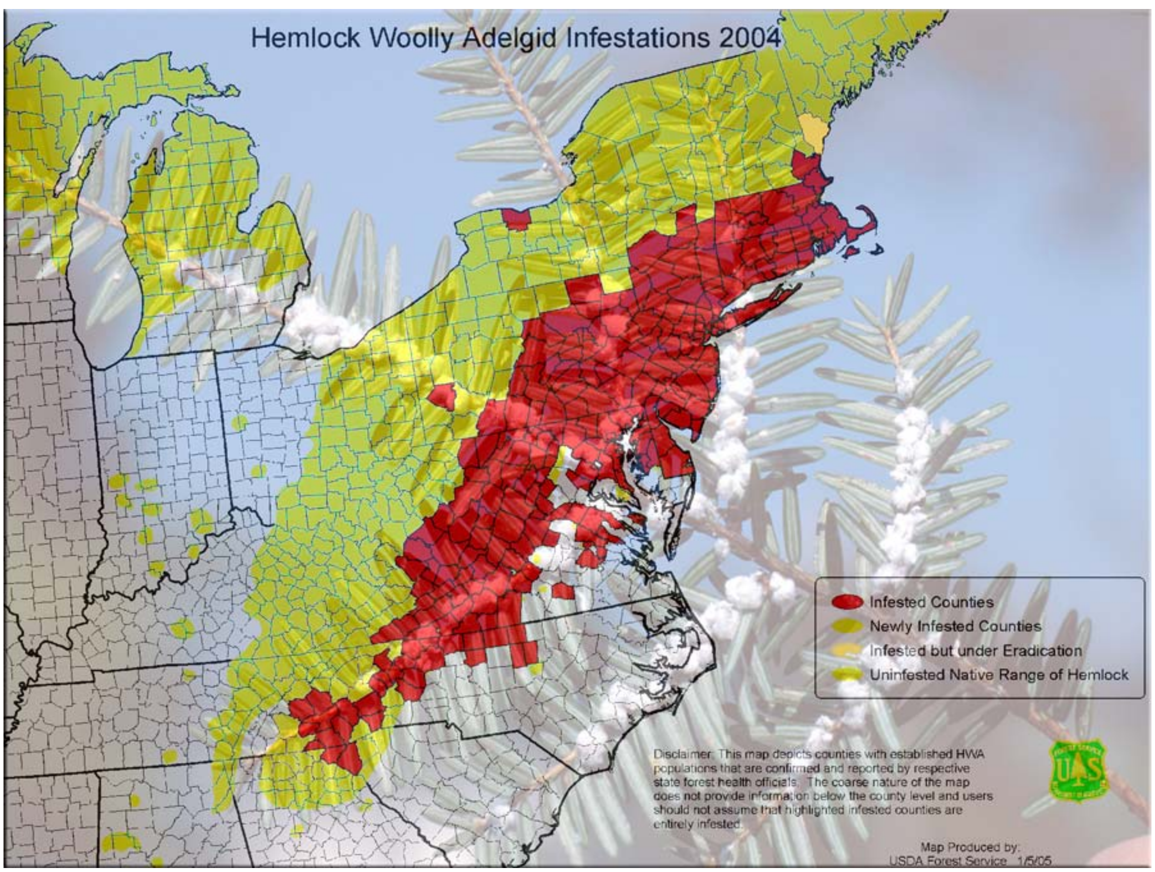
Forest Health Technology Enterprise Team

TECHNOLOGY
TRANSFER

Hemlock Woolly Adelgid

THIRD SYMPOSIUM ON HEMLOCK WOOLLY ADELGID IN THE EASTERN UNITED STATES

ASHEVILLE, NORTH CAROLINA
FEBRUARY 1-3, 2005



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Third Symposium on
Hemlock Woolly Adelgid In the Eastern United States

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Renaissance Asheville Hotel
Asheville, North Carolina

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FOREWORD

Eastern hemlock, *Tsuga canadensis* (L.) Carriere, and Carolina hemlock, *T. caroliniana*, are shade-tolerant and long-lived tree species found in eastern North America. Both survive well in the shade of an overstory, although eastern hemlock has adapted to a variety of soil types and now extends from Nova Scotia across southern Ontario to northern Michigan and northeastern Minnesota, southward into northern Georgia and Alabama, and westward from central New Jersey to the Appalachian Mountains. Carolina hemlock is a relict species limited to a small area in the southern range of eastern hemlock.

Hemlock stands create an environment with many unique ecological and aesthetic characteristics, although due to their long life, they are susceptible to a number of insect pests, including hemlock woolly adelgid (*Adelges tsugae* Annand), elongate hemlock scale (*Fiorinia externa*), hemlock looper (*Lambdina fiscellaria*), and hemlock borer (*Melanophila flvoguttata*).

In recent years (from the 1980s to the present), hemlock mortality and widespread preemptive logging caused by the hemlock woolly adelgid has roused the issue of the future of hemlock. These hemlock forests will be replaced by hardwood species, and resulting local changes to the environment will have detrimental impacts for terrestrial and aquatic ecosystems.

In response to this threat to hemlocks in eastern North America, the U.S. Department of Agriculture Forest Service and numerous state, university, and private organizations have responded with the development of a coordinated effort to manage the hemlock woolly adelgid and other insect pests associated with hemlock.

There have been two recent symposia addressing all aspects of hemlock woolly adelgid: in 1995, First Hemlock Woolly Adelgid Review, Charlottesville, Virginia; and in 2002, Hemlock Woolly Adelgid in the Eastern United States Symposium, East Brunswick, New Jersey. The latest meeting, held in February of 2005, the Third Symposium on Hemlock Woolly Adelgid in the eastern United States, Asheville, North Carolina, also included the presentation of information on the biology and impacts of elongate hemlock scale and the balsam woolly adelgid. Articles and abstracts in these proceedings represent the range of recent and current studies addressing this ongoing concern.

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Overview of the Third Hemlock Woolly Adelgid Symposium (including balsam woolly adelgid and elongate hemlock scale)

Fred P. Hain

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ABSTRACT

While the emphasis of this symposium is the hemlock woolly adelgid (HWA), there are presentations on a closely related insect, the balsam woolly adelgid (BWA) and on an insect frequently found infesting the same trees as HWA, the elongate hemlock scale (EHS). The conference begins with a discussion of the foundation of our science and the challenges of adelgid systematics. Other sessions will discuss economic and ecological impacts, survey and detection, research and technology development, and management. The final presentation will provide a discussion of where we are now and where do we need to go in the research and development of HWA.

ELONGATE HEMLOCK SCALE (*FIORINIA EXTERNA*)

Elongate hemlock scale (EHS) was first reported in 1908 on Long Island, NY (Sasscer 1912). It was introduced from Japan, where densities are much lower than those reported in this country. There are at least 57 host species noted in Connecticut by McClure and Fergione (1977) and McClure (1979), including species of hemlock, cedar, fir, pine, spruce, and yew. The scale is found only on undersides of needles, but the damage is visible from above. EHS is found from North Carolina northward to southern New England and westward to Ohio. The damage includes yellow banding on top of needles and premature needle drop. Concurrent infestations of scales and adelgids hasten hemlock decline, and hemlock borer or *Armillaria* root rot may attack weakened trees.

Presentations on EHS at this symposium will cover classical biological control; natural enemies in the southern Appalachians; management with entomopathogenic fungi; effects of systemic insecticides, a growth regulator, and oil on EHS and associated natural enemies; and reproductive success, mass release and recovery of *Cybocephalus* sp. on EHS.

BALSAM WOOLLY ADELGID: *ADELGES PICEAE* AND HEMLOCK WOOLLY ADELGID: *A. TSUGAE* (HOMOPTERA: ADELGIDAE)

It is interesting to compare the initial significance and spread of hemlock woolly adelgid (HWA) and balsam woolly adelgid (BWA). BWA was found in natural stands of balsam fir in 1908 and in Fraser fir of the southern Appalachians in 1955. Severe mortality was immediately apparent. HWA was found on ornamental eastern hemlock in 1952 or '54 in Richmond, Virginia, and was not considered a serious pest because it was easily controlled with pesticides. HWA became a pest of concern in the late 1980s when it had spread to natural stands. Since then it has caused widespread mortality. Neither adelgid is considered a pest in its native range.

BWA attacks all fir species, but Fraser fir is one of the most susceptible. Usually, mature trees in natural stands are attacked, but trees in Christmas tree plantations are also attacked. The insect can be found on all parts of the tree, but it primarily infests the trunk. Old-growth Fraser fir stands are virtually eliminated, but individual trees still survive. In many cases, vigorous Fraser fir reproduction has replaced the old growth, begging the question what will happen to these trees as they approach the age of maximum susceptibility to BWA. Early research on BWA emphasized biological control. Six European predators are known to be established. They are *Laricobius erichsonii* (Coleoptera: Derodontidae), *Pullus impexus* (Coleoptera: Coccinellidae), *Aphidecta obliterata* (Coleoptera: Coccinellidae), *Aphidoletes thompsoni* (Diptera: Cedidomyiidae), *Cremifania nigrocellulata* (Diptera: Chamaemyiidae), and *Leucopis obscura* (Diptera: Chamaemyiidae). However, there has been no clear demonstration that any of the predators have had a significant impact on BWA populations.

Current research on BWA is emphasizing host factors. BWA presentations at this conference will deal with impacts in the southern Appalachians, host interactions, chemical composition of wood and infested bark, metabolite profiling and microarray analysis of infested and uninfested fir species, and an artificial feeding system development for both adelgid species.

Unlike BWA, HWA will attack all ages of its host in natural stands and, consequently, represents a more serious threat to hemlock than BWA does to fir. Eastern and Carolina hemlock are very susceptible to HWA, while the western and Asian species are not. The basic challenge that we face is to understand why the western and Asian hemlocks are not impacted by HWA the way Eastern and Carolina hemlocks are: is it biological control, host resistance, a combination of the two, or something else? Perhaps the information presented at this conference will begin to answer this question.

The vast majority of presentation will deal with various aspects of HWA. They will include impacts on residential landscapes, recreation areas, and headwater streams; various sampling schemes, use of hyperspectral technology, satellite imagery, and landscape scale models; GIS-based risk assessment, biological and chemical control, host resistance, gene conservation, population dynamics, and use of mitochondrial DNA for determining the range of HWA. During the last session of the conference, management strategies of the various landowners will be covered.

The final topic of the conferences will be a discussion of where we are now and where do we need to go. This will be an assessment, based upon the information presented at this conference, about the HWA Strategic Plan, what has been accomplished, and what still needs to be accomplished.

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A Historical Review of Adelgid Nomenclature

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ABSTRACT

Adelgids are known worldwide as pests of various conifers. Despite their pest status, the nomenclature of adelgids is in a state of disarray. Different classification schemes abound worldwide making information retrieval and communication among biologists, foresters, and taxonomists tedious and difficult. Historically, different adelgid workers have accepted either a two-genus or a multiple-genus system (or variations of each). Phylogenetic analyses of this small family of insects may help bring clarity to the situation.

KEYWORDS

Adelgids, Adelgidae, Hemiptera, taxonomy, nomenclature.

INTRODUCTION

Adelgids (Hemiptera: Sternorrhyncha: Adelgidae), the pine and spruce aphids, are small, soft-bodied insects that feed exclusively on coniferous plants using piercing-sucking mouthparts. Adelgids are primarily Holarctic in distribution (although found worldwide if counting exotic introductions), and 50 species have been described (Carter 1971 and Foottit and Richards 1993). According to Foottit and Richards (1993), the distribution of adelgids mirrors closely the distribution of spruce, a primary to exclusive host to many adelgids.

Adelgids are considered by many workers to be the most primitive members of the aphidoid group of insects (Ghosh 1983), having arisen in the Carboniferous when coniferous trees dominated the landscape. They are separated from aphids (in a strict sense) by the absence of cornicles, short antennal segments, reduced wing venation, a glandular body surface, and oviparity in both parthenogenetic and sexual forms (Carter 1971 and Ghosh 1983). Adelgids are differentiated from phylloxerans, their closest relatives, by a distinct chitinous ovipositor, separated CuA and CuP veins, four to five abdominal spiracles, antennae with three to five segments, and winged forms having three large sensoria at the tips of the antennae (Annand 1928 and Stoetzel 1998). In addition, phylloxerans are only found on deciduous hosts (Stoetzel 1998). Wingless forms of adelgids usually secrete a dense woolly mass from dorsal wax glands. This woolly mass surrounds the female and protects her and her eggs from various environmental elements. Like aphids, adelgids have extremely complex life cycles involving different hosts (usually spruce) and alternation of sexual and parthenogenetic generations (Stoetzel 1998).

Numerous adelgid species, most notably the balsam woolly adelgid, *Adelges piceae* Ratzeburg, the hemlock woolly adelgid, *Adelges tsugae* Annand, and the pine woolly adelgid, *Pineus pini* Linnaeus, have attained pest status around the world. The two former species have become destructive pests in the United States causing widespread host mortality (McClure 1987 and Wallace and Hain 2000). As a result of these threats, numerous studies in recent years have examined the potential effectiveness of various adelgid natural enemies for use in biological control, particularly for the hemlock woolly adelgid (Cheah and McClure 1996 and 1998, Zilahi-Balogh et al. 2003).

Despite their importance as pests, there exists a great deal of instability and difference of opinion in adelgid nomenclature and taxonomy. Different workers around the world use various classification systems at all taxonomic levels, with the exception of the species group. Even the author and date of the family name is ambiguous. The continued use of different names for adelgids worldwide makes retrieval of information and communication among applied researchers and forest managers tedious.

Unfortunately, it is very difficult to regulate many of the different classification schemes via the International Code of Zoological Nomenclature (1999), as the choice of a scheme is largely based on "taxonomic license" and personal preference (Blackman and Eastop 1984). At its heart, the problem comes down to whether one agrees with the lumping of adelgid species into two genera or splitting them into more than two genera. Due to their importance as economic and ecological pests worldwide, universal acceptance of appropriate names for adelgids at all levels—species group, genus group, and family group—should have a high priority. Furthermore, before detailed searches and examinations of the effectiveness of potential natural enemies of pest adelgid species can take place, it is important to know the name of the adelgid they feed on.

This report reviews the two major published generic classifications of adelgids. It is important to understand the history of classification and why authors chose the names they did in order to make sense of what is happening today.

REVIEW OF CLASSIFICATION SCHEMES

Traditionally, there have been two classification schemes of adelgid genera used worldwide: the North American/Great Britain two-genus system and the German (and others) multiple-genus system. The formation of these two classifications is the result of each system focusing on different morphological characters from various life stages to distinguish taxa. The primary difference between them is that the two-genus system distinguishes genera primarily on the number of abdominal spiracles in adults while the German system relies on the morphology of the first instar nymphs of the sistens stage (Annand 1928). There have been modifications to both systems over time, but workers worldwide citing adelgid names have primarily used one of the former schemes.

Along with the different generic name systems, the use of adelgid family group names has varied. Workers have frequently created new family level classifications over time with few of them being accepted universally. Adelgid biologists today use interchangeably the superfamily names Adelgoidea (Dolling 1991), Phylloxeroidea (Heie 1980, 1987, and 1999; Heie and Pike 1992; Foottit and Richards 1993; and Maddison 1998), and Aphidoidea (Blackman and Eastop 1994 and Stoetzel 1998). The use of different superfamily names may add to the confusion in adelgid nomenclature, but most workers agree that placing adelgids in either Aphidoidea or Phylloxeroidea is acceptable: it is simply a question of preference.

Although workers have disagreed on what genus, tribe, subfamily, family, and superfamily to place adelgids in, they have for the most part agreed on the species names. For example, *Dreyfusia piceae* Ratzeburg, 1843, and *Adelges piceae* Ratzeburg, 1843, are two different generic names used by different workers for the same species.

GENERIC CLASSIFICATION SCHEMES

The first mention of adelgids was by the Dutch botanist Clusius in 1853 when he made observations on galls, although the genus *Chermes* was erected by Linnaeus (1756) (Annand 1928). The family group name Adelginae is attributed to Annand (1928). The first major taxonomic workers of adelgids were the Russian entomologist Cholodkovsky (1896) and the German entomologist Börner (1908), both of whom made valuable and independent contributions to adelgid biology in the discovery of alternate hosts and in taxonomy (Annand 1928 and Carter 1971).

One of the first major monographs on adelgid taxonomy was by Börner in 1908 (Annand 1928). Börner placed adelgids in the family Chermesiden and superfamily Aphidoidea. A major theme in this work was his disagreement with Cholodkovsky's definition of how taxonomic species should be separated. Börner believed that species should be separated by morphological differences rather than relying heavily on their biological differences. Subsequently, Börner synonymized many species (combined many species into one) based on morphological differences of the nymphal stages. He used chaetotaxy of head and thorax sclerites and gland features of 1st instar nymphs to differentiate adelgid genera (Shaposhnikov 1964).

Börner (1928) modified his existing classification by listing adelgids in the family Adelgidae. Heie (1980), although citing the year 1930, gives credit to Börner for the authorship of the family name Adelgidae from Börner's 1928 publication. Börner, along with his counterparts, stopped using the name Chermesidae (or variations thereof) at that time due to confusion with the use of a similar name for psyllids. Adelgidae, according to Börner, included the subfamilies Pineinae and Adelginae. Adelginae had two tribes: Dreyfusiini and Adelgini. Pineinae contained *Pineus* Shimer, 1867, and *Pineodes* Börner, 1926 (new genus). *Dreyfusia* Börner, 1908, and *Aphrastasia* Börner, 1909, were included in the tribe Dreyfusiini. The genera *Cholodkovskya* Börner, 1909; *Adelges* Vallot, 1836 (senior synonym of *Cnaphalodes* Macquart, 1843); *Gilletteella* Börner, 1928 (new genus); and *Sacchiphantes* Curtis, 1844, were included in the tribe Adelgini. Of noted absence was the genus *Chermes* Linnaeus, 1756, which Börner also stopped using, similar to the family name Chermesidae. He subsequently assigned the species in this former genus to new genera, current genera, and the reinstated genus *Sacchiphantes* (type species *Chermes abietis* L.)(Börner 1928). This work would provide the foundation for all subsequent adelgid classifications that followed the multiple-genus classification.

Annand (1928) described the biology and taxonomy of the North American adelgids. It was the first work to adopt the two-genus system using *Adelges* and *Pineus*. In his work, adelgids are listed in the subfamily Adelginae (formerly Chermesinae) of the superfamily Aphidoidea and family Phylloxeridae. Annand likely placed adelgids in the phylloxeran family due to their close physical resemblance and similar biology (Carter 1971). Annand believed that the genus *Pineus* should stand by itself, similar to Börner. However, Annand combined the remaining current genera in Börner's classification into the genus *Adelges*. *Adelges* Vallot, 1936, is the oldest name of all adelgid genera and therefore has priority in zoological nomenclature (Article 23.1, International Code for Zoological Nomenclature 1999). Annand used the number of spiracles on the adult female abdomen (five pairs in *Adelges*, four pairs in *Pineus*) to distinguish the two genera. He stated that *Pineus* was likely derived from *Adelges* citing the greater number of abdominal spiracles in *Adelges* as evidence of a primitive character (Annand 1928).

Annand believed Börner had separated genera based on species-level characters resulting, in his opinion, in the reduction of genus to a rank equivalent to species. He also thought that the genus was an artificial group often based on subjective characters but at the same time a valid phylogenetic entity existing in nature and having definable characters that separated it from other such groups. According to Annand, by creating numerous genera for a similar number of species, Börner had lessened the practical value of the genus group taxon and its significance in elucidating evolutionary relationships (Annand 1928). Furthermore, Annand determined that some of Börner's characters were not applicable to all adelgid life stages. For example, Börner based the genus *Cnaphalodes* on the fused cephalic and prothoracic plates and the absence of glands in the first instar nymph of the sistens generation. However, these characters are not relevant to the progrediens stage. Annand believed generic characters should be based on adult characteristics and rarely nymphal characters. He largely based his "lumping" of adelgids into two genera, *Adelges* and *Pineus*, on these beliefs.

FURTHER CLASSIFICATIONS

Following the publications of these two major workers, most taxonomic works on adelgids did not follow Annand's recent work (Silvestri 1939, Börner 1944, Börner and Heinze 1957, Heinze 1961, Shaposhnikov 1964, and Steffan 1968). Most workers followed Börner's classification or a variation of his scheme. The reason for this is unclear; it could have been due to workers not knowing about his work, not accepting it, or thinking it was only applicable to North American genera.

In 1965, the confusion associated with *Chermes* and higher-level uses of the name was put to rest by the International Commission for Zoological Nomenclature. According to a ruling by the Commission (International Commission for Zoological Nomenclature, Opinion 731, 1955), the generic name *Chermes* Linnaeus, 1758, and the family group name *Chermides* Fallen, 1814, were declared invalid due to the confusion associated with their dual use with psyllid insects. This would make any subsequent use of these names in the literature invalid. Apparently, adelgid workers like Annand and Börner realized the confusion long before the Commission did—both stopped using these names in their 1928 works. Nevertheless, many other adelgid workers did not (Silvestri 1939 and Bodenheimer and Swirski 1957).

Carter's manuscript (1971) on the conifer woolly aphids in Britain was the first to recognize and use the two-genus system of classifying adelgids since Annand (1928). Carter placed the Adelgidae in the superfamily Aphidoidea as Börner did. Like Annand, Carter believed adelgids should be placed into either *Pineus* or *Adelges* based on the number of abdominal spiracles of the adult. Carter subsequently reduced in rank all of Börner's genera to subgenera within *Adelges* and *Pineus* and used characters from the antennae (shape, length, and characteristics of the sensoria) and abdominal spiracles of the winged forms to help distinguish adelgid genera.

A few other aphidoid taxonomists have supported Annand and Carter's classification for a number of reasons (Ilharco and van Harten 1987 and Blackman and Eastop 1994). They believed that the separation of Adelgidae by Börner into numerous genera was based on very slight differences (i.e., differences more appropriate for species) and that splitting of taxa into numerous lower groups created greater confusion. Foottit and Richards (1993) provided descriptions and a key for Canadian adelgids, using the two-genus system of *Adelges* and *Pineus*. They stated that, although current European classifications may have merit, they preferred the two-genus system of North America and Great Britain. They said to apply the European system to the North American adelgids "would require extensive revision."

USE OF NAMES TODAY

Unfortunately, classification of adelgids above the species level has become a question of preference (Blackman and Eastop 1984). There has been little consistency in the adelgid nomenclature; recent and past publications on adelgid biology and taxonomic publications have used the various higher-level classification systems interchangeably. Most European authors have used, and continue to use, Börner's generic classification (Eichhorn 1989, Alles 1994, Roversi and Binazzi 1996, Dragan 1999, and Sato 1999). Eichhorn (1968 and 1969), however, used the genus name *Adelges* in various publications. Nonetheless, Carter's and Annand's generic classification is followed by many aphid biologists today (Blackman and Eastop 1984 and 1994, Heie 1999), by most adelgid applied biologists in the New World (McClure 1987, Cheah and McClure 1996 and 1998, Soria et al. 1996, Wallace and Hain 2000, and Zilahi-Balogh et al. 2003; also see this proceedings), and some in the Old World (Szklarzewicz et al. 2000).

DISCUSSION: CAN THERE BE STABILITY IN THIS SYSTEM?

The use of different classification systems at the generic level in the family Adelgidae continues to present day. It has become a problem of personal taxonomic license; just because someone publishes synonymies of certain taxa, it doesn't mean that everyone will follow it. Comparing figures and descriptions of 1st instar nymphs shows that the species entities that each system describe are the same (Annand 1928, Shaposhnikov 1964, and Carter 1971), as discussed previously in this paper. *Dreyfusia piceae* Ratzeburg in Börner's work is the same species as *Adelges piceae* Ratzeburg in Annand's work. Where the classifications disagree is how they should be classified at the generic level.

Consistent classification of adelgids has most likely been hampered by the inherent difficulty in describing the morphological variation in the group and the complexity of their life cycle. Misidentifications of adelgid species are very common in the literature. Researchers at first may have encountered only one stage of the life cycle and named it as a new species unaware of the other life stages. It is therefore important to thoroughly document the biology of the adelgid species in question before assigning it a new name. Furthermore, different adelgid species use the same host at the same time of year, making it even more of a challenge to distinguish species (Annand 1928). As pointed out by many authors (Annand 1928, Carter 1971, and Foottit and Richards 1993) different adelgid workers have historically disagreed on what are the informative generic characters for the family. Instability is also a problem in this family because the ratio of workers to adelgid species is so high (Foottit and Richards 1993). Therefore the number of opinions created via taxonomic license is substantial.

Unfortunately, the confusion in adelgid classification is not unique to this family. People around the world tend to study the taxa unique or endemic to their region, often creating a different classification than their peers around the world. These differences are likely due to poor communication among workers and poor means of information retrieval in isolated areas. Often, workers will also tend to focus on species level problems while ignoring higher level classification. The need for rigorous phylogenetic analyses which sample taxa from all regions and that take into account the worldwide fauna is very important in standardizing a classification that most workers will follow.

With the recent revolution in molecular phylogenetic techniques, it is now possible to collect and use two kinds of data in phylogenetic analyses: molecular and morphological. The only way to determine which and how many genera the 50 species should group into is by performing a rigorous phylogenetic analysis, preferably using both types of data collection, on all adelgid species. Morphological analyses should include characters from immatures and all stages and body regions of the adults. Species that cluster together at the tips of a phylogenetic tree are thereby taken as the most closely related. For example, if all the species in *Adelges* and *Pineus* clustered into two separate natural (monophyletic) groups respectively, it would support Annand and Carter's reasoning that Adelgidae should be split into two natural entities. If each of Börner's genera, with their respective species, clustered separately within the analysis, it would lend support Börner's multiple genus system. If however, two species within *Dreyfusia* were to fall out on opposite ends of the tree, it would be evidence that *Dreyfusia* was not a natural group.

It is important for this systematic research on Adelgidae to continue. Numerous pest adelgids threaten the well-being of forest ecosystems around the world. In order for taxonomists, applied adelgid biologists, and forest managers to communicate, formulate management plans, and attempt to solve these problems, they should all be using the same names.

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The Economic Impacts of Hemlock Woolly Adelgid on Residential Landscape Values: Sparta, New Jersey Case Study

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ABSTRACT

In this paper, we provide preliminary estimates of the impacts of the hemlock woolly adelgid on residential property values in Sparta, New Jersey, using the hedonic property value method. The literature on the aesthetic perceptions of forest landscapes is briefly reviewed to provide guidance in formulating economic hypotheses based on the assumption of an informative relationship between forest aesthetics and economic value. The hedonic property value literature regarding the ornamental and landscape value of trees is also reviewed. The empirical results show that healthy and lightly defoliated hemlocks contribute positive value to residential properties, and that moderately defoliated hemlocks reduce property values. Value 'spillovers', or externalities, are also observed where hemlock health has an impact not only on individual parcels containing hemlock resources, but also on neighboring property values. The implications of our results for forest managers are discussed.

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae*, is an exotic insect causing severe decline and mortality to forests of eastern and Carolina hemlock (*Tsuga canadensis*, *Tsuga caroliniana* Engelm.) throughout their geographical range. The HWA is currently established in 15 eastern states from Georgia to Maine and is perceived as a threat to the remaining states with eastern and Carolina hemlock resources. Hemlocks play a unique role in eastern forest landscapes. Accordingly, the loss and damage of hemlocks may potentially result in enormous ecological impacts that may be similar to the dramatic decline of eastern hemlock forests approximately 4,800 years ago (Orwig and Foster 1998).

The economic impacts of the spread of the HWA have not been systematically quantified. Quantification of the economic damages due to HWA is important for a variety of reasons. First, overall estimates of these impacts may be used by policy-makers to justify expenditures on control or mitigation of HWA. Second, detailed information on the spatial distribution of these impacts may assist policy makers in determining how and where funds should be allocated to the management of this forest pest. Contrasting these economic impacts with the costs of control allows for policy-makers to evaluate management actions and to establish priorities in terms of targeting control efforts.

It is hypothesized that economic damages from HWA may be large because hemlock forests provide a variety of ecosystem services that are valued by people. Ecosystem services derived from hemlock forests include the protection of riparian habitat supporting coldwater species such as trout, the aesthetic value of old growth hemlock stands, particularly on public land where trees may exceed 500 years of age, and the aesthetic value of ornamental and native hemlocks in private residential landscapes. The research reported here examines the economic impact of changes in the health of hemlock stands on the value of residential landscapes.

The economic valuation of landscape aesthetics is in a formative stage. However, if it is assumed that aesthetic values uncovered in psychological studies of human preferences for landscape characteristics are related to economic values, then the scientific literature regarding what is known about the aesthetic perception of landscapes can provide guidance in the specification and interpretation of economic models.

In the next section of this paper, we review and summarize the scientific literature regarding what is known about the perception of scenic beauty of forest stands and, in particular, how changes in forest health may impact forest aesthetics. Then we review the literature on the economic valuation of the arboreal landscape, which has been focused primarily at the parcel level scale, but which recently has come to include the larger landscape scale. Next, we describe the data obtained for a case study of the impact of changes in hemlock health on the value of residential properties in a township in northern New Jersey that has experienced severe hemlock mortality resulting from HWA infestations. In the subsequent section we provide an empirical analysis of how hemlock health has impacted private property values in this township. Finally, we present a summary of our findings and present the implications for forest policy and management.

AESTHETIC PERCEPTION OF FOREST LANDSCAPES

The primary approach to studying the relationship between forest landscapes and aesthetic perceptions is based on psycho-physical methods. These methods seek to identify quantitative relationships between a visual stimulus (often using photographs) and a perceptual response. A widely used psycho-physical model is the scenic beauty estimation (SBE) method, as pioneered by Daniel and Boster (1976). This method typically uses linear regression methods to isolate the impact of singular forest attributes on perceptions of scenic beauty. Ribe (1989) provides a good overall review of research on the aesthetics of forestry and forest management, particularly what has been learned using psycho-physical methods.

The perception of what constitutes a scenic landscape may be traced back to antiquity and, in its most primitive form, has been suggested to reflect Arcadian pastoral idylls that seek a peaceful balance between raw nature and human influence (Parsons and Daniel 2002). Some researchers have hypothesized that aesthetic landscape preferences result from human evolution in savanna environments, leading to preferences for landscape elements conferring opportunities for prospect and refuge—that is, the ability to see but not be seen. This perspective is exemplified by prescriptions for silvicultural practices that create well-lit, park-like forest environments (Brush 1976).

Among the factors influencing the aesthetic perception of forest landscapes, a few are particularly relevant for our analysis. First, a number of studies have found that species diversity can increase scenic beauty (Cook 1972, Daniel and Schroeder 1979, Brown and Daniel 1984). Because hemlocks are typically a relatively minor species in forest composition, their presence contributes to the visual diversity of forest landscapes. Thus, we would anticipate that, if a monotonic relationship exists between scenic beauty and economic value, the presence of healthy hemlock trees would increase private property values. Conversely, if hemlock mortality induces the regeneration of more common species, such as black birch (*Betula lenta*) and other hardwoods (Orwig and Foster 1998, Kizlinski et al. 2002), we would anticipate that loss of the hemlock component would decrease property values.

Second, psycho-physical research has shown that aesthetic perceptions of forests are influenced by forest health. Buhyoff and Leuschner (1978) found that people disliked stands damaged by southern pine beetle (*Dendroctonus frontalis*) and that scenic preference values decreased more precipitously when they were informed about the source of the damage. Further, they estimated that the scenic impacts of insect damage increased rapidly up to about 10 percent of the visual area, above which additional damage had a relatively small impact on scenic preference. Similar results were obtained in a later replication of the experiment (Buhyoffet al. 1980). These studies suggest that a conservative estimate of economic losses from HWA can be obtained by considering a 10 percent increase in hemlock decline, and this level of change is used in the computations below.

Finally, it is noteworthy that Brush (1979), in a study of the perceptions of forest landowners in Massachusetts for twenty different forest sites, found that old hemlock stands were rated, on average, above all other sites for scenic beauty. Thus, hemlocks may possess unique scenic attributes not shared by other species.

ECONOMIC VALUE OF ORNAMENTAL TREES AND FOREST LANDSCAPES

A number of methods are available for estimating the economic impacts of changes in forest health, including the contingent valuation method, the averting behavior method, and the hedonic property value method. The contingent valuation method asks people how much they are willing to pay for changes in environmental quality, and a good review of the application of this method to forest health problems is contained in the study by Kramer et al. (2003). The averting behavior method investigates how much money homeowners actually spend for protection of environmental attributes. This method has been applied to analysis of gypsy moth protection programs by Moeller et al. (1977).

The hedonic property value method uses linear regression to estimate the empirical relationship between real estate prices and environmental attributes after controlling for a suite of relevant housing attributes. This method is methodologically similar to psycho-physical measures of landscape amenities where preference ratings or rankings are replaced by prices. Although the hedonic property value model is useful for estimating the private benefits of environmental attributes, the method relies upon market prices for value inference. Other dimensions of economic value that are not revealed by market prices, such as the value of ecosystem services to future generations, cannot be estimated using hedonic valuation methods.

An early study of this type was conducted by Morales et al. (1976), who examined 60 "comparable" houses in three neighborhoods in Manchester, Connecticut. They found that good tree cover added an average of about 6 percent to the property value. Anderson and Cordell (1988) examined the relationship between the presence of front yard trees and property values of single-family houses in Athens, Georgia, and found that trees in the front yard increased housing prices by roughly 3-5 percent relative to houses without trees. Dombrow, et al. (2000) investigated the contribution of mature trees to the market value of single-family homes in Baton Rouge, Louisiana, and found that that mature trees contributed about 2 percent to housing values.

A recent innovation in conducting hedonic property value studies is to use remote sensing data derived from satellite imagery. Geoghegan et al. (1997) used GIS data to test the hypothesis that the value of land parcels in residential areas is affected by the pattern of surrounding land uses. Using data obtained within a 30-mile radius of Washington, D.C., they found that people in residential areas care about landscape features such as open space, landscape diversity, and fragmentation. Further, they found that landscape context matters: that is, the degree to which landscape features are capitalized into property values depends on whether parcels are located in a highly developed area, a suburban area, or a relatively rural area. Paterson and Boyle (2002) examined how property prices in a relatively rural area of Connecticut (Simsbury and Avon) are affected by the extent of different land use patterns within a 1-kilometer radius around each property. They found that people enjoy the amenities associated with nearby forestlands, but prefer views of other types of cover, as a view of too much forest could lead to a feeling of being "closed-in." A study by Mansfield et al. (2002) in the Research Triangle Park region of North Carolina used a "greenness" index for forest cover to evaluate the importance of tree cover on property values in this rapidly urbanizing area. Their results showed that houses closer to institutional and private forests had greater sales prices and that parcels with a larger proportion of forest cover also had a higher value.

We were only able to identify one study that used the hedonic price model to examine the economic losses to residential property value from tree mortality caused by insect damage (Payne and Strom 1975). The valuation method employed was indirect in that it used the hedonic property value technique to estimate a relationship between the number of trees on a lot and the value of the lot. Then, using this information, estimated losses were simulated for varying degrees of potential tree loss from gypsy moth. The method is limited in that it does not directly estimate the impact of gypsy moth mortality on property value, and it does not consider the lost value from trees that are unsightly or unhealthy as a consequence of gypsy moth infestations.

CASE STUDY DATA – SPARTA, NEW JERSEY

In this paper, we report a hedonic property value analysis for the town of Sparta, in Sussex County, New Jersey Murphy 2005). The 39-square-mile township is located in the northwestern New Jersey highlands and has a population of 17,500. The area is know for its many lake communities and is located 45 miles from New York City. Housing data were obtained from the town clerk of Sparta, New Jersey, and a data set was compiled by an independent computing firm. The raw data had 5,108 house sale records over the period 1970 to 2003. After cleaning the raw data, there were 3,379 usable observations. Available in the data were sales prices and the date each residential property was sold. Structural housing characteristics collected for the hedonic estimation include: square footage of living area, number of bedrooms, number of bathrooms, the size of the parcel in acres, the year the house was built, and whether the basement and/or attic had been finished. Digital tax parcel maps for Sparta were provided by the township's engineering office. Among the salient housing characteristics, the average sale price was $382,180 (adjusted to constant 2002 dollars), and the average lot size was just under 1 acre.

Environmental variables were constructed for each individual parcel and three different spatial scales around each parcel centroid. These variables include measurements of hemlock health change, as well as land cover types, publicly owned open space, golf courses, and water bodies. Various spatial buffers around parcel centroids were examined to investigate the relationship between landscape features and property values across spatial scales. In particular, it is hypothesized that environmental amenities found on any particular parcel will "spillover" and influence the value of neighboring parcels. As there does not seem to be any consensus in previous spatial hedonic studies as to what buffer zone radius to use, a range of distances were chosen for analysis: 0.1 km, 0.5 km and 1.0 km from the parcel centroid. Land cover variables were measured as the number of pixels falling within individual parcels and respective spatial buffers and then converted to proportions of the total number of pixels within those measurement units.

The environmental variables were created at the Center for Remote Sensing and Spatial Analysis, Rutgers University. Landsat satellite data were used to create the environmental variables used in this analysis. The level of resolution was 30m, so classification of hemlock land cover does not represent individual trees, but rather represents hemlock stands. Royle and Lathrop (1997 and 2002) previously used Landsat imagery and change detection techniques to model and map hemlock canopy condition for over 8,000 ha of hemlock stands throughout northern New Jersey for the years 1984, 1992, 1994, 1996, 1998, and 2001. A subset of these data was used in the hedonic property value analysis for Sparta. Four hemlock health classes were created: (1) a combination of healthy and lightly defoliated hemlocks (less than 25 percent defoliation), (2) moderately defoliated hemlocks (25-50 percent defoliation), (3) severely defoliated hemlocks (50-75 percent defoliation), and (4) dead hemlocks (greater than 75 percent defoliation).

In general, hemlock stands in Sparta were patchily distributed and constituted about 3 percent of the land area. Although most hemlock stands are thought to have been healthy in 1984, the health of hemlock stands declined rapidly during the 1990s. In 1998, only about 30 percent of the hemlock area was classified as being either healthy or lightly defoliated. By 2001, nearly all of the hemlock area was classified as being either severely defoliated or dead. Hemlock health variables were created to represent proportions of land area.

Several land cover types were used in this study. Some land cover types were combined, as previous research indicates that people only distinguish about seven land types at any one time (Palmer 2004) and to reduce statistical problems associated with collinear explanatory variables. The land cover classifications used in the analysis include percent highly developed, percent forested, percent wetland, percent covered by streams, percent in public space, distance to the nearest lake, and distance to the nearest golf course. The land cover data were obtained for three different points in time: 1985, 1995, and 2000.

Table 1. Hemlock parameter estimates at various spatial scales (t-statistics in parentheses).

Hemlock Health Class	**Spatial Scale of Model**			
	Parcel	0.1 km	0.5 km	1.0 km
Healthy & Lightly Defoliated	0.68(3.01)	1.13(3.35)	3.95(4.54)	7.39(6.16)
Moderately Defoliated	-0.95(-3.26)	-1.44(-4.35)	-3.62(-3.65)	-6.10(-4.43)
Severely Defoliated	-0.11(-0.65)	0.16(0.71)	0.07(0.30)	-0.09(-0.24)
Dead	0.13(0.45)	0.05(0.14)	0.09(0.22)	0.86(1.52)

EMPIRICAL METHODS

In the basic hedonic property value model, the price of individual properties is regressed on a vector of explanatory variables including housing characteristics, lot characteristics, and environmental variables for the surrounding area. Although Ordinary Least Squares (OLS) regression is often used, interpretation of empirical results may be confounded by spatial dependence. Spatial dependence may arise either in the dependent variable (leading to 'spatial lag' dependence) or in the equation errors (leading to 'spatial error' dependence). If spatial lags are ignored in the analysis, OLS will give biased and inconsistent parameter estimates. If spatial error dependence is ignored, OLS will have a biased variance estimate, resulting in inefficient parameter estimates. In this study, empirical methods are used to correct for both types of spatial dependence in the data. The equations are specified with the logarithm of sales price as the dependent variable. As the land cover variables were measured as proportions of the respective measurement units (parcels and spatial buffers), parameter estimates are interpreted as the proportionate response of housing price to a marginal change in the proportion of land area in a particular land cover type.

RESULTS

The spatial dependence regression models fit the data well, and the R^2 values were roughly 68 percent for each of the four models we estimated. Estimates for the hemlock parameters are shown in Table 1. The other parameter estimates in the models are not shown to simplify the presentation.

The parameter estimates for healthy and lightly defoliated hemlocks were statistically significant at greater than the 1-percent level at all spatial scales and show that healthy hemlock stands contribute positive economic value to property values in our study area. Although we cannot be certain of the reason that healthy hemlocks confer positive values to residential properties, this result is consistent with findings from the scenic beauty literature that species/ structural diversity is a valued component of forest aesthetics. This result is also consistent with the findings reported by Brush (1979) that hemlocks have a special aesthetic appeal.

The significance of the parameter estimates in the spatial buffer models indicates the presence of 'spillover' effects (or 'externalities'). Healthy hemlocks on any particular parcel convey value not only to that parcel but also to other parcels in the neighborhood. This spillover effect is also observed for moderately defoliated hemlocks: moderate hemlock decline decreases property values both for the parcel and for lots in a rather large radius around the damaged parcel.

It is important to understand the interpretation of the magnitude of the parameter estimates. Due to the way that the model was specified (recall that the dependent variable is the logarithm of housing price and hemlock variables are measured as proportions), the parameter estimates show the proportionate change in housing price in response to a proportionate change in the hemlock variables. Thus, a one percent increase (or decrease) in the area of healthy hemlock increases (or decreases) housing price by about 0.7 percent. Similarly, a one percent increase (or decrease) in the area of moderately defoliated hemlocks decreases (or increases) housing price by about 0.95 percent. The similarity in the value of the parameter estimates for healthy/lightly defoliated stands compared with the (absolute value of the) parameter estimates for moderately defoliated stands across all spatial scales indicates that the loss of healthy hemlocks is approximately equal to the gain in unhealthy hemlocks.

An estimate of economic damages to houses at risk can be obtained by calibrating the parameter estimates shown in Table 1 for the relevant area in each of the spatial scales. Parcels classified as containing moderately defoliated hemlock pixels had, on average, about 20 percent of their land area in this land cover classification. In comparison, parcels classified as containing moderately defoliated hemlock pixels within 1 km had, on average, about 1 percent of their land area in this classification. Thus a 1 percent increase in the area of moderately defoliated hemlocks at the parcel level would change the relevant average area in this land cover type from 20 to 21 percent. This represents a 5 percent increase in the relevant average area (1 out of 20). Using this frame of reference, a 1 percent increase in the area of moderately defoliated hemlock within 1 km of a parcel nearly doubles the relevant average area (1-2 percent). Given this perspective, a 10 percent increase in the relevant average area of moderately defoliated hemlocks at the parcel level (20-22 percent) reduces property value by about $7,261 per house at risk. A 10 percent increase in the relevant average area of moderately defoliated hemlock within 1 km of a parcel (1-1.1 percent) reduces property value by about $2,331 per house at risk. Although the reduction in property value is lower for houses in the neighborhood of damaged parcels, as would be expected, there are a greater number of houses at risk in the neighborhood.

It is somewhat surprising that the parameter estimates for severely defoliated and dead hemlocks were not statistically significant. This might be explained by a number of reasons. Because hemlocks in the study area tend to grow in discrete patches and because areas experiencing moderate defoliation tend to be located close to areas with severe defoliation and dead trees, the absence of statistical significance may be due to the statistical problem of multicollinearity. This effect may be compounded by the relatively few observations in each of the hemlock health classes.

DISCUSSION

In this study, we have used remote sensing techniques, concepts drawn from both the landscape ecology and the forest landscape aesthetics literature, and economic theory and methods to estimate the economic impacts of HWA on property values. This research has allowed us to provide the first estimates of economic damage to private landowners resulting from this exotic pest. We emphasize that these damage estimates are valid for a single housing market, and it is not obvious how they can be directly applied to other areas. However, if the results found in our study area are typical of other regions experiencing hemlock decline, then the total economic damages to property owners in the eastern U.S. may be very large. A more definitive assessment of how typical our study results are of damages in other regions would require replication of the procedures described here in other housing markets.

Results of our case study make it very clear that forest managers need to either prevent infestations by HWA altogether or to manage stands in such a fashion that they only become lightly defoliated in order to prevent economic losses to property values. As our results show, economic damages begin to occur when stands become moderately defoliated (25-50 percent defoliation). Although hemlocks may be able to survive many years of moderate decline, particularly in regions where cold winters reduce HWA populations, maintaining stands in these conditions can result in economic losses to residential property values.

While the costs and efficacy of biological control strategies for the HWA are not yet known with precision, the presence of landscape-level externalities suggests that the formation of neighborhood or community groups to combat this insect may be a viable strategy to protect housing values in this and similar markets. This sort of complementary group response would seem to work particularly well for the case of hemlock resource owners, because the resource is often patchily distributed. Further research should be conducted to determine the viability of HWA control strategies that would take advantage of spatial patterns of resources at risk that could capitalize on complementary group behavior at the neighborhood or community level.

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Impacts of Balsam Woolly Adelgid on the Southern Appalachian Spruce-Fir Ecosystem and the North Carolina Christmas Tree Industry

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ABSTRACT

The balsam woolly adelgid, an exotic aphid-like insect from Europe, has wreaked considerable ecological havoc on the boreal red spruce-Fraser fir ecosystem endemic to the Southern Appalachians. During the last 50 years, the adelgid has decimated the Fraser fir stands that exist on a handful of island-like high-elevation ridge systems, and has imposed significant economic costs on the regionally important Christmas tree industry. The virtual elimination of mature fir trees from their natural stands has altered the plant and animal communities unique to the red spruce-Fraser fir forest type. While firs appear to have regenerated well since the initial wave of adelgid-caused mortality, it is unclear whether future infestations will occur and, if they do, how they will impact the species. A mathematical model indicates that Fraser fir populations may be large enough, and the species' life cycle long enough, to avoid the extensive loss of genetic diversity over time unless the populations experience regularly repeated adelgid infestations. Work is under way to identify and propagate Fraser firs resistant to adelgid infestation and to conserve the existing genetic diversity of the species. These activities could allow for the eventual reintroduction of firs into their native stands, should such an effort become necessary.

KEYWORDS

Balsam woolly adelgid, Fraser fir, ecosystem, impact, genetic conservation.

INTRODUCTION

The high-elevation red spruce-Fraser fir forests of North Carolina, Tennessee, and Virginia together encompass a unique boreal ecosystem endemic to the southeastern United States. Never very extensive, this forest type has been reduced by poor forest management practices, and could face elimination as a result of global climate change. Currently, the most serious threat to its continued existence, however, is an insect that measures less than a millimeter in length – the balsam woolly adelgid (*Adelges piceae* Ratz.). During the last 50 years, this exotic invader from Europe has decimated mature natural stands of Fraser fir (*Abies fraseri* [Pursh] Poir.), causing significant changes to the spruce-fir ecosystem. It has also posed problems for North Carolina's $100 million annual Christmas tree industry, which relies almost entirely on plantation-grown Fraser fir.

In natural Fraser fir stands, this economically and ecologically important conifer occurs almost entirely above 1,300 meters, usually in association with red spruce (*Picea rubens* Sarg.), but it becomes the dominant tree species above about 1,800 meters (Busing et al. 1993, Cain 1935, Whittaker 1956). It exists in six major island-like populations (Figure 1): the Great Smoky Mountains in North Carolina and Tennessee; the Black Mountains, the Balsam Mountains, and Grandfather Mountain in North Carolina; Roan Mountain on the Tennessee/North Carolina border; and Mount Rogers in Virginia. Additionally, three minor populations exist in North Carolina: the Plott Balsams, Cataloochee Balsam, and Shining Rock. Whitetop Mountain in Virginia, near Mount Rogers, has red spruce but no Fraser fir.

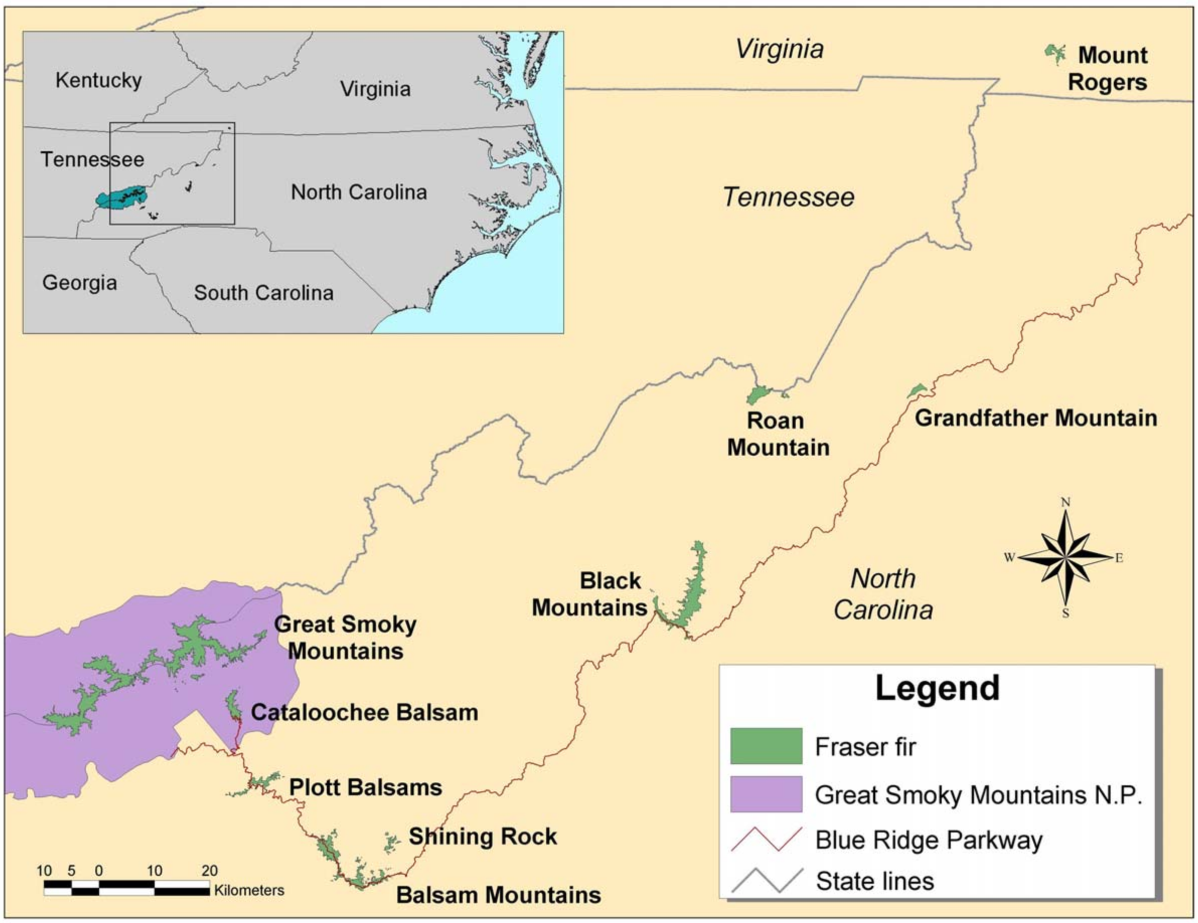

Figure 1: The distribution of Fraser fir in the Southern Appalachians.

These populations are relicts of a boreal forest that extended across much of the Southeast during the peak of the most recent late-Wisconsin glacial period, from 18,000 years to 12,500 years before present (Delcourt and Delcourt 1987, Whitehead 1973, Whitehead 1981). At the full-glacial maximum, this boreal forest may have covered 1.8 million km^2 from Missouri to the Carolinas (Delcourt and Delcourt 1984). By 8,000 years ago, warming climate conditions eliminated lower-elevation spruce-fir stands in the Southeast. Much of the distribution of fir shifted north, following the retreating ice into Canada, New England, and the Northern Appalachians; the fir species now occurring in those areas is balsam fir (*Abies balsamea* [L.] Miller), which is closely related to Fraser fir.

Since the late nineteenth century, logging and slash fires have dramatically reduced the distribution of Fraser fir and red spruce in the Southern Appalachians (Pyle 1984, Pyle and Schafale 1988, Saunders 1979). Failed regeneration caused by logging-site degradation may have reduced the extent of the highest spruce-fir forests, those above 1,670 meters, to less than half their former historical area, from 14,277 hectares to 6,881 hectares (Saunders 1979). Logging and fire in the Great Smoky Mountains, which encompass nearly three-fourths of all Fraser fir-red spruce forest (Dull et al. 1988), reduced the forest type across all elevations from 17,910 hectares to 13,370 hectares (Pyle 1984).

With nearly all the Southern Appalachian spruce-fir forest now occurring on public lands (Dull et al. 1988), poor management practices were no longer a serious concern by the 1950s. Since that time, however, the balsam woolly adelgid (BWA) has inflicted severe mortality on old-growth Fraser fir forest. Introduced into the northeastern United States in the early 1900s, probably on imported nursery stock, the adelgid was first detected on Mount Mitchell in the Black Mountains in 1957, but may have been present in the area as early as 1940 (Eager 1984). Within a few years, the infestation spread from the centrally located Black Mountains to all the other populations: Roan Mountain and Mount Rogers by 1962, the Great Smoky Mountains and Grandfather Mountain by 1963, and the Balsam Mountains by 1968 (Amman 1966, Dull et al. 1988, Eager 1984). By the 1980s, reproductively mature Fraser fir trees had experienced 67 percent mortality throughout the species' range, including 91 percent mortality in Great Smoky Mountains National Park (Dull et al. 1988), site of the largest Fraser fir population.

The adelgid, which has a complex life cycle in its native range, reproduces asexually through parthenogenesis in North America, where the species consists of only females. Having an egg stage, three larval instar stages, and the adult stage, an adelgid spends its entire life immobile except during the first instar, or "crawler," stage, during which it is often dispersed by air currents (Eager 1984). Amman (1966) concluded that wind was responsible for moving the adelgid up to distances of 64 kilometers in the Southern Appalachians. The adelgid feeds by inserting its stylet into cracks in the bark of older fir trees, and remains fixed at the same location throughout its adult life. The attack of thousands of the insects on a tree results in the production of abnormal xylem that conducts water poorly; the tree typically dies within two to nine years of infestation (Amman and Speers 1965, Eager 1984, Hollingsworth and Hain 1991).

The atmospheric deposition of acidic sulfur and nitrogen compounds from power plants and other facilities west of the Appalachians may increase the susceptibility of Fraser fir to the adelgid, although the impact of such pollution remains uncertain even after decades of research. Hain and Arthur (1985) hypothesized that atmospheric deposition, mostly from fog at high elevations, might predispose fir to adelgid mortality, or could be the final source of stress to already infested trees.

Pesticide application and extensive biological control efforts were both ineffective in combating BWA infestation in Fraser fir natural stands. The application of pesticides, including Lindane, potassium salt of oleic acid, and insecticidal soap, was of little practical use in controlling BWA at large scales, since the bole of each tree must be sprayed to the point of saturation for these chemicals to be effective (Dull et al. 1988, Eager 1984, Johnson 1980). During the 1960s, researchers conducted several biological control experiments that introduced dozens of potential BWA predators (Table 1) into infested fir stands in the Southern Appalachians (Amman 1970, Amman and Speers 1971), the Pacific Northwest (Mitchell and Wright 1967), and eastern Canada (Brown and Clark 1960). All the predators failed to reduce BWA populations, however, because of climatic differences between the predators' new and old environments, poor prey acceptance, ineffective synchronization of predator-prey cycles, poor searching ability by the predators, the high reproductive capacity of the BWA, and the rapid death of the host tree (Amman and Speers 1971, Eager 1984, Mitchell and Wright 1967).

ECOLOGICAL IMPACTS OF BALSAM WOOLLY ADELGID INFESTATION

The elimination of most old-growth Fraser fir by the balsam woolly adelgid has dramatically altered the character of the Southern Appalachian spruce-fir forests, an uncommon ecosystem that has considerable ecological, aesthetic, and economic value. In addition to the importance of Fraser fir to the Christmas tree industry, the spruce-fir forest type is a central attraction of several popular visitor destinations, including the Blue Ridge Parkway, Great Smoky Mountains National Park, Mount Mitchell State Park, and the private Grandfather Mountain preserve. It plays an important role in protecting the high-elevation portions for the watersheds of several major rivers. Perhaps most importantly, the Southern Appalachian spruce-fir ecosystem is rich in rare and endemic animals and plants, including one bird species, one mammal, six invertebrates, and 12 plants considered at risk of extinction (Table 2). Of these, two are federally listed as endangered and 10 are federal species of concern (Southern Appalachian Man and the Biosphere 1996).

While three frog species, 13 salamanders, and eight snakes occur in Southern Appalachian spruce-fir forests, only two species of herpetofauna – the pygmy salamander (*Desmognathus wrighti*) and the imitator salamander (*Desmognathus imitator*) – are endemic to this forest type (Mathews and Echternacht 1984). The deterioration of the spruce-fir forest could result in the loss of these species, but more research is needed in this area. The patchy high-elevation spruce-fir forests of the Southern Appalachians have historically hosted a distinctively boreal and unique bird community (Rabenold 1984). This community has changed significantly since the onset of the BWA infestation in the Great Smoky Mountains: 10 of 11 breeding species declined between 1974 and the 1990s, with some near local extinction. Five of the most strongly affected birds – black-capped chickadee (*Poecile atricapilla*), blue-headed vireo (*Vireo solitarius*), red-breasted nuthatch (*Sitta canadensis*), black-throated green warbler (*Dendroica virens*), and golden-crowned kinglet (*Regulus satrapa*) – are canopy- and subcanopy-nesting species characteristic of spruce-fir forests throughout the Appalachians. Meanwhile, bird species characteristic of open, disturbed forests – chestnut-sided warbler (*Dendroica pensylvanica*) and eastern towhee (*Pipilo erythrophthalmus*) – became abundant (Rabenold et al. 1998).

Table 1. Arachnids and insects tested as possible balsam woolly adelgid predators

Order	Species	Origin	Citation*
Acarina (ticks and mites)			
	Allothrombium michelli Davis	North Carolina	2
	Anystis sp.	North Carolina	2
	Leptus sp.	North Carolina	2
	unidentified bdellid	North Carolina	2
Coleoptera (beetles)			
	Adalia luteopicta Mulsant	India	3
	Adalia tetraspilota Hope	India/Pakistan	1,3
	Adonia variegata Goeze	India	1
	Aphidecta obliterata	Sweden/Germany	3
	Ballia diana Mulsant	India	3
	Ballia eucharis Mulsant	India/Pakistan	1,3
	Calvia sp.	India	1
	Chilocorus kuwanea Silvestri	Japan	3
	Exochomus ssp. (2 species)	Pakistan	3
	Harmonia breiti Mader	India/Pakistan	1,3
	Laricobius erichsonii	Germany/Czechoslovakia	3
	Leis dimidiata F.	India	3
	Mulsantina hudsonica Casey	North Carolina	2
	Oenopia sauzeti Mulsant	India	1,3
	Pullus impexus	Germany	3
	Scymnus pumilio Weise	Australia	3
	Synharmonia conglobata L.	India	3
Diptera (true flies)			
	Aphidoletes thompsoni Mohn	Germany/Czechoslovakia	2,3
	Cremifania nigrocellulata	Germany/Czechoslovakia	3
	Leucopis ssp. (3 species)	India	1
	Leucopis obscura	Europe	3
	Metasyrphus lapponicus Zett.	New Brunswick	4
	Neocnemodon coxalis Curr.	New Brunswick	4
	Pipiza sp.	North Carolina	2
	Syrphus torvus O.S.	North Carolina/New Brunswick	2,4
	unidentified syrphid	North Carolina	2
Hemiptera (true bugs)			
	Tetraphleps ssp. (3 species)	India/Pakistan	1
	Tetraphleps sp.	India/Pakistan	3
Neuroptera (lacewings)			
	Chrysopa ssp. (2 species)	India/Pakistan	1,3
	Hemerobius sp.	India	1
	Hemerobius ssp. (2 species)	North Carolina	2
	undetermined Hemerobiidae	India	3

* 1 = Amman and Speers 1971, 2 = Amman 1970, 3 = Mitchell and Wright 1967, 4 = Brown and Clark 1960

Table 2: Federally listed species and species with viability concern, from the red spruce-Fraser fir forest type

Species	Common Name	Type	Status*
Accipiter gentilis	northern goshawk	bird	FSC
Glaucomys sabrinus coloratus	Carolina northern flying squirrel	mammal	E
Cleidogona hoffmani	Hoffman's cleidogonid millipede	invertebrate	VC
Cleidogona lachesis	a millipede	invertebrate	VC
Hepialus sciophanes	a ghost moth	invertebrate	FSC
Mesodon clingmanicus	Clingman covert	invertebrate	FSC
Microhexura montivaga	spruce-fir moss spider	invertebrate	E
Semiothisa fraserata	Fraser fir geometrid	invertebrate	FSC
Abies fraseri	Fraser fir	plant	FSC
Aconitum reclinatum	trailing wolfsbane	plant	VC
Bazzania nudicaulis	liverwort	plant	FSC
Brachydontium trichodes	peak moss	plant	VC
Cacalia rugelia	Rugel's ragwort	plant	FSC
Chelone lyonii	purple turtlehead	plant	VC
Gymnocarpium appalachianum	Appalachian oak fern	plant	FSC
Leptothymenium sharpii	Mt. Leconte moss	plant	FSC
Plagiochila corniculata	liverwort	plant	VC
Solidago glomerata	goldenrod	plant	VC
Sphenolobopsis pearsonii	liverwort	plant	FSC
Stachys clingmanii	Clingman's hedgenettle	plant	VC

* E = endangered; FSC = federal species of concern; VC = species with viability concerns

White (1984) catalogued 132 vascular plant species that occur in the spruce-fir forests of the Great Smoky Mountains. Of these, eight species were endemic to high peaks in the Southern Appalachians and six were species that also occur at or above timberline in the high Northern Appalachians of New England, relict alpine flora that indicate that the southern mountains may have been cold enough for a tree line 12,000-20,000 years ago (Delcourt and Delcourt 1984). Additionally, old-growth Southern Appalachian spruce-fir stands sometimes host a conspicuous and diverse bryophyte cover that contains northern disjunct species (Southern Appalachian Man and the Biosphere 1996). Thirty-six species of mosses and liverworts frequently grow on the bark of Fraser fir, so the decimation of mature fir stands poses a major threat to the diversity and abundance of Southern Appalachian bryophytes, probably causing the decline and extirpation of some rare species (Smith 1984). Similarly, Fraser fir is the preferred substrate tree of roughly 20 of the 100 epiphytic lichen species that occur in these spruce-fir forests, so the loss of mature Fraser firs to BWA may make these species vulnerable to local extinction (Dey 1984).

Before balsam woolly adelgid infestation, the stand composition of old-growth red spruce-Fraser fir forests resulted from a natural disturbance regime dominated by small treefall-created canopy gaps (White et al. 1985). Dramatic Fraser fir mortality, however, quickly opened up large areas of the forest canopy, altering both overstory and understory dynamics in the spruce-fir forest community. The live basal area of Fraser fir was reduced considerably in the Great Smoky Mountains between the 1960s and 1980s as nearly all the firs in the canopy died, while the basal area and density of red spruce stems either increased slightly or remained the same (Busing and Clebsch 1988, Busing et al. 1988). Overall, stand basal area decreased 28 percent (Busing et al. 1988). The density of live fir has been shown to be positively associated with the time since the major wave of mortality in the Great Smoky Mountains (Smith 1997, Smith and Nicholas 1998). At the same time, the opening of the canopy has allowed an increase in sub-shrub coverage, most notably blackberry (*Rubus canadensis* L.) (DeSelm and Boner 1984, Rabenold et al. 1998, Smith 1997), and for a decrease in bryophyte coverage (DeSelm and Boner 1984, Smith 1997). The impact on the herbaceous and shrub layers are less certain, with Smith (1997) seeing an increase in herbaceous and shrub density in opened canopies, and Rabenold et al. (1998) finding a decline in herbaceous cover and in the abundance of shrubs, including hobblebush (*Viburnum alnifolium* Marshall) and mountain cranberry (*Vaccinium erythrocarpum* Michaux).

Fraser fir stands suffering extensive mortality from the balsam woolly adelgid apparently have been able to regenerate with vigorous and numerous offspring (Busing and Clebsch 1988, Witter and Ragenovich 1986), many of which are now 20 to 30 years old – the age of viable seed production for open-grown and overstory Fraser fir trees. The question now is whether these trees will live long enough to produce the next generation of fir before they, too, are severely infested by the adelgid.

Some researchers believe that large numbers of Fraser fir will continue to exist in the high-elevation forests of the Southern Appalachians. Based on research at Mount Mitchell in the 1970s, for example, Witter and Ragenovich (1986) predicted that viable seeds would continue to be produced before the firs are extensively damaged by the balsam woolly adelgid, and that, as a result, Fraser fir should continue to be an important component in the fir, spruce-fir, and spruce-fir-hardwood forest types. Additionally, adelgid populations are currently lower in the Great Smoky Mountains than in the early outbreak years of the 1970s and 1980s, and their reproduction rates are lower (Kristine Johnson, personal communication).

Other research may signal cause for concern about the future of Fraser fir. Pauley and Clebsch (1990) found that 26 percent of fir seedlings at Mt. Collins in the Great Smoky Mountains had adelgid-caused gouting – a number that increased to 39 percent for seedlings older than 5 years. Additionally, they noted that blackberry plants may be inhibiting the establishment of fir seedlings in areas where the adelgid caused severe overstory fir mortality. As a result, they note, future fir establishment could be limited to shaded slopes and downed logs, where blackberry is unable to gain the upper hand. In a study of five summits in the Great Smoky Mountains, Smith and Nicholas (2000) found that firs may be growing faster under the open canopy caused by adelgid mortality, and therefore may reach reproductive maturity at a younger age. However, they predicted that as Fraser fir continues to regenerate, it will be re-infested by the balsam woolly adelgid. This could initiate a regeneration-mortality cycle that slowly reduces the size of fir populations because the adelgid may allow few trees to reach reproductive maturity, and that results in a decreasing number of even-aged patches in different stages of regeneration and mortality (Smith and Nicholas 2000). Interestingly, Fraser fir mortality rates have recently declined, indicating that the initial wave of adelgid-caused mortality is virtually complete, although a new wave of mortality may have begun in the first generation of post-adelgid fir at Mount Mitchell, the first site impacted by the pest (Smith and Nicholas 1999).

MODELING THE IMPACTS OF BWA ON FRASER FIR GENETICS

A significant concern for the future of Fraser fir is that the BWA-caused demographic bottleneck could intensify the effects of genetic drift in the smallest Fraser fir populations. Genetic drift is the random fluctuation of allele frequencies as a result of "sampling errors" during reproduction; while it occurs in all populations, its effects over a small number of generations are most evident in very small populations (Hartl and Clark 1997). It can cause a loss of genetic variation within a population, eventually resulting in the fixation of alleles, which occurs with the elimination of heterozygosity in a gene system (Ellstrand and Elam 1993). Some of the alleles lost as a result of drift could be low-frequency alleles that might allow the population to adapt to environmental change. Decreased genetic variation and inbreeding depression, in turn, may increase a population's risk of extinction from pests, climate change, random events, reduced inbreeding viability, and demographic uncertainty (Barrett and Kohn 1991, Huenneke 1991, Lande 1999). Further, the loss of genetically differentiated populations could result in a serious loss of genetic diversity for the species, with fewer small populations remaining to represent its gene pool (Rajora and Mosseler 2001). However, the migration of genes among populations, in the form of long-distance pollen dispersal in the case of Fraser fir, may counter the disruptive effects of inbreeding and genetic drift by transferring genetic variation among populations (Hamrick and Nason 2000).

Predicting the long-term genetic impacts of balsam woolly adelgid infestation on Fraser fir stands is a complicated proposition for two reasons. First, it's unclear whether BWA infestations will continue to occur as existing young trees mature to an age at which they are susceptible to mortality from the adelgid. Second, both infestation-recovery cycles and intergenerational genetic drift occur over many decades, making them difficult to study over the course of a single career. To address these challenges, we have developed a mathematical model that allows us to test hypotheses about the impact of differing BWA infestation scenarios.

Our stage-structured population matrix model for Fraser fir is unique in that it simulates genetic dynamics in a long-lived forest tree species with overlapping generations. The specific objectives of the stochastic model are: 1) to investigate genetic drift and allele fixation in a two-allele, single-locus gene system in a series of hypothetical fir populations of varying sizes, initial allele frequencies, and number of BWA infestation bottlenecks, and 2) to compare the effect of drift to the impact of natural selection resulting from the presence of a hypothetical BWA-resistance gene in actual Fraser fir populations. We based the stage structure and reproductive characteristics of the modeled Fraser fir populations on field data, including age class distribution in the Great Smoky Mountains in the 1950s before the introduction of BWA (Oosting and Billings 1951) and remote sensing estimates of the spatial extent of Fraser fir populations (Dull et al. 1988). When data for other characteristics, such as pollen dispersal, were not available, we chose parameters to produce biologically plausible behavior.

The models of hypothetical Fraser fir populations showed that allele fixation is more likely with smaller populations, lower initial allele frequencies, and greater number of genetic bottlenecks (in the form of BWA infestations). These models revealed greater genetic drift in populations that are smaller (Table 3) and experiencing more infestations (Figure 2).

Models of real populations indicated that selection (in the form of a BWA-resistant genotype) had a considerably larger impact on allele frequencies and heterozygosity than genetic drift, even after a single infestation-caused bottleneck. With one or no infestation-caused bottlenecks, genetic drift in the smallest real population (Shining Rock, with roughly 5,900 mature trees) was almost indiscernible even after 10,000 years, while repeated bottlenecks resulted in large amounts of drift. In other words, taking into account overlapping generations, Fraser fir populations may be large enough and the species' life cycle long enough to avoid genetic drift unless the populations experience repeated BWA infestations. Other forces, such as natural selection, may be more responsible for existing differences in genetic diversity among populations.

Table 3. The standard deviation of change in recessive allele frequency after 1,000 years – a measure of the potential for genetic drift – increases with smaller hypothetical adult population size and with the number of infestations. (From 100 runs of each size-infestation number combination, with initial recessive allele frequency of 0.1).

Hypothetical Population Size	Number of Infestations		
	0	1	11
100	0.0036	0.0103	0.0644
1,000	0.0010	0.0032	0.0232
10,000	0.0004	0.0009	0.0071

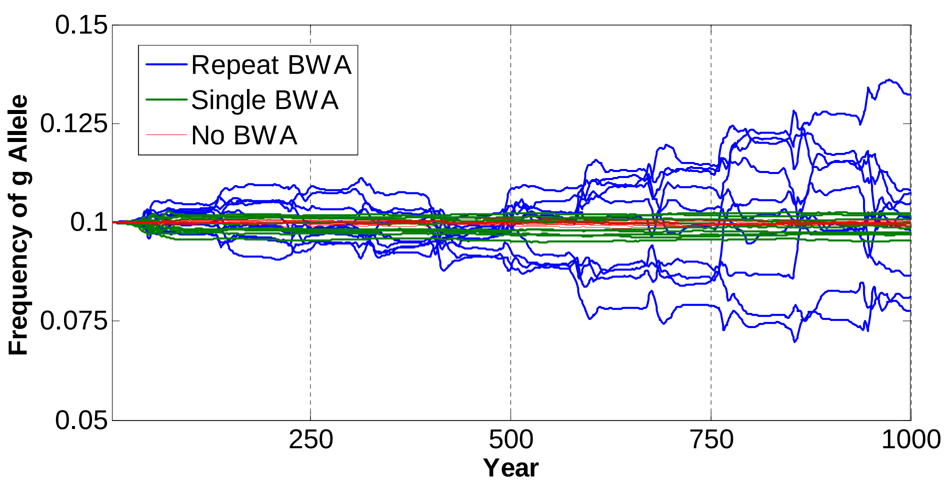

Figure 2. Genetic drift increased considerably with increasing numbers of adelgid-infestation bottlenecks. (From 20 model runs of each infestation level for a hypothetical population of 1,000 mature firs.)

THE SEARCH FOR GENETIC RESISTANCE

The continued existence of Fraser fir in its natural stands may depend on whether the species has genetic resistance to BWA, or the ability to develop it. Trees in the Mount Rogers population, for example, may have this resistance (Eager 1984, Rheinhardt 1984) as they have suffered less mortality than other populations despite having been exposed to the adelgid since 1962 (Eager 1984, Nicholas et al. 1992).

If genes for resistance to the balsam woolly adelgid exist in only a few scattered trees in any of the populations, they may be more likely to successfully produce offspring that are also less susceptible to death from adelgid attack. After several generations, many trees surviving in a stand could be resistant if the infestation is severe and continuous enough to kill off the more vulnerable firs. If such resistance exists and if Smith and Nicholas (2000) were correct that Fraser fir population size could be diminished by the adelgid, it is interesting to speculate which would happen first: the extirpation of smaller Fraser fir populations or the development of populations entirely resistant to the pest. It is unlikely we will know the answer to that question any time soon.

Efforts are underway by forest geneticists and entomologists at North Carolina State University to find and breed firs that are resistant to adelgid attack. With help from rangers at Great Smoky Mountains National Park, North Carolina, state researchers have collected branches from old-growth Fraser fir trees that may have survived the initial balsam woolly adelgid attack. These branches were grafted onto fir trees at the North Carolina Division of Forest Resources' Linville River Nursery and have been transplanted to the N.C. Department of Agriculture/NC State University Mountain Research Center at Laurel Springs. When they are older, these trees will be further multiplied via grafting and infested with adelgids to determine whether they are, indeed, resistant.

This project eventually could allow for the reintroduction of adelgid-resistant Fraser firs into their natural stands. It could also assist Christmas tree producers by facilitating the breeding of Fraser firs that are naturally less vulnerable to attack by the pest, saving growers the time and money required to spray their Christmas tree farms with pesticides.

CONSERVING FRASER FIR GENETIC DIVERSITY

Even if all nine major and minor Fraser fir populations survive depredation by the balsam woolly adelgid, they may face at least one more serious threat: global climate change. A recent model (Delcourt and Delcourt 1998) predicts the elimination of Southern Appalachian Fraser fir-red spruce forest with a possible global mean temperature increase of 3° C caused by greenhouse warming. Fraser fir is listed in North Carolina as an imperiled species, as a species of concern federally, and as a species imperiled and vulnerable to extinction globally (Ameroso and Finnegan 2002). Clearly, the limited distribution of Fraser fir and the threats to its survival in a natural setting are compelling reasons for the systematic conservation of its gene pool (Nicholas et al. 1999).

We are developing a gene conservation plan for Fraser fir that would help facilitate the restoration of the species to its natural stands if BWA or climate change makes such action necessary and would ensure the continued existence of a genetic resource base for Christmas tree breeding. While almost all Fraser fir stands are managed for the continued existence of the species, this is no guarantee that it will be able to evolve in response to rapid environmental changes. An *ex situ* (off-site) conservation plan will help ensure much of the genetic material of these populations is preserved in the event of their loss and could allow for the evolution of genes that, if introduced into natural Fraser fir populations, would make them better able to evolve and survive drastic environmental changes (Eriksson et al. 1993).

The plan will integrate existing Christmas tree breeding and gene conservation efforts with additional measures to archive Fraser fir genetic resources and to expand the amount of genetic variation included in off-site conservation efforts. Its components are likely to include: 1) a seed bank representing both major and minor Fraser fir populations and seeds generated by breeding efforts and in conservation plantings, 2) existing tree breeding elements (provenance and progeny tests, seed orchards, and clone banks), and 3) conservation plantings.

THE IMPACT OF ADELGID ON THE CHRISTMAS TREE INDUSTRY

North Carolina accounts for more than 12 percent of real Christmas trees produced in the United States and ranks second in the total number of trees harvested and first in the dollars made per tree. Nearly all the Christmas trees grown in the state are Fraser fir. In 1999, 1,600 North Carolina growers sold 3.7 million trees worth a reported value of more than $92 million (North Carolina Department of Agriculture and Consumer Services 2005). Virtually all Fraser fir Christmas trees in western North Carolina have to be treated for BWA one or more times during a 5- to 10-year rotation. This results in an annual cost to the industry estimated at $1.53 million. This estimate, however, does not include additional costs such as losses due to BWA damage or increased miticide control costs associated with BWA treatments.

Generally, the only Fraser fir Christmas tree plantations escaping BWA infestations are those isolated from natural stands, other Christmas tree production areas, and Fraser firs grown as yard trees. Producing Christmas trees near infested yard trees or abandoned Christmas tree plantations greatly increases insect pressure. Growers are encouraged to scout for the BWA yearly, and 68.5 percent of growers did so in 2000. The first and most easily recognized symptom of a BWA infestation is the loss of apical dominance in the affected tree, meaning that the tree produces a crooked top rather than a straight one. When trees with crooked tops are found in the field, the trunk can be examined for the presence of the insect. Growers may also find BWA infestations when they harvest trees: trees infested with BWA produce hard, red-colored reaction wood, which makes the trees harder to cut down.

The threshold for treating a Fraser fir plantation is a single infested tree, although culling symptomatic trees may slow the spread in some instances. Several general predators feed on BWA, including the *Harmonia* ladybeetle, but will not eliminate an infestation. Fraser fir is extremely sensitive to adelgids, and even a few of this pest will impact tree quality. With the introduction of the exotic hemlock woolly adelgid (*Adelges tsugae* Annand) into the eastern United States, there is increased interest in predators and parasitoids of adelgids, which could result in greater natural controls of the BWA in the future.

In treating a Christmas tree plantation for BWA, insecticides must be applied with a high-pressure sprayer using 300 to 800 gallons per acre, depending on tree size and density. The entire tree must be wetted with the insecticide because the adelgids can occur on buds, shoots, and branches in addition to the trunk. Trees are treated from two directions as the applicator pulls 200 to 300 feet of hose up and down rows. Only two or three rows can be treated at a time. About half the Christmas tree growers own this type of equipment, but others must hire someone to treat their trees at a cost of $300 to $500 per acre. Because of the cost and difficulty of these treatments, growers hope to achieve up to three years of BWA control with one treatment.

Materials used for control include:

Lindane 20 EC (lindane): Lindane has traditionally been the primary chemical used to control BWA. Lindane was used on 21.7 percent of Christmas tree acres in 1994 and 23.8 percent in 2000. It is no longer being manufactured, however, and its use is declining.

Asana XL (esfenvalerate): Asana, the primary replacement for Lindane, was used on 16.6 percent of Christmas tree acres in 2000, up slightly from 11.8 percent in 1994. Growers have not felt that they are receiving as long-lasting control with Asana as they had with Lindane. Additionally, growers have reported having more problems with hemlock rust mite during the year following Asana applications.

Thiodan 3EC (endosulfan): Because of the concerns associated with Asana, a 24(c) Special Local Need label was granted for two years for Thiodan 3EC. This chemical cannot be used within 300 feet of surface water because of concerns about its effects on aquatic life. In 2000, it was used on 2.2 percent of acres, but that number has since increased.

Horticultural oil: Horticultural oil is currently used on only 0.1 percent of Christmas tree acres. Treatments with oil to control the BWA are only effective during the winter, when no eggs are present. Oils can burn Fraser fir foliage depending on the rate and the time of year used.

Other Insecticides: Provado 1.6F (imidacloprid), Metasystox-R (oxydemeton-methyl), and Lorsban 4E (chlorpyrifos) are labeled for adelgid control, but are not frequently used. Control has not been long-lasting with these materials. Growers are currently interested in two other synthetic pyrethroids: Astro (permethrin) and Talstar (bifenthrin). Astro has provided up to 18 months of control in pest-control trials.

The use of broad spectrum insecticides for BWA control results in outbreaks of spruce spider mites (*Oligonychus ununguis*) and hemlock rust mites (HRM) (*Nalepella tsugifoliae*). Since the mid-1990s, when the use of synthetic pyrethroids such as Asana started to become more common for BWA, rust mites have become an increasing problem. In 2000, 20.2 percent of growers used insecticides and/or miticides to control HRM, compared to fewer than 2 percent in 1994. In fact, if Fraser fir in western North Carolina did not require BWA treatment, growers could use more biological control measures for spider mites, rust mites, and the balsam twig aphid (*Mindarus abietinus*), which are all cosmetic pests. Many native predators exist for these pests, but their numbers are reduced with BWA control.

CONCLUSIONS

Over the course of a few decades, balsam woolly adelgid has drastically altered a boreal relict ecosystem endemic to the Southeastern United States. While it appears that Fraser fir will remain a part of this forest community, it is uncertain whether repeated adelgid infestation would reduce its genetic diversity and its status as a codominant canopy species in this ecosystem. Additional research is needed to determine whether a second wave of adelgid mortality is beginning, and to quantify the impacts of adelgid infestation on the flora and fauna of the red spruce-Fraser fir forest type. Efforts to locate and propagate adelgid-resistant firs and to conserve the genetic diversity of the species could become important in any future plans to restore or augment Southern Appalachian spruce-fir forests in which Fraser fir becomes diminished. These measures could also help reduce the time and financial costs borne by the economically important Christmas tree industry to control adelgids in Fraser fir plantations.

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Long-term Effects of Hemlock Forest Decline on Headwater Stream Communities

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ABSTRACT

We conducted a comparative study to determine the potential long-term impacts of hemlock forest decline on fish and benthic macroinvertebrate assemblages in headwater streams in the Delaware Water Gap National Recreation Area. Hemlock forests throughout eastern North America have been declining due to the hemlock woolly adelgid, an exotic insect pest. We found aquatic invertebrate community structure to be strongly correlated with forest composition. Streams draining hemlock forests were more diverse but less productive than streams draining mixed hardwood forests. In addition, there were distinct differences in macroinvertebrate trophic structure, with predators more common and grazers less common in hemlock-drained streams. In contrast, forest-type differences in fish assemblage structure were less pronounced, although trends suggested that species richness was higher in streams draining hardwood and functional diversity was higher in hemlock-drained streams. We also found important distinctions in terms of fish species composition. Brook trout (*Salvelinus fontinalis*), an important fishery in the park, were three times more likely to occur and four times more abundant in streams draining hemlock than in those draining hardwood forests. Also, fish trophic structure varied between forest type, with insectivores more common in hardwood-drained streams and piscivores more common in hemlock-drained streams. Analysis of stream habitat data indicated that streams draining hemlock forests had more stable thermal and hydrologic regimes. Our findings suggest hemlock decline may result in long-term changes in headwater stream ecosystems that are comparable in scope to effects observed in terrestrial ecosystems.

KEY WORDS

Ecological effects, aquatic communities, hemlock forest decline, headwater streams, habitat stability.

INTRODUCTION

Eastern hemlock (*Tsuga canadensis*) forests have declined substantially in the last two decades as a result of the hemlock woolly adelgid (*Adelges tsugae*). The ecological impact of losing this important climax forest species is poorly understood, but has the potential for significant disturbance to biotic communities by changing the energy availability, microclimate, and distribution and abundance of habitat. The importance of hemlock to many terrestrial assemblages has been documented (reviewed in McManus et al. 1999). In contrast, the influence of hemlock on aquatic assemblages has received little attention, and relatively few studies have evaluated the long-term consequences of changing forest composition on stream communities (see Molles 1982, Stout et al. 1992).

There have been numerous reports of significant changes in headwater stream habitat and aquatic community structure associated with forest disturbances. However, most of these studies have been designed to evaluate the responses of stream ecosystems to the relatively short-term changes that take place between the death or removal of existing riparian forests and the maturation of new overstory canopies. Of particular importance during this period is the shift in the stream energy base associated with large changes in the amount and timing of leaf litter and large woody debris inputs entering the stream and altered light, temperature, and hydrologic regimes (Likens et al. 1970). Such changes have been shown to have significant effects on available habitat (Naiman et al. 1988), nutrient dynamics (Webster et al. 1992), channel morphology (Gregory 1992), and ultimately, the trophic structure and productivity of aquatic assemblages (Wallace and Gurtz 1986, Sheldon 1988). There is no reason to believe that similar short-term changes would not accompany the decline of hemlock forests in Delaware Water Gap National Recreation Area (DEWA). However, considerably less is known about the long-term consequences to headwater stream ecosystems of pest-induced changes in forest composition.

The objective of this study was to compare macroinvertebrate and fish community structure and stream habitat in streams draining hemlock and mixed hardwood forests in DEWA. We limited the study to headwater catchments so that forest-specific effects could be isolated from other confounding factors that are present in larger watersheds. Results should yield information useful in predicting the long-term consequences of hemlock decline on aquatic assemblages. Since hemlock regeneration following hemlock woolly adelgid-induced mortality is poor, and overstory recruitment is largely limited to mixed hardwood species in affected stands (Fuller 1998, Orwig and Foster 1998), we believe our predictions reflect realistic assumptions regarding long-term changes in forest composition.

METHODS

STUDY AREA

Delaware Water Gap National Recreation Area (DEWA) is located in northeastern Pennsylvania and western New Jersey. The park encompasses 27,742 hectares of forested hills, ravines, and bottom lands straddling the Delaware River. Hemlock stands in DEWA are patchily distributed, occurring largely on relatively cool, moist sites. Though stands containing significant amounts of hemlock comprise only about 5% of the forested landscape in DEWA (1130 hectares), where they occur, hemlock often dominates, comprising as much as 77% of the basal area in stands (Sullivan et al. 1998). Recent evidence suggests that prior to Euro-American settlement hemlock represented a greater component of many northeastern U.S. forests than it does today, and that present-day hemlock stands are often restricted to permanent woodlots with reduced disturbance histories (Foster et al. 1998). In DEWA, many hardwood stands, especially in ravine terrains, are younger on average than hemlock stands and contain a large number of cut, decayed hemlock stumps, suggesting that hemlock was likely more abundant in DEWA in the past.

Hemlock woolly adelgid (HWA) infestations were first detected in DEWA in 1989 and a subsequent survey conducted in 1995 indicated that over half of the Park's hemlock stands were infested (Evans 1995). Nevertheless, at the time of this study, HWA-induced mortality was limited to a few small stands, mostly in the south-eastern portion of the Park. Since this study was conducted, impacts from HWA have increased dramatically (R. Evans, personal communication).

LANDSCAPE ANALYSIS AND SAMPLING DESIGN

Our approach was to focus the study on smaller, headwater catchments because larger streams drained areas containing both hemlock and mixed hardwood forest, making forest-specific comparison intractable. In addition, most of these larger watersheds were impacted by humans (e.g., impoundments, agriculture, quarries) that could confound our assessment of the influence of hemlock. Even after limiting the study to headwater catchments, other possible confounding factors remained; we controlled for landscape variability (i.e., terrain and stream size) through the sampling design and we excluded others (i.e., minimum catchment area, beaver activity) through site selection. Because the primary objective was to detect a forest-type effect, we chose to study the end points represented by streams draining forest that were either dominated by hemlock or dominated by mixed hardwood. In this way, we maximized the opportunity to detect a forest-type effect if one existed.

We used geographic information systems (GIS) to characterize the landscape in DEWA and to provide the basis for the terrain-based, paired-site sampling design used in this study. Our goal was to sample across the range of landscape variability in the park and control for the effects of terrain and stream size prior to comparing aquatic invertebrate community measures between forest types. Ultimately, we selected 14 pairs of watersheds that were similar in terrain (defined by channel slope, aspect, solar radiance, channel shape, and elevation) and stream size but varied in forest species composition. Specifically, one watershed in each pair was comprised mainly of hemlock and the other watershed in each pair was comprised mainly of mixed hardwood species. See Young et al. (2002) for a complete description of the study design and GIS methods used to stratify sampling.

FIELD SAMPLING

Invertebrate sampling was conducted during the early spring of 1997. Sample reaches were defined by a distance of 80 m for 1st order streams and 160 m for 2nd order streams. These distances represented approximately 40 mean stream widths and encompassed a minimum of three riffle-pool sequences. We took a total of twenty 30-second kick samples with a D-frame kick net at random locations within each stream reach. See Snyder et al. (2002) for a complete description of methods used to collect and process macrinvertebrate samples.

Fish sampling was conducted in July 1997 during base flow conditions. However, because of the abnormally dry summer, 16 of the 28 initially selected streams dried totally. The 12 streams that remained wetted (7 in hemlock and 5 in hardwood) were sampled using a backpack electroshocking unit. Sampling reaches were defined the same as for macroinvertebrates. See Ross et al. (2003) for details regarding fish sampling.

Instream habitat measurements were taken within the same stream reaches used to collect macroinvertebrates and fish. We collected information on water chemistry, flow, water temperature, and stream channel morphology. Water chemistry included dissolved oxygen, pH, specific conductivity, nitrates, nitrites, ammonia nitrogen, total phosphates, and orthophosphates. Flow was measured during spring high-flow conditions and temperature was measured every hour with Optic StowAway™ temperature loggers. Stream channel measurements included 1) microhabitat diversity, 2) large woody debris (LWD), and 3) the extent to which each stream dried during the summer. See Snyder et al. (2002) for detailed descriptions of instream habitat sampling methods.

STATISTICAL ANALYSES

We compared four measures of invertebrate assemblage structure between streams draining hemlock and hardwood forests: taxa richness, Simpson's evenness index, total density, and number of rare taxa (defined as taxa that occurred at fewer than four sites). Making use of the terrain-based, paired-site design (Young et al. 2002), we tested the null hypothesis that mean difference in each community structure metric between site pairs was equal to zero. For each metric, we calculated the difference between individual hemlock-hardwood site pairs by subtracting the value measured in the hardwood site from that in the corresponding hemlock site. We used a paired t-test to test the statistical significance of the effect of forest type on each measure.

Likewise, we compared three measures of fish assemblage structure between hemlock- and hardwood-drained streams: species richness, species diversity (Shannon-Weiner), and trophic diversity (Ross et al. 2003). However, for fish, we were unable to use the paired site approach to test for differences in community structure because over half of the 28 selected sites dried up prior to fish sampling. Instead, we used a 3-way ANOVA to assess effects of terrain, stream size, and forest type and their interactions on the three fish community structure metrics.

We also compared the taxonomic and trophic composition of both fish and invertebrate assemblages between forest types. For taxonomic composition, we conducted an odds ratio test (Agresti 1990) to determine the association between the presence of each taxon and forest type. For trophic composition, we used multi-response permutation procedure (MRPP) to analyze forest-type effect on the proportion of individuals in multiple trophic groups. For invertebrates there were four groups (shredder-detritivores, collector-detritivores, grazer-algivores, and predators) and for fish there were three groups (piscivore, insectivore, and omnivore). MRPP is a non-parametric procedure designed to test for differences in multivariate responses among groups and has the advantage of not requiring multivariate normality and homogeneity of variance that are seldom met with ecological community data (Zimmerman et al. 1985).

We evaluated the effect of forest type on stream habitat using the same methods as those used to compare aquatic invertebrate assemblage structure responses. We compared temperature patterns (means, maxima, and minima) of streams in hemlock and hardwood forest types graphically using the hourly temperature data collected with temperature loggers. We used an alpha value of $P = 0.10$ for all tests of significance.

RESULTS

AQUATIC COMMUNITY STRUCTURE

From the 28 sites, we collected a total of 53,868 invertebrates from 151 taxa. The number of invertebrate taxa collected at any one site ranged between 21 and 66. A total of 64 taxa were considered rare (i.e., occurred at fewer than four sites), and the number of rare taxa ranged between zero and 10. Total density ranged between 116 - 4698 individuals · m^{-2}. In terms of fish, we collected a total of 1,406 individuals from 15 species and seven families. However, fish sampling was limited to the 12 sites that remained wetted when sampling occurred in July. Most sites harbored between one and four fish species though we collected 12 species from one site.

Despite large variances observed within forest types, we found statistically significant differences in all four invertebrate assemblage response variables. Streams draining hemlock supported on average about 1.5 times more total invertebrate taxa (mean difference = 14 taxa) and nearly 9% higher Simpson Evenness indices than streams draining hardwood forests. In contrast, streams draining hardwood forests were over 2.7 times more dense (mean difference = 838 individuals · m^{-2}) and supported more rare taxa (mean difference = 2 taxa) than streams draining hemlock (Figure 1).

In contrast, none of the three fish assemblage structure measures were significantly different between forest types (ANOVA, $p > 0.25$) although the reduced sample sizes compromised our power to detect differences considerably. Trends suggest that species richness may have been higher in mixed hardwood sites and functional diversity may have been higher in hemlock sites (Figure 2).

Results of odds ratio tests for each invertebrate taxa indicated that forest-specific differences in taxa richness were associated with specific taxa and not simply a random subset of the community. Eleven taxa (7.3% of total) showed strong associations with hemlock (Odds ratio test, $p < 0.10$) while no taxa were strongly associated with hardwood forests (Figure 3). An additional 17 taxa showed weaker associations with hemlock (Odds ratio test, $p < 0.30$), whereas only five taxa showed weak associations with hardwood forests. Of the 28 taxa that showed either strong or weak associations with hemlock, three were found to occur exclusively in hemlock-dominated watersheds while no taxa were found to occur only in streams draining hardwood forests (Figure 3).

Results of odds ratio tests indicated that occurrence patterns were not significantly different for any of the 11 fish taxa. However, again, statistical power was poor because of reduced sample size. Two species showed forest-specific trends. Bluegill (*Lepomis* *machrochirus*) was found more often in streams draining hardwood forests (14% of hemlock sites compared to 60% of hardwood sites), whereas, brook trout (*Salvelinus fontinalis*) was found more often in streams draining hemlock forest (57% of hemlock sites compared to 20% of mixed hardwood sites).

