide a better understanding of actual predatory expectations in the field. In addition to providing an assessment of predatory performance and survival, this technique permits the monitoring of long-term impacts of these predators on HWA and tree health. Whole-tree canopy enclosures provide a tool to acclimate introduced natural enemies to field situations and conditions, as well as a mechanism to establish natural enemies in the field. Recoveries of established populations of *S. tsugae* using standard release protocols generally occurred five to seven years after release in the Great Smoky Mountains National Park and, in some areas, have not been recovered (Grant 2008, Hakeem et al. 2010); however, introduced predators were recovered from hemlock trees after a single release in only two to three years using whole-tree canopy enclosures. Future studies will address the use of these cages to assess complexes of predatory species and to rear large numbers of natural enemies for re-release. Whole-tree canopy enclosures should enhance our knowledge of the establishment and effectiveness of introduced predators of HWA to improve their use in management efforts.

KEYWORDS

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

ACKNOWLEDGEMENT

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MANAGEMENT OF HEMLOCK WOOLLY ADELGID IN GREAT SMOKY MOUNTAINS NATIONAL PARK

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The following is an excerpt from the annual Forest Insects and Disease briefing provided to National Park Service administration by GRSM Resource Management Staff: Though it does not delve into great detail on any one item of the HWA management program it does provide vital knowledge of "on the ground" operations on a large scale attempt to control this forest pest.

Hemlock woolly adelgid (*Adelges tsugae* (Annand)) was first detected in the park in 2002 and has rapidly spread throughout all areas of the park that contain eastern hemlock (*Tsuga canadensis* (L.) (Carriere)). HWA was considered an unimportant pest on ornamental hemlocks when it was first discovered in Richmond, VA in 1952. It subsequently spread into natural forest stands throughout the Mid-Atlantic States and has become one of the region's most serious forest insect problems, with infestations extending south into Georgia and north into New England. HWA attacks several species of hemlock, but eastern hemlock is the only hemlock species found in the park. The insect is easily dispersed by birds and wind or infested horticultural material, and all ages of hemlocks from seedlings to old growth are vulnerable to ecological extinction.

HWA feeds at the base of the tree's needles, reproducing exponentially and sometimes causing tree death in as little as two years. Insecticidal control is possible in landscape settings but is difficult in natural stands. GRSM currently manages HWA with insecticides and release of biological control insects. Biological control holds the best hope for long-term control of HWA at levels that will allow hemlock trees to survive. Early results of biological control with several Asian species of beetles that feed exclusively on HWA show promise. Virgin hemlock stands in the park are particularly vulnerable since they are aging and not vigorous; these stands have been a high priority for treatment with systemic insecticides and biological controls.

Vegetation mapping shows a large hemlock resource in the park. Nearly 1500 acres of old growth hemlock have been documented with some trees in excess of 500 years old, six feet across, and 170 feet tall. The park's total hemlock resource has been mapped at more than 137,000 acres with over 14,000 acres of hemlock-dominated forest. The hemlock forests provide unique habitat for plant and animal species, including stream edge (riparian) species. Hemlock is a key component of many forest types in the park, and stands where hemlock is dominant are not uncommon. No other evergreen species is capable of filling the ecologically critical role of hemlock.

A Finding of No Significant Impact was prepared in December, 2005 in response to the public scoping process and affected agency review of a draft environmental assessment (EA) of Hemlock Woolly Adelgid Control Strategies in GRSM. The EA offered five alternatives: No Treatment, No Action (no change from current level of treatment), Chemical Control Only, Biological Control Only, and Both Chemical and Biological Control. The option of "Both Chemical and Biological Control" was chosen as the "Preferred Alternative." The Hemlock Program in the Park continues to see wide support from the public with over $30,000 dollars raised in t-shirt sales alone to date.

Work to preserve eastern hemlock trees and forests in FY 2010 progressed successfully despite increased tree health decline and obvious mortality of trees throughout the park. Areas of particular focus have been in the Park's eastern areas near Cosby, Big Creek and Cataloochee. Some untreated higher elevation stands over 4,500 ft. continue to survive, perhaps because of colder winter temperatures and/or rime ice. All front country areas received an annual foliar application of insecticidal soap or oil totaling 600 acres. All trees in the front country campgrounds and heavily visited areas were retreated with systemic insecticides if needed. These were initially treated five years ago when the trees were, for the most part, very healthy and HWA populations were low. Service contracts completed in 2009-2010 used a new systemic treatment (Safari/dinotefuran) that has shown rapid results in controlling HWA on some of the largest old growth trees in poor health.

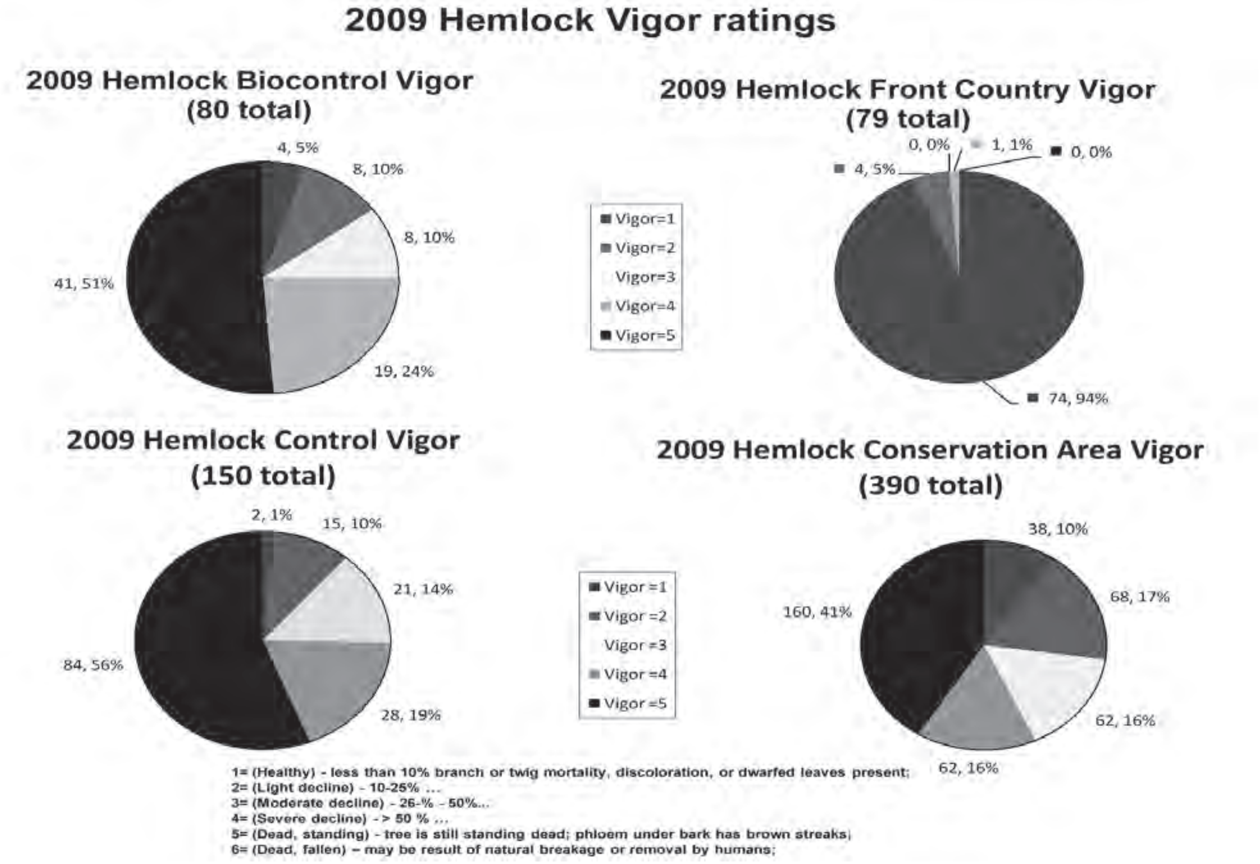
Work in the Conservation Areas (CAs) consisted of applying a second treatment to the very large trees (=>25") based on research, establishing new CAs, and expanding existing areas. Of the 81 existing conservation areas, 50 have received a second treatment. Of the over 132,000 trees systemically treated since the project started over 20,000 of these have been treated in FY 2010 to date. With the returning rains of 2009 many treated trees have shown a positive response to past treatments and untreated trees will presumably respond as well if treated within the next two years.

Some of the new CAs of interest are located:

- Adjacent to the Gabes Mtn Trail in Cosby, TN, adding 56 acres
- Adjacent to State Route 284 in Cataloochee, NC, adding 68 acres
- Adjacent to the Enloe Creek trail in NC, adding 249 acres
- To date, 12 new CAs have been created in FY 2010, totaling 700 acres.

In addition to chemical controls, Park resource managers have been releasing biological control insects for HWA control since 2002. *Sasajiscymnus tsugae* (Sasaji and McLure), or St, is a beetle native to Japan. *Laricobius nigrinus* (Fender), or Ln, is native to the Pacific Northwest. *Scymnus sinuanodulus* (Yu and Yao), or *Ss,* is native to China and has seen limited release for HWA control in the Park. There have been 56 releases of the St predatory beetle totaling 56,669, five Ln releases totaling 1134 beetles, and one Ss release totaling 55 beetles. Although biological controls are expected to take ten years or more to demonstrate effectiveness, positive recovery of beetles has been made in multiple Conservation Areas this year from releases as far back as 2002, though overall success has yet to be determined and will play out over the coming years. Since 2002 the park has released over 500,000 predator beetles as part of the overall control effort.

In 2005 nearly 100 monitoring plots were installed throughout the park to determine effectiveness of treatments against control plots receiving no treatment. Data collected from monitoring consists of Crown Health based on U.S. Forest Service FIA Crown Health Analysis. Monitoring has been showing the positive effects of the treatments compared to control areas with no treatments (Figure 1). Review of the plots is scheduled for on a yearly basis.



1= (Healthy) - less than 10% branch or twig mortality, discoloration, or dwarfed leaves present;
2= (Light decline) - 10-25% ...
3= (Moderate decline) - 26-% - 50%...
4= (Severe decline) -> 50 % ...
5= (Dead, standing) - tree is still standing dead; phloem under bark has brown streaks|
6= (Dead, fallen) – may be result of natural breakage or removal by humans;

Figure 1. Monitoring efficacy based on Vigor Class.

CURRENT RESEARCH AND MANAGEMENT FOR FY2011

Sampling of hemlock canopy needles to determine effective length of systemic treatment was the focus of a recent pilot project in the Fall of 09'. In this study, trees that were treated up to seven years ago with a lower label dose rate of the active ingredient imidacloprid were found to be adelgid free with effective control levels of imidacloprid in the lower canopy, and its metabolite olefin (an effective pesticide) at levels in the upper canopy sufficient to control adelgid. This initial study has developed into the creation of a CESU Agreements Research between the Park and The University of Tennessee that will investigate in depth the minimum systemic treatment needed for the maximum control period. This study will give the park and their neighbors a more precise understanding of proper long term management of the tens of thousands of trees that have been successfully.

Management goals for FY2011 will incorporate innovative application techniques with systemic controls using more stem injection and basal application treatments. The focus of the biocontrol program will be to augment past release sites and/or release larger numbers at one site initially. The technique of caging beetles until they establish on certain trees in one area will also be attempted to maximize biocontrol effect.

The park continues to share information and experience acquired during the program with neighbors such as state park managers, private landowners and national forests in a cooperative effort to preserve this unique tree. Even though great loss of hemlock in the forest continues, the HWA program in Great Smoky Mountains National Park will preserve many valuable areas for decades to come.

ACKNOWLEDGMENTS

GRSM has been fortunate to draw on a dedicated funding source for HWA control. The following are some of the other sources that have made this program possible:

US Forest Service, Forest Health Protection

Friends of the Smokies

NRPP-National Resource Preservation Program

The many visitors and friends to the Park who continue to ask what they can do to save this beautiful tree.

HEMLOCK WOOLLY ADELGID AND HEMLOCK ECOSYSTEMS AT DELAWARE WATER GAP NATIONAL RECREATION AREA

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ABSTRACT

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

HWA infestations reached peak "outbreak" levels in 2007 and 2008, but were very low in 2010. The HWA infestation level one year strongly affects the amount of new growth produced the following year: on average, every 10% increase in HWA infestation level results in a 7.3% decline in the amount of new growth produced the following year. Hemlock mortality is 30% to 40% in many stands in the park, but ranges from 15% to 42%. Despite the fact that 93% of surviving park hemlock trees were in moderate to severe decline in 2010, they still managed to produce abundant new growth in response to low HWA levels and good growing conditions (relatively cool and moist).

A series of recently published studies conducted at DEWA revealed significant interactions among hemlock decline, deer herbivory levels, and exotic plant invasions that have important implications for park management. Taken together, the studies indicate that failure to address the "triple threat" of hemlock decline, deer herbivory and invasive plants in an integrated manner could result not only in the loss of hemlock forests, but forests full of exotic plants. The park has continued and expanded its efforts with biological control of HWA, chemical suppression of HWA, suppression of invasive plants in declining hemlock stands, and excluding deer from selected areas. While all these efforts help to slow and manage the impacts of hemlock decline, they have not been able to prevent them.

KEYWORDS

hemlock, adelgid, invasive, deer, 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 more than 5% of the forested area of the park, and occur on spatially patchy, isolated ravines, steep slopes, or 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.

HWA 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.

MONITORING METHODS

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 plot trees have been assessed annually using the "Visual Crown Rating Methods" developed and used by the US 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 was 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 trees sampled in each stand.

In 2009 monitoring HWA infestation levels and amount of new twig growth at the permanent plots was discontinued because there were no longer enough hemlock trees with enough branches within reach to sample. In 2010 we instead randomly sampled 40 to 140 trees in the 14 hemlock stands that had previously been selected for landscape-scale ecological studies (Young et al. 2002). Sampling these stands enabled us to provide park-wide estimates of HWA infestation levels, hemlock new growth, and hemlock crown conditions ("vigor").

RESULTS

HWA POPULATIONS AND HEMLOCK TREE NEW GROWTH

Figure 1 shows annual average HWA infestation levels and amount of new twig growth at four hemlock stands from 1995 to 2008. HWA infestations reached peak high levels in 1999 at three of the stands, but remained low at Adams Creek until a first peak in 2002. HWA infestations reached peak high levels at all four stands in 2007 and 2008. The very high HWA infestation levels at these sites in 2007 and 2008 indicates that the biological controls released in previous years (see below and Evans and Shreiner 2008) had not been effective as of 2008.

Prediction of hemlock new growth based on HWA infestation level: As demonstrated in Figure 2, HWA infestations strongly reduce the growth potential of hemlock trees. Without any HWA infestation, new growth within a stand ranged from 60% to 100%, and was typically 70% to75%. As HWA infestations increase, hemlock trees generally produce less new growth the following year. The equation describing this relationship is:

$$\text{Predicted New Growth (in year "Y+1")} = 72 - 0.73(\text{HWA infestation level in year "Y"})$$

Every 10% increase in HWA infestation level reduces hemlock tree new growth by 7.3% the following year. While the trend of the effect of HWA on new growth is strong and clear, considerable variability exists so the predictive ability is limited for individual hemlock stands.

Hemlock crown conditions: In 2010, 59% of surviving hemlock trees were either in "severe decline" or "substantial decline" and an additional 38% were in "moderate decline" (Figure 3). Only 3% remained in "light decline" and virtually no "healthy" trees remained.

HWA infestation levels in 2010 were generally very low park-wide and hemlock trees produced abundant new growth (Figure 4). Despite the fact that 97% of surviving hemlock trees in 2010 were in moderate to severe decline, they were still able to produce high levels of new growth; generally from 60% to over 70%.

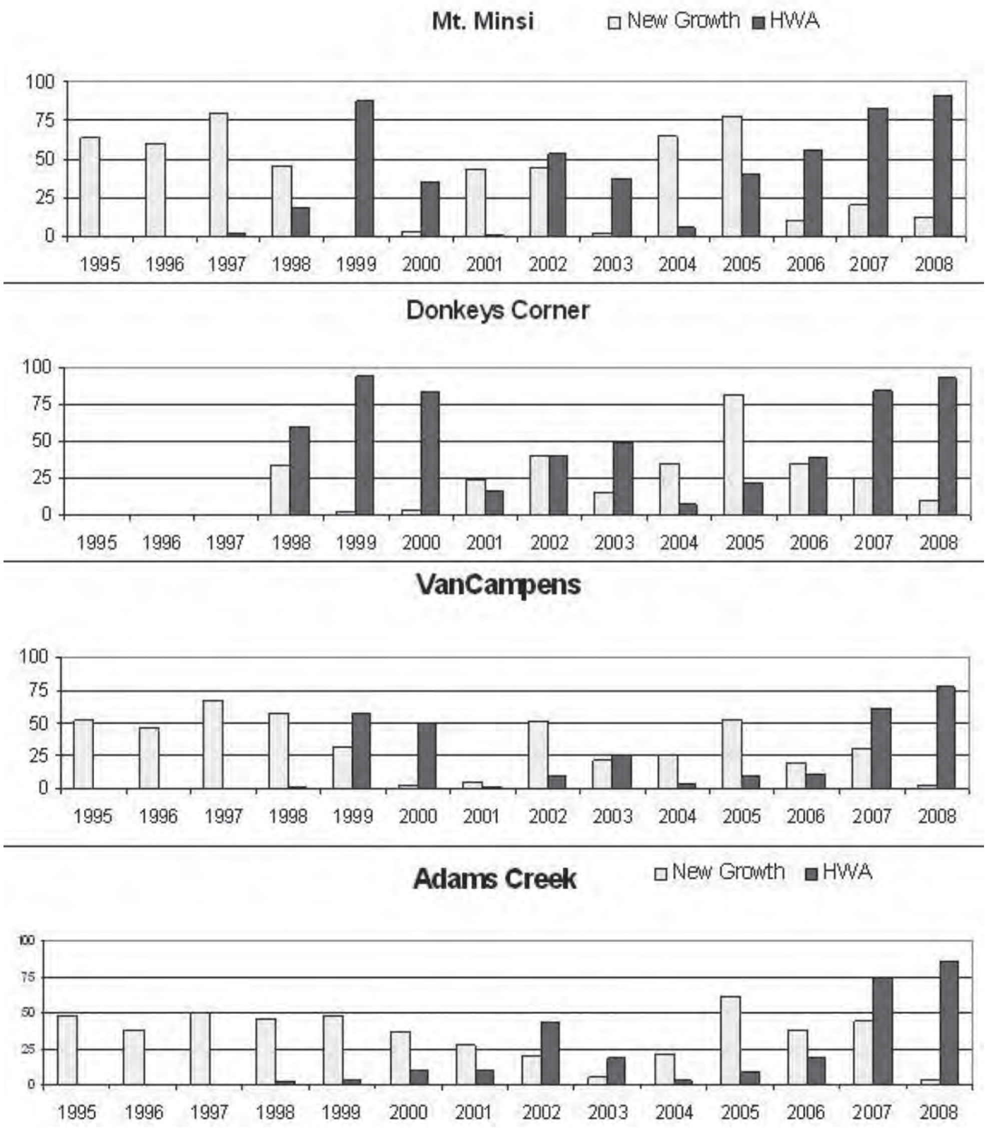

Figure 1. Annual average hemlock new growth (percent of twigs producing new growth; light bars) and HWA infestation levels (percent of twigs infested with HWA; dark bars) at each of four monitoring sites from 1995 through 2008. Data were not collected at Donkeys Corner before 1998.

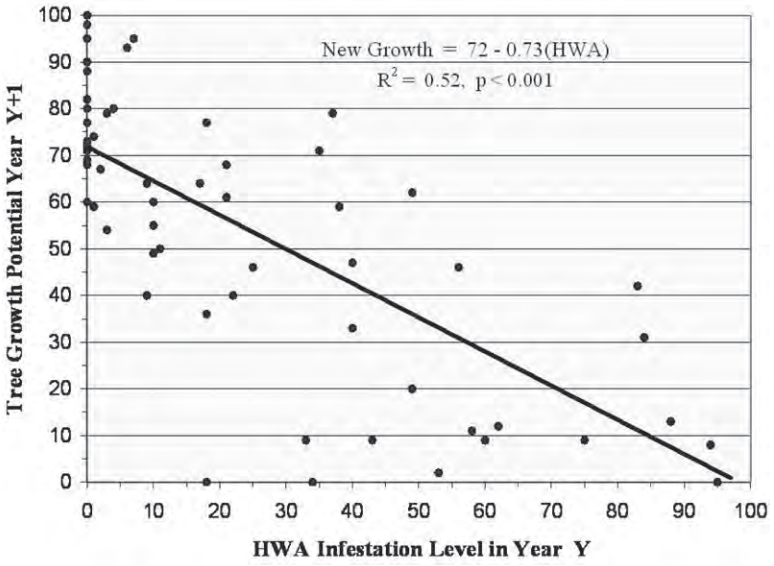

Figure 2. HWA infestations reduce the growth potential of hemlock trees. The higher the HWA infestation, the less growth of hemlock trees the following year; every 10% increase in HWA infestation level reduces hemlock tree new growth by 7.3% the following year. Each data point represents one hemlock stand in one year; data from six hemlock stands from 1995 – 2008 (N=61). (Hemlock growth potential was determined by the 90th percentile of hemlock tree growth in a stand; that is, only 10% of the hemlock trees in a stand produced more than this amount of new growth.)

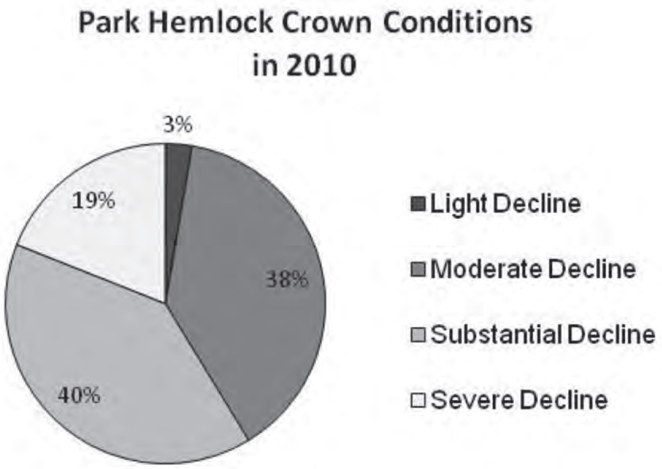

Figure 3. Park hemlock crown conditions ("vigor" ratings) in 2010 as indicated by samples from the 14 representative hemlock stands selected by Young et al. (2002).

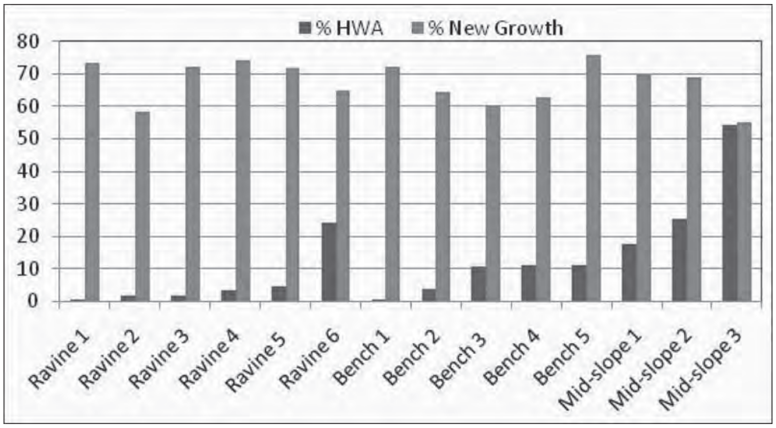

Figure 4. Average hemlock new growth (lighter bars) and HWA infestation levels (darker bars) in 2010 at 14 representative hemlock stands (Young et al. 2002).

Hemlock mortality rates at DEWA: Figure 5 indicates that hemlock tree mortality rates can differ considerably in different stands. At VanCampens the annual hemlock mortality rate is about 0.7%/year, while at Adams it is about 2.8%/year (Figure 5). The higher mortality rate at Adams is puzzling, since VanCampens has been infested with as high or higher levels of HWA for longer than Adams has (Figure 1).

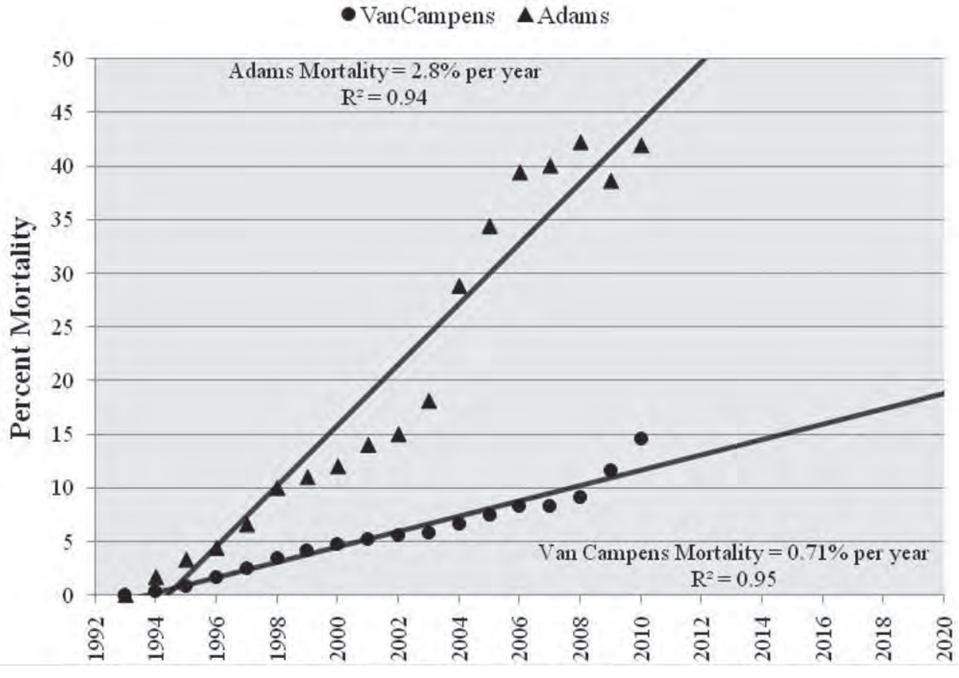

Figure 5. Hemlock mortality in permanent plots at Adams Creek and VanCampens Brook from 2003 to 2010. While mortality averages 2.8%/year at Adams, it is only 0.71%/year at VanCampens. The apparent drop in total mortality from 2006, 2007, and 2008 to 2009 is due to sub-sampling error in 2006, 2007, and 2008; in 2009 and 2010, all trees in all plots were sampled.

HEMLOCK DECLINE, DEER, AND INVASIVE PLANTS

Many parks in the eastern United States are experiencing combinations of hemlock decline, high deer population densities, and exotic plant invasions. A series of recently published studies conducted at DEWA has revealed significant interactions among these factors that have important implications for park management. Taken together, these studies indicate that failure to address the "triple threat" of hemlock decline, deer herbivory and invasive plants in an integrated manner could result in the unintended and undesirable consequence of forests full of exotic plants.

METHODS

Researchers Anne Eschtruth and John Battles of the University of California – Berkeley studied ten of the 14 hemlock stands at DEWA selected by Young et al. (2002) for four years, from 2003 to 2006. All of these stands had been infested with HWA prior to 2003 and exhibited various levels of hemlock decline. In each hemlock stand Dr. Eschtruth established 18 vegetation plots, 20 deer pellet plots (as an index of deer density within each stand), and 40 deer exclosure plots (fenced) with paired control plots (unfenced). She measured the amount of sunlight that could pass through the forest canopy as well as the types and abundance of plants in each of the 400 pairs of deer exclosure and control plots in 2003 and again in 2006. The researchers also estimated seed availability (propagule pressure) of three species of exotic plants at each of the plots: garlic mustard (*Alliaria petiolata*), Japanese stiltgrass *Microstegium vimineum*), and Japanese barberry (*Berberis thunbergii*).

RESULTS

Deer herbivory alters forest response to hemlock decline (Eschtruth and Battles 2008). Deer herbivory had a relatively severe impact on black gum and eastern hemlock saplings even at relatively low deer densities. Black gum and hemlock were more severely impacted by deer herbivory than the other tree species. The negative effects of deer herbivory on red maple, sugar maple, black birch, and chestnut (rock) oak saplings increased exponentially – not linearly – with increasing deer density (Figure 6). Hemlock decline (increasing light availability) magnified the negative impact of deer herbivory on a number of native tree species (Figure 6). The researchers concluded that "deer herbivory may significantly alter forest successional trajectories" following hemlock decline, and that "deer densities should be lower in declining hemlock stands than in healthy stands to maintain the same level of herbivory impact."

Deer also had significant, exponentially positive effects on the abundance of the exotic plants studied (Eschtruth and Battles 2009a). Garlic mustard benefited most positively from deer, stiltgrass benefited least. The researchers concluded that "deer can accelerate the invasion of exotic plants, and hemlock decline interacts with herbivory to magnify the effect."

Overall, exotic plant invasion increased exponentially with increasing seed availability, hemlock decline, and deer herbivory (Eschtruth and Battles 2009b). Hemlock decline magnified invasion at a given seed availability. For example, garlic mustard invasion was much greater with a combination of high propagule pressure and hemlock decline (high light levels) than with either of those factors alone (Figure 7). Healthy hemlock dominated forests (with low light levels) are resistant to invasion by Japanese barberry.

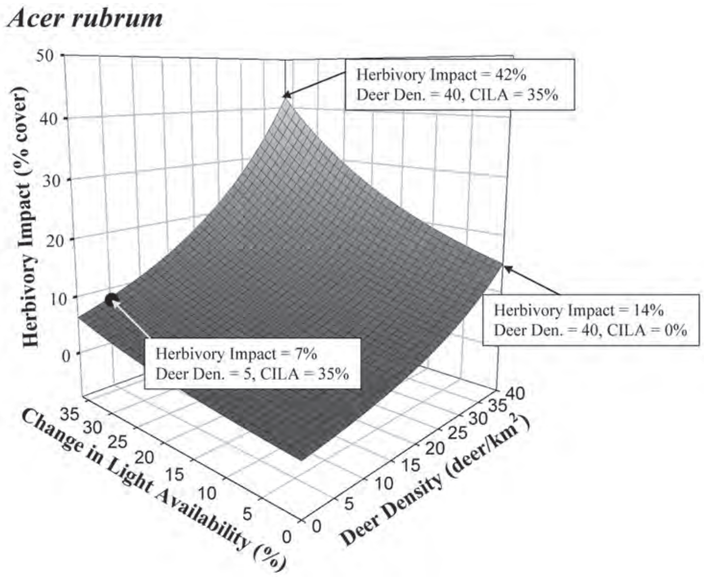

Figure 6. Effects of deer density and change in light availability (CILA) on herbivory impact on red maple. Herbivory impact increases much more dramatically when change in light and deer density both increase than when either increases alone. Figure modified from Eschtruth and Battles (2008).

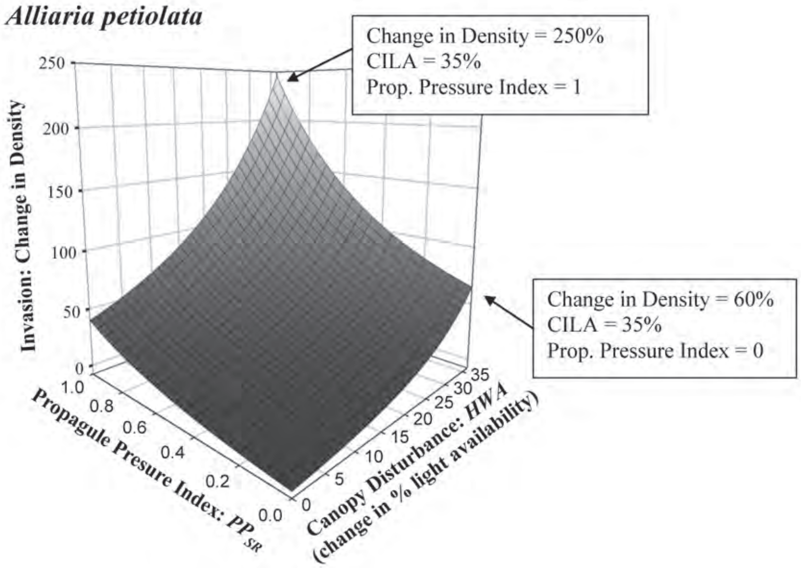

Figure 7. Positive effects of deer density, hemlock decline (change in % light available; CILA), and propagule pressure (seed availability) on the invasion of garlic mustard. Garlic mustard invasion was much greater with a combination of high propagule pressure and hemlock decline (high light levels) than with either of those factors alone. Figure modified from Eschtruth and Battles (2009b).

MANAGEMENT EFFORTS

The park has continued and expanded its efforts with biological control of HWA, chemical suppression of HWA, suppression of invasive plants in declining hemlock stands, and the reforestation demonstration project described by Evans and Shreiner (2008). To date approximately 10,000 hemlock trees in the park have been treated with imidacloprid by soil injection, CoreTect tablets, or stem injections. Many (~2,000) of these trees required stem injections because they were immediately adjacent to streams and surface waters. As of September, 2010, approximately 4,150 *Laricobius nigrinus* beetles have been released in the park. While all these efforts help to slow and manage the impacts of hemlock decline, they have not been able to prevent them.

ACKNOWLEDGEMENTS

The efforts and support of many agencies, universities, and individuals have contributed greatly to the HWA-Hemlock Program at DEWA. I especially thank Brad Onken and Karen Felton of the USDA Forest Service, whose support in the form of funding, labor, and technical assistance, has been critical to this program. I thank Mark Mayer for releasing biological controls in the park and monitoring the release sites for them.

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THE NATIONAL FORESTS IN NORTH CAROLINA: HEMLOCK WOOLLY ADELGID (HWA) MANAGEMENT UPDATE; HAVE WE ACCOMPLISHED OUR GOALS?

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ABSTRACT

The National Forests in North Carolina (NFsNC) began treating eastern and Carolina hemlock in 2004/2005 with insecticide and biological treatments. The treatment areas were identified within the NFsNC hemlock conservation area design. The goal of this design was to identify and protect critical areas of hemlock for ecosystem function and to serve as a genetic reserve of hemlock on the forest. There are currently 71 active eastern hemlock conservation areas and 32 active Carolina hemlock conservation areas, with treatment areas ranging in size from approximately 1 to 10 acres in each conservation area. These treatment areas reflect a valuable stand against the HWA, but additional conservation opportunities remain in selected areas. Locating these areas of remaining viable hemlock has been a large obstacle, but has been abated through cooperative efforts with several local conservation groups. The NFsNC has resolved to take advantage of these remaining opportunities by increasing treatments before it is too late. The increased treatments will primarily utilize insecticides before a future transition to biological treatments. The recent completion of a new environmental analysis has provided additional treatment tools (insecticides in all treatment areas, Safari™, additional biological controls) as well as allowing increased treatments across the Nantahala and Pisgah National Forests, both in the number of sites and the size of sites. The current goal is to increase the treatment of viable and ecologically important hemlock across the Nantahala and Pisgah National Forests before this fleeting opportunity has past.

RESPONDING TO HEMLOCK WOOLLY ADELGID IN MAINE

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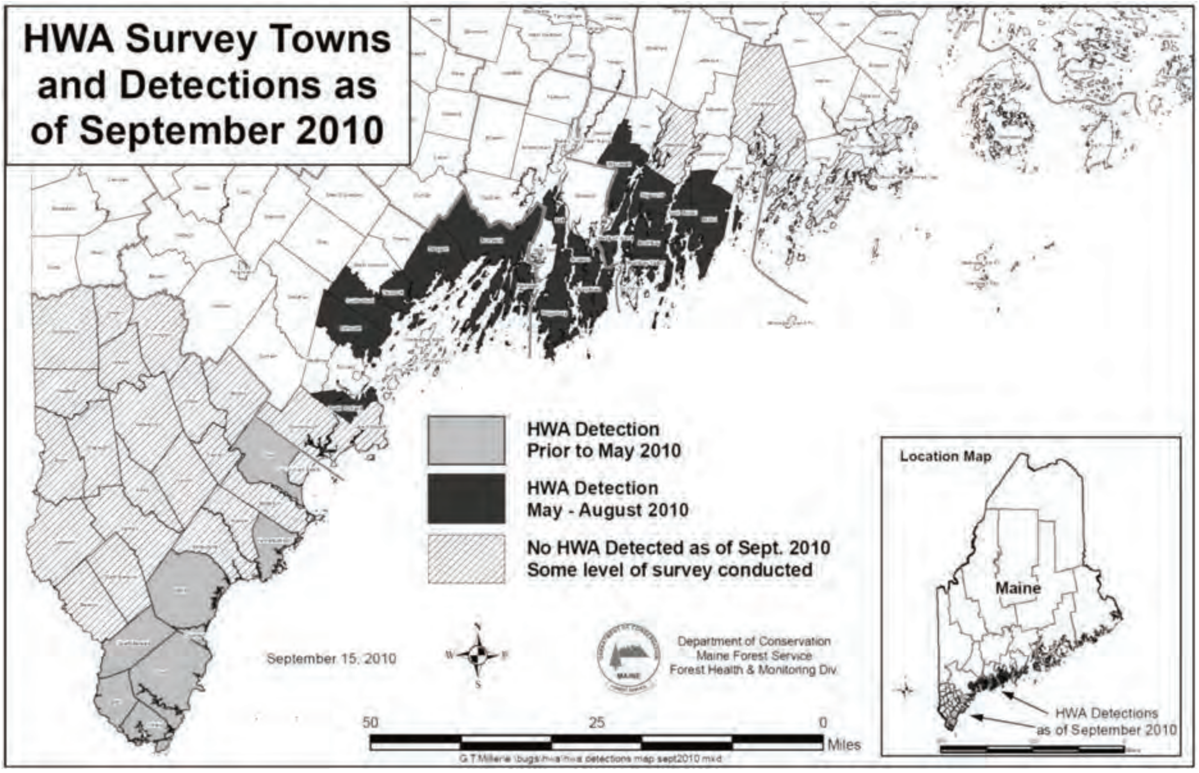
THE SITUATION

The understanding of the hemlock woolly adelgid (HWA) situation in Maine has dramatically changed in the last several months. In April 2010, eight towns were known to have HWA infestations in native hemlock trees. In September 2010, with surveys still underway, 26 towns are known to be infested (see map). Although management principles and tools remain largely the same, they will need to be implemented differently over this larger landscape of infested forest and ornamental trees.



In addition to more locations with known HWA, Maine hemlocks are at risk from elongate hemlock scale (EHS). To date, EHS has not been detected in forest hemlocks. However, two introductions on nursery stock have been found and treated. In both cases the plants had been in the landscape for almost ten years. In one instance the scale had spread to native hemlock and fir surrounding the planting. Elongate hemlock scale has been found in neighboring New Hampshire and detection of natural spread to Maine forests is most likely imminent. In this region, balsam fir provides an additional, favored host for the pest.

