Genetics and Conservation of Hemlock Species Threatened by the Hemlock Woolly Adelgid

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Abstract: Throughout the eastern United States populations of eastern (*Tsuga canadensis*) and Carolina (*Tsuga caroliniana*) hemlock are being decimated by the Hemlock Woolly Adelgid (HWA, *Adelges tsugae*). As part of an integrated approach to management of this exotic forest pest, Camcore (Dept. of Forestry & Environmental Resources at N.C. State University) and the USDA Forest Service Forest Health Protection are cooperating to conduct genetic diversity assessments and establish *ex situ* conservation reserves for these ecologically important forest species. The overall goal of this cooperative effort is to secure genetically diverse seed reserves and maintain viable populations of eastern and Carolina hemlock in perpetuity so that genetic material will be available for restoration efforts in areas where HWA eliminates these species from the forest. We report general findings from amplified fragment length polymorphism (AFLP) and isozyme studies of hemlock genetic diversity and provide an update on the progress of the *ex situ* conservation efforts in the southern region.

Keywords: *Ex situ*, Gene Conservation, Genetic Diversity, Hemlock Woolly Adelgid, *Adelges tsugae*, Eastern hemlock, *Tsuga canadensis*, Carolina hemlock, *Tsuga caroliniana*.

INTRODUCTION

The hemlocks (*Tsuga* spp.) are slow-growing, long-lived trees that are among the most shade tolerant of all species in the Pinaceae (Farjon 1990). Most are medium to large-sized trees at maturity and found in regions with maritime or sub-continental climates. Nine species occur world-wide with distributions restricted to three regions. Five species occur in eastern Asia, growing in China and the Himalayan range (*T. chinensis, T. dumosa,* and *T. forrestii*) and in Japan (*T. sieboldii* and *T. diversifolia*). Two species (*T. heterophylla* and *T. mertensiana*) are found in the Pacific Northwest region of the United States and Canada (Means 1990; Packee 1990). Of concern here is the largest distribution of hemlock species which occurs in eastern North America (Figure 1). Accounting for much of this distribution is eastern hemlock (*T. canadensis*) which ranges from Nova Scotia south to northern Georgia and Alabama and west across eastern Canada and the Upper Midwest to Minnesota (Godman and Lancaster 1990). Carolina hemlock (*T. caroliniana*) has a much smaller distribution that is restricted to a relatively small number of populations in the Southern Appalachian Mountains of Georgia, Tennessee, Virginia, and North and South Carolina (Farjon 1990; Jetton et al. 2008a).

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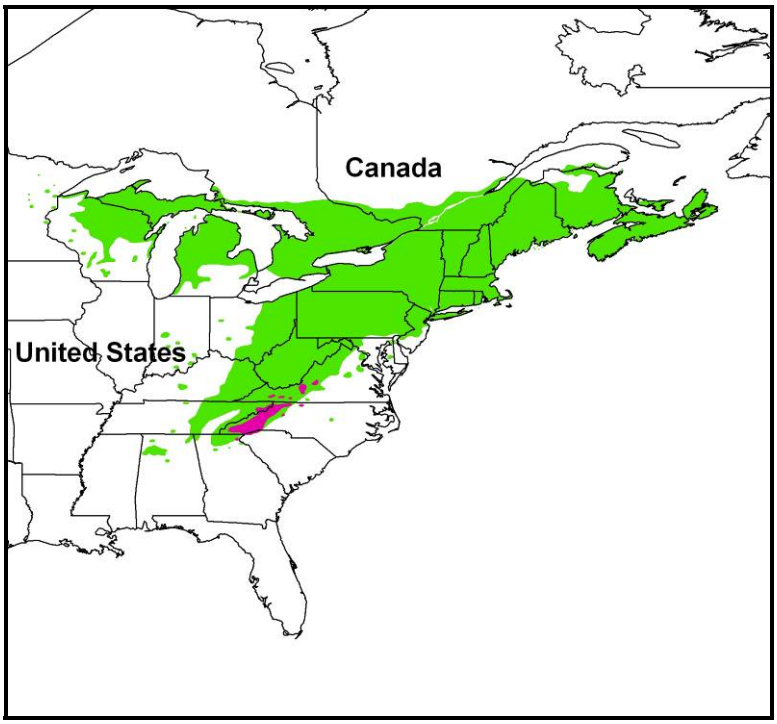

Figure 1. The natural distribution of eastern hemlock (green) and Carolina hemlock (red) in eastern North America.
(Map by Camcore using data from USGS 1999)