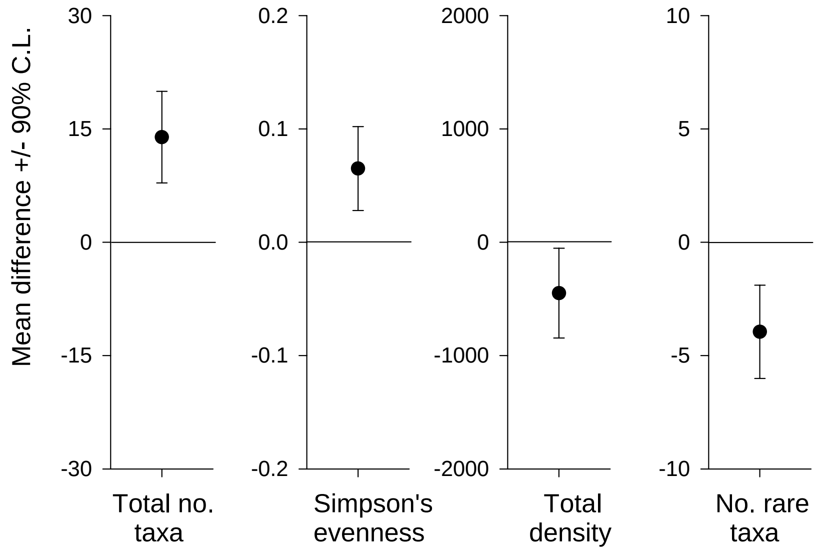

Figure 1. Average difference (± 90% CL) in four aquatic macroinvertebrate assemblage metrics between hemlock-hardwood site pairs. Positive values indicate higher means for hemlock. Cases where 90% confidence limits do not overlap 0 indicates statistical significance (p<0.10).

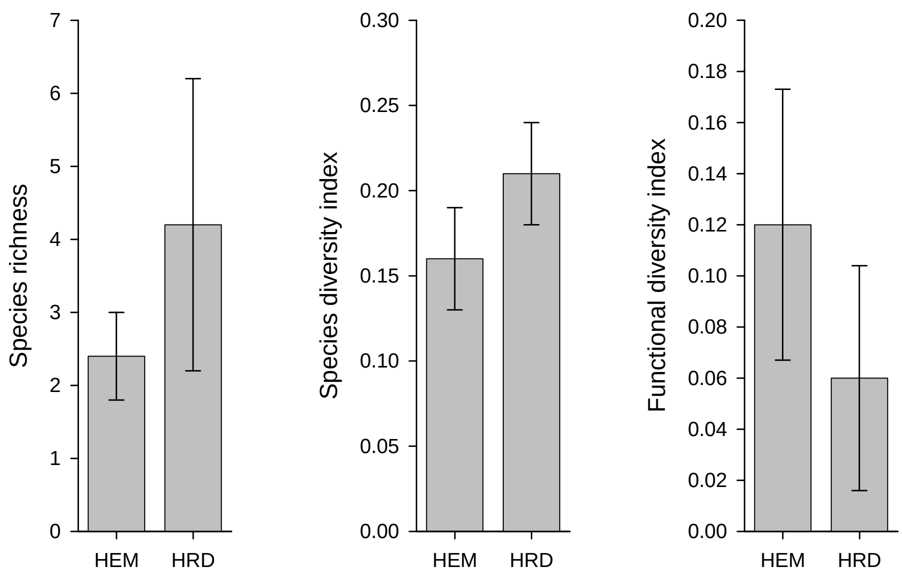

Figure 2. Comparison of three fish assemblage structure metrics between streams draining hemlock and streams draining hardwood forests. Graphs show means ± SE.

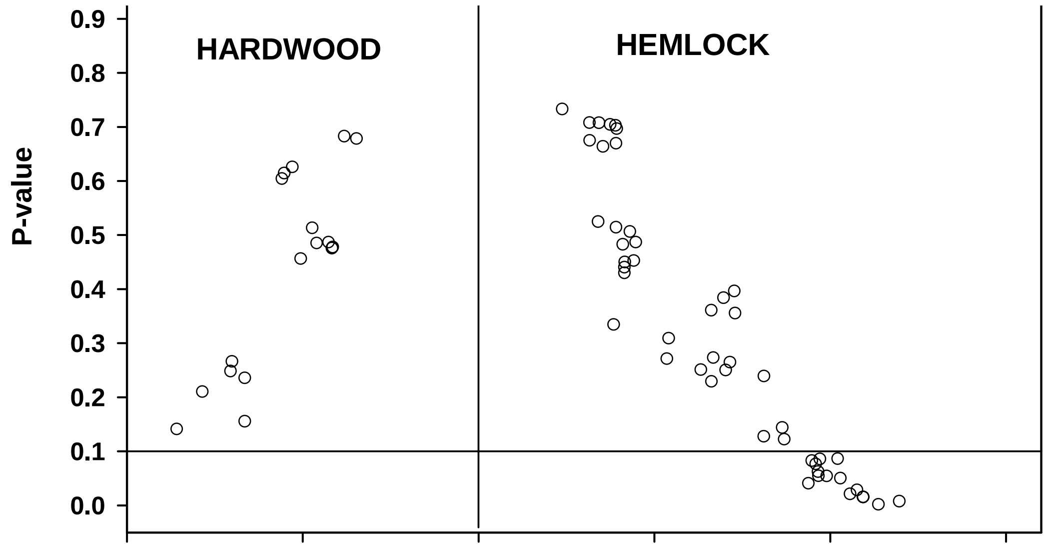

Figure 3. Results of odds ratio test to determine taxa-specific associations with vegetation types. For each taxa, ratio of occurrence in streams draining hemlock to that draining hardwood was used, and therefore, positive values indicate a preference for hemlock. Figure 3 shows the significance as a function of the odds ratio. Species with p-values less than 0.1 were deemed strong associates and those with values between 1.0 and 0.3 were deemed weak associates.

Trophic composition of invertebrate assemblages (i.e., the proportion of individuals in each of the four trophic groups) also differed between streams draining hemlock and hardwood forests (ä = 2.18; p = 0.009). Predators comprised a significantly larger fraction and grazer-algivores a significantly smaller fraction of the invertebrate assemblage in hemlock-drained streams (Figure 4). The proportion of individuals as shredders and collectors did not differ between forest types.

We also observed forest-specific differences in trophic composition for fish (Figure 5). On average, insectivores comprised a significantly larger fraction of the fish community in mixed hardwood sites (mean proportion = 0.77) than in hemlock sites (mean = 0.34) (ANOVA, F = 3.39, p = 0.09), whereas piscivores were proportionally more abundant in hemlock sites (mean = 0.61) than in hardwood sites (mean = 0.21) (ANOVA, F = 2.36, p = 0.15).

STREAM HABITAT

Water chemistry was highly variable among DEWA streams, with several variables exceeding an order of magnitude in range. However, mean differences between hemlock-hardwood site pairs were not significantly different from zero for any of the eight water chemistry variables we measured. Similarly, forest-type did not affect spring discharge, microhabitat diversity, or the frequency of large wood debris.

Median daily stream temperatures at hemlock sites tended to be cooler in the summer, warmer in the winter, and less variable throughout the year. Perhaps even more important to the species that inhabit these streams are the extremes in temperatures in which they are exposed. In particular, we found that summer daily maxima were lower and summer daily minima were higher in hemlock-drained streams. For example, summer daily maxima in streams draining hardwood forests exceeded 20°C over 18% of the time compared to less

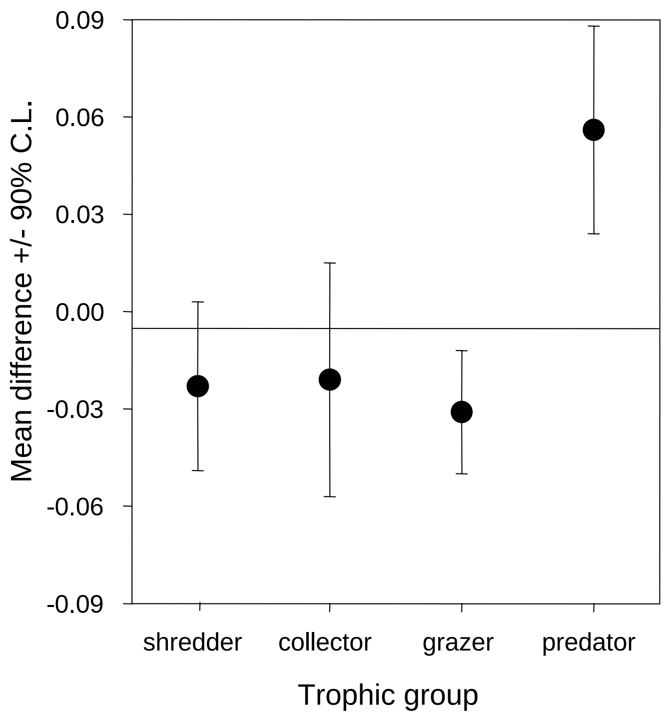

Figure 4. Comparisons of mean differences (± 90% CL) in macroinvertebrate trophic composition between hemlock-hardwood site pairs. Positive values indicate higher means for hemlock.

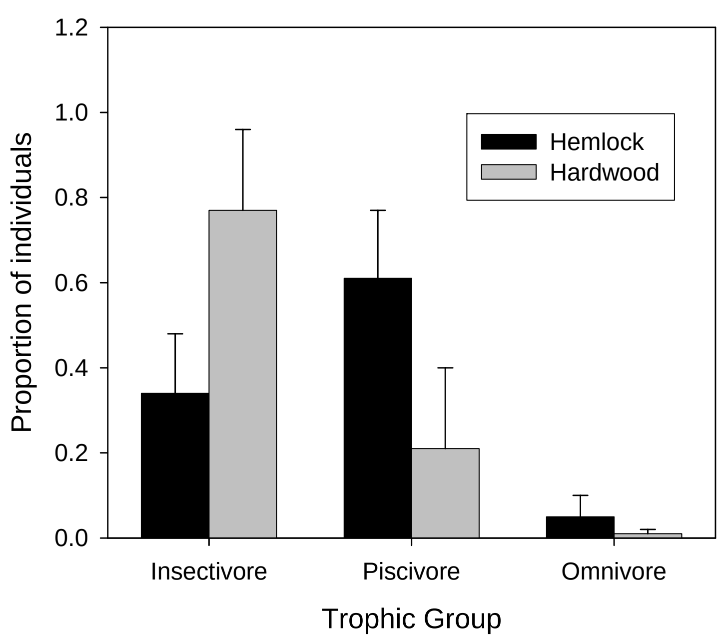

Figure 5. Comparisons of fish trophic composition between steams draining hemlock and hardwood forests. Graph shows mean proportions ± SE.

than 3% of the time in streams draining hemlock, and minimum daily temperatures dropped below freezing 8% of the time in hardwood sites compared to only 0.2% of the time in hemlock sites (Figure 6).

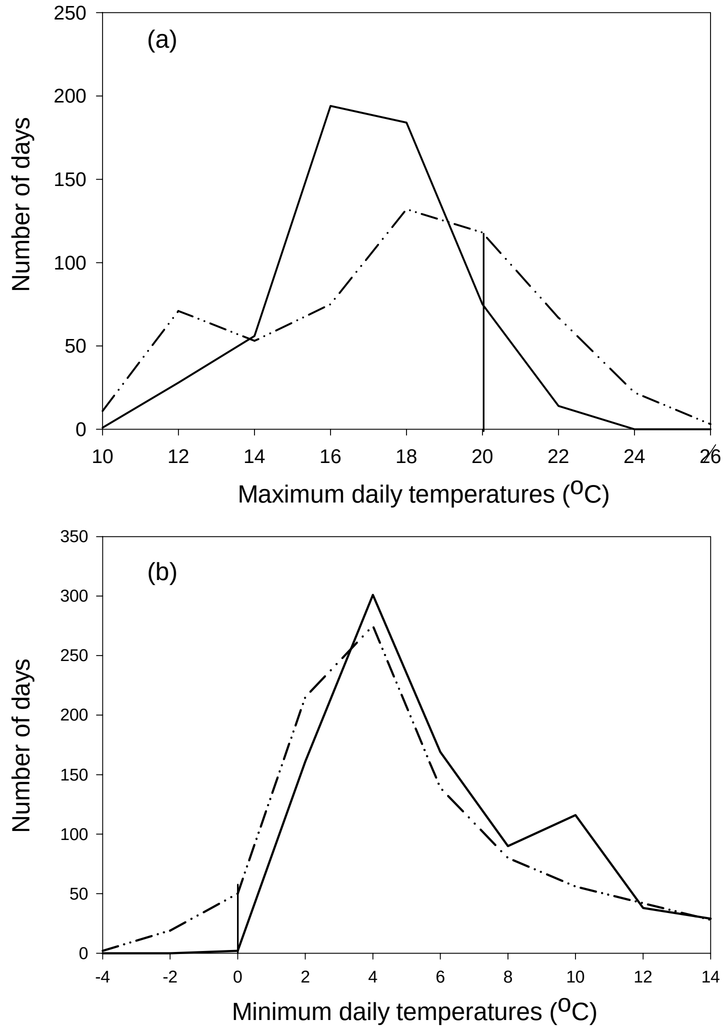

Figure 6. Comparisons of the distributions of (a) summer (July-September) maximum temperatures and (b) winter (October-February) minimum temperatures between streams draining hemlock and hardwood forests. Solid lines represent hemlock forests and dashed lines represent hardwood forests. Vertical lines represent arbitrary thresholds for comparison.

We used flow data from the USGS gaging station on the Bushkill River to characterize long-term rainfall patterns in the Park. We found stream flows to be normal (i.e., close to the long-term average) during the spring of 1997, when invertebrate sampling was conducted. However, the summer of 1997 was very dry, with stream flows during the months of July and August falling well below what would be considered normal for that time of year (see Table 1). Specifically, over the 90-year period between 1908 and 1997, only 5.5% of the average flows in July and 6.6% of the average flows in August were as low, or lower, than those observed in 1997. The result of the dry summer in 1997 was that a substantial portion of our study streams dried completely. We observed forest-specific differences in the extent to which streams dried. In the summer of 1997, nine out of 14 (64%) of streams draining hardwood forests became dry or mostly dry, while two out of 14 (14%) of streams draining hemlock dried partially. None of the streams draining hemlock dried completely compared with four out of 14 (29%) of the streams draining hardwood. Weather patterns during the summer of 1999 were even more severe, with the month of July being one of the driest on record. At our request, DEWA personnel revisited our study sites in July of 1999 and determined which sites remained wet (i.e., contained some water and positive flow). They found six out of 14 (43%) of streams draining hardwood dried completely, compared to only one out of 14 (7%) of the streams draining hemlock (R. Evans, personal communication).

Table 1. Qualitative assessments of drying for all 28 streams (14 hardwood and 14 hemlock) taken in July 1997, during a period of prolonged drought. Drying classes were based on the proportion of stream channel that remained wetted. The table shows the number of streams (percentage of total in parentheses) for each forest type.

Drying Class	Hemlock	Hardwood
Dry (0%)	0 (0%)	4 (29%)
Mostly dry (<50%)	2 (14%)	5 (36%)
Mostly wet (>50%)	3 (21%)	0 (0%)
Wet (100%)	9 (64%)	5 (36%)

DISCUSSION

After accounting for differences in terrain and stream order, we found aquatic invertebrate community structure was significantly different between streams draining hemlock and mixed hardwood forests. Streams draining hemlock forests supported more total taxa than streams draining hardwood forests, and over 7% of the taxa showed strong associations with hemlock, including three taxa that were found exclusively in hemlock streams. These patterns suggest that both within-site and park-wide diversity of aquatic invertebrates were enhanced by hemlock or by factors correlated with hemlock. In addition, invertebrate taxa were distributed more evenly (i.e., higher Simpson's Evenness values) in hemlock-drained streams, indicating that higher richness values were not associated with the chance occurrence of taxa represented by relatively few individuals. In contrast, the number of rare taxa and total densities were lower in streams draining hemlock, suggesting that diversity differences were not related to stochastic factors associated with sampling (e.g., richness related to number of individuals collected or chance occurrence of rare species) and that streams draining hardwood forests may have been more productive.

In contrast, forest-specific differences in fish assemblage structure were not as pronounced although there was some evidence (though weak) that species richness was higher in streams draining mixed hardwood and functional diversity was higher in hemlock-drained streams. We also observed weak associations between two fish species and forest type: brook trout were more likely to occur and more abundant in hemlock-drained streams than hardwood and blue gill were more likely to occur in mixed hardwood drained streams. Statistical associations may have been weaker for fish because of the lower sample size. However, it is also possible that the status of fish species and assemblages are less directly associated with forests surrounding streams because they are able to move in and out of streams. In this case, synoptic information on occurrence patterns may not be definitive because individuals may visit (e.g. for foraging) suboptimal habitats but still require other habitats to complete their life cycles and persist in the longer term. For example, summer stream temperatures may be too warm in streams draining mixed hardwood to support brook trout breeding but individual trout may frequently forage in hardwood-drained streams. If so, over time, widespread hemlock decline would likely have a significant impact on trout populations. In this case, a more intensive research effort would be required that evaluated fish movement patterns and time spent in various habitats in order to determine forest type effects.

We observed differences in stream habitat that could explain differences in benthic macroinvertebrate diversity and fish species composition observed between forest types. Diel and seasonal thermal regimes were more moderate in hemlock-drained streams, and streams draining hardwood forests were more prone to drought disturbance than those draining hemlock. Higher frequency of sub-zero temperatures observed in hardwood-drained streams could promote the formation of anchor ice, which has been found to a major factor regulating benthic assemblages by reducing taxa richness and limiting reproductive habitat (Miller and Stout 1989). Moreover, more stable thermal regimes may help minimize exposure of fish and benthic communities to temperatures outside the optimum range for many component species. More moderate thermal regimes would likely have positive effects on both invertebrates and fish, but may be particularly important in explaining higher brook trout occurrence and abundance patterns in streams draining hemlock. Specifically, brook trout prefer stream temperatures of 14-16°C and spawning is virtually restricted to water of 15°C and below. Furthermore, the upper lethal limit of hatchlings is 20°C, and adults are rarely found in streams where summer temperatures exceed 21°C (Jenkins and Burkhead 1993). The fact that summer maxima exceeded 20°C over 17% of the time in streams draining hardwood forests compared with 3% of the time in streams draining hemlock strongly supports the conclusion that a hemlock-induced effect on moderating stream temperatures was responsible for the distribution and abundance patterns of brook trout that we found. Likewise, there is evidence that benthic assemblages in headwater streams are more diverse in cooler, more thermally static streams (Kamler 1965).

Greater stability of summer baseflows in hemlock-drained streams may have contributed to forest-type differences in benthic diversity. Fewer streams draining hemlock forests dried up during two separate drought years. There is little doubt that stable base flows would afford, at least, short-term benefits to stream invertebrate communities. Since most aquatic insect species require at least one year to complete their life cycles (Wallace 1996), stream drying would likely kill or displace a large fraction of the benthic community leading to lower densities and diversity. Although disturbances such as floods and droughts may have positive effects on diversity patterns in the longer term (e,g., Resh et al. 1988), it is clear that disturbances may cause lasting reductions in the diversity of stream communities if they are severe enough to significantly depress exposed populations and widespread and frequent enough to limit recolonization from unaffected areas (Yount and Niemi 1990).

Hemlock and mixed hardwood forests also differ in their effect on autochthonous and allochthonous sources of energy, and these differences may explain observed differences in total invertebrate density and trophic composition observed between forest types. Streams draining hardwood forests receive more sunlight on an annual basis than those draining hemlock forests because of reduced shading associated with seasonal leaf-off periods, and even during leaf-on periods hemlock forests filter out more sunlight than mixed hardwood forests (Hadley 2000). Because light appears to be the primary factor limiting primary production in these forested headwater stream environments (Wellnitz et al. 1996), increases in incident light would stimulate more algal production, potentially increasing the total energy inputs and broadening the food base for stream invertebrates (Lamberti and Steinman 1997). Our observation that grazing algivores represented a larger fraction of the benthic community in hardwood-drained streams suggests an assemblage response to higher primary production. Smock and MacGregor (1988) showed that similar changes in trophic composition may have occurred following changes in forest composition due to the chestnut blight of the early 1900s.

Although there is some evidence that hemlock and other conifers contribute more allochthonous inputs annually than mixed hardwood forests (Anderson and Sedell 1979, Molles 1982), much of that energy is not available as food for aquatic macroinvertebrates (Webster and Benfield 1986), and so hemlock forests may provide less-usable allochthonous energy than mixed hardwood forests. Thus, in addition to more autochthonous-derived energy, greater contributions of high quality leaf litter may also contribute to the higher total abundances of benthic invertebrates we observed in hardwood-drained streams.

Taken together, the results of the present study indicate that headwater streams drained by hemlock and mixed hardwood forests support substantially different benthic communities, and notable differences in fish assemblages, though fish results are less clear. Further, our results suggest that forest-type differences in light and temperature regimes, stability of summer baseflows, and quality of allochthonous inputs are proximate causes of observed differences in benthic assemblage structure and composition. Based on these associations, we predict that pest-induced declines in hemlock will have long-term consequences for aquatic assemblages including a reduction in invertebrate diversity, a change in fish and invertebrate trophic structure, and possibly a decline in brook trout populations. Concern over trout fisheries may be even more pronounced in more southern areas where hemlock may be even more important in maintaining cooler summer temperatures required by trout.

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BINOMIAL SAMPLING PLAN FOR *ADELGES TSUGAE* ON EASTERN HEMLOCK

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ABSTRACT

The hemlock woolly adelgid is an exotic insect pest of eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *T. caroliniana* Engelm., in the eastern United States. Despite a significant amount of research and management of *Adelges tsugae* (Homoptera: Adelgindae), no statistically based sampling method exists for this tiny and numerous pest. Binomial sequential sampling is currently very popular because it is one sampling plan that gives significant savings of cost and time. We developed an adelgid binomial sequential sampling (HWA BSSP) plan for *individual hemlock trees* at action thresholds of 10% and 30% of new shoots infested with at least one sistens. The HWA BSSP was validated with 210 datasets (trees) sampled across the eastern United States. Compared to a whole sample method (avg_n = 228 shoots per tree) that would be used in lieu of the HWA BSSP, the average number of shoot samples inspected to give acceptable levels of accuracy was 81% and 86% lower at the 10% and 30% thresholds, respectively. The plan requires from 12 to 15 minutes to complete per tree.

We also developed a method of assessing *A. tsugae* infestations *in situ* with the original HWA BSSP. This method involves randomly selecting four lower crown branches per tree and counting the number of shoots infested on the first five new shoots on each branch. This count is compared to the HWA BSSP and infestation levels are classified accordingly if the count is lower or greater than the lower and upper stop sampling boundaries, respectively. If the count remains within the boundary, the number of new shoots infested by at least one adelgid must be counted on the next five new shoots on the same branches, etc. After each round of counts, the total value is compared to the appropriate threshold plan and the infestation is classified (low, high, or continue sampling) accordingly. If 80 new shoots have been examined (20 per branch) without reaching a classification decision, the infestation is classified as indeterminate and should be re-evaluated in six months. We have sampled 48 trees with this method, and infestations were classified with 100% accuracy. This method required an average of two minutes to classify infestations on individual trees, provided lower crown branches were accessible and had sufficient new growth.

KEYWORDS

Binomial, sequential sampling, hemlock, *Adelges tsugae*.

Sampling for Detection and Monitoring of Hemlock Woolly Adelgid within Hemlock Stands

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ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae*) has been spreading through eastern United States for over 50 years, destroying hemlock forests and dramatically changing the landscape. However, the lack of an efficient sampling plan for detecting new infestations and quantifying the percentage of infested trees hampers foresters and researchers. A survey of 1,700 trees in 17 sites with a wide range of infestations forms the basis of the hemlock woolly adelgid (HWA) sampling plan. This is a statistically based plan that allows for defined reliability of population estimates. Yet the plan is relatively straightforward in its execution and is flexible enough to accommodate various sampling goals: the forest practitioner surveys two ground-level branches per hemlock tree for the presence or absence of white woolly masses of adelgid on 1-meter terminal sections; between eight and 100 trees are sampled depending on how many positive trees are found. This allows determination of the percentage of trees infested at the specified precision level (0.25). No sampling plan can ever confirm that a hemlock stand is free of HWA. However, after sampling 100 trees and finding no HWA, the conclusion made with 75% reliability using this plan is that HWA infest less than 2% of the trees. Various levels of assuredness of detection are outlined relative to sampling effort.

KEYWORDS

Binomial, sampling, detection, monitoring, range.

INTRODUCTION

For over 50 years, the hemlock woolly adelgid (*Adelges tsugae*) has been spreading through eastern United States (Cheah *et al.* 2004), and yet no statistically based sampling plan has been developed that allows for the efficient detection and characterization of infestations. A standardized plan would facilitate monitoring of range expansion, aid in directing and gauging the success of management efforts, and serve as a valuable research tool. The primary goals of the hemlock woolly adelgid sampling plan (HSP) are to determine if HWA are in a forested stand (detection) and, if present, the level of infestation (characterization). Many sampling plans are created and never widely adopted. For a plan to be adopted, foresters expect the plan to be simple, practical, and have a defined reliability.

Routine sampling of branches above the lower crown (outside of arms reach) is inefficient when relatively large numbers of trees must be examined. For this reason, the HSP is based on sampling lower branches. However, the question might be asked whether or not these lower branches are representative of HWA presence throughout the canopy. Fidgen (personal communication) examined both lower and mid-crown branches of 78 hemlock trees for the presence or absence of HWA. He found that, in over 60% of the trees, both crown strata were infested. When only one stratum was infested, it was invariably the lower crown, which tended to occur with lower density populations. Therefore, sampling lower branches, when available, should give an indication of a trees infestation status. Although counting adelgid on hemlock trees is not impossible under field conditions, routine counting for sampling is not practical because of their small size and the presence of multiple life stages.

PLAN DEVELOPMENT

A binomial sampling plan where the presence or absence of HWA white woolly masses is assessed forms the basis of the general sampling plan structure. Development of the HSP was facilitated through funding from Harvard Forest Summer Research Program in Ecology. The intern, Joseph Brown of Holyoke Community College, collected an extensive dataset that formed the foundation for creating the HSP. A standard statistical approach was taken in which progressively larger areas of the lower branches of hemlock trees were sampled (i.e., five branchlets on each of two branches, a 1-meter terminal section of two lower branches on opposite sides of the tree, and all the lower branches of a tree). Additionally, counts of white woolly masses were taken from each of the branchlets examined. This data was collected so that the proportion of infested trees could be related to the relative size of HWA populations within a stand. Four blocks of 25 trees were examined within each of 17 sites for a total of 1,700 trees surveyed. Trees within a stand were randomly selected for sampling by taking 25 paces in random cardinal directions and selecting the closest available hemlock tree.

A thorough treatment of the foundation data is not the subject of this paper. However, mention of certain results is necessary for clarity and to instill user confidence in the framework from which the HSP was developed. Infestation levels within the 17 stands examined ranged from 0 to nearly 100% trees infested, with the three sampling methods providing similar results (Figure 1). While sampling of all lower branches tended to indicate higher infestation levels, the two branch sample was selected to create a more uniform sample among trees of different sizes and to give the sampler a finer focus in searching for adelgid white woolly masses, especially when densities are low.

A variety of equations were either applied to the data or used to define sampling parameters. Taylor's power law (Taylor 1961) analysis of the variance to mean regression of the summed count data averaged by tree found a significant ($P \leq 0.05$) relationship of these parameters (Taylor's $a = 7.96$ and $b = 1.499$ with $r^2 = 0.96$). When the Taylor values were incorporated into the Wilson and Room (1983) model for predicting percentage trees infested, a

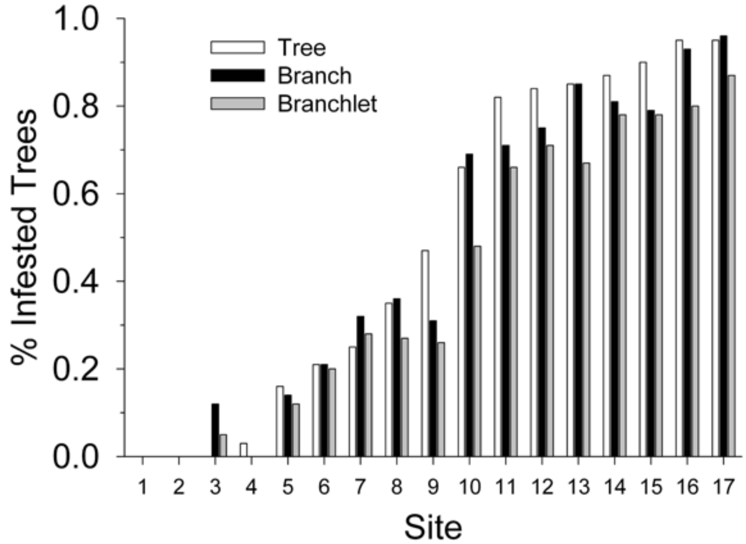

Figure 1. The percentage trees infested in each of the 17 sites used for development of the hemlock woolly adelgid sampling plan when sampled using either branchlets, the terminal meter of two branches, or all lower canopy branches.

significant relationship ($P \leq 0.05$; $r^2 = 0.91$) was found between the predicted percentage of trees infested based on the count data and observed percentage of trees infested found in each site using branch samples. This suggests a predictable relationship between count data and percentage of trees infested.

More relevant to the goals of sampling for detection and characterization of adelgid populations are calculations of minimum detection thresholds and optimum sample size. Minimum detection thresholds are purely probabilistic and don't require use of the field data (Venette et al. 2002). Originally developed for quality control, they are applied to define the probability of detecting the subject of interest at a given level of frequency and specified number of samples, whether it be infested trees, bad light bulbs on an assembly line, or pests in shipments of imported bananas. Table 1 portrays the number of trees required to detect an HWA infested tree at various probabilities and infestation levels. Optimum samples sizes were calculated for binomial sampling at a precision level of 0.25, which is standard for purposes of assessment (Karandinos 1976). The values for optimum sample size were then used to calculate sampling stop points based on the number of infested trees being found and a relative precision of 0.25. The stop points are inserted on the sampling data sheet (available upon request).

HEMLOCK WOOLLY ADELGID SAMPLING PLAN (HSP)

The HSP allows for the standardized detection and characterization of HWA infestations at prescribed levels of reliability and precision. By sampling up to 100 trees, HWA infestations within a stand can be detected with 75% reliability when nearly 2% of trees are infested. Efficient determination of the percentage of trees infested can be accomplished by sampling from eight to 100 trees depending on the number of infested trees being found. A complete, step-by-step description of the sampling plan, including a data collection sheet, is available upon request. The outline provided below provides an overview of the plan's principal components.

Table 1. The maximum number of samples required at different percentage probabilities of finding a single infested tree to provide the minimum detection thresholds indicated (minimum percentage of infested trees).

Minimum % Infested Trees	Maximum # Trees to Detect an Infested Tree at Probability (%)			
	50%	75%	95%	99%
0.5	138	277	598	919
1	69	138	298	458
2	34	69	148	228
3	23	46	98	151
5	14	27	58	90
10	7	13	28	44
20	3	6	13	21

STAND SELECTION

Only limited stand information is required in selecting sites to sample. Although aerial photos would be useful for initial identification and delineation of stands, they are not essential. Generally, more available information (geo-referencing and site characteristics) enhances the ultimate value of the result. Stands four hectare or more in area with a somewhat strong hemlock component are desirable because they provide sufficient trees to sample. Much smaller sites or those with sparse populations of hemlock can be examined, but the selection of trees and thresholds for stopping sampling may not be as appropriate. Obviously there is a point when you would no longer benefit from the plan (i.e., when there are only a few trees or the majority of trees would have to be examined). In this case, a more rigorous assessment of individual trees may be practicable.

WHAT TO SAMPLE

After a brief reconnaissance, the stand is roughly divided into four blocks, but no measurements of stand or block sizes are required. The first hemlock tree to be sampled is then selected. No consideration of tree health is made in the selection except that sample branches are not completely devoid of needles. Trees are selected simply on the basis that they have branches within reach. The underside of the terminal 1 meter of the first branch encountered is examined for any evidence of white woolly masses indicative of HWA. Survival status of the HWA is unimportant and no counting is required. If no HWA were found on the first branch, a second branch on the opposite side of the tree is selected for examination. Evidence of HWA may include large ovisacs, immature HWA, or even scars of woolly masses. The white woolly masses are located at the base of needles and are readily distinguished from other organisms on hemlock, such as spittle bugs, caterpillar and spider webbing, scale insects, etc. Findings of questionable identification should be bagged for later microscopic examination, especially when infestations in new locales are found. The number of HWA on a tree is lower when fewer trees are infested. A few trees may have many HWA (as in the typical photos), but most trees will have few or no HWA, particularly when few trees are infested. Visually scanning along smaller twigs is an useful tactic for espying HWA.

Once the infestation status of the first tree is determined, a list of semi-random cardinal directions is consulted, the sampler takes 25 paces in the appropriate direction and then selects the nearest suitable tree. There is flexibility in selecting trees to cover a stand. The primary objective is to get a representative sample and, in many cases, the size, shape, and lay of a stand needs to be the guide. However, using a prescribed approach such as cardinal directions and fixed intervals ensures sufficient trees are examined in a time-efficient manner. After 25 trees are sampled in a block, the surveyor moves to the next block and samples additional trees.

HOW MANY SAMPLES

For simple detection of HWA within a stand, a single tree that is positive for HWA would suffice to establish its presence in an area. However, if no HWA are found, as many as 100 trees must be examined, depending on the specified infestation threshold and desired assurance of detection (Table 1). Often, data on the level of infestation within a stand are wanted. For estimation of the percentage of trees infested, the HSP calls for sampling from eight to 100 trees for the presence or absence of HWA depending on how many positive trees are found. As the sampler moves from tree to tree, a running tally is made of positive trees found, and this sum is compared with a threshold on the sampling datasheet. Once the threshold is achieved sampling stops. The percentage of trees infested is simply calculated by dividing the number of positive trees by the number examined trees and then multiplying by 100.

WHEN TO SAMPLE

This plan is intended for use when HWA with a white woolly coat can be observed. The optimal sampling period is from early winter, when HWA begin putting on their woolly coat, until early summer, when HWA sistens settle on new growth. During summer the sisten generation is in the first instar and lack a woolly coat, and are therefore difficult to see. They aestivate until mid fall when they resume growing and develop a woolly coat. The utility of HSP for sampling during the aestivation period is under investigation.

PLAN ADOPTION

The first workshop for training forestry personnel on use of the HSP was conducted during the spring of 2004 in Massachusetts. Representatives from Massachusetts, Maine, New Hampshire, New York, and Vermont attended and rated the usefulness of the plan as “high.” However, the success of any sampling plan is gauged by its adoption. Already, the HSP has been used on at least 97 sites in New York, and the U.S. Forest Service has examined 92 stands in Alleghany National Forest and 50 stands in Monongahela National Forest. The 92 Alleghany stands took three crew members two weeks to complete. A survey of properties associated with Harvard Forest, Petersham, Massachusetts, found relatively low populations in stands previously thought to be uninfested.

The availability of a standardized sampling plan offers many opportunities for its incorporation into activities associated with HWA detection, surveillance, and management. Although plan validation and characterization are ongoing, the general utility of the HSP is already evident, state and federal agencies are collecting geo-referenced data and using handheld computers for data management. At Harvard Forest and elsewhere, its utility as a research tool is becoming evident. Currently, no decision thresholds for initiation of management are tied to the plans results. The apparent flexibility and straightforwardness of application of the HSP should allow it to be widely employed.

ACKNOWLEDGEMENTS

I would like to thank Joe Brown for his dedication in working on development of this plan. I am also indebted to Dave Orwig and Aaron Ellison of Harvard Forest for their encouragement and financial support for initial development of the sampling plan and to Brad Onken and Dennis Souto for their support through the US Forest Service for its continued development and deployment.

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A Randomized Branch Sampling Method for Hemlock Woolly Adelgid

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ABSTRACT

An invasive exotic insect, the hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) has caused widespread mortality of eastern hemlock trees (*Tsuga canadensis* (L.) Carrière) and threatens to extirpate the species from North American forests. Neither HWA population densities nor their distribution in forests is well understood, hampering the ability of forest managers to respond to the pest. In addition, standard monitoring methods have inestimable bias and provide limited results. Lacking better information, land managers often have to assume that HWA is evenly distributed and saturates the environment. This paper explains a design unbiased sampling system appropriate to the biology of HWA and presents results of sampling HWA densities in New England forests.

KEYWORDS

Monitoring, population density, crown distribution.

INTRODUCTION

An invasive exotic insect, the hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) has caused widespread mortality of eastern hemlock trees (*Tsuga canadensis* (L.) Carrière) and threatens to extirpate the species from North American forests (McClure et al. 2001, Orwig et al. 2002). HWA-induced mortality is a concern because hemlocks provide important forest structure, habitat, economic benefits, and aesthetic values (Beatty 1984, Kelty 1989, DeGraaf et al. 1992, p. 92, Snyder et al. 2002). Unfortunately, lack of information about HWA impedes efforts to save forest hemlocks.

Information about the distribution of HWA within tree crowns and stands remains a gap in HWA research, in part because sampling methods for HWA are not well established. HWA is difficult to sample because of its small size, tree crown habitat, and lack of attractants. Current sampling for HWA often only includes the lowest portion of the crown adding inestimable bias to population estimates (McClure and Cheah 1999, Adams et al. 2002, Casagrande et al. 2002, Mayer et al. 2002). Before 2005 no studies had produced crown distribution estimates (Gray et al. 1998), although new research is underway (see Fidgen et al. and Costa in this volume). Thus far, population estimates have been general at best, and infestation levels are often just assumed from a decline in hemlock health (Bonneau et al. 1999, Orwig et al. 2002, Tingley et al. 2002). Without a good method to sample and monitor populations in the field, it is difficult to measure effectiveness of control efforts, catch new infestations, identify mortality risk factors, or even find resistant stands. This research details a sampling system designed for HWA and reports the results of monitoring HWA densities in New England forests.

METHODS

The goal of this study is to test randomized branch sampling (RBS) to estimate populations of HWA in a managed forest. The two main areas of interest are differences in HWA densities between heights within the crown and between nearby stands. Estimates of HWA populations within tree crowns may help future sampling efforts and estimates of differences between stands may aid HWA mitigation efforts. RBS is predicated on a design-based approach rather than a model-based approach to inference (Gregoire et al. 1995, Gregoire 1998). In other words, estimators generated from RBS of the mean, total, and variance require no assumptions about the HWA population sampled and are design unbiased (Gregoire et al. 1995). RBS treats a tree as a series of paths from the ground to each terminal shoot. Under RBS, the researcher randomly selects a path to a terminal shoot, and the characteristics of interest—the HWA and needles encountered along this path—become part of the sample. The RBS path can be terminated at any branching node to allow sampling of entire branches. The path is created by a series of random selections at each node. In other words, at each fork in the branch the researcher randomly chooses which branch to follow. The researcher can adjust the probability of selecting a branch to increase the likelihood of sampling more of the quantity of interest so long as the probabilities at any particular fork sum to one. No design bias is introduced if the selection remains probabilistic. The inverse of the product of the unconditional selection probabilities for a sample is used to inflate the sample to an estimate for the whole tree.

RBS provides an operationally efficient mechanism for unbiased estimation of the total, mean, and variance of the quantity sampled. Researchers have used RBS to estimate fruit production (Jessen 1955), tree weight (Valentine et al. 1984), total foliar area (Gregoire et al. 1995), stem length and surface area (Gregoire 1996), needle mass (Gaffrey and Saborowski 1999), tree biomass (Good et al. 2001), coarse woody debris (Gove et al. 2002), and floral distribution (Chen et al. 2003). This study is a new implementation of RBS to estimate insect populations.

I tailored the implementation of RBS in this study to the problem of estimating the number of HWA per needle in hemlock crowns. First, I fell the sample trees to permit access to the full crown. Felling damages the crowns, but comparisons of branches broken in the fall and all other branches shows no significant difference. Anecdotally, I have noticed that branch tips, which are the majority of the samples, are flexible enough to avoid damage in felling. After felling the sample tree, I stratify the crown into thirds and take at least three samples from each third.

This RBS scheme uses the simple random selection of the first node to more efficiently deal with the branching pattern of hemlock while retaining an unbiased design. I measure the diameter of all branches at each subsequent node in order to use the branch basal area as the selection probability of each branch. A field computer, a palm pilot with a custom application, records the selection probabilities, generates a pseudorandom number, and selects the branch to be included in the RBS path.

When a suitably small branch (<30cm in length) is selected, I clip it, place it in an envelope and return it to the lab for counting. I record the length of old and new growth, number of old and new needles, HWA ovisacs, sistens, and scale (*Fiorinia externa* Ferris and *Nuculaspis tsugae* Marlatt). In addition, I catalog stand, tree, and branch attributes such as stand basal area, tree height, crown height, diameter, branch direction, and branch height. These variables allow an investigation of the sources of variance in HWA within and between trees. Samples from the first nine trees included only counts of HWA ovisacs. The more recent 218 samples include the sistens generation of HWA on new growth as a more time-sensitive measure of HWA density, in addition to HWA ovisacs (Ward et al. 2004). All sisten counts are reported as the number of sistens per hundred needles so that the counts can be compared between branches of different health.

In 2004 I added ground-based sampling of the lower crown to the survey. These samples used a pole saw to select branches below 7.5m. I still selected branches using simple random selection and followed the RBS procedure after cutting them down so that estimates of the lower crown would be unbiased. In these samples the upper crown was not included in the sampling universe so unbiased estimators of the whole tree population are not available.

All sampled stands are hemlock or hemlock/hardwood mixtures about 80 to 100 years of age. Most stands are located on the Yale Myers forest in Union, Connecticut, within 5km of each other. I also sampled stands at Great Mountain Forest in Norfolk, Connecticut, and a forest in Sandisfield, Massachusetts. During this sampling effort I have counted over 300 branches on more than 80 trees over two seasons in 15 stands from three forests yielding nearly 140,000 needles and 7,000 adelgids.

RESULTS

In the fall of 2004, the population of HWA at Yale Myers Forest appeared to be much lower than it had been in 2003. The RBS for HWA documented a dramatic reduction in the estimated density of HWA in the three stands sampled in both 2003 and 2004. Figure 1 shows a graph of confidence intervals for three stands in both years. I used the mixed model procedure in SAS to analyze stand as a fixed effect, trees as a random effect, and branches as repeated measures (Littell et al. 1999, SAS Institute Inc. 2002). The mixed model deals appropriately with the correlation between trees in the same stand and constructs confidence limits for the least-squares means of the differences between stands. The unbiased estimators of total numbers of sistens per tree from RBS provide the data used in the mixed model of stand effect.

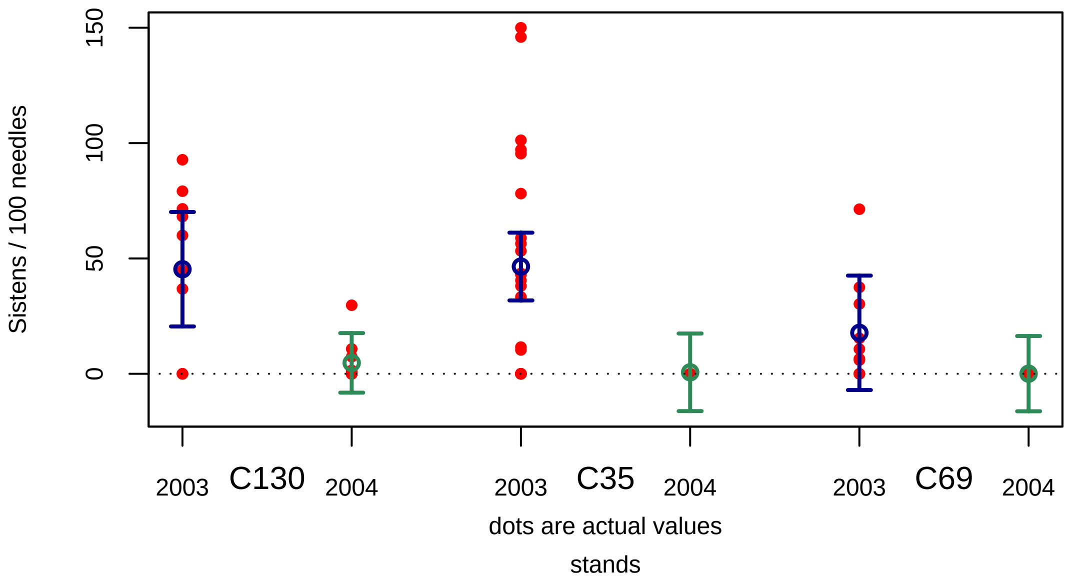

Figure 1. 90% confidence intervals for sistens per hundred needles in each of three stands for 2003 and 2004.

The differences between 2003 and 2004 are significant at the 90% level in both stands C35 and C130. Visual surveys and discussions with managers suggest that this reduction in densities is consistent across the forest.

In 2003 I sampled 174 branches from 16 trees in order to better understand how HWA is distributed throughout the crown. Figure 2 is a plot of the height at which the sample was taken versus the number of sistens per hundred needles recorded in that sample.

The plot of sistens versus sample height does not suggest any pattern. In the 2004 plot there is a cluster of values between 4 and 7.5m above the ground because many samples were collected with a pole saw that could only reach to 7.5m. I divided the crown in thirds and compared the number of sistens per 100 needles in each third in order to test for a pattern in branch height and HWA density (Figure 3).

Not only were there no significant differences between the crown strata estimates, but it seemed that further sampling might even show that the lowest crown stratum has more sistens per hundred needles than the top stratum. In part because of these results, I took fewer samples per tree and more samples across the stand during the second field season. Many samples were taken with a pruning saw and so can provide an unbiased estimate only for the first 7.5 meters of the crown. In addition the population reduction between 2003 and 2004 meant that many more samples were free of HWA. In some cases, sampled trees were almost completely free of infestation, and no RBS samples included sistens. On these trees, I made a purposeful sample of any sistens I could find to document the presence of infestation. Figure 4 includes purposeful samples as noted, although they are not included in the construction of confidence intervals.

Again there is no significant difference between the strata, although in 2004 the trend may be different from the previous year. I took too few samples from the middle and top

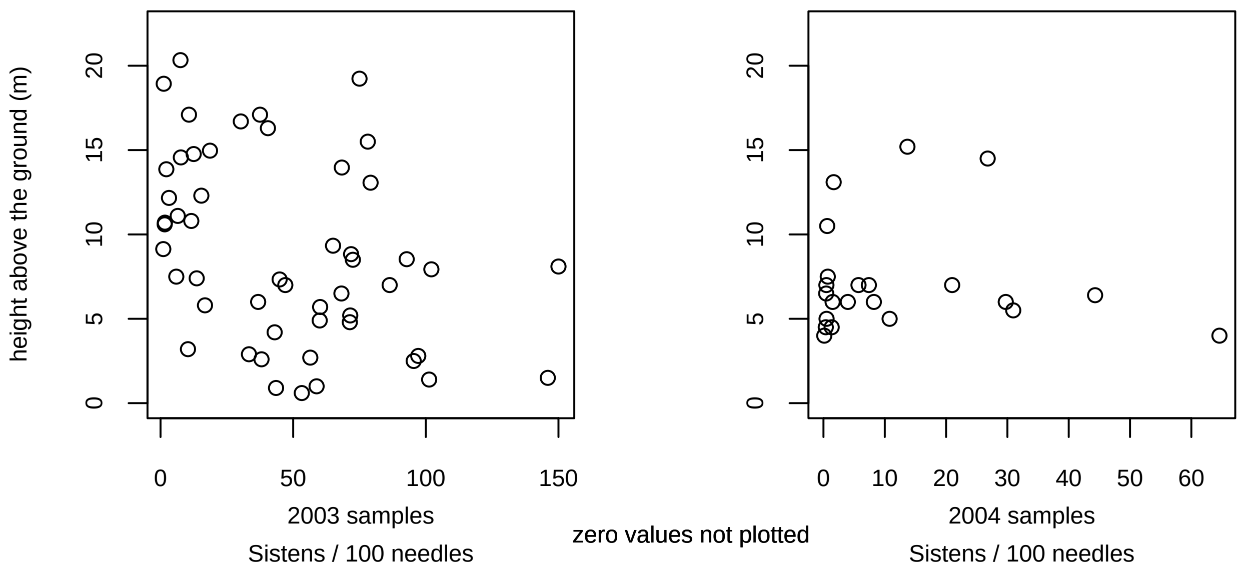

Figure 2. Height above the ground of each sample versus number of sistens per hundred needles.

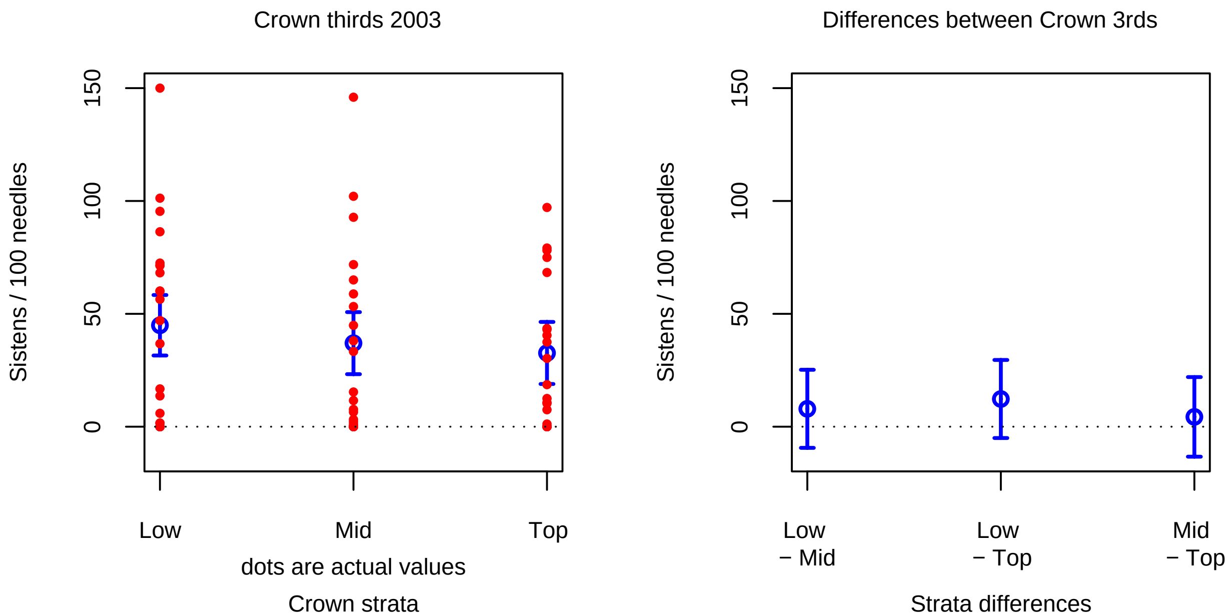

Figure 3. 90% confidence intervals for 2003 crown thirds and differences between crown thirds.

strata to be sure of any trends. Looking at the crown strata from all the 2004 samples together obscures the fact that many of the samples were only selected from the lowest 7.5m of the tree. One way to understand the potential bias in using only the lowest 7.5m is to investigate the number of samples with infestations in just the middle or top stratas. In 2003, 19% of the 16 infested trees sampled did not show infestations in the lowest portion of the crown. In 2004, none of the infestations were visible in the lower crown, although all three trees from

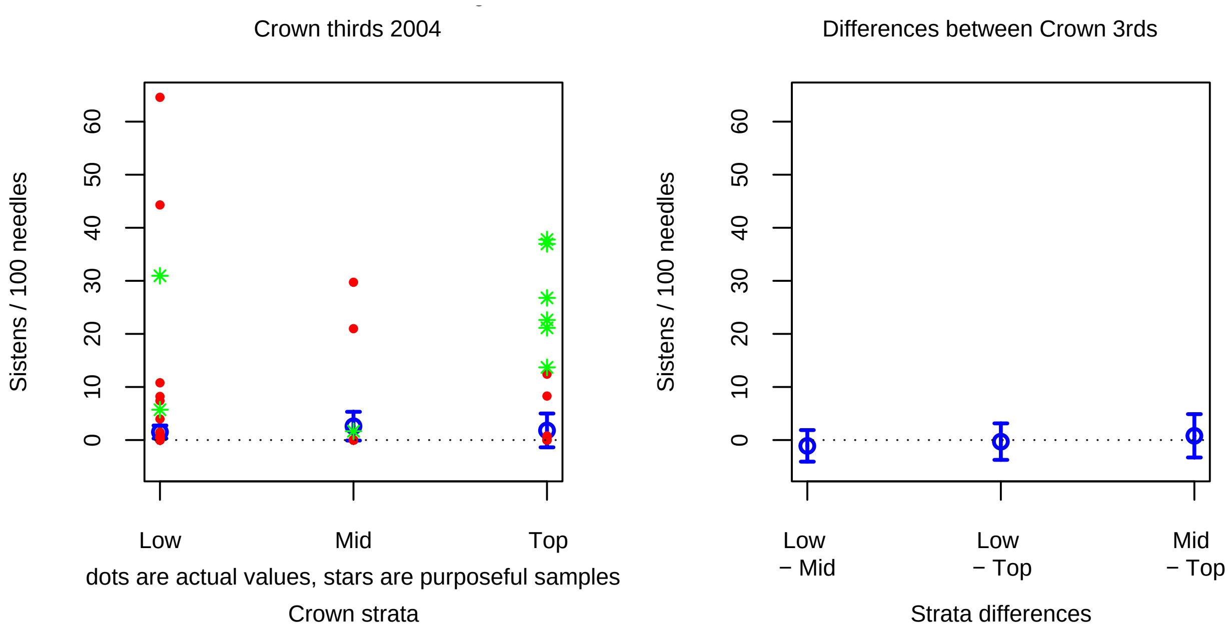

Figure 4. 90% confidence intervals for 2004 crown thirds and differences between crown thirds.

multi-strata samples were infested. A logging operation in stand C32B provided a second opportunity for understanding the bias of sampling only the lowest stratum. In stand C32B, I sampled the lower crown of six trees before logging and then, immediately after logging, I sampled six felled tops. The lower crown samples shown no infestations, but samples from the felled tops revealed very high densities of HWA.

Initial samples suggested there might be some differences between stands. In fact, analysis of HWA ovisacs per hundred needles showed stand C130 to be significantly different from the other stands at the 90% level. The sisten data for stands in 2003 does not show the significant difference that the ovisac data does because there are fewer samples per stand. Figure 5 graphs both ovisacs per hundred needles and sistens per hundred needles in the stands sampled in 2003. The sampling in 2003 focused on a greater number of samples per tree and fewer trees per stand, which made it hard to verify the existence of differences between stands.

I had hoped to be able to demonstrate differences between stands through a combination of branch samples from the lower crown and multi-strata samples. It would be possible to generate design unbiased estimates of the number of sistens per hundred needles in the stand by randomly selecting some trees for lower crown and some for multi-strata sampling. Unfortunately, in most stands I could only sample the lowest stratum because of the difficulty of safely felling trees. Therefore, I could only estimate the numbers of sistens on branches below 7.5 in these stands unbiasedly. Ironically, the bias of estimating the whole stand from lower crown samples may be much greater in 2004 than it would have been in 2003 because of the anecdotal evidence of differences between strata in 2004. Figure 6 depicts biased estimates of number of sistens per hundred needles in the stands based on pole saw samples but not purposeful samples.

The 2004 data shows no significant differences between stands, but this may be due to the bias of using lower crown samples. In addition, the low numbers of sistens in 2004 in comparison to 2003 make stand differences more difficult to pinpoint. At the lower number of sistens per tree in 2004, the sampling intensity would have to be increased to ensure detection of infestation. For example, tree 71 had a small HWA infestation on one branch 14.5m above the ground out of 112 branches, based on a visual census after felling.

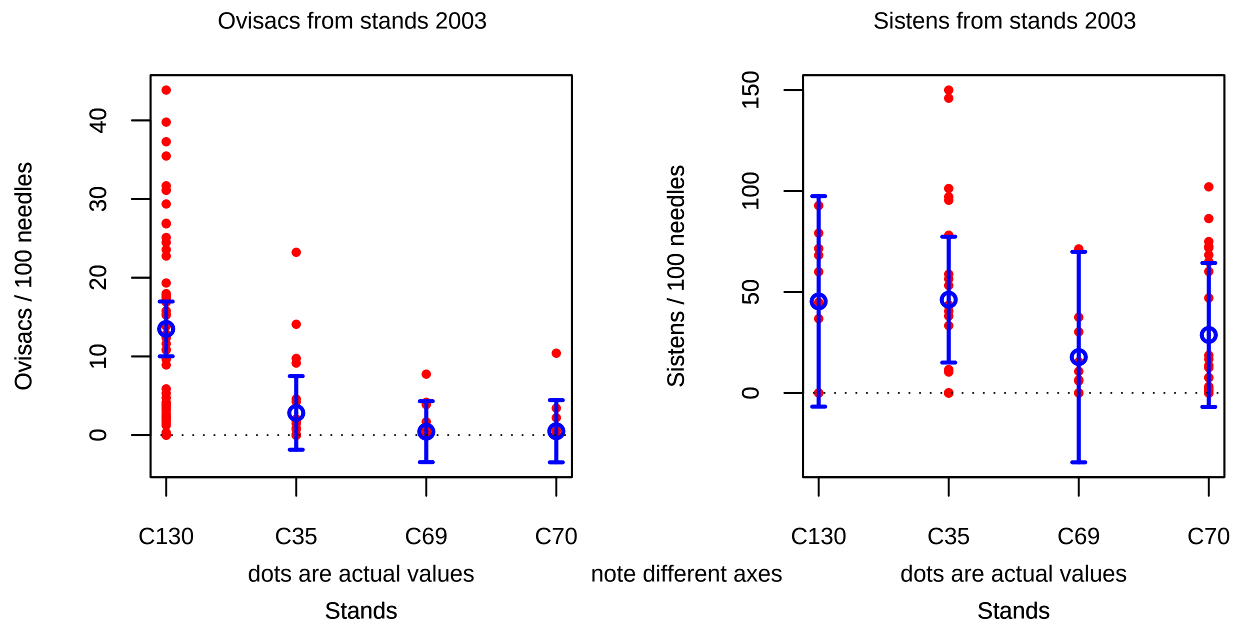

Figure 5. 90% confidence intervals for ovisacs and sistens from stands in 2003.

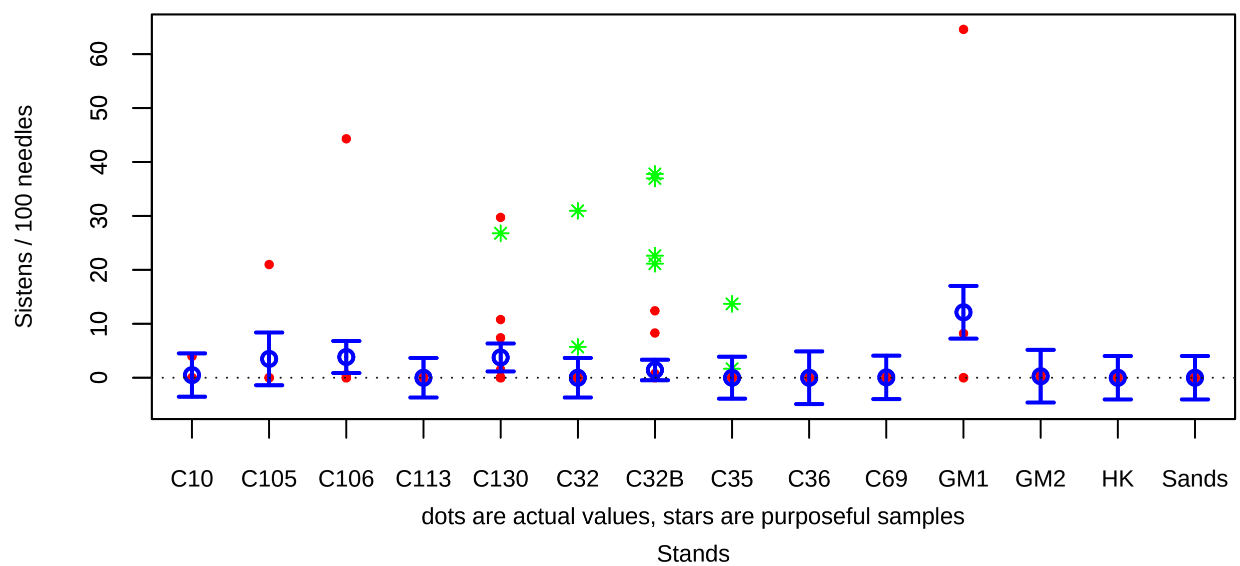

Figure 6. Biased 90% confidence intervals from stands in 2004.

CONCLUSIONS

This research shows the possibilities for using RBS to generate design unbiased estimators for an insect population that is difficult to monitor. The RBS survey at Yale Myers Forest shows large decline in the HWA population in the three stands sampled. Although populations declined, infestations were still present. 2003 data shows some differences in numbers of HWA ovisacs between nearby stands, while data from 2004 is inconclusive. The number of HWA ovisacs per hundred needles was significantly different in C130 from C69 and C70 in 2003. In 2003, there was no significant difference between crown strata. Anecdotal evidence from sampling in 2004 suggests higher HWA populations in the upper crown than the lower crown. It may be that, at high population densities, the difference between upper and lower crown strata is less than the difference between crown strata when HWA densities are low. Further sampling from all crown strata is necessary to better understand the densities of HWA throughout the crown.

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Using Hyperspectral Technologies to Map Hemlock Decline: Pre-Visual Decline Assessment for Early Infestation Detection

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ABSTRACT

Hyperspectral remote sensing technology can help monitor hemlock health across large areas of the landscape. This study examines the capability of a commercially available sensor (Specim's AISA Eagle) to map hemlock decline due to hemlock wooly adelgid (HWA) infestation in the Catskill Mountain area of New York. The AISA Eagle was able to classify hemlock health at the tree level into an 11-class rating system with a one-class tolerance accuracy of 88 percent. The ability of this instrument to predict decline below class 4 (when dieback and transparency reach levels first noticeable in the field) is based upon "pre-visual" changes in chlorophyll content and function that are typical of incipient HWA infestation and early stress. This technology will enable land managers to assess and monitor detailed changes in forest health across the landscape so that integrated pest management programs can be effectively implemented.

KEYWORDS

Forest health, AVIRIS, remote sensing, hemlock woolly adelgid.

INTRODUCTION

One of the most pressing forest health issues currently facing North American forests is the widespread decline of eastern hemlock (*Tsuga canadensis* Carriere) due to the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. The potentially severe consequences and large scale of the HWA infestation requires that land managers be familiar with the actual location of the hemlock resource, as well as its health and infestation status.

Most assessments of decline involve time-consuming field based methods. Although these methods are valuable in monitoring gross changes over time, they are not able to identify trees in the very early stages of decline (Sampson et al. 2000) or assess large acreages, both crucial to the development of integrated pest management strategies aimed at managing the hemlock resource.

Remote sensing technologies are the most viable option to assist land managers in health assessment and monitoring at a regional scale. To date, remote sensing of forest health has been limited to the classification of coarse defoliation classes using aerial photography or multi-spectral resolution visible/NIR space-based sensors, such as Landsat Thematic Mapper (TM) (Lambert et al. 1995; Royle and Lathrop 1997 and 2002, Royle et al. 1995). When measuring decline solely as a function of defoliation, earlier signs of stress such as reductions in photosynthesis and chlorophyll content are not detected. Instruments with higher spectral resolution are needed to accurately detect such changes in vegetation condition (Treitz and Howarth 1999).

There is mounting evidence that hyperspectral instruments have the capability, not only to assess defoliation, but also to identify the early signs of stress—in some cases before visual symptoms are apparent (Cibula and Carter 1992, Mohammed et al. 1995, Zarco-Tejada et al. 2000a and 2000b). This can be explained by the tendency of stressed leaves to undergo reduction in photosynthetic activity and to lose chlorophyll. These changes alter reflectance at chlorophyll-sensitive wavelengths (Vogelmann and Rock 1988, Rock et al. 1988, Vogelmann et al. 1993, Gitelson and Merzlyak 1996, Carter and Knapp 2001).

Chlorophyll$_a$ and $_b$ content are particularly good detectors of stress because of their direct role in photosynthesis. Narrow wavebands near 700nm where changes in chlorophyll absorption are easily detectable have been recommended for early detection of forest damage (Hoque et al. 1990 and 1992) and were able to detect decreased vigor, before visual symptoms were apparent, in pine seedling canopies (Cibula and Carter 1992). Because changes in chlorophyll function typically precede changes in chlorophyll content, chlorophyll fluorescence has also been shown to be a useful tool in identifying pre-visual strain (Zarco-Tejada et al. 2000a and 2000b).

Preliminary work by the authors using an ASD FieldSpec Pro FR field spectroradiometer (Analytical Spectral Devices) highlight several indices and wavelengths that are able to track hemlock stress, including pre-visual symptoms. This work has resulted in the development of equations capable of predicting a 10-class hemlock health scale on independent data with 96% one-class tolerance accuracy (Pontius et al. In press-a). Additional work by the authors using remotely sensed hyperspectral imagery from NASA's Airborne Visible Infrared Imaging Spectrometer (AVIRIS) produced a hemlock abundance map that correctly identified hemlock dominated pixels (>50% basal area) with 88% accuracy. Reflectance at a chlorophyll sensitive wavelength (683nm) coupled with a water band index (R970/900) was able to predict plot level decline with 100 percent one-class tolerance accuracy. The extreme accuracy at the low (0-4) end of the range indicated that these wavelengths might be used to assess early decline, before visual symptoms are apparent (Pontius et al., In press-b).

This study was designed to determine if similar hyperspectral techniques from a commercially available remote sensing platform could be used to predict early hemlock decline symptoms in the Catskills State Park, New York. The hyperspectral instrument used in this study was the airborne AISA Eagle, measuring 130 contiguous bands from 400nm – 970nm, with 3nm spectral resolution, and 2m spatial resolution. Our objectives are to: (1) present the key wavelengths and/or stress indices most strongly correlated with hemlock decline, (2) use this information to develop a simple linear equation to predict decline using a minimal number of variables, and (3) discuss the potential of commercially available hyperspectral sensors.

METHODS

Ground truth data from 65 canopy-dominant hemlocks in the Catskills (Figure 1) were collected using methods specifically designed to quantify the various sequential symptoms of decline that follow adelgid infestation. This included the percent of terminal branchlets with new growth, percent transparency (quantified using a concave spherical densiometer), percent fine twig dieback, and live crown ratio (USDA Forest Service Crown Rating Guide). Raw health data was normalized by assigning a decline value to each measurement (Table 1). This normalized data was then averaged for each tree to determine the decline rating that best described the trees' overall status (where 0 = perfect health and 10 = dead). Species data, GPS location and canopy position were recorded across the imagery for an additional 465 trees for species mapping, including over 20 species. Geographic location data was collected for all trees using a Trimble GeoXT global positioning system with sub-meter accuracy.

On September 3, 2004, hyperspectral data from Helicopter Applicators, Inc.'s AISA Eagle was obtained for a 25,000 acre region of the Catskills State Park (Figure 1). Atmospheric corrections were conducted in house by Helicopter Applicators, Inc. Individual passes were mosaicked together and geometrically registered to USGS 1m resolution digital orthoquads using a polynomial degree 2 warping method (ENVI 4.0 software, Research Systems, Inc). Reflectance spectra were then extracted for pixels corresponding with the ground truth data locations. A mask of all shadow resulting from cloud cover, steep northern aspects, and canopy geometry was applied before application of predictive equations.

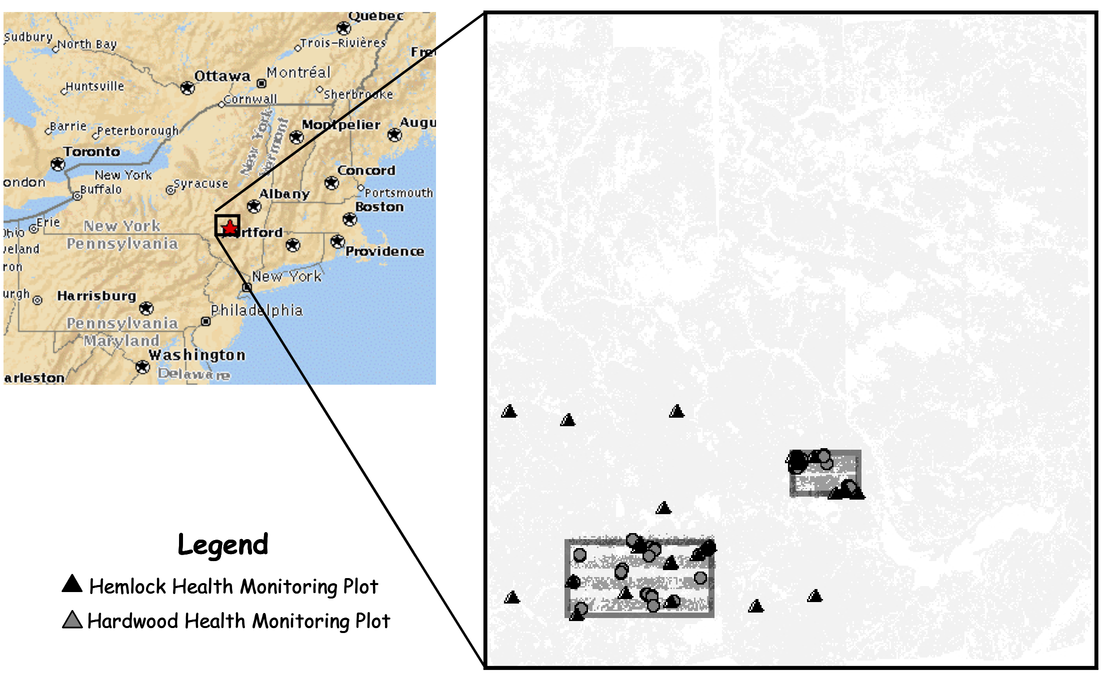

Figure 1. Grey rectangles represent the 2004 HAI study area, comprising a 25,000-acre subset of the original 2001 AVIRIS Catskills imagery (large black square). A series of hemlock health and hardwood plots were establised for ground truth in health calculations and species mapping.

Table 1. A summary of the typical values observed for each measured characteristic by decline class. The best-fit categories for each of the individual measurements were averaged to determine one overall decline rating for each tree.

Decline Class	Health Status	Characteristics
0	Perfect health	100% new growth Negligible canopy transparencey Negligible fine twig dieback Greater than 90% photosynthetically active canopy
1	Very healthy	Over 90% new growth 6-9% canopy transparency Negligible fine twig dieback 80-89% photosynthetically active canopy
2	Healthy (typical forest co-dominant)	Over 85% of branches produce new growth 10-14% canopy transparency Less than 5% fine twig dieback 70-79% photosynthetically active canopy
3	Earliest decline	80-85% of branches produce new growth 15-19% canopy transparency 5-10% fine twig dieback 65-69% photosynthetically active canopy
4	Light decline	75-79% of branches produce new growth 20-24% canopy transparency Approaching 10% fine twig dieback 60-64% photosynthetically active canopy
5	Light to moderate decline	70-74% of branches produce new growth 25-29% canopy transparency 10-15% fine twig dieback 50-59% photosynthetically active canopy
6	Moderate decline	60-69% of branches produce new growth 30-34% canopy transparency Up to 15% fine twig dieback 40-49% photosynthetically active canopy
7	Moderate to severe decline	40-59% of branches produce new growth 35-39% canopy transparency 15-20% fine twig dieback 30-39% photosynthetically active canopy
8	Severe decline	20-39% of branches produce new growth 40-44% canopy transparency Up to 20% fine twig dieback 20-29% photosynthetically active canopy
9	Death imminent	Less than 20% of branches produce new growth Greater than 45% canopy transparency Greater than 25% fine twig dieback Less than 20% photosynthetically active canopy
10	Dead	100% defoliation

Previously established stress detecting wavelengths and indices (Pontius et al. In press-a; Table 2) were related to decline data to determine the strongest stress correlates (Kleinbaum et al 1998). These were then entered into a stepwise linear regression with conservative significance cutoff limits to avoid over-fitting (probability to enter = 0.250, probability to leave = 0.01). Mallow's Cp and PRESS statistics were used to compare the predictive abilities of various models (Kozak and Kozak 2003). Full double-cross validation (jackknifed residuals) were used in lieu of independent validation to assess predictive abilities (Kozak and Kozak 2003). After establishing the best-fit hemlock decline model, the resulting equation was applied to all non-shadowed hemlock pixels within the imagery.

Table 2. A list of existing indices included in our analyses that are known to have strong relationships with stress-specific physiological responses.

Index	Formula	Primary Absorbance Feature	Citation
Carter and Miller Stress	$CMS = \frac{R694\ nm}{R760\ nm}$	Chlorophyll content	Carter and Miller 1994
Curvature Index	$CI = \frac{R683\ nm \bullet 2}{R675\ nm \bullet R691\ nm}$	Chlorophyll a & b content; chlorophyll fluorescence	Zarco-Tejada et al. 2002
Derivative Chlorophyll Index	$DCI = \frac{FD705\ nm}{FD723\ nm}$	Chlorophyll fluorescence	Zarco-Tejada et al. 2002
Chlorophyl Fluorescence	$CF = \frac{FD690\ nm}{FD735\ nm}$	Chlorophyll flurorescence; photosynthetic activity	Mohammed et al. 1995
Normalized Difference Vegetation Index	$NDVI = \frac{R800\ nm - R680\ nm}{R800\ nm + R680\ nm}$	Chlorophyll content and energy absorption	Deblonde & Cihlar 1993; Gamon et al. 1997; Myneni et al. 1995; Rousse et al. 1974
Photo-chemical Reflectance Index	$PRI = \frac{R531\ nm - R570\ nm}{R531\ nm + R570\ nm}$	Xanthopyll Cycle Activity	Gamon et al. 1990; Gamon et al. 1997; Rahman et al. 2001
Red Edge Inflection Point	$REIP = \lambda FD\ max$	Chlorophyll a content; green vegetation density	Gitelson et al. 1996; Rock et al. 1988; Vogelmann et al. 1993
Ratio Vegetation Index	$RVI = \frac{R800\ nm}{R680\ nm}$	Chlorophyll content	Pearson and Miller 1972; Royal and Lathrop 2001
Water Band Index	$WBI = \frac{R970\ nm}{R900\ nm}$	Canopy water content	Carter 1993; Penuelas et al. 1997; Tucker 1980

RESULTS AND DISCUSSION

An examination of the average spectra for various decline classes highlights the spectral changes that accompany hemlock decline (Figure 2). Although the full spectrum is obviously different, our goal was to identify a smaller subset of variables that may account for the maximum variability in spectral signatures. Building off of the key variables identified in previous benchtop hyperspectral work (Pontius et al. In press-a), several wavelengths and stress indices were significantly correlated with hemlock decline using the AISA Eagle sensor (Table 3). Of these, only R680, R760, SD737, and RVI were retained in the final stepwise, linear regression model to predict hemlock decline (Table 4). This model predicted decline on the 41 sample ground truth data set ($R^2 = 0.75$ and RMSE = 0.81). Treated as a class variable, declining trees could be identified within one health class with 88% accuracy (Figure 3).

While this model works well on an empirical basis, it is also important that there be a theoretical framework for the relationships witnessed. In the predictive model presented here, two of the four terms are wavelengths of known chlorophyll absorption (Mohammed et al. 1995, Carter and Miller 1994, Pearson and Miller 1972, Zarco-Tejada et al. 2002a, Carter and Knapp 2001). Miller et al. (1990) identified leaf chlorophyll content, as one of the most significant factors affecting plant vigor.

Chlorophyll$_a$ content, captured by R680 and RVI, is a particularly good indicator of stress because of its direct role in photosynthesis. Such narrow wavebands are sensitive to early stress induced decreases in leaf chlorophyll content (Carter 1993) and have been recommended for early detection of forest damage (Hoque et al. 1990 and 1992). In hemlock, Royle and Lathrop (1997) used RVI calculated from Landsat TM to predict and map four hemlock defoliation based damage classes with 64% accuracy.

Chlorophyll fluorescence sensitive wavelengths were also retained for the final model, including: 680nm and 760nm. Fluorescence is inversely related to photosynthetic rates, (D'Ambrosio et al. 1992, Schreiber and Bilger 1994, Larcher 1994) making it a good measure of relative photosynthetic activity. Because changes in photosynthetic function typically precede changes in chlorophyll content, chlorophyll fluorescence has been shown to be a useful tool in identifying pre-visual strain in other studies as well as the predictive equation presented here (Zarco-Tejada et al. 2000a and 2000b).

The SD737 was the final key term in predicting hemlock decline. This location has a strong -OH absorbance feature (Osborne and Fearn 1986). Although -OH bonds are found in many structures, one of the most obvious and most common in plant tissues is water. Water sensitive wavelengths have been identified in early ASD and AVIRIS decline surveys (Pontius et al. In press-a). It is possible that reflectance at this location is picking up slight differences in the canopy water content of subject trees.

Relative susceptibility of hemlock to HWA has been linked to various site and landscape factors related to water availability (Bonneau et al. 1997, Onken 1995, Royle and Lathrop 1999). Drier conditions stress already weakened tress, making them more susceptible to HWA

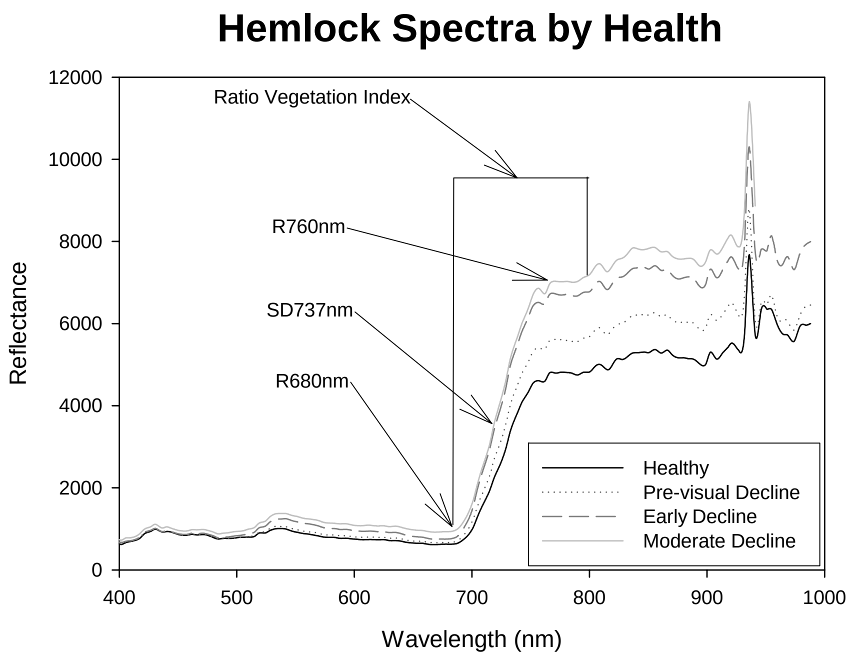

Figure 2. A close look at the average spectra for various decline stages highlight those wavelengths found to be significant in predicting hemlock decline.

Table 3. Previously developed ASD- and AVIRIS-based decline equations were applied to decline data with significant correlations (p<0.0001). Pairwise correlations between key AISA Eagle variables and hemlock decline are listed in order of correlation strength.

Variable	Correlation*	Absorbance Feature	Citation
R680**	**0.70**	$Chlorophyll_a$ & Fluorescence	Mohammed et al. 1995
R552	**0.53**	$Chlorophyll_a$	Penuelas et. al. 1997
PRI	**-0.53**	Xanthophyll Cycle Activity	Gamon et al. 1990; 1997
CMS	**0.49**	Chlorophyll Content	Carter and Miller 1994
NDVI	**-0.33**	Chlorophyll Content	Deblonde and Cihlar 1993
Fluorescence	**0.30**	Chlorophyll Fluorescence	Mohammed et al. 1995
R760**	**0.27**	Chlorophyll Fluorescence	Carter and Miller 1994
SD737**	0.17	OH Bonds	Osborne and Fearn 1986
RVI**	-0.12	Chlorophyll Content	Pearson and Miller 1972

***Boldface** indicates significant correlations at the 0.1 level.
**Signifies a significant variable retained in the final predictive stepwise model.

Table 4. The final AISA Eagle based linear regression equation for predicting hemlock decline, where RVI = R800/R680.

Term	Estimate	Standard Error	Prob>|t|
Intercept	-7.249	1.840	
R680	0.012	0.002	<0.0001
R760	-0.001	0.000	0.0016
SD737	0.007	0.002	0.0020
RVI	1.169	0.271	0.0001

Rsquare = 0.75
RMSE = 0.81
Terms = 4
N = 41

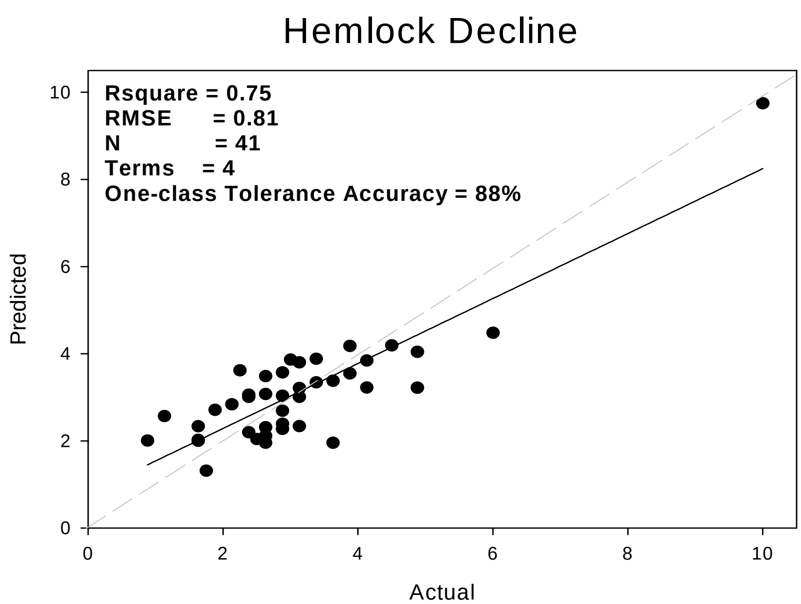

Figure 3. Using the four-term linear regression equation based on R680, R760, SD737, and RVI, decline rating was predicted with an R^2 = 0.75 and RMSE = 0.81. Converting this data to a class variable showed 88% one-class tolerance accuracy. The accuracy below decline class 4 indicates that this technology could be used to identify trees in the very early stages of decline.

and decline. There is also evidence that HWA injects toxic saliva at feeding sites (McClure et al. 1996): it is postulated that the toxic effects of this saliva may include a constricting effect on xylem, which could lead to leaf dehydration following infestation (Shields et al. 1995). Although we did not directly measure leaf water content, it is plausible that trees experiencing the most significant decline and highest infestation levels may be suffering from water stress, leading to significance at water-sensitive wavelengths.

Applying this equation to the full extent of the AISA Eagle imagery, more severe decline is evident in the eastern region, coinciding with the area along the Hudson River and Ashokan Reservoir where HWA has the longest infestation history (Figure 4). Average jackknifed residuals of 0.13 indicate that this equation should also work on independent data within the same range of decline (Kozak and Kozak 2003). While these results are promising, a more rigorous validation covering the full range of decline symptoms in the Catskills with independent validation is required to adequately test how robust this model is.

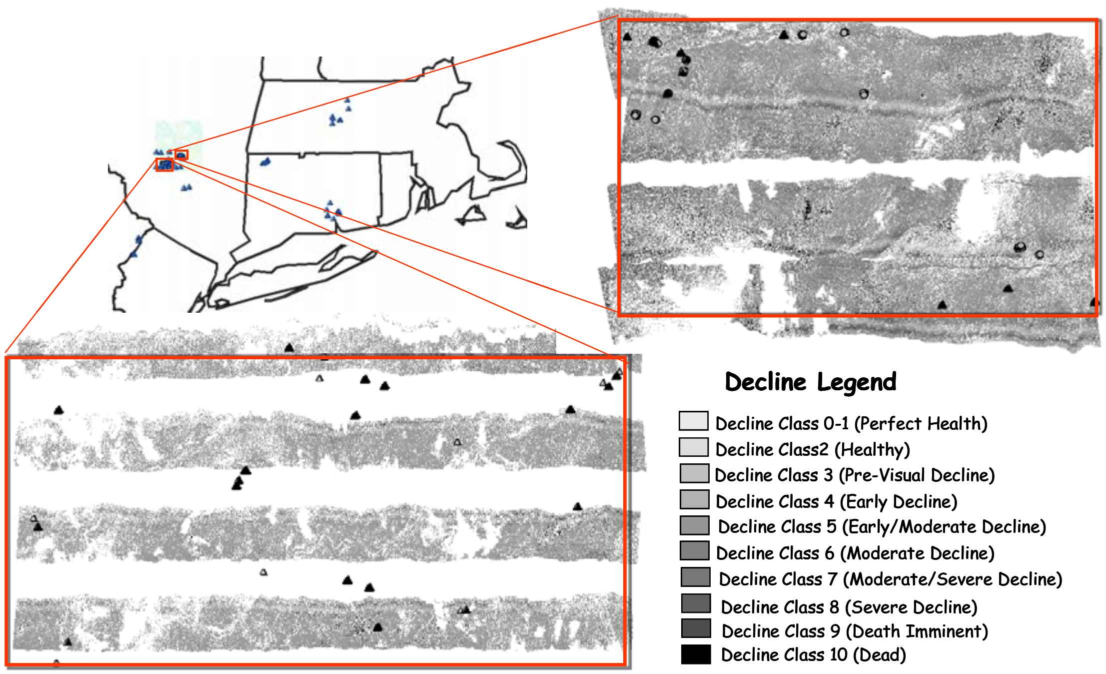

Figure 4. Applied to all pixels, the decline prediction highlights more severe decline symptoms in the eastern portion of the Catskills where HWA has the longest history in hemlock stands. Other stressors are not excluded from this analysis. The obvious gaps in coverage and spectral artifacts (striping) highlight data acquisition problems to be aware of when purchasing imagery.

IDENTIFICATION OF PRE-VISUAL DECLINE SYMPTOMS

Early symptoms of stress in forest species include reductions in photosynthetic activity and chlorophyll content (Mohammed et al. 1995). Such changes are not readily detectible from the ground in mature stands. This is why most forest health ratings rely on defoliation measurements such as transparency or dieback (USDA Forest Service Crown Rating Guide). Up until decline class 4, both of these measurements are below what is typically first categorized as decline in the field (fine twig dieback below 5% and transparency below 15%). Therefore, any results that are successfully able to differentiate between healthy samples (decline class 0) and samples in decline category 4 are most likely picking up changes in chlorophyll content and function before defoliation symptoms are apparent. Prediction accuracy in the low end of the decline range (0-4) was within a one-class tolerance 92% of the time. These results indicate that this technology can be used to detect tree health before visual symptoms are apparent across the landscape.

CROSS-INSTRUMENT APPLICATION

In order for this technology to be applicable on a large spatial and temporal scale, the relationships presented here must also be shown to work on other datasets. The initial selection of wavelengths for examination with the AISA Eagle imagery was based on results from previous ASD and AVIRIS work. All three instruments demonstrated similar relationships between key wavelengths and decline. We believe this indicates that the work presented here will prove robust enough for application to other narrow-band sensors from multiple remote sensing platforms.

Because the ratios or pairs of wavelengths used to calculate indices highlight significant features while correcting for geometrical and background effects (Baret and Guyot 1991), cross-instrument application could be direct. Such simple transformations have been closely correlated with plant characteristics without the sensitivity to external variables such as sun angle or instrument variability (Pinty et al. 1993). Ongoing work will focus on indices and ratios in order to speed processing time.

However, this is not to say that ground truth data will not continue to be necessary in hyperspectral work. The input of known spectra to any predictive model will always increase accuracy and ensure that predictions are as robust as possible. In addition, ground truth data will enable land managers to know the accuracy of their maps and limitations of predictive coverages they are using in their management plans.

CONCLUSIONS

These results indicate that a simple four-term linear regression model based on chlorophyll$_a$, fluorescence, and water absorption features is able to accurately predict a detailed hemlock decline rating system (88% one-class tolerance accuracy). The one-class tolerance accuracy at the extreme low end of the decline scale (0, healthy to 4, pre-visual decline) was 92%, indicating that hyperspectral sensors could be used to detect trees in the very early stages of decline.

There is little evidence that these technologies can diagnose causal agents, as stress may be related to a variety of factors. However, our ground truth data suggests that most declining hemlocks in this region are currently impacted by HWA. These techniques would provide a much-needed tool for the early detection of stressors such as HWA infestation, and will allow forest land management agencies to focus biological control efforts on incipient infestations before trees are severely impacted.

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Operational Use of Hyperspectral Imagery for Forest Health Mapping

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ABSTRACT

Hyperspectral remote sensing imagery has been used to map foliar chemistry (N and Ca), forest productivity, species, and tree health at the landscape scale (25,000 acres to 840,000 acres). Most of this work has been accomplished by research teams using sensors and techniques that are not generally available for use by forest land managers. Recently, hyperspectral remote sensing imagery has become commercially available, making this technology more accessible. However, the techniques for processing this imagery and producing usable maps are complicated and still relatively new. This paper will provide information and guidelines to help forest land managers understand, contract for, and utilize hyperspectral remote sensing imagery to produce landscape scale maps of eastern hemlock health.

KEYWORDS

Remote sensing, hyperspectral, species mapping, hemlock health.

INTRODUCTION

The spread of the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand) across the northeastern United States continues to threaten the eastern hemlock (*Tsuga canadensis* Carriere) resource. The potential loss of eastern hemlock from the forest ecosystem forces land managers to consider a wide array of possible treatment and management options. In order for land management agencies to begin to prioritize and design a focused management plan in response to HWA infestation, it is necessary to efficiently identify the location and health status of the eastern hemlock resource.

Currently, rough species type maps are created using aerial photography, aerial sketch maps, and on the ground surveys. In order to track hemlock health and HWA infestation field surveys are required and detection is limited to sampled plots. Existing remote sensing techniques using broad-band remote sensing imagery are limited to identifying trees only in severe states of decline. Such survey techniques cannot easily identify incipient infestation or early health problems.

Remote sensing technology has been available for landscape scale mapping of forest ecosystems for several decades. The primary users of this technology have typically been researchers studying ecosystem dynamics at a landscape scale. Hyperspectral remote sensing imagery has been used to map foliar chemistry (N and Ca), forest productivity, species, and tree health at the landscape scale (25,000 acres to 840,000 acres)(Smith et al. 2002, Ollinger et al. 2002, Martin and Aber 1997, Pontius et al.In press-a and -b). Most of this work has been accomplished by research teams using sensors and techniques that are not generally available, for use by forest land managers. Recently, hyperspectral remote sensing imagery has become commercially available making this technology more accessible to forest land managers. However the techniques for processing this imagery and producing usable maps are complicated and still relatively new. This paper will provide information and guidelines to help forest land managers understand, contract for, and utilize hyperspectral remote sensing imagery to produce landscape scale maps of hemlock health.

USEFUL TERMS

1. **Spatial Resolution:** Spatial resolution is most often expressed in meters and refers to the area on the ground represented by a single image pixel. For example, a spatial resolution of 1 meter means that a single pixel will contain data from a 1 m by 1 m area on the ground. An image taken at 1 meter resolution is sufficient to show individual tree crowns in a forest. A spatial resolution of 20 meters will contain less detail and is appropriate for gaining stand-level information. For airborne instruments, spatial resolution is largely dependent upon the altitude at which the instrument is flown.

2. **Spectral Resolution:** Number and spectral width (in nanometers) of the bands of reflected light that can be recorded by the sensor. Many narrow bands covering a broad range of the spectrum are characteristic of hyperspectral instruments. The spectral resolution needed will depend on the questions to be answered. For example, identifying areas of severe defoliation will require significantly fewer bands than picking up pre-visual decline symptoms in newly infested stands.

3. **Geo-registration:** Geographic registration or geographic rectification is the spatial referencing of an image to a geographic coordinate system (e.g., latitude/longitude, UTM, State Plane). Once the image data is collected, it is important to be able to link each pixel in the image to the location it came from on the ground. The accuracy of geo-registration has a direct bearing on the usefulness of the final maps and the accuracy of ground truth input and validation.

WHAT IS HYPERSPECTRAL IMAGERY?

Hyperspectral remote sensing imagery is characterized by the large number of narrow bands of reflectance data collected across a broad range of the electromagnetic spectrum. Figure 1 shows a typical spectral signature from a vegetated pixel. NASA's hyperspectral Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) instrument collects data from 400 to 2400 nm. Commercial vendors typically utilize hyperspectral sensors that collect data from 400 to 1000 nm, although there are some full-range (~400-2500 nm) instruments available. By contrast, Landsat TM is classified as a multi-spectral instrument because it collects data in 6 broad bands of reflected light.

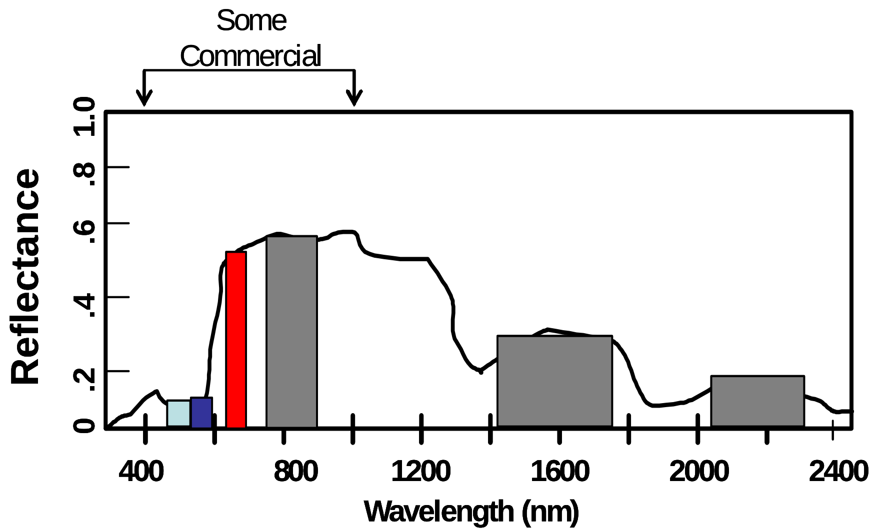

Figure 1. Typical spectral signature taken from a vegetated pixel. The AVIRIS instrument collects information from 400 to 2400 nm. Landsat TM collects information from the six broad regions designated by the shaded rectangles. Some commercial hyperspectral instruments collect data from 400 to 1000 nm.