Complicating the management of hemlock health in the face of two exotic insect threats is the presence of a fungal shoot blight. Managers first noticed dying tips on eastern hemlock in Maine in 2006. Fruiting bodies proved elusive, but were collected in late summer of 2009. The fungus was identified as *Sirococcus tsugae* in December 2009 by USDA APHIS Pathologist Dr. Joseph F. Bischoff . Field reports indicate it is widespread in the state and some pockets of severe understory hemlock decline have been found associated with the tip blight.

In 2009, the Maine Forest Service (MFS) Forest Health and Monitoring Division and our counterparts in New Hampshire and Vermont were awarded a US Forest Service redesign grant for management of HWA. A strong partnership between the organizations existed prior to the grant, but the strengthened partnership and increased communication and collaboration resulting from this grant has been a valuable outcome. This is especially true with newer staff in the face of an expanded threat.

MANAGEMENT APPROACH

The MFS and Maine Department of Agriculture manage 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 Divisions of Plant Industry (Department of Agriculture) and Forest Health and Monitoring (Department of Conservation, Forest Service). The divisions' management tools include biological, chemical and physical control, public outreach, regulation, research, and survey and monitoring.

Maine was proactive in establishing an external quarantine on potentially infested hemlock materials. The quarantine first went into effect in 1988, and has been revised several times since. 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. Quarantine boundaries will need to go through a formal Rule-Making process in order to reflect the current known extent of HWA within Maine. Maine's quarantines work towards the principles of exclusion and containment.

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 quarantine, displays at garden shows, posters for trailheads and other public places, a series of Web-pages dedicated to HWA, a fact sheet and wallet card, door-to-door campaigns associated with other management activities, public service announcements, press releases and articles for newsletters. The MFS pioneered a "Take A Stand" volunteer initiative in which citizen scientists are trained to survey for HWA in adopted hemlock stands. To date, Take a Stand volunteers have detected nine infestations of HWA. MFS has also included HWA in the target pests for the Forest Pest Outreach and Survey Program volunteers trained with the Maine Department of Agriculture. The volunteers are important partners in spreading the word about HWA.

The MFS employs several control tactics including biological, chemical and physical control. Two species of predator beetles have been released in southern York County. As of May 2010, 32,700 *Sasajiscymnus tsugae* and 5270 *Laricobius nigrinus* had been released across 17 sites. In 2010 a grant of $21,450 from USDA Animal Plant Health Inspection Service, Plant Protection and Quarantine allowed MFS to purchase 9000 *S. tsugae* from a private company. The last of the beetle shipments coincided with the confirmation of HWA in Maine's Midcoast region allowing a release at the leading edge of the known HWA distribution. *S. tsugae* has been recovered at several sites, and *L. nigrinus* at one release site. Chemical control is targeted to sites where there is a high risk of human-adelgid interaction and high potential for artificial spread. Physical control, including tree removal and pruning is used in outlying areas 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 HWA help define the management problem. Detection surveys historically have been 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. In response to the detection of HWA in the midcoast of Maine, an intensive survey of hemlocks in coastal towns was initiated; this survey is on-going. The geographic area covered by the current known extent of HWA in Maine will necessitate a change in the intensity of survey conducted in infested counties. To keep with guidelines developed in the USFS redesign project region, this will include at a minimum a survey of all towns bordering known infested areas. Survey and monitoring help define the problem, assess management effectiveness and guide future actions.

The MFS cooperates in research activities as resources allow. Partners in research efforts have included the US Forest Service, the Connecticut Agricultural Experiment Station and the University of Maine. Through research and support of research we hope to better understand the problem and inform management decisions as well as develop new tools for managers facing the reality of HWA in Maine.

IMPACT OF FERTILIZER AND IMIDACLOPRID ON *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE)

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ABSTRACT

Healthy hemlock trees, *Tsuga canadensis* (L.) Carrière, and adelgid, *Adelges tsugae* Annand (Adelgidae), populations should favor retention and population growth of adelgid predators like *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji and McClure) (Coccinellidae). Eastern hemlock trees between 15.2 and 38.1 cm diameter at breast height (dbh) were treated with 0, 10 or 25% of 1.5 g imidacloprid (Merit 75WP) per 2.5 cm dbh [untreated check (UTC), 0.1× and 0.25× dosages, respectively] and were either fertilized or not, in a 3 × 2 factorial design. After two years, more ovisacs and eggs were found on trees receiving imidacloprid in the order UTC > 0.1× > 0.25× dosage. Fertilized trees had greater adelgid fecundity, which was positively correlated with total foliar N in both winter generations. In February 2009 (27 months after imidacloprid treatment), higher imidacloprid dosages to unfertilized trees resulted in reduced adelgid fecundity. Tree growth parameters such as numbers of new shoots or needles and length of new shoots per unit length of branch exhibited a dose response (0.25× > 0.1× > UTC) to insecticide in June 2009 samples. Concentrations of N, P and K were higher in the foliage of trees treated with insecticide while foliar aluminum concentrations were consistently lower in trees with higher insecticide dosages. Trees treated with low rates of imidacloprid were healthier than untreated trees, but only trees treated with the 0.1× dosage had sufficient adelgids to possibly sustain predators over extended periods of time.

A COMBINED TREATMENT STRATEGY USING IMIDACLOPRID TO FACILITATE HEMLOCK RECOVERY IN HEMLOCK WOOLLY ADELGID INFESTED TREES

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ABSTRACT

Due to the widespread establishment of hemlock woolly adelgid [HWA], hemlock may be infested for years prior to treatment. Tree symptoms of prolonged infestation include extensive dieback and thin canopies. Imidacloprid, a systemic neo-nicotinoid insecticide, labeled for use against HWA in forests and woodlands was selected for study. Mature, large diameter trees in poor condition were treated to determine whether tree recoveries were possible. Trees were treated once, via trunk and/or soil injection in Asheville, NC, USA. After the application, changes in tree growth, HWA numbers and imidacloprid residues were monitored. The results of this study suggest that, low-rate tree injection alone or combined with soil application provided a high level of protection and residual activity. The results from imidacloprid residues (LC_{50} HWA > 0.120 μg/g) suggest woodland hemlocks may be protected using these methods for 3-5 years before retreatment becomes necessary.

KEYWORDS

Imidacloprid, Tree injection, Soil Injection, Residues, Tree Recovery

INTRODUCTION

Eastern hemlock (*Tsuga canadensis* L. Carriere) covers a wide range of the United States, from New England, New York, Pennsylvania and the Mid-Atlantic States, expanding westward from central New Jersey to the Appalachian Mountains and continuing south into northern Georgia and Alabama. In the Midwest, hemlock occurs predominantly in eastern Ohio, northern Michigan and Wisconsin (USFS. Silvics Manual. Vol. 1. *Tsuga canadensis*). First identified in the early 1950s in Virginia, USA Hemlock woolly adelgid (*Adelges tsugae* Annand) (Homoptera: Adelgidae) is an exotic insect that infests hemlock trees. Symptoms of HWA infestation include canopy thinning, twig dieback, tree decline and ultimately death. HWA has spread and now infests approximately half of the Eastern native range of hemlock.

Low temperatures may play a role in HWA mortality, thereby limiting its northward spread. Although the cold temperature sensitivity of HWA varies seasonally, substantial mortality occurs at temperatures of -25 to -30 °C (-13 to -22 °F) (Costa et al., 2004). Such low temperature extremes may occur infrequently, or not at all, in the southern extent of the hemlock range. At least one study suggests that hemlock decline is more rapid in southern Appalachia compared to northern areas (Nuckolls et al., 2008).

HWA has piercing-sucking mouthparts and feeds within the xylem parenchyma cells (McClure, 1987) on twigs at the needle base. The xylem parenchyma is living symplast that is rich in carbohydrates and other essential nutrients; as adelgid feed on these food resources, less is available to the tree for life functions (e.g., growth, metabolism, reproduction, storage and defense) (Shigo, 1989). As infestation progresses, twig lengths decrease, followed by loss of tip growth altogether. McClure suggested an inverse relationship between HWA population and hemlock growth (1991). This growth loss occurs with increasing HWA pressure and may take at least three to four years. Ward (1991) suggested an HWA threshold of 25 – 30 HWA/100 needles as negatively impacting twig growth. The threshold used here is 2.0 – 4.0 HWA/cm twig growth (Doccola et al., 2007).

This study is focused on imidacloprid treatment efficacy in the southern part of the hemlock range, where the negative impact of HWA has been devastating. Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine), a neo-nicotinoid insecticide, is labeled for use in managing Hemlock woolly adelgid. Imidacloprid is available in water soluble packets [WSP], in soluble tablets or in liquid [SL] formulations, and may be applied to the foliage, soil or injected directly into the tree. The latter two methods rely on systemic movement of insecticide from the point of application upward into the canopy. Soil and tree-injected formulations are efficiently applied to trees in forested and woodland settings. For example, the Kioritz® (Kioritz Corporation, 7-2, Suehirocho 1 –Chome, Ohme, Tokyo, 198 Japan) soil injector, and the Arborjet QUIK-jet (Arborjet, Inc., Woburn, MA, USA) tree injection equipments are lightweight, portable and simple to use. While only one application method is generally used, there may be advantages to combining soil and tree injection as a strategy for short-term therapeutic and sustained tree protection.

The feeding habits of adelgid yield strong implications toward systemic applications. For systemic activity, the insecticide must move upward in the vascular tissues, thereby concentrated at the growing points at which insects feed. Soil-applied imidacloprid must be in solution to be absorbed by tree roots before systemic activity can occur. Trunk-injected imidacloprid is directly introduced into the sapwood, and must remain in solution (that is to say, not precipitate upon injection) for movement upward into the canopy.

Methods of soil application include basal drenches, soil injection or the use of soluble tablets. The availability of soil-applied imidacloprid is dependent upon soil moisture, which may be inconsistent in woodland environments. A possible disadvantage to these techniques is the inability to act quickly. The imidacloprid applied to soil is only slowly solubilized by soil moisture, especially in soils with high organic content or high Cationic Exchange Capacity [CEC] to which imidacloprid binds (EXTOXNET-PIP/Imidacloprid). Advantages to soil-applied treatments include residual activity over several years (Cowles et al., 2006) and simple, quick application methods. Disadvantages of tree injection include that a drill hole needs to be made into the tree and the higher volume applications take some time to apply in hemlock trees. Advantages to tree injection are less active ingredient (10 – 20% compared to soil application) is used, the formulation is introduced directly into the vascular tissue (thus acting more quickly) and a lower risk of environmental exposure (critical near streams and waterways).

Forty-eight Eastern hemlock trees were selected for treatment: large diameter, mature trees located on the Biltmore Estate in Asheville, NC, USA (latitude 35.567N, longitude -82.5448W). The trees were continuing to decline from their already poor condition, with thin, sparse foliage and twig dieback. Tree recovery was dependent upon immediate and sustained adelgid control, based on the report of Webb et al (2003) that the recovery and new growth of hemlock in poor condition occurred following imidacloprid treatment, although at a slow rate.

These woodland trees were dependent upon natural rain events alone for moisture, which could be highly variable. This was of particular concern, because shoot growth, which is critical to tree recovery (Onken, B.P., unpublished), and root uptake of soil-applied imidacloprid are dependent upon adequate soil moisture.

The objective of this study was to evaluate tree responses to tree injection of imidacloprid with and without soil-applied imidacloprid, as compared to soil-applied imidacloprid alone. To evaluate the effectiveness of the techniques, bioassays were conducted of HWA populations and twig growth, as well as extracted imidacloprid from hemlock needles over a period of two years.

METHODS

Forty-eight hemlocks were treated with the insecticide formulations containing the active ingredient (a.i.) imidacloprid for HWA infestations. Six treatments were assigned in randomized blocks, each of which was replicated 8 times. The trees all showed an approximately 25% live crown ratio, and ranged from 26.5 to 86.8 cm (10.6 to 34.7”) DBH with a mean DBH of 49 cm (19.6”). The treatments were untreated controls [UTC], IMA-jet (Arborjet, Inc.) low dose, IMA-jet high dose, IMA-jet low + MERIT 75 WSP (Bayer Environmental Science) soil injection, IMA-jet high + MERIT soil, and MERIT soil alone. Soil applications were applied using the Kioritz® soil injector; applications were made 30-90 cm (12-36”) away from the tree bole, to target the highest concentration of fibrous roots. Each MERIT 75 WSP 45.4 g (1.6 oz) packet was mixed in 960 ml (32 oz) of water, as per label instructions. Prepared in this manner, the mixture supplied 34.1 g (1.2 oz) of a.i., sufficient to treat a 58.8 cm (23.5”) DBH tree at the 1.45 g a.i. /2.5 cm (inch) DBH rate. The Arborjet Tree I.V. and the QUIK-jet injector were used to make the tree injections. Low doses of imidacloprid were applied at 0.15 g a.i. /2.5 cm (inch) DBH, high doses were applied at 0.30 g a.i. /2.5 cm DBH, and soil injections were made at 1.45 g a.i. /2.5 cm DBH. Doses using the IMA-jet formulation were applied at rates lower than the current label dosage of 0.4 g a.i. /2.5 cm DBH. Tree injections of imidacloprid were applied as formulated (neat). The configuration of the injection site was standardized using a 9 mm (0.375”) Brad point bit, drilled 3.75 cm (1.5”) into the sapwood to create a 2.5 cm^3 capacity site to mitigate for slow tracheary uptake. The number of application sites into the sapwood varied depending upon the method used. The Tree I.V. is equipped with 4 injector tips, and sites were located every 20 cm (8”) of stem circumference. The Tree I.V.

delivered the higher volume dosages into trees. The QUIK-jet applied the lower volume dosages and sites were located every 15 cm (6”) of stem circumference. Treatments were applied on August 29-30, 2007 at the Biltmore Estate. The imidacloprid treatments were timed to anticipate the resumption of adelgid activity following summer aestivation. The amount of time for each treatment to be applied was recorded.

Branch samples were taken from trees in November 2007, 2008 and 2009 (at ~ 70, 435 and 800 days after treatment) for bioassay and imidacloprid residues (Table 1). Four branches were cut, each between 40 and 60 cm (16-24”) in length, from the mid-tree canopy in four sectors by aerial lift truck, and shipped to Arborjet, Inc. for bioassay evaluation. Each branch was cut into shorter branchlets generating 16, 40 and 80 samples (in 2007, 2008 and 2009, resp.) per replicate. Samples were held at 4.5 °C (40 °F) for the extent of the bioassay assessments; samples for imidacloprid residues were placed into labeled, plastic bags, sealed and placed in a freezer at -18 °C (0 °F) until imidacloprid residues were run.

Table 1. Treatments, imidacloprid residues in µg/g (ppm), percent tip growth and HWA infestation per 100 needles and per centimeter of branch.

Year	Treatment	µg/g IMI*	% Growth	HWA/100	HWA/cm
2007	Untreated Controls	0.01	7.00	4.10b	0.28b
	IMA-jet Lo	0.20a	6.07b	2.51b	0.17b
	IMA-jet Hi	0.17a	3.41b	0.00	0.00
	IMA Lo + MERIT	0.22a	13.04b	3.09b	0.21b
	IMA Hi + MERIT soil	0.86a	7.74b	0	0
	MERIT soil	0.14a	1.62b	1.57b	0.11b
2008	Untreated Controls	0.02	4.20b	31.50b	2.57b
	IMA-jet Lo	1.75	12.54b	33.10b	2.70b
	IMA-jet Hi	3.79	19.60b	6.20a	0.51a
	IMA Lo + MERIT soil	1.32	30.58a	15.10b	1.23b
	IMA Hi + MERIT soil	3.27	33.57a	10.20a	0.83a
	MERIT soil	0.33	2.61b	66.0b	5.35b
2008	Untreated Controls	0.09	30.00b	29.30b	2.39b
	IMA-jet Lo	2.04b	72.27a	0.66a	0.05a
	IMA-jet Hi	3.61ab	70.29ac	1.01a	0.08a
	IMA Lo + MERIT soil	3.64ab	81.70a	0.40a	0.03a
	IMA Hi + MERIT soil	6.54a	79.77a	0.71a	0.06a
	MERIT soil	3.14ab	41.70bc	1.59a	0.13a

N=8. Means followed by the same letter are not significantly different at $p < 0.05$. % data transformed (asin (sqrt (x/100)))*57.3 prior to ANOVA. Actual means are presented in table. *Imidacloprid residues reported are for the parent molecule plus metabolites.

Residues reported are from foliage combined from each sampled quadrant in the tree canopy for a mean value. Imidacloprid residues were determined either by liquid chromatography/mass spectrometry [LC/MS/MS] by high-pressure liquid chromatography [HPLC] or by enzyme-linked immune-sorbent assay [ELISA] following drying, grinding and solvent extraction in any given year. For the LC/MS/MS and HPLC residues, values for imidacloprid, olefin and 5-hydroxy-imidacloprid metabolites are reported here as imidacloprid residues. It should be noted that the olefin metabolite has been documented to have higher insecticidal activity in plants than the parent molecule (Nauen et al., 1998). The ELISA residues are semi-quantitative; the imidacloprid residues represent the parent molecule and (unspecified) metabolites. HPLC (Bayer method) and ELISA residues in hemlock tissues correlate closely (Frank J. Byrne, UC Riverside, personal communication). Imidacloprid residues are reported in µg/g (ppm). Two or three untreated hemlock samples were typically reported for the residue analyses, and were therefore excluded from statistical analyses.

Digital scans were made of the branch samples, using a Canon Color Image Scanner (CanoScan 8800F) and identified by treatment and year. The digital images recorded the visible changes in tree condition with time. Tip growth was determined on a percentage basis, adapting the method suggested by Webb et al. (2003). Percent was determined by number of tips with new growth divided by the total counted, multiplied by 100. The mean of the four branches per replicate was used for percent tip growth.

HWA infestations were assessed by microscopic examination. The number of HWA on twigs with needles was counted, and values of HWA /cm and HWA /100 needles were calculated.

Onken (unpublished data) 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 following year. Monthly weather summaries were obtained from online data provided by the National Weather Service Forecast Office, Greenville-Spartanburg, S.C. (NOAA). Monthly precipitations for the period of 2006 to 2009 compared to normal are presented in chart form.

Statistical analyses were conducted using MINITAB (Version 15, Minitab, Inc. State College, PA, USA). Percent data was arsine transformed (asin (sqrt (x/100)))*57.3 prior to ANOVA. Statistical significance was accepted at the 95% confidence interval and $p < 0.05$. T-tests were used to determine mean separation.

RESULTS

Application time varied with the method used, although all three methods were relatively efficient. The QUIK-jet was fastest, the Kioritz was intermediate and the Tree I.V. was slowest; mean application times were 2, 5 and 10 minutes, respectively. The tree injections delivered different volumes (e.g., Tree I.V. was greater than QUIK-jet) which accounted for the difference in application times. The Kioritz injector, on the other hand, delivers 5 mL per stroke, and requires numerous pump strokes to complete an injection. For example, to deliver 960 mL required 192 pump strokes, which accounts for the intermediate time of application.

A total of 192 digital scans conducted from 2007 to 2009, which aided in the quantification of changes in percent twig growth (Figures 1a-b). At the beginning of the study, 51.6% of the hemlock had terminals that died back, 31.3% had no new growth and 17.1% had some growth. Percent tip growth per treatment was not significantly different

Figure 1. Digital scans of hemlock terminals from the untreated control compared to the combined tree injection low dose plus soil applied imidacloprid two years following application: A, of twigs from untreated tree with severe needle loss and tip dieback; and B, of twigs from the combined imidacloprid treatment showing recovery and marked increase in growth.

from the untreated controls: the hemlocks were similar, but in very poor condition (Table 1). At 435-d, twig growth in two treatments (IMA-jet low plus MERIT soil and IMA-jet high plus MERIT soil) were statistically different from the untreated controls, results which were encouraging for tree recovery. At 800-d, the IMA-jet treatments alone (low and high) and combined with MERIT soil were statistically different from the controls.

In 2007 (70-d), HWA bioassay assessments were limited to 5 tips per replicate due to the poor condition of the trees. HWA /cm (and adelgid per 100 needles) of the imidacloprid treatments were not statistically different from the untreated controls. The means of HWA /cm and per 100 needles were very low (i.e., 0.15 and 2.23, resp.), taken together with tree condition, were indicative of a long, protracted infestation. In 2008 (435-d), trees recovery was observed as was a concomitant resurgence in adelgid. HWA /cm (and per 100 needles) was statistically different from the untreated controls for IMA-jet high and IMA-jet plus MERIT soil. The untreated controls, IMA-jet low and MERIT soil treatments had HWA populations high enough to negatively affect tree recovery (i.e., HWA /cm >2.0 and HWA /100 >25). In 2009 (800-d), all imidacloprid treatments were significantly different from the controls. Only the untreated trees had HWA numbers large enough to negatively impact tree growth.

Imidacloprid residues were extracted from hemlock at 70, 435 and 800-d. The values presented in are in ppm (i.e., µg of compound per gram of dried needles) (Table 1). At 70-d, the imidacloprid residues observed in treated trees were from 0.14 to 0.86 µg/g. At 435-d, the residues observed increased from 0.33 (MERIT soil) to 3.79 (IMA-jet high + MERIT soil). At 800-d, the imidacloprid residues recovered were from 2.04 (IMA-jet low) to 6.54 (IMA-jet high + MERIT soil) µg/g.

The normal monthly mean precipitation for Asheville, NC was 9.81 cm (3.92”). The 2006 mean monthly precipitation for Asheville, NC was not significantly different from the normal value [i.e., 10.06 (4.0”) v. 9.81cm (3.92”), resp.]. Lack of precipitation was not a cause for the reduction in growth observed in 2007. The monthly mean precipitation in 2007, however was significantly below normal [7.16 cm (2.86”), *p* = 0.015]; which could have negatively impacted tree recovery in 2008. The monthly precipitation in 2008 was below normal, but was not statistically significant [7.42 cm (2.97”), *p* = 0.068] (Figure 2). Below normal rainfall in 2008 probably did not limit tree growth in 2009.

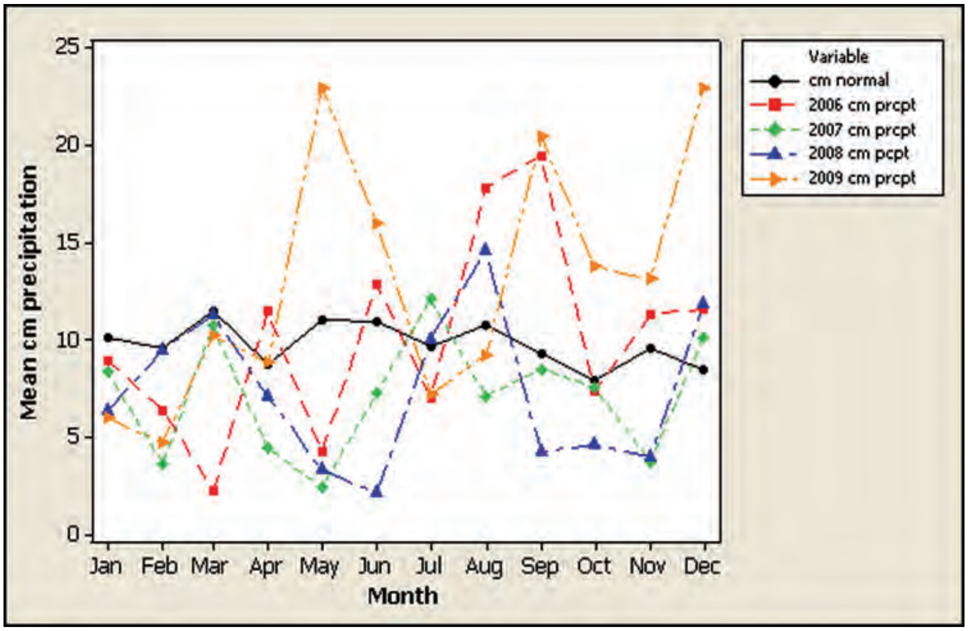

Figure 2. Mean month precipitation in Asheville, NC from 2006 to 2009 with monthly norm.

CONCLUSIONS AND DISCUSSION

This study examined HWA-infested trees in the southern part of the hemlock range. At the time of treatment, the hemlocks had very thin canopies and significant dieback. Even though precipitation was adequate the previous year, 51.6% of the trees were in decline. Of the 17.1% of branch samples with new foliage, the adelgid numbers were found to be very low. Terminal dieback and low numbers of adelgid was indicative of a protracted infestation. This protracted infestation (~ 7 years) left the trees in poor condition.

Could trees in such poor condition recover? The trees were treated in late summer 2007 to coordinate with resumption of HWA feeding. Hemlock in poor condition still moved imidacloprid systemically. Imidacloprid residues were recovered from needles of trees treated at 70-d. Although 2007 precipitation was statistically below normal, there was sufficient moisture for epicormic (adventitious) growth in 2008. As trees recovered, a concomitant resurgence of adelgid numbers was observed. Four of the treatments showed statistically significant increases in adelgid, three of which were great enough (> 2.0 HWA /cm or >25 per 100 needles) to potentially impact hemlock growth. Adelgid recovery followed tree growth, pointing to a need for continued protection. Significant growth in 2008 was observed in the combination imidacloprid treatments only. Imidacloprid residues in the treatments were observed to increase over time. The highest residues obtained in 2008 were in the tree injection treatments and in the combined tree injection and soil treatments, and were correlated with statistically significant growth in the following year. Interestingly, the residues from soil injection alone did not result in statistically significant growth in any year studied. Tree responses were positive, but slow, consistent with that reported by Webb et al. (2003), the greatest growth occurred in the second year following treatment. These observations support the role of tree injection in tree recovery, particularly in compromised trees.

While the adelgid numbers remained consistently high in the untreated trees, their numbers dropped to negligible levels in the imidacloprid treatments in 2009, demonstrating continued insecticidal efficacy. Cowles et al. (2006) report the LC_{50} value for HWA of >120 ppb (0.120 µg/g) protected trees for two years. In this study, values of 0.140 to 0.860 µg/g were observed in branch samples taken 70-d following treatments. At 435-d, the imidacloprid residues recovered were from 0.330 to 3.79 µg/g. At 800-d, the imidacloprid residues observed were from 2.04 to 6.54 µg/g. The level of imidacloprid recovered at 800-d, strongly suggests continued protection into the third and potentially, fourth year with no need for retreatment. This extended residual activity is in part attributed to needle retention (~ 3 years) in hemlock and to the insecticidal activity of imidacloprid metabolites, particularly olefin (Schöning and Schmuck, 2003, Nauen et al., 1998).

These data suggest that even low rates of tree injected imidacloprid could be administered once every four years in woodland trees. Such an extended re-treatment cycle is important from an economic standpoint, particularly if large numbers of woodland trees need to be treated for HWA. Further, the use of the QUIK-jet micro-injector resulted in fast (~2 minutes), efficient application in hemlock. A one-two combined strategy may afford even greater (5+ years) long term protection of hemlock, but to validate this hypothesis will require continued tree monitoring.

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THROUGH SPACE AND TIME: AN EVALUATION OF THE TRANSLOCATION OF IMIDACLOPRID IN EASTERN HEMLOCKS

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ABSTRACT

Imidacloprid is a primary chemical compound used to control hemlock woolly adelgid in both urban and limited forest environments. Two studies were initiated to evaluate the translocation and persistence of imidacloprid using different application methods and different application timing regimes. The first study consisted of 24 trees used to evaluate soil drench, soil injection, and tree injection application methods. No differences in imidacloprid concentrations were found between fall and spring applications. Imidacloprid concentrations peaked in both extracted sap and combined twig and needle tissue 12 months after treatment, and were highest in the bottom strata across all application methods and all times sampled. Concentrations in imidacloprid decreased from the bottom strata to the top across all application methods through the time sampled. Soil drench had the highest level of imidacloprid translocated throughout all strata. Imidacloprid concentrations found in twig and needle tissues in trees treated with a soil drench and have been correlated with a high degree of hemlock woolly adelgid suppression through month 48 post-treatment. The second study consisted of 60 trees used to evaluate soil injection and CoreTect soil pellet application methods. No differences in imidacloprid concentrations were found between fall, winter, spring, and summer applications. Imidacloprid concentrations in both sap and combined twig and needle tissue peaked 12 months after treatment. Concentration in both sap and twig and needle tissue was highest in the bottom strata across all application methods. Trees treated with CoreTect pellets had the highest concentrations of imidacloprid translocated throughout all three strata, through 36 months post-treatment in both the sap and twig and needle tissue. Imidacloprid concentrations found in twig and needle tissues in trees where imidacloprid was delivered using CoreTect and soil injection have been correlated with a high degree of hemlock woolly adelgid suppression through month 36 post-treatment.

KEYWORDS

Imidacloprid, hemlock woolly adelgid, tree injection
soil injection, hemlock health

INTRODUCTION

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), has proven to be detrimental to both eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, throughout eastern North America (McClure 1990, 1991; Souto et al. 1996; Royle and Lathrop 1997; Danoff-Burg and Bird 2002). Imidacloprid is one of the primary insecticides used to control hemlock woolly adelgid. Imidacloprid is primarily delivered as a soil drench, soil injection, tree injection, or as a soil pellet, and treatment rate varies depending upon application type and tree diameter.

Translocation of imidacloprid over a three month period in tree injected and soil injected trees has been documented to occur in eastern hemlock (Tattar et al. 1998); but, the length of time that the compound remained in sufficient concentrations to effectively control the target pest was not determined. Because the hemlock woolly adelgid settles and feeds throughout the canopy, uniform distribution of effective concentrations of imidacloprid throughout the tree is an important factor for the successful control of this invasive pest. The effects of treatment timing and application method on translocation of imidacloprid throughout the canopy, as well as the quantity and persistence of imidacloprid translocated to specific areas of the tree, are unknown.

Two studies were initiated to evaluate the translocation throughout the tree canopy and persistence of imidacloprid using different application methods (soil drench, soil injection, tree injection, and CoreTect soil pellets) and different timing regimes (fall, winter, spring, summer).

METHODS

STUDY 1

Eastern hemlocks (n = 24) were selected at Indian Boundary in the Cherokee National Forest in southeastern Tennessee on 5 November 2005 to evaluate the effects of treatment timing and application method on concentration levels within the canopy. The experimental site was arranged in a split-split plot 2 x 4 factorial complete randomized block design with three replications. Three blocks with eight trees each were established. These trees were arranged in four pairs with one tree in the pair treated in the fall (29-30 November 2005) and the other in the spring (16 April 2006). To monitor translocation of imidacloprid within the tree, each tree was divided into approximately three equal sections or strata (bottom, middle, and top) with each strata representing ca. one-third of the tree. Basic tree characteristics were documented on 25-26 November 2005 and consisted of: tree height, canopy transparency and density, crown class, dbh (diameter at breast height), foliage color, overall appearance, crown condition, and percent of hemlock woolly adelgid on tree. Tree pairs were selected based on how closely two trees matched morphologically with regard to these characteristics. Selected trees ranged in dbh from 16 – 26 cm, and in height from 15 – 30 m. All three blocks were located in a shortleaf pine-oak (type 76) forest (Eyre 1980).

The three application methods evaluated were tree injection, soil drench, soil injection, and the untreated control. The tree injection system consisted of the Mauget$^{\text{Ò}}$ 3 ml 10% imicide capsules and feeder tubes (J. J. Mauget Co., Arcadia, CA). The tree injection was applied at a rate of 1.5 g ai / 2.5 cm dbh. Soil drench was applied using a FMC high pressure hydraulic sprayer (FMC Corporation, Jonesboro, AR). Merit® 75 WP (Bayer, Kansas City, MO) was applied at a rate of 1.5 g ai / 2.5 cm dbh. The soil injection was made using the Kioritz® soil injector (Kioritz Co., Tokyo, Japan). Merit® 75 WP insecticide (Bayer, Kansas City, MO) was diluted to 1 g ai / 2.5 cm dbh in 60 ml of water for application.

Two 24 cm branch clippings were taken at each stratum (bottom, middle, and top) of the test trees at every three months post-treatment through month 48 using a 10 m pole pruner or an articulating boom. Concentrations of imidacloprid were determined for both sap extract and twig and needle tissue. Sap was extracted using a PMS pressure chamber (PMS instrument Co., Albany, OR). Twig and needle tissue was prepared by using 12 cm of the same branch used for sap extractions pulverizing the tissue using a coffee grinder. Imidacloprid residues within the branch sap and twig and needle tissue were quantified using a commercially available enzyme linked immunosorbant assay (ELISA) kit (EnviroLogix 2003). ANOVA and Least Significant Differences (LSD) procedures were conducted on chemical concentration data ($P < 0.05$) in SAS (SAS Institute 2005). ANOVA mixed model type 3 test of fixed effects was used to determine interactions between treatment timing, strata level within the canopy, and application method.

Study 2

Eastern hemlocks (n = 60) were selected at Indian Boundary in the Cherokee National Forest in southeastern Tennessee on 2 May 2008 to evaluate the effects of treatment timing and application method on concentration levels within the canopy. The experimental site was arranged in a split-split plot 4 x 3 factorial complete randomized block design with five replications. Five blocks with twelve trees each were established. Each tree was marked with a numbered identification metal tag. These trees were arranged in three groups of four, with one tree in the group treated in the fall (21 November 2008), one in the spring (22 April 2009), one in the summer (7 July 2008), and one treated in the winter (16 January 2009). To monitor translocation of imidacloprid within the tree, each tree was divided into approximately three equal sections or strata (bottom, middle, and top) or sections with each strata representing ca. one-third of the tree. The basic tree characteristics were documented on 2 May 2008 and consisted of: tree height, canopy transparency and density, crown class, dbh, foliage color, overall appearance, crown condition, and percent of hemlock woolly adelgid on tree. Tree pairs were selected based on how closely two trees matched morphologically with regard to these characteristics. Selected trees ranged in dbh from 17 – 28 cm, and in height from 28 - 35 m. All five blocks were located in a shortleaf pine-oak (type 76) forest (Eyre 1980).

The three application methods evaluated were CoreTect soil pellets, soil injection, and an untreated control. CoreTect pellets were applied at a rate of 1.5 g ai / 2.5 cm dbh. Soil injection was made using the Kioritz® soil injector (Kioritz Co., Tokyo, Japan). Merit ® 75 WP insecticide (Bayer, Kansas City, MO) was diluted to 1 g ai / 2.5 cm dbh in 60 ml of water for application.

Three 24 - cm branch clippings were collected at each stratum (bottom, middle, and top) every three months post-treatment through month 36 using a 10 m (32.8 ft) pole pruner or an articulating boom. Concentrations of imidacloprid were determined for both sap extract and twig and needle tissue. Sap was extracted using a PMS pressure chamber (PMS instrument Co., Albany, OR). To determine the amount of imidacloprid in twig and needle tissue, 12 cm of the same branch used for sap extractions was pulverized using a coffee grinder. Imidacloprid residues within the sap and twig and needle tissue were measured using liquid chromatography coupled with tandem mass spectrometry (LC/MC/MC). ANOVA and Least Significant Differences (LSD) procedures were conducted on chemical concentration data ($P < 0.05$) in SAS (SAS Institute 2005). ANOVA mixed model type 3 test of fixed effects was used to determine interactions between treatment timing, strata level within the canopy, and application method.

RESULTS

STUDY 1

Significant interactions among application method, months post-treatment, and strata for imidacloprid concentrations in branch sap ($F = 3.2$, $P = 0.0007$) and combined twig and needle tissue ($F = 1.19$, $P < 0.0001$) were documented using the mixed proc ANOVA test. The soil drench and soil injection application methods produced higher concentrations of imidacloprid in the branch sap and the twig and needle tissue than the tree injection at the bottom, middle, and top strata of the canopy for entire evaluation period. Imidacloprid concentrations were higher in the bottom strata of the trees across all application types in both branch sap and the twig and needle tissue. All chemically treated trees had translocated imidacloprid by the first 3-month post-treatment sampling period and continued to translocate imidacloprid over the 48-month sampling period with imidacloprid concentrations peaking around month 12 in samples of both branch sap and combined twig and needle tissue. Timing of application was not an interacting factor for imidacloprid concentrations in branch sap and combined twig and needle tissue concentrations.

STUDY 2

Significant interactions among application method, months post-treatment, and strata for imidacloprid concentrations in branch sap ($F = 4.2$, $P = 0.0004$) and combined twig and needle tissue ($F = 2.63$, $P < 0.0001$) were documented using the mixed proc ANOVA test. The CoreTect application method produced higher concentrations of imidacloprid in the branch sap and the twig and needle tissue than the tree injection at the bottom, middle, and top strata of the canopy for entire evaluation period. Imidacloprid concentrations were higher in the bottom strata of the trees across all application types in both branch sap and the twig and needle tissue. All chemically treated trees had translocated imidacloprid by the first 3-month post-treatment sampling period and continued to translocate imidacloprid over the 36-month sampling period with imidacloprid concentrations peaking around month 12 in samples of both branch sap and combined twig and needle tissue. Timing of application was not an interacting factor for imidacloprid concentrations in branch sap and combined twig and needle tissue concentrations.

DISCUSSION

Documentation of the concentration levels of imidacloprid in the sap at various locations in the canopy provides the opportunity to develop a baseline for implementing applications of the insecticide for control of the hemlock woolly adelgid. In these two studies, three general trends were observed relative to insecticide concentration translocation. First, concentrations of imidacloprid in branch sap and the twig and needle tissue in all treated trees progressively decreased from the bottom to the top strata of the canopy, with the highest concentration of the compound over time in the bottom strata. Secondly, the soil drench and CoreTect treatments consistently provided the highest concentration of imidacloprid in the sap of both the branches and the twigs and needles across all strata on the tree. Imidacloprid concentrations >120 ppb have been found to maintain a high degree of suppression (Cowles et al. 2006). CoreTect, soil injection, and soil drenched trees maintained imidacloprid concentration levels in twig and needle tissue >120 ppb. Imidacloprid concentrations in twig and needle tissue in CoreTect treated trees were >120 ppb through the 36 months and soil drenched trees had concentrations that were >120 ppb through 48 months. Imidacloprid concentrations in injected trees were the least uniform throughout the tree strata, especially at the top of the tree. In other field applications, soil drench and soil injections have been found to be effective at controlling the hemlock woolly adelgid (Steward and Horner 1994; Rhea 1995; Steward et al. 1998; Fidgen et al. 2005; Cowles et al. 2006), and in addition to CoreTect, had the most uniform distribution throughout the canopy in our study. Thirdly, application time was not a significant factor in the uptake of imidacloprid; this may be due in part to year round transpiration of eastern hemlocks in the southern Appalachians.