There are a number of factors that threaten the long-term stability of hemlock ecosystems in eastern North America including exotic insects, severe drought, widespread wildfire, suburban development, and climate change. Among these, the Hemlock Woolly Adelgid (HWA, *Adelges tsugae*) is of the most concern and threatens to eliminate both eastern and Carolina hemlocks from their native range (McClure et al. 2001). The adelgid is an exotic insect that was introduced from Japan to the eastern US sometime between 1920 and 1950 (Stoetzel 2002), likely on *T. sieboldii* (Southern Japanese Hemlock) nursery stock planted in the Richmond, Virginia area. HWA is currently found in 18 eastern states from Maine south to Georgia where it infests approximately 50% of hemlock ecosystems. The adelgid feeds primarily on stored nutrients and other resources in xylem ray parenchyma (Young et al. 2005), most probably by extra-oral digestion of solid materials. The symptoms of this feeding include abortion of vegetative and reproductive buds, reduced new growth production, and needle loss (McClure et al. 2001). HWA can kill trees in as little as four years and has caused widespread decline and mortality of both eastern and Carolina hemlock.

In 2003, the USDA Forest Service Forest Health Protection entered into a three-phase cooperative agreement with Camcore (N.C. State University) to preserve seeds of both hemlock species threatened by HWA in the eastern U.S. (Tighe et al. 2005; Jetton et al. 2008b). The objectives of the agreement are to: 1) develop a framework plan for hemlock gene conservation, 2) describe patterns of hemlock genetic diversity across the geographic range of both species, 3) collect seeds from hemlock populations distributed across the geographic range of both species, 4) place hemlock seeds into long-term cold storage at Camcore facilities and those maintained by the National Germplasm Repository, and 5) establish national and international conservation

seed orchards for both hemlock species. The first phase of this effort began in 2003 with seed collections from populations of Carolina hemlock across its Southern Appalachian range. The second phase began in 2005 and is focused on seed collections from eastern hemlock populations distributed throughout the southeastern U.S. portion of the species' geographic range. The third and final phase will follow in late 2009 with a four-year effort to make seed collections from portions of the eastern hemlock range in the northeastern and Midwestern regions of the U.S. The overall goal of this cooperative program between Camcore and the USDA Forest Service is to secure genetically diverse seed reserves and maintain viable populations of eastern and Carolina hemlock in perpetuity so that genetic material will be available for restoration efforts in areas where the hemlock woolly adelgid eliminates hemlock from the forest. The following is a brief synopsis of our project accomplishments to date.

HEMLOCK GENETIC DIVERSITY

A preliminary amplified fragment length polymorphism (AFLP) study indicated that, compared with other conifer species, Carolina hemlock has moderate levels of genetic diversity and that the general trend is for decreasing diversity moving from south to north across the species geographic range (Camcore 2006). Using allozyme markers, eastern hemlock had overall low diversity in the southeastern U.S. portion of its range, with diversity decreasing from east to west (Potter et al. 2008). Currently, we are expanding on our genetic studies of eastern hemlock with a microsatellite (SSR) marker study to describe patterns of genetic diversity within and among approximately 70 populations throughout the species' entire geographic range in the U.S., including both main range interior populations and disjunct island populations. Final results are expected in late 2009.

HEMLOCK SEED COLLECTION

The genetic studies serve as important guidelines for the conservation effort, allowing targeted seed collections in portions of the geographic range that will result in capture of maximum levels of diversity for both species. Using this data, we have made good progress between 2003 and 2009 with seed collections from surviving hemlock stands in the southeastern U.S. (Camcore 2004, 2005, 2006, 2007, 2008). Seeds have been sampled from a total of 97 open-pollinated mother trees in 13 populations of Carolina hemlock in Georgia, Tennessee, Virginia, and North and South Carolina (Figure 2). Eastern hemlock collections have sampled 195 open-pollinated mother trees from 26 populations in Georgia, Kentucky, Tennessee, Virginia and North and South Carolina (Figure 3). In 2010, we expect to reach our seed conservation goals for eastern and Carolina hemlock in the southeast and plan to initiate eastern hemlock collections in the northeast and Midwest.

Hemlock seed collections by Camcore and the USDA Forest Service are in support of the *ex situ* conservation effort, and the Camcore seed bank in Raleigh, NC currently serves as the primary repository for all accessions. Utilizing recommendations from the Woody Plant Seed Manual (USDA FS. 2008) and results of ongoing studies at Camcore in cooperation with the USDA Forest Service National Tree Seed Laboratory (NSL), we are developing optimal seed storage

protocols for both species. Small amounts of Carolina hemlock seed have been submitted to the NSL and the National Germplasm Repository in Fort Collins, CO, and we will do the same with eastern hemlock in the future.