The usefulness of each type of imagery is dependent on the information that is required for management decisions. For example, in order to map foliar N or Ca, the full spectral range shown in Figure 1 is necessary, including wavelengths across the near-infrared spectrum. However, hyperspectral data from 400 to 1000 nm may be sufficient for mapping hemlock health and distinguishing between different tree species (see Pontius et al. in this proceedings). Multi-spectral Landsat TM data has been shown to be capable of mapping hemlock health in broader categories and at a larger spatial scale (Royle and Lathrop 1997).

In order to create a usable map from hyperspectral imagery, there are several image processing steps that need to be completed. These steps are analogous to taking an aerial photograph, developing the film, having someone interpret the photo, and having a map made of species, wetlands, defoliation, etc., based on that interpretation. Figure 2 diagrams the process that is necessary to create a usable map of hemlock health from hyperspectral imagery.

WHAT CAN HYPERSPECTRAL REMOTE SENSING IMAGERY DO FOR YOU?

The benefit of hyperspectral data over traditional detection and monitoring techniques is the ability to detect minute changes in forest condition such as reductions in photosynthetic activity and chlorophyll content. This type of information is most useful in determining the very *early* signs of stress —stress that may not be visible on the ground or in aerial photography. For mature stands of hemlock where infestations are hard to detect, this may be the best way to track the spread of HWA and to target management activities where they have the best chance of success.

Hyperspectral remote sensing imagery can provide detailed information about the location and health of the hemlock resource with a high degree of accuracy. In addition, changes over time can be tracked using imagery from multiple years. Information of this nature can be used to provide focus for more intensive field surveys or activities. For example, instead of

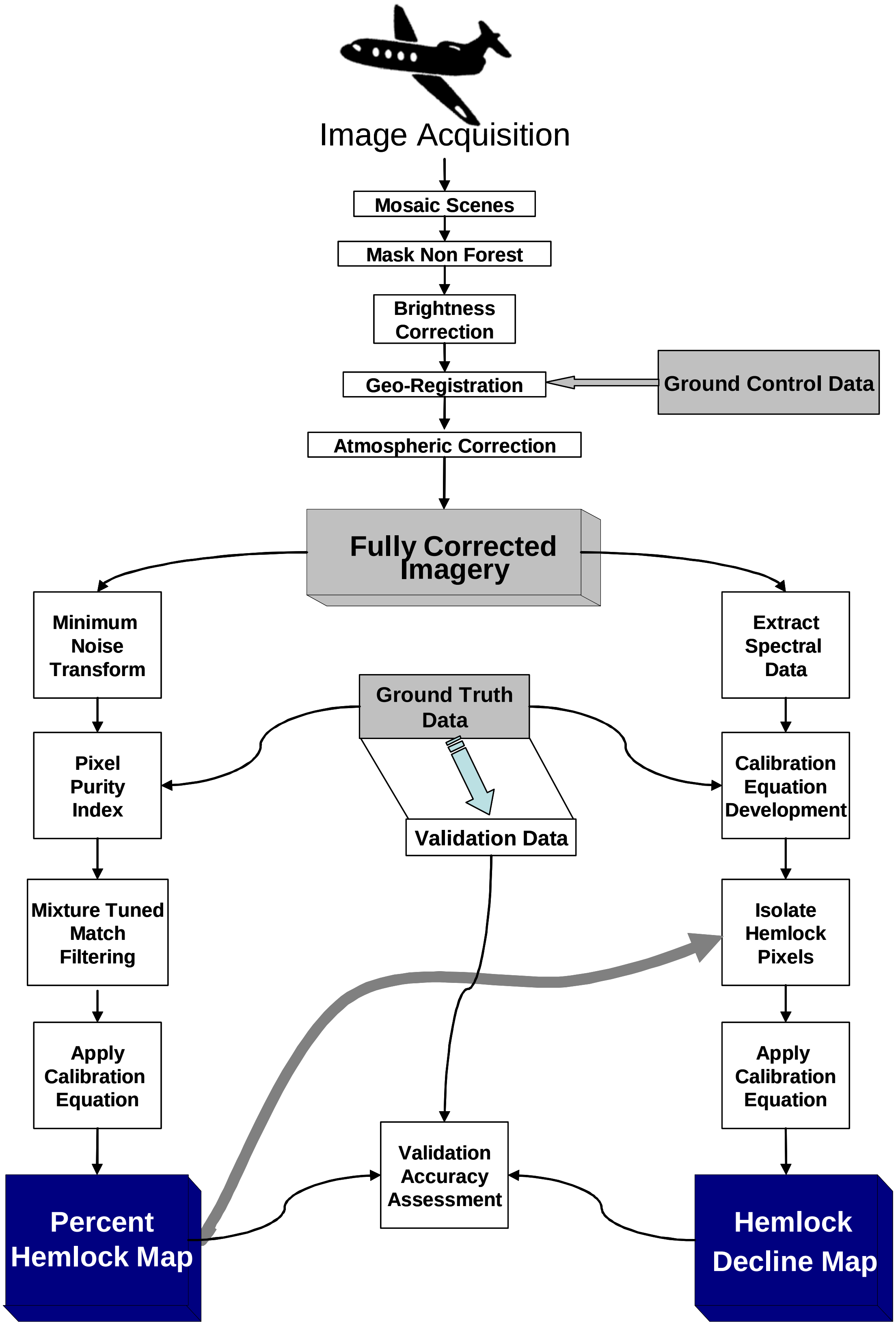

Figure 2. Flow chart of the image-processing steps typically required to derive useful data from hyperspectral imagery.

using field crews to conduct a field plot survey over large areas of the landscape to detect any new HWA infestation, remote sensing technology could be used to identify areas that appear to be unhealthy. Field crews could then be sent to areas identified as unhealthy to determine whether the source of the stress is HWA infestation.

The success of bio-control efforts is also dependent on identifying areas of moderate infestation, where trees are still relatively healthy. Continuous coverages of hemlock health would facilitate the location of appropriate release sites for predatory insects. Other remediation efforts, such as pesticide application are also dependent on locating infested areas with moderately healthy trees. Being able to provide landscape scale information to these management activities should improve their success rates.

IS HYPERSPECTRAL IMAGERY RIGHT FOR YOU?

The use of commercial use of hyperspectral imagery by forest land managers is new, and image acquisition and processing can be quite complicated and expensive. For these reasons, it is advisable to consider the management decisions you are facing in the context of whether the maps produced from hyperspectral imagery will provide new information, more detailed information, and/or, unique information about the landscape you are managing. In some cases you may find that existing methods and technologies are more efficient and/or cost effective. For example, if you are interested only in areas of severe hemlock decline, then Landsat TM data, aerial sketchmapping, and aerial photo-interpretation may be more cost effective options. However, if your management strategy depends upon early detection of decline and accurate mapping of the hemlock resource, then data products derived from hyperspectral imagery may be preferable.

WHERE CAN I GET HYPERSPECTRAL IMAGERY?

Hyperspectral data can be obtained from a number of commercial vendors (Table 1). It is important to note that the data products provided by these vendors will vary. In some cases, a vendor may provide final data products, such as maps of species or stand/tree health, whereas in other cases, the data product may be image data of surface reflectance in a varying number of spectral bands, covering a varying spectral range; in the latter case, it will be necessary to transform that spectral data into the data product required for your management decisions. Land managers may choose to transform spectral data into these final products in-house by applying analysis techniques and algorithms to the imagery or to contract with a third party to generate these products.

Table 1 includes some key variables that can help evaluate whether the instrument is suitable for your needs. The name of the actual instrument a vendor uses is important because different vendors may use the same instrument to produce their image data. The spectral range and resolution are important in determining the suitability of data in generating different products: the narrower the spectral resolution, the more refined predictions of forest health and species mapping will be. To date, the identification of early decline symptoms has been accomplished with up to 10-nm resolution spectra across the visible and near infrared wavelengths (400-800 nm). The location of the company may have a bearing on cost as it can be expensive to ferry the instrument long distances, although this may be mitigated somewhat if the company has other jobs in your area.

Table 1. List of potential sources of hyperspectral imagery. All except AVIRIS are currently commercially available.

Vendor	Instrument	Spectral Range (nm)	Spectral Resolution (nm)	No. of Bands	Platform	Location	Website
NASA	**AVIRIS**	**400 to 2400**	**10**	**224**	**Fixed wing**	**USA**	**http://aviris.jps.nasa.gov**
Helicopter Applicators Inc.	AISA Eagle 1K	400 to 1000	2.9	272	Helicopter	MD	www.helicopterapplicators.com
Flight Landata	HDHIS	446.5 to 906	2.5	240	Fixed wing	MA	www.flidata.com
Earth Search Sciences Inc.	PROBE-1	440 to 2400	14	128	Fixed wing	MT	www.earthsearch.com
Hyvista Corporation	HyMap	400 to 2500	15	126	Fixed wing	Australia	www.hyvista.com
SpecTIR	HyperSpecTIR	450 to 2450	12	227	Fixed wing	NV	www.spectir.com
Galileo Group Inc.	AISA+	400 to 1000	2.9	244	Helicopter/ fixed wing	FL	www.galileo-gp.com
Galileo Group Inc.	AISA Hawk	1000 to 2400	8	256	Helicopter/ fixed wing	FL	www.galileo-gp.com
ITRES	CASI 2	400 to 1000	1.9	288	Fixed wing	CO	www.itres.com

SPATIAL RESOLUTION

Imagery collected from an airborne platform can have variable spatial resolution. Flying closer to the ground will yield a more detailed image, sometimes reaching resolutions of less than 1 meter.

Careful consideration should be given to the level of detail that is required to answer your specific management question. It may not always be better to have a higher level of detail in your image: high spatial resolution images can be more expensive to collect because a smaller area on the ground is covered with each pass of the instrument, and so require more passes (and expense) to capture the area of interest. In addition, the data collected can be more difficult and time-consuming to process not only because of increased data dimensionality, but also because of the increased pixel variation (e.g., individual pixels that capture gaps in the canopy, variation in canopy structure, etc).

The size of the area to be mapped will also have a bearing on the level of detail necessary. For example, if you are mapping 100,000 acres, you may not need or want information for every tree (which is what 1-2 meter spatial resolution will yield): a map created with 20-meter spatial resolution will be sufficient for assessment of tree health and species abundance over large areas of the landscape. On the other hand if you are collecting imagery from urban areas where information on individual trees is essential, highly detailed spatial data (e.g., 1- to 4-m resolution) are necessary to accurately map vegetated and non-vegetated areas.

CONTRACTING FOR HYPERSPECTRAL IMAGE ACQUISITION AND PROCESSING.

There are several items that you may need to provide a vendor. These vary with the data product provided by the vendor, and include the following:

1. Boundary map
   A map of the area that you want flown. The map should be provided to the vendor's specifications. Some vendors require an Arc shapefile while others may only need the coordinates of the corners of the area to be flown.
2. Ground reference data
   Any hyperspectral imagery should have a set of ground truth data as input for the creation of predicted health and species mapping layers. This allows the image processor to train on areas of known infestation, health, and species composition and is necessary in order to maximize the utility of the imagery. In order to collect accurate ground reference data, you or the vendor will need a high-precision GPS unit capable of 1- to 2-meter accuracy
   a. Calibration and validation plot data (60 plots per image)

      Calibration and validation data are field plot measurements collected for species health or abundance. The plots should be spread evenly across the area to be flown. They should cover the full range of health or species abundance found in the area. The data collection should take place concurrent with the image acquisition in order to ensure identical conditions.

      If a vendor provides image data that needs to be processed into a final product, you must collect calibration and validation data. For example, forty plots could be used to calibrate the imagery during the processing phase (e.g., transform the hyperspectral data into the final product) while an additional 20 plots would be used for validating the final product. We recommend this number of plots to ensure the creation of robust prediction equations.

If a vendor provides a final data product, such as species maps or health assessment maps, you may still need to provide the vendor with plot-level data for processing the imagery, as well as for reporting an estimate of accuracy for the final product (validation).

b. Ground control points (50 per image)

These plots are used to geographically register the image so it is possible to relate data from a point on the image to the same point on the ground. If the vendor does not collect ground control data, you will need to provide it yourself. Ground control points are field targets, such as road intersections that can be easily located on the image. For high spatial resolution imagery, isolated tree canopies may be used, while lower spatial resolution imagery would be limited to large road intersections, buildings, or cover type changes. A precise location on the ground is obtained using a high precision GPS unit (1-2 meter accuracy) and can be linked to pixels in image processing and GIS software programs. Fifty plots are often sufficient, while for some images, more or fewer plots are required. To ensure accurately geo-referenced data, these targets should be evenly distributed throughout the geographic extent of the image.

Alternatively, ground control points can be obtained through the use of a geo–registered digital ortho quad (DOQ) maps of your area from the United States Geological Survey. These can be used to extract latitude and longitude for points on the ground that corresponds to points visible in the image. Although easier, this method will yield less accurate results because there is some inherent error associated with the DOQ locational data. Ideally, ground control points obtained in the field would be used in conjunction with registration to DOQ imagery to provide the highest level of accuracy across an entire image.

3. Image processing and/or GIS software

Whether you will be processing the hyperspectral image data or receiving a final data product, you will likely need to be familiar with image processing and/or geographic information systems (GIS) software. This will facilitate the viewing and use of the final product.

In order to protect both the land-manager and vendor, a detailed contract should be developed. In developing this contract the following items be considered:

1. Complete image data coverage of the specified area
2. Spatial resolution requirements
3. Image data pre-processing

- All airborne image data collection is influenced by the atmospheric conditions at the time of flight. It is therefore necessary to convert imagery to reflectance using an atmospheric correction. Multiple programs currently exist (e.g., ACORN and ATREM) that are capable of transforming calibrated at-sensor radiance data to reflectance with minimal inputs, such as date and time of flight, altitude, humidity, etc.
- Depending on the sensor, and resolution, other pre-processing steps may be necessary, such as a view angle correction for scanning spectrometers or shadow removal for high spatial resolution imagery (Figure 2).

4. Documentation of spectral and radiometric instrument calibration procedures and results
    - This ensures that the instrument is being maintained properly and the data obtained from these procedures can help with image processing.
5. Image collection to meet following requirements:
    - Close to solar noon (i.e. ± 2 hours)
    - Collection under cloudless conditions is optimal. Given that portions of the image covered by clouds and their shadows are unusable, you should negotiate acceptable conditions in the contract.
6. Image geo-registration negotiated with vendor
    - At a minimum, a vendor should provide you with imagery that has had inertial navigation system (INS) correction. This corrects for the flight variables, such as roll, pitch and yaw, and must be performed for the imagery to be in a useable form. While this INS correction creates an image similar to an aerial photo, it does not assign geographic coordinates to the imagery: this requires a full geo-registration, which is offered by some vendors, or can be completed in-house. In areas of severe terrain, an orthorectification may also be required to maximize the accuracy or registration. If a vendor is to provide full geo-registration, the expected accuracy should be stated in the contract. Typically one can expect registration accuracy to within 2-3 pixels.
7. Image format
    - The format of the product delivered to you (whether reflectance data or a final prediction coverage) should be negotiated with vendor and match your in-house data-handling capabilities.
8. Final product with at least 80% accuracy
    - If the vendor is to provide a final prediction coverage to you, comparisons to your validation ground truth data should meet some specified accuracy level. It is not uncommon for hyperspectral sensors to predict health ratings and species classification with greater than 80% accuracy.

CONCLUSION

Hyperspectral remote sensing imagery is commercially available and has proven applicability for forest land managers who are making management decisions based upon the location and health of the hemlock resource. However, acquiring and processing this imagery is complicated, requires a high level of technical expertise, and can be expensive. Issues to consider before contracting for hyperspectral imagery include the added value of the expected maps, the level of spatial detail required, and the feasibility of utilizing digital mapping information. Careful attention needs to be given to insuring the spatial and informational accuracy of the final maps. Finally, it should be noted that even though remote sensing technology can produce detailed information for large contiguous areas of the landscape it does not eliminate the need for collecting field-based plot data.

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HYPERSPECTRAL REMOTE SENSING FOR VEGETATION SURVEYS

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ABSTRACT

Hemlock woolly adelgid (HWA), *Adelges tsugae*, and other forest pests, including invasives species, are a problem for our forests and ecosystems. Surveying and monitoring these problems are done at great cost in both money and time. With the use of hyperspectral remote sensing, vegetation identification and stress can be analyzed remotely, saving time and surveying a larger area. Hyperspectral Remote Sensing is the process of gathering spectral signatures remotely. Everything has its own signature, similar to that of a fingerprint, making each unique. Helicopter Applicators Incorporated utilizes an AISA Eagle 1K sensor system to gather and analyze these spectral signatures.

Helicopter Applicators Incorporated (HAI) was established in 1974 as an aerial application company and has since branched into the field of remote sensing. The AISA Eagle 1K has a wider swath ability, which allows it to be flown higher and thereby gather more data in less time and at the same or better resolution than other sensors. With customized hardware and software, HAI offers a full end product.

To demonstrate this, Helicopter Applicators was involved with two vegetation management projects in 2004. HAI flew 400 linear miles in the Kiski-Conemaugh Watershed for invasives identification. West Virginia University gathered spectra via a handheld spectrometer. The mission was to search for 13 invasives species and then compare results to volunteer data throughout the watershed. HAI conclusively located mile-a-minute weed (*Polygonum perfoliatum*), tree of heaven (Sumac) (*Allanthus altissima*), garlic mustard (*Alliaria petiolata*), purple loosestrife (*Lythrum salicarla*), Norway maple (*Acer platanoides*), and common reed (*Phragmites australis*).

The second mission was in the Catskill Mountains in New York in cooperation with The University of New Hampshire (UNH). HAI flew two polygons to determine eastern hemlock (*Tsuga canadensis*) locations and hemlock health, based on GPS ground truthing by UNH. Results are still pending, but preliminary results are encouraging, with both classification and health distributions being qualitatively appropriate.

Despite the lack of conclusive results from these two missions, both have shown that hyperspectral imaging will be valuable for detection and delineation, especially with the bands and flexibility of this sensor flown by Helicopter Applicators Inc.

KEYWORDS

Hyperspectral, remote sensing, detection, aerial survey.

INTRODUCTION TO HELICOPTER APPLICATORS AND HYPERSPECTRAL REMOTE SENSING

Helicopter Applicators Incorporated (HAI) began in 1974 as an aerial application operation. Since then, it has established bases in Pennsylvania, Florida, Delaware, and Mississippi. Currently it has over 30 employees, and owns and operates 15 aircraft. In 2002, HAI branched into remote sensing. It now operates three remote sensing systems including a Radiometric Infrared system, a Three-Chip Digital Daytime camera, and a Hyperspectral Sensor system. All systems are housed in gyro-stabilized gimbals, which are mounted on helicopters.

HAI utilizes an AISA Eagle 1K Hyperspectral Sensor (Figures 1 and 2). It was manufactured in Finland by Specim. The system covers a spectral range from 400 to 1000nm in 272 bands. The sensor is housed in a gyro-stabilized ball with a GPS/INS unit accounting for roll, pitch, and yaw. It is capable of collecting a 1024-pixel swath that can cut flight time by 50% when compared to other sensors. The sensor is capable of sub-meter resolution and has adjustable bandwidth down to 2.5 nm. This allows HAI to collect only areas of the spectrum pertinent to the mission, reducing data size and thereby saving time and money by reducing processing time. The data acquisition hardware consists of a laptop computer, Magma PCI frame grabber, inverter box, hot-swappable disc chassis, and C-MIGITS III GPS and IMU.

The AISA Eagle 1K is classified as a "pushbroom" sensor: as the aircraft moves forward, the sensor collects "lines" or "frames" of data to build an image, each line 1024 pixels wide and one pixel tall. The customized hardware for this system allows for storage and acquisition of large datacubes. The AISA Eagle 1K is a next-generation sensor manufactured by Specim, having one distinct advantage over other sensors: it can be flown at twice the altitude of other sensors, thereby doubling the swath width while still maintaining the same spatial resolution. This allows HAI to cut flight times in half, saving both time and money.

Figure 1. HAI Hyperspectrsl System.

Figure 2. System Mounted on MD 500.

The AISA Eagle 1K spectral range spans from the edge of ultraviolet through the visible and into near infrared of the electromagnetic spectrum. Being able to detect this area of the electromagnetic spectrum is what allows HAI to distinguish among species of vegetation and/or identify levels of vegetative stress. Each species of vegetation has a distinct spectral signature, similar to a fingerprint, making each species unique. As a species becomes stressed, the amount of absorption/reflectance changes altering its spectral signature, which results in the ability to identify vegetative stress. The AISA Eagle 1K can monitor plant health and detect invasive species.

MISSIONS IN VEGETATION MANAGEMENT

The applications of this technology are abounding, but HAI has the most experience with vegetation management. HAI has used spectral signatures to distinguish hemlock trees, classify hemlock health, and identify native and non-native invasives.

INVASIVES PROJECT

One such opportunity was in a study area of the Kiski-Conemaugh watershed near Johnstown, Pennsylvania. During this mission, data was collected at an altitude of 1,162 feet with an airspeed of 45 knots and 245 meter sensor swath, resulting in 0.5 meter spatial resolution. Data was collected from the following streams: Kiski, Loyalhanna, Conemaugh, Blacklick, Little Conemaugh, and Stoney Creek.

West Virginia University collected the ground truth for this mission through use of hand- held spectrometers. The flight objectives for this mission were to locate the following invasive species using the AISA Eagle 1K Hyperspectral Sensor: Japanese knotweed (*Polygonum cuspidatum*), giant hogweed* (*Heracleum mantegazzianum*), tree of heaven (sumac) (*Allanthus altissima*), multiflora rose (*Multiflora rosa*), purple loosestrife (*Lythrum salicarla*), kudzu (*Pueraria montana*), common reed (*Phragmites australis*), hydrilla (*Hydrilla verticillata*), and Eurasian watermilfoil (*Myriophyllum spicatum*) (Aquatics)*, mile-a-minute weed (*Polygonum perfoliatum*), bush honeysuckle (*Lonicera* spp.), Canada thistle* (*Cirsium arvense*), Japanese stilt grass* (*Microstegium vimineum*), Norway maple (*Acer platanoides*) and garlic mustard (*Alliaria petiolata*).

No ground spectra were collected for those invasives with asterisks.

Once the data was collected, HAI was able to apply specific bands to the raw imagery in order to create false color images and true color images. The raw imagery was corrected spectrally and atmospherically. Afterward, the data were geo-rectified. Once the data was analyzed, it was compared to ground results collected by volunteers.

Both HAI and the volunteers located garlic mustard, mile-a-minute weed, Norway maple, and tree of heaven. The volunteers also located Japanese knotweed, and while HAI was able to locate this particular invasive, the results did not conclusively prove detection. HAI also located purple loosestrife and common reed, which the volunteers did not. A second iteration is being performed to verify the presence of Japanese knotweed.

Each of these invasives were then divided into Regions of Interest (ROIs) in ENVI, then overlaid on the imagery. These were able to be displayed individually. The individual ROIs are then converted to GIS vectors. This allows for each to be "on or off" and compared from year to year. The imagery is also converted to a GIS format to be viewed as well (Figure 3).

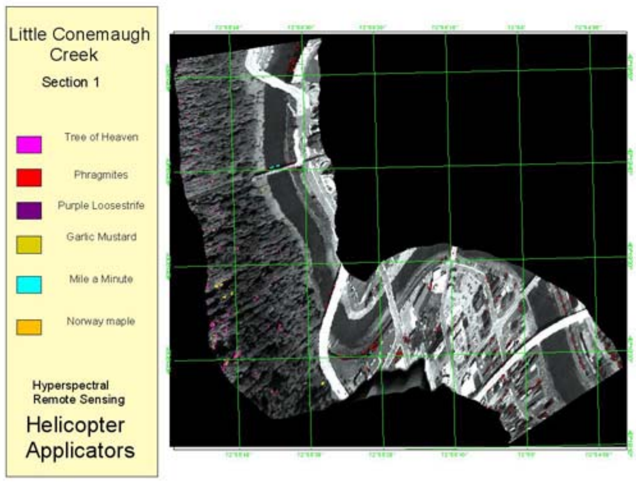

Figure 3. Geo-rectified image with invasive locations in GIS.

HEMLOCK PROJECT

HAI also flew several missions relating to the management of hemlock woolly adelgid. HWA infects the eastern and Carolina hemlock and causes ultimate mortality to hemlocks of all ages. Correct treatment is imperative in rehabilitating hemlocks, as incorrect treatment can accelerate death. Early detection is vital in determining the correct form of treatment and mapping the spread of HWA. In September of 2004, HAI flew a mission in the Catskill Mountains of New York in cooperation with the U.S. Forest Service (USFS) and The University of New Hampshire (UNH).

The goal of the mission was to locate hemlock trees as well as determine their levels of stress. Two blocks were chosen as the study area. The large block was 17,784 acres, and the small block was 4,940 acres. Ground control points were gathered by UNH. The flight time for gathering data for this mission was approximately 45 minutes. The flight parameters for this mission were as follows: data was gathered from an altitude of 4,647 feet at an airspeed of 70 knots. This resulted in an 864-meter swath at a spatial resolution of 2.0 meters.

The mission was for UNH to supply HAI with ground truthed latitudes and longitudes, from which spectra were pulled to identify hemlocks. The first iteration of data processed by the GBC Transform resulted in the location of hemlock trees, which were displayed as green on the image (Figure 4). Once the hemlock locations were identified, the GBC Transform was able to classify four different stress levels within the health range of collected hemlocks. These stress levels were 'Healthy', 'Slight Decline', 'Moderate Decline' and 'Unhealthy' (Figure 5). Both hemlock presence and health classification data could be made into GIS vectors for each ROI.

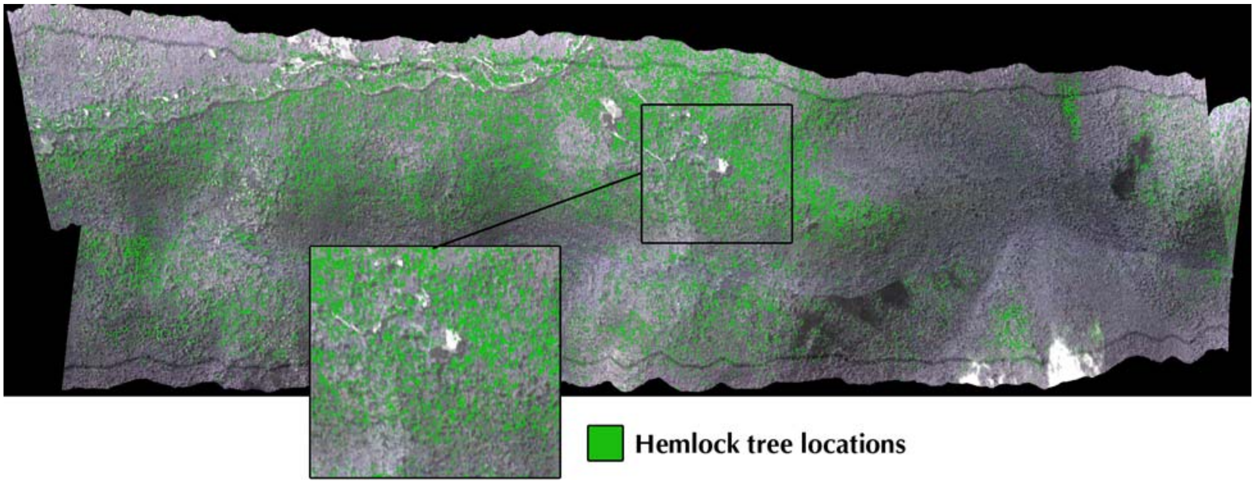

Figure 4: Hemlock locations: small pass 3-4.

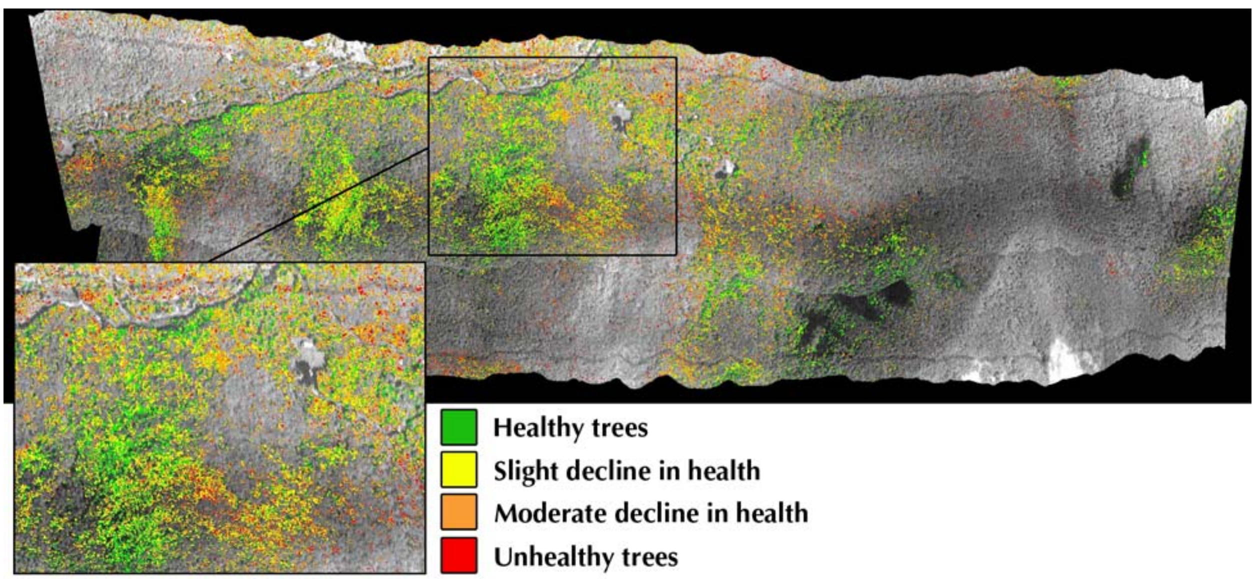

Figure 5: Hemlock health levels: small pass 3-4.

RESULTS OF CATSKILLS CASE STUDY

The preliminary results of the validation study in the Catskill Mountains of New York are promising. HAI partnered with the U.S. Forest Service, The University of New Hampshire, and Remote Measurement Services, LLC, and work is ongoing. Preliminary results are encouraging with both classification and health distributions being qualitatively appropriate, with qualitative verification continuing. Although validation work continues, spatial registration errors within the data have not allowed a quantitative estimate of accuracy.

METHODS AND PROCESSING SOFTWARE

HAI utilizes proprietary processing software designed by Dr. James Sokolowski of Remote Measurement Services (RMS),LLC, known as Hyperspectral Data Processing Software (HyDaPS). HyDaPS is designed for high–volume cubes, which is important for hyperspectral processing. This software calibrates radiance, atmospheric and spectral corrections, and produces reflectance cubes. HAI also utilizes CaliGeo, geo-rectification software, designed by Specim. CaliGeo performs radiometric correction, and geo-rectification. This software also adjusts for elevation through the use of Digital Elevation Models (DEM) or WGS84 Planar Surface elevation. The GBC transform, also a proprietary software designed by Dr. Sokolowski, is used for analysis. This software was specifically designed for the analysis of vegetation. It is capable of differentiating species from species, classifying within a species, and delineating spectral signatures.

In order to perform hyperspectral flights and determine pricing, HAI is given GIS vectors to create a flight plan for the pilot. These vectors are imported into the aircraft's AgNav computer system and display the appropriate flight lines. Once these flight lines are established, a data collection flight is performed. After the data is collected, it is transferred to computers in HAI's processing lab where HyDaPS correction is performed on the data. Next the imagery is geo-rectified with CaliGeo. Imagery spectra are then analyzed by the GBC transform to produce requested results. Finally, new GIS vectors from ENVI ROI's are created, allowing HAI to offer the possibility of a complete end-product.

Prior to each data collection flight, flight parameters are inserted into a "Flight Calculations" spread sheet. This performs the necessary calculations in order to determine resolution, altitude, speed, swath, frame rate, approximate flight time, and amount of data to be collected.

PREVIOUS EXPERIENCE

In a relatively short time, HAI gained substantial experience in the field of Hyperspectral Remote Imaging. HAI has conducted the following data-collection missions:

- October 2003: Perry County, Pennsylvania, Hemlock Study
- November 2003: Perry County, Pennsylvania, Hemlock Study
- November 2003: Appoquinimick Creek, Deleware, Phragmites Study
- March 2004: Perry County, Pennsylvania, Hemlock Study
- May 2004: Department of Defense, Chicken Little Sensor Week, Classified
- June 2004: Kennedy Space Center, Florida (Indian River Lagoon), Submerged Vegetation
- June 2004: City of Houston and EPA, Water Pollution Survey
- June 2004: Department of Homeland Security, Texas, Target Identification
- August 2004: Kiski-Conemaugh Watershed, Pennsylvania, Invasive Species Study
- September 2004: Catskills, New York, Hemlock Study

CONCLUSIONS AND FUTURE DEVELOPMENTS

In conclusion, Helicopter Applicators, Incorporated, is proud to be able to offer hyperspectral remote sensing. Based on our experience, we can provide a complete end-product that includes sub-meter image resolution, classification of vegetation health, differentiation of vegetation species and analysis of data. These full end products of hyperspectral remote sensing can help forest health administrations to identify potential health threats to the forest and take appropriate action. Through the identification of invasives, eradication efforts can be planned. With regards to health levels, proper treatment and priority sites may be established.

HAI is currently completing enhancements to its remose sensing capabilities, including upgrading computer systems for faster processing and upgrading the gimbal system to allow for more accurate data collection. New methods are also being implemented for the collection of ground spectra, as accurate ground truthing is imperative: one such method is for HAI to receive ground coordinates of the species from which spectral cubes can be gathered aerially. This will be more accurate then trying to pull spectra from coordinates in processed data. To further accuracy, HAI is working in cooperation with Research Systems Incorporated (RSI) in order to develop new geo-processing software that will allow for geo-rectification and geo-referencing of high volume cubes. The software will also allow the output of GIS shapefiles, which will allow for identification of individual species and comparison from year to year. Helicopter Applicators strives to keep pace with ever-changing technological advancements in order to meet the needs of the customer.

ACKNOWLEDGEMENTS

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Mapping Hemlocks via Tree-Based Classification of Satellite Imagery and Environmental Data

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ABSTRACT

Within the last few years, the hemlock woolly adelgid (HWA) has made significant inroads into the southern Appalachians. Since the region's native hemlock species are not resistant, timely application of control measures is critical to minimizing hemlock mortality. Unfortunately, hemlock stands in the region are incompletely mapped, and general characteristics of their distribution present serious mapping challenges. One approach for improving classification is to integrate medium-resolution satellite imagery (Landsat, ASTER) and ancillary environmental data. We tested such an approach using images from eastern and western study areas in Great Smoky Mountains National Park. First, we created maps for masking out non-evergreen pixels via unsupervised classification (i.e., cluster busting) of winter images. We then applied the masks to corresponding summer images so we could separate hemlock and non-hemlock evergreens under optimal image conditions. We extracted a large (>14,000) random sample of points from the masked images, stratifying the sample according to an aerial photorapy-derived vegetation map of the park. At each sample point, we recorded the vegetation label as well as image data and values for a suite of topographic, environmental, and proximity variables recorded in a geographic information system (GIS). We applied a series of tree-based classifications to this training data set to create a set of decision rules that most accurately retains the input class of sample points. Our most successful tree had 79 total "leaves" (i.e., distinct decision-rule pathways). We applied these decision rules to the images to develop hemlock maps of the study area. Thematic accuracy assessment of these maps, based on field survey and photo-derived points, indicated 85% overall accuracy in the eastern study area and 69% success at capturing hemlocks in a partial assessment of the western study area. Additional accuracy assessment may offer an opportunity to refine the rules. However, our decision rules can currently be applied elsewhere in the southern Appalachian region for management planning purposes.

KEYWORDS

HWA, Landsat, ASTER, tree-based classification, southern Appalachians.

INTRODUCTION

Hemlock woolly adelgid (*HWA), Adelges tsugae* Annand, is a non-native pest threatening the southern Appalachians. In the past few years, it has made significant inroads into the region, particularly in the Great Smoky Mountains and along the Blue Ridge Parkway. HWA affects both hemlock species native to the region, eastern hemlock (*Tsuga canadensis* [L.] Carr.) and Carolina hemlock (*T. caroliniana* Engelm.). Neither is resistant, though biological control via introduced predator insects holds promise as a method for combating HWA in natural stands. Unfortunately, any counter-measure faces a couple of significant challenges. First, to understand HWA's likely distribution as it spreads throughout the southern Appalachians, the distribution of its host species must be characterized. Second, there must be some way to predict where HWA is most likely to appear next, allowing managers to prioritize their HWA management strategies. Here, we present an approach to the first challenge of mapping hemlocks in the region.

In the northeastern U.S., satellite images have been used successfully for several HWA-related mapping efforts (e.g., Bonneau et al. 1999a and 1999b, Royle and Lathrop 1997 and 2002). These studies have employed time series of Landsat TM data to characterize change in hemlock health through time with considerable accuracy. However, HWA is a relatively recent arrival in the southern Appalachians, so adelgid-induced damage is likely indistinguishable in multispectral satellite imagery. More basically, simply mapping hemlocks from satellite imagery is difficult. Hemlocks are generally found in isolated stands, often in moist valleys, coves, steep ravines, or on north-facing bluffs (Delcourt and Delcourt 2000) and are distributed throughout heterogeneous forests that include other evergreen species from which hemlocks are difficult to separate (Royle and Lathrop 1997).

Nevertheless, given a large region to survey, satellite imagery is still the most feasible tool for hemlock mapping. Incorporation of ancillary data in tree-based classification offers an alternative to the limited distinguishing power of traditional, spectral-based classification. A growing body of literature has highlighted several different approaches for tree-based methods (Murthy 1998). All start with a sample of data representing each level in the class of interest (e.g., the vegetation classes in a vegetation map) and containing values for a number of continuous or categorical variables. This input training sample is subjected to statistical partitioning techniques that identify key variables and split values to most accurately capture the classes of the input training sample points. The resulting product is a tree of decision rules that can then be implemented in GIS or image processing software as an "expert classifier". Tree-based classification approaches have a number of advantageous features. They are typically non-parametric, so conditions of normality are not critical. Furthermore, they algorithmically select variables to be included in the final tree and ignore any extraneous variables. While the commonly used classification and regression tree (CART) method is limited to binary (i.e., two-way) splits, other methods, such as chi-squared automated interaction detection (CHAID), allow for a greater number of splits (Murthy 1998). To develop our expert classifier, we used SAS Enterprise Miner software, which allows implementation of several tree-based classification techniques. We used ERDAS Imagine and ESRI ArcGIS for image processing and GIS analysis, respectively.

STUDY AREA

We selected images covering portions of Great Smoky Mountains National Park (GRSM) in North Carolina and Tennessee. The park has a significant eastern hemlock presence in many areas (Taylor 2002). Originally, we developed our classifier using an ~482 km2 image subset from the eastern side of the park (Figure 1). To strengthen the classifier—via a process that will be explained below—we added a second, ~108 km2 study area on the western side of the park (Figure 1). This area is characterized by gentler topography than the other study area.

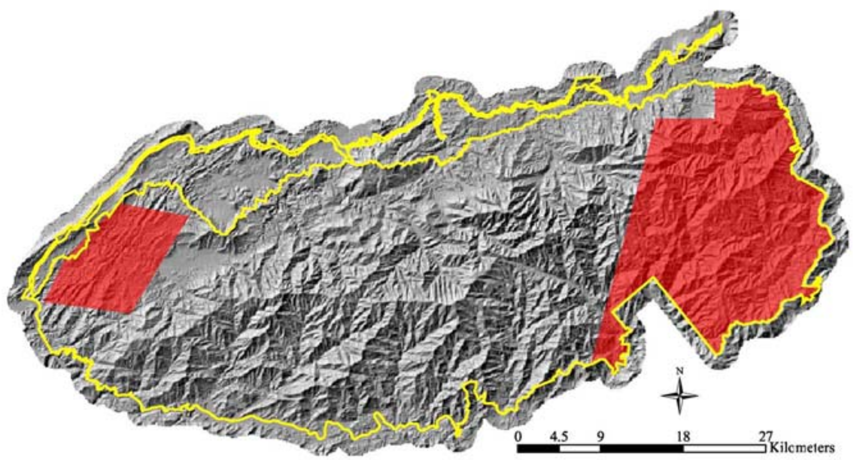

Figure 1. Eastern and western study areas in Great Smoky Mountains National Park.

METHODS

INITIAL ANALYSIS

We acquired a leaf-off October 2001 Landsat ETM+ image from the Global Land Cover Facility (*http://glcf.umiacs.umd.edu/index.shtml*) that covered our eastern study area. We also acquired a leaf-on September 2000 Terra ASTER radiance scene through the NASA Earth Observing System Data Gateway (*http://edcimswww.cr.usgs.gov/pub/imswelcome/*). We chose to use ASTER imagery because of the sensor's good spatial (15-m in the VNIR versus 28.5-m or 30-m for Landsat) and radiometric (12-bit versus 8-bit for Landsat) resolution. After converting the Landsat image to radiance, we fused the 28.5-m multispectral image with its corresponding 14.25-m panchromatic image using an algorithm developed by Halil Cakir (North Carolina State University). This brought the two images closer in spatial resolution. We geometrically corrected the fused Landsat image using a third-order polynomial equation and 92 ground control points (GCPs) collected from color-infrared digital orthophoto quarter quads (DOQQs) of the area (RMSE = 4.1420 m). The ASTER image is actually a combination of two separate image files, a 15-m resolution, three-band image in the visible and near infrared (VNIR), and a 30-m resolution, six-band image in the short-wave infrared (SWIR), merged into a single 15-m image. Though automatically subjected to preliminary geometric correction upon import to ERDAS Imagine, we further corrected the merged ASTER image using a fourth-order polynomial and 80 GCPs (RMSE = 6.4694 m). This better aligned the ASTER image with the Landsat image. We clipped each image to fit our eastern study area.

A major obstacle when using remotely sensed data of mountainous regions is the highly variable level of ground illumination and radiance due to topographic relief, which can result in loss and alteration of image information (Jensen 1996). A number of topographic normalization equations have been proposed that correct pixel values using models of the Earth's surface to account for terrain-induced variation. Based on the recommendations in several studies, we topographically normalized both images using the C-correction method (Teillet et al. 1982). We calculated coefficients for the C-correction equation by regressing image radiance values on illumination values, which were based on a digital elevation model (DEM) as well as the solar azimuth and elevation at the time of image capture.

The leaf-off Landsat image served as a means to mask out all non-evergreen pixels in the study area. We separated the image into evergreen and non-evergreen vegetation classes via cluster busting (Jensen 1996). The cluster busting process required several iterations of unsupervised classification, where each new iteration focused only on pixels that could not be clearly distinguished at the previous iteration. The results of all iterations were merged into a single evergreen/non-evergreen map. When assessed based on DOQQs, this map had an overall accuracy greater than 85%. After resampling this map from 14.25-m to 15-m resolution, we used it to remove all non-evergreen pixels from the ASTER image.

Summer (or leaf-on) images offer good spectral conditions for species separation, and have less topographic shadowing than winter images (Jensen 1996). We used the masked ASTER image to separate hemlock and non-hemlock evergreen classes. To do this, we created a training data set suitable for application in tree-based classification. Our guiding source for the set was a GIS-based vegetation map for GRSM developed primarily from large-scale (1:12,000) aerial photographs (Welch et al. 2002). This map represented the best available source of information on hemlock distribution. It provided four classes of hemlock presence, recorded as unique polygons: dominant, co-dominant, secondary component, and inclusion. In a GIS, we generated a large random sample of points for each of these classes, as well as a random sample of points from areas outside the hemlock polygons but still in the masked ASTER image (i.e., a non-hemlock evergreen class). Notably, large samples are required for tree-based classifiers to perform well (Murthy 1998). Making sure no image pixel was sampled by more than one point, we scaled the sample sizes to match the proportion of the image each class occupied: ~1,000 points for the co-dominant class, ~1,500 points for the dominant, secondary component, and inclusion classes, and ~3,000 points for the non-hemlock evergreen class. For each sample point, we extracted a number of variables derived from individual raster data layers in the GRSM GIS database (Table 1). We also recorded pixel data from the ASTER image. To minimize potential image-specific bias in the pixel values, we used normalized band ratios rather than per-band pixel values.

We used SAS Enterprise Miner to analyze the input training sample. Accepting that a tree fit to five different classes would be unwieldy, we simplified the training sample by combining the dominant with the co-dominant and the secondary component with the inclusion class. (We used additional trees to subdivide these classes in later iterations, but have not reported on them here.) Exploiting the flexibility of the software, we tested trees with 2-, 3-, 4-, and 5-way splits, then chose the one with the lowest misclassification rate. Tree-based classifiers are susceptible to over-fitting of the training data and thus can be less successful at classifying subsequent data sets (Murthy 1998). To avoid over-fitting, we employed automatic pruning of the trees based on Chi-squared testing at a significance level of $\alpha = 0.20$. We also set a minimum of 10 data points for any output node. Although all four trees tested had very similar misclassification rates, the 3-split tree performed the best, with an initial sample accuracy of 61% and 75 "leaves" (i.e., distinct decision rule pathways). This initial sample accuracy is only a partial reflection of the tree accuracy once applied in the expert classifier, but may be seen as a minimum accuracy threshold under the strictest assessment conditions (i.e., not accounting for mitigating factors such as image resolution and the positional accuracy of field data). Table 1 shows which of the eligible input variables were included in the tree.

The "leaves" from the 3-split tree were imported into the Expert Classifier module of ERDAS Imagine. This module allowed us to construct a set of rules for assigning every evergreen pixel in the study area to an output class based on raster layers for each input variable. We created a final output map by merging the expert classifier result with the non-evergreen pixels identified in the cluster-busted Landsat image.

ENHANCED ANALYSIS

As previously noted, tree-based classifications are susceptible to over-fitting of the training data. While we employed automatic pruning, a single training data set may not represent the full variation of conditions for a phenomenon of interest. To enhance the success of our classifier, we developed supplementary training data points using a study area in the western portion of GRSM. This area is quite different from the eastern study area topographically, but is typical of certain portions of the southern Appalachians.

Our processing methods for the western study area were similar to those for the east. We procured two ASTER radiance images, captured in June 2000 (leaf-on) and November 2003 (leaf-off). The images were geometrically corrected with polynomial equations (39 GCPs and RMSE = 6.1546 for June 2000; 24 GCPs and RMSE = 4.6436 for November 2003), clipped to the study area extent, and topographically normalized via C-correction. We generated an evergreen/non-evergreen mask from the November 2003 image via several iterations of cluster busting, and then applied this mask to the June 2000 image. From the remaining evergreen portion of the image, we generated random sample points in proportion to the area each hemlock class occupied in the western study area: ~800 points from dominant, ~1,500 points from co-dominant, ~50 points from secondary component, and ~500 from inclusion, as well as 2,800 points from non-hemlock evergreen areas. For each sample point, we extracted values for the input variables from raster layers in the GRSM GIS database. We then combined these sample points with our eastern area training data set, yielding a substantially larger set of more than 14,000 sample points across five classes.

Table 1. Variables tested in tree-based classification. Plus signs (+) denote variables actually used in initial classification tree; asterisks (*) denote variables used in enhanced classification tree.

Variable	Resolution (m)	Description
Aspect+*	10	Slope direction based on DEM
Curvature	10	Convexity/concavity based on DEM
Elevation+*	10	Elevation from DEM
Landform Index*	10	Index based on DEM (McNab 1993)
Slope+*	10	Degree slope based on DEM
Topographic Relative Moisture Index+*	10	Dryness-wetness index based on DEM (Parker 1982)
Proximity to Streams+*	10	Grid based on GRSM stream layer
Disturbance History	90	Harvested or cleared land (GRSM data)
Fire Frequency	90	Reoccurring burns, 1920s-80s (GRSM)
Fire History	90	Decades of fires, 1920s-80s (GRSM)
Geology	90	General bedrock formations (GRSM)
ASTER Ratios:		Normalized difference indices to allow generalization of any image-based rules in the output trees. Particular ratios were chosen based on band-to-band correlations. Indices calculated as: (band a - band b)/(band a + band b).
Band 3/Band 1+*	15	
Band 1/Band 2+*	15	
Band 4/Band 5+*	15	
Band 4/Band 6+*	15	
Band 4/Band 7*	15	
Band 4/Band 8*	15	
Band 4/Band 9+*	15	

We repeated testing of 2-, 3-, 4-, and 5-split trees in Enterprise Miner in this enhanced data set, again combining the dominant with co-dominant and secondary component with inclusion hemlock classes to simplify the output tree. We used the same settings for tree-based classification. As in the earlier analysis, the 3-split tree performed best, with an initial accuracy of 62% and 79 “leaves.” In fact, all test runs in this enhanced analysis actually performed better (1-3% higher in initial accuracy) than their counterparts in the first analysis. We imported the “leaves” from the 3-split tree into the Expert Classifier module of ERDAS Imagine and used the resulting decision to classify all evergreen pixels in both study areas. We created final output maps by merging these results with the non-evergreen pixels identified by the cluster-busted October 2001 Landsat (for the eastern study area) and November 2003 ASTER (for the western study area) images.

ACCURACY ASSESSMENT

We completed an accuracy assessment of the eastern study area maps—for the original and enhanced classifiers—using 170 reference points gathered from field surveys or by viewing the CIR DOQQs where appropriate. Based on these data, we were only able to judge hemlock presence/absence, so we simplified our assessment to three classes (hemlock, non-hemlock evergreen, and non-evergreen). We examined map pixel values within a 22.5-meter radius of each reference point (approximately equivalent to a 3 x 3 pixel window). If the map class of the reference point corresponded to any pixel falling within the window, then the classification was judged to be correct. We chose this approach to accommodate positional accuracy limitations of the image geometric correction process (± 7.5 m RMSE) and the reference data points, which were largely recorded with recreational grade GPS units (± 15 m). For each map, we created error matrices and calculated an overall value for Cohen's kappa statistic. Cohen's Kappa statistic indicates how much of an improvement a classification effort is over a completely random classification of the same area (Jensen 1996). It can range from 0 to 1, with 0 being the least possible improvement and 1 being the most. We did not have enough field data to perform a full accuracy assessment of the western study area. However, we did have a small set (n = 32) of hemlock survey points that provided some indication of how the enhanced classifier might perform in this sort of region. We used the same 22.5-m radius window for judging accuracy.

RESULTS

The original and enhanced error matrices (Tables 2 and 3) for the eastern study area are similar, with identical overall accuracies (85.3%; 90% confidence interval of 80.5 to 90.1%). Kappa values for the two matrices were nearly identical: 0.765 for the original and 0.767 for the enhanced classifier. More specifically, the two classifiers performed similarly in mapping hemlocks: the enhanced classifier yielded a higher producer's accuracy but a lower user's accuracy for the hemlock class. The partial accuracy assessment for the western study area indicated that 22 out of 32 hemlock survey points were correctly identified, for an accuracy of 69%. Of the ten misclassified points in the western area, one was mistakenly labeled as non-evergreen; the remaining nine were classified as non-hemlock evergreen.

The hemlock presence maps (Figures 2 and 3) reflected very different levels of hemlock presence. In the western study area, hemlocks appear to be limited to narrow riparian corridors, while they are more broadly distributed in the eastern study area.

Tables 2 and 3. Error matrices based on accuracy assessment of the eastern study area – original classifier (top) and enhanced classifier (bottom).

Classification Totals	Reference Totals: Hemlock	Non-Evergreen	Non-Hemlock Evergreen	Total	User's Accuracy
Hemlock	62	4		66	93.9
Non-Evergreen	10	49		59	83.1
Non-hemlock Evergreen	9	2	34	45	75.6
Total	81	55	34	170	
Producer's Accuracy	76.5	89.1	100.0		**85.3** Overall Accuracy

Classification Totals	Reference Totals: Hemlock	Non-Evergreen	Non-Hemlock Evergreen	Total	User's Accuracy
Hemlock	64	6	2	72	88.9
Non-Evergreen	6	49		55	89.1
Non-hemlock Evergreen	11		32	43	74.4
Total	81	55	34	170	
Producer's Accuracy	79.0	89.1	94.1		**85.3** Overall Accuracy

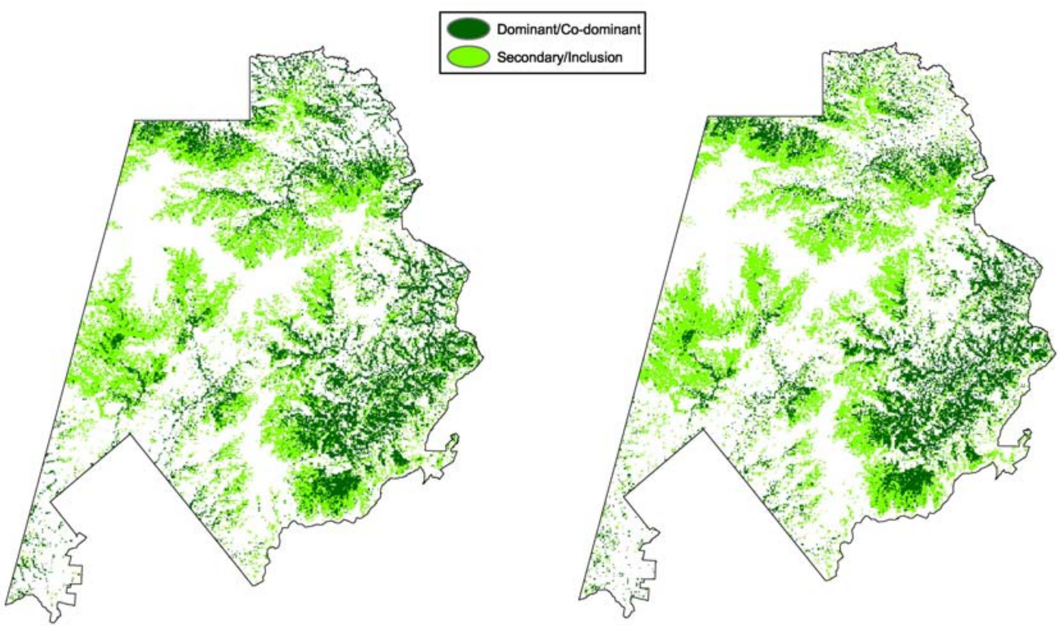

Figure 2. Hemlock distribution maps for the eastern study area – original classifier (right) and enhanced classifier (left).

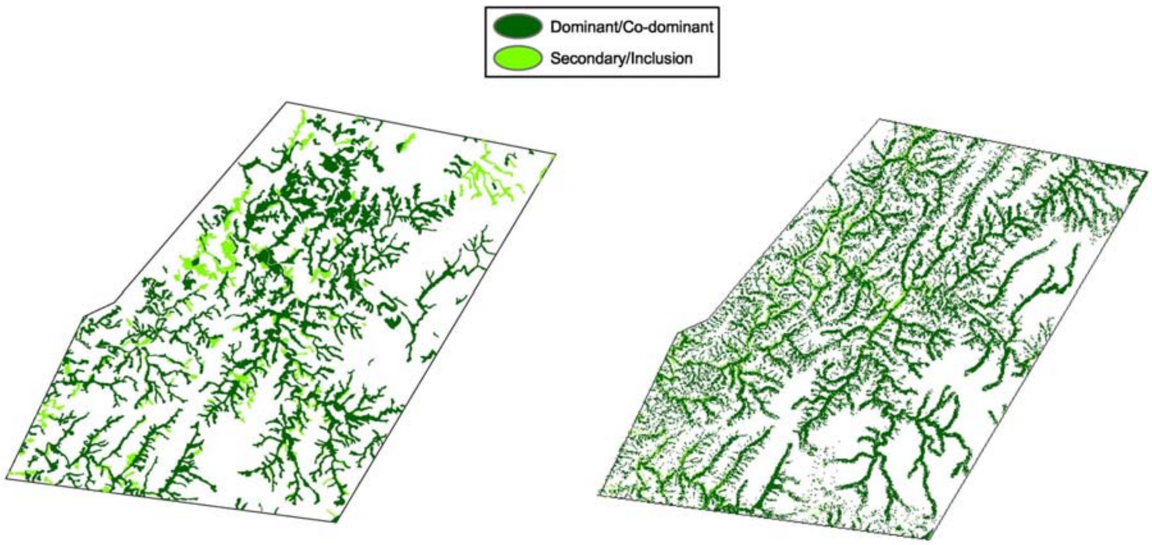

Figure 3. Hemlock distribution map for the western study area (right); photo-derived hemlock polygons used to guide sampling in the western study area (left).

DISCUSSION

The enhanced classifier captured hemlocks fairly well, particularly in the eastern study area. The addition of training data points from the western study area did not substantially alter the enhanced classifier's success in the eastern area, based on the similar accuracies of the original and enhanced classifiers. It is also worth noting that no per-class accuracy value for the enhanced classifier is less than 74.4%, although this assessment only looked at three-class maps. Based on this conservative assessment, the accuracies achieved for the eastern study area meet typical accuracy standards for remote-sensing-derived map products (Congalton and Green 1999). Unfortunately, we do not have enough assessment data to fully judge the enhanced classifier's success in the western study area. However, the hemlock classification accuracy for the west can be reasonably compared to the producer's accuracy for hemlock from the eastern error matrix, and is obviously lower (69% vs. 79%). This may be because so few sample points were available. It may also reflect characteristics of the training data: the photo-derived GRSM vegetation map restricts hemlock to riparian corridors in the western study area, and our enhanced classifier appears to mirror this (Figure 3). This may explain why several of the hemlock survey points in the western area were mistakenly classified as non-hemlock evergreen. Elevation (and more broadly, topography) is less of a factor in the western than in the eastern area, where it more strongly demarcates certain evergreen vegetation types. In locations such as the western study area, hemlocks may be found in small inclusions throughout a forest with numerous other evergreen species. Even a photo-derived vegetation map is likely to miss some of these sparsely distributed inclusions; in fact, they may be smaller than the minimum mapping unit. Such inclusions could be located on the ground and added to the training data set, but this is a potentially expensive proposition that may not result in a significant improvement in accuracy.

We intend to collect additional points in both the eastern and western study areas to expand our accuracy assessment. Ultimately, though, the enhanced classifier will be best served by testing it with data from other parts of the southern Appalachian region. Despite our best efforts, the enhanced classifier may still be over-fitted to the GRSM training data. This can be easily remedied. Though our classifier has too many rules to depict here, its first split logically stratifies the input data into three classes based on elevation. Subsequent splits divide these three elevation-based classes into ever-smaller groups. Notably, any of these finer splits can be manually pruned from the classifier if they are found to be problematic through testing in other parts of the southern Appalachians.

During the testing process and afterwards, the classifier we have described can be used to map hemlock distribution throughout the region. The classifier's rules use topographic and proximity variables that can be calculated from readily available DEMs and stream data. ASTER imagery can be procured for free or at a nominal cost through the Earth Observing System Data Gateway. While the ASTER sensor has only been in service since 1999, much of the southern Appalachian region has already been covered, and it is possible to request satellite tasking that would capture any areas that have not been covered.

The approach outlined here is only one component of an HWA "early warning system" that we have been developing for the southern Appalachians. We have also been working on GIS-based models to predict what areas in the region are at most risk of early HWA infestation. These models yield probability maps that can be used to rank areas at the highest risk of HWA infestation. By overlaying these risk probability maps with classifier-derived maps of hemlock distribution, forest managers can target specific areas for their HWA control efforts, substantially reducing the territory they must cover.

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A GIS-based Risk Assessment for Hemlock Woolly Adelgid in Southern Vermont

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ABSTRACT

A pilot-phase geographic information system (GIS)-based risk assessment was developed for southern Vermont as part of a coordinated effort to reduce the spread of hemlock woolly adelgid. The model incorporated a literature search, interviews, and historical documentation in identifying: 1) factors associated with the risk of HWA introduction and establishment ("susceptibility"), and 2) potential impact of HWA infestations ("vulnerability/ resistance").

Results indicated that although cold temperatures may currently be a limiting factor for northward spread of HWA, cold hardiness zones in which the pest is already established extend well into Vermont. Hemlock is ubiquitous in the state, presenting ample opportunity for natural spread, and historical introductions of the insect highlight risk factors such as nurseries and seasonal residences in conjunction with potential movement corridors. In addition to compilation of these susceptibility factors in a GIS database, a spatially referenced resistance index was created based on soil moisture, slope aspect, and site productivity.

The resulting pest risk assessment model can help focus prevention, monitoring, early detection and rapid response efforts. Susceptibilty factors are being used to help prioritize surveying efforts and incorporating the results of those efforts. Results obtained for the vulnerability/resistance index are untested for generality and require application to a more extensive area for validation, but information garnered offers a means for quantification through statistical correlation with field data. The model is easily modified and will continue to incorporate advances in research, with a goal of closing the loop between research and application by making the results accessible to land managers and forestry practitioners.

Assessments of Biological Control of Hemlock Woolly Adelgid with *Sasajiscymnus tsugae* in Connecticut and New Jersey

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ABSTRACT

The health of hemlocks in *S. tsugae* release sites in Connecticut and New Jersey are compared to non-release sites in an attempt to assess the efficacy of introductions of *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae* (Coleoptera: Coccinellidae) for hemlock woolly adelgid control and management and to remediate hemlock decline. Foliage transparency emerged as an important variable for assessing hemlock crown conditions. In Connecticut, mean foliage transparency was significantly better in release than in non-release sites. Significant improvements in foliage transparency of hemlocks were recorded in *S. tsugae* release sites but not in non-release sites from 2003 to 2004 in New Jersey. Foliage transparency and hemlock mortality in Skyland release sites were significantly lower than in comparable non-release sites. Patterns of hemlock health, *S. tsugae* recoveries, and the impact and interaction of abiotic and biotic factors such as drought, winter mortality of adelgids, and concurrent elongate hemlock scale infestations are also discussed.

KEYWORDS

Adelges tsugae, hemlock woolly adelgid, *Sasajiscymnus (Pseudoscymnus) tsugae*, foliage transparency, eastern hemlock, biological control.

INTRODUCTION

While New Jersey is approximately 1.5 times the land area of Connecticut, both states share almost equivalent forest cover and some of the most densely populated areas in the U.S. Forests cover nearly 1.9 million acres in Connecticut, and eastern hemlocks, *Tsuga canadensis* Carriere, are concentrated in Litchfield County, in the northwestern corner of the state (Wharton et al. 2004), where hemlock stands of more than 1000 acres predominate (Hurlock, personal communication). New Jersey has 1.8 million acres of forested land of which 26,000 acres comprise eastern hemlock stands, also located primarily in the northwestern corner of the state in Sussex, Passaic, Warren and Morris counties (Anonymous 2001). Portions of the northwestern corner of Connecticut are also linked geologically to the northwestern corner of New Jersey. Both the New Jersey Highlands and the Housatonic Highlands in Connecticut are part of the Highlands Province, composed of the oldest Precambrian metasedimentary rocks (Stoffer 2003). Climatically, the Highlands and Skylands regions of New Jersey lie within USDA Zone 6a (minimum winter temperatures between –5 to -10°F) and 6b (0 to –5 °F), which is similar to much of central and southern Connecticut. The northwestern and northeastern corners of Connecticut are part of Zone 5b, where minimum winter temperatures range between –10 to –15 °F.

Connecticut and New Jersey also share a common history with some of the first extensive areas of hemlock decline associated with the initial invasion and spread of *Adelges tsugae* Annand, hemlock woolly adelgid (HWA), in the mid to late 1980s (Ward et al. 1992). In New Jersey, the Highlands region has been heavily infested with HWA since the late 1980s while the northernmost high elevation parts of Sussex and Warren counties, known as the Skylands region, has only more recently been heavily infested, as of the late 1990s. In Connecticut, northwestern Litchfield county is also the most recently infested, while much of the rest of the state has experienced adelgid infestations since the late 1980s. The two states have also cooperated closely in biological control implementations involving *Sasajiscymnus* (formerly *Pseudoscymnus*) *tsugae* Sasaji and McClure, originally imported from southern Honshu, Japan, beginning with the shipment of a starter colony of *S. tsugae* from the Connecticut Agricultural Experiment Station in 1997 to the New Jersey Philip Alampi Beneficial Insect Laboratory. Research on *S. tsugae* in Connecticut, funded by the USDA Forest Service, has continued with studies on the biology and behavior of *S. tsugae*, and hemlock health assessments and monitoring of *S. tsugae* release sites. In New Jersey, the Philip Alampi Laboratory has also mass-reared *S. tsugae* for releases and starter colones in other affected states while also maintaining an extensive release program in New Jersey's infested hemlock stands. To date, 298,160 *S. tsugae* have been released in 70 sites in New Jersey from 1998-2004, while 172,020 have been released in 21 sites in Connecticut from 1995-2002. Many of the release sites selected in Connecticut had moderate to high pre-release adelgid populations, as did release sites in New Jersey—in particular, sites in the Highland region. Although the establishment, field reproduction, synchrony of life cycles with *A. tsugae* and overwintering ability of *S. tsugae* has been previously documented in Connecticut (Cheah and McClure 2000 and 2002) and New Jersey (Mayer et al. 2002a), recovery rates have not been consistently high. Thus, much of the ensuing discussion will center on recent comparative assessments of hemlock health in *S. tsugae* release and non-release sites in the two states.

This paper seeks to identify patterns and summarize encouraging results from the release programs in Connecticut and New Jersey after 6-9 years of monitoring and assessments of *S. tsugae* release sites and to highlight important factors influencing the recovery of hemlocks in adelgid-infested stands.

METHODS

CONNECTICUT

In 2003 and 2004, annual summer evaluations of hemlock health in selected release sites were expanded to include a minimum of 10 to 15 trees per site. Trees evaluated were representative of the age classes at each site and comprised mostly intermediate and co-dominant hemlocks.

At a few sites, the number of trees rated exceeded this as transects to 200m were also included in site evaluations. In addition, fall 2003- winter 2004 surveys (n = 15 trees/site) was conducted in northwestern Connecticut in 28 hemlock stands by the Connecticut Agricultural Experiment Station plant inspectors. All hemlock health ratings followed the standard Forest Inventory Analysis (formerly Forest Health Monitoring) criteria of live crown ratio, crown density, foliage transparency, percentage crown dieback, and live branches (5% classes). In addition, crown estimates of new shoot production and the overall level of adelgid infestations were estimated with binocular inspection in classes of 0, <10%, 11-50%, 51-75%, and >75% (after Tigner, unpublished). Levels of elongate hemlock scale, *Fiorinia externa* Ferris, infestation were also rated visually as none, very light, light, medium, high or very high. At each *S. tsugae* release site, adelgid-infested hemlock tip samples from healthy branches in the lower crown from a minimum of 10 trees were randomly selected during site visits in late February through April, 2003 and 2004, for estimates of winter mortality of the *A. tsugae* sistens. Counts were made under a dissecting microscope to determine the proportion of live and dead adelgids (minimum of 1,000 adelgids/site) per sample. Soil types for each release and non-release site and corresponding woodland suitability groups, which estimate site quality for forest growth, were determined from county-level soil survey maps for all seven Connecticut counties, compiled by the USDA Soil Conservation Service in cooperation with the Connecticut Agricultural Experiment Station and the Storrs Agricultural Experiment Station from 1966-1983. Woodland suitability groups ranged from 1 to 11: 1 being the best site and 11, the poorest. This ranking was used as a method to account for possible site differences before statistical comparisons.

Sampling for predators at selected release sites continued with lower crown sampling of infested branches using 1 meter^2 beating sheets to catch dislodged debris and insects. Thirty infested hemlock tips per site (18-24") were also sampled randomly at 1.5-5m intervals from five selected release sites and surroundings that showed patchy and limited resurgence of adelgids in 2004 and inspected under dissecting microscopes for signs of predation.

NEW JERSEY

Crown ratio, foliage transparency ratings and assessments of hemlock mortality were conducted in 2003 and 2004 at 23 selected *S. tsugae* release sites according to the above Forest Inventory Analysis (Forest Health Monitoring) criteria (Mayer et. al 2002a). In 2003 and 2004, hemlock crown ratings performed at 38 matching non-release sites were also conducted for comparisons. These non-release sites consisted of the most proximal non-release hemlock stands that had similar topographical and hemlock characteristics to those of release stands. All sites rated (n = 20 trees/site) were classified by region (Highland or Skyland) to account for differing histories, intensity, and duration of HWA infestation for statistical analyses. Predator sampling of the lower crown was also conducted at release sites as described above. In addition, a bucket truck was used to sample at increasing heights in the crown in one Skyland site in 2001 to investigate distribution of *S. tsugae* four weeks after initial release in comparison to a simultaneous lower crown survey.

STATISTICAL ANALYSES

Statistical analyses on Connecticut and New Jersey data were performed using the Number Cruncher Statistical System 2000 computer program (Hintze 1998). Range estimates for new shoot production, adelgid and scale infestations from Connecticut sites were transformed into ranks for statistical comparisons (0-4 for new shoot production and adelgid levels, and 0-5 for elongate hemlock scale infestations). Data were checked for normality and equal variances, and where appropriate, the Equal Variance T-Test or the Aspin-Welch Unequal Variance T-Test was used. Non-parametric statistical analyses (the Mann-Witney U-Test and the Wilcoxon Signed-Rank paired t-test) were used for comparisons of non-normal distributions of foliar transparency and hemlock mortality. Linear regressions were also performed to investigate relationships between variables.

RESULTS

CONNECTICUT

Eleven release sites were rated for crown health and infestation levels in 2003 and 16 in 2004 (Table 1). Foliar transparency ratings in 2003 and 2004 were not significantly related to woodland suitability groups. Foliar transparencies in 2003 and 2004 were similar ($p > 0.05$) as were levels of new shoot production. In 2003 and 2004, average levels of new growth were between 50 and 75% of the crown in release sites. Foliage transparency in 2004 was significantly correlated to new growth in 2004 (Figure 1a; $r^2 = 0.2783$, $p = 0.0357$). Levels of adelgid in release sites in 2003 were low (<10%) in eight sites, while in three sites, there was patchy resurgence (10-50%). In 2004, 14 sites had <10% levels of HWA while only two sites showed very patchy resurgence. Comparisons of mean HWA levels in release sites in 2003 and 2004 showed no overall increase in 2004 and remained low overall. Both winters of 2003 and 2004 were severe and resulted in heavy mortality of the adelgid in *S. tsugae* release sites. Mean overwintering mortality of HWA in *S. tsugae* release sites in 2003 was 83.1 ± 7.7% and 87.7 ± 10.9% in 2004, which accounted for much of the subsequent depression of adelgid populations in release sites. Release sites were also infested with *F. externa*. In 2004, 31% of sites had high elongate hemlock scale infestations, 56% of sites had light to moderate infestations, and only 12.5% of sites had negligible scale infestations. In 2004, foliage transparency in release sites was slightly correlated to ratings of scale infestations, although the relationship was not significant (Figure 1b; $r^2 = 0.2235$, $p = 0.0644$). There was also no significant relationship between scale infestations and foliage transparency in 2003.

In non-release sites surveyed in fall 2003 and early winter 2004, foliar transparencies in sites that had indications of adelgid infestation were also not related to woodland suitability groups ($p > 0.05$) (Table 2). Seven of the 28 sites surveyed in pristine areas of high elevations and remote locale were identified as having negligible adelgid or scale infestation. Foliar transparencies from 105 trees from these seven sites were used to develop a baseline mean

Table 1. 2003 and 2004 hemlock health assessments in selected *S. tsugae* release sites (1995-2002) in Connecticut. For HWA and new growth levels: 1 = < 10%; 2 = 11-50%; 3= 51-75%; 4 = > 75% for crown infestation. For EHS levels: 0.5 = Very Light, 1 = Light; 2 = Medium; 3 = High; 4 = Very High.

Year	# Trees	# Sites	Woodland Suitability Group	Means				
				Foliar Transparency	HWA on Crown	New Growth	EHS	Cumulative % Hemlock Mortality
2003	202	11	1-9	33.4 ± 9.5	1.3 ± 0.4	3.1 ± 0.7	1.7 ± 1.1	-
2004	300	16	1-11	37.5 ± 9.3	1.1 ± 0.4	3.3 ± 0.6	2.4 ± 1.3	7.6 ± 13.1

Table 2. Hemlock health assessments in non-release sites in northwestern Connecticut in fall 2003 and early winter 2004. For HWA and new shoot production levels: 1 = < 10%; 2 = 11-50%; 3= 51-75%; 4 = > 75% for crown infestation. For EHS levels: 0.5 = Very Light; 1= Light; 2 = Medium; 3 = High; 4 = Very High.

Non-release Sites	# Trees	# Sites	Woodland Suitability Group	Means			
				Foliar Transparency	HWA on Crown	New Growth	EHS
HWA + EHS	315	21	5-11	47.9 ± 5.2	1.0 ± 0.5	3.7 ± 0.4	2.1 ± 1.5
Negligible HWA/EHS	105	7	2-9	37.0 ± 7.1	0.01 ± 0.04	3.8 ± 0.4	0.1 ± 0.2

foliar transparency that was reflective of healthy, relatively uninfested forest hemlocks growing under normal environmental and climatic conditions in northern Connecticut. Both summer and fall ratings for foliar transparency are still valid for comparisons as both measured the amount of 2003 foliage on the crown, which included the new growth for that same growing season in a non-drought year.

Foliar transparency ratings from all sites were not influenced by woodland suitability groups and allowed direct statistical comparisons of 2003 foliar transparencies of release and non-release sites. Mean foliar transparency in release sites (34.4%) was lower than in non-release sites (47.9%) (Mann Witney U Test; $Z = -3.7901$, $p = 0.000082$). Foliar transparency in release sites compared very favorably ($p > 0.05$) with the baseline foliar transparency (37.8%), while foliar transparencies of infested non-release sites were higher than in baseline sites (Equal Variance t-test; $t = 4.0013$, $p = 0.00023$). Healthy 2003 new shoot production in release sites (50-75%) was slightly lower as compared to non-release sites (>75%) (Mann Witney U-Test; $Z = -2.8992$, $p = 0.00187$), but still reflected recovery. Adelgid levels were not directly comparable as ratings were for different generations of HWA. Hemlock mortality assessments were conducted in 2004 at 16 sites. Mean cumulative hemlock mortality was 7.6 ± 13.1% (3-40%) recorded at six sites (37% of sites), all of which had hemlock borer activity (Table 1). This mortality represented trees that had initially died in 2000 and 2001. However, even in sites with hemlock mortality, hemlocks that survived often showed good recovery and healthy refoliation.

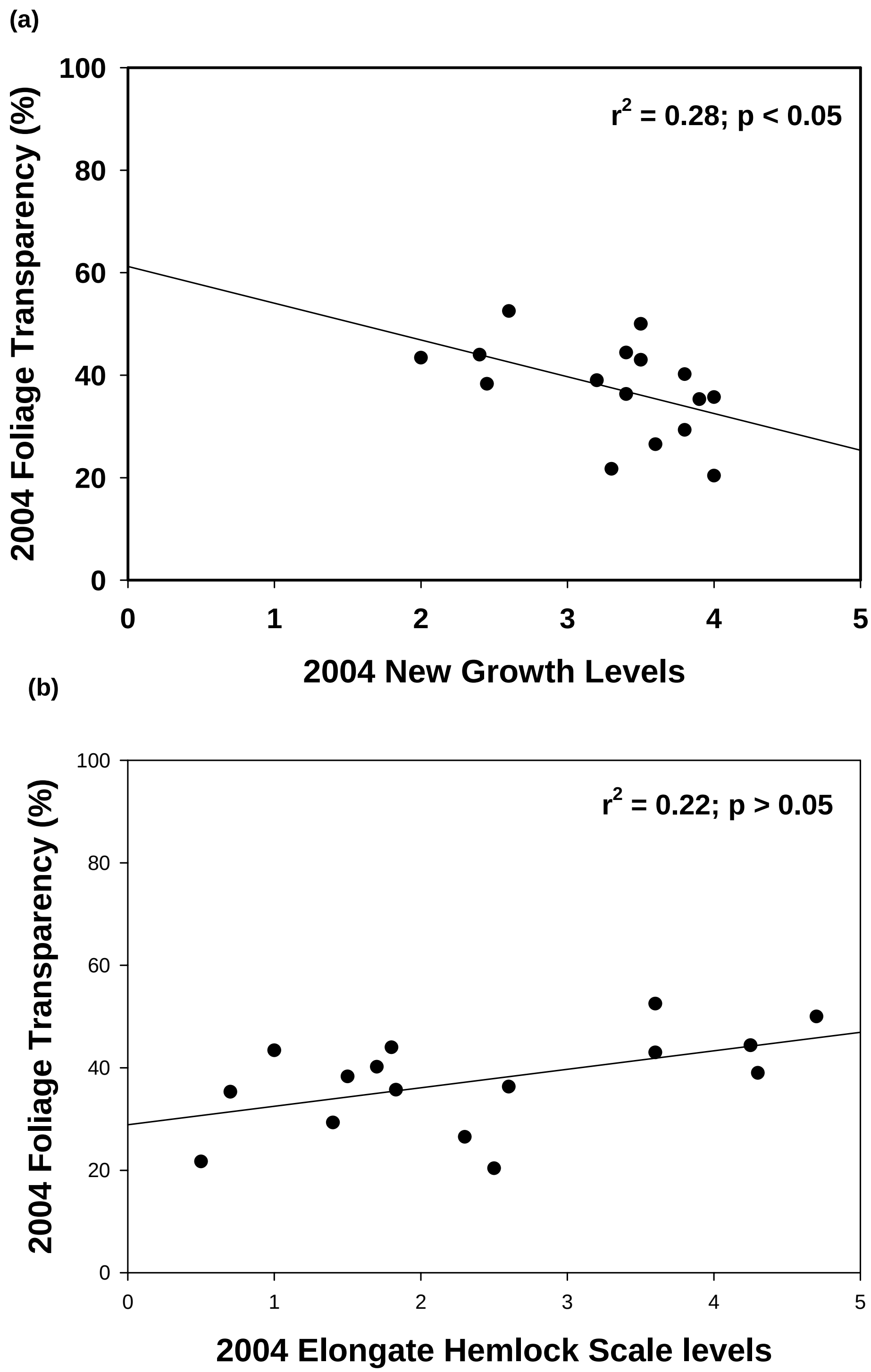


Figure 1. The relationships between foliage transparency and (a) new growth (b) elongate hemlock scale levels in 2004 at Connecticut *S. tsugae* release sites.

In four selected sites where *S. tsugae* had been recovered in previous years, trends in foliage transparency, HWA, and new growth levels on the crown through 2004 are shown in Figures 2(a)- (c). In these sites, mean HWA levels have not risen beyond 50% of the crown over the site. In combination with severe winter mortality in 2003 and 2004, adelgid levels at release sites have remained very low, at less than 10% on the crown. Foliage transparencies have also improved through 2004 from the higher transparencies observed in 2002. Crown levels of new growth have also surged in 2004 at all sites, even in northern sites such as Burr Pond State Park, which had shown severe defoliation and decline just the year before.

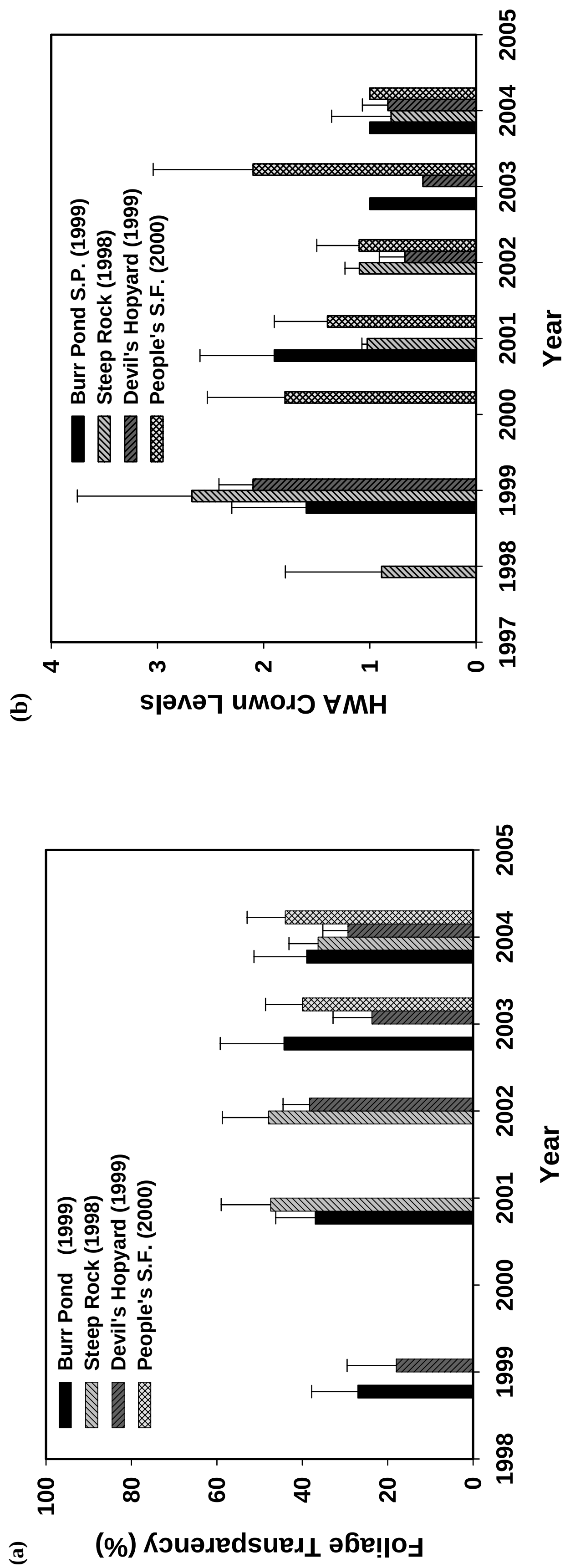
Predator surveys were conducted at a few sites where there was very patchy and light resurgence of adelgids by dislodging insects from lower crown branches into a collecting sheet and by branch tip sample collection for examination under a dissecting microscope. Ground surveys in late June and July at five sites did not recover any *S. tsugae* stages. However, examination of adelgid-infested foliage samples revealed one pre-pupating *S. tsugae* larva, in a web of dead needles, recovered in early June 2004. Dispersal had occurred from the top of the knoll approximately 700-1000m from the original 1999 release area in northeastern Connecticut. This was the first recovery at this site since recoveries of adults and larvae of *S. tsugae* in 2000. To date, recoveries of *S. tsugae* adults and larvae have been made in 13 Connecticut sites (65% of release sites) ranging from 1-6 years after the initial release.

NEW JERSEY

In New Jersey, foliar transparencies and hemlock mortality in release sites were compared by region (Table 3). Mean 2003 foliar transparency in Highland release sites (n = 11; 76%) was higher than in Skyland sites (n = 12; 65.8%) (Equal Variance T-Test; t = 2.5877; p = 0.00859). Hemlock mortality in Highland sites in 2003 was also higher than in Skyland sites (Mann-Witney U-Test; Z = -2.6434, p = 0.00410). In 2004, this trend was repeated with higher mean foliar transparency in Highland as compared to Skyland sites (Mann Witney U-Test; t = 3.2643, p = 0.00055). However, within regions, Highland sites showed significant improvements in foliar transparency from 2003 to 2004 (Paired t-test; t = 3.6829, p = 0.00253), as did Skyland sites (Paired t-test; t = 6.3358, p = 0.00003).

Non-release sites were also compared by region (Table 3). Mean 2003 foliar transparency in Highland sites was higher than in Skyland sites (Equal variance t-test; t = 2.5877, p = 0.00859). In 2004, foliar transparency was also higher in Highland than in Skyland non-release sites (Mann Witney U- Test; Z = 3.2643, p = 0.00055). There were no differences in hemlock mortality between Highland and Skyland non-release sites (Mann Witney U-Test; Z = -1.4902, p = 0.1361). Within regions, foliar transparency in Highland sites did not differ from 2003 to 2004 (Equal Variance T-test; t = 1.1751, p = 0.12256). Foliar transparency in Skyland sites also showed no differences from 2003 to 2004 (Equal Variance T-test; t = 0.5395, p = 0.59433).

Comparisons were made of foliar transparencies and hemlock mortality between *S. tsugae* release and non-release sites by region. Mean 2003 foliar transparency in release sites in the Highland region was similar to that in non-release sites (Equal Variance T-test; t = 0.4913, p = 0.62617). Mean 2003 foliar transparencies also showed no differences between Skyland release and non-release sites (Equal Variance T- Test; t = -0.2692, p = 0.79071). In 2004, foliar



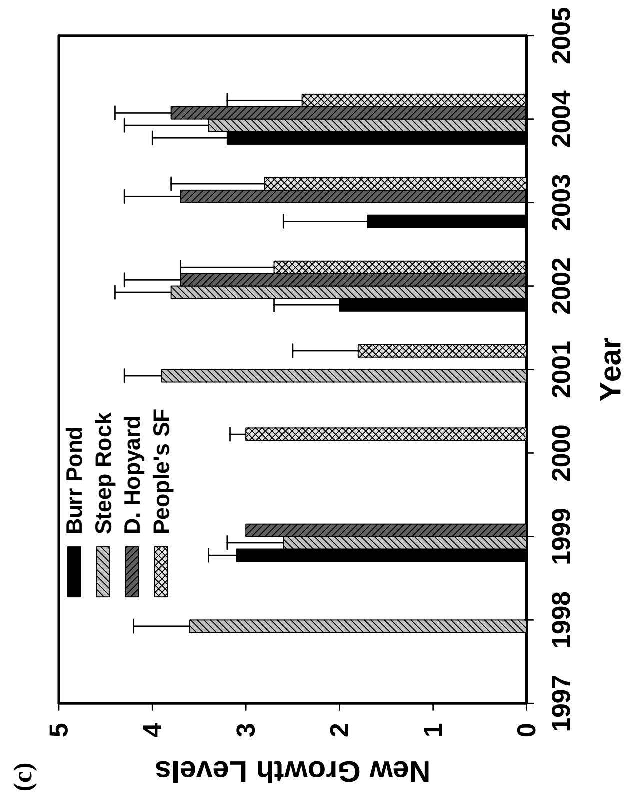


Figure 2. Trends in (a) foliage transparency (b) hemlock woolly adegid crown levels (c) new growth crown levels at selected Connecticut sites where *S. tsugae* had been previously recovered. Year of *S. tsugae* release is shown in parentheses.

Table 3. Foliar transparencies and hemlock mortality in 2003 and 2004 from selected New Jersey *S. tsugae* release sites (R) 1998-2002 and non-release sites (NR) in Highland (H) and Skyland (S) regions.

Year	Region	Type	# Trees	# Sites	Mean Foliar Transparency	# Sites	Mean % Hemlock Mortality
2003	H	R	220	11	76.0 ± 10.9	19	25.3 ± 26.1
	S	R	240	12	65.8 ± 7.7	21	6.1 ± 11.9
2003	H	NR	540	27	74.1 ± 11.1	26	27.9 ± 24.0
	S	NR	180	9	66.9 ± 10.6	8	16.7 ± 19.7
2004	H	R	220	11	67.7 ± 12.2	-	-
	S	R	240	12	55.5 ± 8.0	-	-
2004	H	NR	580	29	70.8 ± 1.0	-	-
	S	NR	360	18	64.4 ± 12.6	-	-

transparencies in Highland release sites did not differ from that in non-release sites (Equal Variance T-Test; t = -0.8017, p = 0.42771). However, in Skyland sites, mean foliar transparency was significantly lower in release sites than in non-release sites (Mann Witney U-Test; Z = -1.9689, p = 0.02448). Similarly, hemlock mortality was lower in Skyland release sites than in non-release sites in 2003 (Mann Witney U-Test; Z = -3.1763, p = 0.000746), while mortality in Highland sites did not differ between release and non-release sites (Mann Witney U-Test; t = -0.8818, p = 0.37787). Eighty-four percent of Highland sites assessed had hemlock mortality while mortality was observed in only 24% of Skyland sites. In contrast, 2003 hemlock mortality was recorded in 96.3% on non-release Highland sites and 66.7% of non-release Skyland sites surveyed. Hemlock borer activity was recorded in sites with hemlock mortality.

An adult *S. tsugae* was recovered in a 1998 Skyland site in 2004 where previous recoveries had also been made in 1999, 2000 and 2002. This site was also one that had heavy adelgid densities in 2003. In the 2001 site sampled with a bucket truck four weeks after a single release of 2500 adults on the lower branches, no *S. tsugae* were recovered from the lower crown (≤3m) during a simultaneous ground survey. In contrast, adults were readily recovered at heights of 5-12m in the canopy of the release and adjacent tree (Figure 3). From 1998 to 2004, *S. tsugae* adults and larvae have been recovered in 20 New Jersey release sites (29% of the release sites), the majority by ground surveys.

DISCUSSION

Connecticut has witnessed the overall recovery of hemlocks on a statewide scale in 2004 in both release and non-release sites. The hemlock recovery in *S. tsugae* release sites, which represented some of the heaviest adelgid-infested stands in Connecticut from 1996-2001, has even surpassed that in non-release sites surveyed in 2003 in the northwestern corner of the

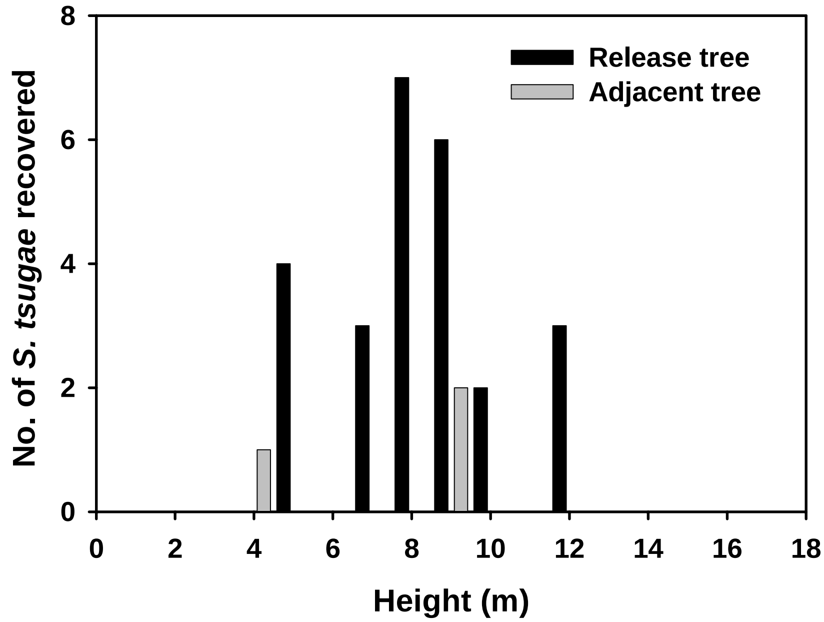


Figure 3. *Sasajiscymnus tsugae* recoveries in New Jersey by bucket truck sampling at different canopy heights on release and adjacent trees four weeks after release in 2001.

state. This region, which is home to some of the most extensive and dense (>50%) hemlock stands in the state, was also moderately infested with adelgid prior to 2003. In addition, approximately 50% of these release sites have also been moderately to heavily infested with elongate hemlock scale for several years, which has also compromised the hemlock crown through its direct feeding impact on the needles. The foliage transparency criterion proved to be most informative for depicting trends in hemlock health over time, as it measures the fullness of the crown in terms of the amount of skylight visible through the foliated portion of the crown. A high rating for foliage transparency indicates defoliation and a thin crown and thus, poor tree health. Transparency ratings of 30% or less are considered the norm for most tree species (Anonymous 2002). Ratings conducted under the Forest Health Monitoring Program from 1996-1999 at 18 Connecticut plots indicated that 4.5% of eastern hemlocks assessed had the highest transparency rating category of 51-100% with 54% showing significant damage and 4.5% with high dieback ratings of 21-50% (Anonymous 2002).

Foliage transparencies in 2004 were significantly lower in release sites as a whole as compared to the non-release sites and previously thin hemlock crowns in release sites have recovered to fullness levels observed in uninfested hemlock stands at high elevations in isolated locations. This recovery comes on the heels of the effects of an extreme drought in 2002 (Table 4) and significant droughts in 1999 and 1998, which were followed by cool, abnormally moist growing seasons in 2000, 2003 and 2004 (data from the Northeast Regional Climate Center). These environmental conditions have facilitated remarkable hemlock refoliation across all woodland suitability sites assessed, even in the poorest sites such as in Washington, Connecticut's Steep Rock Preserve, proving that adelgid-damaged hemlock stands can recover under the right conditions. Woodland suitability groups did not influence hemlock recovery in 2003 and 2004 but this is not unexpected as moisture was not limiting in 2003 and 2004 and pest levels had been reduced. Moisture capacity of different forest soil types in

Table 4. Periods of two months or more of severe and extreme droughts in northern New Jersey and Connecticut (data from the Northeast Regional Climate Center).

State	Climate Division	Severe/ Extreme Drought Period	Duration (in months)
NJ	Northern	7/1999 - 8/1999	2
	Northern	12/2001- 5/2002	6
CT	Northwest	1/2002 - 4/2002	4
	Central	2/2002 - 4/2002	3
	Coastal	7/1999 - 8/1999	2
	Coastal	1/2002 - 4/2002	4

Connecticut is probably much more of a factor affecting tree health and growth in drought years (Lunt 1948).