These general trends can be used by land owners and managers to make informed decisions on what type of treatments have the most potential for effectively treating hemlock woolly adelgid over longer periods of time. Future research is needed to determine if reduced concentrations of imidacloprid will be as effective against the hemlock woolly adelgid and determine the precise time period the compound persists and provides protection within the tree. A possible reduction in imidacloprid concentration would result in greater financial savings and potentially lessen the effect on non-target species (Dilling et al. 2007; Dilling et al. 2008).

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PRESENTATIONS: HEMLOCK CONSERVATION, PRESERVATION AND GENETIC RESISTANCE

BREEDING NOVEL HEMLOCK HYBRIDS WITH RESISTANCE TO HEMLOCK WOOLLY ADELGID

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ABSTRACT

The native eastern hemlock, *Tsuga canadensis*, and the Carolina hemlock, *T. caroliniana*, suffer injury and death following infestation by the hemlock woolly adelgid (HWA), *Adelges tsugae*, an introduced pest from Japan. Asian species of hemlock have shown high tolerance or resistance to the pest. The U.S. National Arboretum initiated a hemlock improvement program in the 1990s with the goals of developing HWA-resistant hemlocks via hybridization between susceptible native hemlocks and resistant eastern Asian species (*T. chinensis, T. diversifolia,* and *T. sieboldii*). Hybrids were recovered between *T. caroliniana* and *T. chinensis,* and among the Asian species (*T. chinensis, T. diversifolia,* and *T. sieboldii*), however, no hybrids with *T. canadensis* were recovered. In order to determine the nature of the incompatibility, crossing experiments were conducted to examine this hybridization barrier. Reciprocal controlled pollinations were made in 2008 and 2010 between *T. chinensis* and *T. canadensis,* with cones harvested at 0, 3, 5, 7 and 9 weeks after pollination. Cones were examined using histological techniques to check for presence of pollen grains, pollen germination, and pollen tube growth. In general, pollen grains were present in all pollinations, with pollen germinating in all crosses by 5 weeks, with pollen

tubes reaching the micropyle between 7 and 9 weeks post-pollination. Both *T. canadensis* and *T. chinensis* pollination mechanisms are similar to that previously observed in *T. heterophylla* from western North America. The incompatibility mechanism appears to be post-zygotic with aborted embryos at 9 weeks. In addition to crossing studies, a two-year artificial inoculation study was begun on container grown hemlocks to establish new protocols for pre-screening hybrid progeny for resistance to HWA. Preliminary results are similar to previous published data on artificial inoculations of field-grown, maturing hybrid hemlocks, indicating that pre-screening container-grown hemlocks may accelerate the evaluation and identification of HWA-resistant hemlocks.

STATUS OF GENE CONSERVATION FOR EASTERN AND CAROLINA HEMLOCK IN THE EASTERN UNITED STATES

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ABSTRACT

Since 2003, Camcore (NC State University) and the USDA Forest Service have worked together to conserve the genetic resources of eastern (*Tsuga canadensis* Carriére) and Carolina (*T. caroliniana* Engelmann) hemlocks threatened by the hemlock woolly adelgid (*Adelges tsugae* Annand). The objectives of this 3-phase cooperative effort are to make genetically representative seed collections from populations distributed across the geographic range of each species, establish *ex situ* conservation plantings both inside and outside the United States, and place seeds into long-term cold storage. The overall goal is to maintain viable populations in perpetuity until hemlock restoration is possible. Here we present an update on our progress with seed collections and conservation bank establishment.

KEYWORDS

ex situ gene conservation, germplasm, *Tsuga canadensis*
Tsuga caroliniana, Adelges tsugae

INTRODUCTION

Camcore (International Tree Conservation and Domestication, NC State University) and the USDA Forest Service Forest Health Protection have been cooperating since 2003 on an east-wide effort to conserve the genetic resources of eastern (*Tsuga canadensis* Carriére) and Carolina (*T. caroliniana* Engelmann) threatened by the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand). The objective of this project is to maintain, in perpetuity, viable *ex situ* populations and seed reserves of both hemlock species for breeding and restoration activities once effective HWA management strategies are in place in the eastern United States. These *ex situ* reserves will also serve as an insurance policy against the “worst-case” scenario where the adelgid completely eliminates hemlock from eastern forest ecosystems.

The strategy and rationale for hemlock gene conservation have been presented in past HWA Symposia proceedings (Tighe et al. 2005; Jetton et al. 2008). Restated briefly, the goals of this 3-phase, 10-year project are to:

1) collect seed from up to 10 mother trees in a many Carolina hemlock populations as can be located (Phase 1)
2) collect seed from up to 10 mother trees in 60 populations (600 trees) of eastern hemlock in the Southern Region (Phase 2)
3) collect seed from up to 10 mother trees in 60 populations (600 trees) of eastern hemlock in the Northern Region (Phase 3)

Some seeds are being used to establish *ex situ* conservation plantings/breeding orchards both inside and outside the United States (Chile and Brazil), while the remaining seed will be catalogued in seed banks at Camcore (NC State University) and the National Tree Seed Laboratory (Dry Branch, GA). This article reports on progress made through the first seven years of the project.

PROGRESS ON SEED COLLECTION

Carolina Hemlock: A total of 26 Carolina hemlock populations have been identified in the Southern Appalachian region. Between 2003 and 2009 seed was collected in 18 of these from a total of 126 mother trees (Figure 1, Table 1). The number of mother trees sampled ranges from a few as 1 to as many as 12 trees per population (Table 1). In the fall of 2010, we plan to collect seed from one additional population located on Looking Glass Mountain in the Pisgah National Forest, North Carolina. We will also confirm 5 additional populations that have been reported in North Carolina at Bat Cave, Cloven Cliff, Kelsey Tract, Shope Creek, Big Fork Knob, and Young's Ridge.

Eastern Hemlock: Between 2005 and 2009 seed has been collected from 34 populations of Eastern hemlock, 33 that are located in the Southern Region and one in the Northern Region, Cook Forest State Park in Pennsylvania (Figure 2). These represent collections from 232 mother trees ranging from 1 to 24 trees sampled per population (Table 2). Seed collections planned for the Southern Region in the fall of 2010 include Chimney Rock State Park (Poole's Creek area) in North Carolina, Hemlock Falls and Anna Ruby Falls on the Chattahoochee National Forest in Georgia, and Paint Creek Campground on the Cherokee National Forest in Tennessee. In late August 2010 we will also explore populations located at Mammoth Cave National Park in Kentucky and the Bankhead National Forest in Alabama. As of the writing of this article plans for seed collections in the Northern Region for 2010 have not been finalized but will be attempted.

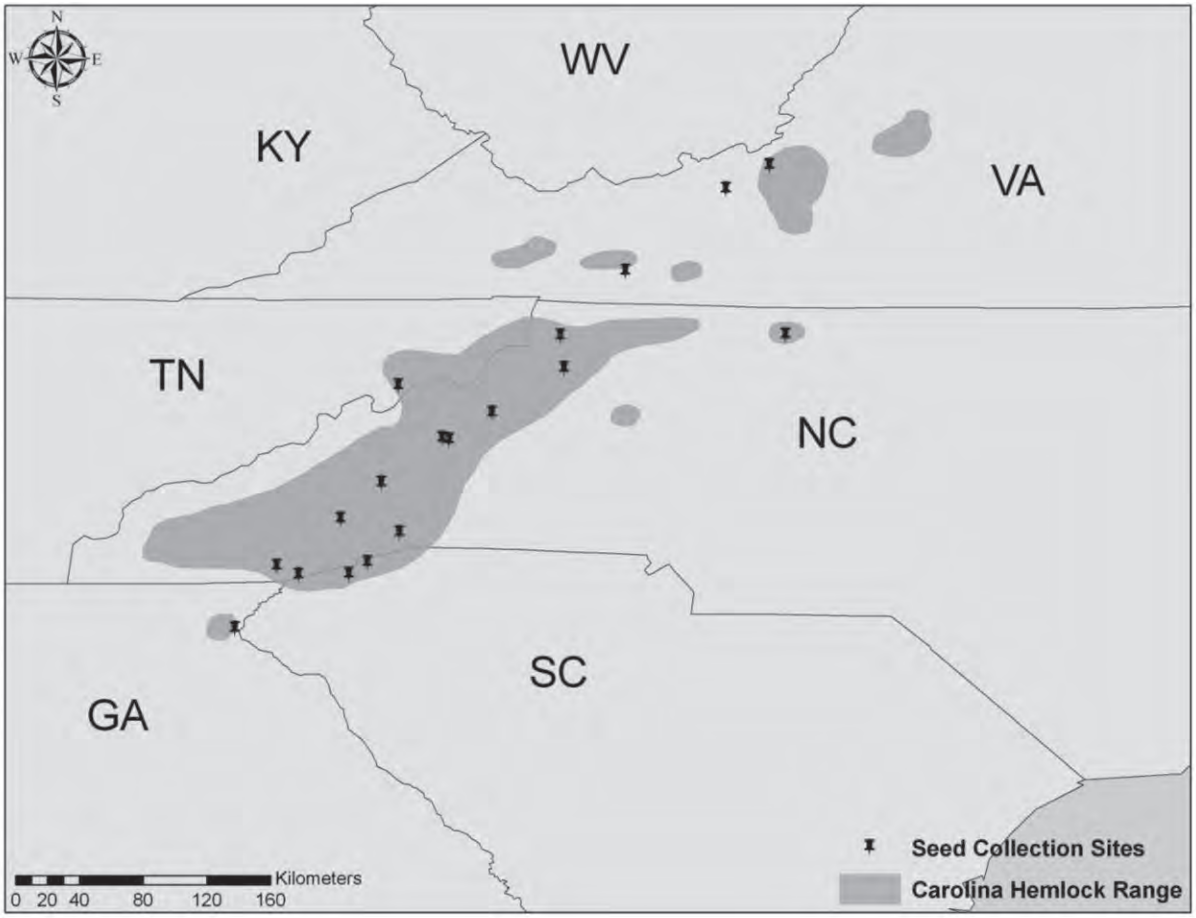

Figure 1. Map of Carolina hemlock populations in the Southern Appalachian Mountains where seed has been collected between 2003 and 2009.

PROGRESS ON CONSERVATION BANK ESTABLISHMENT

Carolina hemlock conservation banks have been established in Chile and North Carolina. The Chile planting was established by Camcore Cooperative member Bioforest-Arauco in September 2008 at Predio Cuyimpalihue in the Los Alamos region of the country. A total of 1,400 seedlings were planted representing 9 populations and 56 families (mother trees) from across the Carolina hemlock geographic range. As of June 2010, survival in the planting was 85%, and Bioforest-Arauco is currently preparing to propagate cuttings from the planted seedlings to be used in the establishment of a second conservation bank in Chile.

The North Carolina planting was established by Camcore in 2008 at the NC State University/NC Department of Agriculture Upper Mountain Research Station in Ashe County. Four hundred seedlings representing 9 populations and 53 families from across the Carolina hemlock range were planted, and as of June 2010 survival in the plot was 91%. We plan to establish an additional Carolina hemlock conservation bank in North Carolina with 411 seedlings, representing 8 populations and 31 families, currently being cultivated in pots at the Upper Mountain Research Station.

Table 1. Summary of seed collections from Carolina hemlock populations in the Southern Appalachian Mountains 2003-2009.

Population	County	State	Lat. (D.d)	Long. (D.d)	Elevation (m)	Mother Trees (#)	Collection Year(s)
Biltmore	Buncombe	NC	35.55	-82.55	650.00	6	2007
Bluff Mountain	Ashe	NC	36.38	-81.54	823.20	8	2003
Caesar's Head	Greenville	SC	35.11	-82.63	933.30	7	2003, 2006, 2009
Carl Sandburg	Henderson	NC	35.27	-82.45	668.00	6	2009
Carolina Hemlocks Cpgd	Yancey	NC	35.81	-82.20	823.20	11	2003, 2008
Cliff Ridge	Unicoi	TN	36.10	-82.45	671.00	12	2006, 2008
Crabtree	Yancey	NC	35.80	-82.16	1131.93	6	2003
Cradle of Forestry	Transylvania	NC	35.35	-82.78	1017.40	11	2003, 2008
Cripple Creek	Wythe	VA	36.75	-81.17	696.20	7	2006, 2008
Hanging Rock	Stokes	NC	36.39	-80.27	480.00	12	2003, 2009
Kentland Farms	Montgomery	VA	37.21	-80.60	568.00	6	2009
Linville Falls	McDowell	NC	35.95	-81.92	995.50	10	2003
Sinking Creek	Craig	VA	37.35	-80.36	980.00	6	2009
Table Rock	Pickens	SC	35.04	-82.73	955.50	3	2003
Tallulah Gorge	Rabun	GA	34.73	-83.38	576.00	3	2005
Whitewater Falls	Jackson	NC	35.03	-83.02	790.00	1	2009
Whiteside Mtn	Jackson	NC	35.08	-83.14	1441.00	1	2009
Wildcat	Watauga	NC	36.20	-81.52	975.00	10	2003

Camcore Cooperative member Rigesa (MeadWestvaco) will plant conservation banks for both eastern and Carolina hemlock near Três Barras, Santa Catarina State, Brazil in September 2010. The eastern hemlock plot will be planted with 177 seedlings representing 7 populations and 26 families from the southern portion of the species' native range. The Carolina hemlock plot will be planted with 231 seedlings representing 9 populations and 41 families from across the Southern Appalachian Mountains.

SUMMARY

Camcore and the USDA Forest Service continue to make progress on the genetic conservation of eastern and Carolina hemlock. Hemlock seed has been collected from a total of 52 populations and 358 mother trees (eastern and Carolina combined). Carolina hemlock conservation plantings have been established in Chile and the United States (North Carolina), and conservation banks for both species will be planted in Brazil in 2010. Although we continue to make steady progress, this project is not without its challenges. As conservation efforts expand into the Northern Region far removed from our home base in Raleigh seed collections will be challenging without the support and cooperation of

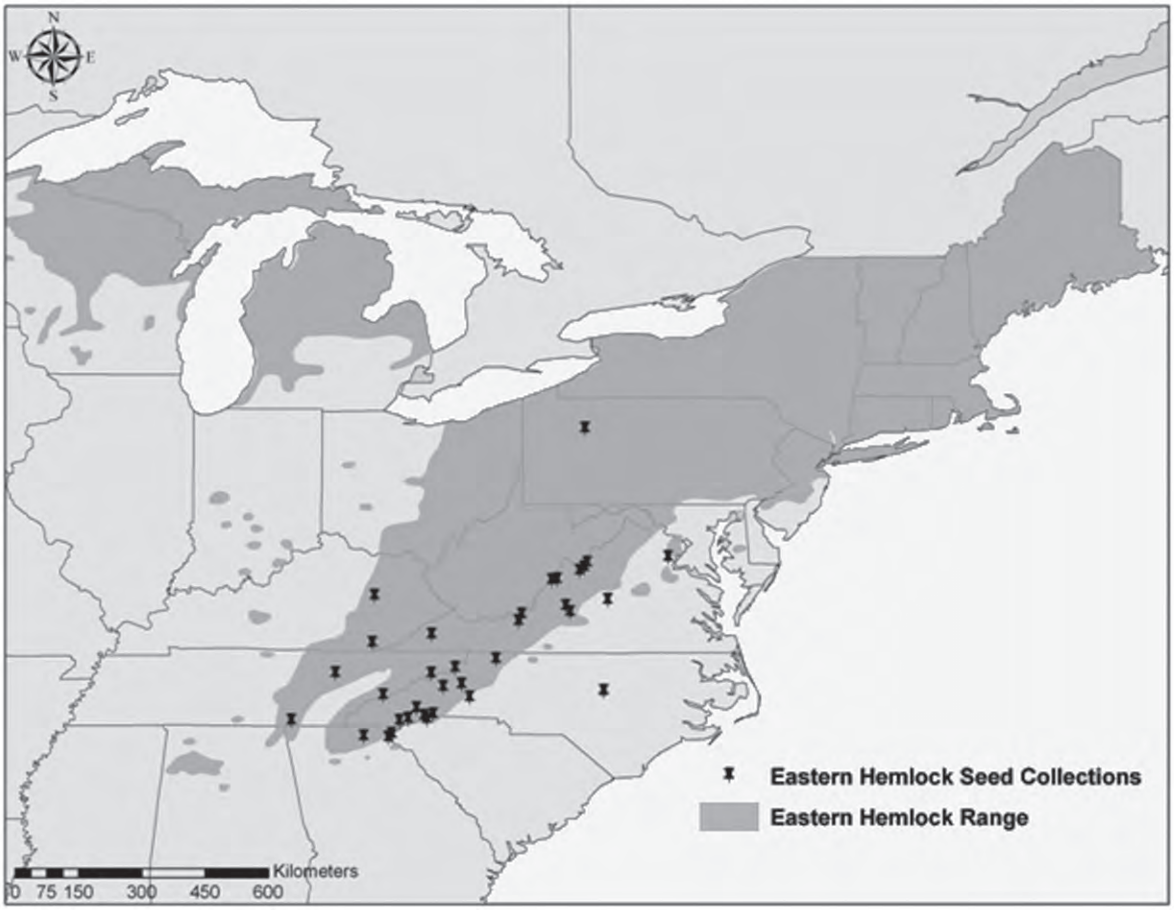

Figure 2. Map of eastern hemlock populations in the eastern United States where seed has been collected between 2005 and 2009.

resources managers in the region. Difficult to predict seed cycles and cone ripening rates and the severity of HWA related decline confound our ability to locate healthy hemlock stands producing suitable amounts of high quality seed. Fortunately, through research on seed biology and stratification treatments we have been able to improve the germination capacity in some of our seed stocks (Jetton and Whittier, unpublished data). Our overseas conservation banks offer the advantage of being far removed and reasonably secure from HWA infestation, but they also carry the disadvantage of distance and being difficult to monitor on a regular basis. Furthermore, world-wide restrictions on the movement of germplasm are becoming more stringent each year, putting future hemlock seed shipments and conservation bank establishment in doubt. This leaves us to wonder if we should consider establishing more chemically protected plantings within the native range.

Table 2. Summary of seed collections from Eastern hemlock populations in the eastern United States 2005-2009.

Provenance	County	State	Lat. (D.d)	Long. (D.d)	Elevation (m)	Mother Trees (#)	Collection Year(s)
Back Creek	Burke	NC	35.86	-81.82	382.30	6	2008
Beech Mountain	Avery	NC	36.22	-81.95	949.15	5	2006
Blowing Springs	Bath	VA	38.10	-79.78	526.00	3	2007
Braley Pond	Augusta	VA	38.29	-79.30	615.00	3	2009
Carl Sandburg	Henderson	NC	35.22	-82.42	714.00	5	2007
Carolina Hemlocks Cpgd	Yancey	NC	35.81	-82.20	767.07	7	2008
Cave Mtn Lake	Rockbridge	VA	37.53	-79.59	371.00	3	2007
Chattooga River	Oconee	SC	34.80	-83.30	333.00	10	2007
Cliff Ridge	Unicoi	TN	36.09	-82.46	620.36	10	2006, 2008
Cook Forest	Forest	PA	41.32	-79.20	365.00	2	2009
Cradle of Forestry	Transylvania	NC	35.35	-82.78	787.73	10	2008
Dupont St. Forest	Transylvania	NC	35.19	-82.62	805.33	10	2006, 2007
Frozen Head	Morgan	TN	36.10	-84.50	499.00	6	2008
GSMNP	Sevier	TN	35.63	-83.48	1223.55	24	2008
Guest River Gorge	Wilkes	VA	36.92	-82.45	612.00	3	2009
Helton Creek	Union	GA	34.75	-83.90	700.00	4	2007, 2009
Hemlock Bluffs	Wake	NC	35.72	-78.78	135.00	3	2009
Hidden Valley	Bath	VA	38.08	-79.90	576.00	4	2007
Hone Quarry	Rockingham	VA	38.46	-79.14	584.95	7	2006, 2007
James River Park	Buckingham	VA	37.67	-78.70	178.00	4	2008
Jones Gap	Greenville	SC	35.12	-82.56	418.00	6	2007, 2009
Kentland Farms	Montgomery	VA	37.21	-80.61	546.00	4	2009
Lake Toxaway	Transylvania	NC	35.13	-82.95	924.00	5	2009
Mountain Lake	Giles	VA	37.36	-80.54	1189.00	10	2009
Natural Bridge	Powell	KY	37.75	-83.67	333.35	10	2008
North Creek	Botetourt	VA	37.40	-79.50	349.00	10	2006, 2007
Pine Mountain	Bell	KY	36.75	-83.72	485.90	7	2008
Prentice	Marion	TN	35.09	-85.44	536.00	3	2007
Quantico	Prince William	VA	38.58	-77.42	41.14	10	2006, 2008
South Mountains	Burke	NC	35.58	-81.64	419.20	10	2007
Stone Mountain	Wilkes	NC	36.40	-81.08	546.00	7	2006, 2007
Tallulah Gorge	Rabun	GA	34.73	-83.38	515.10	13	2005
Todd Lake	Augusta	VA	38.37	-79.21	605.00	7	2006, 2007, 2009
Whiteside Mtn	Jackson	NC	35.08	-83.14	1441.00	1	2009

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GENE DISCOVERY IN CAROLINA HEMLOCK (*TSUGA CAROLINIANA*) TARGETED FOR RESPONSE TO HEMLOCK WOOLLY ADELGID

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ABSTRACT

A cDNA (i.e., expressed genes) library of Carolina hemlock (*Tsuga caroliniana*) was developed and sequenced. Assembled sequences revealed more than 32,000 contigs representing genes that were largely (~65%) of unknown function when annotated through sequence similarity to existing plant gene databases. However, more than 100 hemlock sequence contigs appear to represent genes that play roles in plant responses to either biotic or abiotic stressors. The described DNA sequence dataset with annotation provides an excellent genomic resource for more intensive studies on the biology of hemlock woolly adelgid (HWA)/*Tsuga* interaction.

KEYWORDS

expressed sequence tags (ESTs), next-generation sequencing
sequence assembly, candidate genes, insect resistance

INTRODUCTION

Identifying hemlock (*Tsuga* spp.) genes that contribute to susceptibility and resistance to hemlock woolly adelgid (HWA, *Adeges tsugae*) will aid in the development of biologically-based control strategies. However, little DNA sequence information is available for hemlock species. To address this limitation, we undertook a sequencing project in Carolina hemlock (*T. caroliniana*) that targeted genes responding to HWA infestation. In particular, we generated a large database of expressed sequence tags (ESTs), relatively short

DNA sequence reads, from genes expressed in the sampled tissues. These sequences provide information for developing markers in genes that may be important in determining levels of host susceptibility or resistance. For example, it has been hypothesized that the terpene differences between susceptible and resistant hemlock species may be directly related to the differences in their resistance levels (Lagalante and Montgomery 2003). If this is true, then genes involved in terpene biosynthesis should be useful markers for predicting resistance in population screening or breeding experiments.

METHODS AND RESULTS

To generate as many unique ESTs as possible, we constructed and analyzed a cDNA library from Carolina hemlock using stem and needle tissues from both infested and uninfested branches collected from three unrelated trees. This library was sequenced using massively parallel sequencing-by-synthesis technology as implemented on the 454 Roche GS-20. Final assemblies of the 77.9 million base pairs (Mbp) of DNA sequence (average read length 240.4 bp) resulted in 32,080 contigs (maximum contig length 2,561 bp) and 96,739 singletons. Of these 128,819 EST contigs and singletons, 30,840 had significant sequence hits (i.e., sequences with a high similarity in DNA sequence to other plant species as determined by BLASTX, e-value ≤ 10^{-12}) to the publicly available, National Center of Biotechnology Information (NCBI) protein database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein) (Figure 1).

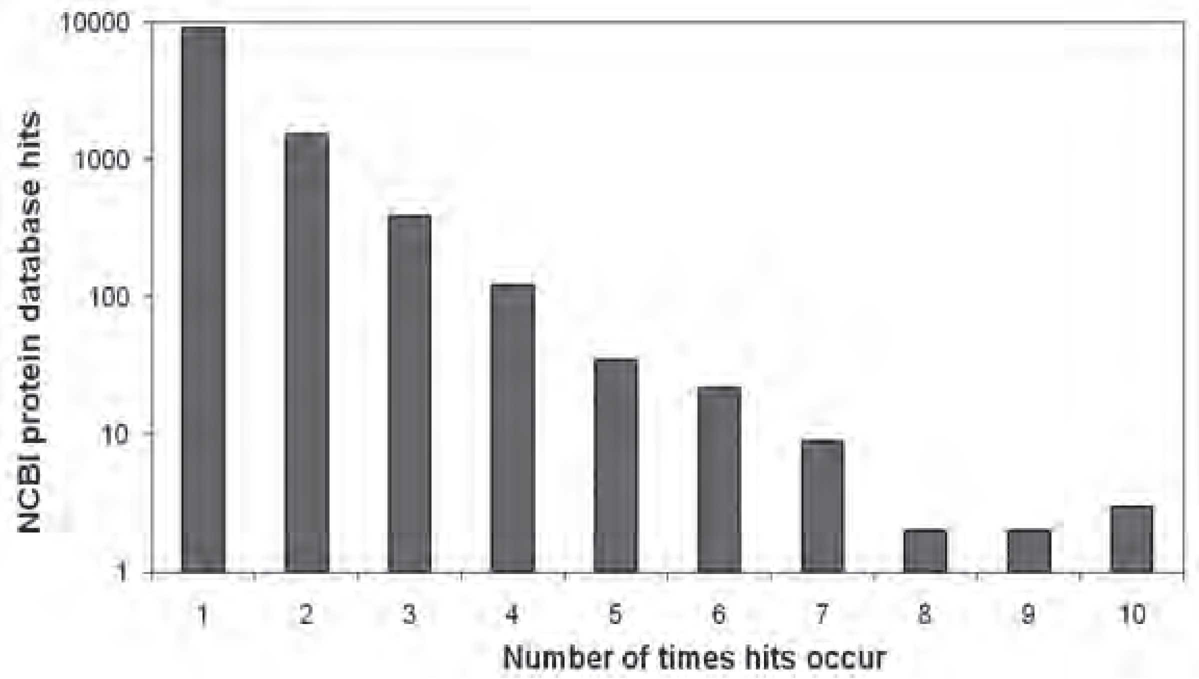

Figure 1. The number of NCBI protein database hits (log scale) that occur once, twice, three times etc., among the 32,080 Carolina hemlock (T. caroliniana) contigs (e-value ≤ 10-12). These data show that the Carolina hemlock EST library contains a large proportion of unique sequences.

Among the significant hits *Picea* sequences were 10 times more frequent than *Pinus*, indicating a closer relationship between *Tsuga* and *Picea* than *Tsuga* to *Pinus*. Even more hits to rice and Arabidopsis were obtained, not because of their close evolutionary relationships to *Tsuga* but due to the much larger numbers of these species' sequences in the databases. Thirty of the Carolina hemlock EST contigs were homologous to genes for plant disease response, and 102 were homologous to genes responsive to biotic or abiotic stressors. These contig sequences represent candidate genes for HWA resistance/susceptibility responses. Perhaps equally important is the large number of EST contigs with either no hits or hits to unknown or predicted proteins (Figure 2). Since there are relatively few conifer sequences in the NCBI database and far fewer *Tsuga* spp. sequences, some proportion of unclassified genes were expected. It is striking, however, that about 80% of the genes showed evidence of sequence homology with other plant genes, but with no evidence for their function (Figure 2). Despite this, these sequence tags can be used to develop genetic markers for experiments aimed at determining which genes condition resistance or susceptibility to HWA.

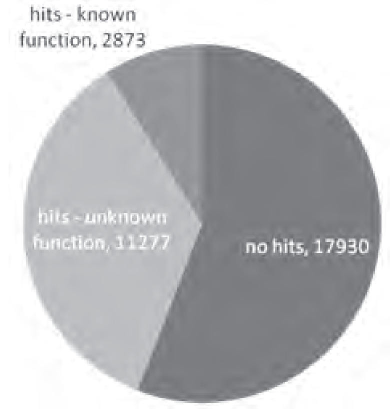

Figure 2. A comparison of sequence similarity hits between 32,080 Carolina hemlock (*T. caroliniana*) contigs and the NCBI protein database indicating that a large number of the Carolina hemlock genes have unknown function.

DISCUSSION AND CONCLUSIONS

Recent advances in high-throughput DNA sequencing technology are providing unprecedented looks into the molecular controls of host reactions to pests and pathogens. The EST database developed for Carolina hemlock provides an initial reference for expressed genes in hemlock, and these sequences can be compared to the growing NCBI database to provide additional information on gene identities and functions. Taken together, this sequence data with annotation provides an excellent genome-based resource for more intensive studies on the biology of HWA/*Tsuga* interaction, including differential gene expression comparisons and genetic mapping experiments. Of special interest is the potential for molecular breeding approaches to develop HWA resistant hemlocks by identifying and backcrossing resistance genes from Chinese hemlock (*T. chinensis*) into the susceptible, yet cross-compatible Carolina hemlock (Bentz et al. 2002; Montgomery et al. 2009).

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OF MICROSATELLITES, HEMLOCK WOOLLY ADELGID AND CLIMATE CHANGE: ASSESSING GENETIC DIVERSITY, AND THREATS TO IT, ACROSS THE RANGE OF EASTERN HEMLOCK

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KEYWORDS

Tsuga canadensis, population genetics, gene conservation, forest threats
genetic risk assessment

ABSTRACT

Eastern hemlock (*Tsuga canadensis* [L.] Carr.) is an ecologically important species facing two serious threats: extensive mortality caused by the invasive hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand), and changing climatic conditions that could render much of its current habitat unsuitable. We are conducting the first rangewide population diversity study of eastern hemlock, using highly variable molecular markers, to (1) help guide gene conservation efforts for the species, (2) assess the genetic effects of isolation on peripheral disjunct populations, and (3) assess regional differences in genetic variation to better understand the biogeographical processes that have shaped the genetic architecture of the species. This study encompasses 61 eastern hemlock populations, of which 9 are peripheral disjuncts, half are from areas north of the maximum extent of the Wisconsinian glaciations, and 17 have been infested with HWA. We selected 13 highly polymorphic and consistent nuclear microsatellite loci to include in the analysis after screening 42 loci that were previously isolated from eastern hemlock, Carolina hemlock (*Tsuga caroliniana* Engelm.) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.). We have conducted a preliminary analysis using eight of the 13 loci.

We found moderate levels of genetic differentiation among populations, with approximately 6 percent of the variation among, rather than within, populations ($F_{ST} = 0.063$). Overall

observed heterozygosity was less than expected heterozygosity (0.619 and 0.532, respectively), and most populations throughout the range of the species had relatively high levels of inbreeding, with the exception of some populations in the southern Appalachian mountains and the Northeast. Based on measures of allelic richness and heterozygosity, the species appears to have two main centers of genetic variation, in the southern Appalachians and New England. Unique alleles, however, were present in the highest numbers in western populations, including disjuncts in Indiana and Kentucky. Northern populations, on average, had more unique alleles than southern populations, while southern populations had slightly higher allelic richness and heterozygosity. Interior populations were more diverse than disjunct populations by nearly every measure. Disjuncts, however, had more private alleles on average, and were much more inbred. Populations uninfested by HWA had slightly higher levels of allelic richness and heterozygosity, and more unique alleles on average, than infested populations.

Using a standard population clustering approach, eastern hemlock populations appear to cluster weakly into regional groups. A Bayesian clustering analysis of individual trees using the program InStruct, meanwhile, suggested that the species contains eight gene pools. Strong geographic patterns were apparent in the arrangement of these gene pools, with all eight gene pools dominant in southern populations, only four dominant in the Northeast, and only one dominant in the Great Lakes region. These findings may offer clues into the location of glacial refugia and patterns of post-Pleistocene movement of the species. Specifically, a glacial refuge may have existed in the South, with a main post-glacial movement into the Northeast, and from there into the Great Lakes region. A separate migratory path may have existed to the populations west of the southern Appalachian crest and south of the Great Lakes.

We are incorporating these results into a genetic risk assessment that takes into account patterns of HWA infestation and potential climate change pressures. We are using the Multivariate Spatio-Temporal Clustering approach to generate maps of currently acceptable habitat for eastern hemlock, and then projecting future suitable habitat in 2050 and 2100 under two scenarios (high and low emissions) for each of two global climate models (Hadley and Parallel Climate Model [PCM]). To quantify the risk of climate change pressure to eastern hemlock populations, we identified currently acceptable habitat that might not be acceptable in 2050 by overlaying the species' current habitat with habitat projected under the Hadley low emissions scenario. While newly acceptable habitat may be available to eastern hemlock north of its current range in Canada, large areas west of the southern Appalachians and in New England may no longer be suitable. These areas contain high levels of genetic variation: higher heterozygosity and allelic richness in New England and unique alleles west of the Appalachians.

The preliminary results of this work suggest the following: (1) Efforts to conserve the genetic variation of eastern hemlock should focus on the areas with the highest allelic richness and heterozygosity (the southern Appalachians and New England) and on areas elsewhere in the range with high numbers of unique alleles. (2) Gene conservation activities also should target disjunct populations. While these appear to be more inbred and less genetically diverse than interior populations, several also contain high numbers of unique alleles. (3) It is not too late to conserve eastern hemlock genetic variation, given that much genetic variation exists in locations that have not yet been impacted by HWA. (4) The potential impacts of climate change should be considered alongside risk of HWA infestation when determining gene conservation strategies for eastern hemlock.

FRAMEWORK FOR GENETIC CONSERVATION OF EASTERN (*TSUGA CANADENSIS*) AND CAROLINA (*T. CAROLINIANA*) HEMLOCK

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BACKGROUND

Native hemlock in eastern US are threatened by a number of biotic and abiotic stressors, but hemlock woolly adelgid is the most severe. Global trade and travel have accelerated the pace of introduction of invasive forest insects and pathogens to an unprecedented level. Carolina hemlock, which has a very limited range, could face extinction within the next decade. Eastern hemlock populations are expected to be drastically reduced and some populations may be completely eliminated. The genetic resources of these species will be irreversibly lost if these drastic reductions in population size occur without careful preparation. Changing climates have resulted in increased risks from drought, fire, and native forest insects and pathogens. Some of our native tree species face the threat of local or range-wide extinctions, along with subsequent negative impacts on the ecosystems within which they reside. Strategies and action plans are needed to guide national gene conservation efforts for the most threatened species. To address these concerns, a workshop was held at the USFS Dorena Genetic Resource Center on October 11-12, 2007. One outcome of the workshop was a General Framework for Genetic Conservation. The framework outlines the steps needed to develop genetic conservation plans for these threatened species or groups of species.

OVERVIEW

Hemlocks are critical components of eastern forests. Their genetic resources are irreplaceable and critical to the maintenance of the species and the ecosystems that depend on them. These genetic resources can be conserved though a variety of approaches, including *in situ* methods in which plants are protected in their native habitats subject to natural evolutionary processes, and *ex situ* methods in which genetic material is stored at off-site locations such as in seed banks, genetic resource plantations (*e.g.* provenance and progeny tests), and seed orchards. A robust gene conservation strategy is being developed for eastern and Carolina hemlocks that combines elements of both approaches based on knowledge of species genetic structure, and the perceived threat to individual species whether from natural disturbance processes, introduced insect and pathogens, or sensitivity to changing climate. These should be supported by effective management policies and strategies to conserve and ultimately restore the species.

STRATEGIC RATIONALE

This framework assumes that a forest tree species (or closely related groups of tree species) has been previously identified as needing immediate attention to conserve genetic resources due to imminent local or range-wide extinction, and that prompt action is needed to conserve genetic resources. Applying this general framework will help ensure that all facets of genetic conservation are considered in the development of species-specific plans.

ORGANIZING FRAMEWORK

The following steps are a general framework for genetic conservation of the hemlock species that are native to eastern US. Swift action on these items is needed to protect the genetic resources of these species. Some additional analyses may continue after initial actions have begun and serve to refine efforts as the work progresses.

1. ORGANIZE WORKING GROUP

The eminent threat posed by the hemlock woolly adelgid across the eastern United State has lead to the formation of a National Working Group to address this threat in a concerted manner. This working group is composed of partners from numerous State and Federal agencies from Maine to Georgia as well many other non-government organizations. These partners have help develop direction and set priorities within the hemlock adelgid arena. The working group has several technical committees one of which deals with hemlock genetics. This technical committee provides overall direction as well as oversight within the realm of hemlock genetics. Within this technical committee is a subgroup whose focus is the conservation of hemlock genetics. The coordination of this effort will fall upon representatives within the US Forest Service along with other partners. This group will take the lead role in the project to develop the conservation plan as well as gather and secure the genetic resources of the two eastern hemlock species. This effort will be cooperative in nature and involve numerous stakeholders from both the public and private sector. For the Forest Service (FS), the Regional Geneticists, Area Geneticists, Research Geneticists and Genetic Resource Centers are key contacts in developing and implementing this plan. Collaboration with other genetic conservation efforts such as the National Genetic Resources Program of USDA Agricultural Research Service (http://www.ars-grin.gov/) and the Center for Plant Conservation at the Missouri Botanical Garden (http://www.centerforplantconservation.org/) will also be pivotal in this process. Rare plant program managers will become involved if either hemlock species is listed (or could potentially be listed) as Threatened or Endangered under the Endangered Species Act.

2. IDENTIFICATION OF THREATS

Risk assessment and evaluation of current and future threats to the long-term sustainability of hemlock species and ecosystems.

- Threats include:
  - **Invasive Insects** Hemlock Woolly Adelgid (HWA, *Adelges tsugae*) is the single greatest threat to eastern (*Tsuga canadensis*) and Carolina (*T. carolin-*

iana) hemlock genetic resources in the Eastern US and is the primary focus of this conservation framework. The adelgid has the potential to cause local and range-wide extinctions of both hemlock species.

The Elongate Hemlock Scale (EHS, *Fiorinia externa*) is also an exotic insect that attacks eastern hemlock. It is not nearly as damaging to hemlocks on its own, but its co-occurrence with HWA can hasten tree decline and death.

- **Climate** Severe drought across the southeastern US during 2007 and 2008 has exacerbated adelgid related mortality in the region. Additionally, drought is one of major factors that limits the establishment and long-term survival of hemlock seedlings and can eliminate the majority of advance regeneration in impacted stands (Godman and Lancaster 1990).
- **Disturbance Events** Severe drought increases the risk of widespread wildfires. While mature hemlocks are reasonably tolerant of fire, seedlings are not. Similar to drought, this can significantly reduce the amount of advance regeneration present in a stand (Godman and Lancaster 1990).
- **Salvage Harvests** Salvage harvests are often recommended to landowners who wish to recover some monetary value from mature stands of hemlocks prior to significant HWA infestation or decline. These may result in the loss of important hemlock genetic resources (Ward et al. 2004).
- **Climate Change** Several climate change scenarios suggest significant reductions in the amount of suitable habitat for hemlock species in the eastern US. McKenney *et.al*. 2007 predict that potential eastern hemlock habitat will be eliminated in the southeastern US and drastically reduced in the northeastern United States by 2100. Iverson and others predict hemlock forest type reductions of up to 40% in the northeastern portion of the country (Iverson et al. 1999, Iverson and Prasad 2002).