Although severe winter mortality in 2003 and 2004 statewide has significantly depressed adelgid populations in subsequent seasons, the continued trend in low adelgid levels is remarkable for its lack of resurgence. Figure 4 shows the dramatic fluctuations in average winter temperatures in Connecticut, New Jersey, and the Northeast region as a whole from 1990 to 2004. While severe winters in 1994 and 1996 (ranked 15 and 39, respectively, since 1896; Northeast Regional Climate Center) were followed by the explosive expansion of HWA in Connecticut, this expected resurgence of HWA has not occurred (ranked 26 and 41 respectively). Significantly, adelgid resurgence also did not occur to any marked extent in 2002 in release sites (Figure 2b) following the warmest winter on record (Northeast Regional Climate Center) where there was negligible winter mortality of HWA. Although there had also been a severe winter drought in 2002, healthy new growth, favorable to adelgid colonization, was also at high levels in monitored trees (Figure 2c) in 2002, so poor hemlock health was not a limiting factor for recolonization by *A. tsugae*.

Could the low levels of HWA be partially attributable to the establishment and impact of *S. tsugae*, acting in concert with other native natural enemies as part of a complex? Figure 5 shows the recoveries of *S. tsugae* in (a) Connecticut and (b) New Jersey in the years following the initial release. Recoveries of *S. tsugae* adults and larvae were readily recorded in the first two years after release using ground surveys. Although recoveries of *S. tsugae* from lower crown sampling have diminished in time, the New Jersey bucket truck study has showed quite conclusively that shortly after release, beetles display a tendency to move upward into the crown and well out of reach of current sampling procedures. Similar results were obtained in another bucket truck survey in Connecticut in June 2001. No *S. tsugae* were detected in a lower crown ground survey a week before but larvae and an adult were recovered at 12- 20m in the hemlock canopy in the year following release (Cheah and McClure 2002). The winters of 2003 and 2004 have also been severe in northwestern New Jersey. One Skyland release site recorded 89% mortality in 2004 (Shields and Cheah unpub.) resulting in very low adelgid levels in subsequent seasons. Concurrently, it is not unexpected that *S. tsugae* and other predators have been hard to find in recent years. Dieback of lower hemlock crown

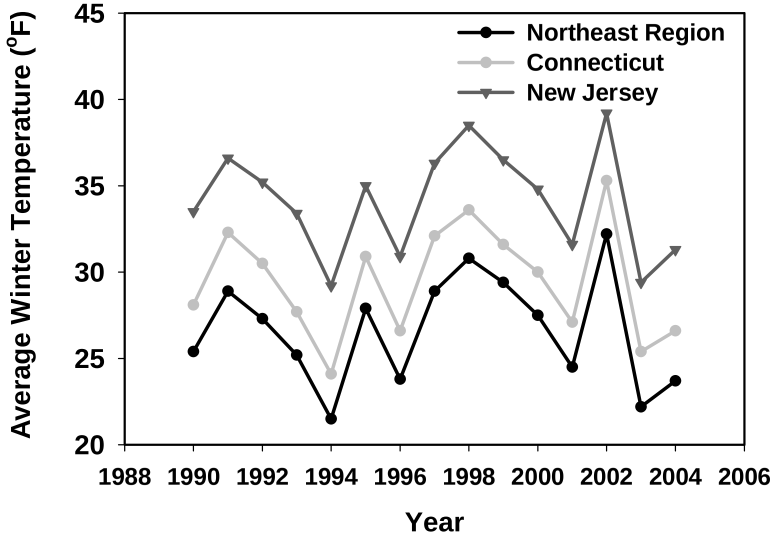


Figure 4. Average winter temperatures in Connecticut, New Jersey and the Northeast region from 1990-2004. Data from the Northeast Regional Climate Center at Cornell University.

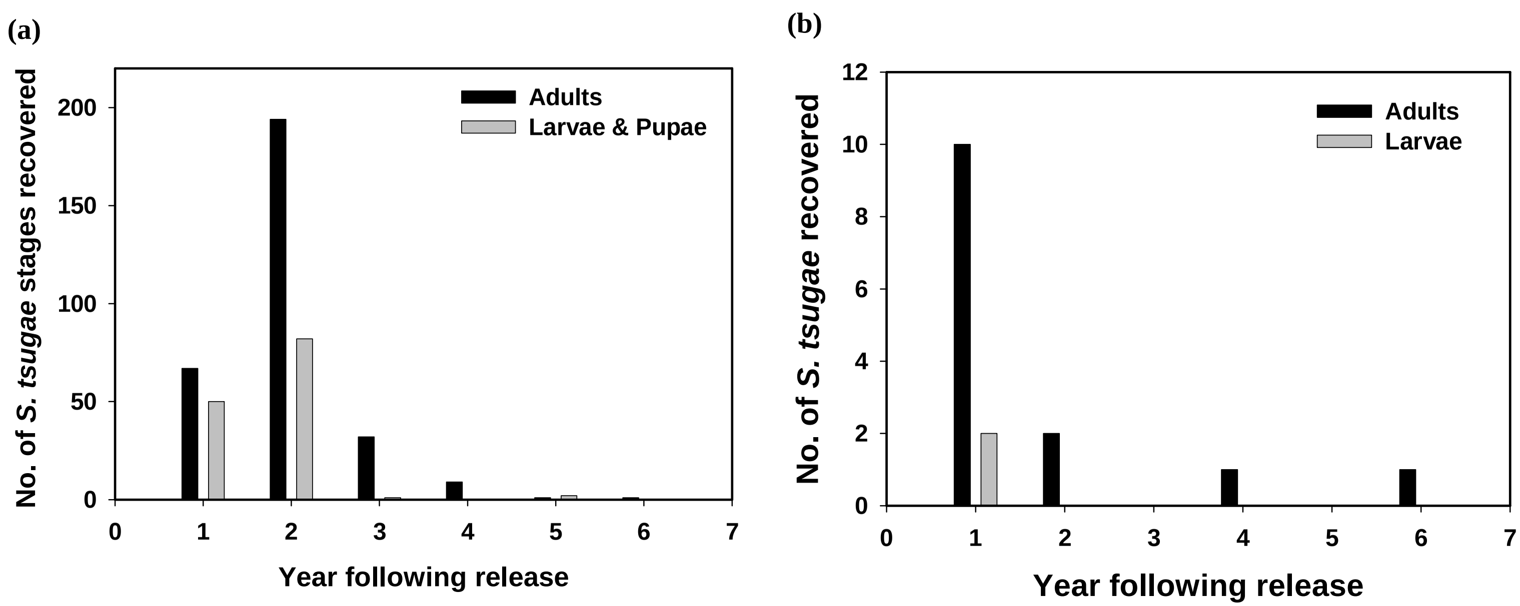


Figure 5. *Sasajiscymnus tsugae* field recoveries following the year of release in (a) Connecticut 1996-2004 and (b) New Jersey 1999-2004.

branches in New Jersey sites has also restricted the ability to sample for predators. Adelgid population rebound should result in better recoveries of *S. tsugae*. However, more efficient and better methods of sampling for *S. tsugae* higher up in the crown need to be developed for monitoring their establishment and impact. The role of *S. tsugae* in biological control of HWA cannot be discounted simply on the basis of the lack of recovery data for the factors outlined above. Since foliage transparency primarily measures the upper foliated crown, it is reasonable to hypothesize that this healthier portion of the hemlock crown may also house predators such as *S. tsugae.*

Previous assessments of HWA damage to hemlock stands in New Jersey have shown that foliage transparency was directly related to the density of adelgid infestation, and that transparencies of >60% have marked a threshold where tree mortality increased significantly (Mayer et al. 2002b). However, adelgid-damaged hemlock stands in New Jersey *S. tsugae* release sites in both Highland and Skyland regions are showing signs of reversing this trend with initial recovery from 2003 – 2004. This improvement in foliage transparency is most evident in the Skyland sites. Skyland release sites have had a more recent history of adelgid population explosion and the initiation of recovery in these sites has occurred at a greater rate than in Highland sites. The Highland region has been heavily infested with HWA and elongate hemlock scale since the late 1980s, a decade more than the Skyland region and has suffered extensive decline from 1984 to 1994 (Royle and Lathrop 2002). In contrast, non-release sites in both regions have not shown improvements in foliage transparencies from 2003 to 2004. Most significantly, comparisons between release and comparable non-release stands in Skyland sites indicated that hemlock recovery, in terms of foliage transparency improvement, was very significantly higher in release sites in 2004. This trend has not yet been detected in Highland sites where foliage transparencies remained similar in release and non-release sites in 2003 and 2004. Hemlock mortality in Skyland release sites was also significantly lower than in non-release sites. Releases of *S. tsugae* appear to be correlated to the reduction of hemlock mortality, at least in the healthier Skyland sites. All release and non-release sites in New Jersey have also been infested with elongate hemlock scale since the late 1980s or earlier. In addition, this northern region suffered extreme drought lasting two months in the summer of 1999 and six months extending into the spring of 2002 (Table 5), and these additional stressors on hemlock, of greater magnitude in New Jersey than in Connecticut, have probably contributed to the greater loss of foliage and hemlock decline in affected stands. As a result, recovery is expected to progress at a slower rate. However, the data appears to indicate that establishment of *S. tsugae* in Highland and Skyland release sites in New Jersey, together with favorable environmental conditions, has helped improve declining hemlock crowns, a trend that has not been paralleled in surveyed non-release sites.

SUMMARY and **CONCLUSIONS**

Connecticut's eastern hemlock stands, which have been under siege in the last two decades from hemlock woolly adelgid, other pests and drought episodes, have shown recent trends in remarkable recovery from a period of decline and damage in the mid-late 1990s. This trend is also correlated with the release of the introduced *S. tsugae* for biological control of hemlock woolly adelgid. This improvement in hemlock health at *S. tsugae* release sites has also occurred at a greater rate than in non-release stands. The ability of affected hemlocks to recover and reverse the trend in defoliation and mortality in just a few years with the intervention of more favorable environmental conditions for hemlock growth is testimony to the species' resilience. In New Jersey, this pattern of recovery has been a little slower to emerge but recent evaluations indicate it is on the right course. The key questions that remain concerning the effectiveness of the introduced biological control agent, *S. tsugae*, is not whether we can detect the beetles numerically with current inadequate procedures, but whether adelgid populations will continue to remain depressed at the current low levels, when winters are not limiting, and whether hemlock decline can be reversed and mortality halted. A prudent strategy might be to augment and reintroduce *S. tsugae* into hemlock stands that show any resurgence of the adelgid, especially after severe winters, as overwintering mortality of *S. tsugae* has not been assessed to date.

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OVERVIEW OF HWA BIOLOGICAL CONTROL ACTIVITIES WITH *LARICOBIUS* SPP.

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ABSTRACT

Laricobius nigrinus, a little known Derodontid beetle, is consistently found associated with hemlock woolly adelgid (HWA) in western hemlock seed orchards in British Columbia (Humble 1994). It turns out that *Laricobius* spp. are prey-specific predators of Adelgidae (Zilahi-Balogh 2004). In collaboration with Forestry Canada, we began studying the potential of *L. nigrinus* as a biological control agent for HWA in the eastern U.S. Field studies conducted in British Columbia showed that the phenology of the predator and the prey were highly synchronized (Zilahi-Balogh et al. 2003):

1. Predator adults are present and active in the winter to feed on developing HWA sistens.
2. Predator eggs are laid in HWA ovisacs, where hatching larvae feed on HWA eggs.
3. Predator larvae drop from the tree into to the soil to pupate and eclose into adults, where they aestivate at the same time and for the same duration as do HWA.
4. The predator adults and HWA sistens emerge from aestivation at the same time.

Laricobius nigrinus adults and larvae both feed almost exclusively and survive only on HWA (Zilahi-Balogh et al. 2002), making them virtually risk free when released. In field-cages, *L. nigrinus* can survive the winter and significantly impact HWA sistens and progrediens densities (Lamb et al. 2005). In these cages, egg densities increase when adult *L. nigrinus* females are added, but impact on HWA density does not (Lamb et al. unpublished data), suggesting optimal predator densities are fairly low.

Rearing this predator is challenging (Lamb et al., this issue), yet most issues have been worked out. One of the last major hurdles has been to overcome the early emergence of adult predators in the insectary. Without adequate food, mortality of emerging predators was very high. Lamb et al. (unpublished data) determined that aestivating adults held at a relatively warm temperature for this insect (19°C), followed by exposure to cooler temperatures (13°C), enables us to extend their dormant period until adequate food (i.e., developing HWA sistens) becomes available.

Beginning in 2003, *L. nigrinus* has been released in numerous sites ranging from Massachusetts to Georgia. David Mausel is studying the colonization, establishment, and spread of the predator (Mausel et al. this proceedings). He is also testing optimal release strategies that can be used on a large scale as more beetles become available.

Recovery of F_1 and F_2 beetles has already been obtained from some sites. One release site was a field insectary in Virginia Tech's Kentland Farms. Established in 2001 (Kok and Salom 2002), this 0.4 ha plantation of young eastern hemlocks infested with HWA, a site where 252 beetles were released in November, 2003. In January 2005, 25 F_1 adults were recovered from branches of release trees on a day when temperatures rose to as high as 18°C.

Other ongoing projects with *L. nigrinus* include an evaluation of potential competitive interactions involving *Sasjiscymnus tsugae*, and *Harmonia axyridis* in lab and field studies (Flowers et al. in this proceedings). We are also investigating the residual effects of imidacloprid treatments for HWA on *L. nigrinus* and *S. tsugae* (Eisenback et al. in this proceedings).

Work with other *Laricobius* spp. include our foreign exploration efforts in China, where two new species were discovered in 2002 (Gatton et al. 2004). Development, reproductive biology, and host-range testing studies for one of these species, *L.* sp. n. *kangdingensis* is being carried out in quarantine (Gatton 2004). Our goal is to get this predator released from quarantine and added to the complex of predators currently being released to control HWA.

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EXPERIMENTAL RELEASES OF *LARICOBIUS NIGRINUS* FOR BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID IN THE EASTERN U.S.

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ABSTRACT

Laricobius nigrinus Fender (Coleoptera: Derodontidae) from the Pacific Northwest was released for biological control of the Asian hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) in the eastern U.S. To date, 7,350 adults have been released at 19 sites in eight states, from Massachusetts to Georgia. The emphasis is on small-scale accretive releases to determine whether such releases will result in establishment. There were ten releases in 2003-20004 and nine releases in 2004-2005 consisting of open release treatments of 75, 150, 300, and 1,200 adults in the fall/winter, 300 adults in the spring, and 300 adults in the fall/winter and spring. Beetles were released at a density of 30, 35, 40, or 80 beetles on either 2, 5, 10, or 15 trees depending on the release size. Sampling of *L. nigrinus*, HWA, and tree health will continue for three years post release. At each site, 30-cm branch sections were marked at the four cardinal points of release and control trees and % HWA infested new shoots (*i.e.* presence/absence), amount of new shoot growth, and amount of shoot dieback were recorded and re-measured annually. Live crown ratio, crown transparency, crown vigor, tree profile photographs, and site characteristics were also recorded. Standardized *L. nigrinus* sampling procedures included beat sheets in the fall/spring and host collection in the spring. In spring 2004, host collection was conducted in Tennessee and North Carolina to recover progeny of the release beetles. Three and 15 adults were reared from Tennessee and North Carolina samples, respectively. In fall 2004, beat sheet sampling was conducted at the 10 release sites from 2003/2004 and F1 adults were recovered in North Carolina (3) and Virginia (1).

CLASSICAL BIOLOGICAL CONTROL OF THE ELONGATE HEMLOCK SCALE, *FIORINIA EXTERNA*: 2004 ACTIVITIES

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ABSTRACT

In 2004, a new project on classical biological control of a hemlock pest, *Fiorinia externa* Ferris (elongate hemlock scale) (Homoptera: Diaspididae), was funded by USDA-Forest Service (FHTET) with work centered at the University of Massachusetts/Amherst. The objectives of the project are to (1) assess current pattern of scale abundance, parasitism, and survival in eastern North America, (2) test the previously proposed hypothesis that host-parasitoid asynchrony accounts for low parasitism and high scale abundance in the northeastern U. S., (3) locate, study, and import new species of parasitoids from Japan and perhaps China as classical biological control agents, and (4) assess host ranges of new parasitoids, followed by their release and an evaluation of their impact on scale density. In 2004, a regional scale survey was conducted, field sites with scale were located, a laboratory scale colony was established, and one trip to Japan made during which scale and parasitoids were found at three locations.

KEYWORDS

Elongate hemlock scale, *Fiorinia externa*, classical biological control, hemlock.

INTRODUCTION

The elongate hemlock scale (EHS), *Fiorinia externa* Ferris, is an armored scale native to Japan that was first recorded in North America on Long Island, New York, in 1908 (Ferris, 1942). Records of EHS exist from Georgia north through Virginia, Maryland, Pennsylvania, New York, New Jersey, Connecticut, Massachusetts, and Rhode Island, and west to Ohio (Scalenet 2005). This scale feeds on a wide range of conifers, including species of hemlock, cedar, fir, pine spruce and yews (McClure 1977 and 1979). Scales are found only on the undersides of needles and crawlers settle principally on the new growth. Scale densities in the United States vary widely, but values from 21 to 164 scales per 100 needles have been reported (McClure and Fergione 1977, McClure 1978). It is possible to find sites with higher densities, but average densities for hemlock stands selected at random have not otherwise been reported. In Japan, densities are much lower: on forest hemlocks, McClure (1986) noted densities as 0.0 to 0.15 scales per 100 needles.

In Japan there is a "landscape" effect on scale density as scales are about 12- to 24-fold more abundant (2.2-3.9/100 needles) on planted hemlocks in landscapes such as temples or parks than in forests (McClure, 1986). In Connecticut, this "landscape" effect either does not exist or is at most much weaker than in Japan, with EHS densities on planted hemlocks being only 1.1- to 4.2-fold higher than on forest hemlocks (McClure and Fergione 1977, McClure 1978). Finally, data from Japan (McClure 1986) suggest that the species identity of hemlock affects scale density very little. Scale density on the eastern North American hemlock *Tsuga canadensis* Carriere, planted in Japan, was 2.9 scales/100 needles, a value that was within the range of scale densities found on native Japanese hemlocks (2.2-3.9) planted in similar landscape settings.

The life-stage phenologies of EHS and its principal parasitoid, *Encarsia citrina* (Crawford), in Connecticut and Japan were determined by McClure (1978 and 1986). In Japan, there is good synchrony between second generation *E. citrina* adults and EHS second instar nymphs (the only stage in which the parasitoid oviposits). In Connecticut, synchrony between these stages was poor. McClure hypothesized that this lack of synchrony in the second generation led to low or fluctuating levels of parasitism in Connecticut instead of the consistently high (>90%) levels seen in Japan. McClure attributed this lack of synchrony to effects of climate, which caused EHS in Connecticut to have less than two full generations per year—in contrast to Japan, where there were two complete generations per year. By extension, this hypothesis predicts better control of EHS south of Connecticut, where two full generations per year occur.

DESCRIPTION OF PLANNED PROJECT

The first goal of this research is to determine if the predicted contrast between the northern and southern parts of the U.S. range of EHS is borne out (i.e., if EHS densities are lower and *E. citrina* parasitism higher in the middle Atlantic states vs. southern New England). The second goal of our research is to study the parasitoids associated with EHS in Japan and determine 1) if the *E. citrina* in Japan is genetically different or not from the population in New England and 2) determine if another parasitoid might be associated with EHS that oviposits in the adult female scale or perhaps in both the second stage female and the adult female (this would be desirable because the adult female is present for much longer and for parasitoids attacking this stage, synchrony issues would be much less critical). In pursuit of such an adult-attacking parasitoid, we plan also to examine populations of *Fiorinia japonica* (Kuwana) in China to see if parasitoids associated with that species might also attack EHS, including adult scales. To meet these broad goals, the following specific objectives were developed for the project:

- Obj. 1. Survey EHS density and parasitism from Maryland to Massachusetts.
- Obj. 2. Match climates of the northeast U.S. to areas in Japan and China.
- Obj. 3. Hire a collector to find *Fiorinia* species on conifers in northern Honshu or Hokkaido.
- Obj. 4. Ship dead Japanese parasitoids to the U.S. for preliminary identification; use molecular tools to compare and distinguish between Japanese and U.S. populations of *Encarsia citrina.*
- Obj. 5. Hire a collector in China to locate potential *Fiorinia*-collection sites.
- Obj. 6. Develop an EHS colony for use in Ansonia FS quarantine lab.
- Obj. 7. Collect live parasitoids from Japan and/or China and ship them to quarantine.
- Obj. 8. Assess the impact of parasitoids on EHS in Japan and China.
- Obj. 9. Start quarantine colonies of all parasitoids recovered.
- Obj. 10. Confirm identity of all parasitoids, estimate likely host ranges, submit petitions for releases, and initiate releases.
- Obj. 11. Establish parasitoids at field sites in Connecticut and Massachusetts.
- Obj. 12. Initiate the field assessment of impacts of introduced parasitoids.

INITIAL RESULTS (FOR 2004)

Work in 2004 addressed Objectives 1-6 only; no work was done on Objective 2. For Objectives 1, 3, 4, 5, and 6, we made the following progress:

Objective 1. Survey EHS density and parasitism from Maryland to Massachusetts. Eight potential EHS survey areas (Figure 1) were identified, four coastal and four inland, stretching from Massachusetts (42 to 42.5 NL) to Maryland-West Virginia (39-39.5 NL). During the survey, hemlock was not found in two of the four coastal zones that we sampled (1: the mid-New Jersey area, and 2: the southern New Jersey-Baltimore, Maryland area). This left six survey regions from which data were collected. These zones were visited in spring of 2004 (April and May) before new hemlock branch growth occurred. Samples for scale density and parasitism were thus based exclusively on needles formed in spring of 2003. Since scales do not fall off needles, a count of scales in early spring of 2004 before new needles were formed provided a summary of the total numbers of scale crawlers that settled in 2003 and how many scales died of parasitism, summed over all scale generations in 2003.

ones 1 and 2: Massachusetts

ones 3: Rhode Island and one 4: Connecticut and New York

one 6: southern Pennsylvania

one 8: Maryland and West Virginia

Figure 1. Regions surveyed in spring 2004 to measure *Fiorinia externa* density and parasitism in the eastern United States between Massachusetts and West Virginia.

Within each region, hemlock stands were located with the assistance of local foresters and entomologists, without regard to the status of EHS at a site. In each of the six survey zones, 10 hemlock stands were located and sampled (except one zone with only nine existing stands). Thus a total of 59 hemlock stands were visited and sampled. At each hemlock stand, the surveyor (Suzanne Lyon) first looked at foliage to see if EHS was present. If scale was detected within a 15-minute search, the site was sampled as described here. If no scale was found on any trees at the stand in a 15-minute search period, the stand was classified as having zero scale and samples were not collected; zero-scale stands were included, however, in the data set from which average scale density in the survey as a whole was calculated.

At sites with scale, we sampled five trees, selecting three branches at random from a zone 1-2 m above the ground and spaced around the tree's circumference. From each of the three sample branches, several apical pieces of foliage (each section ca. 20 cm long) were clipped, bagged, and placed in a cooler and returned to the laboratory at the end of the survey. In the laboratory, sets of randomly selected needles (100 per set) were examined, one set from each branch, by selecting ten branch tips (viewed dorsally so that scales were not visible) and taking ten needles from each tip. Sample needles were taken, starting at the collar on the stem marking the division between 2004 and 2003 wood, taking five needles from each side, moving apically until ten needles were obtained. On these needles, all EHS scales (live, dead, parasitized, in any post-egg life stages) were counted. All needles with scale found in the 15 density samples from a site (5 trees x 3 branches per tree) were pooled and held for further use.

To estimate the rate of parasitism and the proportion of living scales in a sample, 150 scales were dissected per hemlock stand. Scales were initially taken at random from the pool of scale-infested needles held over from the density samples. If fewer than 150 scales were present in these samples, all the foliage collected from a site was examined and all scales collected until 150 were obtained or no more were available. All EHS in the percentage-parasitism samples were dissected and were classified as parasitized if a parasitoid exit hole was found, if a parasitoid pupa was present, or if upon dissection of the scale body a parasitoid egg or larva was detected.

The number of scales per 100 needles across all 59 hemlock stands sampled was determined by averaging scale numbers over all branches in the study, including three zero-density branches credited for each zero-scale site. The average percentage-parasitism and percentage-live scale (in samples of 150 scales per hemlock stand) over all 59 stands was calculated. All three parameters were then graphed versus collection site latitude (Figure 2).

Scale density per 100 needles was highest in inland Connecticut (at 46.5 scales per 100 needles), dropping to 0.0 scales in samples taken from sites further north (in Massachusetts) and from 0.7 (Pennsylvania) to 5.3 (Maryland + West Virginia) at sites further south. In Connecticut and Massachusetts, scale density was negligible at coastal sites (0 to 0.4). In Pennsylvania and Maryland-West Virginia, hemlocks were not found at coastal sites.

Parasitism rates were generally low, averaging 9.5% across all 59 sites. Parasitism was greatest (14%) at coastal Connecticut sites and lowest at inland Pennsylvania sites (4%). Because no EHS scales were found in samples in Massachusetts, it was not possible to estimate parasitism rates.

The percentage of live scales was greatest in the southern part of the sampled range (42%, Maryland + West Virginia; 38%, Pennsylvania) and decreased to 27-28% in New York-Connecticut-Massachusetts. One site in Connecticut (Tunxis State Forest) sampled on May 29, 2004, had only 4.4% live scale.

Objective 3. Hire collector to find *Fiorinia* species on conifers in northern Honshu or Hokkaido. Naoto Kamata was hired to conduct surveys in Japan for EHS. In August of 2004, assistants provided by Dr. Kamata accompanied Suzanne Lyon, who visited sites in the Nagano Prefecture (Mt. Manza, Mt. Shirane, Kamikochi, the Norikura Kogen Highlands, and Mt. Kiso Koma) to look for hemlocks and EHS. Hemlocks infested with EHS were found at three sites: Kamikochi, the Norikura Kogen Highlands, and Mt. Kiso. Scale density was extremely low at all sites. Counts were not made, but only two to three scales were found per tree when small trees were entirely searched. The most promising site visited was Mt. Kiso. Scales were found on *Tsuga diversifolia* Masters at 1,400-1,700-m elevation and held for rearing. Of the approximately 30 EHS collected, five adult parasitoids were obtained and are being held for taxonomic identification and molecular comparison with parasitoids reared from EHS in Connecticut and Massachusetts. It is very likely that other parasitoids died during rearing if they were younger than the pupal stage when the hemlock needles were picked, which kills the host scale. Sampling in March or November would likely give a better estimate of the true rate of parasitism.

Objective 4. Ship dead Japanese parasitoids to the U.S. for preliminary identification; use molecular tools to compare and distinguish Japanese and U.S. populations of *Encarsia citrina*. Five parasitoid adults were reared from the approximately 30 elongate hemlock scales collected in the August, 2004, trip, suggesting a minimum of 16% parasitism. These parasitoids are currently at the University of Massachusetts-Amherst in 100%

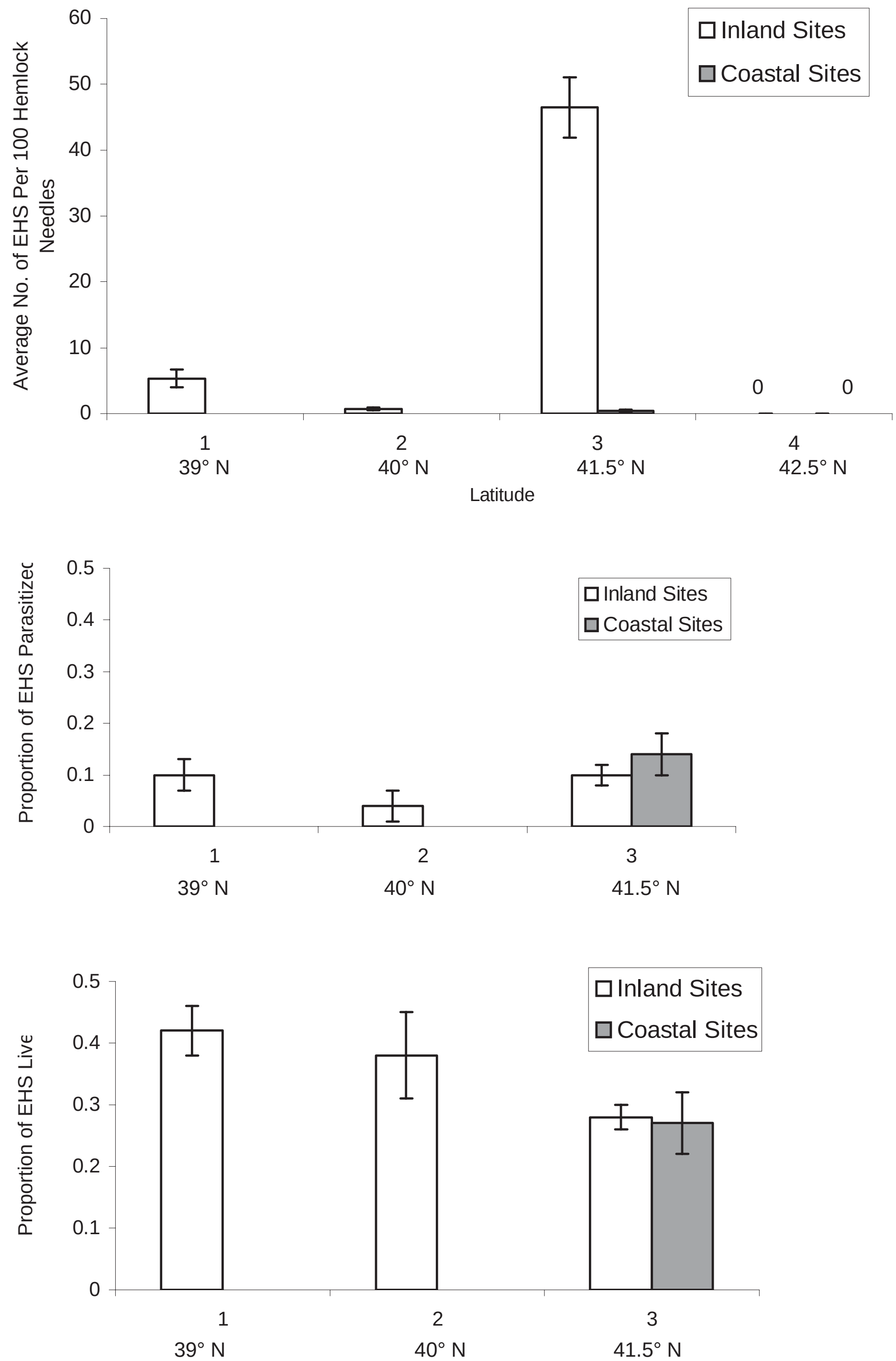

Figure 2. Results of a survey for elongate hemlock scale (*Fiorinia externa*) in the northeastern United States in spring 2004 at four latitudes: one 1 (42.5 NL, Massachusetts), one 2 (41.5 NL, Rhode Island, Connecticut, and northern Pennsylvania), one 3 (40.0 NL, southern Pennsylvania), and one 4 (39.0 NL, Maryland and West Virginia). Top: number (mean ± SE) of scales per hundred needles (avg. 15 samples/site and 9 or 10 sites/zone; hemlocks not found in coastal parts of zones 3 and 4; hemlocks present in zone 1 [Massachusetts], but scale was not detected). Middle: proportion (mean ± 95% CI) scale parasitized (of 150 scale, unless scales were limited)—presumably all parasitism was *Encarsia citrina*). Bottom: proportion (mean ± 95% CI) scales on needles still alive.

alcohol and will be used when more specimens are available for identification and molecular comparisons.

Also, in support of eventual importation to quarantine of live parasitoids from Japan, we applied for and received a permit to import live *Fiorinia* spp. scale and its parasitoids from Japan and China into quarantine. This permit is being held by Kathleen Shields at the Ansonia quarantine laboratory and is ready for use when needed.

Objective 5. Hire a collector in China to locate potential *Fiorinia* collection sites. The occurrence of EHS in China is uncertain as the few existing records may be misidentifications. Consequently, Dr. Wu Sanan of the Beijing Forestry University (a scale taxonomist), the collector hired to search for EHS in China, searched in two ways. One search was based on looking for EHS on hemlocks in parks, temples, or other such sites where hemlock are sometimes planted, as scales at such locations are often much more abundant than on the same tree species in natural settings. This approach was carried out in six locations, including Chengdu Botanical garden (Chengdu City, Sichuan Province), the campus of Sichuan University (Chengdu City), the campus of the Sichuan Agricultural University (Ya'an City, Sichuan Province), Qichengshan Forestry Park (Dujiangyan City, Sichuan Province), the Dendrological Garden at the Sichuan Forestry School (Dujiangyan City, Sichuan Province), and the Jiuzaigou Forestry Park (Jiuzaigou County, Sichuan Province). Specimens of *Tsuga* (hemlock) were found only at the last two sites, and no EHS were detected at either site.

The other approach was to find populations of a related scale, *Fiorinia japonica*. Three sites were examined: (1) Beijing, (2) Zhengzhou City (Henan Province), and (3) Yangling City (Shaanxi Province). At the first site, in a July 25, 2004 collection, about 5% of the scale from *Cedrus deodara* (Roxb.) G. Don. had parasitoid emergence holes. At the second site, no evidence of parasitism was found. At the third site (Yangling City), a high density population of *F. japonica* was found on *Picea asperata* Mast. Parasitoids were not reared from the collected scale but a few did have parasitoid emergence holes.

Objective 6. Develop an EHS colony for use in Ansonia FS quarantine lab. To set up a laboratory colony of parasitoid-free EHS on small plants, we tried several approaches in 2004. First we dug small EHS-infested hemlocks (25-50 cm) in Tunxis State Forest in Connecticut, a site that had high densities of EHS. Efforts to transfer crawlers to new, non-infested hemlock seedlings (dug in Westhampton, Massachusetts) were made in three ways. First, we tried tying the foliage of infested seedlings from Connecticut loosely to that of clean seedlings so that crawlers would move over onto the clean trees. Some did, but most settled on their natal branches. In a second attempt, we used EHS-infested fir branches from a Christmas tree plantation in southwest Connecticut. We tried tying these branches very closely to young hemlock. We also tried placing infested needles in paper cones (about 1 inch deep), which were pinned to branches. We assumed crawlers would crawl up the paper cone onto foliage. We thought this would solve the problem of crawlers settling too soon. However, both of these attempts failed because (1) crawlers settling on the hemlock were scarce and (2) mites and thrips from the field material were numerous and the hemlocks were contaminated by these arthropods. A fourth attempt is currently underway that, while more laborious, is expected to work. Individual female scales with eggs present will be removed from infested foliage and glued onto foliage of fraser fir (about 25 cm tall), which is an excellent host for EHS and shaped to fit well in small cages.

PLANS FOR 2005

Objective 1. Survey EHS density and parasitism in the eastern United States. While our 2004 results partially support the McClure hypothesis (scale density was lower in the southern regions, but parasitism was not higher), the survey needs to be repeated in 2005 at new sites and extended further south. We plan to repeat such a survey in March-May of 2005 covering a region from northern GA to Massachusetts.

Objective 2. Match climates in the northeast U.S. to areas in Japan and China. We need to compare northern Honshu and Hokkaido to Connecticut-Massachusetts to see how important it might be to get parasitoids from Hokkaido. Finding EHS in Hokkaido will require finding it on some non-hemlock host, as hemlocks do not occur on that island. Mike Montgomery and Roy Van Driesche will work together with Kris Abell, the graduate student, to make these comparisons.

Objectives 3 and 4. Collect *Fiorinia* species on conifers in northern Honshu or Hokkaido. Suzanne Lyon and Kris Abell will work with Naoto Kamata to collect more EHS from known sites in Honshu and to find new EHS populations further north. Rearing and dissection of scales from these collections will provide parasitoid adults for taxonomic identification and, through the dissections, information on what host stages each parasitoid species attacks. *Encarsia citrina* specimens will be used for DNA work to determine if they differ from their counterparts already present in the United States. Live specimens of all species will be shipped to the United States to initiate rearing colonies in U.S. quarantine.

Objective 5. Collect parasitoids from other *Fiorinia* species in China. Dr. Wu-Sanan will continue to collect parasitoids from *F. japonica* and determine if these are different species from those found in Japan on *F. externa*. Material from China will be shipped as parasitized scales to the U.S. quarantine laboratory in Connecticut to establish parasitoid colonies for study. Dr. Wu-Sanan will search for *F. externa* on *Abies* or *Cephalotaxus* species.

Objective 6. Develop an EHS colony for use in the Ansonia FS quarantine lab. We will transfer EHS eggs inside female scale covers onto small (20 cm), pesticide-free Fraser fir (greenhouse grown stock from a Connecticut nursery). This species is an excellent host for EHS. These small potted fir trees will be infested by gluing scales with eggs onto fir needles. Scales will be collected by searching foliage of EHS-infested hemlock branches cut from trees at field sites in Connecticut.

DISCUSSION

There are two central questions to answer about a proposed classical biological control program against elongate hemlock scale: (1) Is it possible? and (2) Is it worth doing? We have begun to answer the second question by surveys aimed at establishing the average density of the scale throughout the infested range. In the 2004 survey, elongate hemlock scale was found at high densities primarily in Connecticut. However, heavily infested sites certainly do exist in other states. Dense infestations in the city of Philadelphia in the 1990s were observed by one of the authors (Michael Montgomery), which were believed to have caused a general decline of hemlock in the city. Such high density patches may be of local importance. Further survey work is still needed to determine if the picture that emerged in our 2004 survey is valid. Another concern relative to the importance of this pest is whether it acts synergistically with hemlock woolly adelgid (*Adelges tsugae* Annanad). While it is possible to find these two insects sometimes infesting the same trees, it is not clear at this time if one predisposes the tree to build up in density of the other. Separate Forest Service-funded research on this point is underway by Joe Elkinton of the University of Massachusetts (see Paradis and Elkinton in this volume).

As to the second question of whether or not classical biological control of EHS is feasible, prospects seem good. Many species of armored scales have been successfully controlled by introduction of parasitoids from their native ranges. Data from Japan show EHS there to be at vastly lower density than in Connecticut and show that the change in hemlock species between countries is not sufficient to explain this increase in density. Sheared specimens of the Japanese hemlock *T. diversifolia* in Connecticut have been observed to become heavily infested with elongate hemlock scale (Michael Montgomery, pers. obs.).

Two options are immediately apparent that need to be investigated. First, *E. citrina* in Japan may not be the same species as that name is applied to in the United States. Cryptic species have frequently been discovered in the past when separate populations of seemingly cosmopolitan species of parasitoids are closely compared. Use of molecular tools now makes such comparisons quicker and more robust. However, even if the U. S. and Japanese *E. citrina* populations are found to be the same, another option exists: to import new species of parasitoids. Of greatest interest would be any species able to attack adult female scales, which currently are not attacked in the United States by any parasitoid species. Since this life stage is present for the greatest length of time in the field, a parasitoid attacking it would have few or no problems from a failure of synchrony between parasitoid adults and vulnerable scale stages.

Further work will be required to determine the true importance of this pest and to find the parasitoids associated with it in Japan. However, this effort integrates well with efforts to find additional hemlock woolly adelgid predators in Japan and so the cost of pursuing a project against this pest is somewhat reduced by the overlapping needs of the two projects.

ACKNOWLEDGMENT

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ELONGATE HEMLOCK SCALE AND ITS NATURAL ENEMIES IN THE SOUTHERN APPALACHIANS

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ABSTRACT

The development and distribution of the elongate hemlock scale, *Fiorinia externa*, and its natural enemies on eastern hemlock, *Tsuga canadensis*, were evaluated from forest and urban sites established in eastern Tennessee and western North Carolina. This species is widespread throughout the area and has two overlapping generations annually. A heavy infestation level ranging from 3.7 to 4 on a scale of 0 to 4 was found on eastern hemlocks at four sites. The density of *F. externa* ranged from 2.6 to 7.0 individuals per needle.

The hymenopteran parasitoid, *Encarsia citrina,* parasitized the elongate hemlock scale at all sites. Parasitism rates were variable among the forest and urban sites. Parasitism at the urban sites ranged from 17.9% to 41.3%, while the rate at the forest sites ranged from 20% to 23.9% over the season. Eight predators (neuropterans *Coniopteryx* sp. and *Hemerobius stigma* and the coccinellids *Chilocorus stigma, Harmonia axyridis, Rhyzobius lophanthae, Scymnillus horni,* and two unidentified lady beetle species) were collected and evaluated in feeding behavior, survival, development, and reproduction tests using various densities of the elongate hemlock scale.

KEYWORDS

Elongate hemlock scale, eastern hemlock, biological control, *Fiorinia externa.*

INTRODUCTION

The Southern Appalachian ecosystem represents a sensitive forest system currently significantly impacted by invasions of exotic insect pests. This region includes some of the largest remaining remnants of eastern hemlock, *Tsuga canadensis* (L.) Carriere, in the world with about 3,820 ha located within the Great Smoky Mountains National Park (GRSM) in Tennessee and North Carolina. The exotic hemlock woolly adelgid, *Adelges tsugae* Annand, is a pernicious pest causing destruction to eastern hemlocks throughout the eastern U.S. and now threatens the eastern hemlocks in eastern Tennessee and western North Carolina. Also, the exotic elongate hemlock scale (EHS), *Fiorinia externa* Ferris, has become a significant pest of eastern hemlock in several areas, often co-existing with the hemlock woolly adelgid (McClure 1980b). EHS was first described in 1942 (Ferris 1942) from material collected earlier in Baltimore, Maryland, and Queens, New York (Davidson and McComb 1958, Takagi 1963, Talerico et al. 1967). This exotic species is now established in 14 states throughout the eastern U.S. as well as Canada, Asia, and Europe. EHS was recently discovered infesting eastern hemlocks in the GRSM and in Knox Co., Tennessee (Buck 2004). The host range of EHS includes species of *Abies, Cedrus, Picea, Pinus, Pseudotsuga, Taxus,* and *Tsuga,* with the latter taxa containing common hosts of EHS in the eastern U.S. (Kosztarab 1996, McClure and Fergione 1977). Although scale insect populations normally occur at low levels, changes in conditions and management practices that impact the natural enemy complex often enhance population outbreaks (Rebek and Sadof 2003, Sheffer and Williams 1987). EHS are small, cryptic scale insects capable of inflicting substantial damage to the host tree resulting in loss of plant vigor, dieback, needle drop, or death. Its waxy covering allows EHS to maintain a favorable humidity level, prevents rapid temperature changes, and functions as a protective barrier against chemical applications and natural enemies (Lambdin 2004).

The majority of information on the seasonal development of EHS and the impact of beneficial control agents was developed for populations in the northeastern U.S. (McClure 1977b, 1978b, and 1981). Several differences exist in the recorded information on EHS for the northern states compared to more southern distribution regarding their overwintering habits, number of generations annually, synchrony of parasitoid to host, and degree of parasitism. EHS is reported to have only one complete generation annually in Connecticut (McClure 1980b and 1981), although an occasional partial generation is reported to occur in warmer years (McClure 1978b and 1980b). However, it has two generations annually in Maryland (Davidson and McComb 1958) and Virginia (Kosztarab 1996). This scale insect is reported to overwinter as immatures, adult females, eggs, or in all stages (Kosztarab 1996, McClure 1977b and 1978b, Stimmel 1980). McClure (1977b) reported that EHS overwintered in the egg stage and later (McClure 1978a, Kosztarab 1996) as eggs and fertilized adult females. Also, McClure (1980b) noted that EHS was able to out-compete *Nuculaspis tsugae* (Marlatt), a second exotic species occurring on eastern hemlock in Connecticut. However, in Tennessee, *N. tsugae* has not been reported, but the native hemlock scale, *Abgrallaspis ithacae* (Ferris), has been documented on the needles of eastern hemlock (Lambdin and Watson 1980), and is commonly found throughout the region. While nitrogen fertilization of hemlock trees enhanced EHS population size (McClure 1977a, 1980a), the survival, development rate, and fecundity of EHS were reported to be negatively correlated to an increase in population size (McClure 1979b, 1980a, and 1980b). Control of EHS by insecticides or insecticidal soaps is limited to areas accessible to equipment required for application. Also, chemical applications that did not completely cover the tree were found to result in a resurgence of the EHS, which had a resulting faster developmental and fecundity rate than on unsprayed trees (McClure 1977a).

NATURAL ENEMIES

Biological control is a proven tool for managing certain species of herbivorous pests. However, a limited understanding of invasive pests and their natural enemy complex often hinders our ability to use them effectively. Recorded beneficials impacting populations of EHS include the parasitoids: *Encarsia citrina* (Craw), *Aphytis aonidiae* (Mercet), and *Prospaltella* sp.; and as predators: the mirids, *Atractotomus magnicornis buenoi* Drake and *Phytocorus* sp., a coniopterygid, *Conwentzia pineticola* Enderlein, and coccinellids *Chilocorus stigma* (Say) and *C. kuwanae* Silvestri (Davidson and McComb 1958, McClure 1979a and 1979b). The parasitoid *E. citrina* was reported to reduce populations of EHS up to 72% in Connecticut forests (McClure 1978a, 1978b, 1981, and 2003). When EHS and *N. tsugae* co-existed on the same host plant, McClure (1981) reported EHS out-competes its competitor by increasing their density and by a host shift by *E. citrina* that parasitizes *N. tsugae.* Also, the combination of parasitoids of *E. citrina* and *A. aonidiae* were reported to provide up to 96% control of EHS where two generations annually occur (McClure 1978a, 1979a, 1986) to only 2-55% parasitism in Connecticut and New York where only one generation per year occurs. Also, *C. kuwanae*, an egg predator of *Unaspis euonymi* (Comstock) recently introduced from Asia and successfully established in several parts of the U.S. (Drea and Hendrickson 1988, Lambdin 1995, Nalepa 1992, Van Driesche et al. 1998), is reported to feed on EHS.

OBJECTIVES

The objectives of this project were: (1) assess the densities, distribution, and development of EHS populations on eastern hemlock in forest and urban areas, and (2) identify and assess introduced and established natural enemies of EHS.

METHODOLOGY

OBJECTIVE 1

EHS-infested eastern hemlock trees at forest and urban sites in eastern Tennessee and Asheville, North Carolina were sampled monthly from June to December, 2004. Sites were selected with assistance from Rusty Rhea (USDA Forest Service, Asheville, North Carolina), Bill Hascher (Biltmore Estates, Asheville, North Carolina), and Charles Limebarger (Director, Lynnhurst Cemetery, Knoxville, Tennessee). In eastern Tennessee, one urban site at Lynnhurst Cemetery in Knoxville consisted of over 362 EHS-infested trees. In western North Carolina, one urban site was located on the grounds at the USDA Forest Service Headquarters and one urban and one forest site was located at the Biltmore Estates in Asheville. An infestation rating for EHS (0 = none, 1 = sporadic [1-3 limbs], 2 = light [4-6 limbs], 3 = moderate [7-10 limbs], and 4 = heavy [11+ limbs]) on eastern hemlock was conducted for each tree sampled. At the Lynnhurst and Biltmore forest and urban sites, infested trees were divided into four blocks, each consisting of ca. 50 trees, while the site at the USDA Forest Service Headquarters in Asheville, North Carolina, consisted of three heavily-infested trees. To assess density and development of EHS at each site, four branch samples (15-20 cm long, representing each cardinal direction) were taken monthly from each of three trees per block, placed into plastic bags, labeled, and taken to the laboratory and processed. Data were recorded from 100 needles (50 from new growth and 50 from old growth) per cardinal direction for each tree per block that included stage of development, number of live and dead specimens per stage, and location on new or old growth needles.

To determine development time and number of generations of EHS, specimens from 12 branch samples (15-20 cm long) (one sample per tree per site) were removed every three to five days throughout the development period for each generation and monthly during the over-wintering period. Specimens were observed under a stereoscope, and data recorded for date, tree, block, site, number of specimens per stage, mortality per stage, and fecundity, and entered into Excel files for statistical analysis.

OBJECTIVE 2

Parasitoids were obtained and identified from collections of EHS on 100 needles from each of four branch samples (15-20 cm long from each of the four cardinal directions) from three trees in four blocks at each site, taken to the laboratory, and observed with a stereoscope to determine the number of live and dead parasitoids of male and female EHS. Parasitoid exit holes on the dorsum of the EHS were counted as an indicator of successful parasitoid emergence. From the remaining samples, 100 randomly selected EHS specimens from each site were processed, cleared in Essigs Aphid Fluid, mounted on slides, and examined for parasitoids. Also, two samples per tree (30 cm long) for five trees from five blocks per site were collected, taken to the laboratory, and placed into Plexiglas emergence cages to capture emerging parasitoids to establish the number of emerging parasitoids for each tree per site. Selected newly-emerged parasitoids were processed and observed using an electron microscope to evaluate morphological structures to identify the species. Data recorded included: number and date of emergence, parasitism rates per tree per block per site, seasonality numbers, and percent survivorship of parasitoids. Analysis of variance was performed to assess differences among trees and sites for numbers of EHS and parasitoids obtained, number of parasitoids emerging per host, development period, and survivorship of parasitoids from lab tests.

Established predators were obtained from four beat sheet samples from three trees at five blocks per site. Direct observations (at least one hour per site on each sampling date) of the foliage were conducted to determine predators associated with EHS. Predators collected were returned to the laboratory and maintained in 3.8L glass jars with moistened filter paper inserted to maintain humidity and ventilated by lids covered with polyester mesh. EHS-infested cuttings were placed in Floralife® floral-foam blocks (from Hummert International) with the base wrapped in Parafilm. These species will be evaluated in the future for predatory capabilities against EHS.

RESULTS

From May through December, 2004, 724 samples representing 168,253 EHS specimens on over 64,620 needles were collected to assess the density and distribution of EHS and search for potential biological control agents.

EHS BIOLOGY AND DEVELOPMENT

EHS has two complete overlapping generations per year at sites within the southern Appalachians. The spring peak for crawler emergence occurred in June, while the fall peak occurred in late October into November. Fall peak emergence for males occurred in August, coincident to the highest number of adult females. Gravid adult females were most numerous in late May and October-November. In this dimorphic species, females have three stages of development, while males have additional prepupal and pupal stages. Each female produced 12-16 eggs, which hatched over time.

Crawlers settled on the underside of needles on either side of the mid-vein. They tended to settle under the linear waxy filament produced by the needle to protect the stomates, which continued to develop over the dorsum of the immatures, disrupting the outline of their bodies. Crawlers insert their stylets into the stomata to extract nutrients from the needle. Upon developing into the second instar stage, both male and female lose their legs and eyes, and the antennae are reduced to one-segment. Females produce a grayish-brown waxy covering or test, while males produce a white test with parallel sides. Upon ecdysis, the adult female retains the exuviae of the second instar and continues development within this pupillarial casing. Males complete their prepupal and pupal stages within the test produced during their second stage. Upon maturity, winged adult males back out of their test through a flap located on the posterior end of the test. Males do not feed and live only 24 to 72 hours upon emergence. Although capable of flight, males tend to walk across the needles seeking out females for mating.

EHS POPULATION DENSITY

EHS infestation rating on host trees at the study sites ranged from 3.7 at the Lynnhurst urban site to 4.0 at the other three sites implying a well-established, heavy population. Several of the trees are exhibiting dieback and a few have died from these heavy infestations. While as many as 12 specimens were observed on EHS-infested needles, only 2.5 to 7 specimens survived to maturity. Data from samples evaluated from the four cardinal directions of the host trees at the four sites were variable depending upon site. However, the lowest numbers of EHS per site generally occurred on the northern side of the host tree, while the highest numbers occurred on the western side (Figure 1).

EHS NATURAL ENEMIES

Encarsia citrina was the only parasitoid of EHS recovered and represents the first documentation of this species in Tennessee. Newly-emerged specimens were processed and observed using an electron microscope. This endoparasitoid was widely distributed in both urban and forest sites in eastern Tennessee and western North Carolina. Parasite emergence holes were documented on male and female host tests, although the former represented less than 1% of the parasitism rate. In field samples, the parasitism rate was highly variable at the various sites. Successful emergence of parasitoids as evidenced by exit holes in the host at the three urban sites ranged from 16 to 33%, while the rates at the forest site ranged from 20 to 22% throughout the observation period (Figure 2). An additional 2.0, 6.5, and 8.3% of the hosts at the USDA Forest Service, Biltmore Estate urban, and Lynnhurst urban sites, respectively,

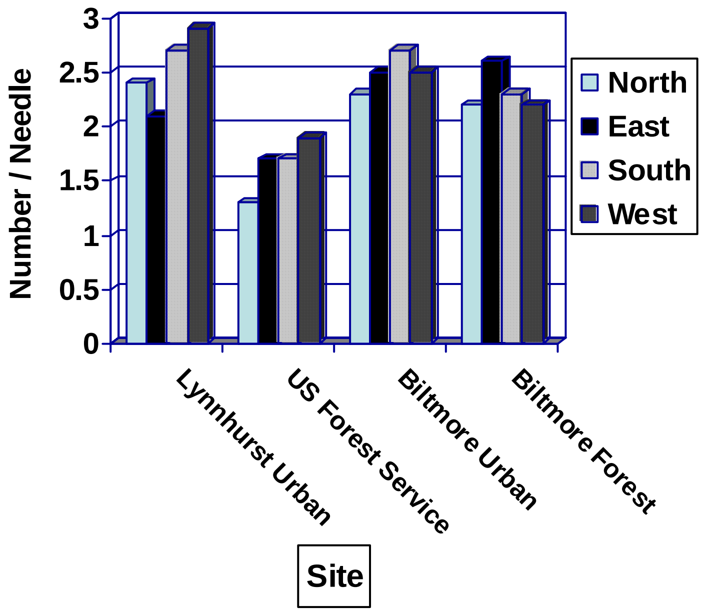

Figure 1. Average number of elongate hemlock scales at each cardinal direction at four sites.

and 2.5% at the Biltmore forest site were killed by parasitoids in the larval or pupal stages, increasing the mortality rate at all sites (Figure 3). Parasitoid peak emergence occurred in August. The population appeared to be lower in the forest sites over the season compared with those in the urban sites.

Female *E. citrina* lays eggs as late first and second instars. Only one adult wasp develops fully from each host. Upon entering the pupal stage, the parasitoid positions itself on its back with its dorsum toward the venter of the host. The adult chews a subcircular hole in the center of its host, emerging by placing its prothoracic legs through the hole followed by its head and pulling itself out of the host. Host finding involves flight to clusters of scales. A short hop-like flight pattern is used to move from needle to needle in search of suitable hosts. Females use their antennae tapping on the scales for host recognition. Emerging parasitoids were found from June through November. The overwintering stage of the parasitoid has not yet been determined.

Eight predators were collected from EHS-infested eastern hemlocks at the four sites from July to December 2004. These include the neuropterans *Coniopteryx* sp. and *Hemerobius stigma* Stevens and the coccinellids *Chilocorus stigma*, *Harmonia axyridis* Pallas, *Rhyzobius lophanthae* Blaisdell, *Scymnillus horni* Gordon, and two unidentified lady beetles. The two neuropteran species and *H. axyridis*, although commonly collected from eastern hemlock, did not feed on EHS during feeding tests. Fifty coccinellid specimens were collected from 180 beat sheet samples from August to December 2004. In three feeding tests with *C. stigma*, only minimal feeding damage to EHS prey offered was recorded. However, *R. lophanthae*, *S. horni*, and the three unidentified lady beetle species did successfully feed on EHS.

Of those predators collected, the lady beetles R. *lophanthae* and *S. horni* are recognized predators of armored scale insects capable of chewing through the hard waxy test of the EHS.

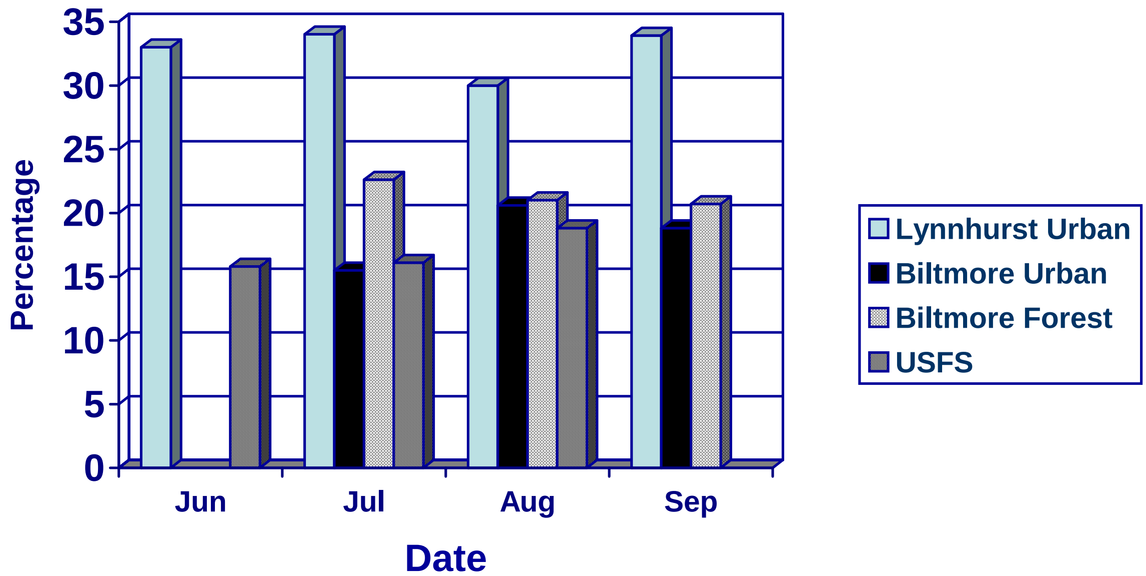

Figure 2. Percentage of paratism of the elongate hemlock scale by *Encarsia citrina* at urban and forest sites.

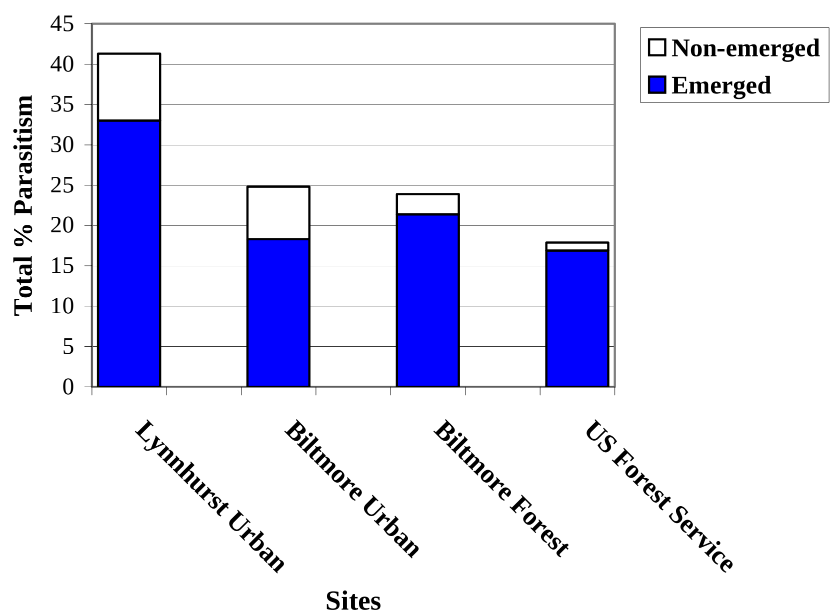

Figure 3. Percentage of emerged and non-emerged *Encarsia citrina* from elongate hemlock scale at urban and forest sites.

From beat sheet and branch cutting samples, both larvae and adults actively fed on prey throughout the summer, fall, and winter. In preliminary prey trials, *R. lophanthae* damaged 93% of adult EHS offered during 19 trials. This predator feeds on all stages of the prey and females lay their eggs singly or in clusters of 1 to 3 near or under the damaged scale tests. Stathas (2000) reported this predator consumed from 426 to 708 prey specimens during its lifespan.

Development and behavioral information for *S. horni* is lacking. From preliminary data, this predator was found to damage 48% of adult scales provided in three trials. Also, one of the undetermined species of coccinellids was observed to damage 17% of the scale specimens offered during three trial periods.

CONCLUSIONS

From June through December, 2004, development of EHS was investigated at four sites and feeding behavior of predators collected was initiated. Although EHS has now been collected from several counties within the southern Appalachians, their presence within a given area appears sporadic. However, populations in infested areas left uncontrolled can build to damaging levels. Populations of EHS are well-established at the study sites and the heavy infestations have killed several trees within the Lynnhurst Cemetery site. The parasitoids and predators established within the sites play an important role in regulating the pest populations. EHS was found for the first time at four locations in Knox County, Tennessee. The discovery of the parasitoid *E. citrina* and the predators *R. lophanthae* and *S. horni* represent new state records.

The discovery of *R. lophanthae*, *S. horni,* and the two unidentified coccinellid species may provide control opportunities against this invasive pest. Of these, *R. lophanthae* has been documented to effectively control diaspidid pests in over nine countries including the US. This predator is reported to have high fecundity (>600 eggs/female) and consumption rates, lacks larval parasitoids, and is active throughout the year (Stathas 2000). The discovery of this established predator presents the opportunity for augmentative releases to control EHS.

The lower population density of EHS per tree at the forest site compared to the urban site at the Biltmore Estates may be a result of a combination of factors, including natural enemies more effectively regulating the pest population. However, the parasitism rate in the forest appeared to be lower than the rate recorded in the urban sites. *E. citrina* emerged throughout the developmental cycle of EHS. Because EHS has two generations within the region, *E. citrina* appears to be highly synchronized with the pest populations. This parasitoid was considered to be the most important natural enemy of oystershell scale in Canterbury, North Carolina, during 1959-1960. It caused an average 40% parasitism from July to December, increasing from 10% in July to 85-100% in November. Future studies will be important in determining the impact of EHS on eastern hemlock in the southern Appalachians. The documentation of potential natural enemies that specifically feed on diaspidids may provide the opportunity for mass releases of these agents to manage the pest.

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Insect-Killing Fungi as a Component of Hemlock Woolly Adelgid Integrated Pest Management

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ABSTRACT

The goal of this project is to develop formulations of insect-killing fungi for application to hemlock forests as part of a hemlock woolly adelgid (HWA) integrated pest management program. Previous studies have identified two strains of *Beauveria bassiana* and a single strain of *Verticillium lecanni* with potential for use against hemlock woolly adelgid. Laboratory and field studies were conducted to develop ultra-low volume (ULV) formulations for delivery of fungal conidia. These studies lead to the development of prototype formulations of conidia in oil- and whey-based carriers. The target density of $1x10^{10}$ conidia/ml formulation was achieved while maintaining sufficient fluidity for spray application. The formulations were used in a small scale forest trial and delivered with an ULV sprayer onto hemlock branches infested with hemlock woolly adelgid. Appropriate controls were included. No significant difference ($P > 0.05$) in droplet densities on upper and lower needle surfaces was found within spray treatments. Nearly 50% of the droplets were in the 100-125 micron size range with the conidia in whey formulation. An unexpected delay of field applications in fall 2004 allowed HWA to molt to a stage that contains a protective woolly coat, which prevented any significant demonstration of field efficacy. Conidia formulated in whey appeared to be persisting at nearly 5 weeks post-treatment, and indications of fungal outgrowth from whey droplets were observed. Fungal growth in whey droplets could recycle fungi in the environment and facilitate development of disease outbreaks. Future research is slated to optimize oil-and-whey based formulations for fungal delivery, persistence, and efficacy against HWA under field conditions.

INTRODUCTION

The hemlock woolly adelgid (HWA) is an invasive pest that is devastating hemlock forests in Eastern United States. A concerted effort is underway to develop insect-killing fungi and other biological agents for use in integrated pest management of HWA (Cheah et al. 2004). The dramatic declines in gypsy moth populations in North America due to the fungus *Entomophaga miamaiga* highlight the potential of insect-killing fungi for forest pest management (Hajek *et al*. 1990). Initially, we directed our research on insect-killing fungi toward collecting numerous isolates and then identifying those with the most insect killing activity (Reid 2003). We also examined their suitability for mass production in anticipation of producing enough fungi for widespread application. Subsequent research examined fungal efficacy against HWA in the field on single hemlock branches to assess the rate and timing of fungal application (Cheah *et al.* 2004). Lab and field trials examined the non-target effects of the fungi on *Sasajiscymnus tsugae*, an introduced predatory beetle of HWA.

The results to date are encouraging for the development of insect-killing fungi as a management tool for HWA. We are actively researching three isolates, a *Verticillium lecanni* and two of *Beauveria bassiana*, because of their positive profiles for efficacy, mass production potential, and compatibility with *S. tsugae*. Field trials between spring 2001 and fall 2003 indicated that significant reductions in adelgid populations occur with fall application of fungal conidia. These fungi, when applied at twice the field application rate, did not negatively affect the predatory beetle, *S. tsugae* (Cheah *et al.* 2004). Currently we are optimizing formulations for ultra-low volume (ULV) delivery, further studying non-target effects and examining fungal persistence. The ability of applied fungi to persist in the environment and have lasting effects on HWA population dynamics influences the selection of deployment strategies for widespread applications.

MATERIALS AND METHODS

A forest trial was conducted in late fall 2004 to examine the spray characteristics of oil- and whey-based formulations and assess their influence on the efficacy of insect-killing fungi against forest populations of HWA. Three fungi (*Beauveria bassiana*: CA-603 and GA082; *Verticillium lecanii*: arsef-6010) were tested in oil formulations and CA-603 was also incorporated into whey (exact components of these formulations are not currently being released). All treatments were delivered using ULV applications of formulated fungal conidia ($1x10^{10}$ condia/ml). There were "no spray" and blank spray (oil and whey) controls. In a hemlock forest in central Massachusetts (Mount Tom Reservation, Holyoke), 1-meter-long branches with greater than 20 branchlets infested with HWA were selected and tagged for treatment. A completely randomized design was used. A pre-spray count of the density, survival, and life stages of HWA sistens was made. This was accomplished by randomly selecting five branchlets/branch that were positive for the presence of HWA and returning the specimens to the lab for microscopic (20-40x) examination.

One milliliter of formulated material was applied with a hand-held ULV sprayer to each of five branches per treatment on October 28, 2004. Post-treatment samples taken as described above were collected five weeks after treatment for comparison to pre-treatment counts to ascertain treatment effects on survival and population density. The data were analyzed for treatment effects using GLM-ANOVA in SAS (SAS 2002) (± = 0.05 for all analysis).

Hemlock foliage was collected directly after spray treatment and examined microscopically to determine the number of droplets on upper and lower leaf surfaces and size distributions of droplet deposits. This was done for the CA-603 treatments formulated in oil and whey and for their respective blank controls. Ten needles were examined per replication within a treatment for the number of droplets within a 0.625 mm^2 microscopic field. Droplet size was examined on a single needle from each replication within a treatment. Droplets sizes were classified in 25 mm increments ranging from 25 to 300 mm by counting the number of droplets within each class. The data on the number of droplets were analyzed for treatment effects using GLM-ANOVA, whereas the distribution of in each size class was examined using Chi-square analysis (SAS 2002). Preliminary examinations of persistence were made from foliage during the post-treatment HWA assessment. This was accomplished by examination of fungi isolated from hemlock needles onto nutritive agar and visual examination of leaf imprints taken using adhesive tape.

RESULTS AND DISCUSSION

No significant differences (P > 0.05) in droplet densities on upper and lower needle surfaces were found within spray treatments (Figure 1). This is a critical finding as HWA are typically located on lower surfaces and therefore difficult to reach with standard spray applications. The total number of droplets found was influenced by the formulation applied, with the oil without conidia having the highest number of droplets. There were significant differences in the distribution of droplet size classes among oil and whey formulations and their controls (Figure 2). For instance, oil without conidia produced a larger proportion of small droplets that probably accounts for the higher number of droplets overall in this treatment. In the case of both oil and whey formulations, when conidia were added, the size of droplets tended to increase. With conidia in whey, nearly 50% of the droplets were in the 100-125 micron size class, and there were none of the larger droplets (>225 microns) found with the oil and conidia formulation, which are indicative of clumping.

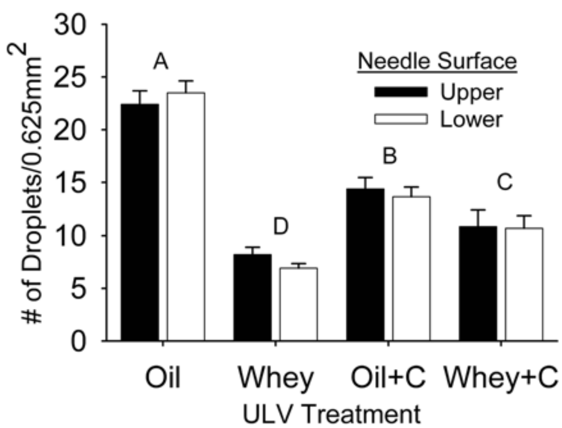

Figure 1. The number of droplets observed on the upper and lower surfaces of hemlock needles after ULV applications of oil and whey formulations with and without conidia of *Beauveria bassiana*. Oil+C and Whey+C are the formulations with conidia. Capital letters indicate significant differences in total counts among formulations. No significant difference was found between needle surfaces.

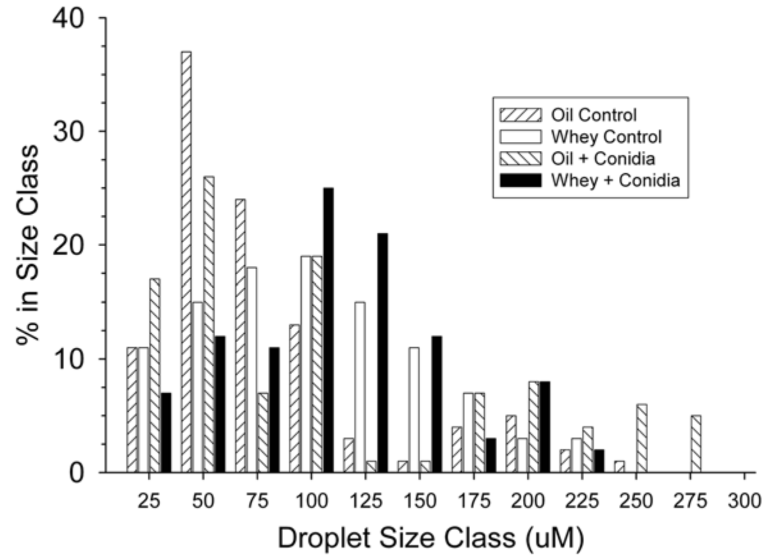

Figure 2. The Frequency of size class distributions of droplets on hemlock needles after ULV applications of oil and whey formulations with and without conidia of *Beauveria bassiana*. Data from both surfaces are combined. There is significant difference in the distribution of size classes among the formulation treatments.

Data on HWA density and survival taken before treatment applications found no significant differences (P > 0.05) in either average density (overall avg. 4.36 live HWA/cm, SE ± 0.24) or mortality (overall avg. 11.8%, SE ± 1.2) of HWA field populations among groups of trees slated for treatment This indicates the overall uniformity of HWA populations within the study site before treatment. However, an unexpected delay of field applications in fall 2004 allowed nearly the entire HWA population (98.4%, SE ± 0.5) to break aestivation and molt to a stage that develops a woolly coat. Nearly five weeks post-treatment, there were no significant differences (P > 0.05) in either average density (overall avg. 4.17 live HWA/cm, SE ± 0.31) or mortality (overall avg. 12.1%, SE ± 1.7) of HWA populations among fungal treatments and the controls. Our previous studies found that fungal applications made with higher-volume formulations during periods when HWA contain a woolly coat were ineffective (unpublished data). This circumstance may have precluded any significant demonstration of field efficacy. Our current strategy is to shift applications six weeks earlier in the year to better avoid the resumption of HWA development in late fall and take advantage of temperatures more favorable for fungal infection.

Conidia formulated in whey appeared to be persisting at nearly five weeks post–treatment and indications of fungal outgrowth from whey droplets were observed (Figure 3). No similar outgrowth was observed on needles treated with oil. Fungal growth in whey droplets could recycle fungi in the environment and facilitate development of disease outbreaks. Future research is slated to optimize whey based formulations for fungal delivery, persistence and efficacy against HWA under field conditions. The compatibility of oil- and whey-based fungal formulations with predatory beetles, adult *Sasajiscymnus tsugae*, will also be examined.

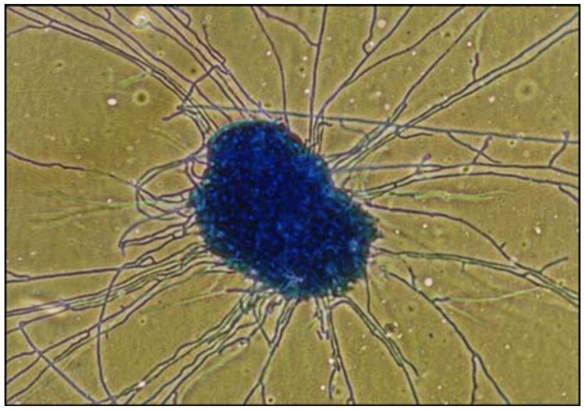

Figure 3. An example of fungal outgrowth found on hemlock needles five weeks after treatment with a whey-based formulation containing conidia of *Beauveria bassiana*. No similar outgrowth was observed on needles treated with oil.

Currently, we are testing fungi against HWA using a methodology in which we select healthy insect populations, apply a dose of fungi selected to allow discrimination between fungi and test formulations, and then examine for treatment effects – commonly referred to as the spray-and-count method. This approach does not reflect the full potential of insect-killing fungi that is often observed under natural conditions. The ability of insect-killing fungi to cause a massive disease outbreak or epizootic is dependent on more than the number of fungal spores in the insects environment. Epizootic potential is also a function of suitable environmental conditions (mostly temperature and water) and insect susceptibility to infection. Insect susceptibility to infection is not static. Developmental changes or response to various stressors, such as low temperature, insect density, and host condition, may cause increased susceptibility to infection. Greater realization of insect-killing potential for impacting HWA populations will likely occur as operational formulations become available for testing under more natural field conditions.

ACKNOWLEDGEMENTS

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Management of Elongate Hemlock Scale with Entomopathogenic Fungi

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ABSTRACT

The artificial dissemination of entomopathogenic fungi, under suitable environmental conditions, may be an important tool for management of an insect pest and, if established, a seasonal control method to maintain the pest population under an economic threshold level. Recently two entomopathogenic fungi were discovered parasitizing the elongate hemlock scale (EHS), *Fiorinia externa* Ferris (Homoptera: Diaspididae). One was found to cause an epizootic within the population of the scale. Exploratory activities in New York showed the expanding range of the epizootic. To assess the potential and viability of this fungus, a set of biological parameters were measures from 26 of the 66 fungal isolates obtained from EHS. Unique growth and sporulation characteristics were found. Both were higher at 25°C (range of growth conditions between 15, 20, and 25°C [± 1°C] spanning 20 days). Conidial germination occurred after 8 hours at 10°C, but it was significantly higher after 6 hours at 25 and 30°C (range of growth conditions between 10, 15, 20, 25 and 30°C [± 1°C] spanning 24 hours). High viability for mass production was observed.

KEYWORDS

Hemlock, *Fiorinia externa*, entomopathogenic fungi, productivity, fungal growth rate.

INTRODUCTION

The eastern or Canada hemlock, *Tsuga canadensis* (L.) Carrière, plays a key role in forest ecosystems by maintaining their stability. It is particularly important along streams and creeks where their shade provides shelter and sustains aquatic ecosystems and a unique microclimate for forest and wildlife (Howe and Mossman 1995, Wydeven and Hay 1995, Crow 1995, Howard et al. 2000). Hemlock is second only to sugar maple in terms of abundance in the northern forest (Curtis 1959). The present decline of the hemlock forest is due to biotic and abiotic factors often acting together. The primary threat consistently reported is the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), found infesting half of the range of hemlock along the eastern seaboard (Knauer et al. 2002). The elongate hemlock scale (EHS), *Fiorinia externa* Ferris (Homoptera: Diaspididae), is becoming important not only as a secondary pest enhanced by an initial stress from HWA but as a parallel stress factor in hemlock decline. A rapid spread of EHS within the area of HWA has been observed. Their highest abundance is within a 300-km radius of New York City (Danoff-Burg and Bird 2002). EHS is present in Pennsylvania, Virginia, Massachusetts, Connecticut, Maryland, New Jersey, Rhode Island, Washington D.C., southern New England and western Ohio (Garrett and Langford 1969, Johnson and Lyon 1988, Hoover 2003, USDA 2004). Strong correlations have been found between HWA and scale infestation levels (Johnson and Lyon 1988, Danoff-Burg and Bird 2002).

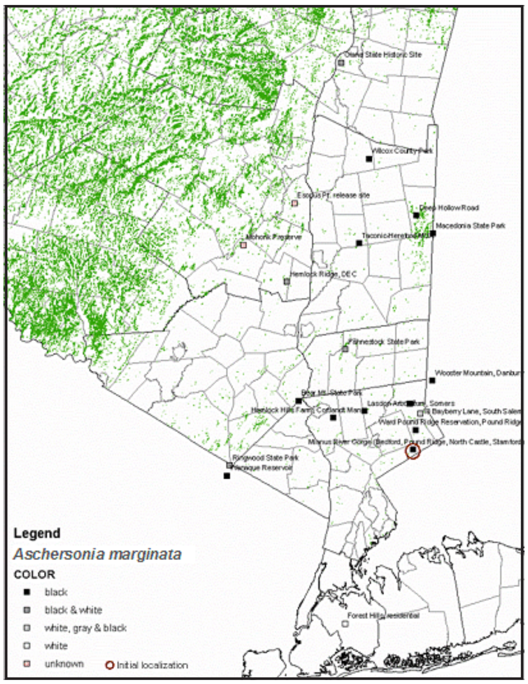
The EHS maybe is a greater problem than HWA, despite its secondary role. Its unique shield-like cover or scale provides protection for the eggs and the adult from contact insecticides, natural enemies and adverse conditions. Losses are soon replaced even with mortality rates of 95% (Baranyovits 1953, Johnson and Lyon 1988). The EHS has probably caused more decline of hemlock in terms of rapid tree mortality than HWA (Greg Hoover, pers. comm.).

A chemical approach to the management of EHS or HWA is not environmentally viable in a forest situation, so alternative measures have been developed: mainly the use of introduced predators. One major factor when considering the use of predators in an open environment is the necessity for a rapid predator population build up before the trees become irreversibly damaged by the pest. In addition, sometimes it is difficult to maintain the population of the predator in levels capable of controlling the pest. The elapse time between the release and achieved biological control is large, sometimes spanning many years.

Scale insects are particularly susceptible to attack by fungi because only one stage is mobile. Quantitative evidence of the importance of entomopathogenic fungi on arthropod populations has been extensively reviewed (Samson et al. 1988), and their impact on armored scales (Homoptera: Diaspididae) has been reported (Helle 1990a and 1990b). An epizootic caused by a "black" fungus within the EHS population in the Mianus River Gorge Preserve in Bedford, New York, was first reported by McClure (2002). Another parasitic 'white fungus' has been recently isolated from EHS at the Entomology Research Laboratory (ERL), University of Vermont.

Because the white fungus was found only occasionally and it was believed that the cause of the epizootic was the black fungus, major emphasis was placed on the latter. This fungus was found in a wide range of EHS sites and was easily cultured, producing high concentrations of spores and different pigmentation when cultured, ranging from whitish-pink to dark black (Gouli et al. 2004). It was first identified by Drs. Svetlana and Vladimir Gouli (ERL) as *Hypocrella* sp., and the species was verified as the anamorph *Aschersonia marginata* Ellis and Ever (Ascomycota: Hypocreales: Clavicipitaceae) by Dr. Zengzhi Li, Head of the Entomogenous Fungal Branch of the Mycological Society of China. Doubts concerning the identification still remain because *Aschersonia* spp. mainly grows in tropical or sub-tropical climates, which are different from that in the northeastern US. It has subsequently been identified as *Fusarium merismoides* by Drs. Humber and Rossman, Cornell Univ. and *Myriangium* sp. by Dr. Nigel Hywel-Jones, National Center for Genetic Engineering and Biotechnology, Thailand. DNA analysis is being done currently to clarify identification of this fungus.

Subsequent expansion from the initial focal point of epizootic observation towards other populations of EHS in New York hemlock stands (Map 1) suggests that an alternative low cost control method may be available. For effectiveness in the management of the scale through the use of entomopathogenic fungi, a thoroughly understanding of the biology of the fungi and their interaction with the host must be determined since they directly affect dissemination, establishment, and their self perpetuation of the pathogen. The research reported herein was designed to assess some of these biological parameters: specifically growth, spore productivity, and germination.



Map 1. Distribution of *Aschersonia marginata* within different counties of New York (2004). Source: New York State Department of Environmental Conservation.

METHODS AND MATERIALS

RATE OF GROWTH AND CONIDIAL PRODUCTION

Assays on the rate of growth and conidial production were done on 26 isolates of the fungus selected randomly from a collection of 66 cultures obtained from different EHS fungal infestation sites. These isolates had been held in long-term storage at -80°C. Ten µl of a $1x10^6$ conidial suspension were pipetted onto a 0.64 cm-diameter sterile disc of filter paper (Scleicher and Schuell, Keene, New Hampshire, Grade 740E) in Petri dishes containing ~20 ml Potato Dextrose Agar (PDA) (Difco®). This medium was used because the fungus starts to produce conidial masses after ~72 hours on a high carbohydrate medium. The Petri dishes were held in the dark in growing chambers and maintained at 15, 20, and 25°C (± 1°C). The trial was repeated twice with four replications per trial. The growth of each isolate was monitored at 5, 10, 15, and 20 days and the outer edges of fungal growth marked at each time period. At the end of 20 days, growth at each time period was measured from the center point to the appropriate mark. Different stages of development of this fungus were present at the same time so the Petri dishes containing the full grown cultures were placed individually in a mixer with 100 ml tap water and blended for 1.5 minutes to obtain a suspension representative of the conidial production per individual colony. The suspension was sub-sampled (0.5 ml) and placed in a test tube with 4.5 ml of a Lactophenol-Cotton Blue Stain (VWR Scientific Products®) to dye spores and stop germination. Subsequent estimation of conidial production per unit volume (1 ml) was assessed with an Improved Neubauer haemacytometer (Propper®).The suspension in the test tube was vortexed for approximately 15 seconds and a drop deposited on each side of the haemacytometer. Under 40x magnification, we randomly selected five squares within each of the two haemacytometer grids and counted the number of spores. The mean number of spores (A) was calculated by multiplying A by the test tube volume dilution factor (10), then by the conversion factor 5,000 (Goettel and Inglis 1997), and finally, by 100 ml (original colony suspension). The data were converted to number of conidia per unit of surface area (cm^2) by dividing the conidial production per colony by the area of growth (Πr^2).

GERMINATION

The quantification of the germination rate under different abiotic conditions (temperatures) was determined by following the conidial development of the 26 colonies (four repetitions) under a compound microscope (40x) after being incubated for 6, 8, 10, 12, 16, 20, and 24 hours on PDA (Difco®) at 10, 15, 20, 25, and 30°C (± 1°C). Germination was present if germ tubes were formed by individual conidia.

STATISTICAL ANALYSES

Data on fungal growth and sporulation were analyzed with an ANOVA (± = 0.05) in SPSS® for Windows® 12.0.2 statistical software to determine differences among treatments per observation period. A Scheffé test (± = 0.05) was employed to identify significant differences among treatments.

RESULTS

The conidial production (Figure 1) did not differ significantly among the 26 isolates ($F = 0.87$; $df = 25{,}130$; $P = 0.6$). The differences were also not significant for the two trials ($F = 3.58$; $df = 1, 154$; $P = 0.06$) but significant for the three temperatures tested ($F = 29.95$; $df = 2, 153$; $P < 0.001$). Differences among all temperatures except for 15 and 20°C were statistically significant (Figure 2).

The average growth/day (Figure 3) did not differ significantly among the isolates ($F = 0.9$; $df = 25$; 130; $P = 0.56$) but did between trials ($F = 4.3$; $df = 1, 154$; $P = 0.04$) and among temperatures ($F = 170$; $df = 2$; 153, $P < 0.001$). The Scheffé test (± = 0.05) showed differences among all temperatures except for 20 and 25°C (Figure 4).

The conidial germination rate varied according to temperature, following a pattern of increase over time. The maximum rate of conidial germination was achieved at the highest temperatures (25 and 30°C) where 100% germination was reached after 8–10 hours (Figure 5). All isolates had reached 100% germination after 24 hours.

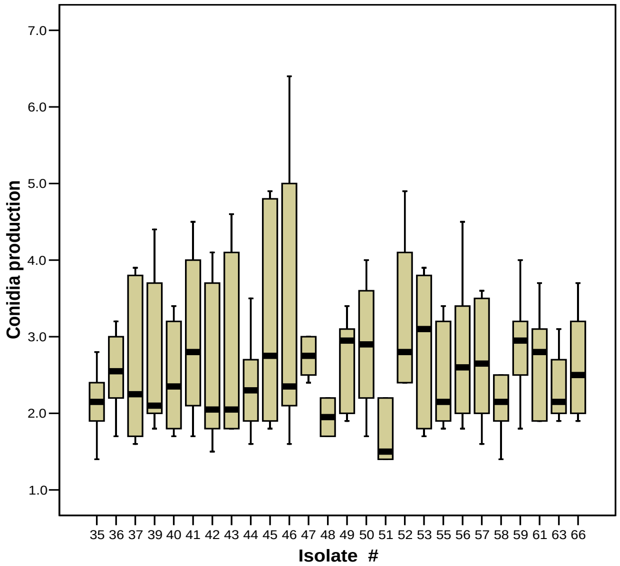


Figure 1. Average conidial productivity per cm² (x10⁷) for the different isolates.

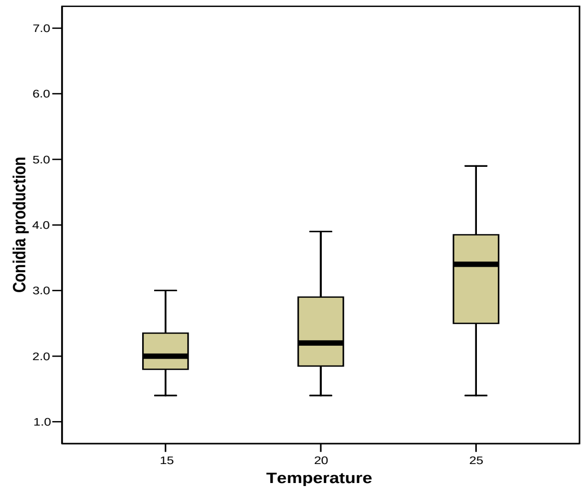


Figure 2. Average conidial productivity per cm² (x10⁷) at different temperatures.

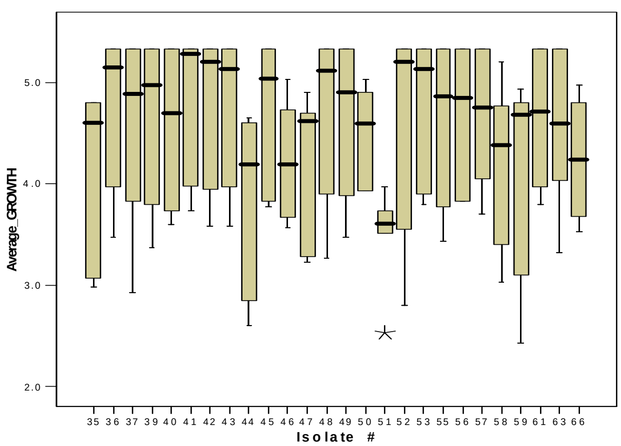


Figure 3. Average growth (mm/day) for the different isolates.

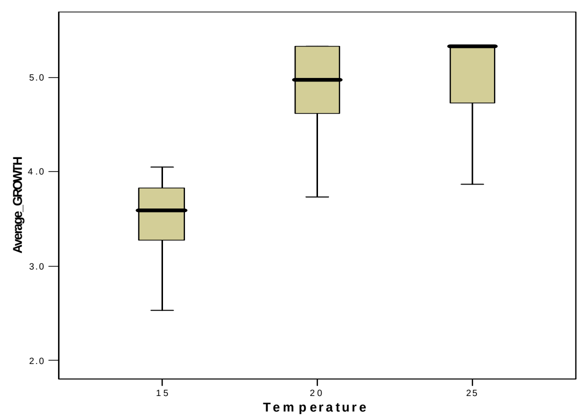


Figure 4. Average growth (mm/day) at different temperatures.

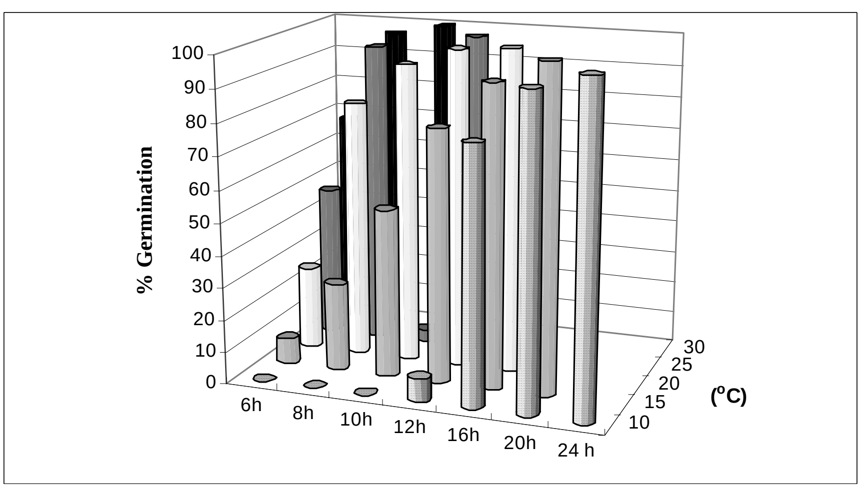


Figure 5. Conidial germination as a function of temperature and time.

Because all isolates did not differ significantly for both growth and conidial productivity within the different temperatures, a ranking system measuring individual performances according to the tested parameters was developed to assess which isolates showed the most promise as a biological control agent. Increasing levels of performance are equivalent to increasing number in the ranking. The values were evaluated for the individual parameters and for an overall measure across parameters (Table 1).

Table 1. Performance of individual isolates for the different parameters tested.

Isolate #	Conidia cm^2 (x10^7)	Growth (mm/day)	Higher germination rate (hour = %)				
			10^o C	15^o C	20^o C	25^o C	30^o C
35	2.1 (± 0.2) ^{2}	4.1 (± 0.4) ^{3}	16h=83% $^{a, 13}$	12h=54% $^{c, 24}$	8h=39% $^{c, 14}$	8h=71% $^{d, 10}$	8h=57% $^{d, 1}$
36	2.5 (± 0.2) ^{5}	4.7 (± 0.3) ^{8}	16h=95% $^{a, 17}$	12h=52% $^{c, 23}$	8h=66% $^{c, 16}$	8h=63% $^{d, 6}$	6h=51% $^{d, 4}$
37	2.6 (± 0.4) ^{6}	4.5 (± 0.4) ^{6}	16h=44% $^{a, 1}$	8h=41% $^{b, 6}$	8h=40% $^{b, 1}$	6h=49% $^{d, 14}$	6h=74% $^{d, 13}$
39	2.7 (± 0.4) ^{7}	4.6 (± 0.3) ^{7}	16h=68% $^{a, 7}$	8h=40% $^{b, 5}$	8h=40% $^{b, 1}$	6h=55% $^{d, 17}$	6h=74% $^{d, 13}$
40	2.5 (± 0.3) ^{5}	4.5 (± 0.3) ^{6}	16h=54% $^{a, 3}$	8h=42% $^{b, 7}$	6h=41% $^{c, 20}$	6h=51% $^{d, 15}$	6h=75% $^{d, 14}$
41	3.0 (± 0.4) ^{10}	4.8 (± 0.3) ^{9}	16h=81% $^{a, 12}$	10h=50% $^{b, 14}$	6h=45% $^{c, 21}$	6h=57% $^{d, 18}$	6h=68% $^{d, 10}$
42	2.5 (± 0.4) ^{5}	4.7 (± 0.3) ^{8}	16h=86% $^{a, 14}$	8h=43% $^{b, 8}$	6h=34% $^{b, 11}$	6h=46% $^{d, 13}$	6h=57% $^{d, 5}$
43	2.7 (± 0.5) ^{7}	4.7 (± 0.3) ^{8}	16h=57% $^{a, 4}$	10h=36% $^{b, 12}$	6h=37% $^{b, 12}$	6h=44% $^{d, 12}$	6h=59% $^{\backslash d, 6}$
44	2.4 (± 0.3) ^{4}	3.8 (± 0.4) ^{2}	16h=81% $^{a, 12}$	10h=52% $^{b, 15}$	8h=78% $^{b, 9}$	8h=64% $^{d, 7}$	6h=66% $^{d, 8}$
45	3.2 (± 0.6) ^{11}	4.7 (± 0.3) ^{8}	16h=78% $^{a, 10}$	6h=33% $^{b, 22}$	6h=43% $^{b, 13}$	6h=57% $^{d, 18}$	6h=77% $^{d, 15}$
46	3.3 (± 0.8) ^{12}	4.2 (± 0.2) ^{4}	16h=58% $^{a, 5}$	12h=35% $^{b, 18}$	8h=63% $^{b, 7}$	8h=60% $^{d, 5}$	6h=50% $^{d, 3}$
47	2.9 (± 0.2) ^{9}	4.2 (± 0.3) ^{4}	16h=78% $^{a, 10}$	12h=32% $^{b, 17}$	8h=56% $^{b, 6}$	8h=48% $^{d, 1}$	6h=78% $^{d, 16}$
48	2.0 (± 0.1) ^{1}	4.6 (± 0.3) ^{7}	16h=63% $^{a, 6}$	8h=50% $^{b, 9}$	8h=50% $^{c, 15}$	6h=54% $^{d, 16}$	6h=72% $^{d, 12}$
49	3.7 (± 0.3) ^{13}	4.6 (± 0.3) ^{7}	16h=79% $^{a, 11}$	12h=45% $^{b, 20}$	8h=49% $^{b, 4}$	6h=55% $^{d, 17}$	6h=68% $^{d, 10}$
50	2.9 (± 0.3) ^{9}	4.4 (± 0.2) ^{5}	16h=43% $^{a, 1}$	8h=34% $^{b, 4}$	8h=48% $^{b, 3}$	6h=68% $^{d, 20}$	6h=81% $^{d, 17}$
51	2.0 (± 0.4) ^{1}	3.3 (± 0.3) ^{1}	16h=86% $^{a, 14}$	12h=52% $^{b, 21}$	8h=45% $^{b, 2}$	8h=68% $^{d, 9}$	6h=44% $^{d, 2}$
52	3.2 (± 0.4) ^{11}	4.5 (± 0.4) ^{6}	16h=94% $^{a, 16}$	8h=32% $^{b, 3}$	8h=77% $^{b, 8}$	8h=65% $^{d, 8}$	6h=85% $^{d, 19}$
53	2.9 (± 0.4) ^{9}	4.7 (± 0.3) ^{8}	16h=44% $^{a, 1}$	10h=46% $^{b, 13}$	8h=78% $^{b, 9}$	8h=53% $^{d, 3}$	6h=67% $^{d, 9}$
55	2.4 (± 0.4) ^{4}	4.6 (± 0.3) ^{7}	16h=72% $^{a, 8}$	8h=28% $^{b, 2}$	8h=76% $^{c, 17}$	6h=57% $^{d, 18}$	6h=66% $^{d, 8}$
56	2.8 (± 0.4) ^{8}	4.6 (± 0.3) ^{7}	16h=72% $^{a, 8}$	10h=29% $^{b, 11}$	8h=54% $^{b, 5}$	6h=60% $^{d, 19}$	6h=86% $^{d, 20}$
57	2.7 (± 0.3) ^{7}	4.6 (± 0.3) ^{7}	16h=77% $^{a, 9}$	12h=24% $^{b, 16}$	8h=56% $^{b, 6}$	6h=55% $^{d, 17}$	6h=69% $^{d, 11}$
58	2.1 (± 0.2) ^{2}	4.2 (± 0.3) ^{4}	16h=77% $^{a, 9}$	6h=66% $^{c, 25}$	6h=74% $^{c, 22}$	6h=89% $^{d, 21}$	6h=95% $^{d, 21}$
59	2.9 (± 0.3) ^{9}	4.1 (± 0.4) ^{3}	16h=77% $^{a, 9}$	10h=28% $^{b, 10}$	8h=84% $^{b, 10}$	8h=81% $^{d, 11}$	8h=66% $^{d, 8}$
61	2.7 (± 0.3) ^{7}	4.6 (± 0.3) ^{7}	16h=52% $^{a, 4}$	8h=32% $^{b, 3}$	8h=66% $^{c, 16}$	8h=50% $^{d, 2}$	6h=69% $^{d, 11}$
63	2.3 (± 0.2) ^{3}	4.5 (± 0.3) ^{6}	16h=79% $^{a, 11}$	12h=36% $^{b, 19}$	8h=78% $^{c, 18}$	8h=57% $^{d, 4}$	6h=63% $^{d, 7}$
66	2.6 (± 0.3) ^{6}	4.2 (± 0.3) ^{4}	16h=88% $^{a, 15}$	16h=32% $^{b, 1}$	8h=84% $^{c, 19}$	8h=63% $^{d, 6}$	6h=82% $^{d, 18}$

a - 100 % germination reached after 24 hours   c - 100 % germination reached after 16 hours
b - 100 % germination reached after 20 hours   d - 100 % germination reached after 10 hours
(± standard error) ranking value

CONCLUSIONS

The 26 isolates tested grew and produced a high number of spores at temperatures ranging from 15 to 25°C. Also, they achieved 100% germination in a short period of time for temperatures ranging from 10 to 30°C. Growth was more affected by temperature than spore production. The rate of growth at 15°C was half that at 20 and 25°C. Although germination of the isolates started at different times 90% of the isolates started to germinate after six hours when the temperature was ≥15°C, and at temperatures 20 and 25° C up to 40-55% germination was achieved for 90% of the isolates after this short period of time. At 30°C, this germination rate was maintained with the exception of some outlier isolates, which reached 80-85% of germination.

Different conclusions on the most promising isolates can be reached depending on the parameters used (Table 1). Isolates 41, 45, and 58 had the highest rankings when considering all the tested parameters together (growth, productivity, and germination). If trying to assess the best isolates to grow under northeastern U.S. environmental conditions, isolates 35, 36, and 45 provided better germination at lower temperatures with good productivity and rate of growth.

Based on these positive results concerning major features required for the potential use of entomopathogenic fungi for insect pest management (high spore productivity, growth, and germination), mass production is critical. Subsequent research will focus in this area and in assessing the virulence of these different strains against the EHS and other pests such as the HWA.

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Comparing Systemic Imidacloprid Application Methods for Controlling Hemlock Woolly Adelgid

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INTRODUCTION

Several studies have shown imidacloprid to have excellent activity for controlling hemlock woolly adelgid (HWA) in a landscape environment (Cowles and Cheah 2002, Doccola et al. 2003, Webb et al. 2003). This study was undertaken to determine which imidacloprid application method would provide the best control of HWA in forests. The methods compared were Kioritz soil injection with (1) placement near the trunk or (2) placement near the trunk and out to the drip line, (3) drench near the base of the trunk with Bayer Tree and Shrub Insect Control, and trunk injection with the (4) Arborjet, (5) Wedgle, and (6) Mauget systems. Along with the untreated check, these treatments were part of a 7 × 2 factorial design, which included a comparison of fall vs. spring application timing.

METHOD

Trees were chosen for this study based on the presence of moderate populations of HWA, the availability of lower branches from which adelgid populations could be observed, and a distance of at least 50 m between study trees. Six replicates were located at five sites in Connecticut: Shenipsit State Forest in Somers, Nathan Hale State Forest in Coventry, Tunxis State Forest in East Hartland, Sequassen Boy Scout Camp in New Hartford, and the Mashantucket Pequot Reservation in Ledyard, for a total of 84 study trees. Insecticides were applied between October 1–29, 2002, and between May 28 – June 6, 2003. The Kioritz-injected imidacloprid treatments used Merit 75W and 1 g of active ingredient per 2.5 cm DBH. Bayer Advanced Tree and Shrub Insect Control (68 ml of product per 2.5 cm DBH, providing 1 g a.i.) was diluted in 3.8 liters of water and drenched outwards from the trunk of the tree to a distance of 45 cm. Trunk injection applications were made of Mauget's Imicide (3 ml of 10% formulation per 15 cm circumference), Wedgle's Pointer (1 ml of 12% formulation every 10 cm circumference), and Arborjet's Imajet (6 ml of 5% formulation every 24 cm circumference) while following each manufacturer's recommended method. The targeted dosages for the Mauget, Wedgle, and Arborjet systems were 0.15, 0.09, and 0.1 g a.i./2.5 cm DBH, respectively. The application rate in the fall with the Wedgle System could not be confirmed, as there is no component to the application device that permits monitoring of active ingredient placement in the tree. Therefore, plugging of the needle orifice (a common occurrence) led to squeezing the handles without actually placing any product in the tree. Two modifications of the Wedgle method were required for successful springtime trunk injections. To prevent plugging of the needle orifice, a 7/64" hole was drilled into the center of the hole left by the bark corer. The injection plug was then inserted as before, and the needle inserted through the plug into the small diameter hole. Unlike the fall application, the application in the spring resulted in easily observable separation of the bark at the cambium layer where imidacloprid suspension was being deposited. Weighing the insecticide reservoir bottle before and after application with a portable electronic centigram balance allowed determination of the amount of product injected into each tree. Calibration marks on the Wedgle device were found to not correctly represent the volume of liquid being injected into the tree, so additional pressurizations (four per injection site) were used to compensate.

Cold temperatures during the winter resulted in HWA mortality at study sites in nearby untreated trees of 85-95%. Therefore, mortality was not evaluated for the overwintering generation but delayed until July 7-15, when following (progrediens) stage had developed. Mortality was also assessed in late November, 2003, and mid-December, 2004. In July, shoots with adelgids were brought back to the laboratory in a cooler and evaluated under a dissecting microscope. Adelgids were probed to determine whether there was movement of legs or mouthparts, and the numbers of living and dead adelgids were counted from a sample of 100 individuals per tree. In the November and December assessments, five shoots were cut from the lower canopy, and five shoots from a height of 20-30 feet. Adelgids were counted on each shoot, up to a total of ten adelgids per shoot. The total for the ten samples then constituted a 1-100 infestation rating.

We used an immunological method to measure imidacloprid residues (EnviroLogix, 2003) to compare with mortality data. Sap from hemlock branches was expressed from 20-50 cm long shoots on May 2-6, July 7-15, and August 20-27, 2003, using a hyperbaric chamber pressurized with nigrogen to ~200 p.s.i. with nitrogen. Sap collected with a pipette required no additional clean-up procedure before being tested with the EnviroLogix ELISA kit. Volumes of 250 – 700 µl were obtained for each sample with 100 µl required for imidacloprid determination. Sap samples were kept frozen once they were brought to the lab.

RESULTS AND DISCUSSION

Site variability and natural mortality affected adelgid survival and obscured insecticide treatment effects in the July assessment. Adelgid mortality ranged from an average of 64% for the Wedgle-treated trees to 80% for the Kioritz, near-trunk imidacloprid placement. Adelgids in the untreated check trees experienced 69% mortality.

November, 2003, and December, 2004, evaluations of adelgid populations determined that fall and spring application timing did not significantly differ. The November 2003 evaluations determined that soil applications resulted in an average population suppression of 79% relative to the untreated check. The Kioritz near-trunk placement of Merit in the fall of 2002 resulted in 100% mortality of adelgids as measured 13 months later. Suppression of adelgids with the soil applications improved further over the next year, resulting in an average of 98.5% reduction compared to the untreated check. Four of the six treatment combinations for soil application resulted in non-detectable HWA populations on the treated trees 18-26 months post-treatment.

In contrast to the soil applications of imidacloprid, trunk injections did not result in significant reductions in adelgid populations, either in the 2003 or 2004 evaluations. Of the trunk injection methods, the Mauget system resulted in populations that were intermediate in value and not significantly different from either the untreated check or the soil application treatments.

The ELISA assay of sap indicated that soil-based application of imidacloprid resulted in good mobilization and persistence in branches. With the Mauget system injections, a relatively short-lived, highly concentrated peak of imidacloprid was found in sap of some branches. Residues from the other two trunk injection methods were of low concentration.

The Mauget System allows visual monitoring of uptake of the formulated product into the tree—however, on many occasions the 3 ml capsules did not empty into the tree and had to be removed in spite of the lack of uptake. Capsules are pressurized, so any material not taken into the tree was lost onto the bark of the tree when the feeder tube was removed, making accurate measurement of uptake impossible. Uptake was very poor in the spring, and better, but variably successful, in the fall.

The Arborjet System provided the most complete feedback to operators regarding the movement of insecticide into the tree at the time of injection. Both the ability of the tree to accept the formulated product and the volume of product applied are easily monitored: the first through the pressure gauge attached to the injection needle, and the second through the injection reservoir calibrated in milliliters.

The imidacloprid test kits have proved to be an effective method for analysis of residues from hemlock sap. Concentrations can be quantified from 0.5 - 5 ppb, requiring considerable dilution and repeat testing for higher concentration samples. Non-specific binding results in values of imidacloprid from sap ranging up to 5 ppb, so at least a 1:10 dilution is required and quantitation of imidacloprid below 5 ppb is not possible with this method. The results have to be considered as semi-quantitative for imidacloprid because some of its metabolites are also detected (though to a lesser degree than the parent compound). It is adaptable for analysis of tissue (needle and twig) samples and the results can be read with a relatively inexpensive scanner and image measurement software.

SUMMARY

Trunk injection methods were less effective for control of HWA than near-trunk soil placement of imidacloprid. Efficacy of injections might be improved if the resulting short duration of mobilization in sap is timed to closely match peak feeding activity of adelgids (e.g., mid-April). The soil applications resulted in long-term moderate concentrations of imidacloprid in the sap, which may be responsible for the reliable, highly effective suppression of HWA populations. The ability of soil application of imidacloprid to provide multiple-year control of HWA must be balanced with the cost of this treatment and its potential to harm non-target aquatic organisms. Analyses of hemlock tissue foliage on untreated trees in this study determined that significant lateral and down-slope movement of imidacloprid

can occur when imidacloprid is applied in water-saturated forest soil (data not shown). Insecticide treatment should be considered a stop-gap measure to preserve trees that are of exceptional value until such time that biological control becomes established.

ACKNOWLEDGMENTS

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DISCLAIMER

Use of a product name does not imply endorsement of the product to the exclusion of others that may also be suitable.

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Potential Concerns for Tree Wound Response from Stem Injection

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ABSTRACT

Stem injection of imidacloprid is an available component of management strategies for hemlock woolly adelgid. Preliminary observations of similar treatments of maple and ash show that the injury sustained by injection warrants investigation of the wound response in eastern hemlock. Such investigations need adequate experimental controls to identify the role of phytoxicity of the active ingredient and the carrier formulation, delivery pressure, seasonality, and tree condition. External indicators such as bark cracks tend to underestimate the amount of cambial dieback. Evaluation of the wound response requires tree dissection. We suggest that the unintended consequences of treatment such as injection injury be considered and incorporated into the management decision process.

KEYWORDS

Stem injection, tree injection, compartmentalization, imidacloprid.

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an aphid-like insect pest that can kill mature eastern hemlock, *Tsuga canadense* (L.) Carr., trees within a few years of infestation. HWA is one of a growing list of introduced insect pests that threaten wild, managed, and urban forests in the eastern United States. Because of the widespread occurrence and importance of eastern hemlock, diverse strategies to manage HWA are being tested, including pest exclusion, roguing of infested individuals, biological control through the release of predators and parasitoids, and the application of chemical insecticides. Although some combination of cultural practices and biological control may eventually protect susceptible forests from HWA, chemical insecticides will likely continue to be used over the next years to reduce HWA severity and spread.

The direct injection of chemical treatments into trees through stem injection has a long history. In recent years, concern over spray drift and the runoff from sprays and soil applications of insecticides has renewed attention to stem injection. Imidacloprid (a chloro-nicotinyl insecticide) in various formulations is the primary insecticide being injected to control HWA and other introduced, invasive pests. Stem injections are part of tests or control strategies for HWA in Pennsylvania, Massachusetts, and Connecticut. The appeal of stem injection lies in the expectation of the delivery of effective doses into targeted tissues (such as branchlets in the case of HWA) throughout the tree translocation system with few or no nontarget effects and a high degree of social acceptance. That appeal, coupled to the potential commercial profitability of the technique suggests that stem injection will continue to be considered as a treatment option for trees infested or threatened by HWA.

The biological tradeoff with stem injection is that the tree is wounded in the course of treatment (Smith 1988). Although the size of the mechanical wound made during injection may be small, the treatment chemical and the application pressures can greatly increase the severity of wound-initiated discoloration and cambial dieback associated with an injection (Shigo et al. 1977).

Tests of efficacy of chemical formulation and injection methods generally focus on the recovery of the treatment chemical or its metabolites from the targeted plant part with some attention given to comparisons of infestation intensity and growth recovery. Rarely do tests of efficacy of injected pesticides include evaluation of the effects of tree injury and wound response, particularly for recently introduced injection technology.

Should the wound response of hemlock injected with imidacloprid or other insecticides be investigated? No critical dissection studies of injected eastern hemlock stems have been published at this time. However, observations of injected and dissected red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marsh.), and white ash (*Fraxinus americana* L.) may focus attention on what should be looked for in future field trials of various injection techniques in hemlock.

METHODS

Sugar maple and white ash dissected for this study were part of a larger trial of the systemic distribution of imidacloprid administered by stem injection at the Mount Greylock State Reservation in Lanesborough, Massachusetts. Trees selected for injection in 2002 were 30-40 cm DBH and growing along the access roadway at the reserve. Injections were made by professional applicators experienced in the methods used. Although the trees were vigorous and apparently healthy, they were likely under moisture stress at the time of injection, making them less than ideal sample trees for testing. Trees were injected in mid August while following the manufacturer recommendations in effect at the time using one of two stem injection methods. The two methods represent two different types of injection schemes. For both systems, injection sites were usually located at the trunk flare, 10-30 cm above ground line. With the Arborjet Viper System (Arborjet Inc., Winchester, Massachusetts), each injection site was prepared by drilling an 8-mm-diameter hole along the stem radius to a depth of about 4 cm into the sapwood. The hole was snugly fitted with a proprietary brass injection port (a prototype of the current plastic Arborplug). Injection sites were distributed along a spiral around the circumference of the lower stem. The number of injection sites was calculated as one-half the stem diameter at DBH, in inches. A proprietary 5% imidacloprid solution was used with an average of 10 to 14 mls of formulation injected through the ports at a delivery pressure of ranging from 300-600 PSI.

Also tested was the Wedgle Direct-Inject System (ArborSystems, Omaha, Nebraska). The Wedgle injection site was prepared by removing a 2-mm-diameter plug to a depth of about 4 mm in the bark, using a special tool. The hole did not extend to the vascular cambium or the wood. The hole was fitted with a hard plastic_injection port filled with silicon_(the Wedgechek). Injection sites were prepared for every 6 inches of trunk circumference. Two proprietary imidacloprid formulations were used to inject the trees: 12 and 20% 'Pointer'. Two ml of insecticide was attempted at each injection site at an unknown delivery pressure.

In September 2003, the injected trees were examined externally, and two sugar maple and two white ash injected by each of the two treatments were felled and bucked. The bolts containing the injection sites were taken to a workshop for examination and further dissection through bark removal, sawing, and splitting.

OBSERVATIONS

After one or two growing seasons, external cracks in the bark were associated with some of the Arborjet and a few of the Wedgle injection sites. When present, the vertical cracks passed through the Arborjet injection sites and 1-2 cm to the side of the Wedgle injection sites (Figure 1).

Stem dissection indicated that the visible extent of external cracks greatly underestimated the amount of cambial dieback, particularly for Wedgle injection sites (Figure 2). Even when there were no cracks evident from the outside of the bark (Figure 2A), the vascular cambium and phloem were killed as seen on the inside of the bark (Figure 2B) and from the wood surface of the dissected tree (Figure 2C) In vigorously growing trees, callus was produced at the margins of the cambial dieback and the cell derivatives had initiated woundwood formation (Figure 2C). Imidacloprid residue around both Wedgle and Arborjet and injection sites was commonly found (Figs. 2B, C and 3).

Internal columns of wound-initiated discoloration were generally well-defined for Arborjet injection sites for both ash and maple (Figure 3). Little wound-initiated discoloration was evident in the Wedgle injection sites.

DISCUSSION

Because of the small sample size, these observations must not be construed to support a preference for one injection technique over another. Similarly, no differences in tolerance between the tree species can be assessed. Because of the short time period between injection and dissection, the long-term effect of single or repeated rounds of injection treatments also cannot be assessed. Although the possible moisture stress at the time of injection may have hindered treatment uptake, the degree of stress was likely well-within the range of what is expected for urban and community trees.

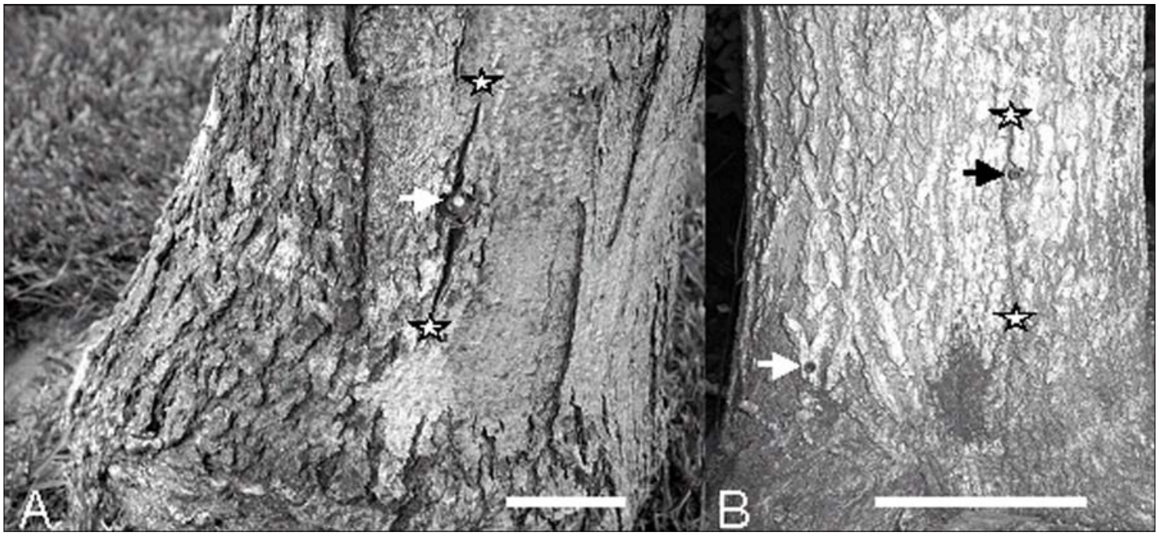

Figure 1. Bark cracks associated with stem injection. The injection sites (arrows) and the vertical limits of the cracks (stars) are marked. (A) Arborjet injection of sugar maple. (B) Wedgle injection of white ash. Scale bars = 5 cm.

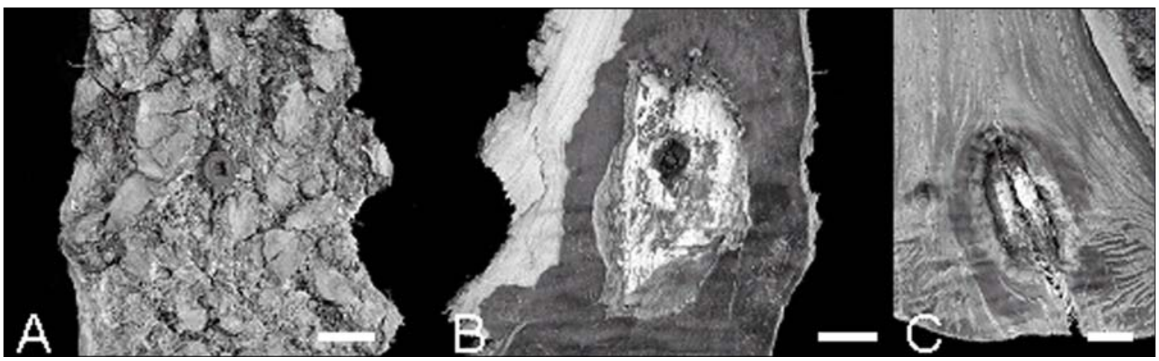

Figure 2. Stem injury from Wedgle injection in dissected white ash. (A) External view of injection site with "arborcheck" plug. (B) Reverse surface of (A) showing plug, killed inner bark, and imidacloprid residue. (C) Stem surface beneath the bark showing the area of cambial dieback, woundwood formation, and imidacloprid residue. Scale bars = 5 mm.

Figure 3. Stem injury from Arborjet injection in dissected sugar maple. Note the wound initiated discoloration and the imidacloprid residue. Scale bar = 10 cm.

We observed that the injection treatments cause a greater amount of injury than is evident from external examination. Dissection studies are needed to assess the extent of cambial dieback and wound-initiated discoloration associated with the injection site. Cambial dieback is a more accurate indicator of the size of the injury than the size of the mechanical injector or external cracking. Given sufficient time in otherwise vigorous trees, woundwood produced at the margins of the cambial dieback will likely close over the wound. Longer-term research is needed to determine if closure results in the desired restoration of cambial continuity around the stem circumference or whether the ribs or rolls of woundwood will press against each other to form stem cracks.

Dead bark and phloem were located to the outside of the killed vascular cambium. To the inside were sapwood cells killed directly by the treatment or indirectly by desiccation and disconnection from living phloem. Compartmentalization is the boundary-setting process that resists the spread of cell death and subsequent infection by microorganisms in wounded wood and bark (Shigo 1984). Frequently, the killed sapwood within compartmentalization boundaries is a different color than healthy sapwood and is referred to as wound-initiated discoloration. More important than the change in wood color is that the formerly alive cells in healthy wood now are dead in discolored wood and incapable of (1) energy storage, (2) active shifts to defensive physiology, and (3) water conduction.

Effective compartmentalization minimizes the volume and resists the spread of wound-initiated discoloration associated with an injury. The Wedgle system induced little wound-initiated discoloration after one growing season. The extensive cambial dieback associated with the Wedgle system is at least in part due to the intentional separation of bark from underlying tissues that is designed to form a reservoir for the injected chemical. Tests using longer intervals of time between injection and dissection are needed to determine if wound-initiated discoloration will develop beneath the cambial dieback.

For these observations, it is impossible to assess whether the injury was due to imidacloprid or the components of the injection method. In addition to longer incubation times, future tests of the wound response from chemical injections need adequate experimental controls. At least until the role of these factors have been well-documented, the simple and interactive effects of the size and type of injector, the delivery pressure, the active ingredient, the vehicle formulation, tree condition, and seasonality of injection should all be tested.

It can be argued that these observations, although recent, no longer reflect the latest technology. Indeed, over the past decades injection technology has swung from using high pressure to low pressure to passive infusion and back again. Formulations have employed high concentrations of active ingredients at low volumes and dilute concentrations at high volumes. New modifications are continually in development. These changes can aid practical management but should not be used as an excuse to avoid critical testing for the effects of injection.

The imidacloprid residue associated with both injection methods needs further examination. Is the presence of residue due to less-than-ideal conditions for uptake of the chemical treatment or is it inherent in the employed techniques? Should the presence of this residue be considered as a nontarget release of the chemical into the environment?

How might these findings be different for eastern hemlock? Previous studies indicate that the tracheid system in conifers can require higher injection pressures for treatment uptake (Sanchez-Zamora and Fernandez-Escobar 2004), causing greater injury. Compartmentalization in conifers usually involves enhanced biosynthesis of terpenes and resin production, which can complicate the assessment of effectiveness of compartmentalization.

We suggest that stem injection as well as other measures to control or manage HWA be considered using a treatment matrix that contains the overlapping dimensions of efficacy, consequences of inaction, the frequency of repeated treatments, direct economic cost, availability of alternative treatments, social acceptance, and unintended consequences of treatments to include the tree wound response.

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Resistance of Hemlock to *Adelges tsugae*: Promising New Developments

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ABSTRACT

Hemlock (*Tsuga)* species native to western North America and Asia are considered to have some degree of resistance to feeding by hemlock woolly adelgid, *Adelges tsugae* Annand. We compared the resistance of seven hemlock species growing in arboreta in the northeastern U.S. to *Adelges tsugae* Annand by artificially infesting trees with *A. tsugae* ovisacs and subsequently counting the number of progrediens (same generation) and sistens (next generation) that developed. Based on this assessment, the most resistant species was *T. chinensis* (Franch.) E. Pritz, followed (in declining order) by *T. diversifolia* (Maxim.) Mast and *T. mertensiana* (Bong.) Carrière; *T. sieboldii* Carrière and *T. heterophylla* (Raf.) Sarg.; and lastly, *T. canadensis* (L.) Carrière and *T. caroliniana* Engelm. Aphids and other insect families in the Aphidoidea, which includes adelgids, are known to have a limited tolerance to some terpenoids. Analysis of terpenoids from the hemlock species under study showed that three interspecific groupings were evident: 1) *T. canadensis* and *T. caroliniana*, 2) *T. chinensis*, *T. sieboldii*, *T. diversifolia*, and *T. heterophylla*, and 3) *T. mertensiana*. Analysis of terpenoids in *T. canadensis* tissues showed that terpenoid concentrations are lower in the tissues in which *A. tsugae* feeds (the leaf cushion) than in the needles, and terpenoid concentrations are higher in developing needles and leaf cushions than in the respective tissues after they have matured. Terpenoid profiles in *Tsuga* may correspond to the relative susceptibility/resistance of species to *A. tsugae* and the insect may decrease its exposure to certain terpenoids by feeding in the leaf cushion and avoiding developing tissues.

The *Ex Situ* Conservation of Carolina Hemlock

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ABSTRACT

Carolina hemlock (*Tsuga caroliniana* Engelm.) is a species represented by several small to moderately sized, isolated populations in the Appalachian Mountains and upper Piedmont from northeastern Georgia to southern Virginia. Over the last several years, there has been great concern about the destruction of Carolina hemlock by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, a pest introduced from Asia into the United States in 1926. Conservation approaches are needed to protect dwindling populations of Carolina hemlock as forest entomologists attempt to find ways to limit the future damage caused by the HWA. In a collaborative effort between Camcore, North Carolina State University (NCSU) and the US Forest Service, seeds of 64 trees from nine populations of Carolina hemlock in North and South Carolina were collected in 2003 as part of *ex situ* conservation attempt to move the species to more protected areas where HWA is not present. Floramap®, a climatic model that predicts where new populations of a species should survive, indicated that central Chile and southern Brazil were potential planting locations. In preparation for seed transport overseas, a small fumigation experiment was conducted. Results indicate that Carolina hemlock seed fumigated at the application rate of 2.5g/m^3 aluminum phosphide for six days at 17-20°C does not harm seed germination and is an acceptable method for seed treatment. With respect to sowing seeds in nurseries in Latin America, research at NCSU suggests that Carolina hemlock should be germinated on moist paper, not moist sand, and that length of stratification at 4.5° C (0 to 90 days) has little affect on germination rates. Plans to enlarge the existing genetic base for *ex situ* conservation failed in 2004 because of poor cone crops in natural stands, but a second attempt will be made to sample additional populations in Virginia, Georgia and Tennessee in 2005. Under this worst-case scenario, genetic material of hemlock from Latin America could someday be returned to the US to repopulate lost Carolina hemlock stands once the technology to control the insect has improved.

KEYWORDS

Ex situ conservation, stratification, germination, fumigation, Camcore.

INTRODUCTION

Carolina hemlock (*Tsuga caroliniana* Engelm.) is represented by several small to moderately-sized, isolated populations located in the Appalachian Mountains and upper Piedmont from northeastern Georgia to southern Virginia (Figure 1). It is commonly found on rocky outcrops and dry exposed ridges from 600 to 1,500 m altitude (Humphrey 1989). Over the last several years, there has been great concern about the destruction of Carolina hemlock by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, a pest introduced from Asia into the United States in 1926. Conservation approaches are needed to protect dwindling populations of Carolina hemlock as forest entomologists attempt to find ways to limit the future damage caused by the HWA.

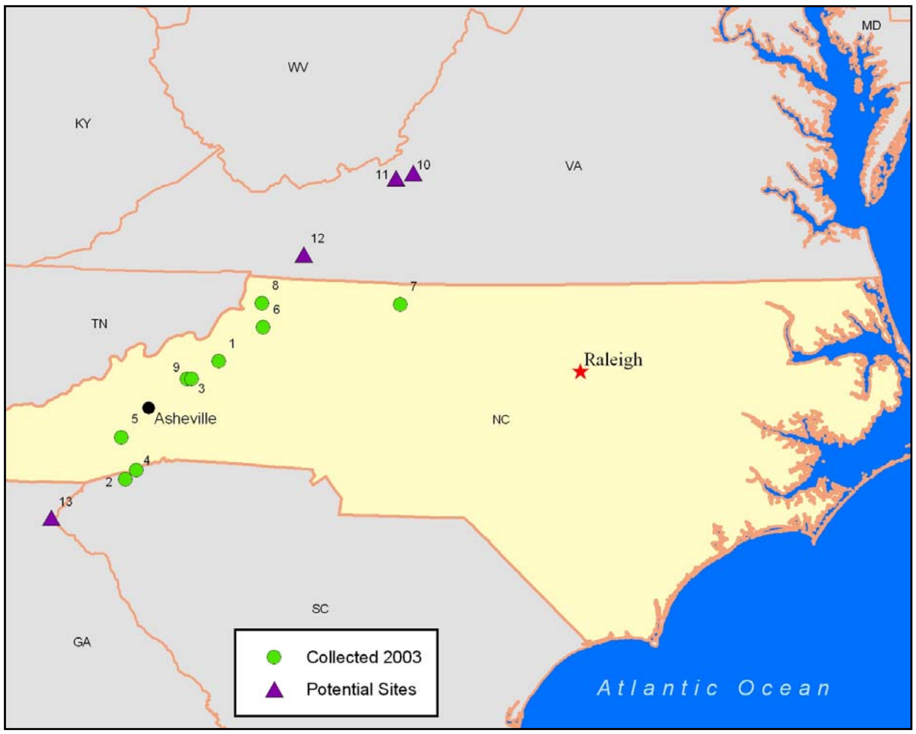

Figure 1. Carolina hemlock collection sites.

Ex situ conservation, or the movement of germplasm from its place of origin to other more protected areas, has often been used to protect tropical forest species from the onslaught of woodcutters and agriculture concerns (Dvorak et al. 2001). The lessons learned from severe and rapid fragmentation in the tropics through human action might help researchers to develop appropriate models to protect Carolina hemlock in the southern U.S. from HWA. For example, to conserve alleles with frequencies of 5% or greater of *Pinus tecunumanii* (a threatened conifer in the Guatemalan highlands), six to 10 trees needed to be sampled per population depending on the size of the population for *ex situ* conservation to be effective (Dvorak et al. 1999). Furthermore, experience with tropical pines indicates that six to eight populations need to be sampled across the species' geographic range to maintain broad adaptability.

Because of the rapid progression of the HWA and the urgent need to protect dwindling gene pools of Carolina hemlock in the southern U.S., Camcore (an international tree conservation and domestication program at North Carolina State University) and the US Forest Service have embarked upon a collaborative program to move representative seed samples of Carolina hemlock to Latin America where the HWA is not present. The goal of the *ex situ* conservation efforts is to plant small field conservation banks as a future source of genetic material to be utilized if the HWA is found to be unstoppable in the U.S. Under this worst-case scenario, genetic material of hemlock from Latin America could someday be returned to the U.S. to repopulate lost Carolina hemlock stands once the technology to control the insect has improved.

For this progressive *ex situ* conservation approach to work, a reasonably sized genetic base needs to be sampled from native populations of Carolina hemlock, good species-site matches need to be made in the new environment, and the knowledge of nursery and field silviculture must be of a sufficiently high level to successfully grow the species in an exotic environment. This paper explores these issues and summarizes initial Camcore/USDA Forest Service efforts to develop a workable *ex situ* conservation program for Carolina hemlock.

MATERIALS AND METHODS

Information about potential Carolina hemlock collection sites was gathered from personnel at the U.S. Forest Service and numerous professional foresters throughout the region. Priority in the first year of collection (2003) was given to natural stands in North and South Carolina. A total of 64 trees in nine populations were sampled (Table 1). Trees were selected within a stand based on availability of cones. A distance of 50 meters was kept between selected trees whenever possible. Selected trees were measured for height and diameter, and then clearly marked with numbered aluminum identification tags. The geographical coordinates for each tree were recorded with a GPS receiver to allow further research or future recollection. Each tree was then rated for cone abundance (High or Low), crown class (Suppressed, Intermediate, Co-dominant, or Dominant), and adelgid presence (None, Low, Medium, or High). The cones were harvested from the lower branches of the trees with a pole pruner and placed in clearly marked cotton bags for transport to NCSU. Upon arrival, cones were transferred into large, lightweight cotton bags and placed in a dry, well-ventilated greenhouse chamber to promote cone opening. After approximately 3 weeks, seeds were extracted from the cones using a research-capacity shaker box, dewinged, and cleaned from debris with a standard seed blower. Seed was then transferred to a laboratory refrigerator and stored at 4.5°C.

To ensure that seeds of Carolina hemlock could be shipped internationally for *ex situ* conservation without problems, two experiments were initiated to better determine seed handling methods. These dealt with a) stratification and seed germination and b) aluminum phosphide fumigation. There was great concern about the response of Carolina hemlock seeds to fumigation prior to international seed shipment, a phytosanitary requirement of many countries in Latin America where the seeds could be sent. The two experiments are described below.

Table 1. Seed collections of Carolina hemlock made by Camcore in 2003 and potential future collection sites.

Prov. #	Provenance	County/State	Latitude & Longitude (Dec. Deg.)	Elevation (m)	Avg. County Rainfall (mm)	# Trees Collected
1	Linville Falls	McDowell, NC	35.94 N 81.92 W	995	1372	10
2	Table Rock	Pickens, SC	35.04 N 82.73 W	956	1422	3
3	C. Hemlocks Campground	Yancey, NC	35.80 N 82.20 W	823	1567	10
4	Caesar's Head	Greenville, SC	35.11 N 82.63 W	933	1364	4
5	Cradle of Forestry	Transylvania, NC	35.35 N 82.78 W	1017	1688	8
6	Wildcat	Watauga, NC	36.20 N 81.52 W	297	1382	10
7	Hanging Rock	Stokes, NC	36.39 N 80.27 W	146	1217	5
8	Bluff Mountain	Ashe, NC	36.38 N 81.54 W	1375	1288	8
9	Crabtree	Yancey, NC	5.80 N 82.20 W	1132	1567	6
10	*Dragon's Tooth	Roanoke, VA	37.37 N 80.17 W	852	1080	---
11	*Sinking Creek	Craig, VA	37.33 N 80.33 W	1009	1001	---
12	*Cripple Creek	Wythe, VA	36.75 N 81.17 W	766	1046	---
13	*Tallulah Gorge	Rabun, GA	34.73 N 83.38 W	576	1859	---

*Rainfall is an annual county average taken from http://www.city-data.com/.

EXPERIMENT 1. STRATIFICATION/GERMINATION

Most references for stratification and germination methods for hemlock are based on experiments with *T. canadensis* (Godman and Lancaster 1990, U.S. Forest Service 1948), *T. mertensiana* (Ruth 1974, U.S. Forest Service, 1948) and *T. heterophylla* (Edwards 1995, U.S. Forest Service 1948, Packee 1990). The only reference to Carolina hemlock seed stratification found for this study suggests that treating seed for 30 days @ 4°C on a moist paper towel, then sowing on a 1:1 coarse sand-potting soil medium, and covering pots with plastic film improves germination (Brown 2002). Recommendations from the past research listed above guided us in the development of seed treatments for Carolina hemlock.

For the stratification experiment, media (sand, paper, 24-hour water, or none) and days in stratification (0, 1, 30, 60, or 90) were evaluated in order to determine the most effective germination method. A seedlot of commercial seed of western hemlock (*Tsuga heterophylla*) from British Columbia, Canada, was included as a control. The addition of western hemlock raised the total number of "provenances" to 10 (nine Carolina hemlock provenances plus western hemlock). After cone collection, seed was extracted and separated by mother tree, and provenance bulk seed packets were prepared. To create the provenance bulks, equal numbers of seed were pooled from all trees collected in one population (e.g., Linville Falls) to reach 160 seeds. This seed was then divided into sixteen provenance bulk packets of 10 seeds each. These 16 packets were then separated into two groups of eight to comprise two replications. Each provenance bulk seed packet (e.g., Linville Falls) was stored at 4.5°C and received one of the treatments listed below:

Replication 1—8 packets

Control (no moisture)	Moist paper 30 days	Moist paper 60 days	Moist paper 90 days
24-hr. water treatment	Moist sand 30 days	Moist sand 60 days	Moist sand 90 days

Replication 2—8 packets

Control (no moisture)	Moist paper 30 days	Moist paper 60 days	Moist paper 90 days
24-hr. water treatment	Moist sand 30 days	Moist sand 60 days	Moist sand 90 days

All seed received cold treatment during storage. Twenty provenance bulk seed packets (10 "provenances" x two replications) received moist sand, and 20 seed packets received moist paper at the beginning of the experiment. Thirty and 60 days later, 20 more packets received the same treatments (10 "provenances" x two replications at each time marker). Upon completion of sand and paper stratification, an additional 20 packets were placed in water stratification for 24 hours, while the remaining 20 packets received no treatment (controls) other than unmoistened cold stratification.

Following stratification, seed was sown and germination data were recorded and processed. Seed was removed from bags and planted in germination trays with a 1:1 coarse sand/Metromix™ media and dusted with vermiculite. Trays were watered and assessed for germination daily for four weeks. Germination data were summed by provenance and treatment, then formatted and arcsine-transformed using SAS® Version 8e to normalize their distribution and satisfy ANOVA prerequisites (SAS Handbook 1999). Western hemlock data was removed from the dataset to prevent biased results. The data from the stratification media study were analyzed using PROC MEANS and PROC GLM at the ± = .05 level.

EXPERIMENT 2. FUMIGATION

Phytosanitary restrictions in many countries require that seeds be fumigated prior to shipment when originating in the U.S. due to specific pathogen risks. After a literature search proved ineffective regarding fumigation effects on the germination of Carolina hemlock seed, a preliminary study was initiated at Camcore to expose hemlock seed to the various fumigation treatments required for shipment to Latin America and to evaluate their effect on seed germination.

Generally, phytosanitary requirements for shipment of seeds to Latin America require a dosage of 2.5g/m^3 of aluminum phosphide or methyl bromide, but differ in the temperature and duration of treatment. The four approved protocols meeting the strictest requirements for seed shipment to Latin America range along a gradient from the most severe of four days at 26°C to the lightest treatment of seven days at 15°C commonly used for sensitive seed. Due to limitations at the fumigation facility, however, the lightest treatment (seven days at 15°C) was not evaluated for this study. The protocols tested are listed in Table 2.

Seed was selected from three of the nine Carolina hemlock provenances collected, representing provenance bulks with high, medium, and low germination in previous tests. Four 100-seed provenance bulks were made from each of the three Carolina hemlock provenances chosen. Each 100-seed bulk had equal representation from all families collected from each provenance in 2003. A *Tsuga heterophylla* control of known germination rate from British Columbia, Canada, was added for comparison. Control packets of *T. heterophylla* contained only 58 instead of 100 seeds due to limited seed availability.

After fumigation, seed was assessed for germination at NCSU. The seed was cold water-stratified for 24 hours, and then placed in germination trays under 24-hour illumination with 100 seeds per dish (only 58/dish for *T. heterophylla*). Germination was assessed daily, and seedlings were transplanted into ray leach tubes after germination for uses in adelgid screening studies at Camcore.

FLORAMAP® TOOL AND SITE SELECTION PROCESS

To help determine where Carolina hemlock could be planted outside of its natural range for *ex situ* conservation, the FloraMap® climatic model was used. FloraMap® was developed at the Center for Tropical Agriculture (CIAT) by Jones and Gladkov (1999) to locate new populations of native agriculture species. This model uses monthly average temperature, monthly diurnal temperature, and average monthly precipitation from sites of known natural populations to predict where similar climates and new populations might possibly occur. The advantage of using Floramap® over other climatic models is that is has an extensive climatic database for Latin America and Africa.

For our purposes we used Floramap® to predict where Carolina hemlock could be planted and grown successfully rather than as a tool to identify where other populations might be found. The latitude/longitude coordinates of the 13 known Carolina hemlock populations in the Appalachian Mountains and Upper Piedmont were entered into the database (Table 1) with temperature weighted slightly higher than rainfall; 1.13 to 0.90, respectively. Temperature was considered more important than rainfall because the species needs a cold period in order to produce seeds while precipitation is not a limiting factor in southern South America. FloraMap® produced a worldwide map denoting pixels (18 sq. km) where the species had a 30% or greater probability of occurring (surviving).

RESULTS AND DISCUSSIONS

STRATIFICATION AND GERMINATION EXPERIMENT

Stratification media was found to significantly ($p = 0.0105$) affect germination in Carolina hemlock (Figure 2). The Paper vs. Sand treatments were significantly different ($p = 0.0002$). Seed stratified in sand had statistically lower germination than with the other media. No significant differences were seen among Paper, 24-hour water, and Control stratification media. Our results indicated that Carolina hemlock seed should not be germinated in moist sand.

Results also suggested stratification length had little effect on germination results (Figure 3). The control treatment received no additional inputs beyond cold storage, as compared to the treatment durations of 24-hr., 30 days, 60 days, or 90 days for the other treatments, but provided statistically similar germination results. Based on these data, cold storage (4.5° C) of seed after collection will provide similar germination results, and additional stratification is not necessary.

FUMIGATION EXPERIMENT

Germination percentages of Carolina hemlock seed subjected to different fumigation rates of aluminum phosphide at 8 weeks are reported in Table 2.

Our results indicate that if Carolina hemlock seed must be fumigated using one of these protocols, the application rate of 2.5g/m^3 aluminum phosphide for six days at 17-20°C should be used. This method was clearly the least damaging to seed germination percentages compared to the controls. It must be noted, however, that the additional protocol for seven days at 15°C was not evaluated for this study due to temperature control constraints, and the trends here suggest that germination percentages for this treatment may exceed the results for six days at 17-20°C.

FLORAMAP® SITE MATCHING

The FloraMap® model predicted that Carolina hemlock could be moved to the west coast of Oregon and Washington of the United States, areas in central Chile, and very restricted areas in southern Brazil. Eastern hemlock has been successfully established in trial plots on the west coast of the U.S., and so the prediction of successful movement of Carolina hemlock to that region seems to make sense.

Predictions for potential planting sites for Carolina hemlock in Brazil include the plateau region of Santa Catarina State from approximately 25 to 26° S latitude. The elevation of the plateau averages approximately 800 m, and the area receives approximately 50 frosts per winter depending on the location. Average rainfall is between 1,500 and 1,800 mm per year and relatively evenly distributed. The area is one of the most productive regions for growing *Pinus taeda* (loblolly pine) in the world.

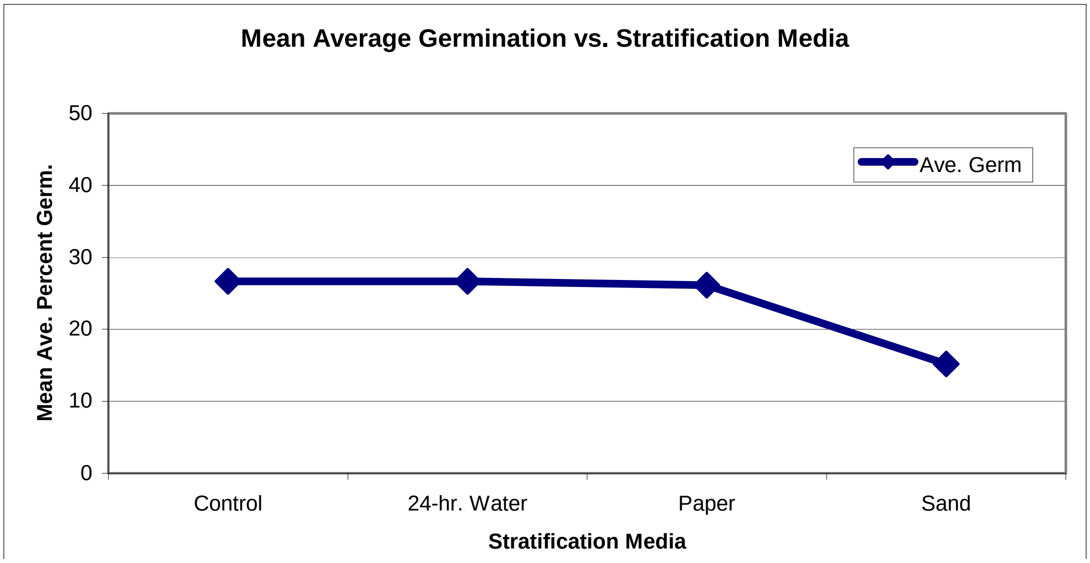

Figure 2. Assessment of stratification media for germination.

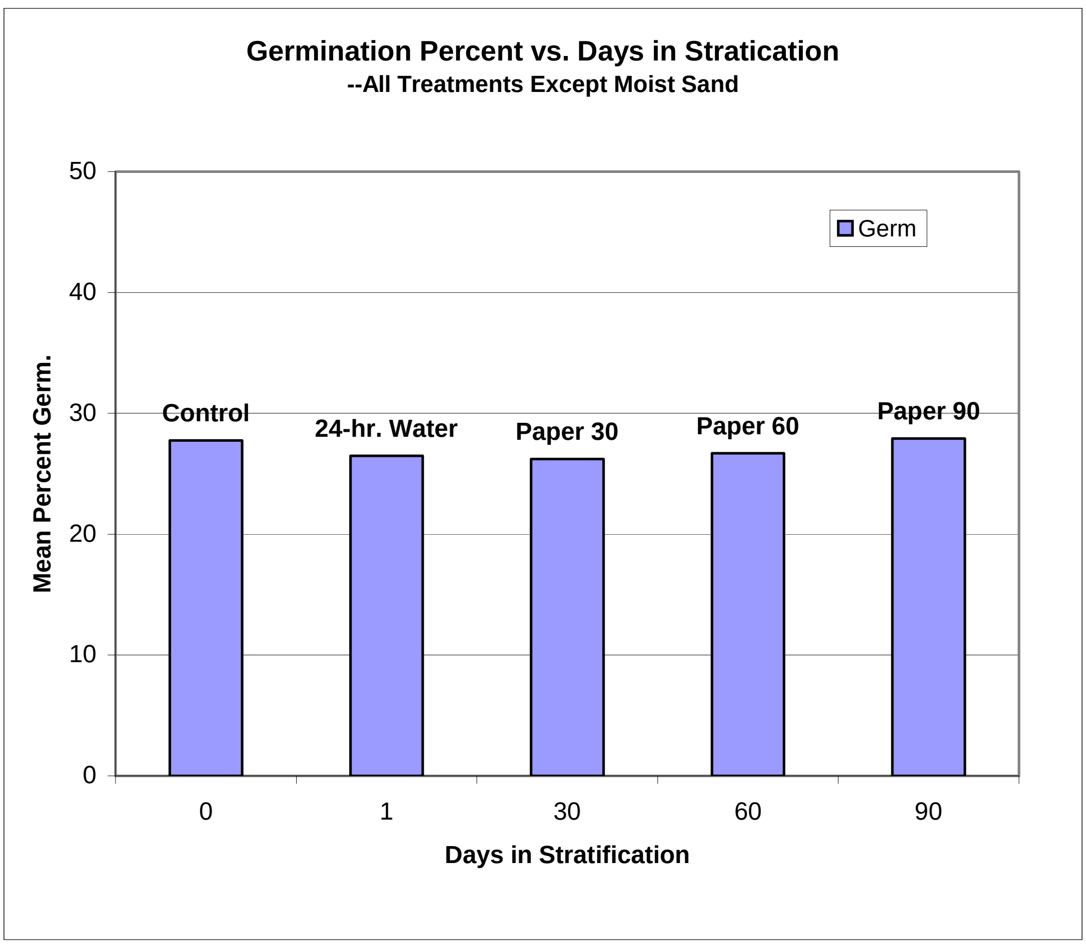

Figure 3. Comparison of duration of stratification time, excluding sand.

Table 2. Germination results of Carolina hemlock seed at eight weeks after fumigation.

Provenance	Treatment	Treatment Length (days)	Treatment Temp. (°C)	% Avg. Germ. After Fumigation
Low Germinator				
Wildcat	fumigated	6	17-20	6
Wildcat	fumigated	5	21-25	0
Wildcat	fumigated	4	26.0	0
Wildcat	control	N/A	4.5	5
Moderate Germinator				
Cradle of Forestry	fumigated	6	17-20	40
Cradle of Forestry	fumigated	5	21-25	0
Cradle of Forestry	fumigated	4	26.0	0
Cradle of Forestry	control	N/A	4.5	62
High Germinator				
Linville Falls	fumigated	6	17-20	52
Linville Falls	fumigated	5	21-25	0
Linville Falls	fumigated	4	26.0	0
Linville Falls	control	N/A	4.5	41
#61133				
T. heterophylla	fumigated	6	17-20	53
T. heterophylla	fumigated	5	21-25	0
T. heterophylla	fumigated	4	26.0	0
T. heterophylla	control	N/A	4.5	81

The general area predicted by FloraMap® where Carolina hemlock should survive in Chile is between Concepción and Valdivia (37 to 39° S latitude). This region of the world, climatically speaking, is a mirror image of the west coast of Washington and Oregon. Planting could occur in the low-lying hills along the coast or in the foothills of the Andes where frosts are more frequent and poorer soils exist. Rainfall in the region is abundant and can range from 2,000 to 2,500 mm, depending on the area. Central Chile is a prime growing region of *Pinus radiata* (radiata or Monterrey pine).

EX SITU CONSERVATION EFFORTS

Our goal is to successfully collect seeds from at least 10 trees in every known population of Carolina hemlock in the eastern U.S., which means re-sampling some stands in North and South Carolina and initiating seed collections in outlier regions in Georgia, Tennessee, and Virginia. Ideally, we would like to plant this material on two sites: one in Brazil and the other in Chile. We believe a sample of 150 trees from approximately 15 populations would adequately conserve the genetic diversity of the species. Assessments of population genetic diversity of Carolina hemlock are being initiated at North Carolina State using molecular markers in a collaborative effort between Camcore and the NCSU Christmas Tree Genetics Program. The number of samples needed per population may be adjusted as we learn more about how genetic diversity is structured between and within populations of Carolina hemlock.

Camcore has identified industrial members in both Brazil (Klabin-Santa Catarina) and Chile (BioForest-Arauco) who are willing to assist agencies in the U.S. in the *ex situ* conservation efforts for Carolina hemlock. These Camcore members in Latin America are experts in plantation forestry of *P. taeda* and *P. radiata*, respectively. However, because Carolina hemlock requires different types of nursery and silvicultural protocols than the pines, success will depend on all partners working closely together to ensure that the best technologies are in place to grow this threatened conifer. If *in situ* conservation and pest management plans are successful in the southeastern U.S., the Carolina hemlock material conserved in Brazil and Chile will never need to be re-introduced into the U.S. However, based on the local experience with American chestnut, the success of seed collections in the U.S. and the subsequent establishment of field trials in Brazil and Chile might be the only hope for Carolina hemlock in the southeastern U.S. in the future.

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PRESERVING EASTERN HEMLOCK GENE POOLS THROUGH *EX SITU* PLANTINGS

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ABSTRACT

Preservation of host species germplasm is a critical component in the overall response to destruction by serious exotic forest pests such as hemlock woolly adelgid. In 1995 and 1996, seed collections of eastern hemlock, *Tsuga canadensis* (L.) Carr., were made in the Great Smoky Mountains National Park (in Tennessee and North Carolina), New York, and Massachusetts to establish *ex situ* plantations of eastern hemlock. The seedlings were grown in containers for three to four years or in containers for two years and transplant beds for two years. In the late winter of 2000, four plantations were established New York (3) and Massachusetts (1). A single plantation was established in the Cumberland Mountains in Tennessee in 2001. Two plantations in upstate New York failed after two continuous years of drought coupled with deer browse. The third New York planting, on the U.S. Military Academy, has good survival and growth, probably related to protection from deer browse. The Massachusetts plantations are surviving, but are infested with hemlock woolly adelgid. Survival and growth are satisfactory in the Tennessee plantation. Challenges in seed collection, nursery production, and planting hemlocks were discussed. The surviving plantations will be eventually managed for seed production to restore locally adapted hemlocks to areas where the species has been decimated by the hemlock woolly adelgid.

KEYWORDS

Eastern hemlock, hemlock woolly adelgid, *ex situ* plantings, germplasm preservation.

INTRODUCTION

The *Tsuga* (Endl.) Carr. genus consists of about 10 species of evergreen, conifer trees native to North America, Japan, China, Taiwan, and the Himalayas. In the United States, eastern hemlock, *Tsuga canadensis* (L.) Carr., has a wide range, occurring naturally from New England to the Georgia mountains and as far west as eastern Minnesota. The species is the most shade-tolerant of all the forest tree species in North America (Godman and Lancaster 1990). For this reason, the species often persists in the under-story or mid-story of the forest for years. Eastern hemlock can take 250-300 years to reach maturity and can live up to 800 years. Approximately 2.3 million acres in eastern North America are dominated by hemlock forests (McWilliams and Schmidt 1999). The trees have a wide variety of habitats, but are most commonly associated with moist forest coves and mountain streams in the southern Appalachian mountains.

Eastern hemlock plays an important ecological role in the eastern forest. It provides a dense and valuable habitat for wildlife such as white-tailed deer (*Odcoileus virginianus* Zimmermann), ruffed grouse (*Bonasa umbellus* L.) and wild turkeys (*Meleagris gallopavo* L.). Several breeding birds are thought to depend on the presence of hemlock for their survival, including blackburnian warbler (*Dendroica fusca* Lath.), black-throated green warbler (*Dendroica virens* Lath.), and blue-headed vireo (*Vireo solitarius* Wilson). Eastern hemlock shades mountain streams, which helps maintain the cool temperatures essential for stream health and the presence of certain aquatic species, such as brook trout. Evans (2004) reported that "the average number of aquatic macroinvertebrate taxa found in hemlock streams was 37% greater than that found in hardwood streams (55 versus 40 taxa). Fifteen macroinvertebrate taxa were strongly associated with hemlock streams, and three taxa were found only in hemlock streams."

In the southern Appalachian mountain region, hemlock glades are critical habitat for many salamander species. This region is considered to be the largest center for salamander diversity in the world. Downed hemlocks provide excellent habitat for salamanders, both in rotting wood and under exfoliating bark. Fallen trees are not only beneficial to salamanders, Ducey and Gove (1999) speculate that coarse woody debris may play an integral role in regeneration of eastern hemlock. Losses of older growth, large trees might not only reduce the seed source, but also reduce the conditions favorable for seed germination and survival. Economically, eastern hemlock was once used as a source of tannin for leather (Godman and Lancaster 1990) and has been important in the pulp and paper industry in modern times. Hemlock is also an important ornamental tree species, often grown by the nursery industry.

Eastern and Carolina (*Tsuga caroliniana* Engelm.) hemlock populations are currently threatened by the exotic insect, hemlock woolly adelgid (*Adelges tsugae* Annand.). Although this adelgid was introduced into the United States approximately 75 years ago from Asia (Annand 1924) it has only become problematic to hemlock populations in eastern forests. The adelgid has caused extensive mortality to eastern hemlock over a large extent of its range. In the United States it has been detected from northeastern Georgia to southeastern Maine and west to eastern Tennessee. It damages trees by inserting piercing, sucking mouthparts into the base of needles and extracting plant fluids. There is also speculation that adelgids insert toxic salivary secretions while feeding. Adelgid activity causes needles to turn brown and drop. Severe infestations can cause death in as few as four years, but trees can often persist for much longer periods of time. The influence of other insects, fungal pathogens, and environmental factors can exacerbate the impact of the adelgid.

Unlike most other coniferous species utilized for pulp and timber, very little is known about hemlock genetics and diversity (cf. Campbell and Schlarbaum 2002). Aside from some genetic plantations in New England that were established in the 1950s (Olson et al. 1959), eastern hemlock has not been included in tree improvement efforts. Correspondingly, no seed orchards have been established.

Although field studies on eastern hemlock genetics have been limited, there have been ecological and biochemical studies on genetic variability in the species. Kessell (1979) addressed the genetic basis for eastern hemlock's distribution. He found two distinct ecotypes of eastern hemlock, a "high response" and a "low response" type, based on external morphology. The high response hemlock is highly moisture sensitive and demonstrates high growth rates and tolerance to low temperatures. The low response hemlock can also be found on moist sites, but is more common on drier slopes. It has a slower growth rate, low sensitivity to moisture, and high sensitivity to temperature. In the northern portion of the range of the tree, the two forms are distinct with very little intermixing. However, in the Allegheny and Southern Appalachian mountains, the forms introgress to produce a multitude of intermediate types, although these populations still have individuals corresponding to the "high" and "low" response eco-types.

Zabinski (1992) tested disjunct populations of eastern hemlock for isozyme variation, but found very few genetic differences. Based on the morphological evidence of Kessell (1979), she recommended that further research on eastern hemlock include a more comprehensive survey of genetic variation that encompasses a range of morphological and physiological differences.

Schaberg et al. (2003) studied rare alleles in eastern hemlock and found that rare alleles either decreased or increased depending on silvicultural treatment. When trees were selectively cut (small and poor form trees were removed), rare alleles decreased. When trees were diameter-limit cut, rare alleles increased. This indicates that if some rare alleles are lost, the species' ability to adapt to environmental changes could be affected. Alternately, if rare alleles increase then the fitness of the stand is compromised.

The threats posed to native trees by exotic, invasive organisms are well recognized, yet most conservation efforts focus on pest management. In the southern Appalachian region, gene conservation or preservation has been minimal (cf. Schlarbaum, et al. 1999; Campbell and Schlarbaum, 2002). American chestnut [*Castanea dentata* (Marsh.) Borkh.], flowering dogwood (*Cornus florida* L.), American beech (*Fagus grandifolia* Ehrh.), and butternut (*Juglans cinerea* L.) are all examples of tree species that have been severely impacted by an exotic organism without systematic efforts toward genetic conservation.

A complete solution to an exotic pest problem involves conservation or preservation of germplasm (Schlarbaum et al. 1999). Reintroduction of a species after the pest problem is controlled should use locally adapted genotypes. *Ex situ* preservation of germplasm is one method to ensure the availability of locally adapted seedlings. With forest tree species, *ex situ* plantations are desirable because of delay in reproductive maturation in many tree species. An existing plantation that is managed for seed production will provide seeds in the volumes needed for reintroduction much quicker than seedlings from stored seed.

In 1995, a cooperative project was formed to collect seeds of eastern hemlock from different locations to subsequently establish *ex situ* plantations for eventual conversion to seedling seed orchards. The seeds from the orchards will be used in reintroduction of local genotypes into areas where hemlock populations had been extirpated by hemlock woolly adelgids.

MATERIALS AND METHODS

COOPERATORS

Initial contacts of interested parties were made by the USDA Forest Service, State and Private Forestry, Forest Health Protection, in Asheville, North Carolina. Participants included: the University of Tennessee's Tree Improvement Program (UT-TIP); Finch, Pruyn & Co., Inc. (FP); U.S. Military Academy (USMA) at West Point, New York; Massachusetts Department of Conservation and Recreation (MDCR); and Great Smoky Mountains National Park (GSMNP). The UT-TIP coordinated the seed collection, growing of experimental material, plantation design and analyses, and distribution of plantations. Finch, Pruyn & Co., Inc., provided the funding to initiate the project.

SEED COLLECTION

Seed collections of eastern hemlock were made in proximity of Glens Falls, New York (FP), West Point, New York (USMA), Amherst, Massachusetts (MDCR), various locations in eastern Tennessee, and in the Great Smoky Mountains National Park. Open-pollinated progeny were collected in the 1995 and 1996 fall seasons. The cones were sent to the UT-TIP and air-dried. Seeds were extracted and processed according to the Woody Plant Seed Manual (USDA Forest Service 1974). The seeds were germinated the spring seasons following the year of collection. The seeds were sown in small planting trays and lightly covered with vermiculite. When the resulting seedlings reached the cotyledon stage, they were transplanted into Cone-Tainers™ or Root-Trainers™ each containing approximately 5 cu. in. of commercial soil mix and medium grade pearlite enriched with organic matter.

SEEDLING PRODUCTION

Seedlings from the 1995 collection were grown for two years in containers and then transplanted to the East Tennessee State Nursery near Delano, Tennessee. At the nursery, the seedlings were placed in a transplant bed for one year and then moved to another transplant bed for an additional year. The seedlings from the 1996 collection were grown in containers for three to four years.

PLANTATION ESTABLISHMENT

Four plantations were established with a combination of transplanted seedlings and containerized seedlings in 2000. Two plantations were established in open fields by FP near Glens Fall, New York; one planting was established in a black birch/ eastern hemlock forest by MDCR near Amherst, Massachusetts; and one planting was established under a forest canopy on the reservation surrounding the U.S. Military Academy. A fifth plantation was established in 2001 on a cleared field, using stock grown in Cone-Tainers™, in the Cumberland Mountains of eastern Tennessee on the University of Tennessee's Cumberland Forest Experiment Station (UT-TIP). The fifth plantation was established at a later date, as the site was not ready for planting in 2000.

The FP plantation had tree mats placed around each seedling to prevent competition that were periodically mowed. The USMA plantation was fenced to protect against deer browse and rub. The UT-TIP plantation was mowed after establishment and herbicide sprayed around the trees to control competing vegetation. The MACR plantation received no maintenance.

STATISTICAL DESIGN AND ANALYSES

The experimental design for the plantations was an incomplete block using three tree row plots. All plantations received a mixture of seed sources. The number of families varied at each location.

Only the USMA and UT plantations were measured for survival and height growth. The USMA plantation was measured in 2002, 2003, and 2004, while the UT-TIP plantation was measured only in 2004. The MDCR plantation was observed annually (except for 2003) for attack by hemlock woolly adelgid and identification of potential resistant trees. A mixed model analysis (SAS 2002) was conducted on data from both plantations. For the USMA plantation, the two types of seedlings (transplanted and container) were first nested within family in growth and survival analyses and then combined in a second group of analyses. Analyses to detect differences among seed sources were also conducted. The UT plantation was analyzed to detect family differences in survival and growth.

RESULTS AND DISCUSSION

NURSERY GROWTH

Eastern hemlock seedlings grew relatively slowly in comparison to various pine species, necessitating a longer nursery period before plantation establishment. It was observed that the seedlings in Cone-Tainers™ were generally smaller and less dense than seedlings grown in Root-Trainers™. The seedlings from the 1995 collection were transplanted to nursery beds due to increased size. Similar treatment of the seedlings from the 1996 collection did not occur due to lack of resources. The seedlings did respond favorably to transplanting and were much larger and more robust than the containerized seedlings at planting.

The variety of containers used and uneven treatment (i.e., transplant vs. containerized) of the seedlings was due to the project's primary objective of germplasm preservation rather than genetic improvement. Additionally, the resources supporting this project were very limited, and the seedlings were placed in whatever containers were available at the time.

FIELD STUDIES/FUTURE PLANS

The FP plantations initially had good survival and satisfactory growth. A two-year drought coupled with deer browse, however, reduced the survival to approximately 10 percent in each plantation. Surviving trees are generally under 1 foot in height due to repeated deer browse. Tree shelters will be erected around the surviving trees to salvage what is left of the plantation.

The MDCR plantation was not browsed by deer, but became quickly infested with hemlock woolly adelgid. The majority (85-95%) of the hemlock overstory at this site was infested with the pest. Seedlings, both bare root and in plugs, from various locations were received and out-planted in black birch/eastern hemlock forest in Amherst, Massachusetts. The survival rate of containerized seedlings (90.9%) was different than transplanted seedlings (58%). As of December, 2004, the containerized seedlings had 32 seedlings that were not infested (12.0%), and the transplanted seedlings had only 7 seedlings (13.7%) that were not infested with hemlock woolly adelgid.

It is unknown why the survival rate of the containerized seedlings was higher than that of the transplanted seedlings. It is interesting to note that the percentage of seedlings that have not been infested is approximately the same for both types of seedlings. Data on annually infestation was not available for this paper, but it will be analyzed in the future to see if any family was more susceptible to early infestation. The plantation will continued to be observed for adelgid infestation and a decision to protect the study will be made when it is clear that there are no genotypes resistant to hemlock woolly adelgid.

Analyses of the USMA plantation revealed no difference in survival and growth among the seed sources. Family (seedling type) and family differences were both evident in survival, but not growth. A net loss of growth occurred between 2002 and 2003, probably due to drought.

The USMA plantation will continued to be maintained and eventually converted into a seedlings seed orchard. Hemlock woolly adelgids have not yet attacked the plantation, and protection will be afforded when it occurs.

The UT-TIP plantation showed no family differences in survival or height. Deer browse has been moderate, but portions of the plantation have suffered minor damage from all-terrain vehicles. The plantation will be maintained and protected against hemlock woolly adelgid until approximately age 10. At that time, the plantation will be converted into a seedling seed orchard and all non-GSMNP genotypes will be removed.

CONCLUSIONS

Ex situ germplasm preservation in plantations is an expensive venture, particularly when approached by widely separated cooperators. The slow growth of the eastern hemlock seedlings caused an extended nursery phase that required constant maintenance in the growing season. If creating seed orchards that will provide seed for reintroduction efforts is the primary objective, then grafting local genotypes from different watersheds would be a more efficient approach to seed production: grafts would produce seeds much earlier than seedlings. In addition, a grafted orchard could be established on a grid, making maintenance and seed collection easier than in a thinned genetic plantation.

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Host Interactions of the Balsam Woolly Adelgid

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ABSTRACT

The balsam woolly adelgid (BWA), *Adelges piceae* Ratz., has caused immense damage to native and planted fir stands for the past century. This paper highlights the current understanding of the initiation of damage by the insect and the host responses to the insect. Although most native North American firs (*Abies* spp.) are highly susceptible to the adelgid, other firs exhibit a range of resistance and some can tolerate even heavy infestations. Possible mechanisms for resistance are discussed.

INTRODUCTION

The balsam woolly adelgid (BWA), *Adelges piceae* Ratz. (Hemiptera: Adelgidae), an inconspicuous insect that feeds on true firs, has caused considerable damage in North American fir stands for the last century. Native stands of mature Fraser fir (*Abies fraseri* [Pursh] Poir.) in the southern Appalachians have come close to elimination (Dull et al. 1988), and the adelgid has altered the composition of the surviving stands (Jenkins 2003). The balsam fir (*Abies balsamea* [Linn.] Mill.) forests of New England and Canada and the mixed fir forests of the Pacific Northwest have been continually plagued by the insect for almost 100 years and the natural stands of Fraser fir in the Southern Appalachians for over 50 years. Grand fir (*Abies grandis* [Dougl.] Lindl.) is gradually being eliminated from low elevation landscapes in the Pacific Northwest and subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.) is being removed as a pioneer tree species in the Cascades (Mitchell and Buffam 2001).

Accidentally introduced into Maine and Nova Scotia around the turn of the 20th Century, (Balch 1952), most likely on imported nursery stock of European silver fir (*Abies alba* Mill.), BWA had become established on balsam fir in Maine by 1908 (Kotinsky 1916). The adelgid was first reported on Fraser fir in the Southern Appalachians on Mount Mitchell in 1957 (Speers 1958) and quickly spread throughout the natural range of Fraser fir. On the West Coast, it was first reported on ornamental firs near San Francisco (Annand 1928). In the Pacific Northwest, it was first reported on grand fir in the Willamette Valley of Oregon (Keen 1952). There, the most frequent hosts are Pacific silver fir (*A. amabilis* [Dougl.] Forbes) and subalpine fir at high elevations and grand fir in valleys (Mitchell et al. 1961). The adelgid was reported in British Columbia on Pacific silver fir and on Vancouver Island on grand fir by the late 1950s (Silver 1959).

SUBSPECIES OF THE BWA

Three geographic subspecies of *A. piceae* Ratz. have been identified in North America (Foottit and Mackauer 1983). *Adelges piceae piceae* (Ratzeburg 1844) has been observed in British Columbia, the Pacific Northwest, and in the native Fraser fir range in the Southern Appalachians. This subspecies corresponds morphologically to Pschorn-Walcher and Zwolfer's *forma typica* (1956) and the 'intermediate group' of Foottit and Mackauer (1980). *Adelges piceae canadensis* (Merker and Eichhorn 1956) is found on balsam fir in Quebec and the maritime provinces of Canada, and in the northeastern United States. *Adelges piceae occidentalis*, subsp. nov. has been observed on Pacific silver fir and grand fir in British Columbia and may be the result of a single introduction. The subspecies are based on the morphological differences of first instars and adults between the three subspecies (*e.g.*, body shape, length, dorsal plates, fusion of pleural and mesial plates, shape of pore fields of mesial plates, number and range of wax pores, etc.).

LIFE HISTORY OF *ADELGES PICEAE*

In North America, *A. piceae* exhibits an anholocyclic mode of generation on fir and does not migrate to spruce (*Picea*), as it does in its native range. The sistens form is wingless and develops on the stem (lenticels, crevices in bark, or callus tissue), branches, or bases of buds (Crystal 1925, Balch 1952). The number of sistens generations per year depends upon temperature and host condition. There is only one hiemosistens generation per year and as many as three or four aestivosistens generations. The sistens—both hiemosistens and aestivosistens—develop through four stages after emerging from the egg: first instar (crawler, neosistens), second and third instar, and adult. The progrediens form, which never overwinters and rarely enters into any period of diapause, can be wingless (aptera) or winged (alata) and develops on the needles of fir (Crystal 1925, Balch 1952, Varty 1956). The progrediens form, observed only in the subspecies *A. piceae canadensis* in North America (Foottit and MacKauer 1983), arises from the first eggs laid by the hiemosistens, differs morphologically from the sistens, and has a shorter embryonic developmental period than the sistens (Eichhorn 1969).

INITIATION OF DAMAGE TO INDIVIDUAL TREES

Balch (1952) described the process of feeding, a summary of which follows. *Adelges piceae* has piercing-sucking mouthparts (stylets) and feeds on parenchyma tissue within the outer 1.5 mm of bark or on twigs at the base of buds. The stylets are inserted through the phellem (epidermis) into the phelloderm (cortex), probing between cells, until a suitable feeding site can be located. Salivary secretions, which can flow into intercellular spaces, are exuded from the tip of the maxillae to form a sheath that lines the path of the stylets. The neosistens inserts its stylets full length before entering diapause. Feeding occurs through repeated partial withdrawal and reinsertion of the stylets in a new direction. The stylets are withdrawn and completely renewed at each molt, with the new stylets inserted near the original point of entry.

The tracks are branched and affect tissue in a 360° pattern. Aphids feed in a similar pattern and are able to assess feeding sites by intermittently ejecting and sucking back up a watery saliva along with soluble material from the host; this watery saliva is diffused into surrounding tissue and can be transported within the host plant (Miles 1965 and 1999). Because of the close relationship between aphids and adelgids, and the many similarities in feeding patterns and capabilities, it is likely that adelgids also secrete the watery saliva, which is then transported through the tree.

The adelgid's salivary secretions appear to create a chemical imbalance within the tree and disrupt the development of normal tissue. Auxin-like compounds (Balch et al. 1964) and pectinase (Adams and McAllan 1958, Forbes and Mullick 1970) have been found in adelgid saliva. Additionally, the gel-like stylet sheath may often form a barrier around damaged parts of parenchyma cells, preventing the disintegration and rupture of cell vacuoles, which would normally be followed by the production of autotoxic defensive compounds in surrounding cells (Miles 1999). The stylet sheath can also slow down or prevent the production of the phenolics that arise from and promote necrosis by adsorbing and immobilizing the phenolics (Miles 1999).

RESPONSE OF *ABIES* SPECIES TO *ADELGES PICEAE* ATTACK

All *Abies* species are susceptible, in varying degrees, to some species of adelgid throughout the world, and susceptibility to *A. piceae* attack varies widely. Firs native to North America (subalpine fir, balsam fir, and Fraser fir) are highly susceptible. Some western species (grand fir, sacred fir [*Abies religiosa* (H.B.K.) Schlecht. et Cham], and noble fir [*Abies procera* Rehd.]) and firs native to central Europe tolerate infestation (Varty 1956, Mitchell 1966), while some Asian species (Veitch fir [*Abies veitchii* Lindl.] and Momi fir [*Abies firma* Sieb. et Zucc.]) appear to be immune to attack, at least in North America (Hall et al. 1971).

Adelgid infestations follow a general trend. The initial infestation begins with a few large trees, generally with deep fissures, and then spreads to nearby trees. The population peaks and when many trees have been killed or are damaged, the population diminishes. This second period may go on for an indefinite period of time and is characterized by increasing gout and gradual dying of trees. Some trees may nevertheless recover. The first outbreak is generally the most severe (Balch 1952), but the infestations can persist for decades, and many stands are gradually succumbing to the stress (Mitchell and Buffam 2001). Stem attack is generally observed in continental climatic zones and twig attack in maritime zones (Greenbank 1970, Schooley and Bryant 1978). Mass stem infestations can cause tree mortality within two to three years. With a crown infestation, the tree will suffer branch dieback and general deterioration, and it can take as long as 10 to 20 years for mortality to occur (Bryant 1974).

Some species exhibit a 'bottom-up' pattern of infestation, wherein the infestation begins on the lower portion of the stem and works upwards towards the crown over time (e.g., in grand fir [Mitchell 1966] and Fraser fir [Amman and Talerico 1967]). Others exhibit a 'top-down' pattern, with the infestations beginning in the crown and working downward (e.g., in balsam fir [Greenbank 1970]). All ages of fir in plantations are susceptible, particularly with

Fraser fir, while in natural stands, older, mature trees are more susceptible. This begs the question as to whether there is something in younger trees—physical or chemical—that protects them from severe infestations.

Infestations are generally classified as "stem" or "crown" infestations. The damage, both microscopic and macroscopic, is fairly consistent among all susceptible firs. With a crown infestation, gouting is apparent. Growth of wood and bark is stimulated at the point of stylets insertion and both hypertrophy and hyperplasia occur. An enlargement of parenchyma cells (up to six or seven times the size of normal cells), including cell walls and nuclei, occurs and these large cells are continually produced, causing swelling of the twigs and in the bark (Balch 1952). Pock-like swellings are often observed on the bark of young trees (Rudinsky 1956). The number of rays increase (Doerksen and Mitchell 1965), as do the number of parenchyma strands (Smith 1967). Large reductions in carbohydrate reserves of needles and twigs have been observed (Puritch and Talmon-De L'Armee 1971). Increasing numbers of these enlarged parenchyma cells disrupt the phloem channels and interfere with the metabolic pathways in the bark (Bryant 1971). Bud growth often ceases and the twig begins to die back from the ends—a flattened top is often observed in infested trees (Balch 1952, Mitchell 1967). In fact, this loss of apical dominance (observed in stem infestations as well as crown infestations) is often the first symptom looked for in Fraser fir Christmas tree plantations when scouting for *A. piceae* infestation (Sidebottom 2004). In addition to gouting, the number and length of branches and the length of the stem are often reduced in a crown infestation (Balch 1952, Schooley 1974).

With a stem infestation, although the stylets of the adelgid can be up to 5 mm from the vascular cambium, xylem production is often compromised resulting in abnormal wood called 'rotholz' or 'redwood'. Rotholz is anatomically similar to compression wood in conifers (Balch 1952). These resultant tracheids are circular rather than rectangular (Timell 1986); are short, thick walled, and highly lignified with small lumens (Doerksen and Mitchell 1965); have a reduced number of conducting pits (Puritch and Petty 1971), higher specific gravity, and greater fibril angle (Foulger 1968); and often have encrusted pit membranes such as those found in heartwood (Puritch and Johnson 1971). Traumatic resin ducts may form in the xylem (Saigo 1976). Sapwood permeability in grand fir has been reduced to 5% of that of normal sapwood (Puritch 1971). Ultimately, these changes throw the tree into physiological drought, reducing the flow of water within the tree and compromising photosynthesis, transpiration, and respiration (Puritch 1973). Heavy infestations can result in tree mortality in one to two years (Balch 1952). Adelgid infestation appears to contribute to premature heartwood formation (Puritch 1977). Rotholz production has also been associated with a significant increase in the proportional area of heartwood (Hollingsworth et al. 1991) and higher percentages of latewood (Doerksen and Mitchell 1965, Smith 1967).

The changes that take place in the bark of the fir trees initially create a more favorable environment for adelgid development, and in the early stages of an infestation fecundity is at its peak; as the trees weaken and the population begins to dwindle, fecundity also decreases (Pschorn-Walcher and Zwolfer 1958, Amman 1970).

HOST RESPONSES THAT MAY AID IN RESISTANCE

Some responses to adelgid attack may aid in the resistance or tolerance to infestation. Many conifers have the ability to form a secondary periderm, consisting of necrophylactic tissue, around a wound (Mullick 1975, Hain et al. 1991). This wound healing mechanism can isolate an area of bark occupied by the adelgid and can effectively protect the underlying bark from further attack for years (Balch 1952, Mullick 1971). It has been hypothesized that the formation of the protective secondary periderm may be inhibited or compromised by chemicals secreted by the adelgid's stylets (Mullick 1975, 1977), though subsequent research has both supported (Hay and Eagar 1981) and failed to support (Arthur and Hain 1985) this hypothesis.

Juvabione or juvabione-like compounds may be produced in response to adelgid attack, as has been evidenced in grand fir and Pacific silver fir (Puritch and Nijholt 1974) and Fraser fir (Fowler et al. 2001). Juvabione-like compounds, which were first discovered in 1965—the famous 'paper factor' of Slama and Williams (1965) isolated from paper towels produced from balsam fir fiber (most likely adelgid-infested balsam fir)—mimic insect juvenile hormones and have been shown to inhibit or disrupt normal development in a number of insects, including *A. piceae* (Fowler 1999). It is possible that, in mature firs that exhibit some resistance or tolerance to BWA, there is a rapid accumulation of chemicals (monoterpenes and juvabione-like sesquiterpenoids) at the site of attack that may interfere with adelgid development. Younger trees or seedlings may be protected by a naturally high accumulation of juvabione-related compounds.

Some firs, particularly grand fir, produce copious amounts of resin in response to wounds. This may serve as a means of resistance, as grand fir is one of the more resistant species of American firs, with only 20-30% mortality associated with adelgid infestation (Mitchell 1966). Many conifers have evolved resin-based defenses, such as oleoresin—a mixture of terpenoids consisting of a terpentine and rosin fraction—to deter insect pests and their symbiotic fungal pathogens. True firs store only small amounts of primary resin in bark blisters, but respond to wounding by producing oleoresins in nonspecialized, adjacent tissues. But increased resin production may be only a short-term solution to BWA. For example, in *A. grandis*, after two to five years of adelgid feeding, a portion of bark below the surface dies, the bark turns black, and this is accompanied by a heavy resin flow. This discourages further attack, but after a few years, the resin hardens and the bark develops fissures, whereupon the adelgids are able to reinfest the same areas (Mitchell 1966).

OTHER FACTORS THAT MAY AID RESISTANCE

The onset of water stress (physiological drought), one of the chief factors in susceptibility to damage, differs among fir species, and those exhibiting a more rapid onset are more susceptible to intense damage (Varty 1956, Mitchell 1967). It follows that species or individual trees within a species that exhibit high levels of drought resistance would also be more resistant or tolerant of BWA.

Production of thick outer bark, as found in European silver fir, has been associated with resistance to or recovery from adelgid attack (Pschorn-Walcher and Zwolfer 1958, Schooley and Bryant 1978).

The texture of the bark may also be an important factor in resistance or susceptibility. Rough or flaky bark may be more preferable for *A. piceae* for a number of reasons, including the interception of airborne eggs and crawlers, stereotropism (the insect is stimulated to grow or change in response to touch), and more easily accessible nutritive areas (*e.g.*, lenticels and bark crannies) composed of young parenchyma (Varty 1956). In fact, lenticel development has been observed to be 'the best single predictor' for BWA population levels on Fraser fir in the Great Smoky Mountains (Hay and Eagar 1981).

Some provenances of Fraser fir appear to respond to adelgid infestation differently than others. For example, BWA infestation was not discovered on Mount Rogers until 1979, although there was evidence that some trees had been under attack for up to 17 years (Haneman et al. 1981), and high mortality was not observed until the 1990s. Mount Rogers' trees become heavily infested but do not appear to suffer rapid mortality (Hollingsworth and Hain 1991, Nicholas et al. 1992). The fir trees on Mount Rogers have been shown to be genetically different (unique allele frequencies) from other natural populations of Fraser fir (Ross 1988), but these differences have not been correlated with susceptibility to adelgid infestation (Hain et al. 1991). Nonetheless, Mount Rogers trees have been shown to form lower levels of rotholz and higher levels of secondary periderm in response to adelgid attack when compared to trees from Mount Mitchell (Hollingsworth and Hain 1992). It has been suggested that these trees may possess the ability to develop secondary periderm more rapidly than those in other locations—this characteristic may aid in adelgid tolerance (Hay and Eagar 1981). Additionally, the trees on Mount Rogers may be less likely to suffer water stress when infested partly due to environmental factors (deep soil, little wind) (Hollingsworth and Hain 1994).

Within the natural range of Fraser fir, although the majority of mature fir trees have been killed, there are remnant populations that have survived multiple decades of adelgid attack. It remains to be seen as to whether these trees have survived as a result of genetic resistance or whether the environment (most are at the highest elevations) plays a more important role.

Among and within all *Abies* species, even those highly susceptible to BWA, trees have been observed time and again to tolerate and even recover from adelgid attack. It is not an uncommon occurrence for balsam fir to recover from adelgid attack after a few years with a replacement of the original leaders and relatively small stem deformities from gouting (Balch and Carroll 1956, Schooley 1976, Schooley and Bryant 1978). Although Mount Mitchell Fraser firs suffered high mortality during the initial wave of infestation, have been shown to be highly susceptible to adelgid damage (Dull et al. 1988), and individual trees cored at Mount Mitchell showed production of rotholz at some point, the trees were uninfested and otherwise appeared healthy (Hain et al. 1991).

CONTINUING THE RESEARCH

Much of the current research focuses on understanding interactions between adelgid, host tree, and the environment. Putative genetic resistance—even within a highly susceptible species such as Fraser fir—is believed to exist, and studies are underway to begin the process of screening across and within *Abies* species for host resistance to BWA. Bark and wood chemicals associated with resistance are being isolated, and studies into the genetic components of resistance continue. The adelgid itself continues to be studied and a method of artificially rearing the insect is currently being developed. Biological control continues to be an area of interest, particularly in Christmas tree plantations at lower elevations.

Balsam woolly adelgid research is as critical now as ever. The insect is a tremendous problem in Christmas tree plantations and costs growers thousands upon thousands of dollars each year. Native stands of Fraser fir exist only in small island populations at the highest elevations in southern Virginia, western North Carolina and eastern Tennessee. Most mature trees were killed during the initial phase of adelgid infestation, but fortunately, many areas were repopulated with young natural seedlings. These regenerated stands have now reached the age wherein they become more susceptible to adelgid attack. The adelgids have continued to exist at low levels in the native stands, and the question arises as to whether the regenerated firs are more resistant to the adelgid than their predecessors. Adelgid populations should be evaluated to determine whether they are increasing, and the compounding effects of air pollution on the physiology of both tree and adelgid should be further assessed.

Researchers continue to delve into the interactions between the balsam woolly adelgid and its host species, advancing the understanding of both insect and host genetics and physiology. The hope remains that host resistance mechanisms can be identified and that, between the inherent resistance in some trees and the breeding or nurturing of resistance in others, the balsam woolly adelgid can be better tolerated in plantations and natural stands and that Fraser fir will remain an important component in the high elevation ecosystems of the Southern Appalachians.

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A Methodological Approach to Elucidate the Effect of Balsam Woolly Adelgid Infestation on the Structure of Wood and Bark

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Traditional analytical methods used in wood chemistry require a significant amount of time and labor to obtain comprehensive information. Better understanding of the mechanisms of pest-host interaction requires new methodological developments to elucidate changes in the chemical structures in components of various parts of the tree (wood, bark, foliage) triggered by infestation. This paper reports development of a rapid and informative approach to elucidate the structures of lignin and polyphenolics with nuclear magnetic resonance (NMR) spectroscopy and its application to track the effect of balsam woolly adelgid (BWA) infestation on the chemical composition of Fraser fir wood and bark. Components of wood and bark have been characterized by wet chemistry methods, correlation 2D NMR techniques (HMQC, HMBC, TOCSY), and quantitative ^{13}C and ^{1}H NMR.

In response to infestation, Fraser fir produces reddish wood (*rotholz*) with a high density and low conductivity that is eventually responsible for the tree death. The *rotholz* wood has a ~15% higher amount of lignin and much higher amount of galactans than that of unifested trees. *Rotholz* lignin has higher amounts of p-hydroxyphenyl (H) units and aliphatic Ohio groups and lower amount of interunit linkages, implying that it is less crosslinked. The degree of condensation of the *rotholz* lignin is not higher than that of normal wood.

Chemical analysis of inner and outer bark samples from infested *A. fraseri*, unifested *A. fraseri,* and resistant *A. veitchii* showed a tendency toward lower amounts of lipophilic extractives and higher amounts of low molecular mass phenols and polyphenols in the bark of the infested trees as compared to the bark of the uninfested trees. An NMR approach developed for comparative analysis of ethanol- and water-soluble fractions allows for the characterization of the phenolic compounds without tedious chromatographic separation. These fractions consist predominantly of flavanoids and condensed tannins with small amounts of phenylpropanoids and lignans. The bark polyphenolics of the infested trees contain much higher amounts of p-hydroxyphenyl moieties and lower amount of guaiacyl (G) aromatic moieties than that of uninfested Fraser fir. The amount of H-moieties in bark polyphenolics of the resistant Veitch fir is also high, but this does not result in a decrease in the G-unit content, in contrast to infested Fraser fir.

USING MITOCHONDRIAL DNA TO DETERMINE THE NATIVE RANGE OF THE HEMLOCK WOOLLY ADELGID

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ABSTRACT

The history of the introduction and spread of the hemlock woolly adelgid (HWA), *Adelges tsugae* (Hemiptera: Adelgidae), is well know for eastern North America where is it causing serious decline of eastern and Carolina hemlocks. However, while it is assumed that these insects were introduced from somewhere in Asia, we do not know their precise geographic origin. Adelgids can also be found on other hemlock species in western North America and east Asia, but these trees are not significantly damaged, suggesting the existence of different varieties of HWA.

The purpose of this study is to use molecular methods to clarify the relationship among hemlock adelgids worldwide, and therefore determine the geographic origin of the introduction to eastern North America. Three mitochondrial genes (COII, ND1, and cytB), providing a total of over 1,500 base pairs of DNA, were sequenced for adelgid samples collected from hemlock in multiple locations in eastern and western North America, China, and Japan to identify their differences.

Phylogenetic analyses indicate that the source of *A. tsugae* in eastern North America was a population of adelgids living on *Tsuga seiboldii* that occurs in the south and at lower elevations in Japan. Adelgids collected in China appear to represent a separate lineage, as do adelgids collected from the other Japanese hemlock species, *T. diversifolia*. It is unclear whether adelgids collected on *T. heterophylla* in western North America represent a native population, or were introduced from an un-sampled population in Japan.

These results indicate that molecular methods can be used to successfully pinpoint the origin of introduced insect pests. In addition, these results can be used to facilitate quarantine efforts by helping to prevent the introduction of other non-native adelgid genotypes, and can help direct resistance breeding and biological control programs.

KEYWORDS

Mitochondrial DNA, phylogeny, native range.

Silvicultural Options for Managing Hemlock Forests threatened by Hemlock Woolly Adelgid

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ABSTRACT

The introduced hemlock woolly adelgid (HWA), *Adelges tsugae*, continues to migrate north into New England, causing widespread hemlock decline and mortality, and an increase in hemlock removal. This outbreak has led to management dilemmas about pre-salvage and salvage logging in hemlock stands: should they be cut down or not? Regardless of the decision made, there is a critical need to review the available options and clearly determine the appropriate goals first, especially if HWA has not reached your forest yet. Although there are various chemical and biological control options for HWA that are effective in ornamental situations, they are not practical or effective at larger scales of stands to landscapes. This paper describes silvicultural options available to help landowners manage their hemlock forests infested with or threatened by HWA.

KEYWORDS

Silviculture, salvage logging, forest management, hemlock forests.

HWA INFORMATION

HWA is widely distributed throughout the eastern United States and moves from 10 to 20 miles per year, transported primarily by wind, birds, and humans. In New England, adelgid movement has been primarily in a northeasterly direction. Tree health and the timing and severity of HWA impacts are influenced by several site and forest factors, including elevation, latitude, topographic position (ridgetop, side slope, hollow, wetland, riparian area etc.), and structure. For example, research has shown that hemlock trees are just as likely to be infested with HWA whether they occur in a hemlock-dominated system or in mixtures with hardwoods or other conifer species (Orwig et al. 2002). It appears that no sites are immune from HWA damage once the insects are firmly established, although hemlock trees growing on ridgetops, on exposed drier sites, or infested with any other secondary pests like scale insects often succumb more quickly to HWA infestation (Bonneau et al. 1999, McClure et al. 2000, Orwig et al. 2002). In addition, extreme cold winter temperatures (below – 5°F or – 20°C) can cause severe HWA population reductions that may temporarily slow the spread and impact of HWA across the landscape (Parker et al. 1998, 1999, Skinner et al. 2003).

SILVICULTURAL OPTIONS

We often desire to manage forests in a way that is most "natural"; however, the current HWA outbreak is novel and many would argue not natural. Harvesting options and related costs differ depending on the unique structure of hemlock in a particular forest and whether the management goal is aesthetics, wildlife habitat, water quality protection, public safety, future successional dynamics, timber revenue, or a combination of these goals. Pre-emptive cutting of uninfested forests is not recommended unless maximizing timber revenue is the main objective, because many questions exist regarding the future dynamics of hemlock and HWA, and cutting could remove potentially resistant hemlock genes. Once a decision has been made to cut hemlock, Best Management Practices (BMPs) should be used to protect forest soils and water quality (Kittredge and Parker 1989, Ward et al. 2004; see also below) during harvesting operations.

For infested hemlock-dominated forests, silvicultural options include:

***Do nothing*:** Infested hemlock trees will die gradually over 4- 12 years depending on site characteristics, and the amount of light reaching the ground will gradually increase. Hemlock mortality will typically result in hardwood establishment, primarily black birch (*Betula lenta*) (Orwig and Foster 1998, Orwig et al. 2002). In Massachusetts and northern New England, white pine (*Pinus strobus*), yellow birch (*Betula alleghaniensis*), oak (*Quercus*), and maple (*Acer*) species may also replace hemlock. In addition, herbaceous plants like ferns and sedges (*Carex* spp.) may establish with the death of hemlock (Orwig 2002). Branches, treetops, and boles will fall over a period of 8 to 15+ years, with little or no scarification (soil disturbance). The dead standing and downed wood will provide valuable wildlife habitat for a variety of bird, mammal, and invertebrate species (Brooks 2001, Tingley et al. 2002). In public areas, doing nothing may require fencing to limit access to hazard trees along trails, roads, and vistas.