  The changes in climate that are predicted will require unprecedented rates of migration by hemlocks. The ability of these species to migrate will be severely compromised by the drastically reduced populations, cone production and rates of natural regeneration caused by HWA.

- Evaluation and status of current, imminent, and future threats
  - Hemlock Woolly Adelgid:
    - **HWA Distribution and Population Levels** As of December 2007, the hemlock woolly adelgid could be found infesting native hemlock stands in 17 eastern US states from Maine to Georgia (Figure 1). Infested counties are noted in brown, but this does not indicate the severity of the infestation. HWA population levels are highly variable from year to year and are impacted by weather patterns (*e.g.* in the Northeast extreme cold winter temperatures typically reduce adelgid density) and host tree health.
    - HWA Genetic Variation and Virulence Mitochondrial DNA analysis indicates that the adelgids that occur in the eastern US have very limited genetic

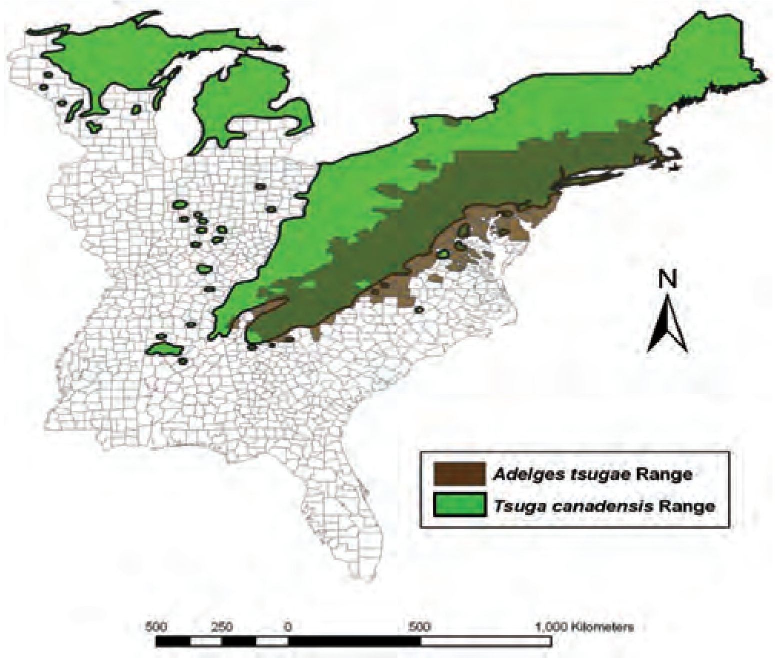

Figure 1. The distribution of hemlock woolly adelgid in the eastern United States as of December 2007. This map was produced by Camcore using USDA Forest Service data found http://www.na.fs.fed.us/fhp/hwa/hwatable_web/hwatable6.pdf.

variation (Havill *et.al.* 2006). This is probably because the current infestation resulted from a single introduction of the pest in Virginia and because adelgid reproduction is 100% parthenogenetic.

Adelgids occur naturally on hemlock species native to East Asia and western North America, but do not cause significant damage or tree decline. Significant HWA related mortality is only an issue on eastern and Carolina hemlocks in the eastern US where the adelgid is an invasive pest (McClure et al. 2001). The source of the US infestation is most likely an adelgid population in southern Japan that infests *T. sieboldii* (Havill et al. 2006).

- **HWA Rate of Spread** The current rate of spread of HWA into uninfested hemlock stands is very difficult to predict. Spread into northern New England has been very slow and is likely related to significant over wintering mortality among adelgids. Spread to the south (North and South Carolina, Tennessee, Georgia) where winter temperatures are warmer and, presumably, HWA over wintering mortality is much less, has been much more rapid. General consensus is that HWA spreads to uninfested areas at an average rate of 10-15 miles per year (Souto et al. 1996).
- **Host Species Silvics, Geographical Distribution, and Known Levels of Resistance or Tolerance** The somewhat rare Carolina hemlock is endemic to the southeastern US where it grows in a small number of isolated populations in Virginia, Tennessee, Georgia, and North and South Carolina

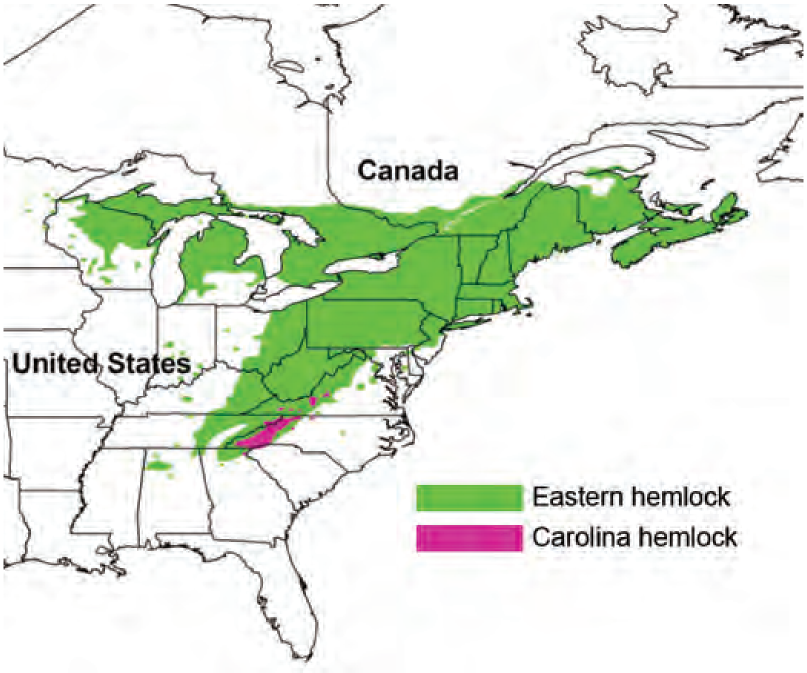

Figure 2. Geographic distribution of eastern and Carolina hemlock in the eastern U.S.

(Figure 2, above). It is typically found growing along exposed ridges and bluffs of the southern Appalachians, but a few populations can be found in higher elevation regions of the Virginia and North Carolina Piedmont. The species is usually described as growing on dry, sandy, nutrient poor soils but recent field reconnaissance and soil analysis indicates that Carolina hemlock occupies a greater diversity of site types than originally thought (Jetton et al. 2008a).

Eastern hemlock is found in eastern North America from Nova Scotia west to Wisconsin and Minnesota, and south along the Appalachian Mountains into Alabama, Georgia, Tennessee, South Carolina, and North Carolina with scattered populations in the western portions of Kentucky, Indiana, and Ohio (Figure 2). It covers a range of elevations from sea level to 1000 meters and displays a high level of phenotypic variation across this range. Eastern hemlock typically grows in moist cool ravines, valleys, and riparian strips and occupies moist, acidic, nutrient rich soils (Godman and Lancaster 1990).

Although there is evidence that Carolina hemlock may harbor some tolerance to HWA, particularly during the early stages of an infestation (Jetton et al. 2008b), there is little indication that either species harbors natural adelgid resistance. In time, both Carolina and eastern hemlocks succumb to HWA infestation.

– **Host/Pest/Environment Interaction** Hemlock woolly adelgid population cycles are driven by a density-dependent feedback cycle that is related to the health of the hemlock host (McClure 1991). Newly infested healthy hemlocks typically support adelgid populations that increase very rapidly and reach maximum densities within one or two years. Once these high HWA densities are reached, the host tree begins to experience twig dieback and very little new growth is produced. This causes a decline in adelgid populations for a one or two year period during which trees experience some recovery. This recovery is associated with the production of stunted new growth, which is quickly colonized by HWA. At this point, trees typically decline to a point where recovery, even in the absence of the adelgid, is unlikely and mortality soon follows.

This feedback between the adelgid and its hemlock host is almost certainly affected by environmental conditions, but this has not been well studied. One theory to explain the reported tolerance of Carolina hemlock during the initial phases of HWA infestation (Jetton et al. 2008b) is that the nutrient and water limited sites that this hemlock species typically occupies make it a poor host, leading to very slow adelgid population growth.

The hemlock woolly adelgid threatens to eliminate both eastern and Carolina hemlocks from large portions of their geographic range. If this were to happen, two ecologically vital tree species would be removed from eastern forests. Similar conifer species that might fill the ecological niche of hemlocks do not exist, and research has demonstrated that openings created by dead or dying trees are filled by black birch in the north and *Rhododendron* in the south (Orwig and Foster 1998). This would have important consequences for other species that inhabit hemlock ecosystems. Hemlocks provide an important source of cover and forage for a number of mammalian and avian species, some that are recognized as hemlock obligates. Eastern hemlock is also an important riparian species throughout much of its geographic range where it stabilizes soils and moderates stream temperatures and habitat conditions for a number of aquatic organisms including amphibians, insects, and fish. Of particular concern is the native brook trout (*Salvenlinus fontinalis*) that is most prevalent in headwater streams draining hemlock forests. The increases in stream temperature and light intensity that would result from the loss of hemlock could eliminate this native fish whose habitat is also impacted due to competition with the introduced brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Evans et al. 1996).

Hemlock woolly adelgid infestations are not restricted to forest hemlocks. Landscape trees and ornamental varieties derived from the native trees are also highly susceptible to the adelgid. Well over 200 cultivated varieties of eastern hemlock have been developed for horticultural use (Swartley 1984), and prior to HWA's widespread dispersal throughout the eastern US, these were worth millions of dollars annually to nursery growers and landscape centers.

The economic impact of HWA should also be viewed from the standpoint of adelgid management. Stem and soil injections of the systemic insecticide Imidacloprid are highly effective at reducing adelgid impacts on individual trees. However, application costs can be as high as $25 to $50 per tree, limiting their use to high value trees in residential landscapes, recreation areas, or easily assessable forest locations. Biological control agents (adelgid predators) are the most promising long-term solution for reducing HWA impacts in the forest environment. Mass rearing programs for many of these predator species have been established throughout the Southeast. They can produce hundreds of thousands of these beneficial insects annually.

Hemlocks are an important component of many federal, state, and municipal parks that occur within their geographic range. The enjoyment that many people derive from visits to these areas is at least partially related to the scenery and environmental conditions created by hemlock forests. Reduced aesthetic values and dangers associated with dead and dying trees as a result of HWA infestation negatively affect this enjoyment and the spiritual connection that some have to these environments. This could lead to indirect economic consequences for the parks themselves or surrounding towns due to reduced tourist dollars.

Concerns about the spread of HWA into currently uninfested areas could also lead to political repercussions associated with the institution of quarantines by individual states to restrict the transport of timber, firewood, and hemlock nursery stock from neighboring states.

3. SUMMARY OF GENETICS KNOWLEDGE FOR EASTERN AND CAROLINA HEMLOCK

- **Hemlock Phylogenetics** Phylogenetic analysis of chloroplast DNA sequences among the nine naturally occurring hemlock species resolved two clades within the genus (Vining 1999; Havill et al. 2008). The two western North America species (*T. heterophylla* and *T. mertensiana*) form one clade and the second includes the Asian species (*T. chinensis*, *T. dumosa*, *T. forrestii*, *T. sieboldii*, and *T. diversifolia*) and Carolina hemlock (*T. caroliniana*) from eastern North American. Eastern Hemlock (*T. canadensis*) is sister to the Asian clade.
- **Carolina Hemlock** The genetic diversity in 15 natural populations of Carolina hemlock has been assessed using amplified fragment length polymorphisms (AFLP). Results suggest that despite its small, disjunct natural distribution, Carolina hemlock has moderate levels of genetic diversity and high amounts of genetic differentiation among populations (Camcore 2006). It appears that populations in the eastern part of the natural distribution are more genetically diverse than those on the western fringes, possibly indicating that these are close to the center of the species' glacial refuge more than 12,000 years ago.
- **Eastern Hemlock** There have been two important studies that assessed levels of genetic diversity in Eastern hemlock using isozymes, one by Zabinski (1992) that

focused on Midwestern populations and a second by Potter et al. (2008) that studied southeastern populations. Both studies found that eastern hemlock exhibited considerably less genetic diversity than most other conifers in the region, but that a greater portion of this small amount of variation is distributed among populations. Potter et al. (2008) found that, among hemlocks in the southeastern US, populations along the eastern periphery and Appalachian interior exhibited higher levels of diversity that those on the western periphery of the geographic range. Both studies support paleobotanical evidence that the refuge area for eastern hemlock during the peak of the Wisconsinian glaciation was located in the Southeast, specifically, to the east of the southern Appalachians. The spatial arrangement of some populations is also likely to influence genetic diversity in this species, particularly when one considers populations that are outliers from the main geographic range and those that occur at different elevations that exhibit phenotype variation. However, these have not been studied in detail.

Genetic variation in adaptively neutral traits such as those that were discussed above often differs from genetic variation in adaptively important traits such as the dates on which growth is initiated or stopped. Relatively little is known about variation in adaptively important traits in eastern hemlock, although a few small studies indicate that at least some exists. Stearns and Olson (1958) reported that hemlock seed collected in Tennessee germinated under different conditions than hemlock seed collected in Indiana or Maine. Nienstaedt and Olson (1961) reported that hemlock seedlings from southern sources tended to continue growing later in the fall than seedlings from northern sources. Eickmeier and others (1974) reported that hemlock grown from seed collected in northeastern Wisconsin were not as tolerant of warm, dry conditions as hemlock grown from seed collected less than 200 miles away in a warmer, drier part of the state.

- **Gaps in Knowledge of Hemlock Genetics** Almost nothing is known about genetic variation in adaptively important traits for either species. This information will be essential for planning hemlock restoration efforts.

  Eastern hemlock population structure and patterns of genetic diversity within the northeastern portion of its US range have not been assessed. Research in this area will be key in developing effective *in situ* and *ex situ* conservation strategies.

4. PLANNING

A. Goals

- o Maintain portions of a relatively small number of populations of mature hemlock *in situ* using insecticides until biological control is feasible.
- o Explore the possibility of maintaining portions of a relatively small number of populations *ex situ* using plantations outside the range of hemlock.
- o Capture enough genetic resources from a relatively large number of populations and individuals in *ex situ* seed collections for use in restoration efforts.

- o Develop biological control methods in order to facilitate reestablish of hemlock populations and maintain them without the use of insecticides.
- o If biological control of hemlock woolly adelgid is not practical, the transfer genes for resistance to HWA to eastern hemlocks may be required.

B. Current Conservation Activities There are currently limited *in situ* conservation activities underway on the Cherokee, Pisgah, Nantahala, Chattahoochee National Forests, and within the Great Smoky Mountains National Park. These NF have selected areas within the native range of hemlock and implemented chemical and biological controls for HWA. These areas have been located in such a manner so that they may exchange pollen and maintain maximum population diversity.

Ex situ gene conservation activities, including identification of existing *ex situ* genetic resources, collection and storage of new populations via seeds or other propagules, and establishment of new gene resource plantations.

- o Identify known domestic and international genetic resources:

Domestic and international *ex situ* genetic resources exist for Carolina hemlock (Camcore 2007). Since 2003, Camcore (NC State University) in cooperation with the USDA Forest Service has been conducting seed collections from all known populations of the species. To date, approximately 22 different populations have been identified and checked in the field. Seeds have been collected from 13 of these and represent samples from 84 mother trees across the geographic range (Figure 3). Populations where seeds have not been collected will be sampled when seed is available. Portions of the collected seed have been sent to cooperators in Chile and Brazil where seedlings are currently be-

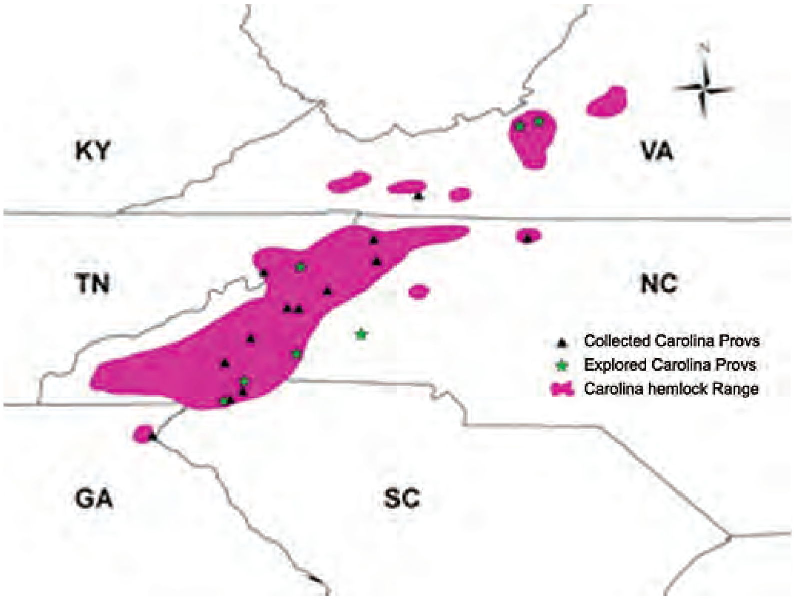

Figure 3. Camcore Carolina hemlock site explorations (shown as stars on map) and seed collections (triangles) 2003 – 2007.

ing cultivated in nurseries for eventual establishment in *ex situ* seed production areas. Seed has also been sent to the University of Arkansas where seedlings are being grown for *ex situ* plantings on the Ozark National Forest. Conservation seed reserves are being held in the Camcore seed bank in Raleigh, North Carolina, and a small working collection of provenance bulks from 3 populations resides at the National Seed Laboratory (NSL) in Dry Branch, Georgia.

In 2005, Camcore began collecting representative genetic material of eastern hemlock for *ex situ* conservation in the 7 state area (Alabama, Georgia, Virginia, Kentucky, Tennessee, North and South Carolina) that comprises the southern portion of the geographic range (Camcore 2007). Priority areas for seed collection to capture the maximum levels of diversity were determined based on a chemical marker analysis from vegetative samples collected from across this 7 state area (Potter et al. 2008). The results from this study were described above in the section on genetics knowledge for species of concern. Restated briefly, this study determined that eastern hemlock exhibits low levels of genetic diversity across the southern portion of its range. Previous *ex situ* conservation efforts by Camcore with other conifers indicate that, for species with low to moderate levels of genetic diversity, a sample size of 6 to 8 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). Given the large distribution of eastern hemlock across the Southeast, the existence of satellite populations that may contain rare alleles, unique adaptations, and phenotypes that vary with elevation, Camcore has decided that sampling seed from 10 mother trees in each of 60 populations (600 trees total) will be sufficient to represent the genetic diversity of the species in the region. Priority will be given to areas of high diversity indicated by Potter et al. (2008) and satellite populations, and seed collections will be stratified by elevation in the Appalachian interior. To date, seed has been collected from 69 mother trees in 14 populations, all located in the eastern portion of the geographic range where high levels of diversity exist (Figure 4). Seed production has been lacking in other parts of the range, most likely due to high HWA impact and severe drought (Camcore 2007). This seed currently resides in the Camcore seed bank where it awaits shipment to Arkansas, Chile, and Brazil for *ex situ* conservation.

Camcore plans to expand its gene conservation work with eastern hemlock to include populations in the northern portion of the geographic range beginning in 2009. Although genetic diversity analyses and population structure have not yet been evaluated, it is assumed that a similar 600 tree strategy will be a good starting point for planning germplasm collections in the 16 state area of Connecticut, Delaware, Indiana, Maine, Maryland, Massachusetts, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, West Virginia, and Wisconsin. The exact number and distribution of mother trees and populations targeted for seed collection can be adjusted to reflect pockets of high diversity and the importance of satellite populations

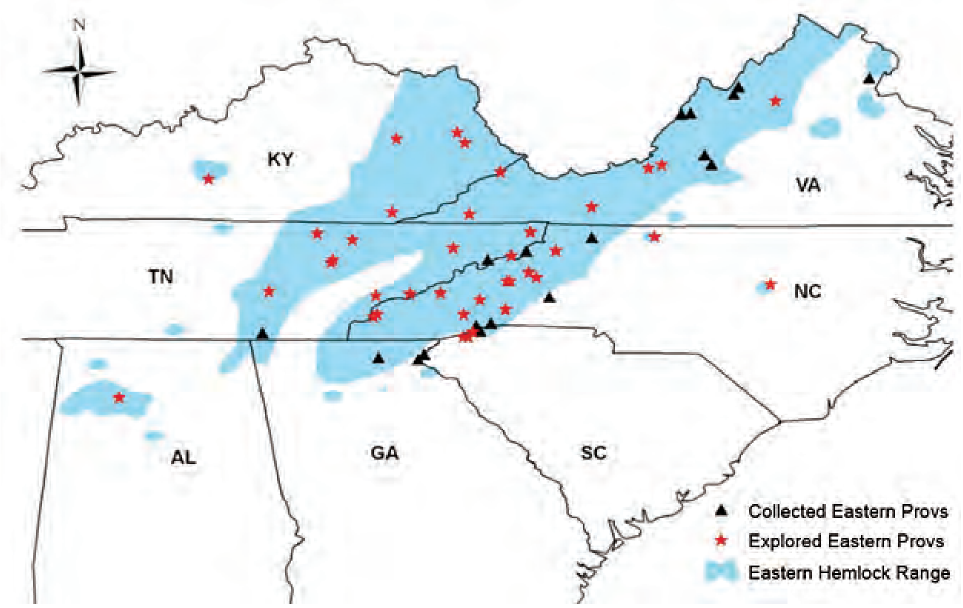

Figure 4. Camcore eastern hemlock site explorations (shown as stars on map) and seed collections (triangles) 2005–2007.

and elevational variation following initial field surveys and completion of diversity studies.

- Establish seed collections within the National Plant Germplasm System through the USFS National Seed Lab (NSL).

Working collections will be maintained at the NSL, whereas long-term collections will be maintained at the National Center for Genetic Resources Preservation in Fort Collins, CO.

Camcore plans to submit both working and conservation collections of hemlock seed to the National Plant Germplasm System when seed is available. Small amounts of Carolina hemlock seed have already been sent to the National Seed Laboratory, and these collections will be expanded now that seedlings for the *ex situ* plantings are established. The small amounts of eastern hemlock seed that have been collected to date are prioritized for the establishment of *ex situ* populations. Camcore anticipates that future improvements in tree health associated with relief from current drought conditions and *in situ* conservation efforts (see above) should increase the level of seed production across the range and the availability of eastern hemlock seed for long-term storage in the National Plant Germplasm System.

- Where possible, establish *ex situ* populations (provenance trials, progeny tests, etc.) of living trees at sites not currently under threat.

Using the FloraMap™ climatic model, Camcore has identified areas in southern Chile and Brazil and the Ozark Mountains of Arkansas that have climates similar to those in populations where Carolina and eastern hemlock seed has already been collected (Jetton et al. 2008a,c). Because hemlocks do not occur naturally in these areas they are ideal for *ex situ* conservation plantations and protecting hemlock genetic resources from HWA. To further refine site selections within these regions that have been identified by FloraMap™, extensive soil sampling and analysis has been conducted in these same hemlock stands to determine optimum soil conditions for seedling establishment (Jetton et al. 2008a). These areas have been targeted for the conservation of Carolina hemlock and southern seed sources of eastern hemlock. Carolina hemlock seedlings are already being grown in forest nurseries at all three locations and the first conservation bank will be planted in Chile in 2008. Additional predictive models from FloraMap™ using data from eastern hemlock populations in the Northeast and Midwest will be necessary to determine if these same regions or others will be most suitable for *ex situ* conservation of northern seed sources.

- Where practical, develop a breeding program to produce locally-adapted, resistant stock through: traditional recurrent selection, interspecific hybridization with backcrossing, and/or genetically engineered resistance or tolerance.

There is little indication that natural HWA resistance exists in either eastern or Carolina hemlock, although there is some indication that the latter is more tolerant of the adelgid (Jetton et al. 2008b) and that this trait may vary among provenances (Camcore 2006). Provenance progeny tests planted within the generally infested region can be used to evaluate this and determine if selection for adelgid tolerance will be possible.

The most promising approach for breeding locally adapted HWA resistant stock might be through interspecific hybridization of eastern and Carolina hemlocks with adelgid resistant hemlock species from the Pacific Northwest and Asia and then backcrossing to the pure species. An approach similar to the American chestnut program would be appropriate. Attempts to hybridize hemlock species have been successful for *T. caroliniana* x *T. chinensis* crosses and the F1 offspring are highly adelgid resistant (Bentz et al. 2002). To date, attempts at other hemlock hybrid combinations have been less successful, but might improve with the use of the broad genetic base made available by current germplasm conservation efforts.

- Establish needed reforestation stock using seed orchards, somatic embryogenesis, or other appropriate technology

When HWA resistant genotypes are available seed orchards and breeding facilities will be developed to produce reforestation stock. Multiple facilities distributed throughout the range of hemlock will be necessary to breed and multiply hemlocks locally adapted to the surrounding areas. Again, a design similar to the research farm system utilized by the American chestnut program is a good guide. However, given the cost of HWA management and the very long breeding cycle of hemlocks (20-30 years per generation), an effort should be made to establish indoor (greenhouse) accelerated breeding houses similar to those used for breeding western hemlock in the Pacific Northwest (Ross et al. 1986; Eastham and Ross 1988).

C. Research and Development Needs

- Studies of genetic variation in adaptively important traits to guide species restoration work, particularly in conjunction with climate change.
- Climatic modeling to better understand of how climate change will affect the current distribution of eastern and Carolina hemlocks in the southeastern US.
- Additional studies on the genetic diversity of HWA in the eastern US to further pinpoint sources of introduction and if the current infestation resulted from a single or multiple introductions.
- Conduct field and greenhouse studies to understand the influence of environmental conditions on the density-dependent feedback between HWA and the host tree.
- Field reconnaissance to evaluate population structures and soil conditions in eastern hemlock stands throughout the northern portion of the geographic range.
- Evaluation of molecular markers within eastern hemlock throughout the northern portion of the geographic range and in disjunct populations such as Hemlock Bluffs in Cary, NC and Mammoth Cave National Park in Kentucky for the presence of rare alleles. This will be critical to identifying pockets of diversity and rare alleles for *ex situ* seed collections.
- Develop breeding techniques for indoor orchards for eastern and Carolina hemlocks. Accelerated breeding could be a key component to the production of HWA resistant stock of reforestation.

C. Rehabilitation and restoration

- Implement strategies to rehabilitate damaged ecosystems:
- Utilize hazard rating systems to prioritize where efforts are most needed and would be most successful;
- Utilize silvicultural methods that enhance natural regeneration opportunities, manage competing vegetation, and create planting opportunities;
- Plant locally-adapted resistant stock (if available);
- Reforest denuded areas;
- Assess efficacy of rehabilitation and restoration strategies over time and adjust activities as need to ensure sustainability;
- Plant diverse species mixes to maintain species diversity and ecological buffering capacity.

D. Policy actions

- o Develop and implement appropriate policies to institutionalize genetic conservation. Include:
    - – Networking – coordinate planning and actions with partners;
    - – Governance – implement effective policies and procedures;
    - – Organization – develop efficient organizational structures;
    - – Advisory groups – engage stakeholders in developing solutions;
    - – Legal considerations and compliance - NEPA, cooperative agreements, intellectual property issues, Executive Orders, ESA, etc.;
    - – Rule-making for management activities – develop and implement appropriate rules and regulations.
- o Consider what needs to be achieved in protection status and establish benchmarks to measure progress.

E. Communication

- o Develop communication plans for effective outreach and information dissemination:
    - – Products – publications, brochures, websites to provide information on genetic conservation programs;
    - – Roll-outs – plan effective public and Congressional information campaigns;
    - – Database(s) – insure that information is available and accessible in information systems such as the Germplasm Resources Information Network (GRIN);
    - – Awareness – build awareness through effective outreach and education efforts.

F. Resources

- o Determine resources need to implement effective conservation plans:
    - – Funding
        - – Consider opportunities for cost-sharing with partners,
        - – Frame high, medium, and low funding level options with clear descriptions of benefits and consequences of each level;
- o Human resources
    - – Identify staffing needs,
    - – Identify volunteer opportunities;
- o Facilities/equipment.

5. MONITORING AND ASSESSMENT

- Develop a Quality Assurance/Quality Control Plan (QA/QC) to monitor and assess progress of genetic conservation plans:
    - Internal review – develop procedures for internal monitoring and assessment of progress towards achieving goals and objectives of genetic conservation plans.
    - External review – develop plans for external reviews of genetic conservation plans.
    - Adaptive management – plan for re-evaluating plans through feedback loops to account for new threats or changes in climate or technologies;
    - Accountability – develop appropriate reporting and accounting procedures.

CONCLUSION

Genetic resources are critical to the maintenance of ecosystems that are productive, sustainable, and resilient to new stresses such as insects, pathogens, and climate change. Developing effective genetic conservation plans for threatened species requires coordinated, consistent approaches to planning and implementation of effective strategies and actions. This general framework will help ensure that all facets of genetic conservation are considered in the development of species-specific plans. The USDA Forest Service will work with partners to implement these plans.

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INVESTIGATING HOST RESISTANCE USING SCANNING ELECTRON MICROSCOPY

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ABSTRACT

This study uses scanning electron microscopy (SEM), a surface-imaging technique, to investigate feeding behaviors of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. Understanding feeding behavior directly relates to the pest-host interactions which can elucidate resistance mechanisms in hemlocks. Salivary sheath material, a viscous product of the salivary glands that hardens upon extrusion, was imaged; insertion site is on the adaxial side of the needle, below the abscission layer and no major differences were noticed between the western population HWA and eastern population HWA. Imaging of stylets within the plant tissue was unsuccessful using SEM and further investigations will incorporate other techniques.

KEYWORDS

Adelges tsugae, feeding behavior, host resistance, scanning electron microscopy

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, has caused massive mortality of hemlocks in eastern North America over the last few decades. After becoming infested, trees can die in as little as four years (McClure 1987, 1991). However, not all trees succumb to the infestations. The seven hemlock species occurring in the native range of HWA exhibit varying levels of resistance (Table 1). This resistance is attributed to innate resistance, a complex of natural enemies, and the scattered distribution of hemlocks (McClure 1992, Montgomery and Lyon 1996). Until recently, literature has stated that eastern hemlock (*Tsuga canadensis* (L.) Carriére) and Carolina hemlock (*T. caroliniana* Engelm.) are the only hemlock species susceptible to HWA. However, following massive devastation of hemlock stands throughout the Appalachian Mountains, reports of individuals and stands surviving or avoiding infestations altogether are surfacing. There is discrepancy between environmental conditions and innate resistance being the cause of such cases. Investigations into mechanisms of resistance are prompted by two observations: resistance varies between the nine species of hemlock that occur worldwide, and resistance varies within eastern and Carolina hemlocks. Therefore, the previously accepted notion of comprehensive susceptibility of eastern and Carolina hemlock is becoming uncertain.

Table 1. Resistance levels and native ranges of hemlocks.

Hemlock species	Range	HWA resistance
Eastern hemlock *Tsuga canadensis*	Eastern North America	Varies (Susceptible to moderately resistant)
Carolina hemlock *T. caroliniana*	Southern Appalachians	Varies (Susceptible to moderately resistant)
Chinese hemlock *T. chinensis*	China	Highly resistant
Northern Japanese hemlock *T. diversifolia*	Northern Japan (high elevations)	Resistant
Western hemlock *T. heterophylla*	Western North America	Moderately resistant
Mountain hemlock *T. mertensiana*	Western North America	Moderately resistant
Southern Japanese hemlock *T. sieboldii*	Southern Japan (low elevations)	Moderately resistant
Himalayan hemlock *T. dumosa*	Himalaya Mountains	Moderately resistant
Forrest's hemlock *T. forrestii*	China	Moderately resistant

Host plant resistance is an important part of agricultural integrated pest management (IPM) and it has been long accepted that tree resistance should be incorporated into forest management as well (Stark 1965, Larsson 2002). A fundamental part in understanding resistance is a thorough knowledge of pest-host interactions, of which insect feeding behavior is a major component (Painter 1951, Davis 1985). Studying the feeding behavior of HWA is an essential stepping stone in reaching a resistant variety of hemlock.

A large part of information regarding the feeding behavior of HWA is based on aphids as a model. Aphids and HWA have similar mouthpart form and function; four stylets (two maxillary and two mandibular) make up the stylet bundle. This stylet bundle forms two channels, the food and salivary canals, and is inserted into the plant to feed. Both insects produce watery saliva, extruded as an aid in feeding, and sheath saliva, which surrounds the stylet bundle (Miles 1999). However, unlike aphids, HWA is stationary and do not feed on phloem. Rather, HWA feeds on the ray parenchyma cells, starch-filled storage cells in the xylem (Young et al. 1995). It is unknown whether extra-oral digestive enzymes, such as amylase, are used to digest these starches prior to ingestion. The sheath material, which is extruded in a bead-like manner as the adelgid explores the host surface, likely plays multiple important roles: 1) to assist in the sucking mechanisms by closing off the ingestion mechanism, preventing leaks, 2) to provide a mechanical advantage in stylet bundle stabilization providing leverage-fulcrum points, 3) to allow easy stylet bundle reinsertion following each molt, 4) to provide better movement through or between cell walls, and 5) protect or insulate the insect's mouthparts against plant defenses. The sheath material is very stable and remains on and in the plant when the insect is removed.

Analysis of stylet pathways may help elucidate mechanisms of resistance (Spiller et al. 1985). Relationships of stylet pathways were also shown to be useful in assessing relative susceptibility of host plants (Cohen et al. 1996, Cohen et al. 1998). Relative susceptibility of different cultivars of cotton was also demonstrated in whiteflies by studying patterns of stylet pathways (Chu et al. 1999). In chinch bugs feeding on resistant cultivars of St. Augustine grass, the sheath material was more abundant than in susceptible cultivars (Rangasamy et al. 2009). In another study, aphids took three times as long to locate internal feeding sites in resistant soybean cultivars (Crompton and Ode 2010). It appears the detection of internal feeding sites is more difficult in resistant plants for these hemipteran insects, evidence which can provide insights to HWA feeding studies. Our hypotheses are that the stylets of HWA will branch more within resistant hemlocks and physical and/or chemical differences in the host surface will contribute to HWA's host selection.

METHODS

Infested hemlock material was collected from Laurel Springs and Crossnore, NC. Branches were kept in buckets of water in an incubator kept at 16 °C and 80% humidity. Small sections of infested branchlets were freeze-fractured, freeze-dried, and mounted for scanning electron microscopy (SEM) observation. SEM is a valuable tool capable of high resolution at high magnifications. It is helpful in observing external stylet habits; fracturing techniques were used in combination as an endeavor to reveal internal stylet movement.

Material from the western population of HWA was sent on ice via overnight shipping by Glenn Kohler (Washington State Department of Natural Resources). Infested branches were kept in quarantine at the Beneficial Insects Lab in Cary, NC. Infested material was fixed in 3% glutaraldehyde prior to removing from quarantined area. Material was post-fixed in 1% osmium tetroxide, dehydrated in a critical point dryer, and prepared for SEM observation.

RESULTS AND DISCUSSION

Several interesting finds were noted at the high magnification SEM provided. Morphological differences between the crawler stage and the adult stage were observed (Figure 1). Aside from being smaller (~0.5 mm), crawlers lacked wax pores, had prominent antennae, and the stylet bundle retracted. Conversely, adults were approximately 1 mm in size, showed prominent pores for wax extrusion, had reduced antennae, and stylets extended. Reduction in antennae is likely related to its transition from the host-seeking stage of the insect. Future work should be done to determine sensory function of the antennae, as they have distinctive morphological characters, such as a pit with finger-like projections and long setae (Figure 2).

Insertion points of the stylet consistently occurred below the abscission layer of the needle on the adaxial side of the needle (Figure 3). This is consistent with the work done by Young et al. (1995). It was also observed that sheath material was produced prior to the stylet bundle's contact with the host surface. This external production allows us to

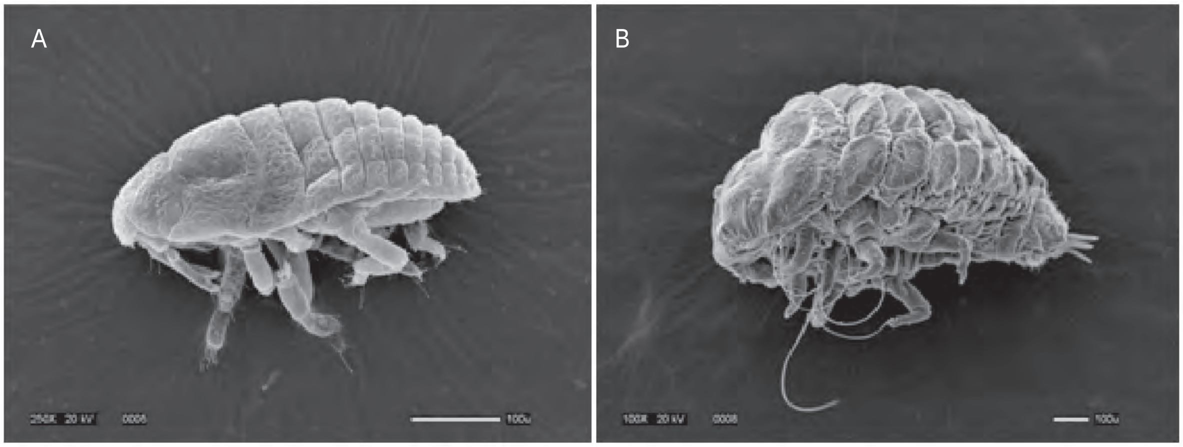

Figure 1. A) crawler; B) adult. Scale=100µ.

Figure 2. Tip of antennae. Scale=1µ.

determine the size and structure we expect to find within the plant material. A break in the sheath material reveals the internal stylets and the bumpy texture of the material is an indicative character (Figure 4).

HWA from the western population were slightly larger and rounder than the eastern population, but there were no major morphological differences noticed. Insertion site between the two populations were alike. Further investigations will include observations of wood anatomy of western and mountain hemlocks.

Parenchyma cells were imaged, but sheath material within the plant tissue was never observed within them. Further investigations into host resistance and feeding behavior will include clearing of hemlock material and staining of sheath material followed by light and confocal microscopy, further SEM of plant material using cryo-SEM, and anatomical studies of the wood of different hemlock species. In addition to observing physical characteristics of different hemlock species, chemical components of the foliar wax will be studied.