***Light selection cut/shelterwood cut*:** This option removes 20 to 50% of the tree basal area, including the dying and heavily damaged hemlock trees throughout the stand or in 0.5- to 1-acre openings. Since more light enters the stand through this treatment than the *Do nothing* option, raspberry (*Rubus* spp.), black birch, and white pine will be stimulated (Kizlinksi et al. 2002), and they can be enriched with plantings (see below). Skid roads and landings used in this treatment can be used for subsequent cuts and/or salvage.

High intensity cutting: This option involves removing more than 50% of the tree basal area and is used if the stand is heavily damaged and/or recovering timber value is the main goal. High light reaches the forest floor, often leading to regeneration of black birch and several weedy species, including raspberry, pokeweed (*Phytolacca Americana*), hay-scented fern (*Dennstaedtia punctilobula*), and—sometimes—invasive species (Kizlinksi et al. 2002, Orwig and Kizlinski 2002). Heavy cutting may also lead to more abundant slash and damage or mortality of residual trees. In many cases, more valuable hardwood species are also removed to increase the value of the timber sale (Brooks 2004), leading to hardwood sprouts from the stumps. The decision to remove species other than hemlock needs to be carefully considered prior to cutting activity. If cutting is done without any regeneration present on steep slopes or near streams, it may pose risks of erosion and nutrient export to streams until newly established vegetation takes up nutrients and impedes overland flows.

For hemlock-hardwood or hemlock-conifer mixes (with or without planting) silvicultural options include:

***Do nothing*:** As with hemlock dominated forests, infested hemlock trees will gradually die over 4 to 12 years, and the stand will convert to a hardwood dominated stand or a mix of hardwoods and white pine. The dead standing and downed wood will provide valuable wildlife habitat for a variety of bird, mammal, and invertebrate species. Often no understory changes will occur if hemlock is a minor component of stand.

***Cut hemlock in groups or throughout stand*:** This option will speed up the conversion to hardwood stands or will facilitate white pine and hemlock regeneration, especially if the stand is not infested or only lightly infested.

If cutting infested hemlock for timber revenue or removing hazard trees is the objective, cutting should begin by the time hemlocks have lost 50 to 75% of foliage, since it is unlikely that they will recover with continued HWA infestation, and they become more hazardous to cut if severely damaged or dead.

PLANTING OPTIONS

Tree planting is not necessary, since trees and other vegetation will reproduce abundantly on their own in the brighter environment caused by hemlock mortality. There are no species that can adequately replace hemlock. However, many species have been planted on sites that have lost or will lose their hemlocks due to HWA or logging (Ward et al. 2004). If conifer trees are desirable, consider planting native species like white pine, red pine (*Pinus resinosa*), or white or red spruce (*Picea glauca* and *Picea rubens*). The exotic Norway spruce (*Picea abies*) has been planted because of its full crown of dark green foliage. If promoting desirable hardwoods is the goal, then various oak species could be planted. If planting in areas of high deer densities (i.e., greater than 20-25/mile^2), seedling shelters and/or fencing may be required to allow the young trees to become established. When planting in logged areas, be aware that black birch and raspberry species will directly compete with any species planted, so planting should immediately follow logging.

HWA/HEMLOCK BEST MANAGEMENT PRACTICES (BMPS)

To reduce the chance that logging activities will spread HWA, consider:

Time of year — HWA has two generations per year and has mobile crawler stages in both late spring and early summer from March through June (McClure 1989). Examine the foliage and logs for the presence of HWA during this time as the pest may be transported on machinery that is moved from site to site, including personal vehicles. If possible, harvest in fall and winter to reduce the risk of transporting the pest and minimize soil disturbance.

Machinery — If harvesting during the months of March through June, power wash logging equipment to remove HWA.

State Quarantines — Vermont, New Hampshire, and Maine currently have quarantines that prevent transportation of hemlock seedlings, nursery stock, logs, lumber, bark, and chips into their states except to pre-approved locations or under specific conditions. Contact individual State Forest Health specialists listed below for details.

Location — Know where your logging is with respect to HWA. Has HWA been identified in the town where logging is taking place? Is it nearby? To find out the current distribution of HWA in your state contact the forest health specialists listed at the end of this paper.

SUMMARY

In conclusion, a variety of silvicultural alternatives are available for forest landowners with hemlock threatened by HWA. The options range from doing nothing to directly influencing vegetation succession with a variety of cutting methods and supplemental plantings, depending on landowner objectives, overall hemlock health, and stand conditions. All options and associated costs should be considered carefully when planning the appropriate management strategies to effectively meet the desired goals.

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STATE FOREST HEALTH COOPERATORS

CONNECTICUT : Connecticut Agricultural Experiment Station, P.O. Box 1106, 123 Huntington Street, New Haven, CT 06504-1106

MAINE: Maine Department of Conservation, Maine Forest Service, 22 State House Station, Augusta, ME 04333-0022

MASSACHUSETTS: Massachusetts Department of Conservation and Recreation, Division of Forests and Parks, Region 4 Headquarters, P.O. Box 484, Amherst, Massachusetts 01004-0484

NEW HAMPSHIRE: NH Dept. of Resources and Economic Development, Division of Forests and Lands, P.O. Box 1856, 172 Pembroke Rd., Concord, NH 03302-1856

NEW YORK: New York Dept. of Environmental Conservation, Division of Lands and Forests, 625 Broadway, Albany, NY 12233-4253

RHODE ISLAND: Rhode Island Dept. of Environmental Management, Division of Forest Environment, 1037 Hartford Pike, North Scituate, RI 02857-1030

VERMONT: Forest Resource Protection, VT Dept. of Forests, Parks and Recreation, 103 S. Main Street, 10 South, Waterbury, VT 05671-0602

For more information on HWA and various control methods, see: *http://www.fs.fed.us/na/morgantown/fhp/hwa/hwasite.html.*

For information about hemlock timber value, see: *http://forest.fnr.umass.edu/stumpage.html.*

The Maryland Hemlock Woolly Adelgid Management Plan

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Hemlocks are a limited resource in Maryland. Although they are relatively common ornamental trees in the Baltimore-Washington urban area, most natural stands of hemlock are found only in the northern and western parts of the state. Hemlocks are normally restricted to riparian areas, and it is estimated that they occur on approximately 50,000 acres in Maryland. Several stands in Garrett and other western counties are valued for their ecological uniqueness and recreational opportunities.

Hemlock woolly adelgids (HWA), *Adelges tsugae*, have been in Maryland since at least the mid-1980s. The first infestations in the state were found in the suburban ornamental hemlocks in the Baltimore-Washington area, and natural stands in the area became infested by 1990. The infestation steadily moved westward and native stands in Frederick and Washington Counties became infested in the early to mid-1990s. Infested hemlocks in Allegany County were found in 1999, and the first infested hemlock in Garrett County (the most western Maryland county) was found in December, 2001.

In areas where HWA has been recorded for 10-15 years, it has resulted in mostly light mortality, but significant hemlock decline. Areas that experienced drought in the late 1990s or have significant infestations of elongate hemlock scale have had the most decline and mortality.

During the past 15 years the Maryland departments of Agriculture and Natural Resources hve been involved in various HWA management activities. Statewide delimiting surveys were conducted in the early 1990s, impact plots were established, there have been several releases of biocontrol organisms, and several trees were injected with an insecticide.

Some high use recreation areas, such as Cunningham Falls State Park, have experienced hemlock mortality and decline. In 2003, a team was assembled to initiate a hemlock management and restoration plan for the Park. More than 50 trees in the Park were injected with imidacloprid, and approximately 50 hazard trees were felled.

In 2003, the Hemlock Woolly Adelgid Task Force was assembled to develop a more unified approach to statewide HWA management. The Task Force included members from the Maryland Department of Agriculture–Forest Pest Management (MDA), Maryland Department of Natural Resources, Maryland Forest Service, Wildlife and Heritage Program, Fisheries, State Parks, USDA Forest Service, and U.S. National Parks Service. After several meetings, the Task Force developed a Maryland Hemlock Woolly Adelgid Management Plan to slow the damage and minimize the impacts of HWA. The objectives of the Plan are to identify HWA infested stands, prioritize stands of highest resource value, and recommend actions in the highest priority stands. Additionally, the Plan is to serve as request for environmental review from the Department of Natural Resources to expedite approval of treatment options.

The first step in the Management Plan is to identify the resource. The experience and knowledge of MDA and other professionals was used to inventory the known hemlock stands throughout the state. MDA staff ground-checked many of the hemlock stands to estimate the hemlock component and describe its health. The Task Force then met to prioritize the hemlock stand treatment based on the recreation, fisheries, wildlife, heritage, and forestry value of each stand. In addition, each stand was assessed for hemlock health and HWA infestation levels. A priority rank was then assigned to each of 150 stands across Maryland. Each stand was digitized into a GIS, and information on rank, hemlock health, HWA levels was added to a database.

By the fall of 2004, the Management Plan was approved by a Department of Natural Resources Management Team. The top ranking 75 stands were then surveyed by MDA staff to assess HWA levels and assess the need for treatment. Two treatment options were selected for stands during the fall of 2004. Part of Rocky Gap State Park was chosen as a site for *Laricobius* beetle release. This part of the Park is inaccessible for other treatment types and is in a designated Wildlands Area. During the fall and early winter of 2004, approximately 150 trees in 15 stands were treated with imidacloprid. These trees were injected using the ArborJet Tree IV system using the IMAJet formulation.

In 2005, additional treatments are scheduled for the high priority sites. Tree injections, soil treatments, and biocontrol releases are treatment options being considered for these sites.

An Overview of Hemlock Woolly Adelgid IPM in Pennsylvania: 1999-2004

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ABSTRACT

Since being introduced into the state in 1969, 42 of Pennsylvania's 67 counties have been infested with the hemlock woolly adelgid. In 2004, Pennsylvania's Department of Conservation and Natural Resources (DCNR) has been using a variety of integrated pest management techniques to manage this destructive insect. We implemented a monitoring program in 2004 that provides township level, georeferenced data on hemlock condition in Pennsylvania. We have had an active biocontrol program for hemlock woolly adelgid since 1999. To date we have released over 186,985 predatory *Sasajiscymnus tsugae* beetles and 600 *Laricobius nigrinus* beetles for use against hemlock woolly adelgid. We have confirmed overwintering and reproduction success of *S. tsugae* in the field. We have identified 86 high value hemlock areas on state forest and state park land that will be chemically treated with imidacloprid in spring 2005. Pre-treatment data was collected on treatment and control trees at eight of the sites in fall 2004. These sites will be used to evaluate the efficacy of chemical treatments in 2005 and 2006. Pennsylvania DCNR is also attempting to manage the introduced elongate hemlock scale. We have released 82,000 predatory *Cybocephalus nipponicus* beetles against this insect since 2003. Our future plans include expanding biocontrol efforts, using remote sensing in uninfested areas, and collaborating with others on hemlock resistance.

KEYWORDS:

Adelges tsugae, Sasajiscymnus tsugae, Laricobius nigrinus, imidacloprid, *Cybocephalus nipponicus.*

INTRODUCTION

Hemlock forest, as classified by Forest Inventory and Analysis (FIA), covers approximately 293,000 acres in Pennsylvania. Hemlock woolly adelgid (HWA) currently occurs in 42 of Pennsylvania's 67 counties, with the current leading edge cutting through the Ridge and Valley Province in the central part of the state (Figure 1). In 2004, Pennsylvania's DCNR developed an integrated pest management plan that outlines our strategies to manage this destructive insect (Appendix 1). Some of the IPM techniques we use to combat the hemlock woolly adelgid include host monitoring using the General Hemlock Survey, biological control, and chemical control of high value hemlocks on state land using imidacloprid.

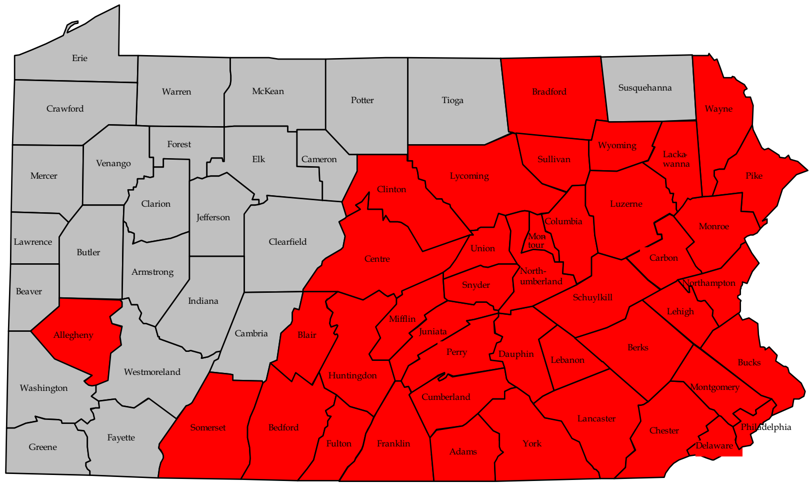

Figure 1. 2004 hemlock woolly adelgid distribution in Pennsylvania.

We currently use *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) and *Laricobius nigrinus* (Coleoptera: Derodontidae) for biocontrol. *Sasajiscymnus tsugae* is a tiny black predator native to Japan that feeds on the spring and summer generations of HWA. *Laricobius nigrinus* is a tiny black beetle that is native to western North America that feeds on HWA; it has one generation per year, and adult *L. nigrinus* feed on HWA from November–March.

The Pennsylvania DCNR's objectives in managing hemlock woolly adelgid are to:

- Identify eastern hemlock sites in Pennsylvania impacted by hemlock woolly adelgid, elongate hemlock scale, *Fabrella tsugae* (Helotiales: Hemiphacidiaceae) needle blight, and other stressors.
- Provide a general overview of hemlock condition in Pennsylvania.
- Release predatory Coleopteran biocontrol agents *Sasajiscymnus tsugae* and *Laricobius nigrinus* in forests infested with hemlock woolly adelgid.
- Assess establishment (overwintering and reproduction) of biocontrol agents at previous release sites.
- Chemically treat hemlock woolly adelgid on selected high-value hemlocks on state forest and state park sites.
- Evaluate chemical efficacy in a subset of treatment sites.

MATERIALS AND METHODS

Pennsylvania's DCNR recently implemented the General Hemlock Survey to provide township-level, georeferenced data of hemlock woolly adelgid infestation, to help focus our biocontrol efforts, and to monitor the statewide condition of hemlocks in Pennsylvania. Evaluators select areas that are a minimum of 1 acre and contain a minimum of 25% *Tsuga canadensis*. Ten branches (collected from three and 10 trees) are randomly selected and inspected for hemlock woolly adelgid, elongate hemlock scale, *Fabrella*, and other stressors, using a hand lens if needed.

Pennsylvania DCNR has been releasing *Sasajiscymnus tsugae* for biocontrol against hemlock woolly adelgid since 1999. Beetles are received from the Philip Alampi Beneficial Insect Laboratory in New Jersey in exchange for infested foliage we provide them. We also purchase beetles from EcoScientific Solutions in Scranton, Pennsylvania. Qualifying release sites are relatively healthy stands infested with hemlock woolly adelgid in central, southern, eastern, and northern areas in Pennsylvania. We usually try to focus releases along the leading edge of HWA infestation. Beating sheet evaluations are used to determine overwintering and reproductive success of these beetles in the field for at least three years post-release.

Pennsylvania DCNR participated in predator efficacy investigations of *Scymnus sinuanodulous* (Coleoptera: Coccinellidae) in 1999, in collaboration with the Connecticut Agricultural research Station (CAES). This involved providing weekly shipments of HWA to CAES in Hamden, Connecticut, and participating in branch bagging experiments.

Our *Laricobius nigrinus* release activities are part of a collaboration with Virginia Polytechnic Institute that began in 2003. The release site includes 15 release trees located in Huntingdon County, Pennsylvania, at N 40.6548, W 77.7371. There is also a nearby control site containing 10 similar trees at N 40.6707, W 77.7080. The release and control areas have healthy hemlocks with low-density infestations of hemlock woolly adelgid. Beating sheet evaluations and sticky panel traps in hemlock canopies have been used in an attempt to recover previously released beetles.

We have identified 86 high value hemlock areas on state forest and state park land that will be treated with imidacloprid in spring 2005. Most sites will be treated through a state-approved contractor, while DCNR personnel will treat a small subset of sites. Trees located on adequate soil that are more than 50 feet from a stream or lake will be treated using soil injections, while those that are closer to water will be treated using stem injections. We will evaluate hemlock condition before and after chemical treatment at eight of the treatment sites (four soil and four trunk injection sites) between 2004-2006.

Pennsylvania DCNR has been releasing *Cybocephalus nipponicus* (Coleoptera: Nitidulidae) for biocontrol against the elongate hemlock scale since 2003. We receive beetles from the Philip Alampi Beneficial Insect Laboratory in New Jersey. We compared scale densities at a control and release tree at one site in Lycoming County Pennsylvania to provide preliminary information on the effectiveness of *C. nipponicus*.

RESULTS

The DCNR surveyed 1,988 sites in 2004 as part of the General Hemlock Survey. Abundant new growth was observed on 80% of hemlocks surveyed, probably due to ample rainfall received during the past two years. 54% of hemlocks surveyed were in light decline, 33% were healthy, 10% were in moderate decline and 3% were in severe decline. HWA was found at 721 sites, while 258 sites contained elongate hemlock scale, 501 sites contained spider mites, and 78 sites contained spittlebugs. Townships with the highest populations of HWA were located in central and eastern Pennsylvania.

The DCNR released 43,890 *S. tsugae* on infested, vigorous hemlocks in central and southern areas of Pennsylvania between March and July 2004 (Figure 2). 186,985 *S. tsugae* have been released since 1999. To date 254 adults and 54 larvae have been recovered at 11 of the release sites using beating sheets in the year following release, including 11 adults recovered in 2004.

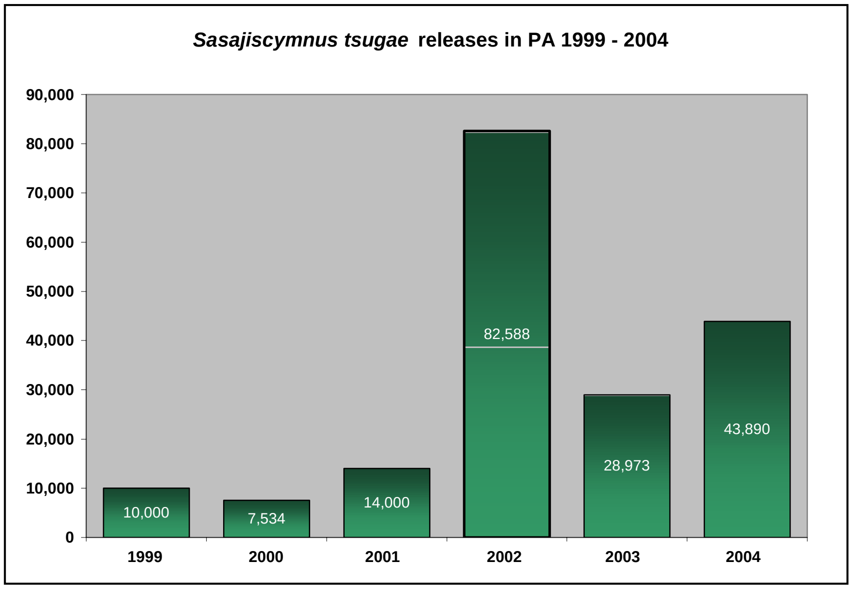

Figure 2. *Sasajiscymnus tsugae* beetles released in Pennsylvania since 1999.

The DCNR released 600 *L. nigrinus* in Huntingdon County, Pennsylvania, in fall 2003 and spring 2004. Evaluations of prior releases of *L. nigrinus* were conducted bimonthly from September to November 2004. No *L. nigrinus* were recovered.

The DCNR has released 82,000 *C. nipponicus* beetles in sites infested with the elongate hemlock scale since 2003. We recovered 40 adult beetles at two release sites in 2004.

This confirms that the beetles are able to successfully overwinter in the field in Pennsylvania. We also noticed decreased scale densities on our release as compared with our control tree at our evaluation site in Lycoming County. However, these results should be interpreted with caution, as our control tree had more new growth than the release tree, and most viable scales were located on new needles. Also, our sample site of one site and one tree are inadequate for the purposes of statistical analysis. However, these preliminary results are encouraging (Figure 3).

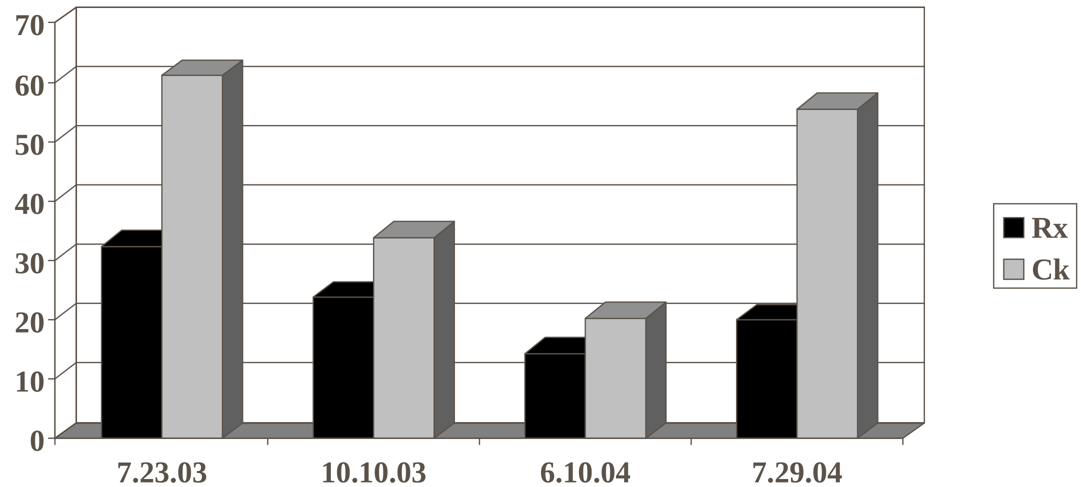

Figure 3. Relative elongate hemlock scale densities. Black bars represent scale density on a hemlock infested with elongate hemlock scale in Lycoming County, where 2,500 *C. nipponicus* were released against *Fiorinia externa* (elongate hemlock scale) in July of 2003. Gray bars represent scale density on a control tree that was located 100 meters away from the release tree.

FUTURE PLANS

Pennsylvania DCNR will continue to explore the use of remote sensing for detecting spot infestations of HWA in northwest Pennsylvania.

Pennsylvania DCNR plans to continue to release biocontrol agents against HWA in future years. We may add additional predators to our current natural enemy complex, including coccinellids in the genus *Scymnus*, as they become available.

Eight sites were established and pre-treatment data were taken in fall 2004. Chemical treatments will be conducted in spring 2005 on all evaluation and regular suppression sites.

We hope to collaborate or assist researchers who are trying to find potentially resistant hemlocks or who are working to develop hybrid species that are genetically resistant or tolerant to hemlock woolly adelgid.

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APPENDIX 1: HEMLOCK WOOLLY ADELGID, *ADELGES TSUGAE* ANNAND, (HOMOPTERA: ADELGIDAE) IPM PLAN

Pennsylvania Department of Conservation and Natural Resources,
Forest Pest Management

DESCRIPTION

The hemlock woolly adelgid, *Adelges tsugae* Annand, was introduced from Japan, where it does not damage *Tsuga diversifola* or *Tsuga seiboldii* due to host resistance and natural enemies. It was discovered in Pennsylvania in 1969. By 2004, 42 counties in the eastern two-thirds of Pennsylvania were infested with this insect (Figure A-1.).

A. tsugae damages *Tsuga canadensis* and *T. caroliniana* in eastern North America by feeding on xylem ray parenchyma. In addition, *A. tsugae* may inject salivary toxins into the hemlock while feeding, accelerating needle drop and branch dieback. Although some trees die within four years, many persist in a weakened state for several years (McClure et al. 2001). Jenkins et al. (1999) found annual nitrification rates to be 30 times higher in sites infested with *A. tsugae,* increasing the potential for nitrate leaching into water and limiting available soil nitrogen.

LIFE CYCLE

A. tsugae populations consist entirely of females that reproduce asexually and complete two generations per year in Pennsylvania. From March to April, sistens (the overwintering generation of adult females) lay 100-300 eggs on cottony masses on hemlock twigs (Figure A-2). In May, immature progrediens crawlers (the spring generation of emerging adelgids) hatch from these eggs and disperse or settle on needle bases to feed. Once settled, immature progrediens nymphs have three instars that mature into adult females by June. Adult females may be wingless or winged. The wingless adults remain on hemlocks, and lay up to 100 eggs in woolly masses on hemlock twigs (Figure A-3). The winged form (sexuparae) disperses to find an alternate spruce host upon which to feed. However, it will die because no suitable host is available in its introduced range. Wingless females are spread by the wind and carried incidentally by birds and other animals. By June-July, crawlers of the sistens generation settle on hemlock (preferring new growth) where they will remain dormant until October. Overwintering sistens nymphs feed and mature on hemlock from October to March (Figure A-4).

MONITORING/SAMPLING PROTOCOL

Look for hemlocks with discolored (grayish) needles and woolly masses on undersides of needle bases. Due to the high variability, high fecundity, and propensity for *A. tsugae* to have an aggregated distribution, Pennsylvania DCNR usually uses presence/absence methods to evaluate populations of hemlock woolly adelgid, rather than absolute population counts.

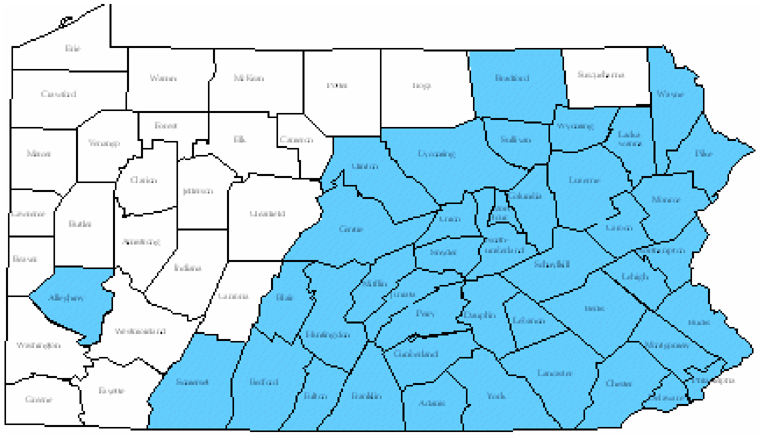

Figure A-1. Infested counties in Pennsylvania.

Figure A-2. *Adelges tsugae* eggs (T.R. Marasco)

Figure A-3. *Adelges tsugae progrediens* adults. Taken from Ravensburg State Park, May 2003 (S.M. Werner).

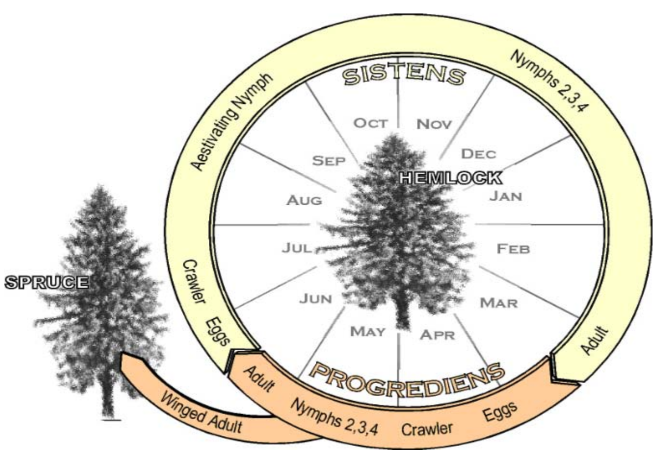

Figure A-4. Life cycle of hemlock woolly adelgid (Vic D'Amico and Mike Montgomery).

Pennsylvania DCNR uses the following method to compare control and treatment trees in our chemical suppression program. The distal 25 shoots of four vigorous branches are selected from the lower crown of a hemlock and tagged with cable ties on each control and treatment tree. All selected branches possess new growth and are infested with live adelgids, as confirmed by the presence of new wool on the branches. Samples targeting sistens on current year's growth will be taken in fall 2004. The same branches, if possible, will be re-evaluated in fall 2005 and fall 2006. On each branch, the number of shoots with new growth, the number of dead shoots, and the number of shoots infested with at least one live hemlock woolly adelgid will be assessed. The presence of other stressors, including *Fiorinia externa*, hemlock borer, and hemlock looper, will also be noted for each tree. The vigor, transparency, and live crown ratio will also be recorded for each tree, each time that *A. tsugae* evaluations are made.

In some cases, it may be necessary to obtain branch samples from tall trees using a shotgun or pole-pruning. Advantages of shotgun sampling are the ability to sample in higher in the canopy where new growth is present. However, it is not possible to count the number of terminals as fragments of branches rather than whole branches are often obtained using this method. Both methods have the disadvantage of being destructive sampling methods, which prevent the evaluator from repeatedly measuring the same branch over time.

Remote sensing technologies are also being evaluated for monitoring *A. tsugae*. Pennsylvania DCNR and Denise Royle, Rutgers University, are working on expanding the algorithm she developed for using Landsat imagery to detect changes in hemlock health in the area from New Jersey to Pennsylvania. Royle and Lathrop (1997) quantified impacts of *A. tsugae* on hemlock health in the New Jersey Highlands using Landsat Thematic Mapper data and a model to compare vegetative index differences in near infrared/red reflectance values. Royle found that, from 1984 to 1994, 47% of the hemlock remained healthy to lightly defoliated, 44% underwent moderate decline, and 9% died. Pennsylvania DCNR and the USDA Forest Service are exploring the use of remote sensing to detect new infestations of *A. tsugae* along the leading edge of infestations and in isolated patches.

MANAGEMENT/CONTROL

Biocontrol Recommended in forest situations, on smaller vigorous trees with moderate to heavy *Adelges tsugae* populations.

Sasajiscymnus tsugae (Coleoptera: Coccinellidae) A tiny, black beetle discovered by Mark McClure in Japan in 1992, *S. tsugae* larvae and adults are only known to prey upon hemlock woolly adelgid, balsam woolly adelgid, and pine bark adelgid. Each larva consumes about 500 eggs or 50-100 nymphs (Cheah and McClure 1998). Adults can live for over a year and consume 50 adelgids/week. *S. tsugae* females lay up to 300 eggs in March and April, during peak egg laying of adelgids. The beetles have a second generation in June around the same time as the second adelgid generation. Adult *S. tsugae* survive on dormant young adelgids during the summer. Pennsylvania DCNR obtains *S. tsugae* from EcoScientific Solutions (Scranton, Pennsylvania) and the New Jersey Department of Agriculture (NJDA), which rear *S. tsugae* on foliage sent to them from Pennsylvania. Releases of *S. tsugae* are focused on *Tsuga canadensis* along the leading edge of the *A .tsugae* infestation in Pennsylvania, on trees that are relatively healthy, and thus have some chance of recovery. At least 2,000 *S. tsugae* are released at sites on sunny days in

May through July. Beating sheet evaluations are used to confirm adult and larval establishment at release sites for at least three years following release. As of 2004, we have released 186,985 *S. tsugae* beetles in Pennsylvania.

Laricobius nigrinus (Coleoptera: Derodontidae) This beetle is found in western North America, where it preys on *Adelges tsugae* on western hemlock, *Tsuga heterophylla*. The genus *Laricobius* feeds only on woolly adelgids. *Laricobius nigrinus* has one generation per year, with adults feeding on HWA sistens in the fall and winter. Adults lay an average of 100 eggs around March 15 on *A. tsugae* sistens ovisacs. Each larva consumes up to 250 *A. tsugae* eggs until mature, when *L. nigrinus* enters the soil to pupate. Adults remain dormant in the soil until fall. Host specificity tests found that *L. nigrinus* preferred to feed on *A. tsugae* over most other woolly adelgids, and it was only able to complete its development on *A. tsugae* (Zilahi-Balogh 2002). Six hundred *L. nigrinus* were released in central Pennsylvania in 2003-2004.

***Scymnus* spp.** Several species in this genus are being investigated for release.

Scymnus (Neopullus) sinuanodulus A univoltine coccinellid native to China, this beetle has a host range restricted to adelgids. Adults lay 130-200 eggs in early spring and feed on all stages of adelgid. Larval *S. sinuanodulus* grow faster and experience lower mortality on adelgid eggs than on nymphs (Lu and Montgomery 2001).

Scymnus ningshanensis Similar to *P. tsugae*, *S. ningshanensis* is found to have greater numerical response in terms of egg laying. Differentiating between males and females is problematic.

Scymnus campodromus Mike Montgomery is investigating this potential predator. It's eggs diapause.

Diapterobates humeralis An oribatid mite that regulates *A. tsugae* in Japan (McClure 1995a), *D. humeralis* doesn't eat eggs but dislodges them when feeding on their wool. But *D. humeralis* is not a good candidate for biocontrol due to low reproductive potential and long generations.

In general, our goal in biocontrol is to pick candidates for release that are effective and as specialized as possible. All organisms go through a testing and approval process with USDA. We may collaborate with that agency in the future to evaluate potential nontarget effects (i.e., on native prey or native competitors) of these biocontrol agents once released into the field.

Chemical Control Practical on an individual tree level for private landowners and public areas with specific high-value trees (e.g., state parks and high use recreational areas).

Imidacloprid For use on large trees after infestation has occurred. Fairly high cost (about 79 cents to $1 per inch of tree dbh) prohibits forest-wide use. Shown to be effective against *A. tsugae*, especially trees with little new growth and no dieback (Steward and Horner, 1994, Webb et al. 2003). Trees under drought stress and those with needle loss and dieback will have difficulty transporting and distributing this systemic insecticide to their canopies (McClure et al. 2001). The 75WP (wettable powder) formulation (Merit) may be applied at mid-concentration (1oz / inch tree dbh) using a Kioritz injector around the base of the tree in areas at least 50' away from waterways (which has advantage of not wounding the tree bark); the chemical is absorbed through the roots, affording at least six months of control. Direct tree bole injections of 5% concentration applied at the rate of ½-1 mL/inch tree dbh should be used for trees closer than 50' to waterways as part of a "best management practice." Preliminary research suggests that soil injection treatments made in the autumn and bole injections made in spring are optimally effective.

1% Horticultural oil More practical for single trees than large areas. May be used to drench trees often < 30' high in April or September and June. This option works by drying and suffocating adelgids and other soft-bodied insects, and is less toxic than conventional insecticides.

WARNING: avoid nitrogen fertilization of infested hemlock! McClure (1991) found **five** times as many *A. tsugae* on fertilized vs. unfertilized hemlocks, and the percentage of surviving nymphs and female egg production was more than twice as high. Results were similar whether fertilization occurred at infestation or 6 months later, indicating that no increased resistance was conferred from fertilization.

Silvicultural Maintain stand vigor by watering area beneath crown dripline during periods of drought to ensure that the tree receives 1 inch of water per week. Selectively remove large trees when heavily infested that act as "reservoirs" for adelgid spread. Replant decimated areas with natives, such as eastern white pine or resistant species, such as the western hemlock (*T. heterophylla)*, which resembles *T. canadensis* in growth form, appearance, and utility (McClure 1995b). Hemlock is known to inhibit its own regeneration through allelopathy. Hemlock litter extracts reduced seed germination by 74% and caused 100% mortality in 6 day old seedlings, but no mortality in seedlings 2 weeks or older (Ward and McCormick 1982).

Cultural *A. tsugae* crawlers often spread through phoresy or "hitchhiking" on birds, plants, mammals, humans, and by wind. Because of this, caution must be used when moving material from infested areas, especially from Mar-June. Spraying infested branches with water in April-June can dislodge eggs and crawlers. Clipping heavily infested branches can also reduce tree populations (McClure 1995b).

Host Tolerance/Resistance/Hybridization It is suspected that one of the reasons *A. tsugae* is so damaging to eastern hemlock is that there is a lack of evolved host tolerance or resistance to this insect's feeding, relative to Asian or even western species of hemlock. One approach to this problem is to identify *Tsuga canadensis* in the field that seem to be surviving *A. tsugae* infestations better than others. This assumes a certain level of intraspecific genetic variation in *T. canadensis*. However, Zabinski (1992) found eastern hemlock to have unexpectedly low genetic variation among seventeen populations examined throughout its range, with the proportion of polymorphic loci being only 0.10 and the number of alleles per locus (among 10 loci examined) being 1.1, significantly lower than many other gymnosperms. Low genetic variation may indicate a population bottleneck during the Pleistocene. Other researchers have found significant differences in photosynthesis, respiration and transpiration among two disjunct populations of eastern hemlock (Eickmeier et al. 1975).

Another option is attempting to hybridize *T. canadensis* with a more tolerant or resistant host. Susan Bentz and Margaret Pooler at the National Arboretum in Washington DC has been involved with hybridization experiments. Attempts to hybridize *T. canadensis* with three Asian species have been unsuccessful (Bentz et al. 2002). Also, attempts to hybridize *T. canadensis* with the morphologically similar western hemlock (*T. heterophylla*) or mountain hemlock (*T. mertensia*) have been unsuccessful because these species are not well adapted to the east coast climate. Brian Maynard at the University of Rhode Island is also testing *T. heterophylla*. His thought is that the seed source for western hemlock has been taken from coastal areas and is not cold hardy north of zone 6. Maynard is targeting seed collected from high elevations in Idaho and southeastern British Columbia.

OTHER FACTORS:

Elevation Hemlock woolly adelgid is not known to occur at elevations over 1,980 feet in its native range (McClure and Cheah 1999). Observations suggest that this is also true in some areas of Pennsylvania.

Weather

Drought Because hemlock is a shallow-rooted species, it is vulnerable to drought and windthrow. Drought can intensify impacts of *A. tsugae* and other stressors. Drought stress over the past five years in Pennsylvania has noticeably increased hemlock decline in several areas. Pollen records show that summer drought in eastern North America that occurred between 5,700 and 5,100 years ago was a major contributing factor in a decline in hemlock that lasted over 1,000 years during that period.

Wind Aerial distribution enhances adelgid dispersal, carrying eggs and nymphs at least 1 km away from infested areas. *Adelges tsugae* crawlers are active earlier in the spring than *F. externa* when winds are stronger and more frequent. Exposure to higher winds (along with its parthenogenic mode of reproduction) is suspected to contribute to *A. tsugae* spreading more rapidly than *F. externa* (McClure 1989).

Heavy rain Precipitation can also affect *A. tsugae* survival by dislodging eggs and nymphs from trees (McClure 1989).

Snowfall/Cold Temperatures High winter mortality can occur in conditions of extremely low temperatures, temperature flux, and high snowfall in North America. Conversely, mild winters in the northeast have resulted in explosive adelgid populations the following seasons.

Negative density dependent feedback (McClure): Like many invasive species, *A. tsugae* can prevent hemlock from producing new growth, and thus ultimately harms its own future survival through increased production of sexuparae and lower fecundity of sistens on old growth.

Other Hemlock Pests: Arthropods—such as elongate hemlock scale (*Fiorinia externa* [Homoptera: Diaspidae]), *Agrallaspis ithacae,* and *Nucleolaspis tsuga),* hemlock borer (*Melanophila fulvogatta* [Coleoptera: Elateridae]), hemlock looper (*Lambdina fiscellaria)*, hemlock needleminer, spider mites, and spittlebugs—and diseases such as Fabrella needle cast, can further weaken trees affected by hemlock woolly adelgid.

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Managing Hemlock Woolly Adelgid and Balsam Woolly Adelgid at Great Smoky Mountains National Park

Kristine Johnson, Glenn Taylor, and Thomas Remaley

ABSTRACT

Great Smoky Mountains National Park (GRSM) was established in 1935 to protect 525,000 acres of the Blue Ridge Mountains in western North Carolina and east Tennessee. It is the largest area in the eastern US managed as wilderness and the most heavily visited National Park. While about 80% of the Park's forests were at least selectively logged (including several billion board feet of hemlock), some of the most extensive old growth forests in the east remain, and a majority of the remote higher elevations were spared (Pyle 1985, Stupka 1964). A 1994 study verified 726 acres of old growth hemlock, with ages over 400 years and tree heights over 160 feet (Johnson 1995 and Yost et al. 1994). Fire suppression has caused an increase in hemlock in the understory of several forest types, including cove and pine-oak, over the past 30 years (Jenkins and White 2002). Hemlocks are a key species as habitat for many upland birds, invertebrates and mammals and shade miles of riparian areas (Shriner 2002).

The Park contains the largest remaining area (48,721 acres) of the southern Appalachian red spruce/Fraser fir forest type (USDA Forest Service 1988). The balsam woolly adelgid (BWA) was discovered in the northeastern section of the Park in 1962; initial management consisted of clearcutting dozens of acres in an attempt to confine the infestation, such as was done to control southern pine beetle. BWA had spread throughout the range of fir in the Park by 1985, when the National Park Service and USDA Forest Service began a program of insecticidal soap spraying in two accessible areas, Clingman's Dome and Balsam Mountain Road. Remnant fir stands have been mapped and bark samples evaluated for juvabione, a juvenile hormone-related compound thought to inhibit BWA reproduction. A genotype preservation plantation was established in cooperation with the University of Tennessee, and a 2001 study delineated remnant stands surviving on several peaks (Kloster 2001). Soap spraying continues on Balsam Mountain Road, and BWA is annually monitored at four locations.

Hemlock woolly adelgid (HWA) was discovered in the Park in April, 2002, near Fontana Lake. The Park had already begun documenting pre-infestation baseline conditions with the 1994 old growth survey and cooperative studies with entomologists at the University of Tennessee and North Carolina State University to inventory invertebrates associated with hemlock (Johnson et al. 1999). A new vegetation map prepared by the University of Georgia (Welch et al. 2002) provided detailed maps of various forest types, which became an important tool in surveying hemlock stands for HWA. This new map shows a total hemlock resource of 87,473 acres in all forest types with a hemlock association, including 5,000 acres of pure hemlock.

Management priorities were established according to tools available (insecticidal soap and oil, systemic insecticides, and biological control), importance of hemlock resources, and accessibility. While the remote old growth stands are the most valuable ecologically, they are the most difficult to treat logistically, and biological control agents are the best possibility for maintaining a viable forest. Roadside and developed areas (campgrounds, picnic areas, and visitor centers) are relatively easy to manage using insecticides. Biological control has been used at a total of 30 sites, and 900 acres of hemlock were treated for HWA with insecticides in fiscal year 2004.

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Don't Color Us In (Yet): Will Regulations and Research Keep Vermont Adelgid-Free?

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ABSTRACT

Vermont is currently free of hemlock woolly adelgid, although natural spread into the state is expected. Recent introductions on nursery stock have demonstrated that inspections in the state of origin cannot reliably exclude the insect and have highlighted the danger of propagating trees outside of the region where they are to be planted. When hemlock woolly adelgid becomes established in Vermont, cold is expected to reduce its impact. Because we do not anticipate "the next chestnut blight," research promises to help us maintain hemlock as a viable species. Recommended actions are to 1) develop reliable survey protocols to delineate infested counties in affected states, including standards for quality assurance, 2) identify strategies to promote local propagation of nursery trees, 3) continue to emphasize biocontrol efforts, focusing on a broad spectrum of agents, and ensuring that safeguards are followed and made widely known, 4) use new technology to produce forest type maps so hemlock stands can be located, and 5) continue research on factors associated with susceptibility and vulnerability and develop management strategies to maintain the values hemlock provides.

DODGING THE BULLET: THE HISTORY OF HEMLOCK WOOLLY ADELGID IN VERMONT

Hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae), is not known to occur in Vermont, although it has spread naturally within ten miles of the state line in Massachusetts. Cold temperatures may have held the insect back, but spread into the state is expected given a period of mild winters. Hemlock occurs statewide and accounts for over 5% of Vermont's trees (USDA Forest Service 2005).

Hemlock woolly adelgid has been introduced via hemlock nursery stock as Vermont is a net importer of hemlock transplants (USDA 1998). The risk of introduction via this pathway was recognized in 1988, when a state quarantine was enacted. Hemlock nursery stock from infested states was admissible only with a declaration that the trees were free from hemlock woolly adelgid (State of Vermont 1988).

In July 1990, hemlock woolly adelgid was detected on tublings that had been planted in a high elevation clearing in the town of Stockbridge. Prior to planting, they had been held in New Jersey near infested trees. Eradication surveys were done twice a year for six years, and all hemlocks located on the site were burned. Live adelgids were found on tublings in 1991, but none were found in subsequent years. This eradication is considered a success, in part because the site is at 2,500 feet elevation, in cold hardiness zone 4, and no native hemlocks occur nearby.

No other introductions are known until May 2004, when infested trees were reported to have come into the state through a wholesale nursery in Hartford, New Hampshire. This nursery had received five hemlock shipments from North Carolina and one from Pennsylvania. All had been certified by state inspectors to be free of hemlock woolly adelgid and were therefore legally imported to Vermont. As a first step in eradicating this introduction, all 413 trees remaining at the wholesale nursery were cut and burned on site.

In subsequent tracing, it was found that 100 trees from the same wholesale nursery were still at retail nurseries, and 160 had been planted in the landscape. An emergency order, issued by the state, required that all of these trees be removed and destroyed by June 25. The chance of a successful statewide eradication was good, since trees had been on-site for only a few weeks. The impact of alternatives, such as no action or a more limited action, could be great, since potentially infested trees had been planted statewide. The state provided no compensation for the trees removed, but landscapers were compensated for their labor if they chose to remove trees for their clients.

Fourteen hemlocks from the wholesale nursery had been sold cash-and-carry from retail nurseries. An attempt was made to find these trees through mass media. Although none of the fourteen were located, many requests were received for tree inspections. These included one for a recent planting of hemlocks from a New Hampshire nursery that proved infested. These trees, and another planting from the same nursery were destroyed.

In all, hemlocks potentially infested with hemlock woolly adelgid had been shipped to or planted in 33 towns (Figure 1). These sites will be monitored to ensure that no adelgids had moved to existing trees. Two-thirds of the planting sites had native hemlocks within 100 feet. The presence of nearby hemlock, plant hardiness zones, and additional information from the GIS-based pest risk assessment project will be used to prioritize monitoring efforts.

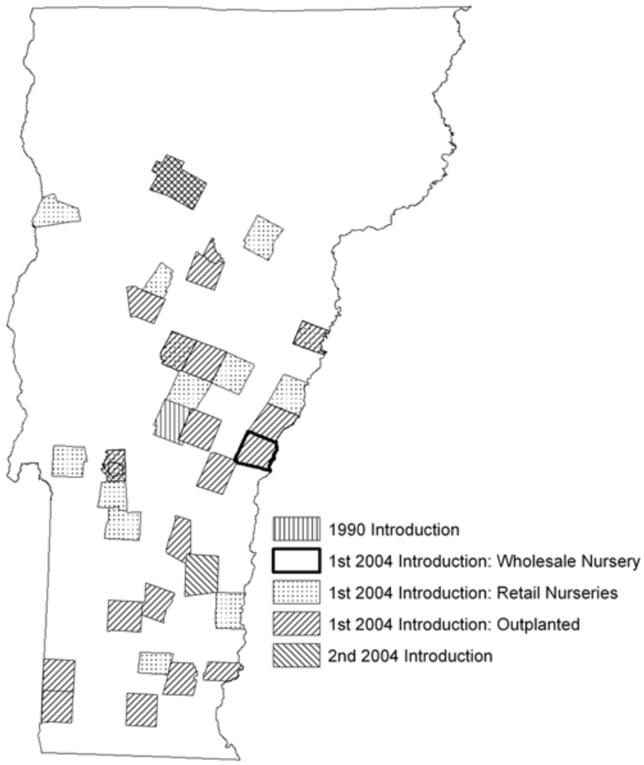

Figure 1. Vermont towns where hemlocks potentially infested with hemlock woolly adelgid were held or shipped, 1990-2004.

WILL REGULATIONS KEEP VERMONT ADELGID-FREE?

Vermont is now the only eastern state in the range of hemlock without an established population of hemlock woolly adelgid. While it is not realistic to count on excluding it forever, delaying its introduction will increase the lead time for research to develop pest management strategies.

The 2004 introduction demonstrated that inspection in the state of origin does not ensure that nursery stock will be free of hemlock woolly adelgid. With its tiny life stages, the insect is difficult to detect at trace levels on densely sheared trees. Vermont is amending its regulations to prohibit all hemlock nursery stock from the area under quarantine, defined as the counties listed in the U.S. Forest Service "List of Counties and States with Known Hemlock Woolly Adelgid Infestations" (USDA Forest Service 2003).

The regulations on hemlock logs, lumber with bark and chips will remain essentially unchanged. These are admissible from areas under quarantine, to sites with a compliance agreement. In fact, there has been little demand for hemlock logs from other states, and no mills have requested compliance agreements.

By contrast, there is a large volume of logs from infested areas being transported through Vermont, mostly to mills in Canada. It is plausible that hemlock woolly adelgid could be introduced on these logs. However, no infestations elsewhere are known to have developed via this pathway, and trucking through the state is not regulated.

The proposed quarantine changes will require that non-quarantined areas adjacent to quarantined counties be surveyed annually and found negative for hemlock woolly adelgid. It is not specified how this is to be done. Quarantine regulations will only work if good protocols with adequate quality assurance are established for delineating the extent of hemlock woolly adelgid on this continent. Developing protocols will be a challenge, since most new infestations are, in fact, located by reports from the public and incidental sightings.

Transport of forest pests on nursery stock is nothing new: that's how white pine blister rust arrived in North America. Although we now appreciate the risk of importing live trees from other countries, restrictions on the state-to-state movement of trees focus on a few pests. Hemlock woolly adelgid, emerald ash borer, and the sudden oak death pathogen have all proved their ability to hitch a ride to new states on nursery trees. To prevent these pests, and species we have yet to recognize from using this pathway, efforts should be made to promote local propagation of nursery trees. Possible strategies might include extension efforts to teach propagation methods, a "locally grown" green certification, and incentives through local economic development groups.

WILL RESEARCH KEEP US ADELGID-FREE?

While research will not keep us adelgid-free, research results may prevent it from eliminating hemlock in Vermont. Once hemlock woolly adelgid has become established in the state, the impact of cold temperatures should slow the rate of hemlock decline. This should provide opportunities to maintain hemlock as a viable species if efforts are guided by research results.

The lesson from balsam woolly adelgid is encouraging, demonstrating that cold-limited insects cause less damage as they reach the northern edge of their range. This insect has been in Vermont for nearly a century, and yet balsam fir remains the second most common tree species in the state (USDA Forest Service 2005). Although balsam woolly adelgid sometimes builds up to tree-killing levels, cold winters are frequent enough to knock any outbreak back before catastrophic damage occurs (Decker et al. 2005). The contribution of introduced predators is unknown; these include *Laricobius erichsonii*, which was introduced to five Vermont towns in 1961-62.

The lesson from gypsy moth is also encouraging, demonstrating that biological control efforts can pay off. Also introduced nearly a century ago, the first gypsy moth outbreak in Vermont lasted over a decade. More recently, outbreaks have followed a pattern similar to the native forest tent caterpillar (Figure 2), thanks to introduced natural enemies.

Diverse biological control efforts are underway for hemlock woolly adelgid and many show promise. While the research community follows protocols aimed at preventing negative

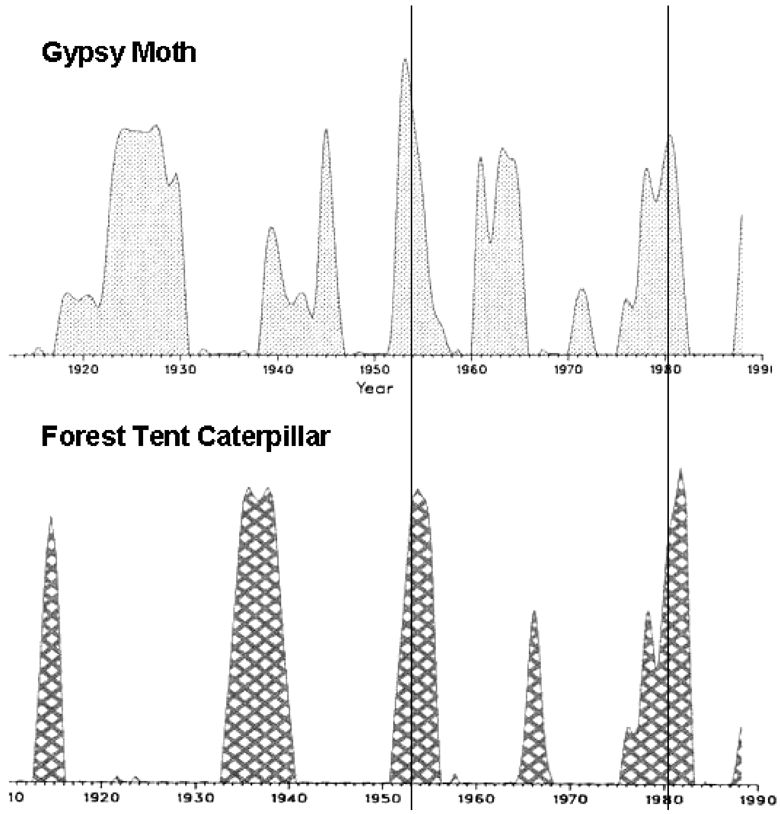

Figure 2. Gypsy moth and forest tent caterpillar population levels in Vermont, 1890-1988. From Parker et al., 1989.

consequences from introduced biocontrol agents, the public remains skeptical. Existing safeguards are not widely known. More transparency in this process would help.

While waiting for hemlock woolly adelgid, existing research results can be used to locate hemlock and prioritize stands for treatment. New technology promises that forest type maps can be produced at a resolution useful on the ground. Like many states, Vermont would need assistance in turning this promise into reality.

Once stands are located, risk assessment maps being developed will allow us to incorporate the expected impact of hemlock woolly adelgid into forest management plans. Continued research into factors associated with susceptibility and vulnerability would help refine these maps. And as hemlock woolly adelgid becomes established, we will benefit from the results of research into strategies which will help us increase stand resistance, maintain crown closure and prevent mortality where possible, and, where necessary, schedule salvage and manage stand conversion.

ACKNOWLEDGMENTS

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Where Are We Now and Where Do We Need to Go?

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ABSTRACT

The Hemlock Woolly Adelgid Management Initiative is an integrated pest management (IPM) plan that develops and implements management strategies that reduce hemlock woolly adelgid (HWA) impacts. This symposium is one product of the HWA Initiative. The manuscripts and posters presented represent a significant increase in our knowledge and demonstrate how this information is used to implement management actions in the field. I commend the authors of both oral and poster presentations for their efforts and sharing this information.

The spread and impact of HWA continues at an alarming rate, particularly in the South, and we cannot afford complacency. We need effective management tools for resource managers faced with the onslaught and aftermath of this devastating pest. We will continue to assess our knowledge, focus on critical data gaps, and accelerate efforts to implement promising management strategies. As part of the HWA Initiative and in response to the information provided at this symposium, the following areas of research, technology development, and management are currently planned or will be considered for further action in the near future.

KEYWORDS

Hemlock woolly adelgid, HWA Management Initiative, management tools, research, technology development.

BIOLOGICAL CONTROL

Biological control is an important component of the HWA Initiative and is crucial to minimize hemlock impacts in forest ecosystems.

PLANNED ACTIVITIES:

1. Continue to rear, release, and evaluate for establishment and efficacy *Laricobius nigrinus*, *Scymnus sinuanodulus, and Sasajiscymnus tsugae.*
2. Accelerate foreign exploration efforts in China and Japan to locate additional natural enemies and streamline the overseas screening process to expedite the shipment of promising species for further evaluation and, if appropriate, their release and establishment.
3. Evaluate the environmental and ecological factors influencing establishment and spread of biological control agents.
4. Continue to evaluate the role pathogens play in controlling HWA populations and assess their potential as a management tool.

PROPOSED ACTIVITIES:

1. Test the hypothesis that a complex of natural enemies is needed to effectively reduce and maintain HWA below damaging thresholds.
2. Accelerate efforts to develop an artificial diet suitable for rearing HWA predators.

CHEMICAL CONTROL

Chemical treatment of individual trees or a group of trees is costly and labor-intensive, but current technology does offer a short-term alternative for protecting high-value trees.

PLANNED ACTIVITIES:

1. Prepare a risk assessment covering the use of imidacloprid in forest environments.
2. Develop cost-effective methods to detect and quantify imidacloprid parent compounds and its metabolites.
3. Continue to evaluate more cost-efficient means of applying chemical treatments.
4. Continue to provide technical and financial assistance to state and federal agencies to suppress HWA infestations on public lands.

PROPOSED ACTIVITY:

- Evaluate hemlock wound response to trunk-injected systemic treatments.

HOST RESISTANCE AND GENETIC PRESERVATION

Many areas have been severely impacted by HWA infestations, and resource managers are now facing rehabilitation and restoration decisions. The genetic diversity of eastern hemlock and Carolina hemlock may be threatened by the continued spread of HWA.

PLANNED ACTIVITIES:

1. Initiate seed collections of eastern hemlock species throughout their range to preserve genetic diversity and provide for reestablishment, if needed.
2. Establish suitable locations for planting collected Carolina hemlock seed outside of the general HWA-infested area.
3. Identify genetic and/or chemical host resistance mechanisms.

PROPOSED ACTIVITY:

- Determine whether existing hemlock species or cultivars have resistance to HWA and can occupy niches currently occupied by eastern hemlock.

SILVICULTURAL MANAGEMENT

Recommendations for silvicultural management of hemlock in advance of and following HWA infestations are limited. Preemptive cutting of eastern hemlock prior to infestation has become a common practice in New England.

PLANNED ACTIVITY:

- Determine whether improving tree vigor through various thinning practices in advance of an infestation can reduce hemlock mortality following an infestation.

PROPOSED ACTIVITY:

- Establish demonstration areas where existing mitigation, restoration, and rehabilitation management strategies can be evaluated.

BIOLOGY

HWA has an extremely complex biology affected by various physiological and ecological factors. Understanding how these factors affect HWA populations may offer new opportunities for management.

PLANNED ACTIVITIES:

1. Continue to assess how winter mortality affects HWA establishment and impacts across the Northeast.
2. Continue regional assessment of HWA genetics.
3. Continue assessment of bacterial endosymbiont diversity associated with HWA.
4. Assess tree and pest response to micronutrient applications.

PROPOSED ACTIVITY:

- Determine the role of microsporidia and fungal pathogens in HWA populations and natural enemies

IMPACT ASSESSMENTS

Eastern hemlock occurs in a broad geographical area scattered throughout the East in forests dominated by hemlock, other conifers, and mixed hardwoods. Currently, few resource managers have adequate inventories or maps of existing hemlock resources, thus making impact assessments difficult.

PLANNED ACTIVITY:

- Continue assessment of hyperspectral and other remote sensing technologies and their utility for mapping and measuring changes in hemlock health.

SURVEY AND MONITORING

Many state forest health specialists have limited knowledge of hemlock resource locations, and detecting new or low-level infestations is difficult because of the small size of the insect and its feeding habits and dispersal patterns.

PLANNED ACTIVITIES:

1. Standardize survey methods to detect and monitor HWA populations.
2. Continue to provide technical and financial assistance to state and federal cooperators.
3. Provide annual regional summaries of hemlock conditions and county-level HWA infestations.

PROPOSED ACTIVITY:

- Determine the utility of hyperspectral technology to detect low-level infestations by focusing on year-to-year changes in spectral reflectance and tree stress.

INTERACTIONS WITH OTHER PESTS

The elongate hemlock scale (EHS) is another exotic pest that threatens eastern hemlock. In many areas, EHS populations have surged on hemlocks previously stressed by HWA, causing further hemlock decline and mortality.

PLANNED ACTIVITIES:

1. Continue assessment of the EHS range and impacts throughout the East.
2. Determine the extent and impact of existing EHS natural enemies.
3. Evaluate the existing natural enemy complex of EHS in its home range (Asia) and classical biological control opportunities in the eastern U.S.
4. Evaluate entomopathogens and their potential for biological control of EHS.
5. Evaluate interactions between EHS and HWA and their impact on hemlock health.
6. Evaluate potential insecticides and application methods for EHS.

PUBLIC AWARENESS AND INFORMATION TRANSFER

PLANNED ACTIVITIES:

1. Continue to update the HWA website with new information and useful publications.
2. Provide funding to state and federal agencies to support public awareness activities.
3. Publish and distribute proceedings of the Third Symposium on HWA in the Eastern United States.
4. Update, print, and distribute the "HWA Pest Alert" to state and federal cooperators.

POSTERS

Effects of Systemic Insecticides, a Growth Regulator, and Oil on Elongate Hemlock Scale and Associated Natural Enemies on Eastern Hemlock

R.G. Ahern, M.J. Raupp, and Stacey R. Bealmear

ABSTRACT

Several species of armored scale, including *Fiorinia externa* (elongate hemlock scale), *Nuculaspis tsugae*, and *Aspidiotus cryptomeriae* (cryptomeria scale) attack, disfigure, and kill *Tsuga canadensis* in the eastern United States. Damage to trees in forest and urban landscapes results in significant economic loss and threatens to eliminate unique sources of germplasm. Our objective was to evaluate practical methods of chemical control for elongate hemlock scale. In addition, we were also interested in the effect of treatments on natural enemies. We evaluated the efficacies of oil, imidicloprid (Merit, Imicide), acephate (Acecap), an undisclosed neonicotinoid using the Arbor-jet system, and the growth regulator pyriproxfen (Distance). Oil, Merit, and Distance significantly reduced scale abundance relative to control levels. Parasitoid emergence showed a similar pattern to that of scale abundance, although parasitoid movement and generalist predator abundance did not differ among treatments. Our results suggest that some chemical controls are effective against elongate hemlock scale. Furthermore, limited disruption of natural enemy communities by chemical application may promote sustainable biological control of scales during times when natural enemy population levels are low or moderate.

This research was funded or supported by the USDA Forest Service's Forest Health Protection program, the International Society of Arboriculture Tree Fund, and the U.S. National Arboretum. Bayer Crop Sciences, Bartlett Tree Experts, Integrated Plant Care, Arbor-jet, Mauget, and Creative Sales provided materials and technical support.

Biological Control of the Hemlock Woolly Adelgid in the Southern Appalachians

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ABSTRACT

Biological control of the hemlock woolly adelgid is thought to be the most feasible long-term solution to controlling this pest. Two species of imported lady beetles (Coleoptera: Coccinellidae) are currently available for release. *Sasajiscymnus tsugae* has been the most widely released, with over 1 million beetles released throughout the range of the adelgid. However, optimum release densities are unknown. Our study showed no significant differences in adelgid density following releases of 300 or 3,000 adult *S. tsugae*, although adelgid densities were significantly higher at control (no release) sites. A sleeve cage study with *S. tsugae* demonstrated significant control of the adelgid when initial densities were between 0-50 ovisacs per branch. A similar sleeve cage study with *Scymnus sinuanodulus* demonstrated some control of the adelgid when densities were greater than 100 ovisacs per branch. No reproduction was observed for either lady beetle during these studies. In addition to these studies, mass releases of 450 *S. sinuanodulus* and 150 *Laricobius nigrinus* (Coleoptera: Derodontidae) were done, representing the first release of these species together and the first release in the state of Georgia. It is hoped that the combination of these three predators will be effective agains the adelgid.

KEYWORDS

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

INTRODUCTION

The hemlock woolly adelgid, *Adelges tsugae* Annand, continues to spread through the southern Appalachians and the southernmost range of eastern hemlock, causing extensive tree mortality. Biological control is considered the most viable, long-term solution to combating the adelgid since chemical and silvicultural control are ineffective or impractical on a large scale. As natural enemies of the adelgid native or present in the eastern U.S. have little impact, recent efforts have been undertaken to release biological control agents imported from Japan, China, and western North America. So far, three beetle that feed on the hemlock woolly adelgid are being released. One species, *Sasajiscymnus tsugae* (Sasaji and McClure)(Coleoptera: Coccinellidae), is widely available, with over 1 million insects released throughout the range of the adelgid. Two other species, *Scymnus sinuanodulus* Yu et Yao (Coleoptera: Coccinellidae) and *Laricobius nigrinus* (Coleoptera: Derodontidae), have seen limited release but will likely be more widely utilized in the future.

Evaluating the success of these predators has been problematic because the beetles are very small and difficult to find a few months after a release. It is unknown whether these beetles will be effective at suppressing the adelgid to non-damaging levels in the long term. Adelgid populations reach much higher densities on eastern hemlock in North America that they do in Asia or western Northern America, where hemlock species are much less susceptible and natural enemies are more abundant. It is thought that more predator species will need to be introduced to produce an effective and stable natural enemy complex.

Although thousands of *S. tsugae* are typically released at a time, the optimum number of beetles to release is unknown; thus, we replicated and repeatedly sample releases of this beetle at five location at typical number and at a 10-fold less number. Because it is difficult to monitor the impact of free releases, we also conducted caged studies of *S. tsugae* and *S. sinuanodulus* at a range of adelgid densities.

EXPERIMENT SUMMARY

We first compared the effects on the local adelgid population when a typical number of *S. tsugae* (3,000) and a lower amount (300) were released. Both the adelgid and beetle populations were monitored at the release sites and at a nearby control site where no release was made. This was replicated three times throughout Rabun County in northeast Georgia during April 2003. For 16 weekly sampling dates (April-August), adelgid density was significantly lower at both release densities compared to the controls. However, during most sampling dates, there was little or no difference between the 300 and 3,000 release densities. This suggests that the efficacy of the higher releases may be offset by more overcrowding and predator dispersal. Although we examined foliage samples using a microscope weekly (72-108 total twigs collected from the upper and lower crown of each of 18 trees), we were unable to document any predator reproduction in the form of beetle eggs or larvae. It is uncertain whether this was due to inadequate sampling intensity or simply the absence of reproduction.

At the Coweeta Hydrologic Laboratory in Otto, North Carolina, sleeve cages were used to enclose adults of *S. tsugae* on hemlock branches infested with variable densities of hemlock woolly adelgid to determine the density levels at which these predators are most effective. Adelgid ovisacs were counted on 40 hemlock branches in early April, coinciding with maximum egg production by the adelgid. Adult predators (one male and two females) were placed in each of 20 of these sleeve cages and 20 cages were left empty. These were left for six weeks: enough time for a new generation of predators to be produced. After this time period, sleeve cages and the enclosed branches were harvested and returned to the laboratory, where adelgid ovisacs and the number of predators were counted. At the lowest initial adelgid densities (0-50 ovisacs per branch), adelgid population growth was greatly reduced compared with controls. At higher densities (>50 ovisacs), there was little or no effect by *S. tsugae* on adelgid population growth. No predator reproduction was documented in any of the sleeve cages, for reasons that remain unknown. Therefore, all of the observable effects on adelgid growth can be attributed to feeding by the three adult predators placed in each sleeve cage.

A similar study was performed using mating pairs of *S. sinuanodulus* produced this spring in the laboratory. In contrast to the multivoltine *S. tsugae*, *S. sinuanodulus* is univoltine and hence was not expected to reproduce during the study. Although some of the sleeve cages with beetles were missing, the cages were set up and harvested three weeks later, and had fewer beetles in each cage; the *S. sinuanodulus* had a greater effect on higher initial adelgid populations than *S. tsugae*.

In addition to the releases of *S. tsugae*, smaller-scale releases were made of *S. sinuanodulus* and *L. nigrinus* were done in Rabun County, in northeastern Georgia. In April 2004, 150 *S. sinuanodulus* were released, and an additional 300 were released in October 2004. These beetles were obtained from the Insect Rearing Facility, U.S. Forest Service, Northeastern Research Station, in Hamden, Connecticut. Also at the same location and on the same day in October, 150 adults of *L. nigrinus*, reared at Virginia Tech, Blacksburg, Virginia, were released.

DISCUSSION

Because *S. sinuanodulus* and *L. nigrinus* only have one generation per year and are not as amenable to mass rearing, quantities available for field release are much more limited than with *S. tsugae*. It is hoped that more efficient rearing techniques will improve the output of these species in the near future. A combination of these three predators may be more effective against the adelgid. Furthermore, the sleeve cage studies reported here demonstrate that S. *tsugae* should be released prior to a large buildup of adelgid populations, and more research is needed to define factors that affect its reproduction following release.

The release of *L. nigrinus* and *S. sinuanodulus* was the first time that these species have been introduced into Georgia and will represent one the the first locations where all three predators (including *S. tsugae*) have been released in the same area (Rabun County). This was the first environmental release of *S. sinuanodulus* and sampling in 2005 will need to be intensive to discern if it has established and reproduced. Over 4,000 *L. nigrinus* have been released recently thoughout the central and southern Appalachians, with year-after recovery reported from several locations.

ACKNOWLEDGEMENTS

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Investigating Genetic Resistance of *Tsuga* to Hemlock Woolly Adelgid

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KEYWORDS

Tsuga chinensis, breeding, hemlock hybridization.

INTRODUCTION

Research on the nature of genetic resistance of hemlock to hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand), at the U.S. National Arboretum focuses on three areas of investigation: 1) create, plant and evaluate hemlock hybrids and parent species for HWA resistance and landscape merit; 2) investigate the nature of the apparent crossability barrier between *T. canadensis* (L.) Carrière, and *T. chinensis*; and 3) Plant and evaluate wild-collected *T. chinensis* germplasm for landscape suitability and HWA resistance.

CREATE, PLANT, AND EVALUATE HEMLOCK HYBRIDS

In 2002, we reported on the extent of self compatability and hybridization from controlled pollination of several hemlock species (Bentz et al. 2002, Pooler et al. 2002). Authentic hybrids were identified from crosses between the *T. caroliniana* (Engelm.) and *T. chinensis* and between the Asiatic species, *T. chinensis*, *T. diversifolia* ((Maxim.) and *T. sieboldii* (Carrière). In 2002, the parents, their hybrids, and self-pollinated progeny were planted in a randomized block design at the USDA's South Farm, Beltsville, Maryland. Beginning in 2004, data collection began on important horticultural attributes of each tree including survival, growth rate, form, phenology, injury from cold, heat or pests, and evidence of natural adelgid infestation. When plants are of suitable size, HWA will be introduced into mesh bags attached to each tree, and data will be collected for such factors as adelgid survival, number of ovisacs produced, number of eggs per ovisac, number of crawlers, and feeding injury. The research will be in cooperation with Michael E. Montgomery, USDA Forest Service, Center for Forest Health Research, Hamden, Connecticut. Data will be analyzed for variation not only among trees of the same progeny group, but also among progeny groups.

INVESTIGATE THE NATURE OF THE CROSSABILITY BARRIER BETWEEN *T. CANADENSIS* AND *T. CHINENSIS*

No hybrids resulted from crosses made between the HWA susceptible *T. canadensis* and the HWA-tolerant *T. chinensis*. In order to determine whether the failure to achieve seed set was due to the lack of fertilization or from zygote/embryo abortion, a series of controlled pollinations was carried out in 2003. Crosses included self-pollinations, intra-species crosses, *T. chinensis* x *T. caroliniana,* and *T. canadensis* x *T. chinensis* cross. Parents were located at the U.S. National Arboretum, Washington, D.C., and Longwood Gardens, Kennett Square, Pennsylvania. Cones were collected at 0, 3, 6, 9, and 24 weeks post pollination from all crosses attempted as well as from normal, open-pollinated controls from the parent trees.

Six weeks post-pollination, donor pollen tubes of sufficient length for fertilization were observed. Even though pollen tubes were of sufficient length, fertilization may not have occurred. Nine weeks post pollination, immature ovules were observed for both viable and unviable crosses. Further ovule development only occurred in viable crosses. Because ovule development occurred in the unviable crosses, these observations suggest that fertilization may have occurred. Rarely does an ovule develop without fertilization. The data suggest that hybrid ovules are aborting due to zygotic failure and not due to lack of pollen germination and subsequent pollen tube growth.

PLANT AND EVALUATE WILD-COLLECTED *T. CHINENSIS* GERMPLASM

Twenty accessions of wild-collected *T. chinensis* varieties and relatives are being planted in a randomized block design in fall 2004-spring 2005 at the USDA's South Farm site. The germplasm collected includes accessions from two explorations to the Peoples Republic of China sponsored by the North American China Plant Exploration Committee (NACPEC) in 1996 and 1999 and accessions collected by Dr. Michael Montgomery. Germplasm planted represents regional and elevation variations which may affect the hardiness and/or suitability of *T. chinensis* in landscape or forestry applications.

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IDENTIFICATION OF PREDATORY INSECTS AND SPIDERS IN *SASAJISCYMNUS TSUGAE* REARING BOXES

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During 2004, Clemson University initiated mass rearing of the biological control agent *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae) for release against the hemlock woolly adelgid (HWA) *Adelges tsugae* Annand (Homoptera: Adelgidae). Several species of predatory insects and spiders were found on eastern hemlock (*Tsuga canadensis* (L.) Carriere) and Carolina hemlock (*Tsuga caroliniana* Englemann) branches infested with HWA in the rearing boxes that were brought in from the field. HWA poses a serious threat to the health and sustainability of the eastern hemlock forests. Native predatory insects and spiders may have an impact on HWA and can cause problems in the mass rearing of *S. tsugae*.

Samples of predatory insects and spiders were collected from rearing boxes and the breakdown of those boxes during the 2004 rearing season. Predatory insects were collected and identified to order, family, genus, and species depending on the taxa. Spiders were identified to order and family.

Predatory insects and spiders from mass rearing boxes of *Sasajiscymnus tsugae* fed with infested branches may or may not be predacious on HWA; however after examination under the microscope, punctures to the eggs of *S. tsugae* were found. Though the specific impact of these predatory insects and spiders have on HWA is uncertain, they can disrupt the life cycle of *S. tsugae* under laboratory conditions and most likely in nature.

The Nature Conservancy's Response to HWA

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ABSTRACT:

The Nature Conservancy has a dispersed structure. In consequence, its staff have adopted a variety of approaches to responding to the threat to conservation goals posed by the hemlock woolly adelgid. This poster describes this varied response.

KEYWORDS

Hemlock woolly adelgid, The Nature Conservancy, portfolio sites, widespread threats.

WHY THE NATURE CONSERVANCY IS ENGAGED WITH HEMLOCK WOOLLY ADELGID

The mission of The Nature Conservancy (TNC) is to preserve the plants, animals and natural communities that represent the diversity of life on Earth by protecting the lands and waters they need to survive.

OVERVIEW OF THE NATURE CONSERVANCY ENGAGEMENT

Nearly 1,340 terrestrial and freshwater ecoregional "portfolio sites[1]" in nine forest types are at risk to damage by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. About 450 of these sites are in counties already infested by HWA; the remaining 890 portfolio sites are within the range of either eastern or Carolina hemlocks (*Tsuga canadensis* (L.) Carr. and *Tsuga caroliniana* Engelm.), but outside of the currently infested counties (see Figure 1). Some sites under threat have great emotional as well as scientific importance: the Mianus River Gorge in New York, for instance, was the organization's first preserve.

The Nature Conservancy's decentralized structure poses a challenge when trying to develop a consistent response to a widespread threat such as that posed by HWA. We find it easier to address more recently-introduced organisms that are still restricted to relatively small areas. Thus, we are actively advocating policies and resources aimed at eradicating the Asian

[1] "Portfolio site" is TNC terminology for a site within an ecoregion determined by our scientific process to be a priority for protecting biological diversity

longhorned beetle, *Anoplophora glabripennis* (Motschulsky), and containing the emerald ash borer, *Agrilus planipennis* (Fairmaire), and *Phytophthora ramorum* (Werres et al.), the pathogen that causes Sudden Oak Death.

A positive aspect of TNC's decentralization is that state chapters may test various approaches to solving a problem. We then replicate those strategies showing most promise.

In the 1990s, TNC changed its focus from small sites which we could protect through purchase or conservation easements to landscape-scale blocks that we have identified as having high biodiversity value (portfolio sites). We now work with public and private partners to improve forest health generally across large forest blocks—each block covering several tens of thousands of acres. We recognize several broad threats that cannot be addressed by our traditional site-based approaches. Among these are invasive species—including insects, pathogens, plants, and aquatic organisms; acid deposition; and global climate change.

We now have staff dedicated to raising awareness and promoting actions intended to minimize the risk from introduced forest insects and diseases. We hope that this project, combined with campaigns to restore fire and other natural disturbance agents to forest management and to improve policies pertaining to acid deposition and global climate change, will together contribute to reducing hemlock trees' vulnerability to introduced insects and other threats.

The hemlock woolly adelgid invasion is extremely challenging. The threat to TNC's mission is undeniably grave: hemlock-dominated ecosystems usually constitute small but distinct segments of the forest matrix we seek to conserve – unique sites that provide habitat for unusual levels of biological diversity and rare species. Hemlock-dominated ecosystems also play a major role in sustaining associated aquatic systems.

Despite our recognition of hemlock-dominated systems' importance, only rarely do hemlock groves or species specifically dependent on hemlock constitute a separate conservation priority. Given TNC's strategy of focusing on threats to identified "conservation targets," this means that the threat to hemlocks can "fall through the cracks." However, even when hemlock stands are specifically identified as targets, TNC staff have sometimes concluded that the biocontrol agent *Pseudoscymnus tsugae* (Sasagi and McClure) offered too little promise to warrant accepting the risk of release.

In determining our response, preserve managers and policy staff have been stymied by several difficulties. First, hemlocks face a myriad of threats; scientists cannot specify the relative importance of HWA in relation to other exotic insects, changing climate, droughts, and soil compaction. Second, few tools are available to control the insect, and they all have significant downsides. Chemical controls are expensive and extremely difficult to deploy in forest systems. Biological control agents have not yet proven effective, and their utilization raises difficult questions about possible non-target impacts. TNC is adapting: we are now more open to dialogue about the benefits as well as the risks posed by biological control in particular circumstances.

Due to the perceived lack of good options, TNC's response has followed a pattern: when HWA is first detected in a state, conservation staff take notice and search for solutions that will protect at least some of the hemlocks on their preserves. Often, they consider the

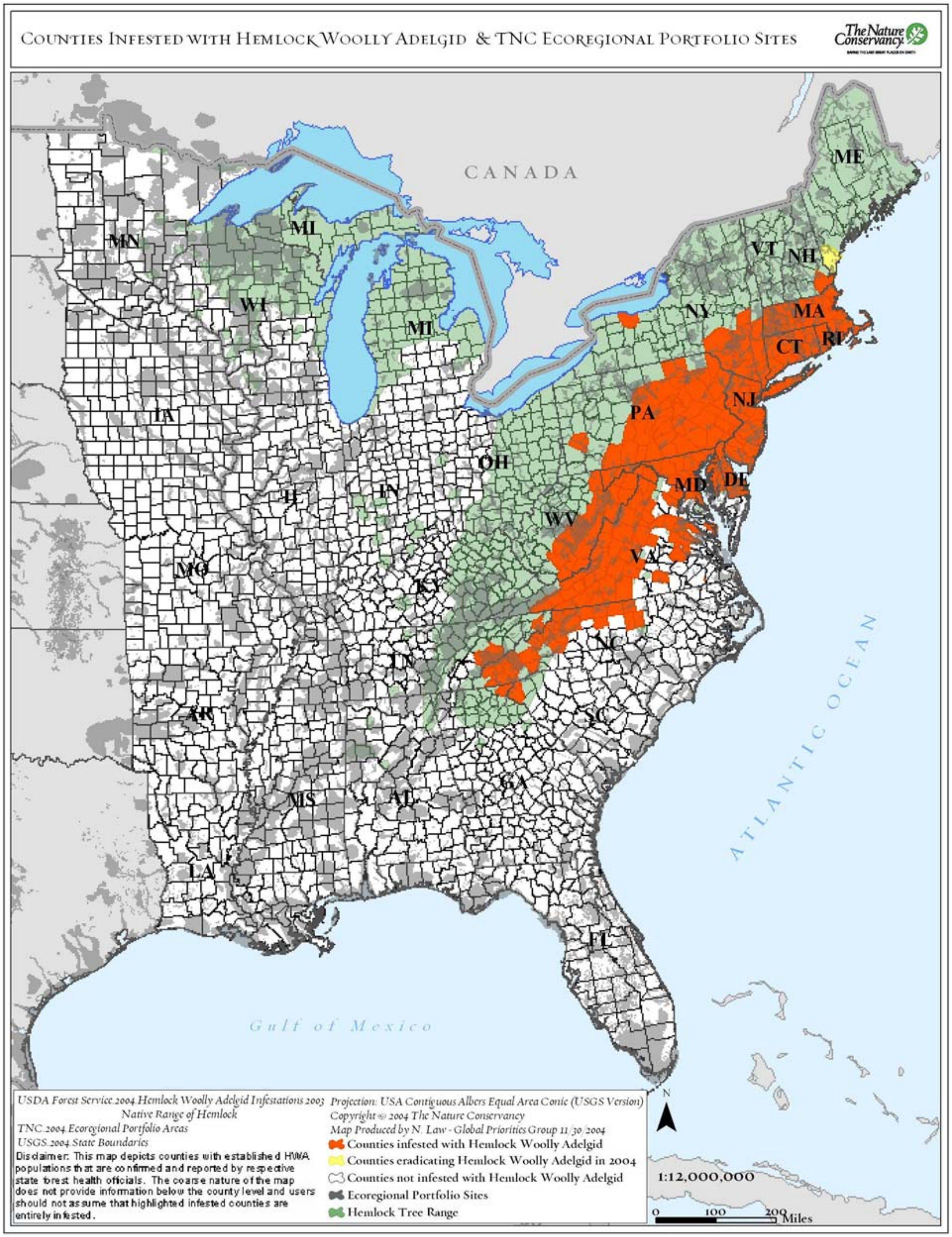

Figure 1. Hemlock range, HWA infestation locations, and portfolio sites of The Nature Conservancy.

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extent and spread of the HWA infestations. Some preserves apply a TNC protocol to determine whether release of biological control agents is permitted: some situations allow such releases; others do not. Once HWA is widespread and TNC staff perceive no effective control solutions, they may focus on other threats against which they believe that they have better prospects (several note that, in terms of minimizing ecosystem impacts, ongoing weed control programs have a higher priority in that they reduce invasions by exotic plants).

TNC's Eastern New York chapter, which is inside the northern reach of HWA, adopted in early 2001 a new protocol that requires supportive answers to nine questions before the Chapter can support release of biocontrol agents. These questions include:

- Establishing the level of threat posed by HWA as distinct from threats posed by other exotic insects, climate change, etc.
- Establishing the potential non-target results of a release.
- Establishing that the benefit will outweigh the costs when neither the efficacy of the biocontrol agent nor its possible non-target effects can be determined when the biocontrol agent is thought unlikely to do more than slow the spread of HWA.
- Having on hand sufficient resources to monitor the spread and impact of both HWA and the biocontrol agent.

In contrast, TNC staff at the southern edge of the HWA infestation in North and South Carolina are actively reaching out to partners and asking TNC's Government Relations staff to help promote effective responses by federal agencies (Figure 2). TNC staff in the Carolinas adopted a Conservation Action Plan for the Southern Blue Ridge in June 2003 that includes, among its action items:

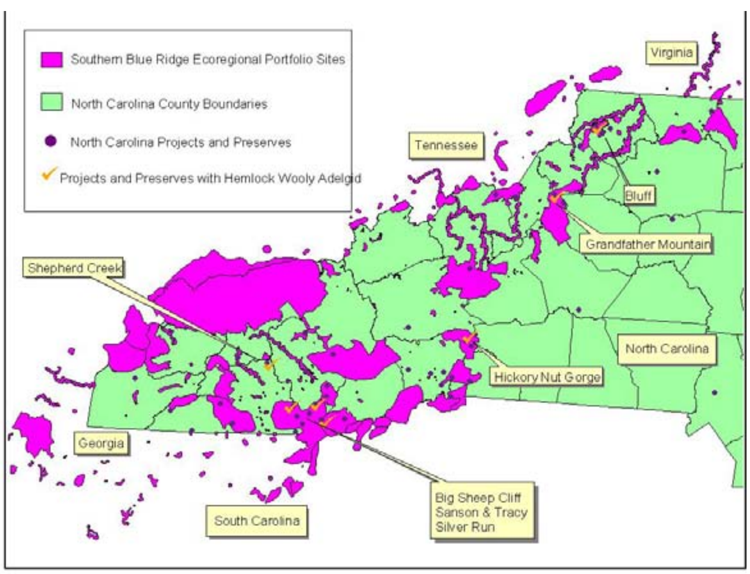

Figure 2. Areas in North Carolina and adjacent areas of TNC concern for HWA.

- Develop a landscape-wide strategy and protocols for treatments and biocontrol agent releases.
- Accelerate mass rearing of agents and research on release techniques.
- Amend any language in TNC easement agreements that hamper release of biocontrol agents.
- Provide assistance in setting priorities for releases.

TNC staff from the eastern states that work on invasive species will meet in April to explore ways to improve our effectiveness; HWA is a focus of the meeting.

In areas that are not yet experiencing infestations, such as Tennessee and Ohio, the staff is in a "watchful waiting" mode. TNC's forest ecologist in Ohio is closely following scientific developments in such areas as biocontrol agents' efficacy and recommendations for forest management practices to restore forest health generally. She is open to the idea of working with partners to conserve genetic material for use in later restoration projects.

At the national level, TNC staff continue searching for useful measures to promote them. In the meantime, we seek to apply to more recently introduced forest pests the lesson from the unfortunate HWA experience; authorities should eradicate such organisms promptly before they spread to threaten important conservation goals.

A SAMPLE OF TNC PORTFOLIO SITES VULNERABLE TO HWA

New England The Berkshire-Taconic landscape reserve on the Connecticut-Massachusetts-New York border is 155,000 acres. It provides habitat for more than 150 rare species. TNC staff's greatest worry is the cumulative impacts of HWA, hemlock looper *Lambdina fiscellaria* (Guenee), and elongate hemlock scale, *Fiorinia externa* (Ferris). TNC staff were disappointed that release of *Pseudoscymnus tsugae* in the Mianus River Gorge of New York reduced HWA populations, but damage by the hemlock looper increased. TNC will prevent exotic plants from invading areas with newly opened canopies through the well-established "Weed it Now" program.

New York The Mianus River Gorge Preserve was TNC's first project and is a Natural History Landmark. The Mianus River is an AA trout stream. The preserve is unusual in that much of its old-growth forest is hemlock. The Mianus River Gorge Preserve is now managed by a separate non-profit consortium. This consortium approved release of *Pseudoscymnus tsugae,* but the results were apparently unsatisfactory.

The nearby Shawangunk Mountains are part of a ridge system that extends through New Jersey to Harrisburg, Pennsylvania. The 90,000-acre Shawangunk bioreserve is a "Last Great Place"–a major landscape rich in biological diversity. There are more than 35 natural communities; the hemlock forests dominate just 5,000 acres. When New York State proposed introducing *Pseudoscymnus tsugae* in the area, the reserve's management (which includes TNC and nine other organizations) could not reach agreement, so it remained neutral on the release, which occurred at one nearby site in 2002. As a result, in part, of this quandary, the Director of Conservation Science for the Eastern New York Chapter developed the more demanding decision protocol for assessing possible releases of biocontrol agents described briefly elsewhere on this poster. As of late 2004, HWA distribution along Shawangunk ridge is still patchy.

New Jersey In 1999, a TNC intern assessed the HWA invasion at all TNC preserves in the state. At that time, 11 of the 40 stands evaluated were experiencing severe defoliation (80 per cent or more), while eight stands were experiencing mild defoliation (less than 19 percent). One stand was described as "healthy." Five years later, TNC staff report that HWA has destroyed or seriously degraded over half of the 26,000 acres of hemlock in New Jersey. The State continues to rear and release *Pseudoscymnus tsugae*, although its effects are uncertain.

Pennsylvania In an effort to protect important ecosystem functions, in spring 2005 TNC staff will begin planting other evergreen trees in the West Branch Preserve in Clinton County. Candidates for planting include eastern white pine *Pinus strobus* (L.), red spruce *Picea rubens* (Sarg.), and even Norway spruce *Picea abies* (L.)(Karst)—which can be removed later if better alternatives become available.

Virginia HWA is affecting every portfolio site at which hemlocks occur. Introduced forest insects and diseases—including HWA—have been identified as the greatest threat in the Alleghany Highlands project area. One preserve, Bottom Creek Gorge, was established to protect the Roanoke River headwaters; it contains the second-highest waterfall and the largest Carolina hemlock in Virginia. The stream provides critical habitat for four species of narrowly endemic fish as well as to the widespread but still rare native brook trout.

West Virginia: TNC's preserve at Ice Mountain protects a site called, in 1845, "one of the greatest natural curiosities of (then) Virginia". The microclimate–created in part by the dense hemlock canopy along the deep rock crevasses which retain ice well into summer–provides habitat for regionally rare, typically boreal plants and uncommon tiger beetles. The preserve approved release of *Pseudoscymnus tsugae* by the West Virginia Department of Agriculture in 2002 and 2004. In addition, USFS researcher Brad Onken injected five trees in the immediate vicinity of the ice vents with imidicloprid in May 2003.

North Carolina and South Carolina: In North Carolina, HWA already is present in five TNC-owned preserves and another five preserves established by easement agreements with partners. HWA threatens at least five portfolio sites.

One—the Blue Wall/Southern Blue Ridge Escarpment—stretches from the Pacolet River north of Spartansburg, South Carolina, west to the Chattooga watershed in Georgia, and from the upper Piedmont of SC to the escarpment ridges of North Carolina. This region harbors more than 300 occurrences of rare species and natural communities. The Blue Wall Escarpment is "a biological hotspot within a hotspot"- the wider Southern Blue Ridge ecoregion. Four Conservation Targets in this region have a strong hemlock component: Gorge Species Assemblage, Carolina Hemlock Bluff, Escarpment Forest Matrix, and Headwater River Systems.

At Grandfather Mountain, a private park with which TNC has both ownership and easement agreements, staff are treating infected hemlocks in the back country with stem injection of Imicide.

Ohio: The Ohio Chapter led TNC planning for conservation of important examples of the Western Allegheny Plateau Ecoregion – an area covering more than 40,000 square miles stretching across portions of Kentucky, New York, Ohio, Pennsylvania, and West Virginia. HWA is not yet present in Ohio, but it threatens six large forest blocks in the West Virginia portion of the ecoregion and five in Ohio. Some extremely rare species are associated with hemlock large patch communities within the overall forest matrix. The federally listed Indiana bat has been found to establish maternity roosts inside dead hemlocks standing in dense hemlock groves (Britzke et al. 2003).

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Monitoring Hemlock Vitality Using Ground-Based Digital Imaging

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ABSTRACT

The vitality of hemlock (*Tsuga* spp.) trees needs to be assessed in order to evaluate the effectiveness of treatments that combat hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae). Ground-based photomonitoring can be used to assess canopy dynamics, which serves as a visual indicator of tree vitality. Here we propose a sampling strategy using a digital camera with a telephoto lens to examine change in needle count over time. The magnification provided by the lens optics reduces some of the complicating effects inherent in other forms of below-canopy photographic methods and enables direct measurement of the objects of interest, the needles.

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), is rapidly infesting and causing widespread mortality of large areas of hemlock (*Tsuga* spp.) forests in the eastern United States. Due to this epidemic, hemlocks are being intensely studied. Branch counts, needle loss, and crown transparency (Mayer et al. 2002, Webb et al. 2003) are often used to assess individual tree responses to HWA infestations. Defoliation and regrowth dynamics are of interest in studies of hemlock response to HWA infestation. Ground-based photomonitoring can be used to assess these canopy dynamics. In this paper we will address some of the issues involved with ground-based photomonitoring using digital cameras and make suggestions on designing an effective system.

GROUND-BASED CANOPY DATA COLLECTION

Due to access difficulties (Barker and Pinard 2001) crowns are often evaluated from a ground-based vantage point. This presents some challenges in the outdoor environment. Hemispherical (fisheye) photography is often used for canopy openness or photosynthetic photon flux density (PPFD) estimation (Englund et al. 2000). Oblique ground-based photography has also been used to monitor foliar change (Curtis and Kelley 1993, Lee et al. 1983, Lindsey and Bassuk 1992). Here emphasis will be placed on ground-based methods of individual tree evaluation. Though this paper will concentrate on hemlock (Figure 1), these methods can be generally applied to other needle-leaved species.

As the HWA attack trees, the hemlock foliage dies and abscises. This can happen at the branch, twig, or even individual needle level. It is desirable to see how many needles are removed, retained, or added over time. Thus, we will set the needle as our object of interest and our minimum scale accordingly. Assuming that sampling is performed at the Nyquist frequency, an optical system must be selected which can resolve one-half of the needle width at a tree height distance. It may be difficult to find such an optical system, but that would be the desired precision. For this repeated measures sampling strategy precise controls are needed to monument and maintain the same optical system parameters over time (Davies 2004, Hall 2001). One advantage of digital cameras lies in their ability to immediately examine the image once it is captured. This can allow the operator to adjust the optical parameters to match previously captured images.

Figure 1. Example of ground-based view of hemlock crown.

OPTICAL ISSUES

There are many environmental and photographic factors that affect ground-based canopy imaging. Here, we discuss several of these factors and their impact on evaluating foliage change over time.

ENVIRONMENTAL EFFECTS

Overcast conditions are favored for ground-based canopy applications (Frazer et al. 2001, Englund et al. 2000), having been empirically determined to be less variable. Some reasons for this are that, as the ambient light is diffuse, penumbral effects, specular reflectance, and the variation of radiant flux density across the scene is more manageable for the imaging sensor. Waiting for overcast conditions can be over-restrictive for our purposes; instead, we suggest a narrow field of view (FOV) to minimize these effects and the probability of imaging the sun directly.