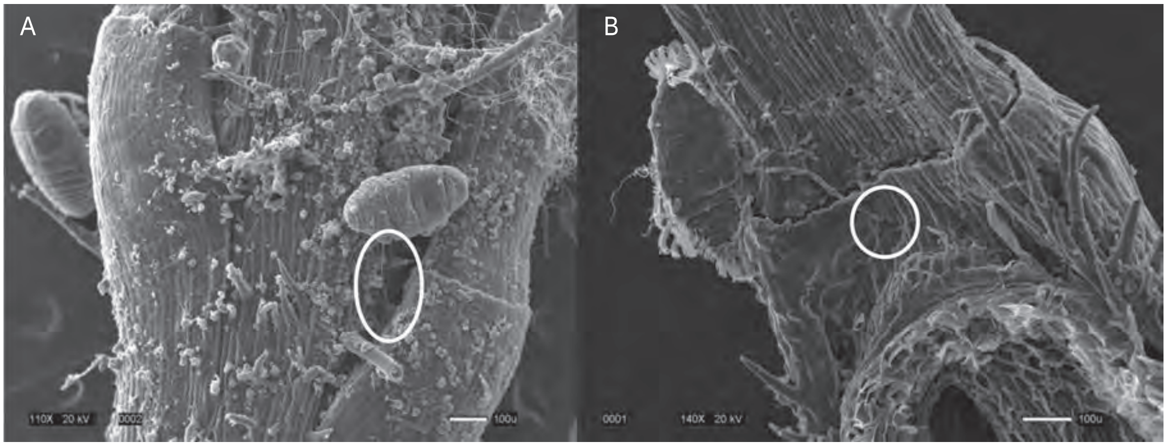

Figure 3. A) Adelgids settled on hemlock, circle indicates stylets leading behind needle to insertion point; B) adaxial side of needle, sheath enclosed stylets leading to insertion point, indicated by circle. Scale=100µ.

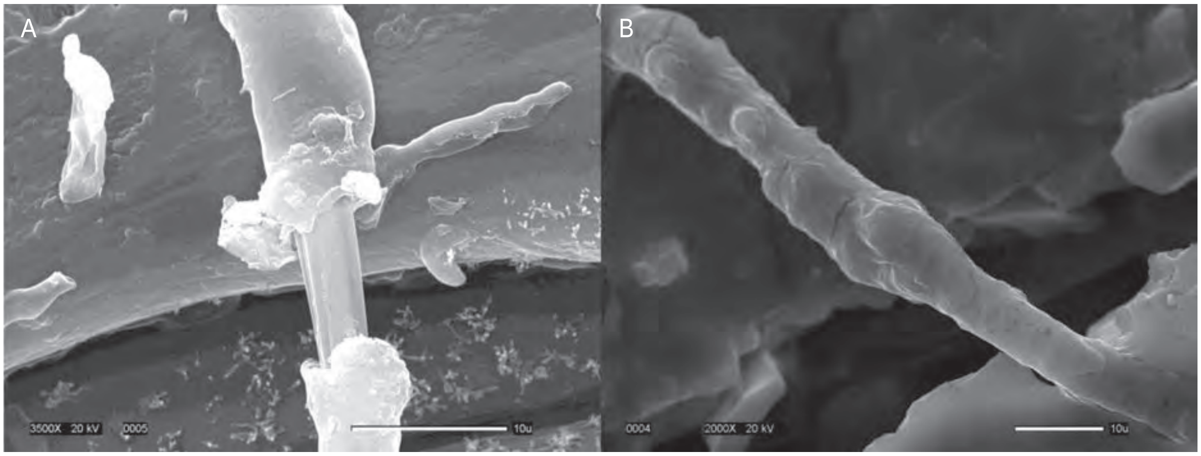

Figure 4. A) Stylets revealed by a break in the salivary sheath; B) uneven, bumpy texture of the salivary sheath. Scale=10µ.

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PRESENTATIONS: PHYSIOLOGICAL IMPACTS AND SPREAD OF HEMLOCK WOOLLY ADELGID

THE EFFECT OF HEMLOCK WOOLLY ADELGID (HWA) INFESTATION ON WATER RELATIONS OF CAROLINA AND EASTERN HEMLOCK: CAN ECOPHYSIOLOGICAL INVESTIGATION OF TREE WATER RELATIONS IMPROVE SILVICULTURAL MANAGEMENT OF THE HWA?

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INTRODUCTION

In eastern North America, hemlock woolly adelgid (HWA; *Adelgestsugae*Annand) is an exotic insect pest from Asia that is rapidly decimating native eastern hemlock (*Tsugacanadensis*(L.) Carr.) and Carolina hemlock (*Tsugacaroliniana*Engelm.). Extensive research has been committed to the ecological impacts and potential control measures of HWA, but the exact physiological mechanisms that cause tree decline and mortality are not known. In Fraser fir (*Abies fraseri*(Pursh.)Poir.), it is known that balsam woolly adelgid (BWA; *Adelgespicea*Ratz.) produces abnormal xylem in response to infestation (Arthur and Hain 1985) and hemlock trees may be reacting to infestation in a similar manner. We hypothesize that this abnormal xylem obstructs water movement within the trees, causing hemlock trees to die of water-stress.

In this study, water relations within 15 eastern and Carolina hemlock trees were evaluated to determine if infestation by HWA was causing waterstress. Water potential, carbon-13 isotope ratio, stem conductivity, and stomatal conductance measurements were conducted on samples derived from those trees. In addition, branch samples were analyzed for possible wood anatomy alterations as a result of infestation (Walker-Lane 2009). Wood anatomy of the branches provided evidence that infested hemlocks are indeed experiencing abnormal wood production in the xylem, which led to reduced water transport capacity in a similar way as in Fraser fir (Hollingsworth et al. 1991). In addition, pre-dawn branch water potential measurements were more negative in infested trees than in non-infested trees. These results indicate that infested eastern and Carolina hemlock are experiencing drought-like symptoms (Arthur and Hain1986), and that plant uptake of soil water by roots is somehow compromised. However, those symptoms are not due to soil moisture limitations but presumably to a hydraulic decoupling at the soil-root interface. Moreover, carbon isotope ratios of the branches were more positive for infested trees, while stomatal conductance was lower in infested trees. This indicates that the significant decrease in reduced conducting sapwood area, terminal branch growth and leaf area in infested trees were sufficient to influence sap flux and whole-tree water use. A soil-plant water transport model (Sperry et al. 1998) showed that even small summer drought would induce total loss of water transport capacity and would induce irreversible hydraulic failure of the conducting tissue, causing tree death.

We conclude that changes in xylem anatomy and leaf area in response to HWA decreased the capacity for water uptake and transport, placing infested trees at a compounding competitive disadvantage (relative to surrounding trees) to assimilate water and carbon, and acquire soil resources. These physiological responses reduced tree capacity to resist further infestation, eventually leading to tree mortality. However, it is not clear how these physiological stressors interact with environmental conditions (e.g., drought, high irradiance, high evaporative demand) to cause tree death. It has been reported that infested trees die more quickly in warmer and drier locations (Ford and Vose 2007). Our study is consistent with these reports showing a link between HWA and accelerated mortality from drought stress. We predict that HWA will cause higher mortality in the Southeast in warmer and drier locations, which will be aggravated by periodic droughts that further stress infested trees. Possible increase in drought frequency due to climate change is then also predicted to increase HWA mortality. In order to manage HWA and reduce its impact to hemlock resources we propose that forest resource managers plant trees in cooler and wetter locations and if possible on soil with high moisture capacity and the greatest amount of plant available water such as silt loam soil.

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NATIVE COMMUNITIES ON AN EXOTIC TREE: THE ARTHROPOD COMMUNITY ON *TSUGA CHINENSIS* IN NEW ENGLAND; A POTENTIAL ECOLOGICAL SURROGATE FOR *T. CANADENSIS*

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ABSTRACT

Invasive species impact forested systems both directly, by altering the abundance and distribution of host species, and indirectly, by altering the community structure and biodiversity of impacted stands. While addressing the direct effects is typically the dominant focus for management efforts, the management of indirect impacts is likely to grow in importance as forests are increasingly managed for the provision of multiple ecosystem functions, properties, and services. Here we evaluated components of the arthropod biodiversity associated with eastern hemlock threatened by the hemlock woolly adelgid, an invasive insect herbivore in the eastern United States. We also surveyed the arthropod communities associated with white pine (a commonly co-occurring native conifer), and Chinese hemlock (*Tsuga chinensis*), a potential ecological surrogate for eastern hemlock. Chinese hemlock (*Tsuga chinensis*) has been proposed as a potential surrogate as it is shade tolerant and suited to the conditions of the eastern U.S. Preliminary findings show that there are approximately 1000 arthropod morphospecies associated with native eastern white pine and eastern hemlock. About half of these morphospecies were found only on the eastern hemlock, suggesting these hemlocks host a large component of biodiversity, and that the community found on hemlock is not likely to be maintained by the continued presence of white pine. The addition of Chinese hemlock to the stand captured approximately half of the species otherwise found only on eastern hemlock, indicating this species may "buffer" the biodiversity of stands, however, little is known about the other ecosystem consequences of this non-native hemlock species. More work is needed to evaluate its potential use as a surrogate species for the maintenance of ecosystem properties in the forests of the eastern United States.

INTRODUCTION

Although the hemlock woolly adelgid was first documented in the eastern United States as early as 1951 (Gouger, 1971), little effort was put into its control beyond landscape settings. Recognition of the adelgid as a landscape-scale threat however changed in the late 1970s as the adelgid moved into naturally forested systems where it led to increased hemlock mortality and changes in stand structure and ecosystem function (Orwig and Foster, 1998; Orwig and Foster, 2000; Kizlinski et al., 2002; Orwig, 2002; Orwig et al., 2008).

Current efforts to manage the adelgid have focused on mitigating the direct impacts of the adelgid (i.e. hemlock mortality) through the use of chemical and classical biological control. However, less effort has been placed on addressing the indirect effects of hemlock loss and adelgid management efforts, particularly the potential impacts of both on the arthropod communities associated with hemlock.

One of the potential tools for mitigating the ecological effects of the loss of hemlock from eastern forests is the use of a surrogate species, a species which can provide some of the ecological roles filled by the threatened native species. Among hemlock species, Chinese hemlock (*Tsuga chinensis*) has the potential to act as a surrogate, as the species is well suited to conditions in the eastern United States, is shade tolerant, grows quickly, and is resistant to the hemlock woolly adelgid. However, little is known about the potential ecological role Chinese hemlock might play in the forests of eastern North America.

To evaluate some of the potential ecosystem systems and properties Chinese hemlock could provide, we took advantage of Chinese hemlocks planted in a New England forest to address three key questions: First, what are the biodiversity and community structures associated with eastern hemlock which are potentially threatened by the hemlock woolly adelgid? For this study, we focused on canopy arthropods as a model system to evaluate community effects. Second, does the presence of white pine, a commonly co-occurring conifer species, host the arthropod species found on eastern hemlock, potentially providing a buffer against the loss of biodiversity found on eastern hemlock? Third, what arthropods are found in association with Chinese hemlock grown in forested settings, and can this species support the arthropod community threatened by the potential loss of eastern hemlock?

METHODS

To evaluate the three questions described above, arthropods were collected from 12-14 trees for each of three species, eastern hemlock (*Tsuga canadensis*), Chinese hemlock (*T. chinensis*), and eastern white pine (*Pinus strobus*) growing in forested settings in the Yale-Myers Forest near Union Connecticut. The Yale-Myers Forest is a working, teaching and research forest owned and managed by the Yale School of Forestry and Environmental Studies.

The eastern hemlock and white pine surveyed were selected from naturally regenerated trees growing amongst Chinese hemlock planted in 2003. White pine and eastern hemlock trees were selected to match the size and apparent age of the planted Chinese hemlock to avoid ontogenetic effects. Arthropods were collected from three branches on each tree once per month for 12 months using a batting-and-beating method. Collections were started in June of 2008, and continued through June of 2009. The study trees were not surveyed in December of 2008 due to weather. The bagging-and-beating method consists of placing a cloth bag over a branch, enclosing a branch of about 1 meter. The bag is then held closed near the base of the branch (near the trunk), while the branch is struck by hand 10-15 times. The bag is then removed, and the contained arthropods are collected using forceps and an aspirator. Collected material was returned to the lab where it was sorted to morphospecies using a dissecting microscope, stored in ethanol (70%

ETOH), and frozen. Examples of each morphospecies were compiled to produce a reference collection. Previous studies have shown that the use of morphospecies is suitable for the evaluation of biodiversity and community composition (Oliver and Beattie 1996, Siemmens et al. 1998), and the term species is hereafter used for simplicity.

Arthropod communities were evaluated to determine three measures of biodiversity including alpha diversity (the average number of species per tree), beta diversity (species turnover, or the detection of new species as new trees are surveyed), and gamma diversity, the (estimated) number of species in the stand. Monthly measures of mean alpha diversity were compared among species using an ANOVA followed by Tukey's for post-hoc comparisons. Beta diversity was estimated by generating rarefied species accumulation curves. Gamma diversity was estimated by fitting the data to a Michaelis-Mentin Function using EstimateS V8.0 (Colwell 2006). Community composition was evaluated using Non-Metric Multidimensional Scaling in the software program PC-Ord V5 (McCune and Mefford 2006), statistical differences were evaluated using a Multiresponse Permutation Procedure.

RESULTS

Arthropod abundance on white pine and eastern hemlock varied seasonally, with peak abundances occurring in the summer months (Figure 1A). Although abundances were very low in the winter, arthropods continued to be detected despite field temperatures as low as 17 °C. Monthly measures of alpha diversity (mean number of arthropod species per tree) also demonstrated a strong seasonal component, with peak species richness values in June, July and August (Figure 1B). During the months of July and August, arthropod species richness was significantly higher on eastern hemlock when compared to white pine.

Species turnover was slightly higher in eastern hemlock when compared to white pine (Figure 2), suggesting a higher overall community complexity. Similarly, estimated gamma diversity was higher for eastern hemlock, with a projected 1302 species, while the projected gamma diversity for white pine is 1049 species

Community composition differed between the hemlock and white pine (MRPP $p < 0.05$, Figure 3). This difference in composition indicates that although species richness was higher on the hemlock, the community found on white pine does not simply represent a subset of the species on hemlock, though there is some overlap as shown by Figure 4.

Seasonal patterns of arthropod species richness and abundance on Chinese hemlock was similar to that shown by both white pine and eastern hemlock (Figures 1A and 1B). Total arthropod abundances on Chinese hemlock were similar to both native species (Figure 1A), and species richness did not differ from either species statistically. However, community composition did differ statistically from both, indicating Chinese hemlock, white pine, and eastern hemlock host distinct communities (MRPP $p < 0.05$, Figure 5), though there is some overlap among the three species and between species pairs (Figure 5).

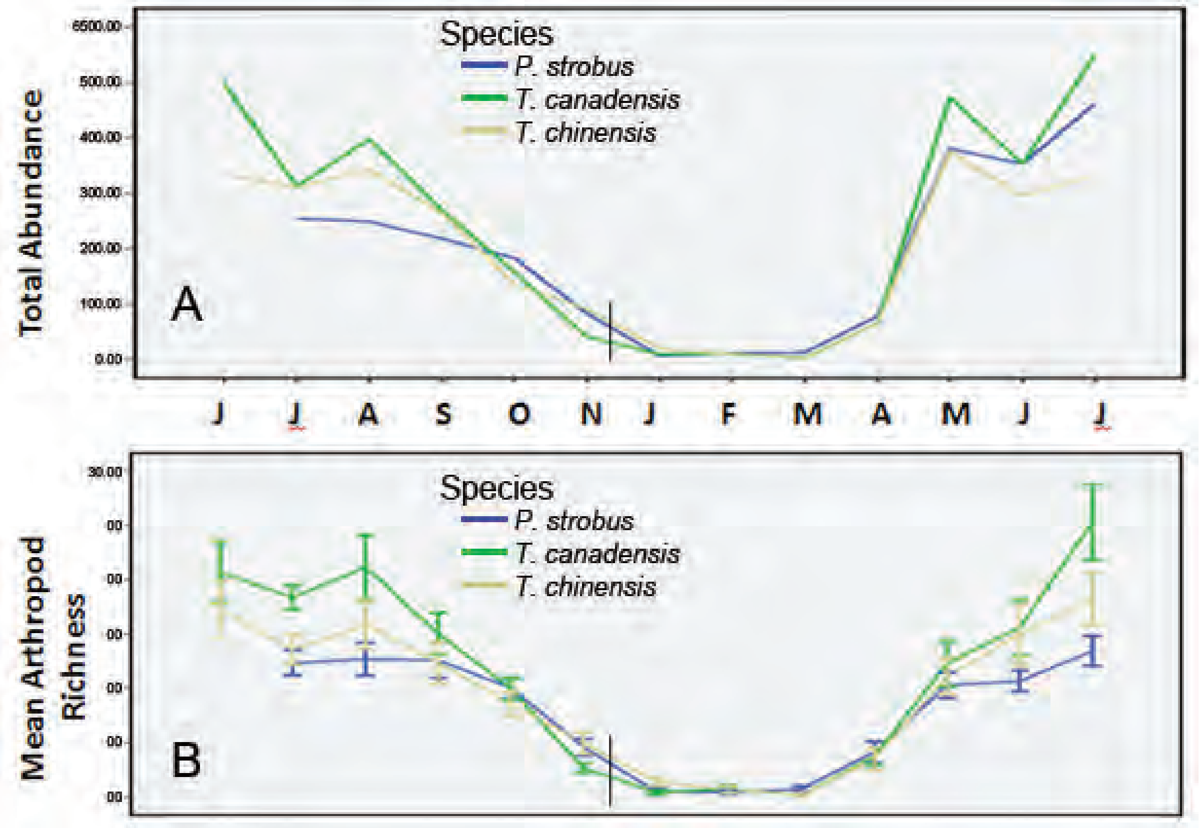

Figure 1. A) Monthly arthropod abundance values for the period of survey. Note the missing month of December, which was not collected due to weather. B) Monthly mean species richness for each of the three conifer species. Statistical differences are limited to both June months, and August, at which time only the white pine and eastern hemlock differ significantly.

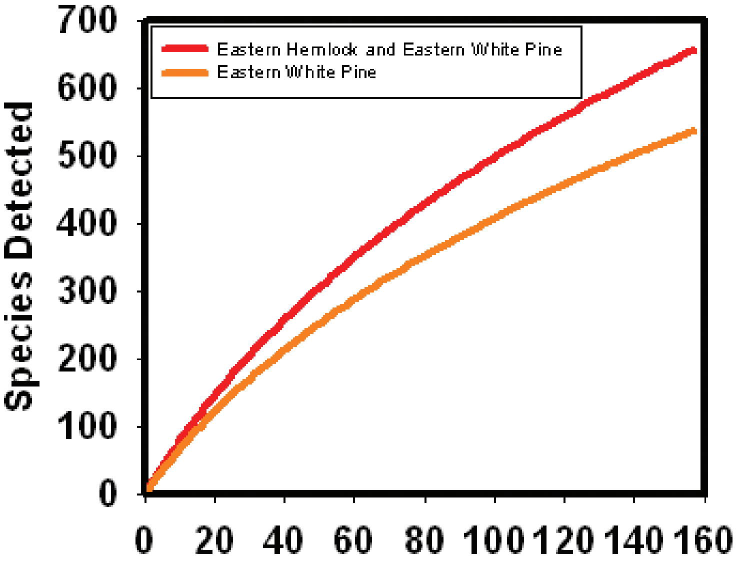

Figure 2. Species accumulation curves for eastern white pine and Eastern hemlock. The x axis denotes survey intensity measured as tree-months surveyed.

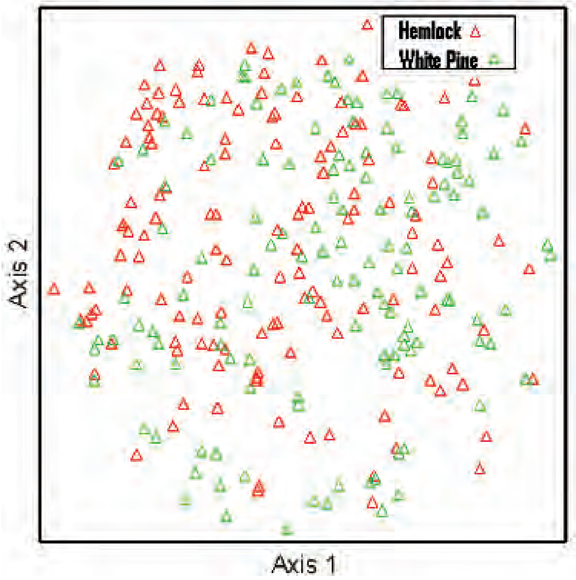

Figure 3. Non-metric multidimensional scaling ordination of the arthropod communities associated with hemlock, white pine, and Chinese hemlock. MRPP analyses demonstrate a statistically significant difference among the three species (MRPP $p < 0.05$).

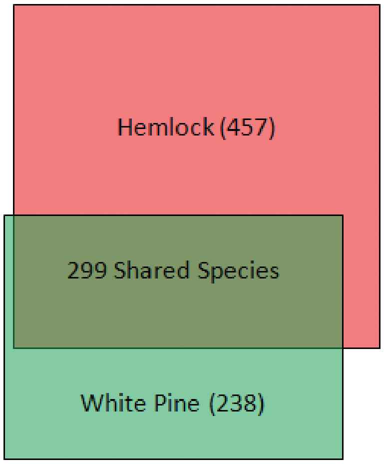

Figure 4. Venn diagram indicating the number of morphospecies found only on eastern hemlock, only on white pine, or both tree species.

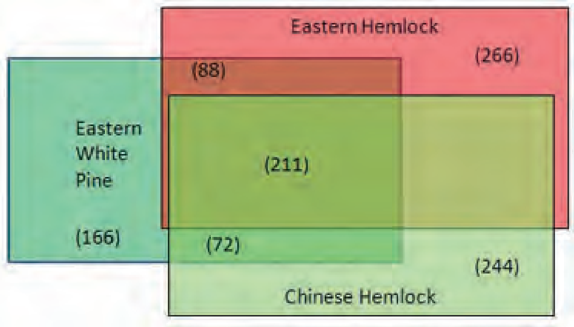

Figure 5. Venn diagram indicating the number of morphospecies found only on eastern hemlock, only on white pine, only on Chinese hemlock, and combinations of the three species.

CONCLUSIONS

The survey of eastern hemlock and white pine growing in northeastern forests yielded nearly 1000 morphospecies. Of these, nearly half were detected only on eastern hemlock, while one-fourth were collected only on white pine. The remaining one-fourth of the overall community was found on both species. Overall, this survey suggests the hemlock stands of eastern North America support a diverse arthropod community, and that a large portion of this community may be limited to eastern hemlock. The loss of hemlock from these stands as a result of infestation by the hemlock woolly adelgid may therefore pose a serious risk to the overall biodiversity of these forested systems. The continued presence of white pine may provide continued ecological support for only half of the surveyed arthropods.

The addition of Chinese hemlocks resulted in the discovery of two key patterns. First, the addition of Chinese hemlock reduced the number of species found only on eastern hemlock to roughly one-fourth of the total number of species. Based only on this very simple analysis, the addition of Chinese hemlock may provide some ecological surrogacy by providing resources for half of the canopy arthropod diversity that might otherwise be lost with the removal of eastern hemlock. The second key finding is that the inclusion of Chinese hemlock in the survey yielded 244 additional species not found on either of the native trees. Although the identity of these species is not yet known (identifications are ongoing), it is likely that the majority of these are native to North American forests, and presumably are not dependant on the Chinese hemlock (with which they lack an co-evolutionary history). The ecological roles of these species (herbivores, predators, parasites, or simply "tourist" species) also remains unknown and merits further study.

Overall, the described survey provides a small snapshot of some of the arthropod diversity found on conifers in northeastern forests, and highlights the unique role eastern hemlock plays in supporting that diversity. The potential reduction or removal of eastern hemlocks from these stands through either infestation by *Adelges tsugae* or through salvage logging practices may threaten the overall biodiversity of these stands, as the loss of the hemlock may lead to the loss of hundreds, and potentially thousands (when the roles of landscape size and heterogeneity are incorporated) of species.

The concept of ecological surrogates as a tool for the maintenance of ecological properties and services is a new one, and there remain a number of critical questions that must be addressed before the approach is applied. With those caveats in mind, there is the potential for Chinese hemlock, which is resistant to infestation by the hemlock woolly adelgid, to provide support for some of the canopy diversity that might otherwise be lost with the removal of eastern hemlocks. This approach may also have potential utility when combined with ongoing management efforts such as chemical and biological control. For example, the use of chemical controls has demonstrated a high efficacy against the adelgid. However, the insecticides currently being used to control the adelgid are systemic and broad spectrum with the potential to impact a large component of the arthropod community. The addition of pesticide-free Chinese hemlocks to chemically treated areas may provide a refuge for some components of the arthropod community while eastern hemlocks are chemically treated. However, little is known about the potential impacts of introducing a non-native hemlock species into the forested stands of eastern North America, and further work remains to be done to evaluate the potential costs and benefits associated with the use of an ecological surrogate.

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POSTERS

DEVELOPING PROCESS CONTROL AND QUALITY CONTROL IN REARING SYSTEMS FOR HEMLOCK WOOLLY ADELGID PREDATORS

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ABSTRACT

We have begun development of process and quality control systems for *Sasajiscymnus tsugae* and *Laricobius nigrinus* mass rearing programs. First, we established the desirable qualities of predators considered highly fit for managing HWA populations after release: survival, search capacity, voracity, fecundity, and longevity. We are using these standards as a basis then establishing secondary standards that are highly correlated with the primary qualities of fitness: weight, sex ratio, protein content, lipid content, carbohydrate content, and free radical scavenging capacity. We are trying to tie output quality of the predators with the processes involved in rearing; thus we are tying **process control** to **quality control**. Next, we developed a preliminary cause and effect diagram (fish bone diagram) to give an overview of the rearing process factors that were pertinent to potential loss of quality (diet, environment, soil {for *Laricobius*}, microbial factors, and handling). Using the Pareto model and past rearing experience, we focused preliminary attention to diet quality and soil. For diet quality, we started with a semi-objective rating system of prey quality (abundance and estimates of prey nutritional value in a scale of 0-5 at 0.5 increments). We are currently tying these estimates to weight per prey item, protein, lipid, carbohydrate, and free radical scavenger contents of prey to enhance objectivity of prey quality assessment. We have also been measuring soil characteristics in soils from under hemlocks and artificial soil mixtures used in mass rearing labs. The characteristics that we are measuring are soil texture profiles, pH, water activity, and organic content. We have also performed tests of *Laricobius* larval (pre-pupal) responses to different soil textures.

Introduction. Several species of exotic and non-indigenous predators are being mass-reared for control of the exotic pest hemlock woolly adelgids. These predators are considered one of the most important weapons in our arsenal against the serious pests that are destroying our eastern hemlocks and threatening Carolina hemlocks; therefore rearing high quality predators in an economically efficient manner is of great importance to forest managers. The laboratories doing the mass-rearing use fairly standardized techniques due to careful coordination efforts by USDA, Forest Service program coordinators. However, because of differences in locations, resources, and personnel, there are some variations in the rearing process, and variations within systems and between systems inevitably lead to variations in quality (Lorraine and Bruzzone 1992 and Cohen 2003). Therefore, our team was funded by the USDA, Forest Service to develop a set of organizing and coordinating protocols for standardizing and improving the process of mass-rearing of HWA predators and helping the rearing groups develop uniform, logical, and objective standards of quality. We make the distinction between process control (PC) and quality control (QC) as the determination if the steps in the process of product production are within acceptable boundaries or limits vs. the outcome of the process or the product itself being of suitable quality to do the job for which it was intended. In our case, we are trying to assure that the steps taken in mass-rearing the predators of HWA are performed within a defined range (temperatures, food quality, soil characteristics, lowest possible instances of disease, intervals of feeding, cage cleanliness, etc.). At the end of production, our goal is to assure that the predators are produced not only in desirable numbers but that they are sufficiently fit so that when released, they will have a search capacity, longevity, voracity, and persistence in infested sites so that they will control outbreaks of HWA.

The optimal practice in development of process control and quality control would be to develop the standards as a function of extensive collection of relevant data as the process is being initially research and developed. However, most often PC and QC are developed after the fact of working out the procedures, and that is the challenge of the current rearing situation, to go back to the rearing laboratories and collect data that will help us retrofit a PC and QC program to the existing rearing programs.

MATERIALS AND METHODS

Having several establish protocols and conditions that have been show to work with varying degrees of success, our first step was to collect data on such factors as predator weights, sex ratios, development periods, longevity and other related biological conditions. We are using these parameters and the judgment of personnel in various laboratories to help define the biological range of the predators. We are also trying to collect data on the quality of prey (HWA) at different seasons, the environmental conditions, the degree of disease (mainly microsporidia infections), and a range of biochemical characteristics (protein, carbohydrate, lipid, and antioxidant contents of predators and their food). We have also been working with soils to measure textures of natural and artificial soils used in Laricobius rearing, water activity, water content, pH, and soil textural distribution (sand/silt/clay).

RESULTS

Using PC/QC graphics: we have been developing Pareto charts to help determine the most important or influential factors in the rearing domain to focus our attention most efficiently on whether food quality, disease, environmental factors (temperature, humidity, light conditions, soil conditions, cage characteristics), personnel, etc. are the most significant factors in the control of the process and the products' quality. We have also used fishbone diagrams in conjunction with the Pareto charts to help organize our research focus. Once we have collected preliminary data about the systems when they are working well, we use these data to develop control charts, which have means, upper control limits and lower control limits to help us know when our process is within or going out of control and needs to be adjusted. We are striving to set up protocols for using these charts on a continual basis to lead to the desired goal of continuous improvement. And we will be developing surveys to help us tie satisfaction of the end-users (stakeholders or forest managers) with our products.

DISCUSSION

So far, though we are very early in or PC/QC research development program, we have found significant information about where the process most often seems to go wrong. For example, our initial Pareto analysis has shown that the primary cause of process failure (mortality of predators) is in the prey quality, including the biomass of prey, protein content, and frequency of food changes (which can be partially offset by using artificial diet when prey are of low quality or not abundant). We have found that balance of temperature and humidity can also be very influential in mold formation, which leads to total failure of the food source. We have also made several important discoveries concerning the soil conditions of Laricobius.

These findings, once they are completed and worked out with the inputs and consensus of the rearing groups, will lead to the production of higher quality, more consistently available, and more fit predators to serve the needs of the forest managers who rely on the predators to find and attack prey with vigor and voracity.

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EGG RELEASES OF HEMLOCK WOOLLY ADELGID PREDATORS TO SUPPLEMENT MASS REARING EFFORTS

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ABSTRACT

In an effort to increase predator release numbers, circumvent periods of high lab mortality, reduce lab rearing space and effort, and develop protocols for releasing HWA predator eggs, we compared field and lab egg releases of *Laricobius nigrinus* (*Ln*) and determined the number of *Ln* eggs per lab ovipositional twig from standardized mass rearing procedures. Survival of *Ln* in sleeve cages was significantly lower than lab controls, but densities of generalist predators were also significantly higher in the field releases and likely impacting *Ln* survival. Count of hemlock woolly adelgid ovisacs is a poor predictor for *Ln* eggs. However, subsampling *Ln* ovipositional twigs throughout the egg-laying period was effective for estimating *Ln* eggs for field releases.

KEYWORDS

egg releases, *Laricobius nigrinus*, generalist predators, Georgia

INTRODUCTION

A suite of natural enemies is desired to prevent mortality of eastern hemlock, *Tsuga canadensis,* from the exotic hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, increasing the importance of mass rearing multiple predators for classical biological control. Mass rearing HWA predators continues to be plagued by high levels of lab mortality, limited predator field establishment, and large requirements for lab space and time. Furthermore, high quality food for rearing HWA predators is increasingly getting more difficult to locate and obtain for labs throughout the range of the HWA infestation. In an effort to increase predator release numbers, circumvent periods of high lab mortality, reduce lab rearing space and effort, and develop protocols for releasing HWA predator eggs, we had two main objectives: 1) compare field and lab egg releases of *Laricobius nigrinus* (*Ln*); and 2) determine the number of *Ln* eggs per lab ovipositional twig from standardized mass rearing procedures.

METHODS

Egg releases of *Ln* were conducted in northeast Georgia on the Chattahoochee National Forest. Egg releases were conducted five times in 2007 and four times in 2008 during February to late April. Ten to fifteen *Ln* eggs each were counted on individual twigs and released into sleeve cages containing high densities of HWA. A total of 108 egg releases were conducted on individually infested eastern hemlocks over the two year study. Lab controls (N=10) were conducted concurrently in 2007 in plastic rearing containers. The same number of *Ln* eggs were released in the lab controls. Assays were terminated after 30 d. Egg release success was determined by the number of mature, C-shaped *Ln* larvae in each screen cage or container. Generalist arthropod predators were counted in each release treatment.

HWA infested twigs containing *Ln* eggs were obtained from the UGA rearing colony in 2009 and 2010. Rearing protocols for *Ln* followed the standardized procedures developed by Virginia Tech. Ten twigs were subsampled from multiple oviposition jars throughout Febuary to June (N=309). Density of *Ln* eggs and HWA ovisacs per twig was determined by direct counts. Linear regression was used to determine the relationship of *Ln* eggs to HWA ovisacs. Counts of *Ln* eggs were also compared between the two years of the study and throughout the egg laying period (pre-, peak, and declining activity).

RESULTS

Percent survival of *Ln* populations in sleeve cages was significantly less (65%) than lab controls (25%; $F_{1, 106}$=9.3; P=0.002). *Laricobius nigrinus* survival declined in sleeve cages during the release period in 2007. A similar trend was seen in caged releases for 2008, but *Ln* survival increased in the 4 April release date. Mean arthropod predator density was significantly higher in sleeve cages (2.2($\pm$0.22)) than lab controls (0.18($\pm$0.45); $F_{1, 106}$=15.3; P=0.0002). Spiders were common generalist predators in the screen cages.

There was a linear increasing trend for HWA ovisacs and *Ln* eggs, but the regression was extremely weak. The density of HWA ovisacs did not effectively predict the number of *Ln* eggs on subsampled twigs ($y=0.0686x+2.3462$; $R^2=0.169$). Mean *Ln* egg densities on subsampled ovipositional twigs did not significantly differ between 2009 and 2010. However, *Ln* egg laying significantly increased during the middle of the egg laying period (mid-March to early May; $F_{2, 306}$=25.9; $P<0.0001$).

DISCUSSION

To by-pass lab mortality, reduce lab effort, lower rearing costs, and decrease large requirements of space and supplies, we assessed *Ln* egg releases in 2007 and 2008. Egg to mature larva survival was significantly lower in sleeve cages than lab controls, whereas generalist predator density was significantly higher in sleeve cages than lab controls. Generalist predator density most likely decreased *Ln* survival in field sleeve cages. Egg releases can supplement adult releases especially early in the spring when predator densities are low in north Georgia.

To easily determine the number of *Ln* eggs on twigs and develop egg release protocols, several measurements were assessed from lab ovipositional twigs. The number of HWA ovisacs per twig was counted, but a poor predictor of *Ln* egg densities. Additional measurements need to be assessed to effectively estimate *Ln* egg densities without meticulously counting eggs. Mean *Ln* egg densities on ovipositional twigs did not significantly differ between 2009 and 2010. However, *Ln* egg laying significantly increased during the middle of the egg laying period. Subsampling ovipositional twigs sequentially during these three periods (pre-, peak, and declining activity) can provide a good estimate of *Ln* eggs for field releases. By-passing the larval period and summer aestivation of *Ln* can allow multiple predators to be reared in a lab by reducing tedious lab labor, cut rearing costs, and reduce the amount of infested foliage required for sustaining mass rearing efforts.

ACKNOWLEDGEMENT

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A VOLUNTEER MONITORING PROGRAM TO ASSESS THE IMPACT OF BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

A program was established that involved volunteers in monitoring hemlock woolly adelgid [HWA] (*Adelges tsugae*) densities in areas where the predators *Sasajiscymnus tsugae* or *Laricobius nigrinus* had been released for biological control of HWA. The pilot phase of the project was conducted at 10 sites in NC and 2 in GA where *S. tsugae* had been released in 2004 or 2005. A second group of 14 sites in the Chattahoochee National Forest (GA) were monitored beginning in either 2008 or 2009 through 2010. This set of sites included 2 where predators had been released 2 years prior to initiation of monitoring, 9 where monitoring began the year of predator release, and 3 where no predators were released.

Each monitoring site consisted of a group of 5 trees, a central tree and 4 surrounding trees located in the cardinal directions approximately 25m from the central tree. Each monitoring site was color coded using a distinct 2-colored chenille (pipe cleaner) twist. Within a site, individual trees were color coded as: central = orange; north = red; east = blue; south = pink; west = black. On each tree, 1 branch in each cardinal direction was selected for sampling and received 2 twists of the same color spaced 20cm apart. Branches were color coded as: north = white; east = red; south = yellow; west = blue. In a photograph, an individual branch was identified as in Figure 1.

Figure 1. Orange and green twist identifies the monitoring site; single pink twist identifies this as the south tree at this site; two red twists spaced 20cm apart identify this as the photograph area on the east branch of this tree.

Sampling was accomplished by volunteers taking a digital photograph with the 20cm segment of each branch marked with the 2 same-color twists roughly centered in the photograph. Volunteers were asked to take photographs monthly beginning in February or March and continuing through at least June during a year. All photographs were sent to me at Clemson University either as email attachments, by posting on a web-based photograph site, or on a CD.

At Clemson, the number of HWA in all photographs for a given year were counted by a single person (J. Culin – 2006), M. Simmons (2007 and 2008), or Brittany Ellis (2009 and 2010). To determine HWA density, all HWA ovisacs visible on the branch between the 20cm twists or on any side branch arising between the twists were counted.

In the pilot study, of the 11 sites monitored in 2006 and 2007 HWA densities were significantly higher in 2007, while at 2 sites HWA densities were higher in 2006. At the site monitored from 2006 through 2008, HWA densities increases slightly from 2006 to 2007, then were significantly higher in 2008. Sampling was terminated at all but 1 pilot study site after 2007 due to almost complete needle loss at all sites on branches low enough for volunteers to photograph. That site was nearly totally defoliated by the end of 2008.

In the Chattahoochee National Forest study, of 4 sites sampled in both 2008 and 2009 there were 2 with higher HWA densities in 2008, 1 with higher densities in 2009, and 1 with similar densities in both years. There were 7 sites monitored from 2008 through 2010. Of these, 1 had highest densities in 2008 with declining numbers in 2009 followed by 2010, 2 had similar densities in 2008 and 2009 with significantly lower densities in 2010, 3 had highest densities in 2009, with lower numbers in 2008 and lowest in 2010, and 1 had highest numbers in 2009 with similar densities in 2008 and 2010.