Other objects can interfere with the clear viewing of the object of interest. Objects in the background can affect the contrast needed to delineate the object of interest and foreground entities can block the view completely. Reasonable precautions can be taken during the first sampling cycle; there may be no remedy other than disregarding the sample for subsequent visits. For this reason, we suggest taking a larger number of samples than initially needed, knowing that some may be discarded in future revisits.

PHOTOGRAPHIC EFFECTS

Resolution

Measurement precision in any sampling space (i.e., spatial, spectral, and temporal) is limited by the ability to segment and distinguish between very small changes. As digital sensors are arrangements of discrete sampling areas, not unlike film emulsions, there are limits to spatial precision. There are also spectral precision limits on the ability to measure specific quantities of light of specific wavelengths over specific time intervals.

Many studies attribute digital camera overestimation to the limited spatial resolution of the image (Frazer et al. 2001, Englund et al. 2000). This is of particular concern for needle-leaved species as the needle arrangement creates many very thin gaps. Thus, the measurement scale should be considered at the outset of the canopy study to determine the minimum gap (or leaf) dimension to be considered.

It is important to have the appropriate spatial and spectral resolution to be able to resolve the object of interest: Figure 2 shows two images (a and b) captured with 35 and 280 mm-equivalent focal lengths, respectively. The interaction between spatial and spectral resolution can have serious affects at the analysis stage. Threshold selection can also contribute to variability when images are analyzed: the binary images (Figure 2, c and d) show how spatial resolution and thresholding can have a combined effect on the classification of foliage or sky.

Lens Factors

Lens systems gather, filter, and direct light rays to the optical sensor and play a large part in the usefulness of the output image. Lens focal length affects the spatial and radiometric properties of the captured image: short focal lengths produce wide-angle views with limited magnification; conversely, longer focal lengths cover more narrow views in greater detail (higher spatial resolution). The broader spatial coverage of shorter focal lengths incorporates a greater radiant flux density, allowing small apertures and faster shutter speeds, but greater variation across the scene. The lower amount of light captured with long focal lengths necessitates greater aperture and/or shutter speed adjustments for adequate exposure. The need to adjust focus will be an added complication when using long focal lengths as the depth of field (DOF) will be drastically reduced.

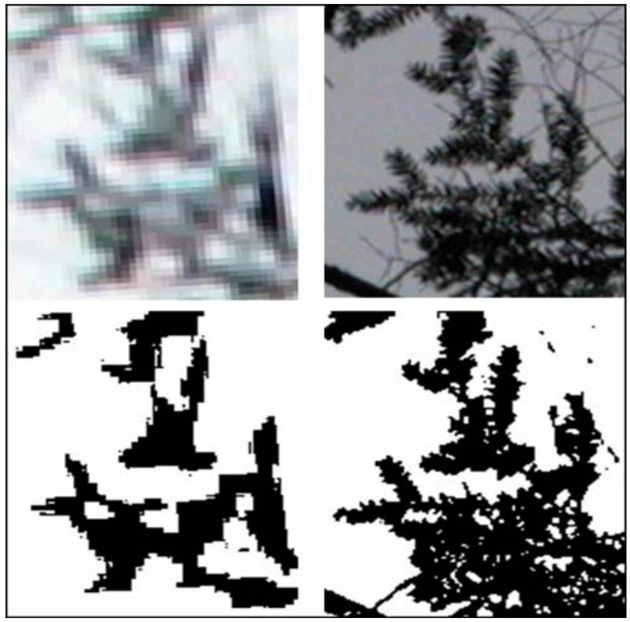

Figure 2. Depiction of a hemlock branch imaged at 35 and 280 mm equivalent focal lengths (a and b) and these same image sections when a global threshold is applied (c and d, respectively).

Short focal lengths tend toward barrel distortion and long focal lengths create pin cushion distortion. Spatial dimensions are not being considered for this crown assessment application so distortion effects are not a primary concern. As repeated measurements are being considered it would minimize variation if the same lens was used for all remeasurements.

Exposure

The understory of a healthy hemlock stand is typically very dark and proper exposure is critical so smaller structures do not get washed out. For taking these zenith-looking images, the aperture should be stopped down as much as possible to improve contrast (Bunnell and Vales 1990). A smaller aperture necessitates a longer shutter speed however if the camera is mounted on a tripod motion blur should be minimal (unless the wind is blowing the tree/needles). Blooming and penumbral effects are reduced in the underexposed image. The stopped down images are more able to detect smaller tree structures amidst a majority sky background (Figure 3).

Digital Camera Factors

Digital cameras have come under scrutiny for below-canopy photography purposes (Frazer et al. 2001, Englund et al. 2000). In addition to their limited and discrete spatial and spectral resolution, other collection and processing anomalies have contributed to the complications of consumer-grade digital cameras. While we recognize these limitations, we believe these difficulties are of limited consequence within our sampling protocol.

Consumer-grade digital cameras are most commonly designed for color image processing using a color filter array applied over the sensing elements. Digital image manipula-

Figure 3. Images captured with different aperture settings. The image on the right is stopped down two f-stops. Contrast is enhanced in the stopped down image and more crown structures are preserved in sky-dominated areas. Blooming is also reduced.

tions including gamma correction, white balancing, sharpening, interpolation, and data compression are then performed to create a visually pleasing output image at a minimal storage size. As the operations performed are normally proprietary methods that differ among manufacturers, it is difficult to determine the actual measured values.

All optical sensors have light collection and recording limitations or dynamic range and significant clipping can occur at very high or very low levels of radiance collection. If a sensing element receives an overabundant amount of light, there is also the potential for blooming (or overflow into adjacent elements). Chromatic aberration, or color blurring, is due to variable refraction of light wavelengths as they pass through the lens optics causing them to focus at different distances (Frazer et al. 2001). These effects are exacerbated at shorter focal lengths.

DISCUSSION

Ground-based photomonitoring provides a cost-effective means of evaluating change in canopy dynamics over time, but is not without difficulties. The sample must be visually unobstructed. The appropriate scale must be selected. After scale determination, the location of the camera and focal length must be determined. For repeated measures sampling the problem of camera relocation must be addressed. As lighting can not be controlled in the outdoor environment, camera settings must be adjusted accordingly.

At low spatial resolutions comparison would have to be made of radiant flux density allowed to pass through the same crown areas. Compensating for the many changing environmental and optical factors at each photo-session would be extremely difficult. It is for these reasons that we propose using a longer focal length such that individual needles can be discerned. At this level, we only need to determine presence or absence of individual needles over time (Figure 4). To do so, we randomly select an azimuth around the stem axis and a random distance between the stem axis and the crown edge in this direction. This point will be marked so the camera setup can be performed easily for subsequent image collection. The camera will be placed over this point with the camera axis facing the zenith. A focal length will be selected such that individual needles can be discerned. Immediately prior to image collection, a copy of the initial image will be used to re-establish identical camera parameters

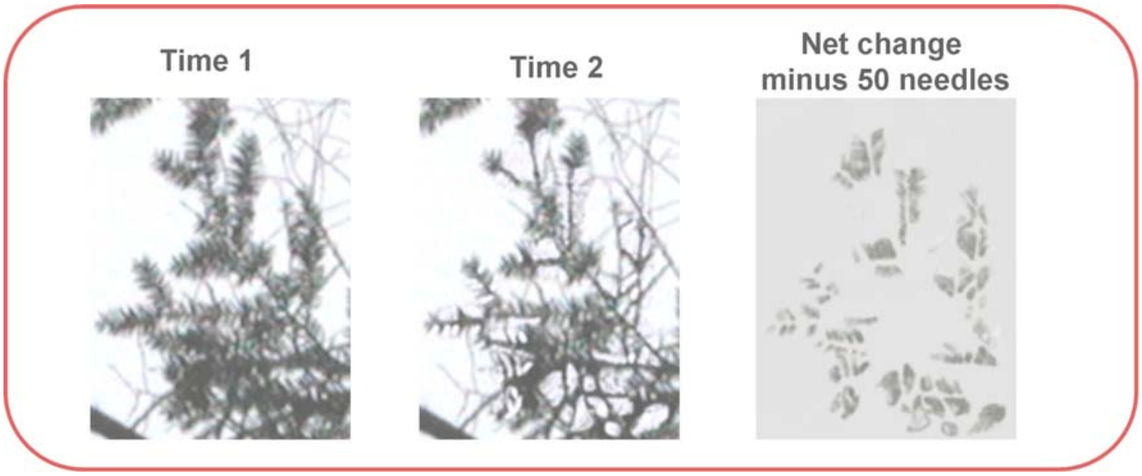

Figure 4. Repeated measures example of foliage change over time.

(i.e., focal length, aperture, position, view angle). Field tests need to be performed at varying levels of foliage change to evaluate the efficacy of this proposed method.

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Update on Rearing *Sasajiscymnus tsugae* at the Clemson Insectary and Field Monitoring of the First Release Site

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is the primary threat to hemlock forests in eastern North America. *Sasajiscymnus tsugae* (Sasaji and McClure), an introduced coccinellid from Japan, is a biological control agent for HWA. In mass rearing *S. tsugae*, our main goals are to maximize adult beetle production, reduce the amount of human labor, and minimize production costs. Comparison studies conducted during the 2004 production year indicated that *S. tsugae* females laid significantly more eggs in oviposition jars oriented horizontally rather than vertically. An adjustment in rearing box design resulted in decreased time for watering and feeding and a mean increase of 147 adults per rearing box. Skilled personnel and attention to detail resulted in the production of 110,000 adults from an initial *S. tsugae* cohort of 200 females and 150 males. Monitoring of the first *S. tsugae* release site indicated the presence of *S. tsugae* larvae, dispersal of beetles over 130 meters from the initial release trees to other HWA-infested hemlocks, and presence of beetles at the release site over a six month period.

KEY WORDS

Biological control, mass rearing, hemlock woolly adelgid.

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an introduced pest that is threatening the health and sustainability of hemlock forests in the eastern United States (Knauer et al. 2002). HWA feeding can produce severe needle loss, bud death, branch dieback, and tree death in as little as four years (McClure 1995, Cheah and McClure 1998, McClure et al. 2001). HWA can cause local tree loss, regional decline, or elimination of the ecologically important eastern hemlock, *Tsuga canadensis* (L.) Carriere (Orwig et al. 2002) and Carolina hemlock, *T. caroliniana* Engelmann (McClure et al.2001).

The multi-agency HWA Steering Committee considers biological control agents to be the most environmentally and economically effective method of controlling HWA in a forest setting. Many native North American predators occasionally feed on HWA, but none have shown any significant impact on HWA populations (Wallace and Hain 2000, McClure 2001). One of the most promising non-native biological control agents against HWA is *Sasajiscymnus* *tsugae* (Sasaji and McClure) (formerly *Pseudoscymnus tsugae*), a small black coccinellid from Japan (Cheah and McClure 1998).

In mass rearing *S. tsugae*, our main goals are to maximize adult beetle production, reduce the amount of human labor, and minimize production cost. One of the most important steps in mass producing *S. tsugae* under environmentally controlled conditions is accurately determining egg production. Palmer and Sheppard (2002) found that cotton gauze can be used to predict egg production. Accurate egg estimates are especially important with *S. tsugae* because larvae are cannibalistic (Blumenthal 2002); consequently, production of *S. tsugae* that reach the adult stage decreases at high larval densities. In 2004, comparison studies were conducted on egg production in oviposition jars that were oriented horizontally versus vertically. Additionally, comparative studies were conducted between conventional-style rearing boxes and modified-style rearing boxes. The first release site was monitored for the presence of *S. tsugae* reproduction, dispersal, and survivorship over a nine-month period.

METHODS AND MATERIALS

The North Carolina Department of Agriculture and Consumer Services mass rearing laboratory in Cary, North Carolina, and the Philip Alampi Beneficial Insects Rearing Lab in Trenton, New Jersey, provided the initial cohort of 350 *S. tsugae* (200 females and 150 males) for the study. Upon arrival, beetles were sexed and separated at a mean ratio of 10 females to 5 males for placement into 3.8-liter glass oviposition jars.

OVIPOSITION JARS

Based on earlier choice tests to determine the most effective gauze type for oviposition, we used Curad® basic care stretch gauze (Belersdorf, Inc., Wilton, Connecticut) for all experiments. Three 5 cm x 5 cm pieces of gauze were randomly placed among six 20-25 cm long HWA-infested hemlock twigs which were arranged into a bouquet. The hemlock bouquet was placed into an oviposition jar, a small amount of supplemental honey (as an additional food source) was spread lightly across the inside of the jar, *S. tsugae* beetles were added, and the jar's lid secured. Each oviposition jar was randomly assigned to a vertical or horizontal test group. Equal numbers of each jar orientation were setup on a daily basis (Figure 1). Oviposition jars were then placed into a controlled-environment (25 ± 1^{o}C, 60 ± 5% humidity, and 16:8 L:D) room.

Hemlock bouquets were removed after seven days from the oviposition jars. The number of viable eggs were counted and recorded by oviposition jar orientation (vertical and horizontal). Trials were conducted from 30 November to 30 December 2004 using 300 oviposition jars. Egg production based on oviposition jar position was compared by standard analysis of variance procedures (ANOVA) (SAS Institute Inc. 2003).

REARING BOXES

Experiments to determine which rearing box style produced the highest number of adult beetles were conducted from 16 February to 6 May 2004 using 30 each conventional (Figure 2) and modified (Figure 3) rearing boxes. Modifications included use of larger wet foam blocks to maintain a more constant humidity and arrangement of hemlock twigs to minim natural orientation on trees. Each rearing box comparison contained similar numbers of eggs on gauze removed from oviposition jars on the same day. The same sequence and schedule of watering, adding HWA-infested hemlock twigs, and adding honey as a supplemental food source was used for both types of rearing boxes. The conventional and modified rearing boxes were located side by side in a controlled environment room. After 35 ± 3 days, the number of adult *S. tsugae* were collected, counted, and recorded from both styles of rearing boxes. Adult beetle production based on rearing box style was compared by standard ANOVA procedures with means separated by LSD (SAS Institute Inc. 2003).

Figure 1. Rearing jars for *Sasajiscymnus tsugae*.

Figure 2. Conventional rearing box.

Figure 3. Modified rearing box.

MONITORING

We monitored the initial release sites along a valley north of Walhalla, South Carolina, leading into the Walhalla Fish Hatchery near position 34’59"N 83’04"W. The release of 6,680 *S. tsugae* adults occurred on 12 March 2004 at a location 2460 feet above sea level. At the first location, 4,430 adults were placed on two HWA-infested hemlocks, and approximately 300 m downstream along the same hillside, 2,250 adults were placed on a single HWA-infested hemlock. The sites were monitored using visual observation to determine the number of adult beetles, larvae, eggs, or pupae in the lower canopy. The number of trees where *S. tsugae* were found and the total search time were recorded.

RESULTS AND DISCUSSION

OVIPOSITION JARS

Sasajiscymnus tsugae females laid significantly more eggs on gauze in the horizontal oviposition jars compared to those in the vertical position ($P = 0.03$) (Table 1). The horizontal oviposition jar allows the twigs in the bouquets to be arranged similarly to twigs on the hemlock trees. When running 100 oviposition jars per week, the horizontally positioned jars will weekly produce an additional 1,900 eggs on gauze per week. Based on this experiment, we now place all oviposition jars in the horizontal position.

It will be interesting to see if there is a difference in the total number of adults that are reared from eggs taken from the two jar positions. The results of this part of the experiment will not be known until the beginning of March, 2005, when this study will be completed.

REARING BOXES

The modified rearing boxes produced significantly more *S. tsugae* ($P = 0.02$) than the conventional rearing boxes (Table 2), and reduced time required for the feeding and watering process. The larger floral wet foam blocks in the modified boxes maintained a more constant moisture level. Additionally, the arrangement of the HWA-infested twigs was in an overlapping pattern along the sides of the blocks similar to how twigs naturally occur on the hemlock tree. With 100 rearing boxes, the modified boxes have the potential to produce an additional 14,700 *S. tsugae* per year. We are in the process of changing all rearing boxes to the modified arrangement to produce as many *S. tsugae* as possible for release against hemlock woolly adelgids.

MONITORING

Sasajiscymnus tsugae released above the Walhalla Fish Hatchery, Walhalla, South Carolina, provided evidence of reproduction in the field with eggs, larvae, and pupae found on HWA-infested hemlock trees at the site. Dispersal from the initial three trees to eight HWA-infested hemlocks at the site was recorded. Beetles were observed at the release site over a six-month period (Table 3). From the initial release to the first observation, there were two light snows and five days with freezing overnight temperatures at the release site.

Table 1. Comparison of *Sasajiscymnus tsugae* mean egg production ± SEM on 5 cm x 5 cm gauze strips from 30 November to 30 December 2004 at the mass rearing facility in Clemson, South Carolina, using 300 3.8-liter glass oviposition jars.

Jar Orientation	**Eggs per Oviposition Jar ± SEM**
Horizontal	139.2 ± 6.9a*
Vertical	120.1 ± 5.4b

*Means in a column followed by the same letter are not significantly different (LSD, P = 0.05).

Table 2. Comparison of the mean number of adult *Sasajiscymnus tsugae* ± SEM with two rearing box styles (conventional and modified) during spring 2004 at the mass rearing facility in Clemson, South Carolina, from 60 rearing boxes.

Style of Rearing Box	***Sasajiscymnus tsugae* Adults ± SEM**
Conventional	434 ± (34)a*
Modified	607 ± (45)b

*Means in a column followed by the same letter are not significantly different (LSD, P = 0.05).

Table 3. Monitoring of the first release site in 2004 of *Sasajiscymnus tsugae* near the Walhalla Fish Hatchery for adults, eggs, larvae, and pupae; dispersal to other trees; and amount of time spent searching for life stages.

Date	***Sasajiscymnus tsugae***				**Hemlocks with Beetles**	**Time**
	Adults	**Larvae**	**Eggs**	**Pupae**		
12 March	6680	-	-	-	3	Release
24 March	120	4	-	-	3	10 min
14 April	47	2	3	-	8	2 hr
17 May	5	1	2	2	5	6 hr
16 June	3	-	-	-	2	12 hr
15 September	2	-	-	-	2	8 hr
1 December	-	-	-	-	0	8 hr

The beetles survived the cold weather and dispersed from the release trees up to 130 m to other nearby HWA-infested trees. The direction of dispersal was primarily downhill from the release trees, which could have been due to the majority of HWA-infested hemlocks being downhill from the release trees. There were indications of reproduction occurring, with observations of larvae, eggs, and pupae detected on both release trees and nearby hemlocks. At the nine-month sample date, no adult beetles were found in the lower canopy of the hemlocks. However, there was a noticeable difference in the HWA-infestation level, with release-site trees having only lightly scattered "wool" compared to heavy "wool" levels on hemlocks 200 m away. We plan to return in March 2005 to assess and compare the condition of the hemlocks at the release site to hemlocks in the next valley based on pre-release HWA-infestation data from 2004.

In the first year of mass rearing, Clemson's insectary produced over 110,000 *S. tsugae* adults and released over 100,000 beetles into the hemlock forests of South Carolina, North Carolina, and Georgia. This year's goal for the facility is to produce 150,000 *S. tsugae* for release into HWA-infested hemlocks. We plan to continue investigations on the dispersal and the long-term impacts of *S. tsugae* on HWA with studies near selected release sites.

ACKNOWLEDGMENTS

We thank Dr. Kathleen Kidd (North Carolina Department of Agriculture and Consumer Services) and Daniel Palmer (Phillip Alampi Beneficial Insect Laboratory, New Jersey) for the initial supply of *S. tsugae.* Thanks to LayLa Burgess, Cora Allard, Wess Klunk, Karen Burton, Carrie Hendrix, Will Reeves, and James Korecki, the hard working technicians and students who have worked at the Clemson Insectary. We also thank Rusty Rhea (Forest Entomologist USDA FS) and Buzz Williams (Chattooga Conservancy) for help in site selection and releasing the beetles. For project funding, we thank the Jackson-Macon Conservation Alliance, the Chattooga Conservancy, the National Forest Foundation, and USDA Forest Service.

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Arborjet Approach and Use of Stem Micro-Infusion Treatments for the Management of Specific Insect Pests and Physiological Diseases in Forest, Landscape and Plantation Trees

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ABSTRACT

Four posters present a cross section of injection methodology by Arborjet, Inc. Poster #1 summarizes a study conducted in 2003-2004 to assess the efficacy of Arborjet's micro-infused IMA-jet (5% SL) with the Tree I.V. system in the management of hemlock woolly adelgid (*Adelges tsugae* Annand). In this study, sixteen (16) 41 cm HWA infested Eastern hemlock (*Tsuga canadensis* Carriere) were injected with a 1.6mL per cm DBH rate (0.8 gm A.I./cm DBH) IMA-jet formulation of imidacloprid. Four 9mm-diameter Arborplugs (differential septa) were set into the active transport (xylem) tissues as the micro-infusion interface. Eight non-treatment trees served as controls. Evaluations were performed in the fall 2004. Eight 45-60cm branch samples were taken from the mid-canopy of the study trees. Five branchlets were cut from each twig sample. HWA mortality was determined by microscopic examination. Viable HWA/linear cm was calculated for each sample examined. Annual twig extension (last three years) was also measured as an indicator of hemlock health. Live HWA pressures on treated trees equaled 0.04/linear cm compared to 1.8/linear cm for untreated trees, a 45X reduction in HWA pressure. Percent mortality on treated trees equaled 98.4%, compared to 26.6% for the controls. Treatment tree growth response was 5.78 cm versus 4.19 cm in the controls, a difference that has biological significance.

Poster #2 summarizes the cooperative work with Michigan State University studying the efficacy of trunk injections in the treatment of emerald ash borer (*Agrilus planipennis* Fairmaire). Peak ELISA imidacloprid residues for Arborjet's 5% IMA-jet treated trees was 350 ppb versus 42 ppb for Mauget's 10% Imicide. EAB adults feeding assay was also conducted to assess the relative efficacy of the injected imidacloprid formulations. Populations of EAB were fed branches from injected trees at 15, 28, 49, 59, and 70 days. EAB adult mortality was consistently higher in Arborjet's 5% IMA-jet treated trees than Mauget's 10% Imicide and/or controls. Observed mortality was highest at 49 days: 90% in Arborjet-treated trees, 50% for Mauget-treated trees, and 40% in the controls.

Poster #3 presents some of the plant health studies performed in the process of formulation development. Current studies include the response of chlorotic pin oak (*Quercus palustris* Muench) to MIN-jet Iron treatments. Of interest are efficacy and duration; 3x, 6x, and 9x dose rates were use to assay dose-rate responses. A pin oak severity rating was developed as a tool to aid in the assays. Physiological disease presents a range of symptomology from mild leaf yellowing to severe interveinal chlorosis, canopy dieback, epicormic dieback and ultimately, death as carbohydrate storage is depleted. Assays of plant health response are sched-

uled in 2005, 2006, and 2007. In a study performed in London plane trees (*Platanus x Acerifolia*) susceptible to defoliation by anthracnose, trees were injected with MIN-jet Copper and compared with two systemic fungicides that are labeled for trunk injection. In this unique study, we are interested in tree health response despite the presence of the causal agent. In previous injection work, we observed trees superior recovery and higher health indices (including more rapid development of woundwood at the injection sites) compared to fungicide treatments.

Poster #4 illustrates the relative wound response in trees using Arborjet technology. A digital assessment was developed to help the practitioner to rate wound response in trees. The scale uses a -1, 0, and +1 rating system, where '-1' indicates wounds sites with cracking, oozing, '0' indicates no observable response, and '+1' is indicative of wound closure. Three factors influence wound response: the tree species (including xylem anatomy and wood density), the nature of the physical injury (methodology), and the formulation used (relative phytotoxicity).

Effects of Systemic, Sublethal Doses of Imidacloprid on Two Predators of Hemlock Woolly Adelgid

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ABSTRACT

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an introduced pest of two native hemlock species, eastern hemlock (*Tsuga canadensis* (L.) Carr.), and Carolina hemlock *(T. caroliniana)*. Currently, chemical control is the most effective way to control HWA infestations in accessible stands and on individual trees. Some of the most widely used pesticides against HWA are imidacloprid-based products. *Laricobius nigrinus* Fender and *Sasajiscymnus tsugae* Sasaji and McClure are two predator beetles being released in the eastern U.S. as biological control agents of HWA. As the biological control agents establish themselves in the forests and as land managers implement chemical control of the pest it will be important to understand the interactions between the two agents and how to best apply them together in forest and landscape settings. This study examines non-target effects of the systemic applications of imidacloprid on two important predators of HWA.

On April 23, 2004, 24 hemlocks on a private residence in Abingdon, Virginia, were treated by the Mauget (J.J. Mauget Co. Arcadia, California) method. There were four treatments: full, half, and quarter rates of Imicide®, and an untreated control. Five weeks after treatments, HWA-infested foliage was fed to beetles in a no choice test, and beetle survivorship and appetite were observed. To monitor HWA populations on the trees, the proportion of 200 shoots infested with at least one HWA were measured before and after treatments. To determine imidacloprid levels in the trees, branches were dried, ground, and the imidacloprid extracted with solvent and measured with an Envirologix (Portland, Maine) ELISA test kit.

Prior to treatment, the proportion of shoots infested did not differ significantly between treatment groups, but were significant six months after treatment. The change of the proportion of shoots infested before and after treatments was significant between treatments. Control and quarter proportions increased (46 and 17 percent respectively), while half and full proportions both decreased 28 percent. Half and full treatments were comparable as they both reduced HWA populations to under 10% infestation.

Differences in beetle survival rates were not statistically significant between sexes, species, or their combination, so data were pooled by sex and species. Although the mean survival rate for both predators was highest in the control treatment, means were not significant among treatments. Mean survival rate after 10 days for *L. nigrinus* was 80% and *S. tsugae* was 86%. Two possible explanations for the lack of significant differences among treatments are that imidacloprid levels were not high enough at the time of the test or that treatments may

not affect beetle feeding and mortality. Branches are being tested to determine their imidacloprid levels at the time of the tests.

The total number of adelgids eaten did not differ significantly among treatments, species, or their combination. The amount of HWA eaten was significantly different between sexes, with females eating more than males. *S. tsugae* males ate less than females but more than *L. nigrinus* males. The amount of feeding seemed unaffected by treatments, although chemical levels at the time of the tests are still to be determined. Several follow-up experiments are planned for spring 2005. Choice tests will help determine whether beetles prefer treated or untreated adelgids. To test if adelgids are a means by which the predators can be exposed, untreated branches will be cut from trees and treated with high concentrations of imidacloprid before beetles are placed on them. Also, predator eggs and larvae will be placed on treated branches to study survivorship and development. These experiments will shed more light on managing infestations and the interactions of the biological and chemical control of HWA.

KEYWORDS

Imidacloprid, *Laricobius nigrinus, Sasajiscymnus tsugae.*

Changes in Ant Community Structure and Composition Associated with Hemlock Decline in New England

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ABSTRACT

Impacts of invasive species on the structure of the invaded communities is an active area of ecological research, but the effects of the hemlock woolly adelgid (HWA) on forest organisms other than economically important timber trees rarely have been examined. To date, studies of HWA impacts principally have assessed mortality rates of hemlock and their subsequent replacement by early-successional hardwoods, and changes in core ecosystem properties. However, if certain taxa are restricted to hemlock forests, or if dominance by hemlock precludes the colonization or occurrence of particular species, then removal of hemlock could result in changes in overall species diversity and composition within HWA-infested stands and across the landscape. We examined this hypothesis for forest ant communities in southern New England. Specifically, we asked how ant species richness and composition differed among intact hemlock stands, HWA-infested and damaged stands, and mid-successional hardwood "controls." In total, 22 species of ants were collected from 16 sites spread across Connecticut and Massachusetts. Average species richness among sites ranged from three to 12 (mean = 7), and was inversely related to the percent of hemlock basal area in the stand. Average dissimilarity among sites was 73%; notably, *Formica* spp. were virtually absent from intact, uninfested hemlock stands. At the stand level, loss of hemlock due to HWA results in an increase in ant species diversity, but this local increase in diversity is offset by homogenization of diversity across the landscape. The rate and impacts of colonization by *Formica* spp. into HWA-infested stands may alter ecosystem structure and dynamics in these forests and merits further study.

KEYWORDS

Ants, diversity, hemlock woolly adelgid, New England, species richness.

INTRODUCTION

Quantifying and predicting the impacts of invasive species on the structure and function of communities is a major focus of ecological research (*e.g.,* Vitousek 1986, Levine 2000, Crooks 2002, Gurevitch and Padilla 2004). To date, studies of the impacts of the hemlock woolly adelgid (HWA) on eastern U.S. forests principally have assessed mortality rates of hemlock and their subsequent replacement by early-successional hardwoods (*e.g.,* Lapin 1994, Orwig and Foster 1998, Bonneau et al. 1999, Mayer et al. 2002). Other studies have focused on ecosystem changes attendant to loss of hemlock due to HWA (*e.g.,* Jenkins et al. 1999, Orwig and Foster 2000, Stadler et al. 2005). However, if other taxa are restricted to hemlock forests, or if dominance by hemlock precludes the colonization or occurrence of certain species, then removal of hemlock could result in changes in overall species diversity and composition within HWA-infested stands and across the landscape. However, studies of the impact of HWA on organisms other than economically important timber trees in eastern forests are rare (Tingley et al. 2002).

Here, we examine the changes in ant (Hymenoptera: Formicidae) community structure and composition occurring as HWA alters hemlock forests. Ants may be a useful indicator taxon for the impact of HWA on other hemlock-associated communities for several reasons. Ants are abundant and diverse in eastern (U.S.) forests (Cole 1940, Gotelli and Ellison 2002); they interact with plants (*e.g.,* Beattie and Culver 1981, Guo and Brown 1996) and invertebrates (Buckley 1987, Cushing 1997); and they can both drive (*e.g.,* Lyford 1963, Folgarait 1998, MacMahon et al. 2000) and respond to (Johnson 1992) ecosystem processes. Ant community structure changes in response to changes in plant community structure in deserts and grasslands (*e.g.* Bestelmeyer 2005), and ants have been used as indicators of ecosystem degradation and successful rehabilitation in arid environments (*e.g.,* Andersen et al. 2002).

As a first step to understanding how ant community structure could be affected by HWA impacts to hemlock forests, we examined how ant species richness and composition varied among intact hemlock stands, hemlock stands in varying states of degradation due to HWA and that have varying amounts of early-successional shrubs and hardwoods, and mid-successional deciduous stands – to which succession will proceed following the loss of hemlock in New England. Our results have implications for managing anticipated changes in local and regional biodiversity associated with the impact of HWA on New England forests. The observed compositional changes in ant communities associated with loss of hemlock also may be associated with substantial changes in soil ecosystem processes.

METHODS

SITES

We sampled eight sites in Connecticut (CT) and eight sites in Massachusetts (MA). The CT sites are a subset of those surveyed by Orwig et al. (2002) for HWA damage in southern New England, and are focal sites for studies of the impact of HWA on ecosystem processes (Jefts and Orwig 2005). These sites were all dominated by hemlock, and mortality due to HWA in these sites ranges from none to nearly 100% (Table 1). The MA sites were all 90 x 90 m plots

Table 1. Site characteristics. Latitude and longitude are in decimal degrees; elevation is in meters above sea level; overstory basal area is in m^2 (in a 20 x 20 m plot at the CT sites and a 30 x 30 m plot at the MA sites; percent hemlock is percent of total basal area that is live hemlock; GSF is global site factor—proportion of total solar radiation above the canopy that reaches ground level (in the center of the sample plot); AntS is number of ant species collected at each site.

Site	Lat.	Long.	Elev.	Overstory Basal Area	% Hemlock	GSF	AntS
Connecticut							
Selden Neck	41.40	72.40	64	41.8	31	0.043	10
Burnham Brook	41.46	72.33	132	8.4	2	0.067	10
Devil's Hopyard	41.47	72.34	72	38.1	65	0.056	10
Sunrise Resort	41.50	72.48	128	32.2	32	0.039	8
Salmon River	41.56	72.44	187	36.7	60	0.041	9
Ash Brook	41.78	72.40	174	20.8	29	0.110	12
Willington Hill	41.87	72.25	178	65.8	87	0.022	4
Crooked Road	41.98	72.27	228	60.2	65	0.065	7
Massachusetts							
Simes 1	42.47	72.22	207	49.6	82	0.073	4
Simes 2	42.47	72.22	210	44.2	68	0.034	3
Simes 3	42.47	72.22	214	40.5	56	0.053	4
Simes 4	42.47	72.21	225	51.4	77	0.038	7
Simes 5	42.47	72.21	220	52.2	78	0.081	4
Simes 6	42.47	72.21	224	71.9	70	0.080	3
Simes 7	42.48	72.21	229	44.8	6	0.083	8
Simes 8	42.47	72.22	220	26.4	0	0.041	11

located within the Simes Tract at the Harvard Forest. These plots are scheduled for experimental manipulations (logging, girdling to simulate slow death due to HWA, or no treatment) in 2005; plots 1-6 are in stands dominated by hemlock, whereas plots 7 and 8 are mid-successional hardwood "controls" (Barker-Plotkin et al. 2004). As of summer 2004, HWA had been found in two of the Simes plots (plots 5 and 6), but no impacts on tree vigor had yet been observed (Diana Barszcz and Scott Costa, pers. obs.).

ANT SAMPLING

We used standard methods for sampling diversity of ground-nesting ants (Agosti and Alonso 2000). At each site, we established a square grid of 25 pitfall traps (20 x 20 m with 5 m spacing in CT; 10 x 10 m with 2.5 m spacing in MA). Each pitfall trap consisted of a 95mm-diameter plastic cup filled with 20 mm of dilute soapy water. Traps were buried so that the upper lip of each trap was flush with the surface of the substrate, and left in place for 48 hours during dry weather. Trap contents were fixed in the field in 95% EtOH. After the pitfall traps were collected, we removed the traps, refilled the holes, and set out a grid of 25 bait stations each consisting of 50 g of Pecan Sandies on a 12.5 x 7 cm index card. Baits were allowed to accumulate ants for 1 hour in the middle of the day, and then representative individuals were collected with a suction aspirator. We also collected 3 1-liter leaf litter samples from each grid and sifted them in the field to collect litter-dwelling ants. Finally, we actively searched in and around each grid for 1 hour and hand-collected any ants that were found on the substrate, in the leaf litter, or on low-growing vegetation. At each site, two complete ant surveys (pitfalls, baits, litter, and hand-samples) were conducted separated by approximately 42 days. The same grids were re-sampled in the second survey. The CT sites were sampled in June–August, 2004, and the MA sites were sampled in July and August in both 2003 and 2004 (with only one sample in 2004). All ants were identified to species; identifications were confirmed by Stefan Cover of the Museum of Comprative Zoology.

VEGETATION SAMPLING

To obtain an "ant's-eye" view of the vegetation at each site, we recorded the number of stems of all herbs and shrubs in 50 x 50 cm square quadrats centered on each pitfall trap. We sampled vegetation in mid-July, the peak of the growing season, to ensure that we recorded the vast majority of both early and late emerging plants. In addition, diameter at breast height (DBH) of all trees greater than 5 cm DBH in each grid was also recorded. Total basal area and percent basal area in hemlock were determined from these data.

CANOPY MEASUREMENTS

Available light levels beneath the forest canopy were estimated from hemispherical canopy photographs, which were taken after full leaf flush of the canopy at each site. Photographs were taken 1 m above ground level using an 8mm fish-eye lens on a Nikon F-3 camera. Although this does not precisely correspond to an ants-eye view at ground level, differences among sites in ground-level light interception are closely correlated with these measurements (Chazdon and Field 1987, Rich et al. 1993). The camera was leveled and oriented to magnetic north for each photograph. Images were digitized at 300dpi and analyzed using HemiView 3.1 (Delta-T, Cambridge, United Kingdom). We summed weighted values of direct site factor

(DSF) (total direct beam solar radiation) and indirect site factor (total diffuse solar radiation) to compute a global site factor (GSF) (total solar radiation) for each forest site (Rich et al. 1993). GSF values are expressed as percent of total possible solar radiation (i.e., above the canopy) during the growing season (April through October), corrected for latitude and solar track.

RESULTS

In total, 22 species of ants in three subfamilies were collected across the 16 sites (Figure 1). Of these, four are common generalist species of eastern forests (*Camponotus pennsylvanicus* (de Geer), *Aphaenogaster rudis* (Enzmann) *s.l.*, *Temnothorax longispinosus* (Roger), and *Myrmica punctiventris* (Roger)) and four are southern species near their northern range boundary (*Prenolepis impairs* (Say), *Acanthomyops interjectus* (Mayr), *Camponotus chromaiodes* Bolton, and *Stenamma schmitti* Wheeler). The average species richness (á-diversity) at a site was 7 species, and ranged from 3 to 12 species (Table 1). The average pair-wise Jaccard dissimilarity (one measure of landscape-level, or β-diversity) among sites was 0.73 (range = 0.2 – 0.93), indicating substantial differences among sites.

Ant species richness was lowest in dense hemlock stands and increased significantly as hemlock basal area declined (Table 1, Figure 2). This change in species richness appears to be an actual effect of hemlock, as ant species richness was not significantly associated with other, potentially confounding factors, including latitude ($P = 0.19$), elevation ($P = 0.33$), or richness (number of species) and diversity (Simpson's index) of understory vegetation ($P = 0.07$).

The increase in species richness in early-successional mixed stands of shrubs and hardwoods (CT stands impacted by HWA) and in mid-successional (80-125 year-old) deciduous stands (MA stands without HWA) was due almost entirely to the occurrence in these stands of *Formica* spp., which were entirely absent in dense hemlock stands. This pattern is independent of subgenera: *Formica aserva* Forel (subgenus *sanguniea*) is an actively-foraging, slave-making ant. The similarly active *Formica neogagates* Viereck (subgenus *neogagates*) forms small colonies. *Formica subsericea* Say and *F. subaenescens* Emery (both subgenus *fusca*) are generalists and somewhat less active foragers. As with the overall relationship between species richness and hemlock basal area, the effect on *Formica* appears to be an actual effect of hemlock, as there was no consistent relationship with insolation ($P = 0.73$) – an otherwise common driver of ant activity (Figure 3).

DISCUSSION

We examined changes in ant diversity and composition in stands with a known history of HWA impact (the CT stands) (Orwig et al. 2002), in stands so far undamaged by HWA (Simes plots 1-6 in MA), and in mid-successional hardwood stands (Simes plots 7-8 in MA) that represent the anticipated stand structure 80-100 years from now, after HWA has eliminated hemlock from our landscape (Barker-Plotkin et al. 2004). At the stand level, a shift from hemlock to hardwood resulted in an increase in ant species diversity (Figure 2). Because geographic (latitude and elevation) or habitat (understory vegetation structure, composition, and insolation) did not significantly affect ant species richness, we conclude that the low levels of

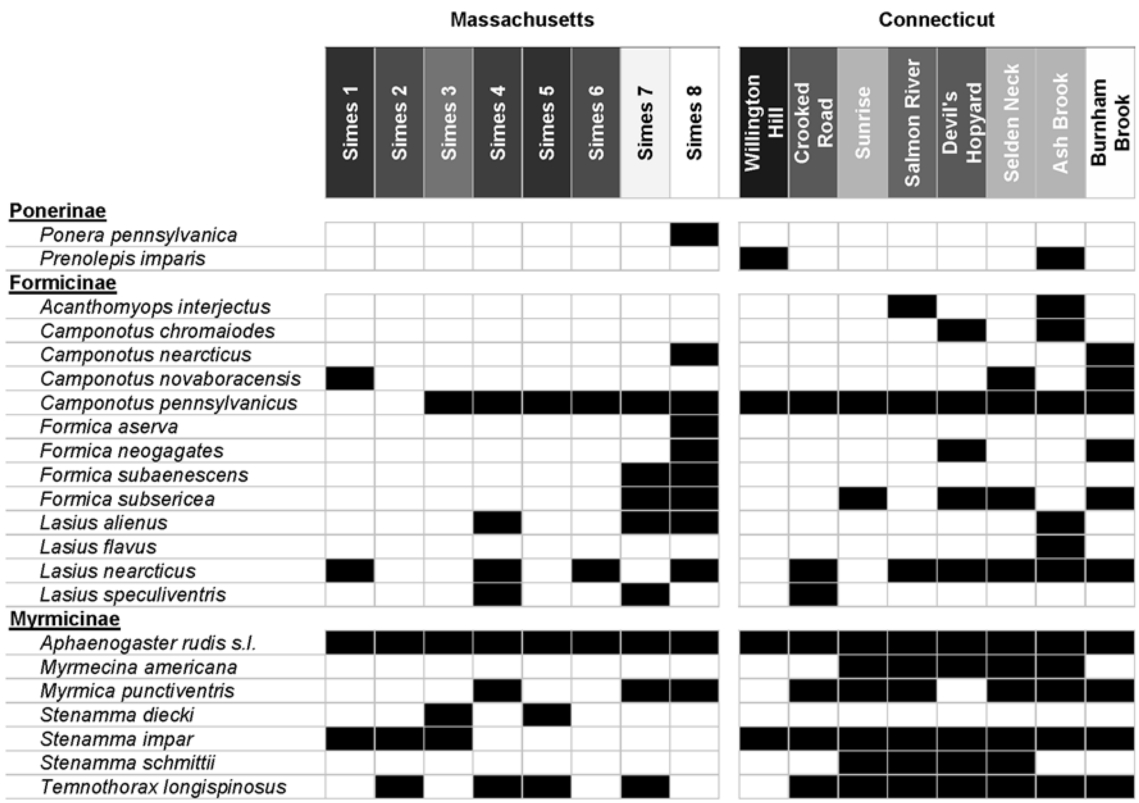

Figure 1. Species by site matrix of ant species at the 16 sampled sites. Shading of the site name is proportional to the percent hemlock cover (black = 100%; white = 0%).

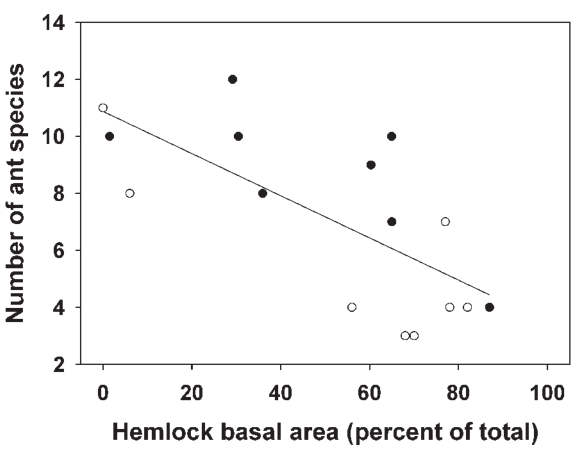

Figure 2. Relationship ($r^2 = 0.50$, $P = 0.002$) between ant species richness and percent of hemlock (basal area) in the eight sampled stands in Connecticut (solid circles) and the eight sample plots in Massachusetts (open circles).

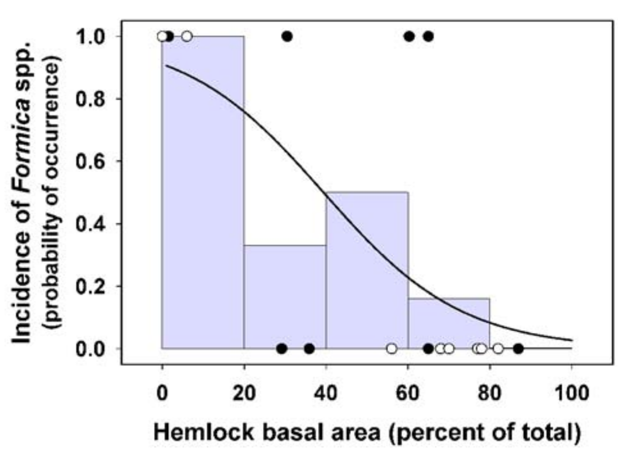

Figure 3. Incidence of *Formica* spp. at the 16 sites. Points are the raw data (solid circles: Connecticut stands; open circles: Massachusetts stands); bars are proportion of sites in each of five basal area classes in which *Formica* spp. were found; the line is the best-fit logistic regression ($P = 0.045$) to the incidence data, and predicts the probability of encountering *Formica* for any given percent of hemlock basal area.

ant species richness observed in hemlock results from effects of hemlock itself on the system. Such effects could include changes in local microclimate or soil properties (Mladenoff 1987, Finzi et al. 1998a and 1998b, Ferrari 1999, Hadley 2000). The increase in species richness observed in early- and mid-successional hardwood stands could be associated with a greater number of homopterans in these forests (Buckley 1987). Homoptera are often tended by formicine ants, and the rate and impacts of colonization by *Formica* spp. into HWA-infested stands may alter homoptera population dynamics, consequent nutrient throughfall (*cf.* Stadler et al. 2005), and ecosystem structure and dynamics in these forests. The proximate and mechanistic causes and the system-wide effects of this compositional shift in ant diversity merit further study.

An oft-professed goal of ecosystem management is to increase biodiversity (*e.g.,* Tilman et al. 1997, Chapin et al. 2000, Garber-Yonts et al. 2004); the increase in ant species richness as hemlock declines superficially suggests that HWA exerts a net positive effect on faunal diversity in New England forests. However, the increase in ant species richness at local (stand) scales would be more than offset by the reduction in regional biodiversity (expressed as dissimilarity among sites). In other words, by converting a regionally diverse assemblage of forests stands, HWA homogenizes our forests – their plants, their ants, and presumably other associated fauna. This biotic impoverishment affects us all.

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Competitive Interactions Among Two Biological Control Agents of Hemlock Woolly Adelgid and an Established Generalist Predator in Southwestern Virginia

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ABSTRACT

Classical biological control using prey-specific predators is currently underway to manage hemlock woolly adelgid (HWA) in the eastern United States. *Laricobius nigrinus* Fender and *Sasajiscymnus (=Pseudoscymnus) tsugae* Sasaji and McClure are prey-specific predators with good phenological synchrony with HWA. *Harmonia axyridis* Pallas, a highly polyphagous and voracious predator, previously introduced for biological control of various homopteran pests, is now commonly found in association with HWA in the southeastern United States. Competitive interactions among species utilizing the same resource at the same time can lead to reductions in predator diversity and decrease the efficacy of biological control. Therefore, we examined interactions among these three species in two hemlock stands in southwestern Virginia.

Adult female predators were enclosed in sleeve cages on infested hemlock branches for 6 weeks (April-May, 2003-04) at a high and low elevation hemlock stand in southwestern Virginia. Ten eastern hemlocks (20-40 year) were selected at each site that had densities of 3-4 HWA/cm. Predators were evaluated during the winter (sistens) generation of HWA, alone and in two- and three-predator conspecific or heterospecific groupings. Predator survival was assessed as the number of live predators at the end of each trial. Consumption of the HWA winter generation (sistens), impact on the spring generation (progrediens), and net reproduction for each predator was evaluated for 200 cm of branch clippings per enclosure. HWA densities on branches without predators were used as a control. Survival was analyzed using logistic regression. Predator feeding, impact, and net reproduction were analyzed using two-way ANOVA. Independent categorical variables for both models included species combination, site, and their interaction. Results were considered significant at the 5% probability level.

Survival by all predator species was not significantly affected by predator combination or site. Intra- and interspecific predation by *L. nigrinus* or *S. tsugae* was not observed and may be due to biological constraints (such as host specificity) or experimental conditions (such as high HWA densities). Intra- and interspecific predation by *H. axyridis* is well documented, but was not observed in our studies. Previous observations suggest it to be inversely related to prey density in this species. Its absence in our studies may be due to high HWA densities, avoidance behaviors, or temporal or spatial asynchrony among these species.

Predator feeding on the winter (sistens) generation of HWA and their impact on the following spring (progrediens) generation was significantly different by species combination and site. Among individual predators, *L. nigrinus* and *H. axyridis* had greater feeding overall than *S. tsugae*. This may due to *L. nigrinus* being adapted to lower temperatures and to the larger body size and voracity of *H. axyridis*. Feeding was significantly greater for all species in two- and three-predator conspecific and heterospecific combinations. This suggests that predator interactions did not significantly interfere with feeding activity. Feeding was greater at the low elevation site, and this may be related to the higher mean temperatures found at that site. Similarly, an individual predator, *L. nigrinus* had much greater impact than *H. axyridis* or *S. tsugae* on reducing the number of developing progrediens in the next generation. This may be attributed to the large number of larvae produced during the trial, which disturbed and consumed many HWA ovisacs. The other species fed mainly on sistens adults that had already laid their eggs. *L. nigrinus* and *H. axyridis* had significantly greater impact in conspecific groupings and with one another, while heterospecific grouping with *S. tsugae* did not increase their impact. However, further research is necessary to determine the density of predators, alone or in combination, that will maintain HWA densities below injury levels. Impact was greater at the high elevation site, and this may be attributed to increased HWA mortality at the lower temperatures found there. All predator treatments had greater feeding and impact than no-predator control branches.

Net reproduction by *L. nigrinus* and *H. axyridis* was significantly different by species combination and site. As an individual species, *L. nigrinus* had very high net reproduction when alone or with conspecifics in comparison to the other species. Immature stages were also further advanced, developmentally, than the other species. Since *L. nigrinus* has a much lower developmental threshold, they may have been able to continue reproduction at the low temperatures of early spring. Low reproductive output by *S. tsugae* and *H. axyridis* may be attributed to higher temperature requirements or prey quality. Competitive interference by conspecifics was observed for all species to varying degrees. For *L. nigrinus* and *S. tsugae*, this was detected by lower-than-expected results with conspecifics. Interference was the most pronounced for *H. axyridis*. Previous studies indicate that indirect interference, in the form of fecal cues or oviposition-deterring pheromones, as well as direct interference, such as through egg cannibalism, occur in this species. Similar mechanisms may be present in the two prey-specific species, but this requires further investigation. In contrast, no significant predator interference was detected in heterospecific groupings. This may be attributed to prey specificity by *L. nigrinus* and *S. tsugae*, the abundance of HWA provided, or the occurrence of different ovipositional strategies that may have served to reduce resource competition. Net reproduction was also greater at the low elevation site, and this may again be related to the higher mean temperatures found there.

The lack of strong seasonal synchrony for these species may limit competitive effects in field populations. For *L. nigrinus*, there may be limited overlap with other predators due to their earlier spring activity and development. However, *S. tsugae* may be at greater risk due to their later spring development, which overlaps with the presence of many generalist predators. Implications for management include applying low-density releases of the biological control agents to reduce the potential for conspecific interference and rapid dispersal. Further studies are warranted to examine both direct and indirect mechanisms of competition, the existence of avoidance strategies, and the degree of natural dispersal (*i.e.* immigration and emigration) of these species in hemlock stands.

KEYWORDS

Laricobius nigrinus, *Sasajiscymnus (=Pseudoscymnus) tsugae*, *Harmonia axyridis*, biological control.

Hemlock Ecosystems and Spatial Patterns of *Adelges tsugae* Infestation in Northwestern North Carolina

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ABSTRACT

Hemlock (*Tsuga spp*.) ecosystems and associated plant assemblages are at risk of decline across the Southern Appalachians. Grandfather Mountain, Linville, Lutherock, and northern Watauga County in North Carolina harbor excellent examples of these ecosystems. If not suppressed, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, may facilitate the destruction of native hemlocks in the region. The goals of this project are: 1) identify biotic and abiotic interrelationships within hemlock ecosystems, 2) assist the surrounding communities in employing an effective system for conserving biodiversity in hemlock ecosystems using a geographic information system (GIS), and 3) develop a methodology for permanently monitoring hemlock ecosystems. Study sites are located within 20 hemlock forests in five localities to maximize combinations of age, elevation, slope, and aspect. Surveys were conducted at these sites using the North Carolina Vegetation Survey methodology, with the addition of woody seedling surveys, arthropod inventories, and age class analysis. The results indicate there were unique plant assemblages within these hemlock ecosystems. Preliminary data suggests that high HWA infestation levels within these ecosystems occurred in areas closest to roads and streams. Plots at the highest elevations had lower HWA infestation levels than those at lower elevations. Results from this study can be used as a reference by researchers, land managers, and conservationists to prioritize Southern Appalachian hemlock forests for conservation and/or restoration.

KEYWORDS

Tsuga, hemlock, adelgid, ecosystem, Southern Appalachians.

INTRODUCTION

The diversity of plant assemblages in the Southern Appalachians is strongly influenced by elevation and moisture (Braun 1950, Whittaker 1956). Within the Southern Appalachians, the hemlock ecosystems of northwestern North Carolina are exceptional because they provide unique niches for a diversity of flora and fauna. Only a few scattered fragments of undisturbed or old-growth ecosystems still remain in the region. Grandfather Mountain, Linville, Lutherock, and northern Watauga County in North Carolina harbor excellent examples of these ecosystems. If not suppressed, the hemlock woolly adelgid (HWA), *Adelges* *tsugae* Annand may facilitate the destruction of these native hemlocks. In order to facilitate the present management techniques and restoration efforts we must understand the composition and design of the hemlock ecosystems in northwestern North Carolina in part by asking these questions: 1) What are some ecological indicators that differ between healthy and declining hemlock ecosystems? 2) How do species richness, basal area, importance values, and soils vary within or among these ecosystems? 3) Are roads and streams the important vectors of HWA transmission? 4) What elevations are HWA infestation levels highest? The specific goals of this project are to: 1) identify biotic and abiotic interrelationships by gathering baseline ecological information within hemlock ecosystems of northwestern North Carolina, 2) Assist surrounding communities in employing an effective system for conserving biodiversity in hemlock ecosystems using a geographic information system (GIS), and 3) Develop a methodology for permanently monitoring hemlock ecosystems.

MATERIALS AND METHODS

This study identified, classified, and mapped the patterns of infestation and mortality of hemlocks due to HWA. This project collected data both in the field and in the laboratory regarding the vegetation structure of hemlock ecosystems. A total of 16 permanent plots at five sites have been analyzed. Surveys on four additional plots will be performed by spring 2005. All sites were located on private lands within Avery and Watauga counties, North Carolina. The five sites surveyed were Linville (LINN), Grandfather Mountain (GFM), Appalachian State University Tater Hill Bog (THB), Appalachian State University Gilley Property (GP), and Lutherock Camp (LR). Plot site selection and data collection followed the North Carolina Vegetation Survey Protocol (Peet et al. 1998) (Figure 1). Individual hemlock trees within permanent plots were monitored for HWA, measured diameter at breast height (DBH) in centimeters, estimated height, and estimated amount of defoliation (Mausel 2003). The composition of hemlock forests was compared to the composition of similar forests in the Southern Appalachians. Infestation was monitored by sampling hemlock limbs in fall of 2004. Sampling will occur again in 2005. Plot environmental variables were derived from 10-meter resolution digital elevation model (DEM) using ArcGIS 9.0 software. We analyzed streams and roads at a scale of 1:250,000 using data from the Southern Appalachian Assessment (SAA) GIS database (Herman 1996). The landscape survey of plots was conducted with rigorous field work, GIS, and remote sensing techniques.

RESULTS

A total of 16 hemlock plots were surveyed (Table 1). Hemlock woolly adelgid (HWA) occurred in 81% of the plots. Infestation levels ranged from 0 (no infestation) to 4 (heavy infestation). The average infestation level for all plots surveyed was 1.94 (light infestation). The elevation of plots ranged from 996 m at GP3 to 1348 m at GFM3. Average elevation was 1175 m. Slope of the plots ranged from 1° at GP4 to 34° in LINN1. Average slope was 17°. The minimum distance to a stream was 73 m at LINN1. The maximum distance to a stream was 1348 m at GFM3. Average distance to a stream was 466 m. The minimum distance to a road was 74 m at LINN1. The maximum distance to a road was 1177 m at GFM3. The

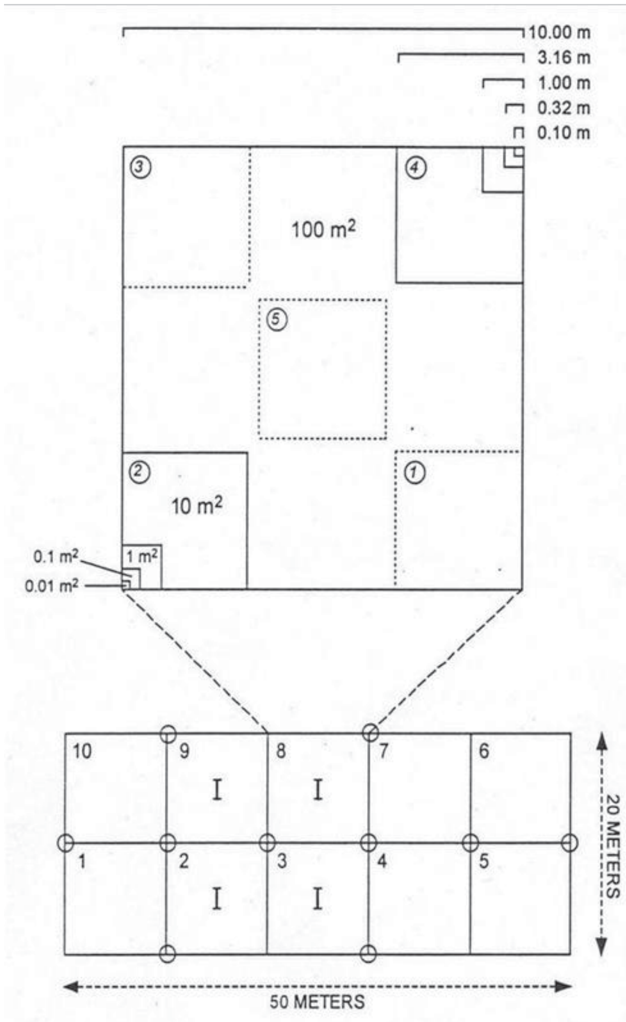

Figure 1. Hemlock permanent plots. Plots were surveyed at the canopy, subcanopy, and herbaceous levels using North Carolina Vegetation Survey methodology (Peet et al. 1998). Layout of 0.1 ha hemlock permanent plot. Each plot was 50 m x 20 m and consists of 10 focus modules. Modules 2,3,8,9 were focal modules with nested quadrats in the corners.

Table 1. Summary of ecosystem characteristics for hemlock ecosystems of Linville (LINN), Grandfather Mountain (GFM), ASU Gilley Research Station (GP), and ASU Tater Hill Bog (THB) within Avery and Watauga counties, North Carolina.

Plot	Aspect	Elevation (m)	Slope (°)	Distance to stream (m)	Distance to road (m)	Infestation Level
LINN1	W	1131	34	73	74	4
LINN2	NW	1111	8	265	505	2
LINN3	W	1100	17	275	134	1
LINN4	E	1179	25	310	261	4
LINN5	NW	1229	21	670	771	3
GFM1	SW	1333	14	1193	171	1
GFM2	S	1347	6	1474	148	0
GFM3	N	1348	31	153	1177	2
GFM4	NE	1234	11	362	412	1
GFM5	N	1220	29	544	120	4
GP1	SE	1038	7	447	456	2
GP2	S	1017	29	380	429	2
GP3	NE	996	20	532	567	1
GP4	NE	1001	1	598	485	4
THB1	N	1259	11	88	441	0
THB2	NW	1252	3	87	586	0
Average		1175	17	466	421	1.94

average distance to a road was 421 m. Preliminary data suggested that high HWA infestation levels occurred in areas closest to roads and streams. Plots surveyed at the highest elevations had lower HWA infestation levels than those at lower elevations.

IMPLICATIONS OF THIS STUDY

Hemlock woolly adelgid (HWA) is responsible for hemlock decline in northwestern North Carolina. Suppression of HWA and the future of hemlock ecosystems in the Southern Appalachians will depend on a mixture of biological control agents and efficient use of insecticides. Early detection and unfavorable climate may assist the suppression of HWA populations in the near future. This study will provide better estimates of habitats vulnerable to HWA infestation and may contribute to protection of this unique ecosystem. Our baseline data can potentially facilitate identification of areas conducive for the release of predator beetles on HWA. The examination of hemlock ecosystems and HWA may furthermore prove constructive for monitoring potential biological impacts of hemlock decline on mammal, avian, amphibian, aquatic, and herbaceous plant communities throughout the Southern Appalachians.

Regardless of our ability to reconstruct forest composition through modeling, there is no substitute for long-term forest data. Data will be used in an effort to facilitate future ecosystem restoration, land and pest management decisions, and to efficiently conserve hemlock forests that are aesthetically and ecologically the most important to the region.

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Assessment of Egg Releases for Establishment of *Sasajiscymnus tsugae* on Eastern Hemlock

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ABSTRACT

Infestations of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), are now regularly found on eastern hemlock, *Tsuga canadensis* (L.) Carriere, in the Great Smoky Mountains National Park. Management programs have been implemented to reduce the impact of this exotic pest on hemlocks. One management tool has been to release an exotic predatory lady beetle, *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae* (Sasaji and McClure), which feeds on hemlock woolly adelgid. Unfortunately, rearing programs to produce large numbers of adult *S. tsugae* are labor intensive and expensive. Thus, alternate release plans (e.g., egg releases) are under investigation to maximize production capacity and minimize inputs. Egg releases would save time, effort, and money. This paper summarizes results of the first year of this multi-year study to assess egg releases as a means to establish *S. tsugae*. Results supported the use of egg releases for field establishment of *S. tsugae* against hemlock woolly adelgid, as eggs hatched, immatures developed through all life stages, and adults were recovered. The timing of egg releases with suitable prey stages is critical and imperative to the successful survival and colonization of *S. tsugae*. Suitable food must be present at the time of release, as well as several weeks after release. Further research will better clarify optimum release conditions and contribute to a more defined release protocol to enable forest resource managers to enhance release and establishment of *S. tsugae* against hemlock woolly adelgid on eastern hemlock.

KEYWORDS

Sasajiscymnus tsugae, biological control, predator, *Tsuga canadensis*, *Adelges tsugae*.

INTRODUCTION

The health and survival of eastern hemlock, *Tsuga canadensis* (L.) Carriere, are now threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), a small aphid-like insect introduced into the United States from Japan and China. Knauer et al. (2002) listed the hemlock woolly adelgid as the single greatest threat to the health and sustainability of hemlock as a forest resource in eastern North America. In the forests of the southern Appalachians, eastern hemlock is an important component and is widely distributed. More than 1,500 ha of hemlock-dominated forests are found in the Great Smoky Mountains National Park (GRSM), and some of these trees are more than 400 years old (Johnson 1995). Of the more than 100 native tree species in GRSM, the eastern hemlock is the only species of hemlock known to occur within its boundaries (Taylor 2002). Eastern hemlock is ecologically and environmentally important as a forest component and is a dominant species in at least five of the 12 vegetation types in GRSM (Taylor 2002).

Unfortunately, management tools are limited in an entire forest, considering its area and diverse systems. Although insecticides may save individual trees, area-wide chemical control is impractical or not economically feasible in the forest. No native natural enemies or pest-resistant eastern hemlocks have been found, but exotic biological control agents have been identified as potential mortality agents of hemlock woolly adelgid (Butin et al. 2002, Cheah and McClure 1996, 1998, 2000, McClure 1995, McClure et al. 2000, Montgomery and Lyon 1996, Wallace and Hain 2000). One of these organisms, a lady beetle, *Sasajiscymnus* (=*Pseudoscymnus*) *tsugae* (Sasaji and McClure), has been released against hemlock woolly adelgid in numerous states (Blumenthal 2002, Casagrande et al. 2002, Cheah and McClure 2002, McClure and Cheah 2002). The release of *S. tsugae* in newly-infested areas, such as the GRSM, before the adelgid becomes widely distributed may lower the adelgid's ability to reach highly damaging levels.

Rearing programs to produce large numbers of adult *S. tsugae* are labor intensive and expensive. Thus, the development and implementation of alternate release plans (e.g., egg releases) would maximize production capacity and minimize inputs. Releases of eggs at field sites would be a tremendous boost to rearing programs by saving time, effort, and money. A multi-year research project was initiated to assess the potential success and benefits of alternative strategies to release and establish *S. tsugae* on eastern hemlock. This research focuses specifically on egg releases and includes the following objectives: 1) develop protocols for egg releases of *S. tsugae* to augment the regional biological control effort, 2) evaluate incidence and establishment of *S. tsugae* one year after field release, and 3) assess development, survival, and colonization of *S. tsugae* released as eggs against hemlock woolly adelgid in sleeve cages in the field. This paper summarizes results of the first year of this study.

MATERIALS AND METHODS

Before the initiation of this project, discussions were held with numerous individuals with experience working with large-scale insect rearing programs or with *S. tsugae*. Based on these discussions and previous research results (specifically, those presented in Palmer and Sheppard 2002), protocols were developed to acquire and maintain large numbers of eggs for shipment (obtained from the New Jersey Department of Agriculture), laboratory confinement, and placement in the field. Eggs were shipped via overnight mail and placed in the field on the day they were received.

In 2003, egg releases of *S. tsugae* were made at two locations (Jakes Creek, 16 trees, and Meigs Creek, six trees) by park personnel in the GRSM. Eggs were placed in the field on April 24 at Jakes Creek (4,000 eggs) and on May 22 at Meigs Creek (5,000 eggs). Post-release evaluations (visual examinations, sweep-net samples, and branch collections evaluated in the laboratory) were conducted several months and one year after egg releases.

In a separate study to investigate the development and survival of *S. tsugae* on eastern hemlock, twigs and gauze containing eggs (ca. 6,500) of *S. tsugae* were placed on twigs using twist ties and covered with screened sleeve cages. Eggs were placed in 21 sleeve cages (ca. 250 eggs/cage) in the field on April 22, 2004, at Elkmont. As a control treatment, eggs also were placed on twigs of eastern hemlock in jars (3.8 l) and maintained in incubators in the laboratory. Three cages were removed each week for seven weeks and taken to the laboratory where the contents were examined thoroughly; the number of damaged and nondamaged woolly masses were counted to assess predator activity. Development and survival of *S. tsugae* in the control jars also was monitored weekly for comparison. Data analyses were performed using SPSS® (2002) to assess differences among seasonal survival and development of beetles.

RESULTS AND DISCUSSION

Low numbers (fewer than 10) of larvae and adult beetles were found several weeks after release at both Jakes Creek and Meigs Creek locations in 2003. However, no larvae or beetles were recovered at either location one year post-release, in 2004. These results were somewhat consistent with adult beetle releases in which predator recovery was low in subsequent years (P.L. Lambdin, unpublished data). Tree health and adelgid vigor and quality on release trees were extremely poor in 2004; these characteristics are not conducive to beetle viability. Results also suggested that the initial egg releases in 2003 may have been made too late in the season for developing beetles to have sufficient food (i.e., crawlers) for an extended duration.

Sleeve cage evaluations suggested that egg releases of *S. tsugae* provide a viable means to attempt to establish populations of this predator. Eggs hatched and all larval instars developed including prepupa and pupa. Adults were recovered (though in low numbers), suggesting that trees could be colonized via egg releases of *S. tsugae*. Poor adelgid quality or lack of suitable stages may have affected adult development.

Predatory activity of larvae on adelgids within sleeve cages was evident. About 43.9% of all woolly masses on twigs inside cages were damaged by *S. tsugae*. Comparatively, only 19.1% of woolly masses held as controls in the laboratory exhibited similar damage. Although these masses were disturbed or damaged, many nondamaged eggs remained. Percentage of damaged woolly masses in the field peaked at 64.9% after five weeks (Table 1).

Adelgid crawlers were numerous when this research was initiated on April 22, and had been extremely abundant earlier. On the release date, crawler densities averaged approximately 15.5/2.5 cm of twig length. Unfortunately, densities fell sharply (ca. 93%) one week later and remained almost nonexistent during the remainder of the study (Figure 1). Because adelgid eggs and crawlers provide nutritional resources for beetle larvae, the low numbers of these prey probably impacted the numbers of beetles that developed successfully to adulthood. In the sleeve cages where suitable prey was unavailable, cannibalism of *S. tsugae* on prepupae and pupae was observed. This cannibalism could adversely affect colonization and establishment of this predator.

SUMMARY

These results are encouraging as they support the use of egg releases of *S. tsugae* for field establishment against hemlock woolly adelgid. Eggs hatched, immatures developed through all life stages, and adults were recovered. It is important to also assess the feasibility of releasing early-instar larvae, which are not as vulnerable to predators and not as susceptible to temperature/climatic fluctuations. As this preliminary assessment demonstrates, timing of egg and larval releases with suitable prey stages is critical and imperative to the successful survival and colonization of *S. tsugae*. Suitable food must be present at the time of release, as well as several weeks after release, for beetle development. Adelgid eggs, in general, are suitable food for larvae of *S. tsugae*. However, in this study, many adelgid eggs were available but were unacceptable as food for *S. tsugae* and never hatched. The reasons for these non-viable and unacceptable eggs are unclear. Further research will better clarify optimum release conditions and contribute to a more defined release protocol. Improved methodologies will enable forest resource managers to release and establish *S. tsugae* over a wider geographical area to reduce populations of hemlock woolly adelgid on eastern hemlock.

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Table 1. Weekly assessment of damaged and nondamaged woolly masses in sleeve cages during seven weeks after field placement.

Week No.	No. Damaged Woolly Masses	No. Nondamaged Woolly Masses	% Damaged Masses
2	2.91 + 0.30 a*	2.80 + 0.54 b	51.0
3	3.10 + 0.28 a	3.83 + 0.46 b	44.7
4	2.60 + 0.30 a	3.22 + 0.29 b	44.7
5	3.11 + 0.22 a	1.68 + 0.19 a	64.9
6	1.48 + 0.28 b	3.43 + 0.43 b	30.1
7	1.77 + 0.24 b	5.11 + 0.43 c	25.7

* Numbers within a column followed by the same letter are not significantly different (p=0.05).

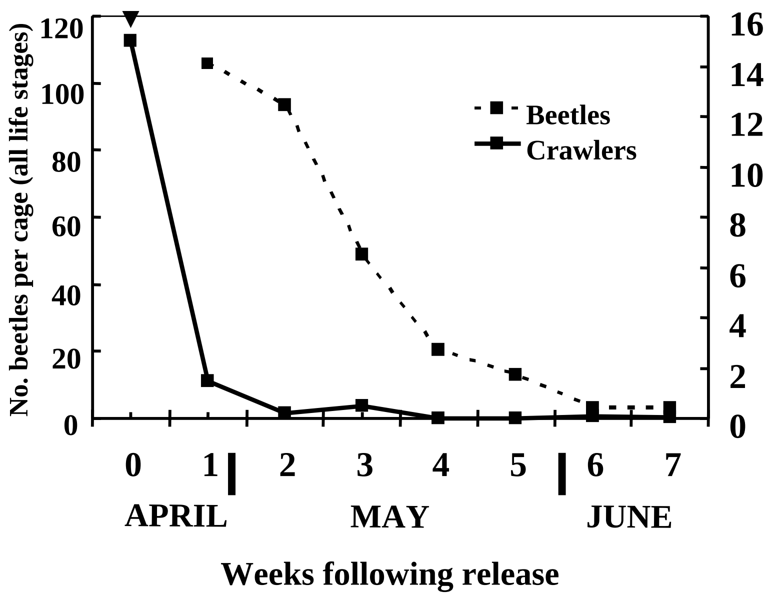

Figure 1. Weekly assessment of predator and adelgid activity in sleeve cages for seven weeks following egg release, Great Smoky Mountains National Park, 2004.

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Preliminary Assessment of the Cold Tolerance of *Laricobius nigrinus*, a Winter-active Predator of the Hemlock Woolly Adelgid from Western Canada

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ABSTRACT

Laricobius nigrinus Fender is a winter-active predator of the hemlock woolly adelgid (HWA), *Adelges tusgae* Annand, native to the Pacific northwest of North America. Adults appear in the foliage of infested hemlocks as the overwintering HWA sistens generation develops (Oct. – Dec) and begin oviposition in the ovisacs of HWA soon after adelgid oviposition begins in January. Thus, multiple life stages of the predator are present during the coldest periods of the Pacific Northwest winter. As *L. nigrinus* is being evaluated as a biological control agent against HWA in the eastern U.S., where winter climates can be more severe than those of its native range, it is important to understand the cold tolerance of the life stages of the predator present during the winter months.

We assessed the cold tolerance of adults, eggs, and larvae of *L. nigrinus* through evaluations of the supercooling points (SCPs) of field-collected adults and laboratory-reared (at 5°C) eggs and larvae. Freezing was fatal to all life stages. The mean SCPs of adults ranged between –16°C and –19°C, while those of overwintering one-day-old and five-day-old eggs were –27.5°C and –26.9°C, respectively. Newly eclosed first instar larvae (L_1) that had not yet begun to feed supercooled to -24°C, while their supercooling capacity diminished slightly (0 = –22.1°C) once feeding was intitated. Supercooling capacity diminished with each successive instar, with the SCPs of the L_2, L_3 and L_4 being –17°C, –15°C and –13°C, respectively.

Survival of eggs and adults after exposure to sub-zero temperatures above their mean SCPs (–10°C and –20°C for eggs; –10°C and –15°C for adults) for increasing durations (to a maximum of 8 hours) was also evaluated. Survivorship was highest for eggs and adults exposed to –10°C. Survival of eggs declined with decreasing temperature and increasing duration of exposure. Survival of eggs exposed to –20°C for 1 hour was 45%, with no survival evident at longer durations. Increasing durations of exposure of adults to –15°C (1°-4°C above mean adult SCPs) resulted in reduced adult survival. However, 40% of the adults tested survived 8 hours of exposure to –15°C. These results indicate that both the duration and extent of extreme winter minima may be determinants of the range of *L. nigrinus* in eastern North America.

The Effects of HWA Outbreaks on Ecosystem Level Changes in Southern New England

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ABSTRACT

The continued spread of the introduced hemlock woolly adelgid (HWA), *Adelges tsugae*, has lead to widespread decline and mortality of eastern hemlock (*Tsuga canadensis*) and initiated intensive hemlock logging. This pest alters the structural and vegetative composition of hemlock forests by transforming them into largely hardwood-dominated forests. Less well understood are the impacts that HWA has on local trophic interactions and how this pest may alter many ecosystem processes. We provide evidence from several studies (Stadler et al. 2005, Orwig et al. and Jefts et al., unpublished data) that highlight the important role that HWA plays in altering stand microenvironment, soil nitrogen (N) availability, soil mycorrhizal associations, litter quality, litter microbiology, and canopy throughfall chemistry. In addition, we compare microenvironmental conditions and nutrient availability associated with HWA infestation and with hemlock logging, one of the primary management responses to HWA outbreaks.

Results from these studies suggest that persistent HWA feeding leads to direct crown deterioration and initiates subtle but important changes in canopy characteristics that have cascading effects on a variety of ecosystem processes. Forests infested with HWA commonly have significantly higher soil temperatures (by 1 - 2 °F) and mineral soil moisture content and lower organic soil moisture content (decreases up to 40%) than uninfested forests. In addition, infested forests typically have higher soil N availability (171 and 92 µg N/g resin in infested vs. uninfested forests) due to several different mechanisms, including: 1) induced microenvironmental changes that favor decomposition due to deteriorating crowns, 2) reduced uptake as trees decline, and 3) enhanced N content of litter, and canopy throughfall. Furthermore, infested forests have significantly lower root ectomycorrhizal (ECM) colonization than uninfested forests (up to 30% less ECM coverage), suggesting that soil N is no longer limiting, and trees are not allocating as much resources to below-ground production—which may in turn also affect changes in soil nutrient availability and cycling. HWA-infested foliage exhibited significantly higher foliar N content (1.9–2.8% infested vs. 1.5–1.9% control) and abundances of bacteria, yeasts, and filamentous fungi than uninfested foliage. Throughfall precipitation collected under infested hemlock branches contained significantly higher concentrations of nitrate, total N, and dissolved organic N than that collected under uninfested branches.

Hemlock logging led to higher soil temperatures and greater available soil N than infested or uninfested forests. Although N availability is highly variable, intensive cutting of hemlock in response to HWA may lead to ecosystem level impacts that are higher in magnitude than the insect itself, further increasing N losses from these systems. Results indicate that introduced pests and selective tree decline can rapidly and dramatically alter ecosystem processes such as energy flow and ion fluxes, even prior to the onset of extensive tree mortality.

KEYWORDS

Ecosystem processes, nutrient cycling, throughfall chemistry, hemlock cutting.

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EVAULATION OF MICROSATELLITE MARKERS IN FRASER FIR (*ABIES FRASERI*)

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ABSTRACT

We tested 20 microsatellite primer pairs from Fraser fir (*Abies fraseri*) for PCR amplification and allelic polymorphism. These primers originated from cloned inserts enriched for DNA sequences containing tandem repeats of (AC)n, (AG)n, and (AAT)n. In total, 30 clones were selected for evaluation. PCR primers for 18 of these clones consistently produced single/simple PCR profiles. From these, nine markers were found to be polymorphic among 13 Fraser fir samples and are apparently robust for use in population genetic or genome mapping studies. These markers are being applied in a population genetic study of Fraser fir to assist in efforts to effectively and efficiently conserve the species' genetic diversity.

KEYWORDS

Microsatellite DNA, population genetics, fir.

Hemlock Woolly Adelgid Research at the Coweeta Hydrologic Laboratory

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ABSTRACT

Hemlock woolly adelgid (HWA) is a non-native invasive pest that impacts eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). Hemlock trees serve important ecological roles in the southern Appalachians as a keystone species in near-stream areas. Scientists at the USDA Forest Service, Coweeta Hydrologic Laboratory and their collaborators have established studies to examine the function of hemlock in riparian areas and the effects of its potential demise through the activity of HWA. We have focused our research activities in four areas: mapping and monitoring, effects, control, and restoration. Utilizing permanent vegetation plots, established in the Coweeta basin in 1934, we can map the extent and monitor the progress of HWA infestation and its effects on plant biodiversity. We have established intensive research plots to measure the effects of infestation on terrestrial and aquatic nitrogen and carbon cycling, forest and stream microclimatology, site productivity, and plant physiology. Future research will explore methods to restore ecosystem function in areas where hemlock is or will be heavily impacted by HWA. Of particular interest is restoring the function of hemlock in terms of providing critical habitat for birds and other animals, shading streams to maintain water temperatures required by trout and other aquatic organisms, and regulating nutrient, carbon, and water pools and fluxes.

GUIDELINES FOR REARING *LARICOBIUS NIGRINUS* FENDER

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ABSTRACT

Laricobius nigrinus (Coleoptera: Derodontidae) is attracting attention for its potential usefulness as a predator of the hemlock woolly adelgid (HWA), *Adelges tsugae Annand*. *Laricobius nigrinus* is a univoltine species with an unusual lifecycle, where adults spend the summer in diapause but are active for the rest of the year. Field and laboratory studies indicate *L. nigrinus* possesses many characteristics deemed favorable for biological control agents. To expedite its establishment and be a viable control option for HWA, *L. nigrinus* must be reared in large numbers for field releases. Procedures that have shown some success in laboratory rearing of *L. nigrinus* at Virginia Tech are described.

KEYWORDS

Hemlock woolly adelgid, biological control, colony rearing, predators.

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae) is an exotic insect that attacks and kills eastern hemlock, *Tsuga canadensis* (L.) and Carolina hemlock, *T. caroliniana* Engelmann trees in the eastern United States (Ward et al. 2004). HWA is an innocuous inhabitant of *Tsuga* spp in Asia, where it is believed to originate (Montgomery et al. 2000). Populations of this insect may be regulated by host resistance and natural enemies (McClure et al. 2000). In eastern North America, HWA populations rapidly reach lethal levels and with few chemical control options, natural enemies may be the most promising method for controlling HWA in the eastern states (Wallace and Hain 2000).

Laricobius nigrinus Fender (Coleoptera: Derodontidae) is a predator found in association with HWA in western North America (Zilahi-Balogh et al. 2003a), where hemlock is not typically injured by HWA (Furniss and Carolin 1977). *L. nigrinus* is being evaluated for its potential as a biological control agent for HWA. Field and laboratory studies have revealed that *L. nigrinus* can play an important role as part of a complex of biological control agents aimed at regulating HWA abundance.

This predator has several attributes that are considered necessary for a successful biological control agent including: synchrony and adaptability with its environment and host, host-searching capacity, ability to increase in numbers; and general mobility (Huffaker and

Kennett 1969). Zilahi-Balogh (2001) concluded that *L. nigrinus* adults and larvae feed selectively on HWA and in larval development tests, *L. nigrinus* completed development only on HWA, indicating that it is unable to survive on other prey species (Zilahi-Balogh et al. 2002). Field studies in British Columbia, Canada have shown that its lifecycle is highly synchronous with HWA, and field studies in Virginia show similar host synchrony in the eastern U.S. (Lamb et al. 2005). In addition, two years after a small field release of *L. nigrinus* in Virginia, F_2 adults were recovered, indicating their potential ability to establish in the eastern U.S. (Lamb et al. unpublished data).

To be a viable biological control candidate, *L. nigrinus* must be successfully mass reared for field releases. Our mass rearing efforts have focused on laboratory propagation and the use of field insectaries. Successful procedures for laboratory rearing *L. nigrinus* have been developed after four years of research. Although there is room for improvement, the following methods have yielded several generations of the beetle. This paper describes the life cycle of *L. nigrinus* and current procedures used for rearing each life stage in the laboratory at Virginia Tech.

LIFE CYCLE OF *LARICOBIUS NIGRINUS* AND *ADELGES TSUGAE*

Field studies in British Columbia (Zilahi-Balogh et al. 2003a) and rearing *L. nigrinus* in the laboratory have revealed a univoltine life cycle that is synchronous with the life cycle of HWA (Zilahi-Balogh et al. 2003b). HWA has a complex polymorphic life cycle that involves two asexual generations annually (McClure 1991). The overwintering generation (sistens) feeds and develops throughout the fall and winter months and deposits eggs within woolly ovisacs in early spring. The second generation (progrediens) lays eggs within woolly ovisacs in late spring, which hatch into sistens crawlers that settle at the base of young needles, feed for a short time, and then enter aestivation (summer diapause) (McClure 1989; Gray and Salom 1996; Salom et al. 2002). Aestivation lasts for several months, and sistens nymphs resume feeding and development in October (McClure 1987; Gray and Salom 1996).

L. nigrinus adults overwinter on the hemlock branches, feeding on HWA sistens. In late January or early February, adult *L. nigrinus* begin ovipositing single eggs directly in sistens ovisacs. Oviposition by *L. nigrinus* is synchronous with the oviposition period of HWA sistens, beginning in January or February and ending in May or June. Eggs hatch and eclosed larvae feed on HWA eggs. Larvae develop through four instars and the mature larvae drop to the ground and migrate to a pupation site within the soil. Development from egg to prepupae takes five to six weeks using the procedures outlined below. At 15°C, *L. nigrinus* remain as prepupae for about 10 days prior to pupation (Zilahi-Balogh et al. 2003b). Pupal development occurs in cells in the soil, and after about two weeks, pupae develop into adults. The adults remain in the soil and enter aestival diapause in early summer. In October, adult *L. nigrinus* emerge from the soil and migrate back to hemlock trees in search of HWA. This is approximately the same time that the HWA sistens are resuming development. Thus, both species enter and complete aestival diapause at approximately the same time.

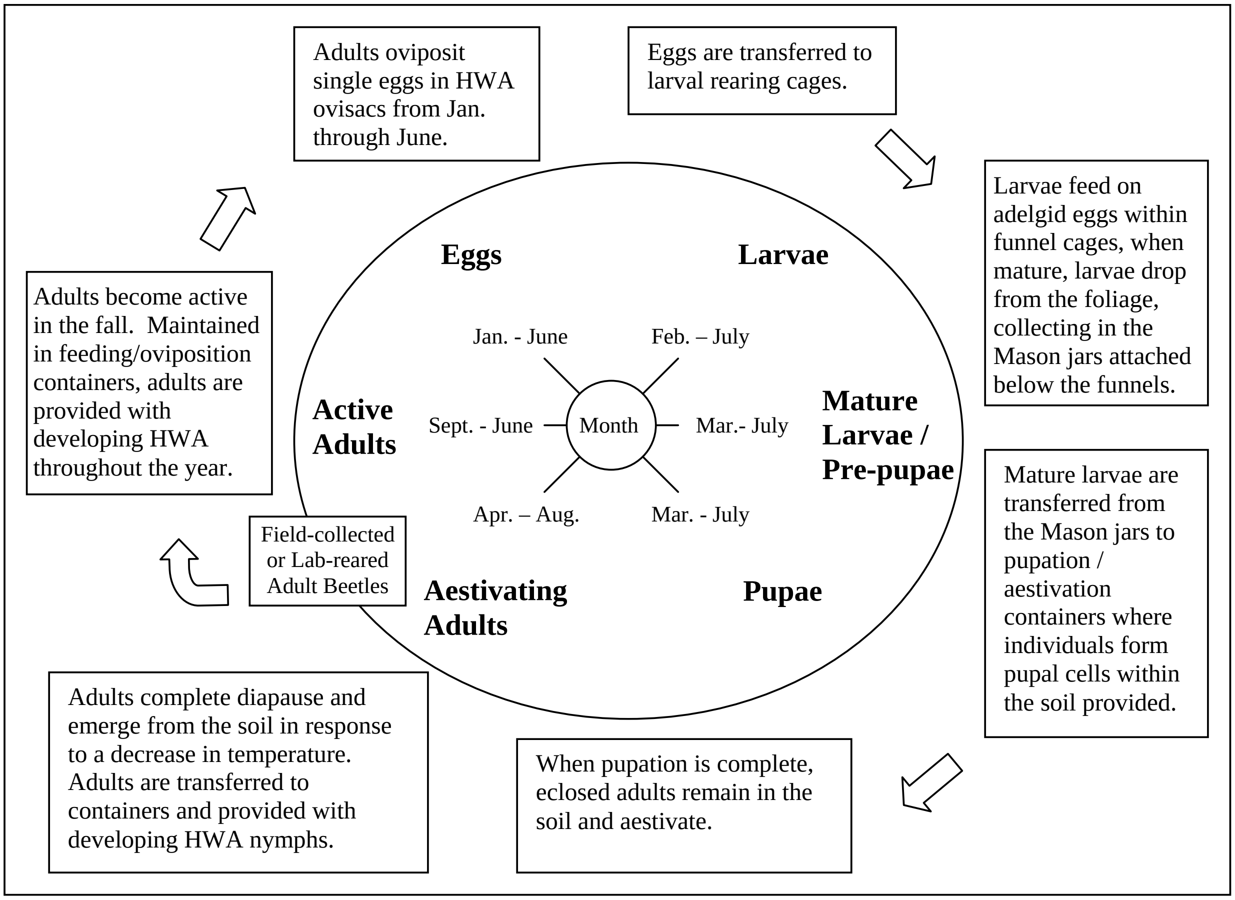

Figure 1. A representation of the univoltine life cycle of *Laricobius nigrinus* and the primary procedures occurring during the rearing of each life stage.

Table 1. Holding temperatures and day-length for *L. nigrinus* adults at different times of their active period.

Time of Year	Day:Night Temp. (°C)[1]	Daylength (h)[2]	Adults per Container	Frequency of Feeding	Destination of Old Hemlock Bouquets
Pre-oviposition (Oct.-Jan.)	4:2	12 to 10	50	Every 2 weeks	Large plexiglass box to recover lost adults
Oviposition (Feb.-Mar.)	6:4	10 to 12	25	Once a week	Inserted in floral foam and placed in funnel cages
Oviposition (Apr.-Jun.)	10:8	12 to 16	25	Twice a week	Inserted in floral foam and placed in funnel cages

[1] Increase in temperature should coincide with peak L. *nigrinus* oviposition and when HWA ovisacs are 50-75% full.
[2] Daylength is gradually increased or decreased over each period.

Figure 1 represents the life cycle of *L. nigrinus* and the general rearing procedures coinciding with each life stage. References made to specific months indicate the average time of year HWA is available in particular stages in Blacksburg, Virginia. The exact time of year *L. nigrinus* begins and ends oviposition is influenced by the stage of the HWA provided to them.

FEEDING *L. NIGRINUS* ADULTS (EARLY FALL THROUGH LATE SPRING)

Laricobius nigrinus adults become active in the early months of fall and require host material upon emergence. They begin feeding on HWA nymphs attached to hemlock branches and feed all winter. In late January, they begin to lay eggs, singly in the HWA wool sacs (ovisacs) and continue until about June (peaks in late March–early April).

The general procedures for storing and maintaining adults are similar throughout the year, however there are minor changes in conditions between the pre-oviposition (Oct.-Jan.) and oviposition (Feb-June) periods (Table 1).

Storage (Adult Feeding/Oviposition Containers)

- Gallon-sized plastic containers (screened holes on the side and the lid) with moistened filter paper cut to fit the bottom.
- Each container holds one hemlock "bouquet" (a floral foam-filled film canister holding 10-15 heavily HWA-infested hemlock branches (approximately 20 cm long).
- Adults are stored in environmental chambers programmed to create conditions shown in Table 1.

Maintenance

- At the end of each feeding period, every adult is recovered from its current container and transferred to a new container with a fresh hemlock bouquet (spray with distilled water).
- Containers are kept at 4°C immediately before adults are fed to reduce flight.
- Adults transfers are carried out under a nylon screen to a table-top cage to prevent flying adults from escaping.

  (a) Pre-ovipositing Adults

  – If all adults are recovered from old containers, discard the old hemlock bouquet.

  – If some individuals are not accounted for, old hemlock branches are placed in a large plexiglass box at room temperature and observed daily for missing adults.

  (b) Ovipositing Adults

  – It is critical to provide heavily-infested foliage with ovipositing HWA to adults to ensure that early instars can access adelgid eggs. Adult *L. nigrinus* should be provided with enough adelgids (preferably excess amounts, ~100 ovisacs per adult beetle) for themselves and the early instars of the progeny they produce.

– To ensure predator eggs are deposited in optimal sites (singly, in full adelgid ovisacs) adults are fed twice a week and the number of adults per container is reduced (Table 1). These modifications also decrease larval competition during their development and increase synchrony of larval maturation.

– The hemlock branches from which adults are removed from at each feeding contain the *L. nigrinus* eggs laid since the time of the last feeding. These branches are transferred to parafilm-wrapped floral foam blocks and are inserted alternately with fresh hemlock branches infested with oviposting HWA. The additional HWA fresh foliage provides host material for the more mobile 3rd and 4th *L. nigrinus* instars. These blocks are placed into funnel cages (Lamb et al. 2002).

DEVELOPING *L. NIGRINUS* LARVAE (DEVELOPING FROM FEBRUARY TO JUNE)

After a few weeks, the *L. nigrinus* eggs hatch within the woolly ovisac and early instars feed on the nearby adelgid eggs. Third and fourth instars are more mobile and feed on adelgid eggs and developing nymphs. The duration for *L. nigrinus* development from egg to mature fourth instar larvae (pre-pupae) varies depending on temperature and food quality. The larvae develop at temperatures ranging between 9° and 21° C; however, pupal development does not occur at the higher temperatures of this range. The optimal temperature for successful development of larvae is likely between 12° and 18°C (Zilahi-Balogh et al. 2003b). After consuming a sufficient amount of prey (200+ eggs) to complete larval development, the prepupal stage is reached. This stage is a non-feeding fourth instar that drops from the hemlock branches and seeks a pupation site in the soil. Larvae are reared in funnel cages, designed to take advantage of this "dropping/migrating" stage so mature larvae (prepupae) essentially collect themselves.

Storage (Larval Funnel Cages)

- Hemlock branches with HWA and *L. nigrinus* eggs are inserted into parafilm-wrapped floral foam blocks, intermixed with fresh HWA-infested branches, and placed into funnel cages. A cylindrical top, made of .7 mm acetate, is placed over the 25 cm funnel base and a black, pint-size Mason jar is attached underneath the funnel. Each Mason jar contains about 2 teaspoons of moistened pupating medium.[1]
- The funnel cages are set up on racks in cold rooms that are maintained at about 13°C and daylength increased at a rate similar to natural conditions. At this temperature and with adequate prey, mature larvae begin collecting in the jars below the funnels after 3-4 weeks. If the temperature increases above 18°C, development rate of larvae increases dramatically, causing funnels to yield both mature and immature larvae. It is possible that the higher temperatures cause the larvae to be more active, resulting in immature

[1] Pupating Medium:
Southland peat moss (sifted through mesh screen with 0.5 cm openings), Mosser Lee long fiber sphagnum moss (ground finely), and Play sand.
Mix materials (2:2:1 peat:sphagnum:sand) and add distilled water until mixture reaches ~50% saturation. Mixture is then sterilized using two treatments of heat and pressure for several hours or steam sterilization for 12+ hours separated by 24+ hours at room temperature.

larvae collecting in the jars below the funnels. This situation can also be observed when larvae are not provided with an adequate amount of HWA eggs within the funnel. Differences in appearance and behavior can separate mature and immature larvae.[2]

Maintenance

- Funnels are set up immediately after adults are fed.
- The foliage in each funnel is sprayed with distilled water twice a month and soil in collecting jars below are moistened every couple of days (in anticipation for maturing larvae).
- Several days before anticipated larval drop, the collecting jars below the funnels are checked by emptying the contents into a Petri dish, and a paintbrush is used to sift through the materials to recover any larvae.
- Collecting jars are checked daily to separate mature from immature larvae; mature larvae become settled for pupation if given enough time (~36 hours), but mortality of immature larvae in the collecting jars is high unless immature larvae are returned to branches with prey promptly.
- Mature larvae (prepupae) are transferred, using a moistened paintbrush, to pupation/aestivation containers for the summer.
- Immature larvae are transferred to HWA-infested hemlock and placed back in a funnel cage.

MATURE LARVAE/PREPUPAE (MARCH – JULY)

Upon being transferred to pupation/aestivation containers, mature larvae burrow into the soil, form pupal cells, and remain in a c-shape within their pupal cells for 10-14 days before development into pupae. Within 36 hours of dropping from the foliage, prepupae will settle into cells within the soil to pupate. It is important that individuals are transferred to a pupation/aestivation container before a pupal cell is formed as they have difficulty moving to a new site to pupate after they have settled.