This program has shown that volunteers can be enlisted to assist in monitoring HWA populations. By doing this we have been able to monitor a larger number of sites, over a much larger geographic area, than we could have done using only personnel from our laboratory.

COMPARING PREDATORY PERFORMANCE AND BEHAVIORAL CHARACTERISTICS OF WILD-CAUGHT *SASAJISCYMNUS TSUGAE* WITH A LABORATORY-REARED POPULATION

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ABSTRACT

Since 1995, millions of *Sasjiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) have been mass reared and released as a biological control agent of the hemlock woolly adelgid (*Adelges tsuage* Annand, or HWA), but with limited recovery and uncertain efficacy. Nearly all *S. tsugae* released in North America have been the progeny of the original founding colony, which consisted of fewer than 100 beetles collected from the Kansai area of Japan in 1994. This project is aimed at identifying and understanding behaviors that may differ between laboratory-reared *S. tsugae* derived from the original founding colony versus a new colony derived from wild individuals recently collected from the native range in Japan. Significant differences in performance between the two populations could reflect the cumulative effect of numerous generations of laboratory rearing without infusion of new wild individuals. Results may be used to inform decisions about future colony maintenance at rearing laboratories, improve rearing procedures, and thereby enhance biological control efforts for HWA. Genetic information could be used to track establishment and spread of successful genotypes so that these can receive rearing priority. The study may also provide insight into why field recovery rates of *S. tsugae* are often low.

INTRODUCTION

Hemlock woolly adelgid (HWA) threatens the sustainability of hemlock (*Tsuga* spp.) in eastern North America, and biological control is considered the most promising long-term management strategy to reduce its impact in forests (Cheah et. al 2004). For the past 15 years, millions of *Sasjiscymnus tsugae* (Coleoptera: Coccinellidae) have been mass reared and released as in an effort to control HWA, but with limited recovery and uncertain efficacy (Salom et al. 2008). Since 1995, nearly all *S. tsugae* released in North America have been the progeny of the original founding colony, which consisted of fewer than 100 beetles collected from the Kansai area of Japan in 1994 (Cheah et al. 2005).

Currently, the University of Tennessee is separately rearing *S. tsugae* progeny from both the original founding colony, and from a more recent colony initiated by beetles caught in Japan in 2008. Limited observations suggest possible differences in their behavior of these two populations. These behavioral differences may indicate 1) low genetic variation in the original founding colony, and/or 2) that inbreeding depression, adaptation to laboratory conditions, or other changes have resulted from rearing *S. tsugae* through numerous (30+) generations in the laboratory without infusing colonies with new wild individuals. If laboratory-reared *S. tsugae* have adapted to laboratory conditions at the expense of fitness or performance in natural environments, this may at least partially explain the poor recovery of *S. tsugae* from field releases. This research is aimed at identifying and understanding the behaviors that may differ between laboratory-reared *S. tsugae* vs. wild individuals collected from the native range in Japan.

The primary objectives of this study are to:

1. Establish a new colony of *S. tsugae* from wild populations in Japan, and characterize the genetic variation in this colony versus existing laboratory colonies.
2. Compare predator survival and performance (feeding and oviposition rates) in laboratory-reared *S. tsugae* to that of wild- caught *S. tsugae.*
3. Compare activity and fitness of laboratory-reared *S. tsugae* to that of wild-caught *S. tsugae (*e.g. time spent walking, resting, feeding, mating, flying, righting themselves).
4. Determine the preferred location of oviposition (HWA infested hemlock foliage vs. cotton gauze) of each population of *S. tsugae* and average survival of their progeny.
5. Compare behavioral responses to olfactory stimuli (e.g. infested and uninfested hemlock, other *S. tsugae* adults) between the two populations.

PROPOSED METHODS AND EARLY PROGRESS

1. Establish a new colony and characterize genetic variation.

S. tsugae adults and larvae were collected using a beatsheet from 2 sites in Japan (Figs. 3-5) in late May and early June, 2010. Individuals were shipped and reared out at Virginia Tech's quarantine facility. Of the 50 adults collected in Japan, 14 are currently alive and ovipositing. Half of the 30 surviving larvae that were shipped developed into adults and are ovipositing. Presently, there are >80 F1 adults and many F1 larvae being maintained at the University of Tennessee.

Genetic variation in both the new and the lab-reared populations will be characterized by sequencing 650 base pairs from the 5' end of the mitochondrial COI gene.

2. Compare predator survival and performance.

Adult females and larvae will be placed on a hemlock twig with a known number of HWA eggs for 3 days. The number of remaining HWA eggs and *S. tsuage* eggs will be compared between the two populations. Survival, feeding, and oviposition will be assessed using four outdoor sleeve cage treatments: open branches, caged branches, branches caged with lab-reared *S. tsugae*, and branches caged with wild-caught *S. tsugae*. Mean number of eggs laid and reduction in HWA density will be compared.

3. Compare activity and fitness

   Males and females (individually and in pairs) of each population will be observed and video recorded in a petri dish for 10 minutes. The time spent walking, resting, feeding, and attempting to fly will compared between populations. Adults from each population will be placed on their dorsal side and the time required to right themselves will be compared between the two populations.

4. Determine the preferred location of oviposition

   Although originally implemented only as a means to estimate the number of eggs laid, insertion of cotton gauze among infested hemlock foliage during oviposition has been retained as part of the protocol for rearing *S. tsugae*. Mated females of each strain will be offered HWA-infested hemlock and cotton gauze individually for 3 days. The number of eggs laid and progeny produced on the hemlock twig and gauze will be compared between populations.

5. Compare behavioral responses to olfactory stimuli

   Males and females from each population will be individually assayed in a six-chambered olfactometer. The first choice and the amount of time spent in the areas associated with each chamber will be recorded for 15 minutes. In one assay, two chambers will be empty, 2 will have HWA-infested hemlock and 2 will have uninfested hemlock. In a second assay, chamber treatments will include 1 opposite sex adult, 6 opposite sex adults, 3 males and 3 females, and 3 blanks in every other chamber.

EXPECTED OUTCOMES

This project will provide important information about the behavior of lab-reared and wild-collected S. *tsugae.* Significant differences in performance between the two populations could reflect the cumulative effect of numerous generations of laboratory rearing without infusion of new wild individuals. Results may be used to inform decisions about future colony maintenance at rearing laboratories, improve rearing procedures, and thereby enhance biological control efforts for HWA. Genetic information could be used to track establishment and spread of successful genotypes so that these can receive rearing priority. The study may also provide insight into why field recovery rates of *S. tsugae* are often low.

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EVALUATION OF HYBRIDIZATION AMONG THREE *LARICOBIUS* SPECIES, PREDATORS OF HEMLOCK WOOLLY ADELGID (ADELGIDAE)

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ABSTRACT

Laricobius nigrinus, native to the PNW and Canada, is a predator of the hemlock woolly adelgid (HWA). It has been released as a biological control agent throughout the eastern United States since 2003. *L. rubidus* is a native predator of pine bark adelgid in the eastern U.S. It is also now commonly found on HWA, and is able to complete development on this host. Recent larval and adult collections from hemlock stands where *L. nigrinus* has been released were found to include hybrids between the two *Laricobius* species. Hybridization between these two species is a concern, as it may result in hybrid incompatibility or genetic assimilation, although heterosis or further genetic divergence are possibilities as well. *L. osakensis* is a predator of HWA native to Japan that has recently been released from quarantine and will be assessed in the field as another potential biological control agent. Due to concerns over potential hybridization among the three *Laricobius* species, we will assess whether the congenerics can mate with each other and produce viable eggs. If viable offspring are produced, we will confirm that they are hybrids using genetic markers, and document their development, fitness, host preference, and morphology relative to our current knowledge of these species. The fitness components of the parents, F_1 and F_2 hybrids that will be measured include: fecundity, the number of eggs produced per cross; fertility, the number of eggs that hatch per cross; developmental time, the average duration of development from egg hatch through pre-pupae; and viability, the number of pupae that develop into adults. We will also use genetic markers to determine where hybridization between *L. rubidus* and *L. nigrinus* is occurring in eastern U.S. where *L. nigrinus* has been released, how hybridization is changing over time, and the host preference of hybrids in a natural setting.

POTENTIAL CHANGES IN TRANSPIRATION WITH SHIFTS IN SPECIES COMPOSITION FOLLOWING THE LOSS OF EASTERN HEMLOCK IN SOUTHERN APPALACHIAN RIPARIAN FORESTS

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ABSTRACT

Eastern hemlock (*Tsuga canadensis* (L.) Carr.) is declining throughout much of its range in the eastern US due to hemlock woolly adelgid (HWA) infestation. The loss of this foundation species will impact the hydrologic cycle in these systems. To estimate the impact on the hydrologic budget, we quantified transpiration over six years for *T. canadensis*, and over three years for co-occurring species *Acer rubrum*, *Betula lenta*, and *Rhododendron maximum* using sapflow probes. These three species represent the likely woody species that will dominate the trajectory of succession following the loss of eastern hemlock. In areas where *R. maximum* is a dominant component of the shrub layer, regeneration of overstory tree species is severely restricted. We developed relationships between transpiration and climate for all species. Given the loss of *T. canadensis* from the ecosystem, we modeled implications on transpiration from two succession scenarios: one in which hemlock is lost from the canopy and a *R. maximum* subcanopy results, and one in which eastern hemlock is replaced in the canopy by *A. rubrum* and *B. lenta*.

Transpiration was shown to decline since 2004 for *T. canadensis*, and no such decline was observed for the other species. We found that with the loss of hemlock leaf area, light levels in the subcanopy increased almost 17-fold, and we estimated that *R. maximum* would increase transpiration by over 4-fold. Although *R. maximum* transpiration increased, this increase was not enough to make up for the loss of *T. canadensis'* contribution to transpiration. For example, estimated sap flow of *R. maximum* during days 142–285 in 2005 was found to be 4.78 g s^{-1} while sap flow of *T. canadensis* under healthy conditions during several days of the growing season in 2004 was 14.78 g s^{-1}. By contrast, if species composition shifted from *T. canadensis* to 100% *A. rubrum*, 100% *B. lenta*, or a 50/50 mixture of the two species, stand sap flow increased by 38%, 71%, and 55% respectively. Although actual post-mortality scenarios are uncertain, the loss of *T. canadensis* will result in changes in the structure and function of this ecosystem.

PHYLOGENY OF THE ADELGID-FEEDING LEUCOPINI (DIPTERA: CHAMAEMYIIDAE): DEVELOPING TOOLS TO ENHANCE ADELGID BIOLOGICAL CONTROL

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ABSTRACT

A successful biological control program depends on the correct taxonomic identification of natural enemies and an accurate assessment of their establishment and spread. Species in several genera of Leucopini (Diptera: Chamaemyiidae) are among the most important natural enemies regulating species of Adelgidae (Hemiptera: Sternorrhyncha) in their native ranges, and have been used successfully in biological control programs in several parts of the world. Examples of adelgids regulated by chamaemyiids in their native ranges include the balsam woolly adelgid, *Adelges piceae* (Eichhorn 1968) and the pine adelgid, *Pineus pini* (Wilson 1938) in Europe, the fir adelgids, *A. knucheli* and *A. joshii*, in the Himalayas (Rao and Ghani 1972), and the pine bark adelgid, *Pineus strobi*, in eastern North America (Sluss and Foote 1973). Chamaemyiids have also been considered as biological control agents for several introduced adelgids species. In fact, the only instances of successful biological control of adelgids utilized chamaemyiids from Europe. *Pineus boerneri* was controlled below economically injurious levels in pine plantations in Chile with *Neoleucopis obscura* (Mills 1990); and *P. pini* was controlled in Hawaii with *N. obscura* (Culliney et al. 1988) and in New Zealand with *N. tapiae* (Zondag and Nuttall 1989).

With the widespread mortality of hemlocks in the eastern U.S. as a results of hemlock woolly adelgid infestation, leucopine species from western North America are being considered for release as biocontrol agents. In western North America, where there is a native hemlock adelgid lineage (Havill et al. 2006), field surveys in Oregon and Washington found that two leucopine species were among the most abundant predators on the adelgid (Kohler et al. 2008). This prompted our studies of adelgid-feeding leucopines, first to develop a robust phylogenetic hypothesis to help predict the most effective candidates for biocontrol, and second to develop COI barcodes to aid in their identification to monitor establishment and spread after release. Accurate species delineation is necessary to distinguish the introduced species from those already present in the introduced landscape when tracking establishment and impact following release, and knowledge of their current range will provide critical information for identifying areas of the introduced landscape where establishment is most likely.

New collections have significantly expanded the known geographical ranges for certain species, as well as the known host associations. The COI, DNA barcode region has been sequenced for species within *Neoleucopis*, *Anchioleucopis*, *Lipoleucopis*, *Leucopis* (both adelgid- and aphid-feeding), and several species of *Leucopis* that will soon be placed in new genera. The phylogenetic work began with COI sequences, and is expanding to include additional mitochondrial and nuclear gene regions (e.g., CAD, EF-1alpha, TPI), as well as morphology. Current analyses confirm the monophyly of each of the included genera. In addition to these analyses, in the course of describing the new genera by the first author, morphological character searching has resulted in the ability to distinguish the larvae and puparia for certain species, and to identify certain genera by their egg stage. In addition, study of the typically ignored female terminalia has clarified species-level characteristics to identify certain species in this group. To expand on this project, the authors will continue adding taxa to develop a hypothesis of relationships among all genera of the family.

KEYWORDS

biological control, Diptera, silver flies, taxonomy, molecular phylogenetics

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COEXISTENCE OF TWO INTRODUCED PREDATORY BEETLE SPECIES OF HEMLOCK WOOLLY ADELGID ON EASTERN HEMLOCK

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ABSTRACT

The invasive insect hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a serious threat to eastern hemlock, *Tsuga canadensis* (L.) Carrière, throughout the eastern U.S., including the southern Appalachians (Ward et al. 2004). In 2002, HWA was first documented in the Great Smoky Mountains National Park (Lambdin et al. 2006, Grant 2008). Although more than 500,000 adult *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) and ca. 7,000 adult *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) have been released on adelgid-infested eastern hemlock in the Great Smoky Mountains National Park (GRSM), as part of an integrated management plan, few, if any, records exist of these species co-occurring or establishing on the same trees in the same location. The purpose of this study was to assess establishment of these introduced predatory beetles after removal of whole-tree enclosures from eastern hemlock.

Fifteen eastern hemlock trees (ca. 8 m tall) were selected in Walland, Blount County, Tennessee, and 12 of these trees were caged using whole-tree enclosures (dimensions: ca. 8.26 m tall, 6.35 m basal diameter, made of lightweight Nylon netting and constructed by Camel Mfg.). Between December 2007 and/or March 2008, *L. nigrinus* (*n* = 190/caged tree, three trees), *S. tsugae* (*n* = 300/caged tree, three trees), and *Scymnus sinuanodulus* Yu and Yao (*n* = 90/caged tree, three trees) were released onto hemlock trees within the whole-tree canopy enclosures. Adults of *L. nigrinus* and *S. tsugae* were obtained from Lindsay Young Beneficial Insects Laboratory, University of Tennessee, and adults of *S. sinuanodulus* were obtained from the University of Georgia and the USDA Forest Service Northern Research Station, Connecticut. Three caged control (no beetles) and three open control (no cages) trees also were established. Cages were removed from trees in July 2009, and weekly beat-sheet sampling (five beats per tree) was conducted from January through July 2010 to assess establishment of these introduced predatory beetles.

Adults of *L. nigrinus, S. tsugae,* and a native species *Laricobius rubidus* (LeConte), as well as larvae of *Laricobius* species and *S. tsugae,* were collected in beat-sheet samples from eastern hemlocks after the whole-tree canopy enclosures had been removed. Both *L. nigrinus* and *S. tsugae* were found on four trees, and these two introduced predator species and the native predator species were also found on one tree. *Laricobius nigrinus*

and *S. tsugae* dispersed throughout the site and were recovered from two and five non-release trees, respectively, within a distance of 50 m of original releases. These two introduced predator species were recovered within two years following releases. However, while both beetle species have been recovered in some of their respective release sites in GRSM, many of the recoveries of *S. tsugae* in GRSM have been within 5 to 7 years after releases (Hakeem et al. 2010). The whole-tree cages may have enhanced the ability of these introduced predators to find food and mate. Thus, releases of these natural enemies, especially *S. tsugae,* in large whole-tree canopy enclosures, placed over hemlocks in a forest may better acclimate the released beetles to field conditions, increasing their establishment and effectiveness.

Recovery of *L. nigrinus* and *S. tsugae* on the same trees indicates that they can coexist and are compatible. This coexistence may be due, in part, to minimal overlap in their biology. These multiple predator species could provide prolonged predation of HWA, and their coexistence may enhance management of HWA using biological control programs.

Keywords

Sasajiscymnus tsugae, Laricobius nigrinus, *Laricobius rubidus*
hemlock woolly adelgid, coexistence, predators, biological control

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ECOSYSTEM IMPACTS OF HEMLOCK WOOLLY ADELGID (*ADELGES TSUGA*) INDUCED EASTERN HEMLOCK (*TSUGA CANADENSIS*) MORTALITY IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

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ABSTRACT

An invasive pest, hemlock woolly adelgid (*Adelges tsuga* Annand) is widely distributed within the GSMNP and is causing widespread mortality of eastern hemlock (*Tsuga canadensis* (L.) Carr.). The ecosystem impacts (both terrestrial and aquatic) from the loss of this tree species are still unknown. The loss of a keystone species like eastern hemlock has the potential to alter species presence, abundance and diversity in the effected systems. A study was initiated during the summer of 2009 utilizing aquatic macroinvertebrates to assess the effects of eastern hemlock mortality on aquatic species presence, abundance, and diversity.

Preliminary macroinvertebrate metrics suggest that eastern hemlock mortality will lead to aquatic system changes. Early results suggest that changing forest composition as a result of eastern hemlock mortality will impact aquatic systems by altering species presence, abundance, and /or diversity, or by creating shifts in functional feeding group dominance.

These changes are likely to lead to a loss of some highly specialized species in these systems, while increasing the abundance of other less specialized species. Further analyses are under way on the samples collected fall 2009, while work continues on sorting and identifying the spring 2010 sample.

KEYWORDS

macroinvertebrates, ecosystem function, eastern hemlock

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SLOW-THE-SPREAD MANAGEMENT OF HEMLOCK WOOLLY ADELGID AT THE NORTHERN EDGE

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ABSTRACT

This project addresses hemlock woolly adelgid management at the northern edge of its distribution in New England. Hemlock landscapes comprise nearly one million acres of forestland in the three-state area. Communication and cooperation is fostered through development of a coordinated program to slow the spread of hemlock woolly adelgid in the northern New England states of Maine, New Hampshire and Vermont. This results in strengthened regional partnerships and increased management efficiency. Activities are focused on eradication of outlying populations, suppression activities at the leading edge and integrated management in the infested area. Funding is provided by a USDA Forest Service Northeastern Area Redesign Grant.

OVERVIEW

Hemlock woolly adelgid (HWA) finds its northern distribution in southern Maine, New Hampshire and Vermont. The three northern New England states have worked together to address the threat of HWA since the 1980s when, lacking a national effort to contain the pest, we enacted parallel quarantines and wood movement protocols. This collaborative approach has continued over the past twenty years, and has been critical in limiting expansion of the insect's range.

The hemlock resource in northern New England covers approximately one million acres and provides critical wildlife wintering habitat, protects riparian areas, has aesthetic benefits and is a significant component of the local wood products industry. Hemlock woolly adelgid is one of the most significant contributing agents to the National Risk Map and losses in excess of 10% of basal area are expected in the region within the next twelve years.

The purpose of the project is to implement an integrated Slow-The-Spread program. The project influences positive change on the ground by shifting our response to HWA from exclusion/eradication into a more complex program that integrates containment and impact mitigation within the generally infested area. Strategies include early detection, biocontrol agents, public awareness, outreach to affected industries, regulatory restrictions, identification of forest management strategies and appropriate use of chemicals. Important components of the project are public awareness, participation of cooperators and mobilization of volunteers.

Specific goals and objectives include:

- Conduct surveys to outline the infestation, detect spread, and monitor eradication.
- Train volunteers to conduct surveys through landowner, "green", and industry groups.
- Create public awareness through outreach.
- Solicit input from stakeholders on HWA management.
- Eradicate or suppress HWA in selected sites using pesticides or tree removal.
- Utilize biocontrols to establish natural enemies and reduce HWA populations.
- Establish assessment plots to monitor infestation dynamics and impacts.
- Maintain quarantines through information, compliance agreements and inspections.

The funding for this grant will conclude in 2011. The capabilities derived from the project will extend beyond the life of the project itself. Hemlock woolly adelgid is now a permanent resident in the northern forest. By implementing a regional strategy, we will develop a framework we will use in the future for managing spread and mitigating impacts.

EASTERN HEMLOCK IN SOUTHERN APPALACHIAN FORESTS AND THE IMPACTS OF HEMLOCK WOOLLY ADELGID ON NUTRIENT POOLS AND CYCLING

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Eastern Hemlock (*Tsuga canadensis*) trees serve an important ecological role in riparian ecosystems in the southern Appalachians. Significant hemlock mortality is occurring due to the hemlock woolly adelgid (HWA), a non-native invasive pest that impacts eastern hemlock and Carolina hemlock (*Tsuga caroliniana*). Our objective was to quantify the impacts of HWA and its associated hemlock mortality on nutrient cycling pools and processes that ultimately determine stream water quality. We established 8 research plots in riparian areas with greater than 50% basal area hemlock and 4 reference plots in riparian areas without hemlock (hardwood plots). Individual hemlock trees were girdled in 4 of the hemlock plots to induce rapid hemlock mortality. Plot measurements included soil and air temperature, soil moisture, soil nutrient pools, soil nitrogen (N) transformations, litterfall amount and chemistry, forest floor amount and chemistry, throughfall chemistry, and soil solution chemistry. Both girdled and control hemlock plots had greater soil moisture content and cooler growing season temperatures compared to hardwood plots, as well as greater surface soil total C. There were no significant differences in soil N mineralization rates among girdled hemlock, control hemlock, or hardwood plots. We found that hemlock and hardwood plots cycle NH_4 and PO_4 differently. Inputs of PO_4 via throughfall were greater in hemlock plots, while hardwood plots leach greater amounts of NH_4 and PO_4 below the rooting zone. Differences in nutrient pools and cycling between hemlock and hardwood riparian areas suggest that the loss of hemlock from riparian areas may alter nutrient cycling processes and increase nutrient inputs to streams.

IMIDACLOPRID APPLICATION IN LOW AND HIGH ELEVATION EASTERN HEMLOCK STANDS IN THE SOUTHERN APPALACHIANS: MOVEMENT IN SOILS AND IMPACTS ON SOIL MICROARTHROPODS

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Soil applied imidacloprid is an effective insecticide to control, *Adelges tsugae* (hemlock woolly adelgid) infestations in eastern hemlock (*Tsuga canadensis*) trees; however, its effectiveness and safe application requires understanding movement in the soil and impacts on non-target species. We quantified the movement of imidacloprid in forest soils in the southern Appalachians following soil injection. We selected 4 trees in each of a low and high elevation site at the Coweeta Hydrologic Laboratory in western North Carolina. The soils on each site differed in profile depth, total carbon and nitrogen content, and effective cation exchange capacity. We applied imidacloprid in a circular pattern 1.5 m from the tree, 5 cm into the mineral soil, using a Kioritz® soil injector, at recommended dosage. We tracked the horizontal movement of imidacloprid through the soil by sampling soil solution and soil at distances of 1m, 2m, and at the drip line of each tree. For vertical movement, soil and soil solution was collected at 20 cm below the surface as well as the bottom of the soil profile, just above the saprolite, 50 cm below the surface in the low elevation site and 90 cm in the high elevation site. Soil solution samples were collected during months 1, 3, 6, 9, and 12 following application. We collected soil samples for imidacloprid extraction 2 weeks, 6 months, and 12 months after application. Imidacloprid was extracted from soils with acetonitrile. Both soil solution and soil extract imidacloprid concentrations were determined by HPLC. Soil microarthropod populations were determined in the surface 5 cm of soil 2 weeks, 6 months, and 12 months after application. We found that imidacloprid moved both vertically and horizontally in both high and low OM soils and that concentration generally declined as it moved downward in the soil profile. Soil microarthropod populations were negatively correlated with imidacloprid concentration in the low elevation site 6 and 12 month after application. Overall we found that imidacloprid movement was limited horizontally and vertically and it did not move beyond the hemlock tree drip line.

MONITORING THE HEMLOCK WOOLLY ADELGID IN NEW JERSEY, 1989-2009

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ABSTRACT

The HWA population appears to be cyclical, in New Jersey. Since 1989, peak populations of HWA have occurred every nine years since 1989 with the lowest populations occurring midway between the peaks, ten years apart. In 2009, there was a slight increase in tree mortality (58% to 59%) in the monitored plots but there has been no substantial increase in tree mortality since 2003. The sharpest increase in tree mortality was between 1999 and 2001 following the second sistens population peak. The crown ratings of ratio and transparency have only marginally weakened during the same period. Past experience has shown that periods of increased new growth are usually the direct result of a decrease in hemlock woolly adelgid (HWA) populations and vice versa

KEYWORDS

Adelges tsugae, hemlock woolly adelgid population, hemlock mortality..

INTRODUCTION

High populations of the HWA in the natural forest were first observed in the mid-1980s in New Jersey and the New Jersey Department of Agriculture established some monitoring plots to observe the effect of the insect. This report is the result of monitoring the permanent hemlock study plots. The objective of this work is to document the impact of the hemlock woolly adelgid (HWA) and associated factors in natural hemlock stands over an extended period. Data collected include stand mortality, HWA population level, crown ratings and percent new growth of eastern hemlock.

MATERIALS AND METHODS

Eleven study plots were set up in 1988 and were chosen as representative of natural hemlock stands and HWA populations. Of the 11 plots selected, nine were infested with adelgid and two were uninfested. The same 11 plots were monitored in 1989. Two of the plots were abandoned in 1990 because they were continually being treated with chemicals and/or fertilized and field personnel were unable to get an accurate record of the treatments. Subsequently, in 1990 two new plots were added to replace the ones that were dropped. These eleven plots had been monitored since 1990 (Ward 1991). Data were occasionally not collected from two plots because permission to enter the property could not be obtained from the new property owners.

Three subplots were established within each plot to ensure that an undisturbed group of trees could be observed from year to year. Subplots were set up using the following criteria: 1) location in the densest parts of the hemlock stands; 2) good accessibility to branches; 3) open areas were avoided because they were not representative of a plot as a whole.

A #10 prism was used to delineate the sample hemlock trees within the subplot. One tree was designated as the center tree and any tree that was observed within the 360° radius of the prism was included in the subplot. The tree lying closest to magnetic North with respect to the center tree was designated tree number 1. All hemlock trees within the prism, moving in a clockwise direction, were numbered sequentially.

HWA POPULATION LEVELS

Previous work (Ward 1991) indicated that the percent new growth in hemlocks declines precipitously when a population of 25-30 HWA at the base of 100 needles is reached. There was no appreciable effect on the percentage of new growth when populations of HWA were less than 25-30 HWA per 100 needles.

Population levels were determined by sampling HWA infested trees just outside of the subplots as in Ward (1991) using a methodology reported by McClure (1977). In McClure's work, the number of *Fiorinia externa* Ferris scales per 100 needles was sampled and although the hemlock woolly adelgid does not infest the needles, there are overwintering sistens at the base of the needles which were sampled. The data was collected in August with only hemlock woolly adelgid sistens counted. Cuttings were made from six different trees within the site (2 cuttings each in the proximity of each subplot. These cuttings were brought back to the laboratory and ten 100-needle sections were randomly selected from each of the six cuttings; 6,000 needles were examined in each plot. All adelgids present in the sections were counted and then an average was obtained for each of the plots.

MORTALITY

Mortality was defined as no cambial activity and no needles on the tree. Trees with any needles at all were considered to be living even though they may have been ecologically dead. Plot mortality was calculated by counting the number of dead trees observed during the crown rating survey and dividing that number by the total number of trees in the plot to determine the percentage.

CROWN RATING

The crown ratings were implemented as in Millers *et al.* (1992). Crown ratio is the percentage of total tree height that supports living foliage. Crown transparency is the amount of visible light going through the live portion of the crown and is also expressed as a percentage. A high percentage means that more light is visible through the crown, which indicates a distressed crown/tree. Crown transparency is akin to percent defoliation.

RESULTS AND DISCUSSION

HWA POPULATION

The HWA prefers current year's growth and is rarely found on older material. After several years of heavy infestation the amount of new growth declines substantially (McClure *et al.* 1996). There are exceptions to this depending on the environmental conditions at the site. Ward (1991) found that in New Jersey as the sistens population reached 25/30 HWA/at the base of 100 needles, the amount of new growth declines. Figure 1 shows the combined statewide average HWA sistens population levels for the plots from 1989-2009.

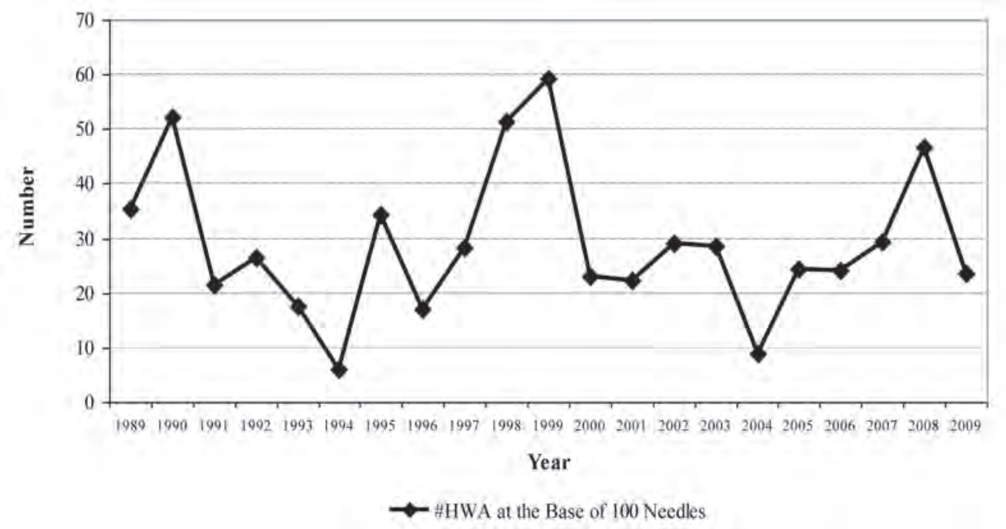

Figure 1. HWA Sistens population in New Jersey, 1989-2009

Interestingly, there is a peak population level for the hemlock woolly adelgid every nine years, in 1990, 1999, and in 2008 with the lowest populations occurring midway between the peaks, ten years apart. There are subsequent smaller peaks but it seems that once the amount of new growth and tree health decline, the HWA population declines precipitously four to five years after the peak sistens populations.

The 2009 data indicate that NJ is at the beginning of a cyclical downward trend in the HWA population for the next three to four years.

TREE MORTALITY CONSIDERATIONS

Figure 2 shows the combined overall average mortality in all of the monitored sites. In the monitored plots, tree mortality ranged from a low of 10.5% to a high of 100% in

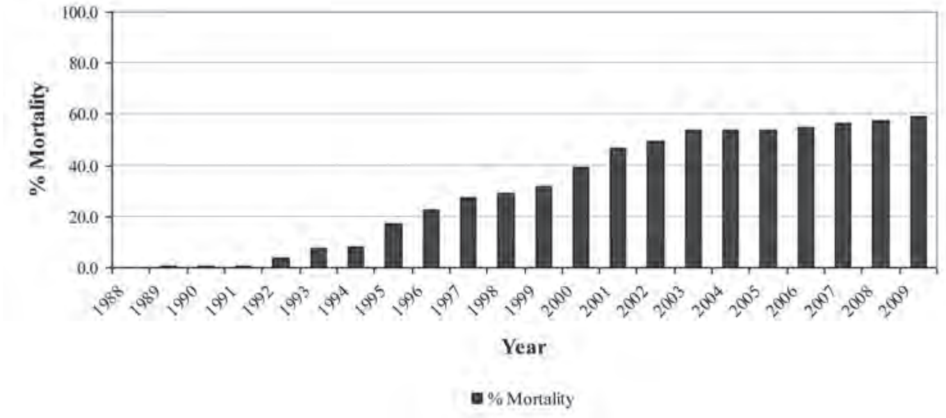

Figure 2. Average % mortality of hemlocks in New Jersey, 1989-2009

2009. Substantial hemlock mortality started to occur in 1995 when it was 17% climbing to 50% by 2002 after the second sistens population peak. The biggest increase in mortality occurred from 1999 when it was 32% to 47% by 2001 immediately after the second peak HWA population of 1999. Since 2003 when the mortality was 54% it has slightly increased to 59% by 2009. It is possible that there may be another larger increase in tree mortality in 2011 as the HWA population peaked for a third time but the data have not shown that. As a measure of the state as a whole then, almost 60% of the hemlock trees have died in New Jersey since the beginning of the hemlock woolly adelgid infestation. The stands where mortality has been the highest were the most susceptible stands and in areas that were the most marginal for the hemlock ecosystem. There are other factors that contribute to the death of the trees such as drought, elongate hemlock scale and in one plot, beavers, but the one factor that stands out consistently is the presence of a heavy HWA population. It is possible that most of the tree mortality in New Jersey has already occurred and that there will be little tree mortality in future years or that it will not occur as quickly as it did after the initial HWA infestation in the mid-1980s.

Orwig (2002) found that after hemlock mortality in infested stands in Connecticut, saplings of black birch, *Betula lenta* L., and red maple, *Acer rubrum* replaced the dead hemlock. He also found Japanese stilt grass, *Microstegium vimineum*, in one of the monitored stands. These observations are similar to those we have made in New Jersey but Japanese barberry, *Berberis thunbergii* and Japanese wineberry, *Rubus phoenicolasius* are also becoming more prevalent in areas where there is substantial hemlock mortality. Japanese stilt grass is present in all hemlock stands in which there is mortality in New Jersey.

CROWN RATINGS

Figure 3 shows the average crown ratios of living tress for all the plots since 1994 with the ratios having declined since that time. Like the tree mortality the average crown ratios have not changed appreciably since 2002 when the average was 28% and in 2009 is 26%. This decline makes it difficult to sample for the HWA or its predators with the majority of the foliage up in the crown where it is unreachable by field crews.

Figure 4 shows the average combined crown transparency ratings of the living trees for all of the plots. The lower or improved ratings from 1995 to 1998 included were primarily due the increase in the amount of new growth on the trees during that time

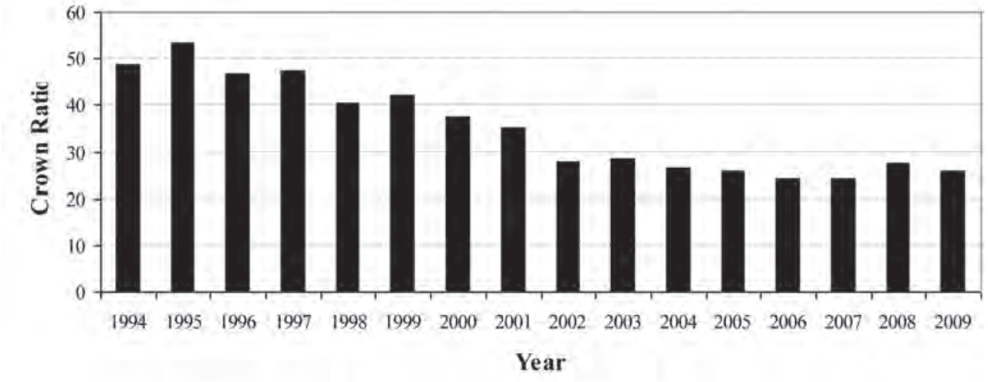

Figure 3. Average crown ratio of hemlock trees in New Jersey, 1994 – 2009

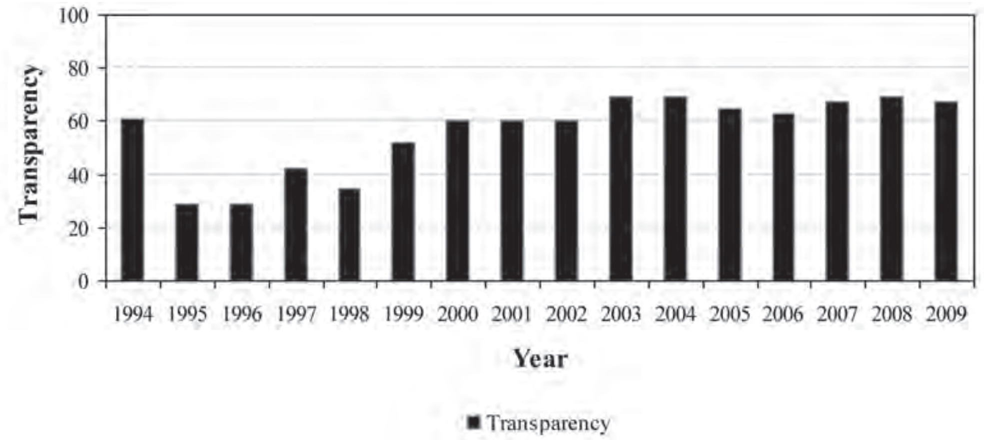

Figure 4. Crown transparency of living trees in New Jersey, 1994 - 2009.

period which shows that the trees can recover. The higher the crown transparency, the less healthy the tree is. As with the hemlock mortality there has been little change in the crown transparency since 2003.

Overall hemlock health has declined in New Jersey but the tree mortality has not approached the level that was initially feared. Not all stands have been affected with some hardly affected at all even with periodic HWA infestations. With what is known now, forest resource managers could use the information to prioritize which stands to log before the trees decline, which stands to use control methods in to attempt to preserve the trees and which hemlock stands to leave alone.

Even with the high HWA population in 2008, the crown ratings and tree mortality did not get appreciably worse in 2009. In New Jersey, tree health may have stabilized for the present and possibly into the future as well.

Forest managers may be able to take this data to determine the level of effort that they may want to put into protecting infested stands. Releases of predators could be planned during the next population peak or salvage operations could be implemented before the HWA sistens populations reach their second peaks after which time stand mortality may increase.

CONCLUSION

The HWA shows a nine year cycle in NJ where there are peak populations and then periods midway between the peaks where the population have declined or collapsed. The HWA continues to be negatively impacting hemlock stands in New Jersey and the percentage of highly stressed trees and mortality in the most heavily infested stands has slightly increased but overall mortality and stand health has been fairly stable since 2003 but the sharpest increase in tree mortality followed the second peak sistens population levels in 1999. Approximately 60% of the monitored hemlock trees in NJ have died since the beginning of the hemlock woolly adelgid infestation in the mid-1980s.