Storage (Pupation/Aestivation Containers)

- Quart-sized and half gallon-sized, clear and opaque, plastic containers with several screened holes for air movement are used as pupation/aestivation containers. Each container contains at least 5 cm of pupating medium; the bottom layer is packed tightly and the upper layer loosely.
- Depending on the size of the container, between 50 and 200 larvae are placed in each container.

[2] Mature larvae have a yellow vental side and sclerotized dorsal side, typically are moving and are negatively phototactic and will drop off the branch if placed back on an HWA-infested hemlock branch.
Immature larvae are smaller in size, dark in color, and often have white wool attached to their dorsal side; attach themselves to objects and do not move; and will not drop off branch if placed back onto HWA-infested hemlock branch.

- Mold within the pupation/aestivation containers has been a recurring problem. Sources of fungal spores are minimized, all containers and the pupation medium are sterilized, and an anti-fungal agent (methyl paraben) is applied to the soil surface weekly.
- Pupation/aestivation containers are maintained at 15°C and long days (16 hours).

Maintainence

- Moisture level of pupation medium should be maintained at about 30-40% saturation by spraying distilled water into containers once a week.

PUPAE/AESTIVATING ADULTS (LATE APRIL THROUGH JULY)

The delicate pupae develop two weeks after burrowing in the soil. *L. nigrinus* pupae are bright yellow and require a narrow temperature regime during this time (Zilahi-Balogh et al. 2003c). At 15°C, pupation is complete in approximately two weeks and the newly eclosed adults remain under the soil surface, and enter summer diapause (aestivation). Adults remain in the soil until they are cued to emerge by a decrease in temperature (Lamb, unpublished data). To prevent premature emergence, aestivating adults are stored at high temperatures until mid-September, at which time the temperature is decreased, and adults emerge several weeks later.

Storage (Pupation/Aestivation Containers)

- Pupation/aestivation containers are kept in the large cold room at 15°C until pupation is complete. Once adults have eclosed, containers are moved to 19°C and long daylength (16 hours) until mid-September, when the containers are moved to 13°C and decreasing daylength at a rate similar to natural conditions.

Maintenance

- Pupation/aestivation containers are watered weekly to maintain about 30% soil saturation.
- Containers are checked every other day beginning in late August to recover adults emerging prematurely
- In September, after the temperature is decreased, containers are checked daily for emerging adults.

EMERGING ADULTS (SEPTEMBER – DECEMBER)

Adults complete aestivation and emerge from the soil in the fall, approximately three weeks after the temperature is decreased. Adults become active and climb up the sides and lids of the pupation/aestivation containers. Premature adult emergence is undesired because emerging adults require developing HWA nymphs to feed on, which do not emerge from diapause until October.

Storage (Feeding/Oviposition Containers)

- Emerging adults are transferred from the lids and sides of the pupation/aestivation containers to adult feeding/oviposition containers with developing HWA nymphs and distilled water.
- Recently emergent adults are kept at 6°C and 12 hours daylength. Temperature is lowered if prey is limited.

Maintenance

- Pupation/aestivation containers are checked for emerging adults several times per day, particularly in the late afternoon and evening.
- Distilled water is sprayed through the screen lids of the feeding/oviposition containers several times per week to ensure adults have adequate water. When temperature is decreased (about November), the frequency of watering decreases.
- Active adults are either released in the field or maintained in feeding/oviposition containers according to the procedures outlined in Table 1.

CONCLUSION

These rearing procedures have produced approximately 44,000 F_1 adults during the past 4 years. Survival through pupation and aestivation has a greater influence on the number of F_1 adults produced than size of the initial colony. In addition, field collected adults seem to be more fecund than lab-reared adults; therefore, starter colonies should be larger ($\geq$2000) if adults are lab-reared and smaller (500-1000) if adults are field-collected.

Several parts of the current rearing procedures need further improvement. Most importantly, the preparation and maintenance of the pupation medium needs to be modified to reduce the high level of pre-pupal and pupal mortality that occurs. In addition, pupation/aestivation containers should be modified to increase the ease and efficiency of adult recovery while they are emerging from the soil. Development of rearing containers that take advantage of the “migratory stage” of mature larvae immediately after dropping from the foliage would reduce the time spent checking funnel jars. The cage should allow larvae, needles, and debris to fall into one level and from there, enable the larvae to move themselves to a pupation/aestivation container, thereby preventing needles and debris from falling into the sterile pupating medium.

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HWA Risk Assessment in Deer Wintering Areas of Southern Vermont

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ABSTRACT

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, an introduced aphid-like insect from Asia, is threatening to eliminate eastern hemlock, *Tsuga canadensis* (L.) Carr., throughout its range. Among its many ecological functions, eastern hemlock provides winter cover and browse for white-tailed deer, *Odocoileus virginianus* Zimmerman, in areas experiencing harsh winter conditions. In central and southern Vermont, eastern hemlock is the basic component of many white-tailed deer wintering areas (DWA) and is thought to reduce snow depths and moderate temperatures. The first objective of this study is to determine the relative risk of HWA infestation to the eastern hemlock dominated DWAs in southern Vermont. The eastern hemlock component of conifer dominated DWAs will be determined and used in a GIS model predicting the susceptibility and vulnerability of DWAs to HWA infestation. A sub-sample of thirty-two DWAs in southern Vermont were inventoried in summer and fall of 2004 for analysis. The second objective is to quantify the effects of eastern hemlock cover on winter temperature and snow depth conditions. Within six site-paired hemlock and hardwood stands, temperatures were recorded half-hourly and snow depths were recorded bi-weekly in the winters of '03-'04 and '04-'05. Preliminary results indicate that extreme temperatures are moderated and snow depth is reduced within eastern hemlock stands as compared to site-paired hardwood stands. The elimination of eastern hemlock from the forests of southern Vermont by HWA would greatly alter the local ecology and will dramatically affect species reliant upon hemlock stands for winter habitat.

KEYWORDS

Tsuga canadensis, *Odocoileus virginianus*, *Adelges tsugae*, winter, risk assessment.

INTRODUCTION

Eastern hemlock, *Tsuga canadensis* (L.) Carr, represents the most important deer wintering area (DWA) cover type in southern Vermont (Reay et al. 1990), and the removal of this cover by hemlock woolly adelgid, *Adelges tsugae* Annand (HWA), has the potential to negatively affect white-tailed deer, *Odocoileus virginianus* Zimmerman, populations and health. It is believed that, for deer living in areas with severe winters, the two main factors leading to winter hardship are cold temperatures and deep snow pack (Verme 1968). Eastern hemlock is considered to be one of the most shade-tolerant species in North America (Goerlich and Nyland 1999) and grows in stands with particularly dense foliage, which is thought to reduce radiant heat loss and intercept falling snow. Thus, it is assumed that temperatures beneath hemlock canopies are moderated and snow depth is decreased, but this has not been statistically quantified. Dominant overstory vegetation replacing dying eastern hemlock tends to be exclusively hardwood species (Orwig and Foster 1998, Orwig and Kizlinski 2002), and therefore current winter conditions within hardwood stands approximate those of HWA aftermath forests.

The methods required to accurately remotely sense eastern hemlock are not yet developed. As a result, a large scale eastern hemlock coverage map of Vermont is not available, whereas an extensive GIS data layer of the DWAs of Vermont is available (VCGI 2003). Thus, the conifer component of the DWA GIS layer is currently the best approximation of an eastern hemlock coverage map for southern Vermont.

The primary objective of this study is to determine the relative risk of HWA infestation to the eastern hemlock dominated DWAs in southern Vermont. The second objective is to compare winter environmental conditions in hemlock stands versus hardwood stands to quantify some of the potential effects of HWA induced defoliation on important winter habitat elements.

METHODS

RISK ASSESSMENT OF DEER WINTERING AREAS

A random subsample of 32 DWAs in southern Vermont was selected for field surveying in the summer of 2004 from three elevations and three size classes. One sampling plot was assigned for every 40 acres of conifer and mixed cover within each site. Plot data included: DBH of trees within a 10-factor prism plot, understory stems and percent cover by species, groundcover stems and percent cover by species, and crown transparency. At the stand level, data will be evaluated for elevation and size class trends in the eastern hemlock component of DWAs. At the plot level, a spatial statistical analysis will be performed relating site attributes (slope, aspect, elevation, edaphic characteristics, and distance from streams) and eastern hemlock occurrence in order to create a GIS model predicting eastern hemlock occurrence in the landscape.

Spatial statistical analysis using the HWA risk assessment GIS layers (Machin 2003) and vegetation data will be done in order to classify the DWAs of southern Vermont into low, medium, and high risk categories based upon the estimated eastern hemlock component and vulnerability and susceptibility to HWA infestation.

WINTER HABITAT ASSESSMENT

To compare important white-tailed deer winter habitat conditions in hardwood versus hemlock stands, three sites were chosen for study in winter '03-'04 and six sites in winter '04-'05. Sites were selected across a range of aspects and elevations in order to account for the effects of landscape diversity within DWAs. Eastern hemlock and hardwood site-pairs were selected within each site with corresponding slope position, aspect, elevation, and proximity to control for between site-pair environmental variability.

Three temperature data loggers per half site-pair were attached at breast height to the north side of the boles of selected overstory trees serving as plot centers within each site. The degree of snow compaction and the extent of snow crust formation were measured on a bi-weekly basis from January-April '04 and December-April '04-'05 using a snow compaction devise based on the design of Verme (1968). Four measurements of effective and true snow depths were measured along randomly assigned directions stretching from plot centers.

PRELIMINARY RESULTS

Temperature and snow depth data from '03-'04 were analyzed for differences between cover types within site-pairs (Figures 1 and 2 and Tables 1 and 2).

CONCLUSIONS

With HWA populations less than two miles south of the border in Massachusetts, the state of Vermont is on the verge of infestation. It is extremely important for Vermont land and wildlife managers to prepare for the inevitable ecological changes that will accompany the mortality of eastern hemlock. Evaluating the potential risk of HWA introduction and establishment in the DWAs of Vermont is important for two specific reasons: 1) the DWA GIS layer is currently the best approximation of an eastern hemlock coverage map for the state of Vermont and 2) eastern hemlock provides invaluable winter habitat to white-tailed deer in the northern portion of their range. Information gleaned from the GIS analysis will be valuable for land and wildlife managers concerned both with hemlock forests and with the wildlife species that depend upon them. In addition, spatial analysis may provide a widely applicable predictive model for creating a region-wide eastern hemlock coverage map. Results of this study will help prioritize eastern hemlock DWA management activities in preparation for the probable infestation by HWA. The snow depth and temperature study will quantify the buffering capacity of eastern hemlock on winter conditions that has long been assumed. Lastly, this study will hopefully promote further scientific inquiry into the ecological implications of eastern hemlock removal on winter conditions.

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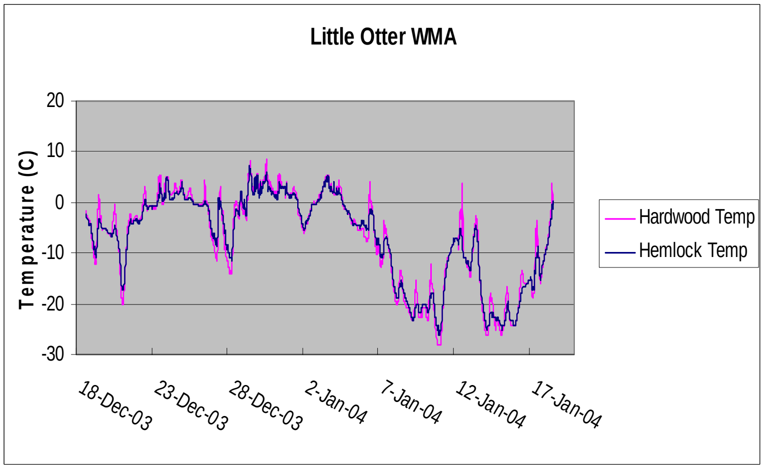

Figure 1. Graph exhibiting the buffering of extreme temperatures under an eastern hemlock canopy in the winter of '03-'04.

Table 1. P-values of a one-way ANOVA testing for temperature differences between hardwood and hemlock stands in winter '03-'04.

	Little Otter WMA	Otter Creek WMA	Gale Meadows WMA
Day maximum temperature	p < 0001	p = .0158	p = .0033
Day mean temperature	p = .005	*	*
Night mean temperature	p = 0.562*	*	*
Night minimum temperature	p = .0314	*	*

* No statistical significance.

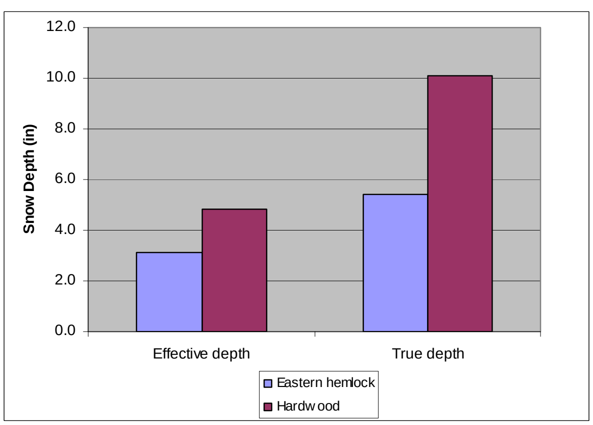

Figure 2. Winter '03-'04 overall average snow depths in eastern hemlock versus hardwood stands.

Table 2. P-values of a t-test assessing the average daily difference between snow depths in site paired hardwood and hemlock stands.

	p-value
Average True	0.002*
Average Effective	0.1025
Between Tree True	0.004*
Under Tree True	0.0025*
Between Tree Effective	0.0841
Under Tree Effective	0.1384

* Significant at $\alpha < .05$

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IMPACT OF IMIDACLOPRID ON HEMLOCK WOOLLY ADELGID (*ADELGES TSUGAE*) AND WATER QUALITY AT MT. LAKE, VIRGINIA

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ABSTRACT

Two groups of hemlock trees were treated with imidacloprid using Kioritz® soil injectors and one group of trees with Mauget stem injection capsules from 2001 to 2003. In one group of soil injected trees the density of HWA was reduced by 35%, 14 months after the first application and two months after the second application. Tree health declined in all years but not as much as for treated trees in this group. HWA density was reduced by 52% in a second group of soil injected trees, three months after the first treatment and 93% one year after the second treatment. Tree health remained unchanged for three years for all trees, treated and untreated. Stem-injected trees showed a 52% reduction in HWA three months after the first treatment and an 87% reduction one year after the second injection. Tree health of these trees did not change and were no different that the untreated trees. Both groups of trees (soil injected and stem injected) had similar health indices, and both treatment methods are providing a similar protection from HWA. Treated trees were located more than 50 m from a stream or the lake. Imidacloprid was detected in lake and spring water in concentrations ranging from less than 0.02 ppb to 1.7 ppb in lake water and 3.5 ppb in spring water. The density of invertebrates in the springs did not change from 2001 – 2003. However, caution must be used when applying imidacloprid near waterways, especially in rocky well-drained soil.

KEYWORDS:

Imidacloprid, hemlock woolly adelgid, *Adelges tsugae*, water quality, tree health, Imicide®, Merit®.

INTRODUCTION

Mountain Lake Resort is located in Giles County, Virginia. This 1,052 ha resort is situated on Salt Pond Mountain (1,000–1,200 m) with a 22 ha natural lake. Hemlock woolly adelgid (HWA) *Adelges tsugae* Annand began infesting *Tsuga* spp. in southwest during the mid-1990s. HWA was first noticed in the resort area in 1999. Old growth stands of hemlocks encircle the lake, with many trees over 100 cm diameter at breast height (DBH). The hemlocks (*Tsuga canadensis* (L.) Carrière) surrounding the lake are the dominant species in this unique climax forest. In order to preserve as many trees as possible the resort contracted the authors and a chemical control program was begun in 2001.

Based on the success of imidacloprid (Steward and Horner 1994, Tatter et al. 1998, Steward et al. 1998, Cowles and Cheah 2002, Silcox 2002, Webb et al. 2003), this product was used to control HWA at Mt. Lake. The effectiveness of imidacloprid treatments on HWA, tree health, migration of imidacloprid into the lake, and impact on stream invertebrates were evaluated.

METHODS

Two methods of imidacloprid injection, soil and stem injections, were used. To minimize imidacloprid migrating into the water table or streams in the area, stem injections were used where treated trees were more than 50 m from the lake, springs, or streams.

Three groups of hemlock trees (84, 26, and 32, respectively) were treated between 2001 and 2003. Groups I and II were treated with imidacloprid soil injections and Group III with stem injections. Based on their probability of survival, trees that were less than 50% defoliated and had new growth were selected. Tree DBH ranged from 5 to 128 cm, with a mean of 17 cm. Groups I and II were treated by soil injection with imidacloprid (Merit® 75 WP) at a rate of 0.75 g/cm DBH (0.55 g a.i./cm DBH) with Kioritz® soil injectors in April of 2001, 2002, and 2003. Soil treatments were made using the Basal System (Silcox 2002). Injections were made around the tree, 10 to 20 cm from the tree base. Trees in Group III were close to a stream (Pond Drain) and were stem injected with imidacloprid (Imicide® 10%) at the rate of one 3 ml Mauget (J.J. Mauget Company, Arcadia, California) capsule/5 cm DBH in April 2002 and April 2003.

A subset of treated trees was paired with untreated trees for each group. The untreated trees selected were similar in size and located up slope and at least 15 m away from its paired treated tree. To determine the density of HWA, the number of live HWA adult sistens in March and adult progrediens in June were counted on 30 cm of the terminal branches on the north, south, east, and west aspects of each treated and untreated tree.

Tree health was evaluated for each treated and untreated tree once during the growing season by recording the percent crown density, live crown ratio, live branches, live tips, and new foliage. These five parameters were summed and divided by 5 to obtain a health index. A value of 100 would be a perfect tree and 0 would indicate a dead tree.

Water samples (1.9 l) were taken at four locations in the lake and from four springs that flowed into the lake to determine if imidacloprid was migrating through the soil and into the lake and water-table at Mountain Lake. Samples were analyzed at Virginia Tech Pesticide Residue Lab using high performance liquid chromatography (HPLC).

To determine the impact of imidacloprid on stream invertebrates, all springs flowing into the lake were sampled in the months of April and June of 2001, 2002, and 2003. Sampling was done each spring between the lake and the forest edge. For each year of sampling, fifty stones were picked up and the invertebrates found were collected at each sample site. The water level in these springs was too shallow to use a net. Due to the rise in the lake level in 2003 the springs that were sampled became submerged and invertebrate sampling was not possible.

Tree health indices for each treatment group and the number of invertebrates recorded from the springs at each sample date were analyzed using one-way analysis of variance (ANOVA) and the Tukey-Kramer HSD test (SAS Institute 1989).

RESULTS

GROUP I

No statistically significant difference in the HWA density was found between treated and untreated trees 10 months after treatment (Table 1, Figure 1). At this sample date (Feb. 15, 2002) there were 1.9 HWA per cm on the treated trees and 1.5 HWA per cm on the untreated trees. This is approximately a 37% drop in the HWA density from the previous year and is likely due to the natural decline in HWA several years after the initial infestation (McClure 1991). Ten months should be a more than adequate time for imidacloprid to enter the hemlock branch tips. Tatter et al., (1998) reported that lethal concentrations of imidacloprid occurred 12 weeks after soil injections. A possible reason for no treatment effect on HWA is that an insufficient amount of imidacloprid reached the branch tips due to below normal rainfall for several months after the first application. The highly organic, loose rocky soil may have resulted in low rates of binding with soil particles. However, after the second application on April 6, 2002, sampling on June 18, 2002, showed a 35% reduction in HWA on the treated trees compared with the untreated trees. As planned, no soil injections were made to this group of trees in 2003 and 2004 and the HWA population was very low on treated and untreated trees.

Due to several years of drought and HWA infestation hemlock health declined significantly in 2002 (Table 2). There was no difference in tree health between the treated and untreated trees until 2003, the second year after the first treatment. The health index of the treated trees was approximately 52 in 2003 and 2004 while the health index of the untreated trees declined from 44 to 20. The reduction in HWA population density caused by imidacloprid may have prevented the treated trees from declining significantly in health. Recovery of tree health following imidacloprid treatments was not as great as reported by Webb et al. (2003), who reported dramatic recovery of tree health following imidacloprid treatments. Their study was done in a residential landscape and differences in soil type may have contributed to the greater recovery in health compared to our study done in a forest with rocky, well-drained soil.

GROUP II

Three months after treatment the number of HWA on the treated trees (1.14/cm) was 52% lower than the untreated trees (2.39 / cm) (Table 3, Figure 1). In 2003 the HWA population was very low on the treated and untreated trees due to the natural decline that HWA exhibits several years after initial infestation. HWA density was also very low in 2004; however, there was a 93% reduction in HWA on the treated trees compared with the untreated trees.

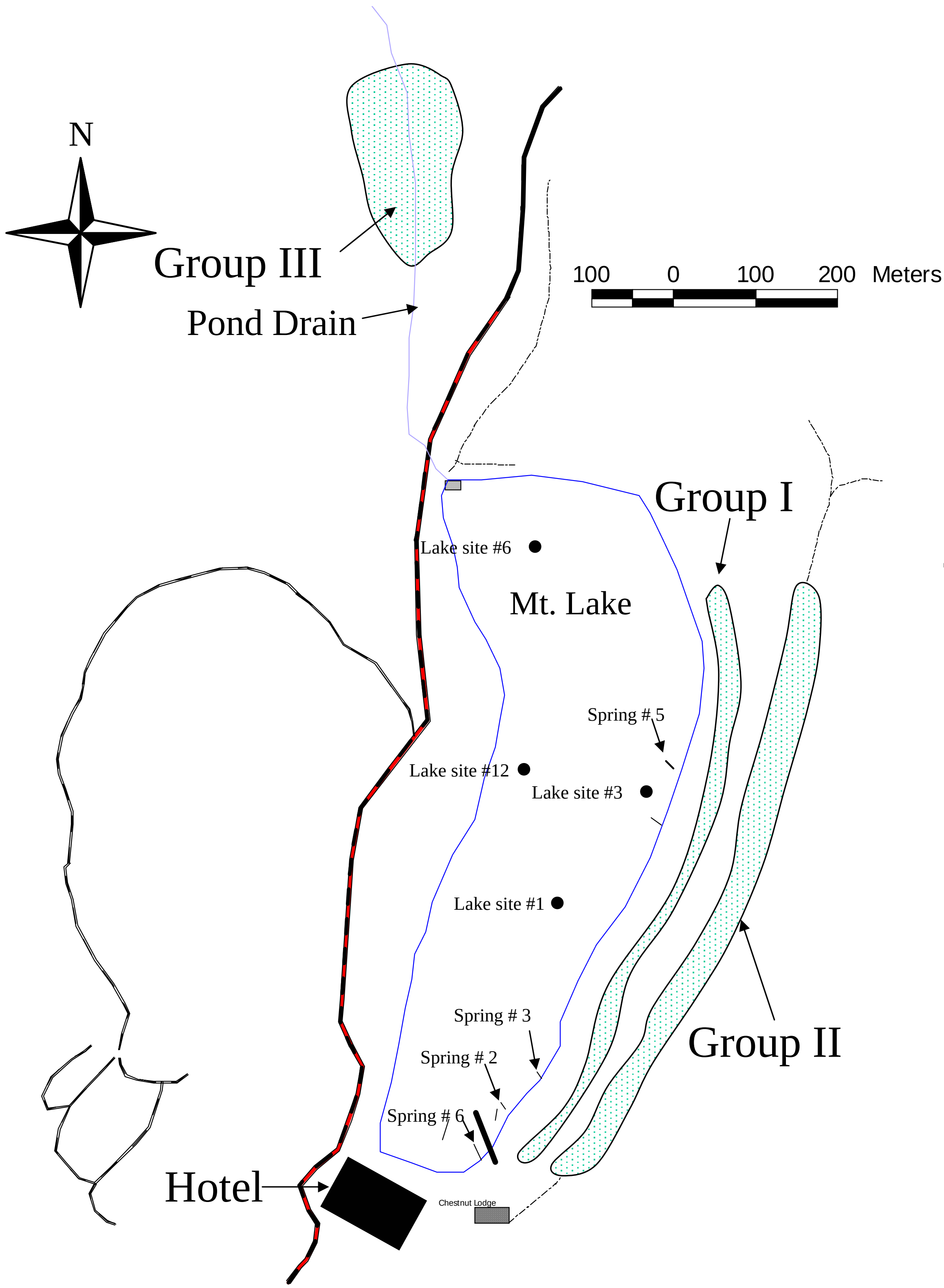

Figure 1. Sampling sites.

Unlike the health of Group I, there was no difference in tree health between the treated and untreated trees and no change in health over the three-year period (Table 4). This group of trees also had a lower density of HWA than Group I in 2002, and this may have resulted in less stress on these trees. No treated or untreated trees have died in this group.

GROUP III.

Eleven weeks (July 9, 2002) after the first treatment (Table 5, Figure 1) the treated trees had 66% fewer HWA (0.59/cm) than untreated trees (1.73/cm). From April 2003 to April 2004, no differences in HWA density occurred. However, in June 2004 there were 87% fewer HWA on the treated trees (0.004 HWA / cm) compared with untreated trees (0.031 HWA / cm). Health of the treated and untreated trees improved from 2002 to 2003, but then declined in 2004 to the levels found in 2002 (Table 6).

MIGRATION OF MERIT

One month after the first soil treatment imidacloprid was found at 3.6 and 1.6 ppb in samples #6 and #12 (Figure 1), respectively (Table 7). No imidacloprid was found in lake water samples #1 and #3. After soil treatments in 2002, no imidacloprid was detected. While imidacloprid was not detected in the lake water three weeks after the 2003 soil injection, it was detected in the spring water.

Three months after this treatment trace amounts were found in the lake water and measurable amounts were found in the spring water. Measurable and trace amounts were also found in the lake and spring water in 2004. The soil at Mt. Lake is very porous and rocky and likely allowed for the migration of imidacloprid into the springs and lake. When soil injections were made, loose rock was often contacted. Soil injections in very rocky soil such as this may not be appropriate due to the potential migration of imidacloprid.

IMPACT ON NONTARGET BENTHIC SPECIES

The number of invertebrates did not change from 2001 to 2003 (Figure 1, Table 8). Although, imidacloprid was found in the spring water in April 2003 (Table 7) at a concentration up to 0.5 ppb, it did not appear to impact the invertebrate population. No sampling was done after 2003 due to the rise in the lake level, so the impact of the higher concentrations of imidacloprid found in 2004 could not be determined. The closest trees to the lake that were soil injected were approximately 50 m. However, many underground springs occur in this area, and it is likely that imidacloprid could travel into the springs and lake through this route.

The LC50 for trout, daphnia, and algae is 211,000, 85,000, and 10,000 ppb, respectively (Extoxnet 2005). The levels of imidacloprid in the lake water were well below detrimental concentrations for these groups of organisms. However, caution must be used when applying imidacloprid in rocky soils near water due to migration protential.

Table 1. Mean number of HWA per cm on soil injected imidacloprid treated and untreated hemlock trees in Group I at Mt. Lake.

	# HWA/cm						
	Treated		Untreated		t statistics		
Date	**n**	***X* ± SD**	**n**	***X* ± SD**	***t***	**df**	***P***
1st treatment April 7, 2001							
April 11, 2001	15	2.78±1.65	15	2.78±1.87	-0.008	28	0.99
June 25, 2001	15	3.09±1.14	15	3.52±1.55	-0.846	28	0.40
Feb. 15, 2002	15	1.97±1.35	15	1.49±1.16	1.032	28	0.31
2nd treatment April 6, 2002							
June 18, 2002	15	2.13±1.14	15	3.29±1.16	-2.769	28	0.01
April 15, 2003	15	0.01±0.02	15	0.03±0.02	-0.873	28	0.39
June 24, 2003	15	0.003±0.010	15	0.038±0.136	-0.997	28	0.34
Jan. 22, 2004	15	0.001±0.002	15	0.074±0.187	-1.533	28	0.14
June 23, 2004	15	0.0	15	0.048±0.144	-1.287	28	0.21

Table 2. Tree health index for soil injected imidacloprid treated and untreated hemlock trees in Group I at Mt. Lake.

	Tree Health Index						
	Treated		Untreated		t statistics		
Date	**n**	***X* ± SD**	**n**	***X* ± SD**	***t***	**df**	***P***
1st treatment April 7, 2001							
2001	15	78±4 a[1]	15	74±7 a	-1.94	28	0.06
2nd treatment April 6, 2002							
2002	15	62±7 b	15	58±6 b	1.60	28	0.12
2003	15	51±7 c	15	44±6 c	2.60	28	0.01
2004	15	52±20 c	15	20±22 d	4.28	28	0.001

[1]Means followed by the same letter in the column are not significantly different at P = 0.05,Tukey-Kramer HSD test.

Table 3. Mean number of HWA per cm on soil injected imidacloprid treated and untreated hemlock trees in Group II at Mt. Lake.

	# HWA/cm						
	Treated		Untreated		*t* statistics		
Date	**n**	***X* ± SD**	**n**	***X* ± SD**	***t***	**df**	***P***
1st treatment April 6, 2002							
July 3, 2002	15	1.14±0.87	15	2.39±0.92	-3.820	28	0.001
2nd treatment April 4, 2003							
March 25, 2003	15	0.006±0.021	15	0.064±0.141	-1.565	28	0.129
June 24, 2003	15	0.012±0.041	15	0.028±0.107	-0.904	28	0.381
Feb. 19, 2004	15	0.008±0.018	15	0.016±0.021	-1.118	28	0.273
June 23, 2004	15	0.005±0.017	15	0.069±0.075	-3.241	28	0.003

Table 4. Tree health index values for soil injected imidacloprid treated and untreated hemlock Trees in Group II at Mt. Lake.

	Tree Health Index						
	Treated		Untreated		*t* statistics		
Date	**n**	***X* ± SD**	**n**	***X* ± SD**	***t***	**df**	***P***
1st treatment April 6, 2002							
2002	15	63±4 a[1]	15	63±6 a	-0.26	28	0.79
2nd treatment April 4, 2003							
2003	15	56±7 a	15	55±7 a	0.34	28	0.74
2004	15	54±2 a	15	54±2 a	0.067	28	0.95

[1]Means followed by the same letter in the column are not significantly different at P = 0.05,Tukey-Kramer HSD test.

Table 5. Mean number of HWA per cm on Mauget stem injected imidacloprid treated and untreated hemlock trees in Group III at Mt. Lake.

	# HWA/cm						
	Treated		Untreated		t statistics		
Date	**n**	***X* ± SD**	**n**	***X* ± SD**	***t***	**df**	***P***
1st treatment April 23, 2002							
July 9, 2002	10	0.59±0.42	10	1.73±0.89	-3.65	18	0.002
2nd treatment April 16, 2003							
April 16, 2003	10	0.015±0.04	10	0.019±0.03	-0.291	18	0.774
July 8, 2003	10	0	10	0.003±0.009	-1.00	9	0.343
April 6, 2004	9	0.001±0.003	9	0.005±0.007	-1.639	16	0.121
June 29, 2004	10	0.004±0.007	10	0.031±0.036	-2.319	18	0.032

Table 6. Tree health index for Mauget stem injected imidacloprid treated and untreated hemlock trees in Group III at Mt. Lake.

	Tree Health Index						
	Treated		Untreated		t statistics		
Date	**n**	***X* ± SD**	**n**	***X* ± SD**	***t***	**df**	***P***
1st treatment April 23, 2002							
2002	10	64±2 b[1]	10	64±4 b	-0.07	18	0.94
2nd treatment April 16, 2003`							
2003	10	73±3 a	10	72±4 a	0.84	18	0.41
2004	10	66±6 b	10	64±9 b	0.57	18	0.57

[1]Means followed by the same letter in the column are not significantly different at P = 0.05,Tukey-Kramer HSD test.

Table 7. Parts per billion (ppb) of imidacloprid found in water samples at Mt. Lake.

Date collected	Lake Site#	Imidacloprid ppb	Spring Site#	Imidacloprid ppb
		Soil treatment on April 7, 2001		
April 10, 2001	6	0		
	12	0		
May 7, 2001	1	0		
	3	0		
	6	3.6		
	12	1.6		
March 14, 2002	1	0		
	3	0		
	6	0		
	12	0		
		Soil treatment on April 6, 2002		
June 27, 2002	1	0		
	3	0		
	6	0		
	12	0		
		Soil treatment on April 4, 2003		
April 22, 2003	1	0	2	0.46
	3	0	3	0
	6	0	5	0
	12	0	6	0.26
July 8, 2003	1	trace[1]	2	0
	3	trace	3	trace
	6	0	5	0.069
	12	trace	6	0
May 11, 2004	1	trace	2	0
	3	trace	3	trace
	6	trace	5	NT[2]
	12	0	6	trace
		Soil treatment on Sept. 30, 2004		
Dec. 12, 2004	1	1.7	2	0
	3	0	3	3.5
	6	0	5	3.3
	12	0	6	0.7

[1]"Trace" indicates that imidacloprid was present but at levels below 0.02 ppb.
[2]NT = not tested.

Table 8. The mean number of invertebrates in springs sampled around the perimeter of Mt.Lake.

Sample Date	Number of Springs	# Invertebrates per Stone $\bar{X} \pm$ SD
April 17, 2001	6	0.43±0.74 a[1]
July 17, 2001	4	0.74±0.36 a
March 27, 2002	7	2.29±5.02 a
June 27, 2002	4	2.62±1.75 a
April 22, 2003	7	3.33±2.89 a

[1]Means followed by the same letter are not significantly different at P = 0.05, Tukey-Kramer HSD test.

CONCLUSIONS

Soil and stem injections of imidacloprid significantly reduced the density of HWA, from 35% to 93%. Tree health did not improve three years after the first treatment of imidacloprid, but did not decline as rapidly as it did in untreated trees, and the treatments have prevented or delayed the death of these trees. Tree Groups I, II, and III were not located in the same area and consequently were not subject to the same biotic and abiotic factors due to the differences in topography and microhabitat. Conclusions on the efficacy of imidacloprid on HWA among these three groups may not be valid. However, all groups of trees (soil injected and stem injected) have similar health indices and both treatment methods appear to be providing a similar protection from the impact of HWA.

Although low levels of imidacloprid were present in lake and spring water, the number of invertebrates did not change over the course of this study and did not appear to be impacted by imidacloprid. However, caution must be used when applying imidacloprid near waterways due to migration potential. More research is needed to determine more definitively a safe distance for treating trees near water, especially in porous rocky soil.

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Reproductive Success of *Cybocephalus* sp. nr. *nipponicus* Enrody-Younga on Elongate Hemlock Scale, *Fiorinia externa* Ferris

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ABSTRACT

A field study was carried out to evaluate the survival and reproductive success of the established predator *Cybocephalus* sp.nr. *nipponicus* Enrody-Younga (Coleoptera: Cybocephalidae) on elongate hemlock scale (EHS), *Fiorinia externa* Ferris (Homoptera: Diaspididae). A sleeve-cage method with the following four treatment combinations was used to examine the impact of *C*. sp.nr. *nipponicus* on EHS: 1) sleeve-caged branches containing hemlock scales at the test density and a mated pair of *C*. sp.nr. *nipponicus*, 2) sleeve-caged branches containing hemlock scale at the test density and without *C*. sp.nr. *nipponicus*, 3) open branch with test scale density, and 4) open uninfested branches. We found that *C*. sp.nr. *nipponicus* can survive and reproduce successfully on EHS and has a significant impact on EHS populations. Overall, reproduction by *C*. sp.nr. *nipponicus* was observed in 75 percent of the treatment combinations. A total of 45 *C*. sp.nr. *nipponicus* males, 75 females and 30 larvae were recovered from the 'sleeve-cage with predators' treatment. We observed 30.18 percent survival of released *C*. sp.nr. *nipponicus* males and 26.41 percent survival of females. A significant difference in the mean number of surviving scales between sleeve-cage with predators and sleeve-cage without predators was observed. We also examined levels of parasitism of EHS by the adventive parasitoid, *Encarsia citrina* Craw (Hymenoptera: Aphelinidae). Significant differences in percent parasitism of EHS were also observed between sleeve-cage with predators and without predator treatments and between open infested branch and uninfested branch treatments. The highest level of parasitism (47.5 percent) was observed in the 'open infested branch' treatment.

KEYWORDS

Elongate hemlock scale, *Cybocephalus* sp.nr. *nipponicus*, reproductive success, *Encarsia citrina*, parasitism.

MASS RELEASE AND RECOVERY OF *CYBOCEPHALUS SP. NR. NIPPONICUS* ON ELONGATE HEMLOCK SCALE, *FIORINIA EXTERNA* FERRIS

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ABSTRACT

Fiorinia externa Ferris, the elongate hemlock scale (EHS), has been a serious pest of hemlock in New Jersey for many years. The Phillip Alampi Beneficial Insect Laboratory has recovered *Cybocephalus* sp. nr. *nipponicus* Enrody-Younga, from nine sites, four of which had never received releases of the beetle, indicating that the beetles are dispersing to EHS on their own. In 2001, *C.* sp. nr. *nipponicus* significantly reduced the EHS population on a release tree (t-test, $p \leq 0.05$), prevented the EHS from increasing in a 2002 release site, and significantly reduced the scale populations at release sites in 2003 and in 2004 (Mann-Whitney Rank-Sum test, $p \leq 0.05$). *C.* sp. nr. *nipponicus* populations are increasing at sites where the beetle was first recovered and the indications are that the beetle has the potential to be a biological control agent on this pest.

KEYWORDS

Fiorinia externa, *Cybocephalus* sp. nr. *nipponicus*, biological control, hemlock.

INTRODUCTION

The elongate hemlock scale (*Fiorinia externa* Ferris) can be a serious pest of eastern hemlock, *Tsuga canadensis* (L.) Carriere and is found from Massachusetts to Virginia and west to Ohio (Kosztarab 1996). It can kill hemlock trees given enough time and works together with the hemlock woolly adelgid to accelerate the decline of hemlock (McClure 2002). It has also been found to kill hemlocks after a heavy HWA infestation has passed through an area, leaving trees with high *Fiorina* scale populations (Danoff-Burg and Bird 2002). The elongate hemlock scale (EHS) has one generation per year in the northeastern United States (McClure 1978) and there are few biological control agents that are effective. In McClure (1986), *Aspidiophagus citrinus* Craw (Hymenoptera: Aphelinidae) and *Chilocorus kuwanae* Sylvestri (Coleoptera: Coccinellidae) were shown to be effective biological control agents in Japan and are present in the United States, but there are two generations of EHS in Japan, providing the beetle more opportunity for predation. In Japan, there is a high degree of synchrony of *A. citrinus* with the host because *A. citrinus* requires two generations per year to complete its life cycle, which allows it to build up to effective levels. In the US, *A. citrinus* completes two generations per year, and its life cycle is asynchronous with *F. externa,* which reduces its effectiveness (McClure 1986). In New Jersey, the coccinellid, *Chilocorus stigma* (Say) fills the same ecological niche as *C. kuwanae* but it is not as effective in controlling the scale populations in most instances. *C. kuwanae* has been released and established in New Jersey, but has never been recovered from hemlock by Phillip Alampi Beneficial Insect Laboratory staff. All *Chilocorus* species recovered from hemlock have been *C. stigma.* In New Jersey all but about 10% of the hemlock stands have been seriously impacted by the HWA and most of them also have a EHS infestation (Mayer et al. 2002). Combined with the drought of 2001, the trees are acutely stressed.

Since 1986, the Phillip Alampi Beneficial Insect Laboratory (PABIL) has been working with a predatory beetle, *Cybocephalus* sp. nr. *nipponicus* Enrody-Younga, (Coleoptera: Cybocephalidae) (Figure 1), a tiny exotic predatory beetle from Korea that has been used as a biological control agent on the euonymus scale, *Unaspis euonymi* (Comstock) (Homoptera: Diaspididae). *C.* sp. nr. *nipponicus* is a predator on scales in the family Diaspididae and feeds on San Jose scale, *Diaspidiotus perniciosus* (Comstock), and juniper scale, *Carulaspis juniperi* (Bouche), as well as euonymus scale. The beetles were shipped to the PABIL from the USDA Beneficial Insect Research Laboratory in Niles, Michigan. From 1986-1993, the PABIL received and released *C.* sp. nr. *nipponicus* on scale-infested euonymus plants throughout New Jersey. In 1994, the PABIL initiated a mass-rearing program in New Jersey that allowed the lab to substantially increase the numbers of *C.* sp. nr. *nipponicus* and the number of euonymus release sites.

Figure 1. *Cybocephalus* sp. nr. *nipponicus*. Adult males (top), adult female (lower left), and larva (lower right).

In 1999, two interesting events occurred that heightened the laboratory's interest in *C.* sp. nr. *nipponicus* as a possible predator of the EHS. In 1999, *C.* sp. nr. *nipponicus* was recovered from hemlock sites at Washington Crossing State Park and at the Freer Nature Preserve in Colts Neck, New Jersey, while surveying for the predator of the hemlock woolly adelgid, *Sasajiscymnus tsugae*. The beetles were not released in those stands but were released on euonymus scale at three sites nearby the Freer Preserve in 1996/1997 and in 1995 at one site near Washington Crossing State Park. The recovery was originally thought to be an aberration, but the collection of the beetles on EHS in succeeding years at the Freer Preserve in Monmouth County and at other sites gave us an indication that the beetle may feed on the scale.

The second event was an innundative release of 300-400 *C.* sp. nr. *nipponicus* on the approximately 200 scale-infested hemlock trees in the exterior hoop/shade-house/cold frame at the laboratory where young hemlocks were kept for experimental purposes in 1999. The beetles rapidly reduced the EHS population and gave a strong indication that the beetles would feed on the EHS.

In 2000, it was decided to expand to some larger trial releases on EHS to see if the beetles established. No data were collected, and only the presence or absence of the *C.* sp. nr. *nipponicus* was noted.

MATERIALS AND METHODS

In 2000, releases were made at Allamuchy Mt. State Park, the Upper Wickecheoke Reserve, and the Princeton Battlefield State Park in an attempt to establish the beetles. The beetles were packed in Fonda 8 oz. containers with excelsior, 500 beetles per container. At the release site the lid was removed and the containers, excelsior, and lids were placed into the branches of the tree. After five minutes, any remaining beetles in the containers were gently brushed onto the infested branches using a soft, 1-inch camel hair paintbrush. Observations of *C.* sp. nr. *nipponicus* were made either by a ten minute walk and examination at each site or by beating branches with a whiffle ball bat below which was placed a 1-m^2 white beating sheet. Any *C.* sp. nr. *nipponicus* recovered in the seasons after release were collected and brought up to the Phillip Alampi Beneficial Insect Laboratory to confirm establishment.

In 2001, 3,750 *C.* sp. nr. *nipponicus* were released onto one tree at the Lower Wickecheoke Reserve in Hunterdon County. The site consisted of central hardwoods with about 15% hemlock and was isolated from previous euonymus scale release sites by about 2.7 km. Two trees with similar EHS populations were selected, 100 m apart, one as a release and one as a control. Twenty randomly selected 3 cm cuttings were selected from the scale-infested parts of each tree in April and in October; using a dissecting microscope in the laboratory, the number of live scales per 3 cm was counted. The scale covers were all removed using a dissecting needle and the scale underneath was punctured to test whether it was alive. A simple t-test was used to analyze the data (Fox et al. 1994).

In 2002, 20,000 *C.* sp. nr. *nipponicus* were released into an isolated hemlock stand in the Minisink Valley section of High Point State Park. The first release of 10,000 was on April 18 and the second on April 25. The site was mixed northern hardwood/central hardwood forest with greater than 50% hemlocks. Twenty trees were selected, 10 as release trees, 10 as controls. 2,000 beetles were released per tree. The release area was 160 m uphill from the control site. The beetles were transported to the site in Sweetheart®, 165 oz., stock number 10T1 paper buckets covered with Sweetheart® 10V19S paper lids. There were 2,000 beetles per bucket, and the buckets were filled with excelsior to increase surface area. At the release site, the lid was removed and the buckets, excelsior, and lids were placed into the branches of the tree. After five minutes, any stragglers in the buckets were gently brushed out onto the infested branches using a 1-inch camel's hair paintbrush. Ten randomly selected 3 cm cuttings were selected from the scale-infested parts of each tree in April and again in October and the number of live scales per 3 cm was counted back at the laboratory. A Mann-Whitney Rank Sum test was performed on the data because the data was not normally distributed (Fox et al. 1994). The data from all release trees was combined, as was the data from the control trees. An additional 12 sites also received releases in order to establish *C.* sp. nr. *nipponicus* in the hemlock forest in as many areas as possible.

In 2003, releases were made on 10 trees following the same protocols as in 2002 except that 3,000 beetles were released per tree—30,000 total, instead of 2,000—and over a longer time frame—from April 23 to June 18. The release location was near the Clinton Reservoir on the Newark Watershed in Passaic County.

In 2004, releases were made on 10 trees as above, but 5,000 beetles were released per tree from April 23 to May 7, totaling 50,000 beetles. The release location was at the Minisink Valley section of High Point State Park approximately 140 m uphill from the 2002 release site.

RESULTS AND DISCUSSION

Table 1 shows the recoveries of *C.* sp. nr. *nipponicus* at sites where field personnel were searching for *S. tsugae*. Four of the six sites, Washington Crossing, Johnson Lake, Freer Nature Preserve, and Lake Valhalla, never received any releases of *C.* sp. nr. *nipponicus* but the beetles were released nearby on *Euonymus* plants infested with the euonymus scale *U. euonymi* and migrated to the hemlock. *C.* sp. nr. *nipponicus* has been established throughout New Jersey on euonymus by the Phillip Alampi Beneficial Insect Laboratory and it has been observed feeding on juniper scale, *Carulaspis juniperi* Boche, pine needle scale, *Chionaspis pinifoliae* (Fitch), and San Jose scale, *Quadraspidiotus perniciosus* (Comstock).

The Phillip Alampi Beneficial Insect Laboratory rears *C.* sp. nr. *nipponicus* on San Jose scale-infested butternut squash and has released a total of 699,010 beetles since 1986. The beetle is found on almost every scale-infested euonymus plant that is checked and is distributed throughout New Jersey (Matadha et al. 2003). Van Driesche et al. (1998) observed that *C.* sp. nr. *nipponicus* dispersed from the original release sites in New England.

Cybocephalus sp. nr. *nipponicus* has considerably reduced euonymus scale, *U. euonymi* populations in landscapes in New Jersey reducing damage to the plants (Mayer et al. 1995, Hudson et al. 2001). The beetles tend to stay on the plants until the food source is exhausted, then leave and return when the scale population has increased. They do not disperse as readily as *C. kuwanae*.

Table 1. Overwintering recoveries of *Cybocephalus* sp. nr. *nipponicus* at *Sasajiscymnus tsugae* release sites.

Site	Year(s) of Recovery
Washington Crossing State Park*	1999, 2002, 2004
Freer Nature Preserve*	1999, 2000, 2001, 2002, 2004
Lake Valhalla*	2002, 2003
Princeton Battlefield State Park	2002, 2003, 2004
Johnson Lake*	2002, 2003
Allamuchy State Park	2002, 2003, 2004
Cedar Pond, Newark Watershed	2004
High Point SP, Minisink Valley Section	2004
Clinton Reservoir, Newark Watershed	2004

**C.* sp. nr. *nipponicus* never released at these sites.

Since 1999, *C.* sp. nr. *nipponicus* has been increasingly found on hemlock, although in low numbers. Of the three releases made in 2000, same-season recoveries were made at two of the sites: Allamuchy State Park and Princeton Battlefield State Park. The recovery at Allamuchy in July 2002 was 420 m from the release site. The numbers were low, but the fact that the beetles were recovered there is encouraging. After the recovery, an additional release of 1,000 beetles was put into the site in September 2002. The beetles have been recovered from the Freer Preserve every year since 1999. In 2004, 79 beetles were recovered at Princeton Battlefield, 26 at the Freer Preserve and 16 at Washington Crossing. This is the largest number of beetles ever recovered at these sites from one survey and indicates that the *C.* sp. nr. *nipponicus* population is increasing on hemlock in those areas.

Table 2 shows the history of releases of *C.* sp. nr. *nipponicus* on EHS. The first trial releases were made in 2000 and the 2001 release was an attempt to show a statistically significant reduction through an inundative release. Table 3 shows the results of the releases in 2001. A simple t-test was run on the data. The results were statistically significant (p d ≤ 0.05) in that the average number of scales significantly decreased on the release tree while there was no significant change in the population on the control tree. Although this is a very small sample and it was not repeated, the indication is that the *C.* sp. nr. *nipponicus* can reduce the EHS population on individual trees. When first releasing a new species, we always attempt to inundate an area to make sure the insects will have an effect. In succeeding years, the number of insects released is reduced until a minimum number is reached that will still be effective. The 2001 release site was isolated from other hemlock stands, and the hemlocks were not a dominant component of the stand. This ensured that the beetles would not disperse, and they were readily found on the release tree throughout the summer.

Table 2. History of releases of *C. nipponicus* on elongate hemlock scale in New Jersey.

Date of Release	Number	County	Location
4/07/00	5,000	Sussex	Allamuchy State Park
5/03/00	2,500	Hunterdon	Wickecheoke Reserve Upper
6/07/00	2,500	Mercer	Princeton Battlefield State Park
Total	10,000		
6/29/01	3,750	Hunterdon	Wickecheoke Reserve Lower
Total	**3,750**		
4/18/02	20,000	Sussex	High Point SP, Minisink Valley
5/02/02	15,000	Passaic	Clinton Reservoir, near PSP
5/031/02	5,000	Hunterdon	Natural Lands Trust Preserve
6/7/02	4,000	Sussex	Sparta Mountain WMA
6/7/02	11,000	Warren	White Lake WMA
6/28/02	4,000	Passaic	Wanaque WMA
7/12/02	3,500	Gloucester	Park Lake
8/29/02	1,000	Gloucester	Arlington Blvd and Commodore Dr
9/12/02	1,000	Sussex	Allamuchy State Park
9/26/02	2,500	Passaic	Clinton Road, Site by boat launch
10/10/02	2,000	Passaic	Wanaque WMA
10/25/02	1,800	Sussex	Kittatinny Valley SP
11/7/02	1,500	Hunterdon	Westcott Reserve
Total	**72,300**		
4/24 - 7/18/03	30,000	Passaic	Clinton Reservoir
9/4/03	4,000	Sussex	Wawayanda State Park
10/15/03	3,000	Sussex	Hamburg Mt. WMA
10/9/03	3,000	Passaic	Dunkers Pond, Newark Watershed
10/14/03	1,500	Sussex	Stokes State Forest, Stoney Lake
Total	**41,500**		
4/23-5/7/04	50,000	Sussex	High Point SP, Minisink Valley
8/20-9/3/04	5,000	Morris	Kay Environmental Center
Total	**55,000**		
Grand Total	**182,550**		

With the results from 2001 in mind, the 2002 release was expanded to include more trees and a total of 2,000 *C.* sp. nr. *nipponicus* were released per tree. Table 4 shows the results of the releases in 2002. There were 10 release trees instead of one, but the total number of beetles released per tree was reduced from 2001. The data here are less clear but the release trees did not sustain an increase in the *Fiorinia* scale population whereas the population median on the control trees significantly increased (Mann-Whitney, p d $\leq$ 0.05). The beetles prevented the EHS population from increasing on the release trees. In 2003, the experiment was repeated, but 3,000 beetles were placed on a tree versus 2,000 and the *C.* sp. nr. *nipponicus* reduced the EHS population significantly (Mann-Whitney, p d $\leq$ 0.05). Table 5 shows the results of the releases in 2003. In 2004, similar results were obtained as 2003 with the release of 5,000 beetles per tree (Mann-Whitney, p d $\leq$ 0.05). Table 6 shows the results of the releases in 2004. Although the trees are still stressed and the number of beetles released is not sufficient to reduce the EHS population in a forest in one year's time, the indications are that the *C.* sp. nr. *nipponicus* can reduce the population on specific trees if sufficient numbers are released. It remains to be determined whether *C.* sp. nr. *nipponicus* can reduce the EHS population in a forest over time; however, the fact that the beetle does feed, reproduce and is recovered on the scale is very encouraging.

One note of interest is that T. Dorsey (co-author) placed *C.* sp. nr. *nipponicus* larvae on EHS infested hemlock twigs and was able to rear them to the adult stage in the laboratory, which proved that the larvae could develop on the scale. No eggs were found on the scale, but this may have been due to desiccation of the scale on cut twigs held in the laboratory. The scale may have died before any larvae could complete their development. Unquestionably, the beetles do eat the EHS and develop on the scale. There is the potential, then, for *C.* sp. nr. *nipponicus* to be a biological control agent but the big question is whether the *C.* sp. nr. *nipponicus* can be an effective control agent. *Chilocorus stigma*, a native coccinellid also feeds on the EHS, but it does not reach population levels sufficient to impact the scale, probably due to the dispersal of the insect in the adult stage. On beech scale, *Cryptococcus fagisuga*, *C. stigma* fed on the scale in all stages but readily dispersed in the adult stage (Mayer and Allen 1983). *Chilocorus kuwanae*, an introduced coccinellid, may also have some potential, but Phillip Alampi Beneficial Insect Laboratory personnel have never recovered it from hemlock. *Cybocephalus* sp. nr. *nipponicus* may be like *C. stigma*, which feeds on the scale but may never attain sufficient numbers to control the pest. However, *C.* sp. nr. *nipponicus* population numbers are increasing in the initial recovery sites, and visual inspection shows that the EHS population is appreciatively reduced.

The impact on the management of the hemlock woolly adelgid and EHS by *C.* sp. nr. *nipponicus* is currently negligible. Although the beetle is increasing its population in New Jersey forests, the increase is slow and the EHS may not be its preferred host. The goal of the New Jersey Department of Agriculture is to establish the beetles in hemlock stands throughout New Jersey. It will take years, possibly a decade before the full impact of the beetle will be known, but the potential for effect is there.

Table 3. 2001 release of *C. nipponicus* on *Fiorina* scale; 3,750 released.

	Mean # of Live Scales/3 cm*	
	April	**October**
Release Trees	26.5a	11.2b
Control Trees	20.3a	17.0a

*t-test - p ≤ 0.05. Numbers followed by the same letter within a row are not significantly different.

Table 4. 2002 release of *C. nipponicus* on *Fiorina* scale; 2,000 released per tree at High Point State Park, Minisink Valley Section.

	Mean # of Live Scales/3 cm*	
	April	**October**
Release Trees	7.0a	8.0a
Control Trees	5.0a	11.0b

*Mann-Whitney rank sum test - p ≤ 0.05. Numbers followed by the same letter within a row are not significantly different. (Release: T = 9977.0, P = 0.8594; Control: T = 8925.0, P = 0.0060.)

Table 5. 2003 release of *C. nipponicus* on *Fiorina* scale; 3,000 released per tree at Clinton Reservoir.

	Mean # of Live Scales/3 cm*	
	April	**October**
Release Trees	27.0a	19.0b
Control Trees	21.5a	23.0a

*Mann-Whitney rank sum test - p ≤ 0.05. Numbers followed by the same letter within a row are not significantly different. (Release: T = 8879.0, P = 0.0042; Control: T = 10665.0, P = 0.1332.)

Table 6. 2004 release of *C. nipponicus* on *Fiorina* scale; 5,000 released per tree at High Point State Park, Minisink Valley Section.

	Mean # of Live Scales/3 cm*	
	April	**October**
Release Trees	8.0a	5.0b
Control Trees	5.0a	3.0a

*Mann-Whitney rank sum test - p ≤ 0.05. Numbers followed by the same letter within a row are not significantly different. (Release: T = 11159.0, P = 0.0068; Control: T = 10605.5, P = 0.1751.)

CONCLUSION

The Phillip Alampi Beneficial Insect Laboratory has been making trial releases of *C.* sp. nr. *nipponicus* onto *F. externa*. The beetles have established at nine sites, four of which have never received releases, and the EHS population has been reduced or prevented from increasing at the 2001, 2002, 2003 and 2004 release sites. Escalating numbers of beetles are being recovered every year, and visual inspection of the recovery sites shows a reduction in the EHS population. The potential is there for *C.* sp. nr. *nipponicus* to be a biological control agent on the EHS.

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Monitoring Hemlock Crown Health in Delaware Water Gap National Recreational Area

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ABSTRACT

Decline of the health of hemlocks in Delaware Water Gap National Recreation Area was noticeable in the southern areas of the park by 1992. The following year, a series of plots were established to monitor hemlock health and the abundance of hemlock woolly adelgid. This poster examines only the health rating of the hemlocks in the monitoring plots.

METHODS

Seven sites were selected on the Pennsylvania and New Jersey sides of the Delaware River which runs through the middle of the park. One site, Grey Towers, is just north of the park boundary. The hemlock stands are associated mostly with watercourses that run to the river, often in steep ravines with spectacular waterfalls. Plots were installed from 1993 to 1995; the Donkeys Corner site was added in 1998 (Figure 1). Dunnfield Creek access was difficult and too many measurements were missed, so this site was discontinued and its data are omitted from analysis.

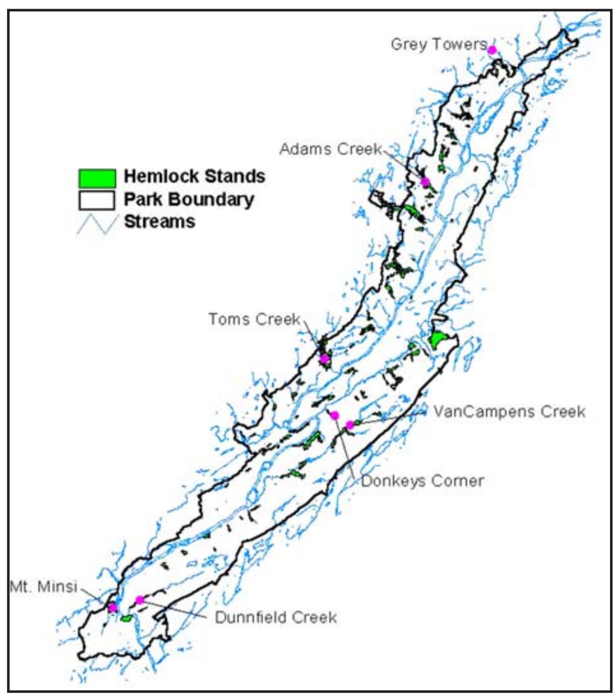

Figure 1. Study sites in the Delaware Water Gap National Recreation Area.

Each site had several plots of 10 living hemlock trees. The number of plots at each site ranged from three to 36, with more plots on larger sites. Diameter at breast height (DBH) and crown position were measured for each tree. Each year (with some gaps), the crown condition was estimated by a trained crew using the "Visual Crown Rating Methods" developed by Forest Service State and Private Forestry (USDA Forest Service, 1998).

<u>**Live Crown Condition Indicators**</u>

Live Crown Ratio — ratio of live crown to total tree height

Foliage Density — an estimate (percentage) of the fullness of the crown, based on the amount of skylight blocked from view by leaves, branches, bole, and fruits

Foliar transparency — an estimate (percentage) of the amount of light seen through the foliage parts of branches

Crown Dieback — the percentage of branches with newly dead twigs in the live crown

Ratings of the crown were done by a two- or three-person crew standing 15–30 m (50–100 ft) from the base of the tree at various angles with an open view of the crown. The consensus visual estimates were recorded to the nearest 5% for dieback, density, transparency, and live crown ratio. Crown diameter was measured with a tape measure on the ground from one edge of the canopy to the opposite edge; a second measurement was made at right angles to the first.

After completing this formal rating of crown indicators the crew scored the overall health of the hemlock crown into four classes: 1 = severe decline, 2 = moderate decline, 3 = slight decline, and 4 = healthy. Trees with dead crowns were recorded as 0.

Measurements were made on 900 trees. The number of trees measured in any year varied from 201 to 801. Although the sampling of trees from year to year was haphazard, trends in health based on the trees measured each year seem to depict the average condition of hemlock in the park reasonably well. The data presented represent averages for all the trees measured each year and have not been interpolated for missing data. Future work will analyze the data with models and statistical procedures.

RESULTS

The crown condition indicators showed little change from 1993 to 1999 except for crown density, which began its decrease earlier. Dieback of branches showed the greatest change, increasing 4-fold from 6% in 1999 to 23% in 2004. Transparency of the crown increased from 25% to 45%. The live crown ratio showed little change. Figure 2 illustrates these trends.

The overall health rating by the trained observers provided a simple, easily understood "report card" of hemlock health. By 2004, none of the hemlocks were rated completely healthy, only 1% were slightly unhealthy, 64% in moderate decline, 11% in severe decline, and 23% were dead. Although the onset and pace of decline in hemlock health in the park varied among the study sites, by 2004 there were no completely healthy hemlocks in any of the sites (Figure 3). Annual mortality during the last five years was about 3%. The report card for all sites is poor with no sign of improvement.

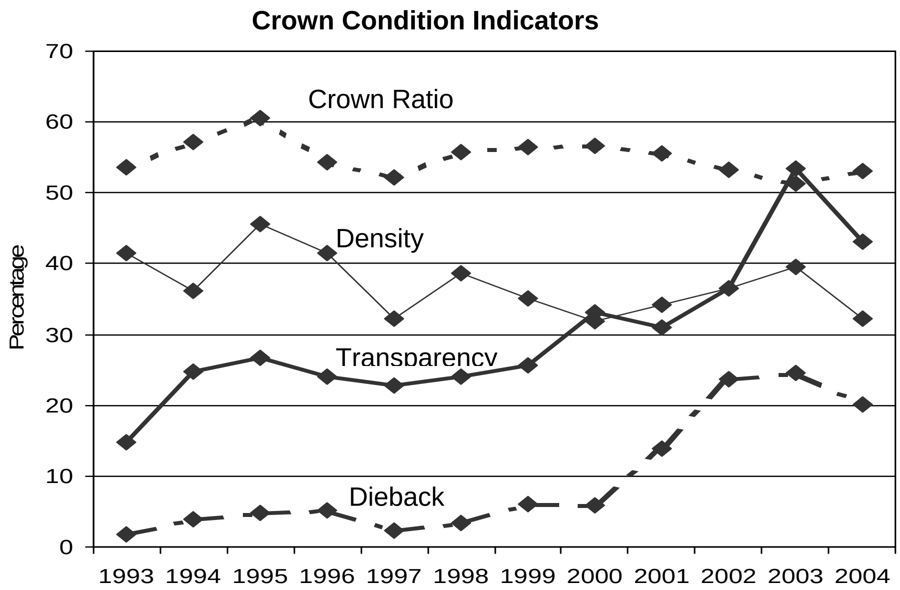

Figure 2. Trends in tree health indicators.

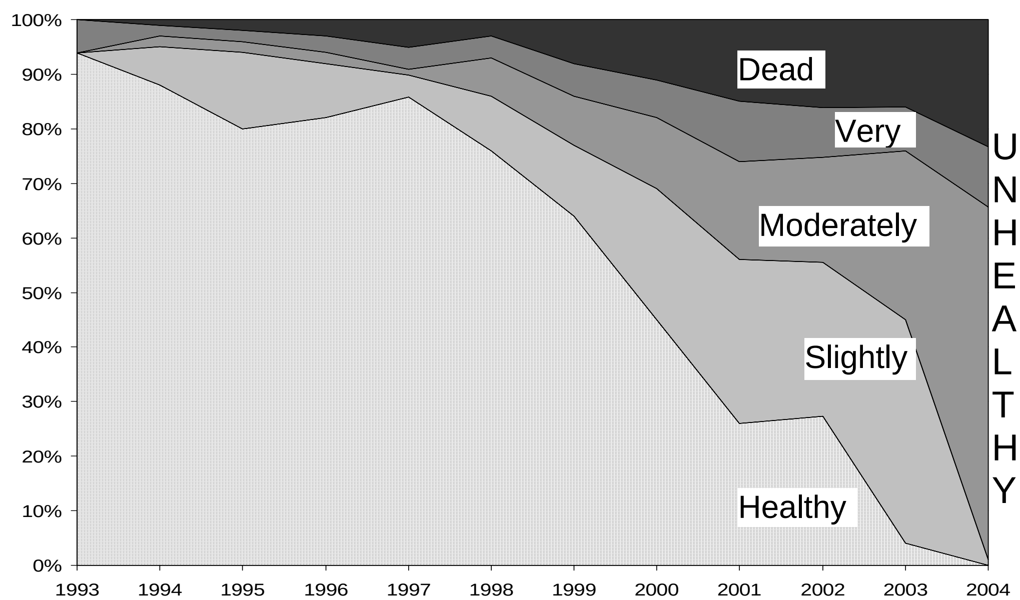

Figure 3. Overall health of hemlocks, 1993-2004.

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Matthew Seese and Karen Felton are thanked for consistent, reliable rating of the crown condition and for managing the data.

Management of HWA and Restoration of Hemlock Health

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ABSTRACT

Hemlock woolly adelgid (*Adelges tsugae*) populations have been dramatically reduced and the health of eastern hemlock (*Tsuga canadensis*) forest stands has been restored in two locations in northeastern Pennsylvania.

Bushkill Falls, Pike County, Pennsylvania has over 30-acres of hemlock-dominated forest. A comprehensive inventory and health assessment was conducted of individual trees in November 2000. Defoliation of 50 percent and significant individual mortality of hemlocks over 20-inches DBH was observed in five (5) hemlock-dominated stands. The economic and ecological importance of 1,500 trees was studied and 1,100 trees were chemically injected with imidacloprid in 2001-2002. The systemic insecticide was applied via soil drench (Merit® 75WSP) with a Kioritz® soil injector and via stem injections (Pointer ®) with a Wedgle® tree injector. Sampling results and visual observations indicated adelgid populations were reduced to less than 10 percent of pre-application levels in the winter 2002-2003. Significant new growth was subsequently observed on all treated trees with exception of hemlocks that had experienced significant defoliation.

A foliar chemistry analysis was conducted in late winter 2003 to determine if elements necessary for optimal photosynthesis were lacking in the needles of previously infested hemlocks. An analysis of 12 micro-elements and crude fiber from samples taken at Bushkill Falls was compared to samples taken from healthy nursery-grown hemlocks. The results indicated hemlocks infested with HWA had deficiencies in micro-elements necessary to maximize photosynthesis and produce new growth in the absence of HWA. Deficiencies were observed in all five stands. In May 2004, a customized mix of chelated nitrogen and micro-elements was applied to the foliage of 30-acres of hemlocks via helicopter spraying. New growth of 3 inches or more was abundant throughout the forest in summer and fall of 2004, and foliar element levels examined after treatment showed significant positive changes.

The Henryville Troutfisherman own land bordering six (6) miles of Paradise Creek in Monroe County, Pennsylvania. Paradise Creek is an high value trout stream which maintains native reproducing brook trout Hemlocks occupy approximately 30 percent of the

forest cover within 100 yards of the streambank. Hemlocks form greater than 80 percent of the forest over some pools and riffles. HWA was observed on greater than 60 percent of the hemlocks along the stream in April 2001. HWA canopy decline was observed and crown densities were 50 percent of normal in many locations. Scattered mortality of mature hemlocks was observed. In May and October 2001, approximately 500 HWA infested trees were treated with a systemic insecticide applied via soil drench (Merit® 75WSP) with a Kioritz® soil injector and via stem injections (Pointer ®) with a Wedgle® tree injector.

Approximately 5,000 *Sasajiscymnus tsugae* beetles were released in May and June 2002 within stands treated with systemic insecticides; however, beetle-release trees were not injected. HWA surveys in the 2003 and 2004 indicated that HWA was observed on less than 5-percent of trees and HWA populations were limited to scattered individuals. New growth was abundant on treated and untreated trees.

GROWTH AND SURVIVAL OF HEMLOCK WOOLLY ADELGID ON THE NORTHERN FRONTIER

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ABSTRACT

The hemlock woolly adelgid, *Adelges tsugae* Annand, was originally introduced to the eastern U.S. in 1954 near Richmond, Virginia, from Osaka, a lowland region of Japan (Havill 2005). The insect reached Connecticut in 1985, and subsequently invaded Springfield, Massachusetts, in 1991. We are attempting to document the impacts of cold temperatures on the survival and fecundity of the adelgid at the northern edge of its range in southern New England. Further objectives are to determine how adelgid density affects overwintering survival, and if there are any Allee effects associated with overwintering mortality. Allee effects can be described as decreases in survival or fecundity as population densities decline to extremely low levels frequently resulting in extinctions of incipient populations. New infestations may arise from a single dispersing individual, and the resulting small populations face genetic bottlenecks, inbreeding depression, and the difficulty of individuals finding mates. While parthenogenesis excludes the adelgid from the latter challenge, there are several other reasons to expect Allee effects in newly colonized adelgid populations. These include the documented benefits of some hemipterans forming feeding aggregations, the possible weakening of tree defenses when adelgid are at higher densities, and increased protection from the environment when woolly masses are clumped and overlapping on branchlets.

In order to study the effects of overwintering temperature and adelgid density on survival and fecundity of the sistens generation, thirteen sites throughout Massachusetts and Connecticut were sampled between March and May, 2004. We found that adelgid mortality significantly increased with decreasing mean overwintering temperature $P=0.050$). The same trend is present using average minimum winter temperature, however the regression is not statistically significant ($p=0.407$). Fecundity also declined at lower minimum temperatures, a trend that was marginally significant ($p=0.066$). Overwintering mortality increases with density even at the lowest densities ($p<0.001$); thus there is no evidence for an Allee effect in overwintering mortality.

We are also collecting data on adelgid survival and fecundity at several sites for both the progrediens and sistens generations to see if we can explain the very slow rate of spread we have observed in Massachusetts over the past four years.

DEVELOPMENT OF A MICROSATELLITE LIBRARY FOR THE EASTERN HEMLOCK, *TSUGA CANADENSIS*

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ABSTRACT

Eastern hemlock, *Tsuga canadensis*, is a vital component of the eastern North American forest, providing important habitat for songbirds and small mammals (Yamasaki et al. 2000, Young et al. 2002), browse for deer and other herbivores (Anderson and Loucks 1979), and temperature moderation important for native brook trout in first and second order streams (Ross et al. 2003). Over the last 20 years, widespread decline and mortality of hemlock in the eastern United States has occurred, particularly in association with hemlock woolly adelgid (*Adelges tsugae*) infestations. In order to mitigate hemlock decline, an important first step is to determine the genetic structure of the existing hemlock populations. This will be accomplished using a library of microsatellite markers recently being developed at our facility.

Microsatellites occur throughout a species' genome and are comprised of segments of repeated DNA with a short (usually 1-6 nucleotides) repeat length, are highly unstable, and mutate at high rates compared to other genetic markers. These differing numbers of repeats at a microsatellite constitute alleles and are inherited in a Mendelian fashion. Divergence of allele frequencies between populations occurs over time due to new mutations and drift while migration leads to a homogenization of allele frequencies. Effects of natural selection may overcome these forces by causing selection of an allele at a specific locus.

Screening individuals with microsatellite markers allows these allele frequencies to be measured and can reveal a multitude of information about the species being studied. Microsatellites have been used for genetic mapping, linage analyses of disease susceptibility genes, paternity and kinship analysis, early cancer detection, and the probability of sample identity at both the individual and population levels. In the study of entire populations, microsatellites are also useful in determining the degree of hybridization between closely related species, interspecies and inter-population variation, estimating effective population size, degree of population structure including both the amount of migration between subpopulations and genetic relationships among the various subpopulations (Murray 1996).

For eastern hemlock, these markers will facilitate identification of native variants that are resistant to infestation effects of hemlock woolly adelgid and allow the effectiveness of recovery strategies, such as genetic engineering of resistance genes and intergression of congeneric genomes into *T. canadensis,* to be monitored.

The Effects of Hemlock Woolly Adelgid Infestation on Breeding Populations of Three Species of Eastern Hemlock-Dependent Songbirds in the Delaware Water Gap National Recreation Area

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ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae*) is currently decimating eastern hemlock stands throughout the northeastern United States. Recent studies have demonstrated that several species of songbirds, including the black-throated green warbler (*Dendroica virens*), Blackburnian warbler (*Dendroica fusca*) and blue-headed vireo (*Vireo solitarius*), are hemlock-dependent species during the breeding season (Benzinger 1994, Howe and Mossman 1996, Tingley et al. 2002, Ross et al. 2004). In this study, line transect detection frequencies and resulting densities for these species were compared among hemlock stands differing in degree of infestation and hemlock condition in order to determine effects of infestation level on breeding populations. Correlation of several measures of infestation level with detection frequency and density estimates varied considerably and was, for the most part, not significant. Measures of infestation utilizing height of the lowest live branch and distance from first dead to first live branch (lower height and smaller distance, respectively, equate to better health as lower branches tend to die off first) were significantly correlated with blue-headed vireo detection frequency ($P < 0.05$) and with black-throated green warbler detection frequency and density estimation ($P < 0.05$) using Spearman's rho correlations. Results suggest a relationship between degree of infestation, hemlock condition, and the population levels of these two species, but not for Blackburnian warbler.

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WINTER MORTALITY IN *ADELGES TSUGAE* POPULATIONS IN 2003 AND 2004

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ABSTRACT

We assessed the mortality of hemlock woolly adelgid populations in the northeastern U.S. after the winters of 2002-2003 and 2003-2004. In 2003, adelgid winter mortality averaged 86.0% at 29 sites in New York and New England, 73.8% at six Pennsylvania sites, and 11.2% at a North Carolina site. In 2004, adelgid winter mortality averaged 93.6% at 17 New York and New England sites, 78.4% at seven Pennsylvania sites, and 21.1% at the North Carolina site. Mortality was positively correlated with degrees of latitude and the minimum temperature recorded at each site.

KEY WORDS

Hemlock woolly adelgid, mortality.

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, a destructive invasive species native to Asia, is a serious pest of hemlock trees in the eastern U.S. It was first found in eastern North America in Virginia in the 1950s and since that time has spread to locations from southern Maine to Georgia. The range of eastern hemlock extends well into Canada, but cold winter temperatures could be a factor in limiting the northward spread of this pest. We assessed the mortality of HWA populations after the winter of 2002-2003 and 2003-2004, the coldest winters recorded in the Northeast in the past decade (National Weather Service data).

METHODS

Between the beginning of March and the end of April 2003, we sampled HWA populations at 36 sites in New Hampshire, Massachusetts, Connecticut, New York, Pennsylvania, and North Carolina. During a similar period in 2004 we sampled populations at 35 sites in ME, New Hampshire, Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Maryland, West Virginia, and North Carolina. At each site, one or two branch tips (30-40 cm long) were cut from 10 eastern hemlock, *Tsuga canadensis* (L.) Carrière, that had new growth, no history of fertilization or insecticide treatment, and light to moderate HWA infestations. Samples were examined within two to five days of collection. The length of new growth and the number of living and dead HWA sistens that were 2nd, 3rd, or 4th stage nymphs or adults were counted. First instar nymphs also were counted but not included in the assessment of winter mortality. Mortality assessments were made by carefully probing each woolly mass and adelgid found on the new growth of branch tips. Where possible, at least 100 HWA sistens, nymphal stage 2 or older, were examined from each of the 10 trees at each site, totaling at least 1,000 HWA per site. In addition, in 2004, a determination was made of the life stage of each living and dead adelgid on the branch tips collected from one of the 10 trees at each site. Latitude, longitude, and elevation were recorded at each site. Highest and lowest daily temperatures for the period November 2002 through March 2003 were obtained from the National Climate Data Center for the weather station closest to each site. Available data were analyzed using the Spearman rank correlation test and multiple regression. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

In the northeastern U.S., HWA sistens aestivate during the summer months as first instars and many do not survive the summer. In October, surviving nymphs begin feeding and resume development through four instars, generally maturing by February or March. Because samples were collected and evaluated in March and April, living HWA were adults, or in some cases 3rd or 4th stage nymphs. Dead adelgids ranged from 1st stage nymphs to adults. Percent mortality of adelgids that broke aestivation was highest in the 2nd and 3rd nymphal stages (32 and 54%, respectively); comparatively few adelgids died as 4th instars (12%) or adults (2%).

In 2003, mortality of HWA sistens that had successfully molted into 2nd stage nymphs averaged 86.0% at 29 sites in New York and New England, 73.8% at six Pennsylvania sites, and 11.2% at a North Carolina site; highest mortality observed was 99.4% at a New Hampshire site. In 2004, HWA winter mortality averaged 93.6% at 17 New York and New England sites, 78.4% at seven Pennsylvania sites, and 21.1% at the North Carolina site; highest mortality observed was 100% at a New York site. Analysis of data from the winter of 2002-2003 indicates that mortality was positively correlated with degrees of latitude ($r = 0.422$, $P = 0.010$), even when the outlying North Carolina site was excluded ($r = .371$, $P = 0.028$), and negatively correlated with mean daily low temperature ($r = -0.626$, $P = 0.03$). There were no significant correlations between percent mortality and plant hardiness zone, longitude, or elevation, but there was a slight negative correlation between percent mortality and the minimum temperature recorded at each site ($r = 0.333$, $P = 0.047$). This may indicate that high mortality is more the result of long-term low temperatures over the course of the winter than of a cold period of short duration.

Compared with sites examined in 2003, the 35 sites sampled in 2004 extended over a greater part of the adelgid's current range and as a result, there was a stronger positive correlation between adelgid mortality and degrees of latitude ($r = 0.590$, $P = 0.0002$). Mortality was negatively correlated with degrees of longitude ($r = 0.624$, $P = <0.0001$) and elevation ($r = 0.395$, $P = 0.0190$). (Verified weather data for 2004 are not yet available.) Based on multiple regression analysis, only latitude accounted for a significant amount of the variance in percent mortality (P = 0.0321). This may be because the more western sites tended to be farther south and at higher elevations.

Although HWA populations are established in the eastern U.S. as far north as the Catskills in New York and southeastern New Hampshire and ME, existing populations are restricted to plant hardiness zone 5A (min. low of -26.5° to -28.8°C), or warmer. Based on the high winter mortality experienced by northern HWA populations in 2003 and 2004, we speculate that cold winter temperatures will limit the rate and extent of its northward spread.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of D. Mikus, R. Hirth, J. Fagan, and K. McManus of the USDA Forest Service, R. Hiskes and R. Cowles of the Connecticut Agricultural Experiment Station, and the many state and federal cooperators who provided us with samples.

BACTERIAL ENDOSYMBIONTS OF *ADELGES TSUGAE* ANNAND: POTENTIAL TARGETS FOR BIOCONTROL?

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ABSTRACT

We report the existence of four morphological forms of endosymbiotic bacteria in *Adelges tsugae* Annand and describe their ultrastructure. The endosymbionts are both intracellular and free-living in the hemocele. Systemic treatment of adelgid-infested hemlock seedlings with the antibiotic rifampicin resulted in degradation of adelgid endosymbionts and eventual death of the insects.

KEY WORDS

Bacteria, endosymbiont, ultrastructure.

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is a devastating non-native pest of eastern hemlocks (*Tsuga canadensis* (L.) Carrière and *T. caroliniana* Engelm.) and a major threat to the health and sustainability of hemlock as a forest resource in eastern North America. HWA is native to Asia, where it infests Asian hemlocks but causes no particular damage. It was first reported in western North America in the 1920s on western hemlocks (*T. heterophylla* (Raf.) Sarg. and *T. mertensiana* (Bong.) Carrière), but these species, like those in Asia, appear to tolerate the effects of adelgid feeding. It is not known with certainty whether native predators in Asia and western North America mitigate impacts of HWA on hemlock in these areas or if genetic differences make eastern hemlocks more susceptible to adverse impacts of adelgid feeding. However, a recent study of terpenoids in worldwide *Tsuga* species found a possible linkage between terpenoid profiles and hemlock susceptibility to HWA (Lagalante and Montgomery 2003).

Terpenoids are volatile compounds found in conifers; they serve in chemical communication between plants and insects and are known to have antibiotic properties. There is evidence of terpenoid interference with the host-symbiont relationship in a number of insects, including Aphidoidea (aphids, phylloxerans, adelgids) (Van den Heuvel et al. 1998; Raguraman and Saxena 1994). Symbiotic microorganisms are common in Aphidoidea, but little is known about such in the Adelgidae. Here we describe bacterial endosymbionts in HWA, and speculate that host plant terpenoids in western and Asian hemlocks may adversely affect these microorganisms and inhibit HWA development on these *Tsuga* species.

METHODS

Hemlock woolly adelgid-infested foliage was collected from *T. canadensis* located in urban and forested settings in CT, PA, and VA, and from *T. heterophylla* in UT and OR. Collections were made over a period of several years and included all developmental stages of the three adelgid morphs (sistens, progrediens, and sexuparae). In addition, a group of eastern hemlock seedlings, naturally infested with aestivating HWA sistens, was treated in the fall with the antibiotic rifampicin via root soak for five days. Control seedlings were treated with water. All specimens were examined using light microscopy and transmission and scanning electron microscopy.

RESULTS AND DISCUSSION

Adelges tsugae harbors bacterial organisms that are found free living in the hemocele as well as bacteria in specialized cells known as mycetocytes or bacteriocytes. Based on their ultrastructure, *A. tsugae* endosymbionts appear to be prokaryotic bacteria with several distinct morphological forms. There are no apparent differences in the forms of bacteria found in the sistens, progrediens, or sexuparae morphs.

Form "A" bacteria are exclusively intracellular, residing in mycetocytes. They are 2.1-2.9 μm in diameter and variable in shape. The bacteria are densely packed in the mycetocyte and their cytoplasm is uniformly distributed and relatively dense. Form "A" endosymbionts are not present in oocytes or eggs prior to formation of mycetocytes, but they are found in all other *A. tsugae* stages except the post-oviposition adult, which lacks mycetocytes. Form "B" endosymbionts occur both intra- and extracellularly. They are spherical, 2.3-3.1 μm in diameter, and less electron-dense than the "A" form. They also are in mycetocytes, both separately and in combination with the "A" form. The "B" form often is seen in mycetocytes with disrupted cell membranes. Form "C" endosymbionts are rod-shaped, 1.5-2.3 μm in diameter and 8.7-14.6 μm long. They are prevalent in the hemocele of all *A. tsugae* nymphs and adults, and in oocytes and eggs, but do not appear in mycetocytes. The "C" form penetrates oocytes, transmitting the organism to the next adelgid generation. As oocytes develop, another morphological form, "D", appears along with "B" and "C" in a bacterial syncytium, which is incorporated into the embryo. As mycetocytes develop, the "D" form disappears and the "A" form appears in the new mycetocytes.

Treating infested hemlock seedlings with the antibiotic rifampicin resulted in inhibition of HWA development while changes occurred concurrently in the appearance of the endosymbionts. Treated adelgids eventually became aposymbiotic, gut tissues became increasingly necrotic, and nymphs died in the second instar. Anomalies similar to those seen in the endosymbionts of antibiotic-treated HWA (i.e., vacuolization, ruptured cell membranes, and necrosis) were occasionally observed in adelgids collected from *T. canadensis* and often observed in HWA on *T. heterophylla.*

Lagalante and Montgomery (2003) speculated that the significant differences in terpenoid profiles in the two eastern North American *Tsuga* species and the Asian and western North American species might correspond to the susceptibility/resistance of *Tsuga* species to HWA. We speculate that the mode of action for resistance may be terpenoid-induced degeneration of the endosymbiont population in HWA resulting in reduced development and fecundity of the insect population feeding on western North American and Asian *Tsuga* hosts. There could be subtle differences between the terpenoid profiles of individual *T. canadensis*, which could account for the degeneration observed in endosymbionts of some HWA and could reflect tolerance of some hemlocks to HWA feeding. Thus, the bacterial endosymbionts of HWA could serve as appropriate targets for biocontrol through systemic inhibition of metabolic pathways or by selecting resistant cultivars based on terpenoid profiles.

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Study of the Utilization Options for Dead and Dying Eastern Hemlock in the Southern Appalachians

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ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae* Annand) is a non-native pest that is decimating the eastern hemlock (*Tsuga canadensis* L.) population in the forests of the eastern United States. The Southern Appalachian region, which falls entirely within the natural distribution of eastern hemlock, has reported adelgid infestations in nearly half of its range. If the infestations continue to spread at the current rate, the entire Southern Appalachian region will be affected within a decade. The majority of the current research effort focuses on preventing the spread of the adelgid. Unfortunately, the damage is already done in many areas and little research has been done on examining the utilization potential for the dead hemlocks. The purpose of this study is to examine the current markets for hemlock, determine at what stages of decline hemlock wood can still be used for various products, determine product yield lost when processing dead material, and to make management and harvesting recommendations based on the findings.

CURRENT INVENTORY AND TIMBER PRODUCT OUTPUT

The U.S. contains more than 2.8 billion cubic feet of hemlock sawtimber, of which approximately 10% (280 million) is found in the Southern Appalachians. The majority of hemlock sawtimber in the Southern Appalachians is found on private land (53.8%) and national forest land (44.0%). North Carolina has the largest inventory of sawtimber-size hemlock trees (9.3 million), followed by Virginia (7.8 million) and Tennessee (7.4 million). Hemlock trees in the Southern Appalachians have an average breast-height diameter of 9.3 inches and an average volume of 13.0 cubic feet (based on trees with 6 inches or greater DBH).

According to Forest Service timber product output (TPO) data, approximately 4.4 million cubic feet of hemlock is harvested annually in the Southern Appalachians. Eighty-five percent of the harvested timber is used for sawlogs and the remainder is used for pulpwood, composite products, fuelwood, and veneer logs (Figure 1).

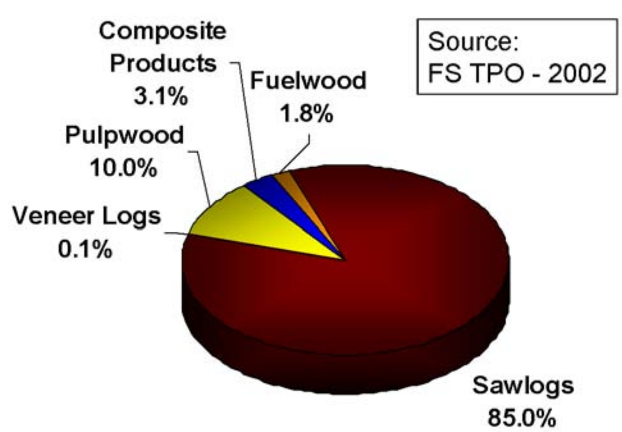

Figure 1. Total Eastern Hemlock Timber Use in the Southern Appalachians.

WOOD PROPERTIES

Wood of eastern hemlock is coarse and uneven in texture, moderately lightweight, moderately hard, moderately low in strength, moderately stiff, and moderately low in shock resistance (Alden 1997). Though these characteristics may appear to be unfavorable, ponderosa and lodgepole pine (highly preferable species) share quite similar properties (Forest Products Laboratory 1999). The most unfavorable characteristic of hemlock is that it's prone to ring shake (a lengthwise separation of wood between and parallel to growth rings) (Gardner and Diebel 1995). Ring shake is probably the most significant deterrent to widespread hemlock utilization.

CURRENT HEMLOCK MARKETS

In order to assess the current markets for hemlock, a localized study area was first established in Virginia, West Virginia, and North Carolina. Primary wood manufacturers in the study area are currently being contacted to determine use of hemlock timber. The following information is being collected from each mill: mill type, volume of hemlock processed, type of products purchased, type of products sold, delivered cost of products purchased, and end-product value. Preliminary results indicate that there is a demand for hemlock lumber (Figure 2) for local construction, and there also appears to be a demand for hemlock logs in the log home industry (Figure 3).

Figure 2. Local use hemlock lumber.

Figure 3. Three year old hemlock farmhouse.

DEAD HEMLOCK DECAY RATE

An important factor to consider when discussing the utilization of dead timber is the maximum length of time between tree death and harvest that will still yield a useable and profitable product. Therefore, an attempt is being made to determine the decay rate of dead hemlock trees measured as the wood's specific gravity loss over time since death. Half-inch increment core samples are first collected from dead hemlock trees within the study area (Figure 4). Core samples from adjacent live hemlock trees are also collected. Other information gathered from each tree includes: DBH, branch and bole structures retained (primary branches, secondary branches, etc.), GPS coordinates, aspect, and slope. Core samples are then brought back to the lab and analyzed to determine specific gravity.

Figure 4. Half-inch diameter hemlock core sample.

For the second part of the decay rate equation, it's necessary to determine the time-since-death for each sample tree. Because stand history is usually not available, an attempt is made to cross-date core samples of dead hemlock trees with core samples from live, adjacent hemlock trees. Cross-dating involves matching the growth ring patterns of the live tree samples with the growth ring patterns of the dead tree samples. Once they are matched, an estimate of time since death can be determined by counting the outer rings missing on the dead tree samples (Figure 5).

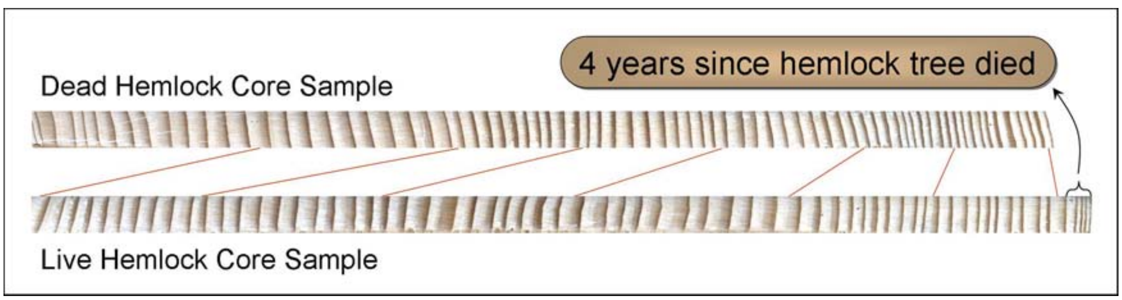

Figure 5. Cross-dating method used to determine time since death for dead hemlock trees.

LIVE AND DEAD PRODUCT YIELDS

Because of the physical deterioration associated with dead trees, the product yields from dead timber are expected to be lower when compared to the utilization of live trees (Cahill 1980, Snellgrove and Cahill 1980). In order to determine the volume of useable material lost when processing dead timber, a yield study will be conducted. Both live and dead hemlock trees will be followed from standing to final product (Figure 6). Final products will include lumber, log home timbers, etc. Volume measurements will be taken at each stage of processing, from bucking the tree length logs to the final product. Tree and log yields will be calculated for both live and dead timber, and the average yield lost will be determined.

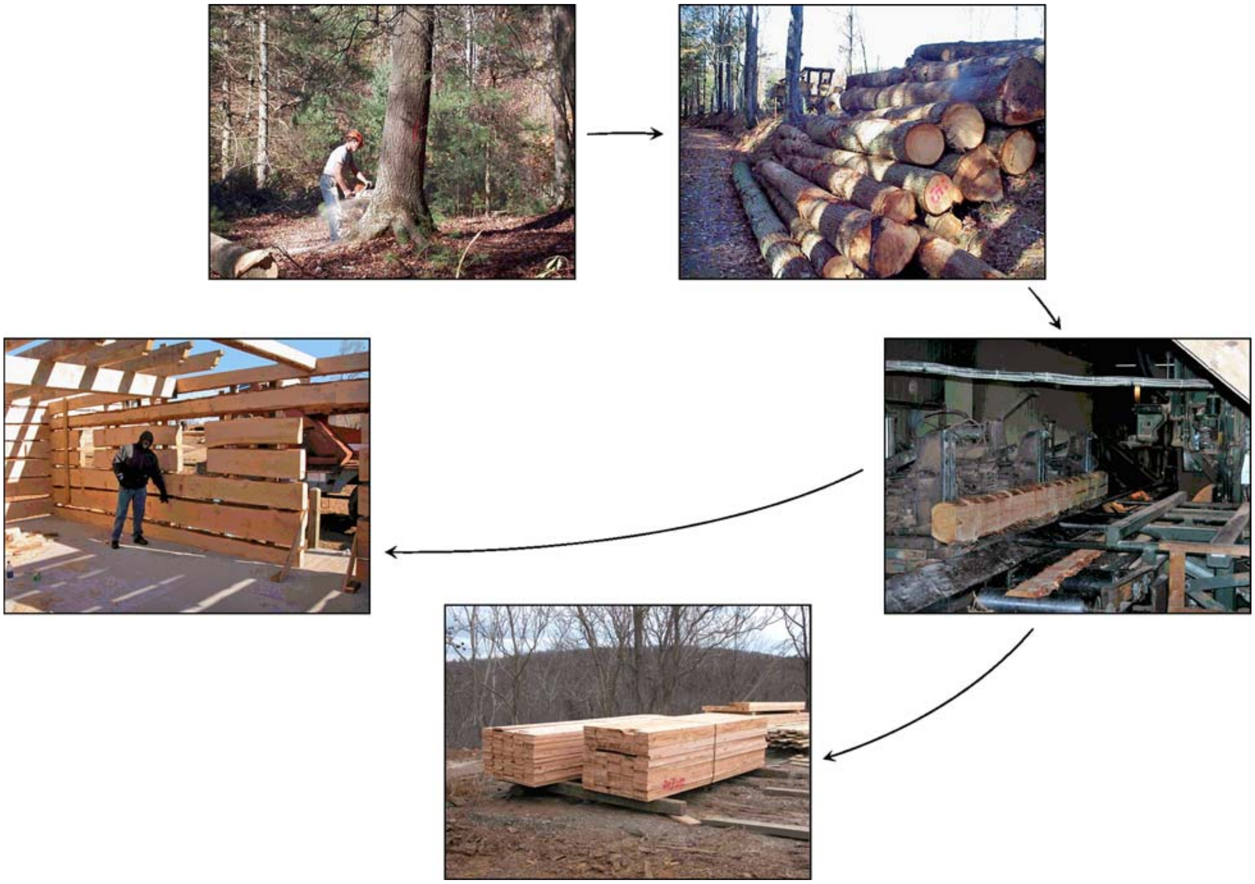

Figure 6. Production process from standing hemlock tree to final product.

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