ACKNOWLEDGEMENTS

We would like to thank the US Forest Service, especially Brad Onken for partial funding over the many years of the project. We also thank the private landowners who allowed us to work on their properties even although they were well aware that their trees would most likely decline. The National Park Service and the New Jersey Department of Environmental Protection, Division of Parks and Forestry also allowed us to work on some of their properties. Thanks go to Fred Douthit and other employees of the NJ Dept. of Agriculture who contributed to the project. Lastly we want to thank Mark McClure of the Connecticut Agricultural Experiment Station who helped inspire the initial work.

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COLLECTION AND REDISTRIBUTION OF 581 ADULTS OF THE HWA PREDATOR, *LARICOBIUS NIGRINUS* FENDER, FROM THE HEMLOCK HILL AREA, LEES-MCRAE COLLEGE, BANNER ELK, NORTH CAROLINA, DURING NOVEMBER 2009 AND APRIL 2010

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- 581 *Laricobius nigrinus* adults were collected by beat sampling from the Hemlock Hill area during 4 days of collecting (November 6th and 9th, 2009 - 381 adults and April 11th and 12th 2010 - 200 adults).
- 816 *L. nigrinus* beetles were collected from the Hemlock Hill area during the past three years (2007-2009).
- Collections are increasing in numbers: 46(2007), 189(2008), 581 (2009 season).
- There is an exponential increase in both numbers and the dispersal of *L. nigrinus*.
- 381 beetles collected during November 2009 were redistributed to an abandoned hemlock nursery behind the Mast General Store in Valle Cruces, NC to start a predator field insectary there. We believe this is the first instance of locally collected L. nigrinus being used to start another field insectary.
- *L. nigrinus* adults are now common over a 1 mile radius from the original release site at Hemlock Hill.
- We are seeing regrowth of hemlocks with predators in the Banner Elk and Elk River areas.
- Made possible by US Forest Service, NC Forestry Division, Virginia Tech, and excellent teamwork. Thank You!

INTEGRATED PEST MANAGEMENT OF HWA ON 1100 ACRES OF HEMLOCKS AT GRANDFATHER GOLF AND COUNTRY CLUB, LINVILLE, NORTH CAROLINA, SINCE 2002

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- HWA was 1st detected in Avery Co. NC, at Grandfather Golf and Country Club in 2002.
- Chemical control (Merit) using the Kiortz soil injector lasted from 2003-2007 over 1100 acres of hemlocks.
- Hemlocks near waterways were injected using the Mauget injector over the same 1100 acres.
- Visibly stressed trees were injected with Stemix micronutrients using existing holes from the Mauget injector.
- Biological control of the HWA at GGCC started in 2008; to date we have made 36 releases of 300 *L. nigrinus* adults for <u>a total of 10,800 L. nigrinus beetles released</u> (12 releases of 300 beetles each year for 3 years).
- April 2008 – summer predator bag study with St and Ss; all St beetle progeny (433) St released at GGCC.
- April 2009 – M. E. Montgomery releases 500 adults of the Chinese summer predator *Scymnus ningshanensis*.
- We recovered progeny and adults of Sn until September '09. No recoveries thus far during 2010.
- Thank you Dr. Montgomery, and the USDA Forest Service for providing summer predators; Gina Davis for sampling data and positive DNA analysis of L. nigrinus larvae.

STATUS OF HEMLOCK WOOLLY ADELGID BIOLOGICAL CONTROL BEETLES IN THE SOUTHERNMOST RANGE OF HEMLOCKS

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ABSTRACT

We conducted a comparative study to test the effects of hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) biological control beetles on both eastern hemlock, *Tsuga canadensis* (L) Carr., tree condition and HWA density levels. Study sites are located in the Chattahoochee National Forest (CNF) in northern Georgia where three species of predatory beetles (> 700,000 individuals) have been released since 2006: *Sasajiscymnus tsugae* Sasaji (St), *Scymnus sinuanodulus* Yu and Yao (Ss), and *Laricobius nigrinus* Fender (Ln). This region represents the southern edge of hemlock's contiguous native range. Sites in the CNF were sampled for predatory beetle recovery and efficacy beginning in April 2010. HWA levels and tree condition ratings were determined for all sampled hemlocks and compared between the two treatment types (beetle release and non-release sites). In addition, twigs were collected from beetle release sites for beetle recovery efforts. Short-term, preliminary results indicate that neither tree condition (P =0.19) nor HWA levels (P = 0.50) appear to be influenced by the presence or absence of biological control beetles. A valid assessment of biological control beetle efficacy will require 2-4 more years of observations. Ten of the fifteen sites sampled had beetle recovery by June 2010, with a total of 141 St adults and 77 larvae (St and *Laricobius* spp.) recovered. It is currently unclear what factors may be regulating hemlock tree condition, HWA levels, and biological control beetle densities in the CNF. Future objectives are to determine HWA phenology in Georgia and heat thresholds for both HWA and Ln.

KEYWORDS

biological control, Georgia, *Sasajiscymnus tsugae, Scymnus sinuanodulus*
Laricobius nigrinus

HOST PREFERENCES OF *SCYMNUS (PULLUS) CONIFERARUM*: AN ADELGID PREDATOR

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The conifer lady beetle, *Scymnus* (*Pullus*) *coniferarum* Crotch (1874), is endemic to western North America (Figure 1). Whitehead (1967) noted that all collection records of S. *coniferarum* were from pine and that he collected large numbers from lodgepole pine and Monterey pine infested with adelgids in California. *Scymnus coniferarum* adults were also collected from western hemlock, *Tsuga heterophylla*, infested with *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA) while collecting the derodontid beetle, *Laricobius nigrinus* Fender, in the Seattle, WA, metropolitan area, during the fall and winter. This article summarizes information we have obtained on the host range of *S. coniferarum* based on field observations and laboratory choice-tests.

Figure 1. *Scymnus* (*Pullus*) *coniferarum*. Photo by Nathan Havill.

FIELD OBSERVATIONS

More than 800 *S. coniferarum* were collected from western hemlock over a 3-year period. Hemlock is a host not previously reported for this lady beetle. We collected 24 *S. coniferarum* from western white pine, *Pinus monticola*, which had an unidentified pine adelgid in dense colonies on branch terminals. We did not collect it from adelgid infested larch, fir, Douglas-fir or Japanese pine. We were not able to sample adelgid-infested lodgepole pine, Ponderosa pine, or Monterey pine—the hosts previously reported in the literature.

Initially, the collection of *S. coniferarum* was incidental to the collection of *L. nigrinus* and the choice of trees to sample was biased to where we believed *L. nigrinus* was most abundant. Generally, *S. coniferarum* was not as abundant as *L. nigrinus* – 0-10 *S. coniferarum* were recovered in a few hours of beat-sheet sampling compared to 100-550 *L. nigrinus* in the same collecting period. The sampling was done primarily in parks, arboreta, golf courses, and hedge borders of parking lots and yards. Sampling in natural forests yielded much smaller numbers of both beetles. The highest numbers of *S. coniferarum* were recovered from trees with mulch over landscape fabric beneath the trees. These trees had relatively few *L. nigrinus*.

Sampling, with *S. coniferarum* the target species conducted 18-21 June 2010, resulted in the collection of 184 *S. coniferarum* adults and 269 larvae. During this season, only three larvae of *L. nigrinus* were collected. Thus, the developmental period of the two predators is separated, with *S. coniferarum* larvae developing when the eggs laid by the progrediens generation are present.

HOST CHOICE TESTS

Preference tests between pine and hemlock adelgids were made using single adult *S. coniferarum* placed in a Petri dish for 20 hr with a piece of pine and a piece of hemlock foliage. The adelgid eggs on each twig were counted at the start and end of the test. The wool covering the eggs was spread apart to see the eggs, and eggs were removed so that each dish contained an equal number of hemlock and pine adelgids. Since it is larger and lays more eggs, usually more *A. tsugae* eggs were removed. In most trials, the adults were removed. In the trials with adults present, many more pine adelgids were consumed than hemlock adelgids. This may reflect the smaller size of the pine adelgids. In two of the trials, there was no statistical significance between the number of pine bark adelgid (*Pineus strobi* (Hartig)) and hemlock adelgid eggs consumed, although more pine adelgid eggs were eaten. A test with pine adelgid on Scotch pine and HWA, both freshly collected in Connecticut, showed a preference for HWA. A test with pine adelgid on Japanese pine and HWA, collected in North Carolina, showed a preference for the pine adelgid. A test where the lady beetle was given larger branches of foliage in a large, 4-liter cage, found that the beetles spent more time on the pine foliage for the first 4 hr, but after 48 hrs more beetles were on the hemlock foliage. Overall, the results of the feeding tests were mixed—*S. coniferarum* feeds readily on both pine and hemlock adelgids.

No-choice tests were conducted with other Homoptera. In these tests, *S. coniferarum* did not feed on the basswood aphid, elongate hemlock scale, or yew mealy bug. Adults fed on all stages of *A. tsugae*, except for diapausing 1st instars.

SIMILAR SPECIES

The appearance, biology, and feeding preference *S. coniferarum* closely resembles *S. suturalis* Thunberg, another lady beetle in the subgenus *Pullus.* The latter is endemic in Europe, but was introduced (apparently both purposely and accidently) and is now widely established in the northeastern United States (Gordon 1985). Larvae of *S. suturalis* were found on eastern hemlock, white pine, and Scotch pine in Hamden, CT, during the months of May and June (Montgomery and Lyon 1995). In laboratory, it also favors *Pineus* spp. adelgids as prey and has been frequently recovered from pines infested with adelgids, in both Europe and the United States (Montgomery et al. 1997).

Scymnus coniferarum can be easily distinguished from the lady beetles imported from China that are in the subgenus *Neopullus*. These *Neopullus* beetles have shown a preference for HWA over pine adelgids in laboratory choice tests, although they will feed on pine adelgids (Montgomery et al. 1997; Butin et al. 2004).

DISCUSSION

Scymnus coniferarum feeds and reproduces on HWA both in nature and in the laboratory. It appears to be a specialist on adelgids, preying on pine adelgids as well as HWA. Because it pupates near its host or under tree bark, soil conditions are not as important as they are for *L. nigrinus* which pupates in the soil or duff. Published collection records show that it is distributed in a wide range of climates in western North America. Recently, it was reported in Chile feeding on adelgids on Monterey pine (Gonzalez 2010). Thus, the climate of the eastern United States does not seem to be a limiting factor to its establishment. Its larval stage is present in late spring, when *L. nigrinus* is mostly in the soil; thus, the larval stages would complement each other rather than complete for the same resources. More information is needed on its life history in order to predict its potential for biological control of HWA in the eastern United States.

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EVALUATION OF REARING TECHNIQUES FOR PREDATORS OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

Data recorded while mass-rearing and during studies of the predators *Sasajiscymnus tsugae* (Sasaji and McClure) and *Laricobius nigrinus* Fender were used to evaluate rearing techniques. Fertility did not differ between young and old *S. tsuga*e females used for mass-rearing early in the rearing season. As density of *S. tsugae* increased in rearing boxes, survival decreases. Regression lines to predict survival at various densities were developed. Survival of developing *S. tsugae* in rearing boxes was not affected by type of adelgid-infested hemlock bouquet used (open foam base vs. enclosed foam base). Although a limited study using three *L. nigrinu*s larval densities in soil aestivation boxes indicated density is negatively correlated to adult emergence from these boxes, data collected during mass-rearing indicated larval density did not affect survival to adult emergence.

KEY WORDS

HWA predators, mass-rearing, fertility, density, survival

INTRODUCTION

Since the 1950s the hemlock woolly adelgid (HWA) has been attacking and killing hemlocks in the eastern US. Although chemical treatments provide control, treating trees over vast areas, such as in forests, is not feasible. Presently, biological control using predatory beetles is recognized as the only viable option for suppressing HWA populations on a large scale. Biocontrol also has the potential to permanently reduce HWA numbers. Several beetle species have been identified as HWA predators, but only a few are mass-reared for release. Of these, the two reared and released in the greatest numbers are the coccinellid *Sasajiscymnus tsugae* and the derodontid *Laricobius nigrinus*.

Several laboratories, including the Lindsay Young Beneficial Insects Lab (LYBIL) at the University of Tennessee, mass-rear these beetles for field release using protocols developed for each species. At LYBIL we have reared >833,000 and >17,000 *S. tsugae* and *L. nigrinus* adults, respectively, since 2004. While rearing these beetles, data were taken and studies conducted to provide information that may improve rearing success. Presented here are results of these efforts.

MATERIALS AND METHODS

Age-related fertility. *S. tsugae* females used for rearing usually fall into two age groups: those held over from the previous rearing season (ca. 5 months old at the beginning of the current rearing season) and those produced during the current rearing season (at least 8 weeks old when they are first used for egg production). To compare the fertility of the two groups, eggs oviposited in 1-gallon, glass jars containing HWA-infested foliage and gauze were removed from gauze and placed individually in petri dishes. Eggs were observed for eclosion. Three replications of 10 eggs each for each age group were used. Also, eggs oviposited on foliage and gauze in oviposition jars were held in plexiglass rearing boxes (0.6 x 0.6 x 0.6 m), and resulting adults noted. Ninety-six and 156 boxes were used for eggs produced by young and old females, respectively. Data were subjected to t-test analysis.

Predator density in rearing boxes. When mass rearing *S. tsugae*, the predator's eggs are placed in rearing boxes at various densities. To examine the effect of *S. tsugae* population density on survival of the predator from egg to adult in boxes, data of percentage survival from February (30 boxes) and April (38 boxes) 2010 were subjected to t-test and regression analysis. Data from these two months were used because they vary greatly in density.

Bouquet type and survival. Bouquets consisting of HWA-infested hemlock twigs are provided to developing *S. tsugae* in rearing boxes. Twigs are held in either open blocks of floral foam or foam enclosed in sealed Nalgene® containers. Wandering *S. tsugae* larvae can enter Nalgene® containers where they perish. Effect of bouquet type on survival of developing *S. tsugae* was determined by comparing survival in boxes containing either open-foam (45 boxes) or enclosed-foam (44 boxes) bouquets during the 2008 rearing season.

Density in aestivation boxes. Mature *L. nigrinus* larvae are placed in 3.1-L plastic containers holding a soil mix in which they burrow to pupate. Adults emerge and aestivate in the soil during summer. Usually, 150 larvae are placed in one container. To examine the effect of predator density on survival, larvae were placed in soil at three densities (50, 100, and 150 per container). Eleven containers were used per density. Survival to adult emergence in the autumn was noted. Survival data from the 2006 rearing season, when various densities were used for rearing, were also analyzed.

RESULTS AND DISCUSSION

Age-related fertility. For eggs observed individually, there was no difference in percentage eclosing (mean±SE) between those produced by young (94.4 ± 3.4) and old (98.0 ± 1.27) *S. tsugae* females (t = -1.02, P = 0.323, df = 17). Similarly, mean survival for rearing boxes containing eggs produced by either young (42.6 ± 2.6%) or old (40.8 ± 1.6%) *S. tsugae* females did not differ significantly (t = 0.61, P = 0.543, df = 250). Results indicate fertility did not differ between young and old females, at least during the first third of the rearing season when these data were taken.

Predator density in rearing boxes. Mean survival for *S. tsugae* from egg to adult, 43.7 ± 4.1 and 18.7 ± 1.8% for February and April, respectively, was significantly different (*t* = -6.0, P<0.0001, df = 66). The relationship between density and survival was negatively correlated for both months (Feb.: Y = 88.5465-0.0488X; *F* = 11.345; *P* = 0.002; df = 1, 26; r^2 = 0.303;

Apr.: Y = 41.7455-0.0118X; *F* = 4.729; *P* = 0.037; df = 1, 35; r^2 = 0.119). Surprisingly, the regression line slope for February, when food quality was better (more HWA eggs available) and *S. tsugae* egg densities lower (933.7 ± 4.8 vs. 1246.8 ± 33.5 for April), was steeper than that for April indicating that increasing density had a greater effect on survival during February.

Bouquet type and survival. Percentage survival of developing *S. tsugae* reared on the two types of bouquets was essentially the same for the treatment groups (open: 40.3 ± 2.3; enclosed: 41.0 ± 2.1) (*t* = -0.22, *P* = 0.825, df = 87). This indicates bouquet type does not affect *S. tsugae* survival in rearing boxes.

Density in aestivation boxes. Survival for containers with 50, 100 and 150 *L. nigrinus* larvae was 35.8 ± 4.6, 32.8 ± 4.4 and 30.3 ± 4.5%, respectively, and was not significantly different among densities (*F* = 0.37; *P* = 0.692; df = 2,30). However, density and survival exhibited a significant, linear relationship (*F* = 16.44; *P* = 0.0003; df = 1,31; r^2 = 0.347). Analysis of the 2006 rearing data revealed percentage survival in containers with relatively low mean larval density (24.8 ± 4.8% surviving, 139.0 ± 7.1 larvae) did not differ significantly from those with high density (23.9 ± 4.1% surviving, 201.8 ± 9.4 larvae) (*t*= -0.13, *P* = 0.897, df = 19). Although results of the study using the three densities indicated there is a significant linear relationship between density and survival, comparison of means from the 2006 rearing data detected no effect. Regression analysis of these data (results not provided here) also failed to detect a relationship.

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EFFECTS OF TWO INTRODUCED PESTS ON FOLIAR TERPENES OF EASTERN HEMLOCK

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ABSTRACT

Hemlock woolly adelgid (*Adelges tsugae*) and elongate hemlock scale (*Fiorinia externa*) are two introduced insect pests of eastern hemlock (*Tsuga canadensis)* that often co-occur on the same trees, but have very different effects on their hemlock hosts. Adelgid usually cause swift and dramatic declines in tree health, often resulting in tree death within a few years, while quite heavy infestations of scale results in only mild declines in tree health. Previous evidence indicates that infestation of eastern hemlocks by the scale has a paradoxically beneficial effect on the tree by protecting it in some unknown manner from the more damaging effects of the adelgid. The role of oleoresin metabolites has been frequently implicated in conifer resistance against bark-beetles, pathogens, and other pests. Here we report preliminary foliar terpene profiles from a manipulative study investigating why the adelgid is so much more destructive and how elongate hemlock scale protects hemlocks from hemlock woolly adelgid. We predicted that the terpene profiles of hemlocks infested with scale or adelgid will differ from uninfested control trees in divergent ways, in agreement with the very different effects of the two insects on their host hemlocks. Forest saplings were transplanted from Pelham, MA to a plantation in Kingston, RI, and were artificially inoculated each spring since April 2007 with adelgid crawlers, scale crawlers, or neither insect. Samples of previous year growth were collected, flash frozen in the field and solvent-extracted. The ten major monoterpene and sesquiterpene resin components were tentatively identified and quantified by GC/FID. Mean individual and total terpene amounts for the three treatment groups were calculated. Although we found no significant differences in concentrations of individual terpenes between the three treatment groups, principal component analysis revealed potentially significant differences between overall terpene profiles of control and infested trees. The current study examined oleoresin in needles of eastern hemlock repeatedly infested with elongate hemlock scale, hemlock woolly adelgid, or neither insect. Studies of other conifers suggest there may be greater changes in oleoresin concentrations induced by herbivory in stem secondary xylem compared to foliage. A parallel study of terpene accumulation in stems and woody tissue is underway. This is especially relevant given that hemlock woolly adelgid, the greatest threat to the eastern North American hemlocks, feeds on hemlock twigs.

EX SITU SEED COLLECTION REPRESENTS GENETIC VARIATION PRESENT IN NATURAL STANDS OF CAROLINA HEMLOCK

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ABSTACT

Carolina hemlock (*Tsuga caroliniana* Engelmann) is a rare conifer endemic to the highlands of the southeastern United States, where it is experiencing significant decline as a result of infestation by the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand). Since 2003, Camcore (NC State University) and the USDA Forest Service have been cooperating to conserve the genetic resources of Carolina and eastern (*T. canadensis* Carriére) hemlocks threatened by HWA. We employed amplified fragment length polymorphism (AFLP) molecular markers to: (1) compare the genetic diversity of a seed sample for *ex situ* gene conservation to the genetic diversity present in a broader foliage sample collected from natural stands, and (2) investigate the genetic relationships among the populations sampled. The results indicate that the *ex situ* collection did adequately represent the genetic variation present within the larger natural stand sample. The populations sampled were moderately genetically differentiated. Some of the populations with the highest genetic diversity were located along the eastern and southern edge of the species range.

KEYWORDS

Adelges tsugae, *Tsuga caroliniana*, AFLP genetic analysis, gene conservation

INTRODUCTION

Carolina hemlock (*Tsuga caroliniana* Englemann) is a rare conifer species that grows at elevations of 600 to 1500 m along dry bluffs and rocky ridges of the Southern Appalachian Mountains in Virginia, Tennessee, Georgia, and North and South Carolina (Humphrey 1989). A small number of populations can also be found growing at elevations of 100 to 600 m in association with isolated mountain ranges of the North Carolina and Virginia Piedmont (Stevens 1976). Historically, this species has been described as occupying dry, coarse, nutrient poor soils (Farjon 1990), although recent field studies have revealed that it is more broadly adapted to a greater variety of soil types than originally thought (Jetton et al. 2008a). Typical forest associates include *Pinus strobus* L., *P. pungens* Lamb., *Quercus* spp., *Acer rubrum* L., ericaceous shrubs such as *Kalmia latifolia* L. and *Rhododendron* spp., and occasionally eastern hemlock (*T. canadensis* Carriére) where the two species occur sympatrically in transition zones between their preferred habitats.

Across its geographic range, Carolina hemlock is beginning to experience significant decline due to the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand), an exotic insect that has also caused widespread mortality among populations of eastern hemlock (McClure et al. 2001). The adelgid is native to the hemlock forests of eastern Asia, Japan, and the Pacific Northwest region of North America (McClure et al. 2001; Havill et al. 2006). It was first reported in the eastern United States in Richmond, Virginia in 1951 (Souto et al. 1996) and was likely introduced on nursery stock imported from Japan (Stoetzel 2002). Since then, HWA has spread to 18 eastern States from Maine south to Georgia, where it can kill trees in as little as four years, threatening to eliminate these ecologically important species from eastern forests (McClure et al. 2001).

The current integrated approach to the management of HWA includes silvicultural tools to maintain tree health and vigor, chemical controls in the form of systemic insecticides, biological control with adelgid predators imported from areas where HWA is native, and the *ex situ* conservation of hemlock genetic resources (Ward et al. 2004; Jetton et al. 2008b). A key factor to the success of gene conservation programs is that seed collections capture a representative number of alleles to adequately protect the gene pools of the species in question (FAO 2004). To do this, one must understand the population genetic structure of a species and how patterns of genetic diversity vary across the landscape (Erikson et al. 1993). For Carolina hemlock, Camcore addressed this issue in a study utilizing amplified fragment length polymorphism (AFLP) molecular markers to compare the genetic composition of seedlings grown from an *ex situ* seed sample with a broader population sample consisting of a foliage sample collected from trees in natural populations. The objectives of the study were to: (1) compare the genetic diversity of a seed sample collected from nine populations in 2003 for *ex situ* gene conservation to the genetic diversity present in a broader foliage sample collected in 2005 from 15 natural stands, and (2) investigate the genetic relationships among the populations sampled.

MATERIALS AND METHODS

Provenance Seed and Foliage Collections: This study included 15 populations of Carolina hemlock in the Southern Appalachian region (Figure 1; Table 1). The *ex situ* seed sample consisted of seed collected in August and September 2003 from 64 mother trees representing nine of the populations located in North and South Carolina. Seed was collected from 3 to 10 mother trees per population, and each mother tree sampled within a population was located at least 100 m from the next. The natural stand foliage samples were made in all 15 populations in May 2005, covering the entire geographic range of Carolina hemlock and encompassing 144 trees. In 13 of these provenances, foliage samples were made from the lower crown of 10 mother trees, located at least 100 m apart. At Dragons Tooth in Virginia, foliage was sampled from 11 trees, and at Tallulah Gorge in Georgia, foliage was collected from three of the four trees known to occur at the site.

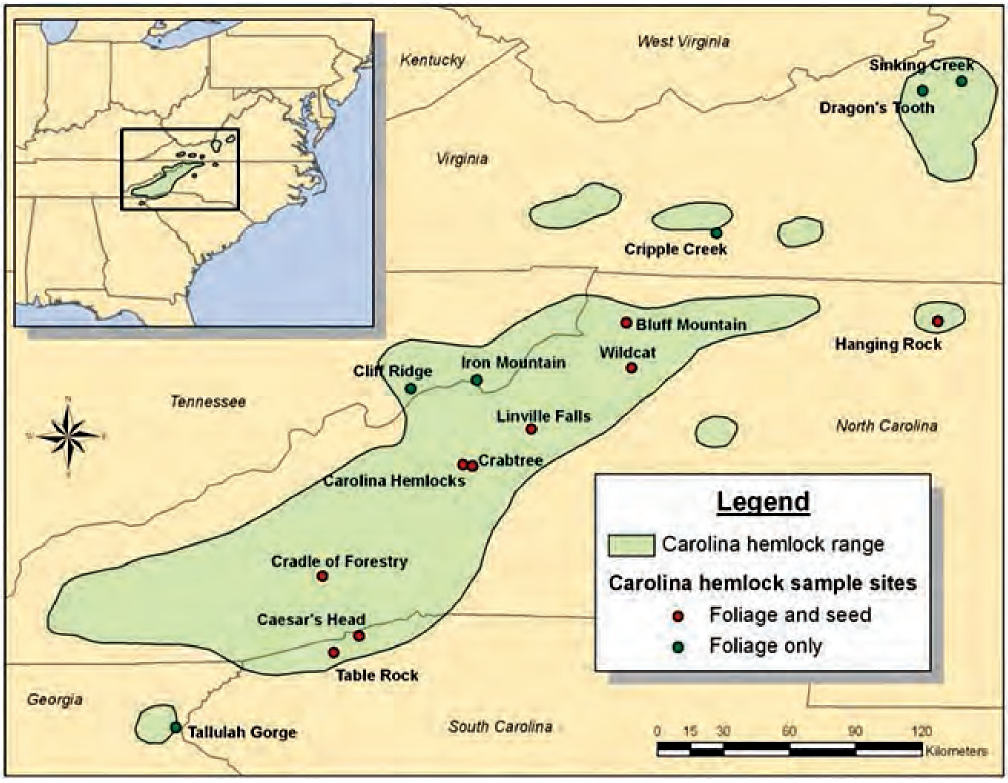

Figure 1. The range of Carolina hemlock and the provenances sampled for the molecular marker analysis.

Table 1. Carolina hemlock populations included in the molecular marker analysis, including coordinates, elevation and numbers of trees sampled for the *ex situ* conservation (seed sample) and from natural stands (foliage sample). Two seedlings were produced and sampled from each seed tree for the molecular marker study.

Population	County, State	Lat. (D.d)	Long. (D.d)	Elev. (m)	2003 Seed Sample Trees (#)	2005 Foliage Sample Trees (#)
Linville Falls	McDowell, NC	35.94 N	81.92 W	995	10	10
Table Rock	Pickens, SC	35.04 N	82.73 W	956	3	10
Carolina Hemlocks Cpgd	Yancey, NC	35.80 N	83.20 W	823	10	10
Caesar's Head	Greenville, SC	35.11 N	82.63 W	933	4	10
Cradle of Forestry	Transylvania, NC	35.35 N	82.78 W	1017	8	10
Wildcat	Watauga, NC	36.20 N	81.52 W	297	10	10
Hanging Rock	Stokes, NC	36.39 N	80.27 W	146	5	10
Bluff Mountain	Ashe, NC	36.38 N	81.54 W	1375	8	10
Crabtree	Yancey, NC	35.80 N	82.16 W	1132	6	10
Cripple Creek	Wythe, VA	36.75 N	81.17 W	766	*	10
Sinking Creek	Craig, VA	37.33 N	80.33 W	1009	*	10
Dragons Tooth	Roanoke, VA	37.37 N	80.17 W	852	*	11
Tallulah Gorge	Rabun, GA	34.73 N	83.38 W	410	*	3
Cliff Ridge	Unicoi, TN	36.10 N	82.45 W	671	*	10
Iron Mountain	Carter, TN	36.15 N	82.15 W	1503	*	10

DNA EXTRACTION AND PREPARATION

For the *ex situ* seed sample, two seedlings from each of 55 mother trees were grown from seed in the greenhouse and needles were harvested. For the natural stand sample, needles were harvested from each of the 144 foliage samples collected. Genomic DNA for all samples was extracted from needle samples using the DNeasy Plant Mini Kit (Qiagen, Chatsworth, California, USA). DNA concentrations were estimated using known concentrations of λ DNA on 1.5% agarose gels, and extracted DNA was stored at -80 °C for long term storage and at -20 °C when in use.

AFLP MARKER GENERATION

Amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995) are molecular markers used in population genetics studies because they are reproducible, require no previous knowledge of genomic sequences, and generate a large amount of information (Glaubitz and Moran 2000). At the same time, they can be time-consuming and technically demanding, and are dominant markers with only two alleles (presence and absence of a given fragment), and therefore cannot be used to determine heterozygosity or homozygosity at a specific locus. The AFLP process involves five main steps: (1) digestion of the sample DNA with restriction enzymes, (2) ligation of adaptors to the restriction digest mix, (3) pre-amplification using polymerase chain reaction (PCR) of the ligated mixture, (4) selective PCR amplification of the pre-amplified fragments, and (5) separation and detection of the selective amplification fragments. For this study, AFLP reactions were performed on a PTC-100 thermal cycler (MJ Research, Waltham, Massachusetts). Restriction digestion and ligation were performed using the 5x reaction buffer, enzyme mix of *Eco*RI (a 6-base pair cutter) and *Mse*I (a 4-base pair cutter), adapter mix and T4 DNA ligase provided in the AFLP Template Preparation Kit (LI-COR Inc., Lincoln. Nebraska). Each adapter ligation reaction consisted of 12.5 µl of restriction digestion mixture, 12 µl of adapter mix and 0.5 µl of T4 DNA ligase. The ligase was then diluted with TE buffer. The restriction digestion, adapter ligation, pre-amplification, and selective amplification PCR steps generally followed the Myburg and Remington (2000) protocol. Because of the large genome size of conifers, pre-amplification was conducted using *Eco*RI+2/ *Mse*I+2 primers, *Eco*RI+AC and *Mse*I+CC. Selective amplification was performed using *Eco*RI+3/ *Mse*I+4 primers. Twenty-four primer combinations were screened across samples from nine Carolina hemlock and six eastern hemlock trees, with the combinations of *Eco*RI +ACC/*Mse*I+CCTG, *Eco*RI +ACG/*Mse*I+CCTG and *Eco*RI+ACT/*Mse*I+CCTG selected for their high levels of polymorphism. Each *Eco*RI primer was fluorescently labeled with a different label for multiplex fragment separation and detection on an ABI Prism 3100 capillary sequencer at the Iowa State University DNA Laboratory (Ames, Iowa). Peaks in the range of 50-560 bp were sized and binned, and then fragment presence called, using GeneMarker 1.51 (SoftGenetics, State College, Pennsylvania, USA). Peaks were tested for replication by comparing trace files from a subset of rerun samples, with 125 consistently amplifying markers selected across the three primer combinations.

AFLP DATA ANALYSES

The program AFLP-SURV version 1.0 (Vekemans et al. 2002) generated genetic diversity and population genetic structure estimates based on the approach of Lynch and Milligan (1994), which uses the average expected heterozygosity of the marker loci, or Nei's genetic diversity, as a measure of genetic diversity. This approach estimates allelic frequencies at each marker locus in each population, assuming they are dominant and have only two alleles (a dominant marker allele indicating the presence of a length fragment, and a recessive null allele indicating the absence of the fragment). These allele frequencies were generated by applying a Bayesian method with non-uniform prior distribution of allele frequencies, a general method that is expected to give the most accurate results (Zhivotovsky 1999). Hardy-Weinberg genotypic proportions were assumed, and 1000 permutations were run in each analysis of population genetic structure (F_{ST}) and genetic distance. We generated a neighbor-joining (NJ) dendrogram to visualize the relationships among provenances using the seed sample (*ex situ*) populations, including only one of the two seedlings from each mother tree. The seed sample populations were used rather than the natural stands because of more consistent fragment amplification across provenances. The Hanging Rock provenance was not included, however, because of an insufficient sample size, while the adjacent Table Rock and Caesar's Head provenances were combined into a single population. The dendrogram was constructed using the NEIGHBOR and CONSENSE components of PHYLIP 3.6 (Felsenstein 2005). Confidence estimates associated with the topology of the tree were assigned based on 1,000 bootstrap replicates, with the eastern hemlock samples included as an outgroup.

RESULTS

GENETIC DIFFERENTIATION BETWEEN THE FOLIAGE AND SEED SAMPLES

The F_{ST} genetic differentiation value between the foliage sample (natural stand population) and the seed sample (*ex situ* conservation population) was relatively small (F_{ST} = 0.037). Additionally, Nei's genetic difference between the two populations was small (0.018) and differences in the expected heterozygosity were minimal, although the percent of polymorphic loci was higher in the seed sample (Table 2). It is possible that some of the differences revealed in these analyses were the result of differences in sampling. However, a comparison of foliage samples and seedlings grown from seed collected from a single provenance, Crabtree in North Carolina, showed no genetic differentiation significantly different from 0 (F_{ST} = -0.001), and highly similar percentages of polymorphic loci (89.8 percent and 92.3 percent, respectively) and expected heterozygosity (0.353 and 0.329).

Table 2. Genetic diversity present within the foliage sample (natural stand) and the seed sample (*ex situ* conservation) populations, based on amplified fragment length polymorphism (AFLP) analysis.

Population	**Trees (#)**	**Populations (#)**	**Polymorphic loci**		**Expected Heterozygosity**
			N	**Percent**	
Foliage (natural stands)	144	15	101	80.8	0.302
Seed (*ex situ* conservation)	110	9	103	90.4	0.331

GENETIC STRUCTURE AND VARIATION IN THE *EX SITU* SEED SAMPLE

Analysis of the *ex situ* seed sample showed moderate genetic differentiation among the populations represented (F_{ST} = 0.0584). The populations themselves showed relatively similar levels of genetic variation, with the exception of Linville Falls, which had a lower percentage of polymorphic loci and expected heterozygosity (Table 3). Interestingly, two populations with smaller sample sizes, Hanging Rock and Table Rock, each had 100 percent polymorphic loci; Hanging Rock is a disjunct population in the northern part of the range (Figure 1). Caesar's Head and Cradle of Forestry, both near the southern edge of the species distribution, also had relatively high polymorphism and heterozygosity.

Table 3. Genetic diversity present within the seed sample (*ex situ* conservation) populations, based on amplified fragment length polymorphism (AFLP) analysis.

Population	Trees (#)	Percent Polymorphic Loci	Expected Heterozygosity
Bluff Mountain	8	92.4	0.3309
Caesar's Head	5	97.1	0.3244
Carolina Hemlocks	10	92.4	0.3113
Crabtree	6	92.3	0.3291
Cradle of Forestry	8	95.8	0.3302
Hanging Rock	5	100	0.3801
Linville Falls	10	77.4	0.2655
Table Rock	3	100	0.3145
Wildcat	10	91.6	0.3289

Population pairwise genetic distance and genetic differentiation values (Table 4) tended to be smaller for populations of closer proximity. For example, both the genetic distance and the genetic differentiation between the southern Cradle of Forestry and Caesar's Head/Table Rock provenances was 0, as were these values between the northern Wildcat and Bluff Mountain provenances. At the same time, the Carolina Hemlocks

Table 4. Pairwise differences among Carolina hemlock seed sample (*ex situ* conservation) populations based on amplified fragment length polymorphism (AFLP) analysis. Upper diagonal: pairwise Nei's genetic distance. Lower diagonal: pairwise F_{ST} estimates.

	BM	CH/TR	CHCG	CT	CoF	LF	WC	EH
Bluff Mountain	.	0.011	0.013	0.007	0.004	0.030	0.000	0.107
Caesar's Head/Table Rock	0.029	.	0.000	0.049	0.000	0.013	0.012	0.154
Carolina Hemlocks C.G.	0.035	0.003	.	0.056	0.008	0.043	0.006	0.128
Crabtree	0.021	0.095	0.118	.	0.061	0.070	0.030	0.087
Cradle of Forestry	0.015	0.000	0.030	0.118	.	0.018	0.001	0.154
Linville Falls	0.072	0.042	0.099	0.141	0.044	.	0.016	0.198
Wildcat	0.000	0.035	0.031	0.061	0.016	0.044	.	0.131
Eastern hemlock	0.146	0.190	0.181	0.131	0.195	0.260	0.175	.

Campground provenance is more closely related to the Cradle of Forestry and Caesar's Head/Table Rock provenances than to the adjacent Crabtree provenance.

A consensus neighbor-joining dendrogram based on Nei's genetic distance (Figure 2) found statistical support (slightly more than ~50 percent of the 1,000 replicates) for a clustering of the two southernmost provenances, Cradle of Forestry and Caesar's Head/ Table Rock, with the central Linville Falls and Carolina Hemlocks provenances. The northern Wildcat population was clustered with this group, with the most northern population in the analysis, Bluff Mountain, clustered with this group with strong bootstrap support. External to this large cluster was the central Crabtree provenance.

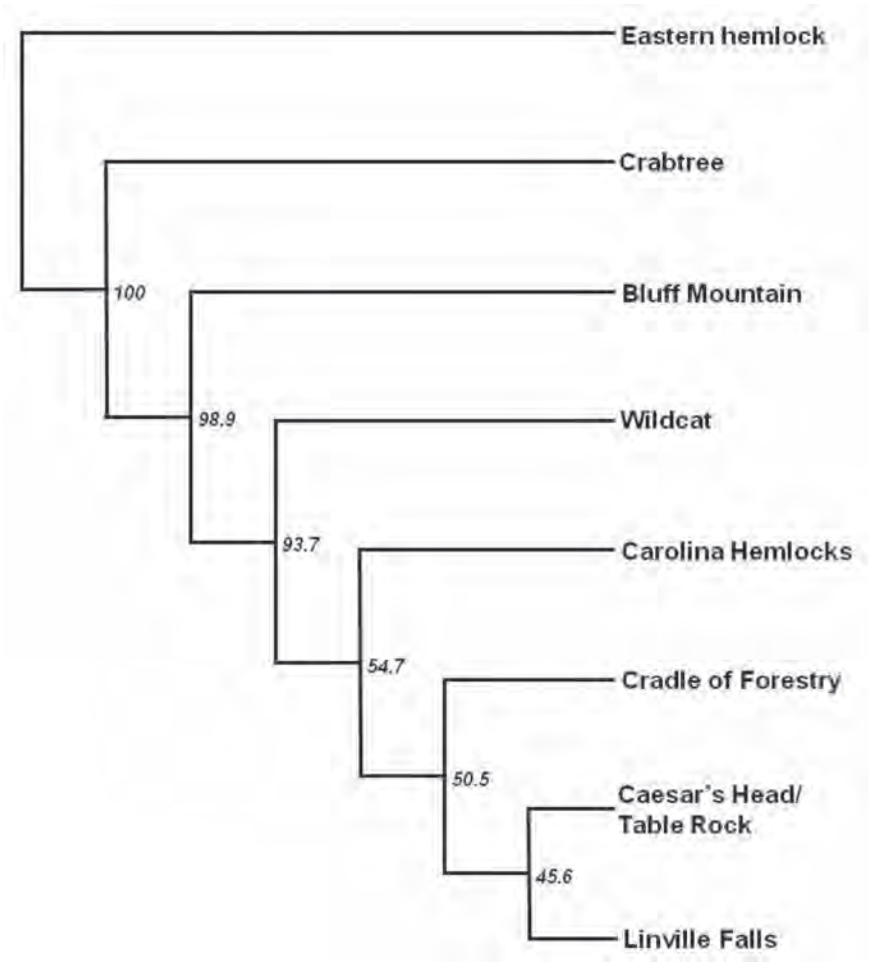

Figure 2. Consensus neighbor-joining dendrogram depicting Nei's genetic distances among the seven Carolina hemlock seed collection (*ex situ*) populations, with eastern hemlock used as an outgroup. The values represent the percent bootstrap support for the nodes over 1000 replicates; those above 50 percent are considered well-supported.

DISCUSSION

Since 2003, Camcore (International Tree Conservation and Domestication, N.C. State University) and the USDA Forest Service Forest Health Protection have cooperated to conserve the genetic resources of Carolina and eastern hemlocks threatened by HWA (Jetton et al. 2008b). Utilizing an *ex situ* approach, seeds are being collected from across the geographic range of each species. Some seeds are placed in long-term cold storage while the rest are sent to other regions of the world with climates suitable for growing hemlock and where the adelgid does not occur. In this case, seeds are being sent to Chile, Brazil, and the Ozark Mountains of Arkansas, germinated, and out-planted into conservation reserves (seed orchards). The goal of this program is to maintain hemlock populations and seed reserves in perpetuity so that gene pools for both species survive and are available for restoration efforts once viable HWA management strategies are available.

Previous genetic conservation studies by Camcore with the Mexican and Central American pines indicate that, based on molecular markers, for species with low to moderate levels of genetic diversity, seed sampled from 10 to 20 mother trees per population in 6 to 8 populations distributed across a species' geographic range will capture alleles that occur at frequencies of 5% or greater (Dvorak et al. 1999). Our results here indicate that Carolina hemlock has overall moderate levels of genetic diversity for a conifer, and that our goal to collect germplasm from 10 mother trees per population in as many populations as can be located should be sufficient to obtain a genetically representative seed sample for *ex situ* conservation. This is supported by the finding that there was relatively little genetic differentiation between the 9 population seed sample collected in 2003 and the broader 15 population foliage sample collected in 2005.

Interestingly, we did find that the level of polymorphism in the *ex situ* seed sample was about 10% higher than in the foliage sample from the natural stands. One explanation for this might be that, by sampling two seedlings from each mother tree in the seed sample, greater pollen-parent contribution was captured relative to the foliage sample. Alternatively, this might also be the result of greater genetic variation in seedlings compared to mature trees, with some loss of variation expected as trees in natural stands mature and some are lost to selection.

Our results also show that some of the populations with the highest levels of genetic diversity are located along the eastern (Hanging Rock) and southern (Caesar's Head, Cradle of Forestry, and Table Rock) edges of the species' distribution. This suggests that the Pleistocene glacial refugia for Carolina hemlock may have been located southeast of the Appalachian Mountains, similar to the pattern we described for eastern hemlock using isozyme markers (Potter et al. 2008). This is supported by paleobotanical evidence, based on pollen sediments, that hemlocks were restricted to the Appalachian, coastal plain, and continental shelf regions of the southeast during the last maximum of the Wisconsinian glacial period (Davis 1981; Delcourt and Delcourt 1987; Prentice et al. 1991). However, additional work incorporating additional populations and using another molecular marker system, such as microsatellites, will be needed to further explore this possibility.

The results of molecular marker studies such as we present here are instructive to the design and success of current and future gene conservation strategies for Carolina and eastern hemlock. The data on the genetic structure and variation among populations help us to understand how best to manage *ex situ* conservation plantings and seed reserves so that a broad and adaptive genetic base is maintained for breeding and restoration efforts. They can also tell us if small, fragmented populations such as the one found at Caesar's Head contribute unique alleles to the gene pool and if those genes might be captured in larger populations elsewhere. We can also use the data to better identify those populations that should receive priority for *in situ* protection (chemical and biological controls) so that high levels of genetic diversity are maintained in surviving populations that will be critical to the restoration of the species.

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WHY DO HEMLOCKS DIE FROM ADELGID BUT NOT SCALE? EVIDENCE FOR A HYPERSENSITIVE RESPONSE IN EASTERN HEMLOCKS

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ABSTRACT

Two invasive herbivores, hemlock woolly adelgid (*Adelges tsugae*, 'HWA') and eastern hemlock scale (*Fiorinia externa*, 'EHS') reach high densities on eastern hemlocks (*Tsuga canadensis*) on the east coast of the US. Although both species are considered pests, HWA has been found to cause more damage to hemlock than EHS. It has been suggested that HWA are 'phytotoxic' to hemlocks and that their feeding induces a hypersensitive response (HR) in the plant. The HR is a plant defense response characterized by elevated levels of hydrogen peroxide (H_2O_2) and tissue death where the herbivore is feeding. We measured H_2O_2 levels in needles of HWA-infested, EHS-infested, and uninfested control trees. HWA-infested trees had significantly higher levels of H_2O_2 than EHS and control treatments. Needles on which HWA or EHS were feeding had elevated H_2O_2 levels relative to the control, suggesting the presence of a localized HR. In the HWA treatment, we also found increased H_2O_2 activity in needles without direct contact to the herbivore, suggesting the presence of a systemic HR in HWA-infested trees. In contrast, there was no evidence for a systemic HR in EHS-infested trees. The presence of a systemic HR in eastern hemlocks in response to HWA feeding suggests that plant damage from this insect extends far from the adelgid's feeding site(s) and may be due to the lack of a coevolutionary history between these two species.

KEYWORDS

Adelges tsugae, *Fiorinia externa,* plant defense, foliar chemistry

ADELGID RESISTANCE IN NATURALLY-OCCURRING EASTERN HEMLOCKS

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ABSTRACT

Eastern hemlock (*Tsuga canadensis*) trees are currently under the threat of extirpation due to the invasive hemlock woolly adelgid (*Adelges tsugae*, 'HWA'). We have developed criteria to identify rare surviving hemlock trees that may possess some level of resistance to HWA. Putatively-resistant trees have been located and propagated along with western hemlocks (*T. heterophylla*, a species of hemlock that is known to be resistant to HWA) and control trees. In two separate HWA inoculation experiments conducted over two years, we found that putatively-resistant trees had consistently lower HWA densities than control trees. These results suggest the presence of some degree of naturally-occurring resistance in these rare survivors. We are in the first stages of a propagation program designed to produce HWA-resistant hemlocks for further experimentation and eventual use in reforestation efforts.

KEYWORDS

Adelges tsugae, plant resistance, propagation, reforestation.

STRATIFIED DISPERSION: TRACKING LONG-DISTANCE DISPERSAL EVENTS IN THE FOREST UNDERSTORY

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ABSTRACT

Understanding the dispersal mechanisms of invasive species is the key to predicting the speed and potential of their spread. The hemlock woolly adelgid (*Adelges tsugae*) is a passively-dispersed invasive hemipteran that infests and kills eastern hemlock (*Tsuga canadensis*) in the eastern United States. The dispersal dynamics of adelgids have remained largely unexplored because of its small size and the difficulties associated with identifying source populations of dispersing propagules. We experimentally simulated adelgid dispersal by dusting mature hemlock trees with fluorescent powders made up of similarly-sized particles to the passively-dispersed crawler phase of the adelgid. We created 'source' populations, by dusting hemlock trees with orange, pink, or yellow powder. Particle movement was tracked using sticky cards, and the amount of powder at each distance was quantified by photographing each card under ultraviolet light and using image analysis software to quantify fluorescing pixels. We fit dispersal functions to these data using likelihood profiling techniques. Powder density was highest on sticky cards within 10-25 m of each source tree, and dropped off sharply (~83% lower) on traps from 33-100 m away. Powder from each source tree was detected on the most distant cards (470 m). Powder-coated insects were captured throughout the trapping array, proposing a potentially important role in the dispersal of adelgid. Interestingly, the functions best describing the dispersal patterns differed between sampling periods, but had the same form within samplings, suggesting that dispersal dynamics may differ by time of year, but less so between locations we sampled. Although the powder density tapered off with distance, its ability to travel upwards of 400 meters in the forest understory implies that wind-dispersed *A. tsugae* crawlers can spread rapidly even in closed-canopy settings.

KEYWORDS

Adelges tsugae, dispersal, colonization

CHANGES IN HEMLOCK FOLIAR CHEMISTRY CAUSED BY ADELGID AND SCALE INFESTATION

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The hemlock woolly adelgid is known to cause death to eastern hemlock in as little as four years. The elongate hemlock scale also affects eastern hemlock although its attack is much less symptomatic. Both insects are rapidly spreading and often coexist in the same trees. A recent study (Preisser and Elkinton 2008) showed that when both are present, the negative effect on new growth is less severe. We aimed to investigate how these two pests interact at the physiological level by studying how nutritional quality (amino acid concentration and composition) varies in response to attack over time. Upon stress, plants have been shown to remobilize nitrogen locked in stressed parts, thereby increasing N availability for other plant functions. According to the plant stress hypothesis (White 1984), herbivores might benefit from this increase in N. We observed a temporary large spike in amino acid content caused by adelgid colonization. This N allocated to new tissue will support new adelgid generations, eventually depleting resources as seen by the gradual total amino acid content decrease over time. Scale, on the contrary, did not have an acute effect on foliage chemistry, but seemed to gradually and slowly deplete nutrients over time. Interestingly, when hemlocks were inoculated sequentially with one insect on the 1st year followed by the other insect on the 3rd year the effect differed to when both were introduced simultaneously. The order of colonization also mattered. This shows that studying effects of other pests is crucial in making predictions about stands health.

KEYWORDS

Adelges tsugae, foliar chemistry, amino acids, tree physiology

DOES SCALE INFESTATION DETER ADELGID SETTLEMENT ON HEMLOCKS? A PILOT EXPERIMENT

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The hemlock woolly adelgid is not the only pest affecting eastern hemlock. The elongate hemlock scale also affects hemlocks and often co-exists with the adelgid. In order to minimize competition with this and other pests, or perhaps to take advantage of the physiological changes caused by this other herbivore, adelgids may be able to detect differences in needle quality by probing the needles before settling. The goal of this pilot study was firstly, to develop a lab-method to study whether adelgids can detect differences in foliage and secondly to see whether they may prefer infested or uninfested foliage within a tree. Foliage from two different sources was placed in petri dishes together with adelgid egg masses. We allowed the eggs to hatch and crawlers to choose between the two choices offered in each case for two and half weeks. The foliage was then placed under a microscope and the crawlers on each of the two foliage choices counted. Our preliminary results suggest that adelgids prefer uninfested hemlocks over infested trees. They were also able to distinguish between scale-infested foliage and uninfested foliage within the same tree, which could be more ecologically relevant given the scope of their active dispersal. Due to the explorative nature of this pilot study additional experiments and more replication is needed in order to generalize these results. Future experiments will aim to optimize all parameters to improve this method and observations in the lab will be used to compare with observations in the field.

KEYWORDS

Adelges tsugae, *Fiorinia externa*, herbivore preference, competition

OCCURRENCE OF *SASAJISCYMNUS TSUGAE* IN JAPAN

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Sasajiscymnus tsugae Sasaji and McClure (called *St* below) is a hopeful biocontrol agent for *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA) in the eastern United States. This lady beetle is native to Japan and we investigated aspects of its biology in Japan. Distribution of *St* is limited to Kanto and Kansai areas of Honshu Island at elevations from 5 to 800 meters. We found *St* on *Tsuga sieboldii*, but not on *T. diversifolia*, which grows at higher elevations and more northern areas. We also found *St* on pine growing near hemlock. It has also been found on pine near the seashore (Matsubara 2000) and on marsh vegetation, far from any hemlock trees (Sasaji and McClure 1997). Despite extensive searching, we did not find it in the high mountain areas of central Honshu or in western Honshu, where Sasaji and McClure (1997) reported it.

Detailed observations of *St* phenology were made on twelve *T. sieboldii* trees, at sites in Kobe, Nara, and Takatsuki. Most *St* were collected from one sheared tree in Takatsuki that was heavily infested with HWA. The larvae of *St* were present in late May and early June whereas larvae of *Laricobius osakensis* Montgomery and Shiyake were present much earlier, from January to late May. The adults of *St* were most abundant during July and August, when HWA was in diapause and few other predators were on hemlock.

Discussion–Records of *S. tsugae* in Japan indicate that it is a predator of adelgids on both pine and hemlock at low elevations on Honshu Island. It appears to have only one generation/yr, which is synchronized with the progrediens generation. *S. tsugae and L. osakensis* likely have a complementary impact on HWA. When *St* larvae are present, many generalist predators are abundant on HWA, including lacewing and syrphid larvae, and adult Elateridae, Cantharidae, Melyridae, and other Coccinellidae (Shiyake et al. 2008). Unlike other predators, adult *St* remain on hemlock during the hot summer months, an aspect of its life history that should be studied further both in Japan and in the USA.

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RELATIONSHIPS OF NONSTRUCTURAL CARBOHYDRATES AND THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

The effect of feeding by *Adelges tsugae* Annand, the hemlock woolly adelgid (HWA) on nutritional carbohydrates in the twigs and needles of eastern hemlock, *Tsuga canadensis* was examined in early June. The experiment compared the current year, new growth and the previous year's growth—on which HWA feeds during the winter and spring—on infested and uninfested branches from the same tree. Nonstructural carbohydrate (NSC), composed of starch and free sugars, and the proportion that is starch were higher in needles than in the twigs, and in the previous year's foliage than in the current year's foliage. There were no significant differences between branches infested and uninfested by HWA; however, there were significant foliage age x HWA infestation interactions in level of starch and total nonstructural carbohydrate—the older growth had higher levels in infested branches whereas the new growth had higher levels in uninfested branches. Our results suggest that the adelgid is able to alter the physiology of eastern hemlock, by inhibiting the transport of carbohydrate from the previous year's foliage to the new growth of hemlock. This makes it possible for HWA to maintain high populations, while its host declines.

KEYWORDS

Adelges tsugae, hemlock woolly adelgid, *Tsuga canadensis*
nonstructural carbohydrates

INTRODUCTION

Adelges tsugae Annand is a non-native, small, sucking insect that is devastating hemlocks in the eastern United States. Commonly called the hemlock woolly adelgid, or HWA, it feeds in xylem ray parenchyma cells in the leaf cushion (Young et al. 1995). Xylem ray parenchyma is noted for storage of starch and transport of nutrients between the xylem vessels and the phloem. Our test hypothesis is that HWA depletes nutrients stored in the previous year's growth on which it is feeding, which deprives the hemlock of nutrients needed to produce new foliage in the spring.

METHODS

We tested the nutrient depletion hypothesis by comparing the starch and sugar content of twigs and needles on terminals of hemlock branches infested or uninfested by HWA. Starch and sugar interconvert readily and compose the non-structural carbohydrate (NSC), along with pectin, which our analysis excluded because it is not a nutrient. We summarize here our analysis of the foliage about one-month after budbreak, when the adelgid is feeding on the previous year's growth and new, flushing spring growth is present, but HWA has not yet migrated to the new growth. This is a time when there is a great demand for the carbohydrate reserves by both the adelgid and its host.

The eastern hemlocks used for this study were 3-5 meters tall and planted four years earlier in a nursery at Lasdon Arboretum, Katonah, New York. They had been artificially infested with HWA two years earlier (Weston and Harper 2009). Infested and uninfested branches were collected from three cardinal directions (north, east, south) on each tree at mid-level at 1:00 PM on June 8, a sunny day. Each branch was separated into four samples, needles and twigs of the newest and next oldest growth, which were analyzed separately for nonstructural carbohydrate. The procedure used extracts starch and free sugars from the tissue and uses amyloglucosidase to hydrolyze starch to glucose, and glucose oxidase reagent to colorimetrically determine the glucose equivalents (Dekker and Richards 1971).

RESULTS

Exploratory statistical analysis of the data indicated that the cardinal location of the branches was not a significant factor (p=0.678). Therefore, cardinal direction was omitted from the final model (Table 1). Since an infested branch and an uninfested branch were selected from each of the three cardinal directions on each tree, we also ran a model where the two branch types from each cardinal direction were paired. This model gave results similar to Table 1. Comparison of variability between trees and branches within a tree indicated that between tree variability accounted for more of the model error.

Two of the three main factors, foliage age and tissue type, were statistically significant (Table 1). Compared to twigs, needles had higher concentrations of starch, total NSC, and the proportion of NSC that was starch (Figure 1). The older, previous-year foliage compared to the new, rapidly expanding current-year foliage had higher levels of Total NSC and starch and lower levels of sugar. There was a Foliage age x Tissue interaction, which reflects the higher levels of starch in new needles than in old needles whereas old twigs had higher levels of starch than new twigs. The carbohydrates were not significantly different overall in HWA infested and uninfested branches; however, there was a significant interaction between foliage age and HWA infestation, because starch and total NSC in the older growth were higher in infested branches than in uninfested branches whereas, in the new growth, infested branches had lower levels than uninfested branches. In other words, NSC levels were increased in the internode on which HWA was feeding and decreased in the new growth, distal to the feeding site.

Table 1. Three-factor ANOVA for June 8 data showing F-values for the model factors and probability not significant in parenthesis.

Factor	df	Sugar	Starch	NSC Total	Starch as % of Total NSC
Block (Tree)	2	5.676 (0.005)	20.579 (<0.001)	20.543 (<0.001)	4.183 (0.020)
HWA infested (Yes/No)	1	0.957 (0.332)	1.430 (0.236)	3.075 (0.084)	0.394 (0.532)
Foliage age (New/Older)	1	46.743 (<0.001)	4.398 (0.040)	4.157 (0.046)	9.556 (0.003)
Tissue (Twig/Needle)	1	3.913 (0.052)	83.913 (<0.001)	60.363 (<0.001)	32.783 (<0.001)
HWA x Age	1	1.429 (0.236)	8.317 (0.005)	12.485 (0.001)	1.889 (0.174)
HWA x Tissue	1	0.511 (0.477)	0.834 (0.365)	1.742 (0.192)	2.145 (0.174)
Age x Tissue	1	0.187 (0.667)	13.491 (0.001)	11.094 (0.001)	4.055 (0.048)
HWA x Age x Tissue	1	0.634 (0.429)	3.343 (0.072)	1.718 (0.195)	0.637 (0.428)
MS Error	62	0.046	0.124	0.129	429.970

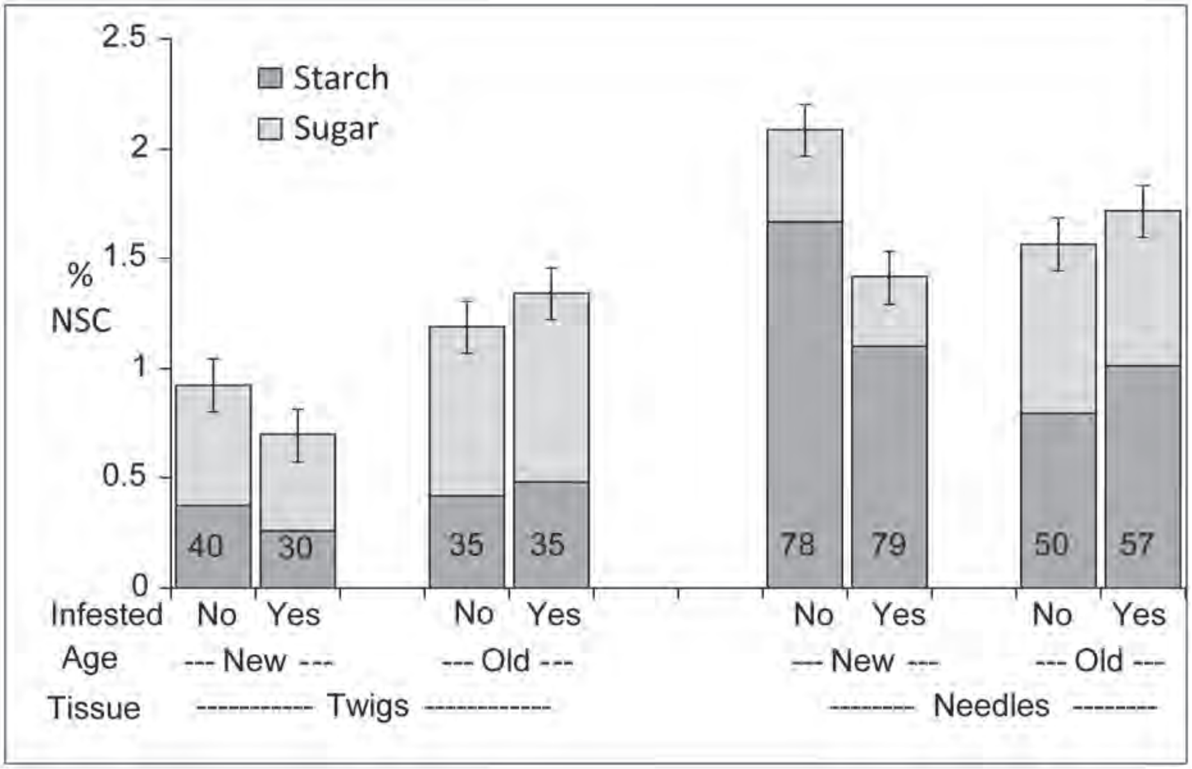

Figure 1. Nonstructural carbohydrate (NSC), composed of starch and soluble sugars, in twigs and needles on June 8 of the new, developing growth and old, previous year growth from hemlock branches infested (Yes) or uninfested (No) by HWA. The error bars are for total NSC; the values inside the bars are the proportion of NSC that is starch.

Discussion

Our hypothesis that feeding by HWA depletes nutritional carbohydrates in the age class of foliage on which it feeds was not supported by the data. The higher level of NSC in the previous year's growth, where it is feeding at the time of budbreak, suggests an effect that is more than a simple depletion of nutrients. The results indicate that HWA is able to manipulate the physiology of its host by inhibiting the transport of carbohydrate from the foliage on which it is feeding, thus depriving the new foliage of nutrients needed for its growth. This makes it possible for HWA to maintain high population densities, while the host is losing vitality.

Adelgids are one of many families of insects that induce plants to produce galls. Insect galls are noted for their high levels of lipids and starch (Shannon and Brewer 1980, Havill and Foottit 2007) and phytohormones (Straka et al. 2010). Although adelgids produce galls only on their spruce primary hosts, they may also cause analogous reactions in their larch, fir, Douglas-fir, hemlock, or pine secondary hosts. A notable example is the reaction of balsam fir to feeding by the non-native *Adelges piceae* (Ratz.), the balsam woolly adelgid (BWA). Its feeding can cause swelling and distortion of the twigs as well as inhibition of bud growth. This "gout disease" is most pronounced on vigorous trees and is believed to be the result of disturbance of the normal balance of hormones in the tree (Balch et al. 1964). The effect can be duplicated by application of the auxin indolyl-3-acetic acid to the stem (ibid.). Unlike BWA, HWA feeding does not cause discernable changes in cell anatomy (Young et al. 1995); however, our data indicate that it may alter the physiology of eastern hemlock. Because BWA and HWA did not co-evolve with balsam fir and eastern hemlock, their feeding may stimulate these hosts to a greater extent than hosts in their native ranges. The hyper-responsiveness of the host initially induces a more favorable nutritional state for the adelgid, but eventually leads to reduced growth, decline in vigor, and even death of the host.

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SEASONALITY OF ESTABLISHED POPULATIONS OF *SASAJISCYMNUS TSUGAE* ON EASTERN HEMLOCK IN THE SOUTHERN APPALACHIANS

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ABSTRACT

The seasonality of adult and larval stages of *Sasajiscymnus tsugae* (Sasaji and McClure) has been documented in its native range in Japan and in the northeastern U.S. (Cheah and McClure 2000, Shiyake et al. 2008). However, the seasonality of this predatory beetle, native to Japan, released against hemlock woolly adelgid, *Adelges tsugae* Annand, in the southern U.S. is unclear. In 2007, a pilot study was initiated to evaluate the feasibility of the use of whole-tree cages to assess survival, interactions, and establishment of introduced predators, including *S. tsugae*, released against *A. tsugae* on eastern hemlock, *Tsuga canadensis* (L.) Carriere. Population monitoring throughout this study demonstrated that *S. tsugae* had reproduced in the field and established field populations within the whole-tree canopy enclosures. After the completion of this study and removal of the cages, further insight was sought on the seasonality of this beetle in an established population in the southern Appalachians.

A study site was selected in Walland, Blount County, Tennessee, and 15 medium-sized (ca. 8 m tall) eastern hemlock were selected for use in the study. Cages (ca. 8.26 m tall, 6.35 m basal diameter), made of lightweight nylon netting and constructed by Camel Manufacturing, were placed over study trees from November through December 2007. Adult *S. tsugae* were obtained from the Lindsay Young Beneficial Insect Laboratory (University of Tennessee) and released (n = 300/cage, 1:1 sex ratio) in three cages in March 2008 (other predators were released in other sets of cages). Cages were removed from study trees in July 2009.

Beat-sheet (71 x 71 cm) sampling (five beats per tree) on trees within the cages was conducted periodically (April, May, July, November, December 2008; February, March, June, July 2009) (i.e., nine times) while trees were enclosed in cages. After removal of cages from the trees, weekly beat-sheet sampling was conducted from 15 January through 15 April 2010 (on only five trees), on 20 April 2010 (all 15 study trees), from 30 April through 18 June 2010 (all trees except one used for another study), and from 25 June through 30 July 2010 (all 15 study trees) (i.e., 29 times). For both periodic and weekly beat-sheet sampling, any adult and/or larval *S. tsugae* collected were counted, and voucher specimens were taken to the laboratory for confirmation of identification. Data from all beat-sheet samples were pooled by sampling date to assess seasonality of *S. tsugae*.

Based on weekly sampling during 2010 after cages had been removed, *S. tsugae* has established in this site and was collected from 12 of the 15 study trees. Based on both periodic and weekly sampling, adult beetles were collected from February through November, and larvae were collected April through July. *Sasajiscymnus tsugae* exhibited similar seasonality observed by researchers in its home range and in other regions of the U.S. where it was released against *A. tsugae*. Shiyake et al. (2008) found *S. tsugae* on hemlocks (*Tsuga sieboldii* Carriere) in Japan in all months except January and February, and Cheah and McClure (2000) found *S. tsugae* on eastern hemlock in all months in the eastern U.S. In the current study conducted in the southern Appalachians, collections of *S. tsugae* during periodic sampling in July 2008 and 2009 and through July 2010 during weekly sampling corroborate findings of Cheah and McClure (2000), in that *S. tsugae* may remain on trees throughout the summer. Additionally, no *S. tsugae* was collected in December or January, and few were collected in November and February, which is similar to what Shiyake et al. (2008) observed. Weekly sampling is continuing through 2010 to more completely assess the seasonality of *S. tsugae* in the southern Appalachians.

Based on results thus far, *S. tsugae* appears less active than *Laricobius nigrinus* Fender, another predatory beetle that has been released en masse against *A. tsugae*, in the coldest months (December through February), and can feed on settled crawlers July through September when *L. nigrinus* is inactive in the soil (Cheah et al. 2004). Because each species is more active at times when the other is less active, *L. nigrinus* and *S. tsugae* should be able to coexist on the same trees and complement each other in biological control programs. Knowledge gained by further assessment of the seasonality of *S. tsugae* will provide more detailed information on when both larvae and adults of this predator are active in the southeastern U.S. This information could aid forest managers through enhancement of existing release protocols by demonstrating the feasibility of tandem releases of *L. nigrinus* and *S. tsugae* at the same sites. The incorporation of this practice in future releases may potentially increase the ability of these predators to reduce the detrimental impacts of *A. tsugae* in an area.

KEYWORDS

Sasajiscymnus tsugae, seasonality, biological control

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ASSESSMENT OF TREE HEALTH CHARACTERISTICS FOLLOWING RELEASES OF PREDATORY BEETLES ON EASTERN HEMLOCK

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ABSTRACT

The health of eastern hemlock (n = 15), *Tsuga canadensis* (L.) Carriere, was evaluated in Walland, Tennessee, where three predators [*Laricobius nigrinus* Fender, *Sasajiscymnus tsugae* (Sasaji and McClure), and *Scymnus sinuanodulus* Yu and Yao] were released against hemlock woolly adelgid, *Adelges tsugae* Annand (HWA). Canopy quality, estimated by density and transparency ratings, was lowest in trees that had been caged with *S. sinuanodulus*, while trees that had been caged with *L. nigrinus* and *S. tsugae* exhibited no change in transparencies between 2009 and 2010. The amount of light reaching the interior canopy, assessed by comparing photosynthetically active radiation on the outside of the canopy to the canopy interior, was lowest in 2009 in trees that had been caged with *L. nigrinus* and in 2010 in trees that had been caged with *S. tsugae*, indicating good canopy health. New growth on the terminals of branches increased between 2009 and 2010 in trees that had been caged with *S. sinuanodulus* and *S. tsugae*, and consistent levels of new growth were maintained in trees that had been caged with *L. nigrinus.* Because *L. nigrinus* and *S. tsugae* became established in the study site, these findings indicate that predation of HWA by *L. nigrinus* and *S. tsugae* may benefit the tree health, and feeding by these predators on HWA may continue to positively impact the health of these hemlock trees in this site where both species of predators have established.

KEYWORDS

Laricobius nigrinus, *Sasajiscymnus tsugae*, *Scymnus sinuanodulus*
tree health, biological control

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an invasive insect, introduced into North America from Asia in 1951 (Cheah et al. 2004). In the eastern U.S., this insect feeds primarily on eastern hemlock, *Tsuga canadensis* (L.) Carriere, and has detrimental impacts on forest ecosystems that contain hemlock (Orwig and Foster 1998). Feeding by HWA can cause death of hemlock trees within 3-6 years after infestation in the southern U.S. (Grant 2008). As tree health declines, new growth at the tips of twigs and branches decreases/ends, and needles begin to drop, resulting in an increasingly transparent canopy (McClure et al. 2001, Cheah et al. 2004).

In 2007, a study involving *Laricobius nigrinus* Fender, *Sasajiscymnus tsugae* (Sasaji and McClure), and *Scymnus sinuanodulus* Yu and Yao was initiated to assess the feasibility of the use of whole-tree cages to assess survival and establishment of predators of HWA. Sampling during the study in 2009 and after the removal of the cages in 2010 documented the establishment of *L. nigrinus* and *S. tsugae*; however, their impact on tree health via reductions in HWA populations is unclear. To investigate the relationship between tree health and the establishment of a complex of predatory beetles, indicators of tree health (i.e., canopy quality and levels of new growth) were assessed in 2009 and 2010.

MATERIALS AND METHODS

Fifteen eastern hemlock trees (ca. 8 m tall) located in Walland, Blount County, Tennessee (bordering the Great Smoky Mountains National Park), were selected for use in the study. Cages (ca. 8.26 m tall, 6.35 m basal diameter), made of lightweight nylon netting and constructed by Camel Manufacturing, were placed over 12 trees from October through December 2007. Predatory beetles [*L. nigrinus* (*n* = 190/ cage, three cages, December 11, 2007 and January 11, 2008), *S. tsugae* (*n* = 300/cage, three cages, March 27, 2008), and *S. sinuanodulus* (*n* = ca. 90/cage, three cages, March 20, 2008)] were released within the cages. Predatory beetles were obtained from either the Lindsay Young Beneficial Insect Laboratory (University of Tennessee) (*L. nigrinus* and *S. tsugae*) or the University of Georgia (*S. sinuanodulus*). Additionally, three trees were caged with no beetles (caged control), and three trees were not caged (open control). Cages were removed from trees in July 2009. Beat-sheet (71 x 71cm) sampling (five beats per tree) conducted in 2009 and 2010 indicated that *L. nigrinus* and *S. tsugae* have established at the site; no *S. sinuanodulus* were recovered in beat-sheet samples in 2009 or 2010.

On 21 July 2009 and 28 July 2010, canopy quality was estimated for each study tree using a crown density and foliar transparency rating card according to the Forest Inventory and Analysis Field Methods for Phase 3 Measurements, Version 4.0 (USDA-FS 2007). Although this method is useful in estimating tree canopy quality, a more quantifiable means of assessing canopy quality (a ceptometer) also was incorporated into this study.

On 17 April 2009 and 30 July 2010, canopy density was quantified using a Decagon AccuPAR ceptometer to measure the amount of photosynthetically active radiation (PAR) penetrating the canopy. For each tree, one reading was taken around the outside margins of the canopy in each cardinal direction with the sensor levelled parallel to the ground and oriented to the south. One reading then was taken inside the canopy by placing the sensor against the west side of the bole levelled parallel to the ground and oriented to the south. The PAR values from the outside margins of the canopy were averaged for each tree, and the inner canopy PAR value was divided by the respective average PAR value from the outer margins. The resulting ratio ($0 \leq n \leq 1$) depicts how similar PAR readings from the inner canopy are to the average PAR outside the canopy, with lower values signifying a denser canopy, higher values signifying a thinner canopy, and a value of one signifying no difference between the inner and outer canopy.

On 21 July 2009, the length of new growth at the terminus of 10 branches per tree for each study tree was measured (cm) and recorded. The 10 branch measurements were averaged per tree. On 28 July 2010, five branches per tree were randomly selected and on each branch the length of new growth was measured (cm) on the five most apical twigs. The five twig measurements were averaged for each branch, and the five branch measurements were averaged for each tree.

To compare tree health characteristics between 2009 and 2010, all tree health factors (i.e., canopy density and foliar transparency ratings, PAR ratios, and length of new growth) were averaged per treatment (caged with each beetle species, caged without beetles, no cage) for each year. Statistical analysis was not conducted, and arithmetic means are presented.

RESULTS

Canopy quality, which was estimated by crown density and foliar transparency ratings, changed little within treatments from 2009 to 2010. No change was observed in crown density ratings for open control trees, but all trees that had been caged with predatory beetles showed small decreases in crown densities (Figure 1A). Foliar transparency ratings increased slightly in caged control and open control trees and in trees that had been caged with *S. sinuanodulus* (Figure 1B). However, there were no changes in foliar transparency ratings for trees that had been caged with *L. nigrinus* and *S. tsugae* (Figure 1B). Trees that had been caged with *S. sinuanodulus* exhibited the lowest crown density ratings during both years and highest transparency ratings in 2010 (Figure 1).

Variation was observed in PAR ratios between 2009 and 2010 within most treatments (Figure 2). The lowest PAR ratios, which indicate a relatively denser canopy, were observed in trees that had been caged with *L. nigrinus* and *S. tsugae* in 2009 and 2010, respectively. PAR ratios decreased in open control trees and trees that had been caged with *S. tsugae*, indicating denser canopies in 2010 than in 2009. PAR ratios increased from 2009 to 2010 in trees that had been caged with *S. sinuanodulus*, indicating thinning canopies. Little or no change in PAR ratios between 2009 and 2010 was observed in trees that had been caged with *L. nigrinus* and caged control trees.

Average new growth on branch tips varied between 2009 and 2010 among treatments (Figure 3). New growth decreased in caged control and open control trees but increased in trees that had been caged with *S. sinuanodulus* and *S. tsugae* between 2009 and 2010. Trees that had been caged with *L. nigrinus* maintained similar levels of new growth from 2009 to 2010.

DISCUSSION

The preliminary results of this study are encouraging. Although eastern hemlock at this location probably has been infested with HWA since 2005, the similarity in the qualitative transparency ratings for trees caged with *L. nigrinus* and *S. tsugae* in 2009 and 2010 indicates that canopy quality has been maintained in these trees. This observation is further supported by decreases in PAR ratios for trees caged with *S. tsugae* and only slight increases in PAR ratios for trees caged with *L. nigrinus*. Trees caged with *L. nigrinus* have maintained consistent levels of new growth, and trees caged with *S. tsugae* exhibited increased levels of new growth. Also, during weekly sampling in 2010, both *L. nigrinus* and *S. tsugae* were collected from five of the six control trees, and *S. tsugae* adults were collected from all three trees formerly caged with *S. sinuanodulus*. These predators may have positively influenced the health of trees on which they had not been released. The feeding of both predatory species on HWA on control trees may have contributed to decreases in PAR ratios of control trees from 2009 to 2010. Additionally, feeding by *S. tsugae* on HWA-infested trees previously caged with *S. sinuanodulus* may have contributed to increases in new growth from 2009 to 2010. Therefore, the establishment and dissemination of *L. nigrinus* and *S. tsugae* throughout the study site may contribute positively to prolonged tree health. Tree health conditions will continue to be monitored through 2011 to further assess the long-term impacts that predators of HWA may have on hemlock health. Continued monitoring and assessment of tree health and predator population levels may help provide quantitative associations between established predators of HWA and tree health in the southern Appalachians.

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INFLUENCE OF HEMLOCK SPECIES AND FERTILIZATION ON FEEDING PREFERENCE OF SPECIALIST PREDATORS OF HEMLOCK WOOLLY ADELGID

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ABSTRACT

Feeding preference of adelgid predators [*Sasajiscymnus tsugae* (Sasaji and McClure) (Coccinellidae) or *Laricobius nigrinus* Fender (Derodontidae)] was tested by providing equal numbers of adelgid eggs developed on various hemlock species [eastern hemlock, *Tsuga canadensis* (L.) Carr. ; western hemlock, *T. heterophylla* (Raf.) Sarg. ; and mountain hemlock, *T. mertensiana* (Bong.) Carrière] that received fertilizer or not, in a small choice arena. After 24 h, *S. tsugae* consumed more eggs that laid adelgids fed on fertilized *T. canadensis* compared to unfertilized *T. canadensis*. Greater than 50% more *A. tsugae* eggs derived from adelgid raised on fertilized *T. canadensis* were consumed by *Laricobius nigrinus* adults than from unfertilized ones. However, both predators did not show any preference between *A. tsugae* eggs developed between fertilized or unfertilized *T. heterophylla* and *T. mertensiana*. No significance in prey preference was noticed when both the predators were given a choice of *A. tsugae* eggs derived from adelgids fed on *T. canadensis* and either *T. heterophylla* or *T. mertensiana*.

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