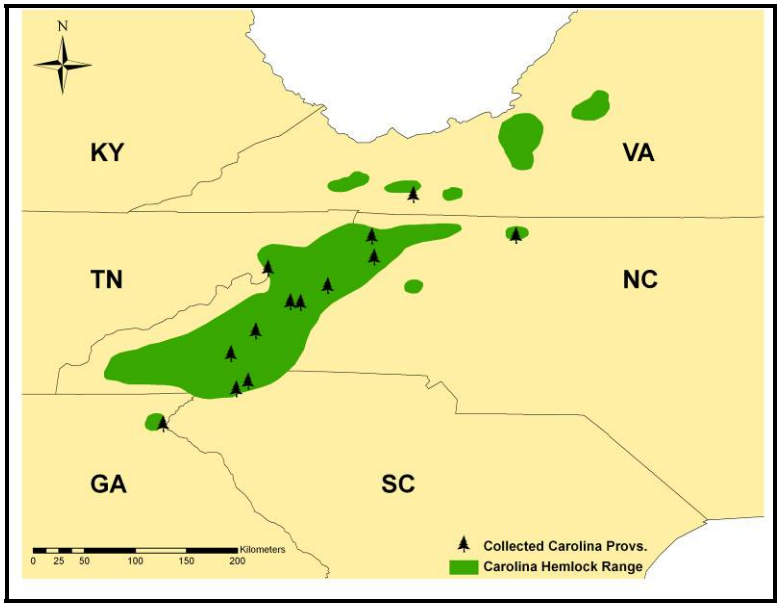

Figure 2. Provenance locations for Carolina hemlock seed collections made by Camcore in the Southern Appalachian Mountains between 2003 and 2008. (Map by Camcore using data from Camcore and USGS 1999)

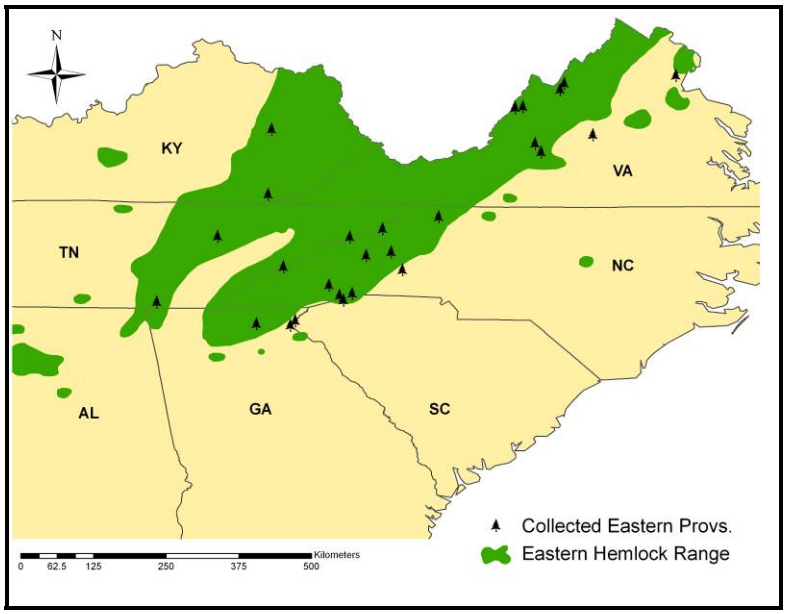

Figure 3. Provenance locations for Eastern hemlock seed collections made by Camcore in the southeastern United States between 2005 and 2008. (Map by Camcore using data from Camcore and USGS 1999)

HEMLOCK CONSERVATION SEED ORCHARDS

Camcore members Arauco-Bioforest and Forestal Mininco in Chile and Rigesa-MeadWestvaco and Klabin SA in Brazil are working with Camcore and the USDA Forest Service to establish hemlock *ex situ* conservation plantings in South America. In the U.S., the University of Arkansas and the USDA Forest Service are working together to establish similar plantings in the Ozark Mountains. Our first operational Carolina hemlock conservation bank was planted in September 2009 at Cuyimpalihue by Arauco-Bioforest in Chile. This planting contains 1,400 seedlings representing 64 open-pollinated mother trees and 9 populations. Additional conservation plantings are planned for 2010 establishment utilizing eastern and Carolina hemlock seedlings under cultivation at forest nurseries in Brazil and Carolina hemlock seedlings being grown at the University of Arkansas. In 2009, all cooperators in Chile, Brazil, and the U.S. will receive additional seeds of both species for future conservation bank expansion.

Camcore is also working to establish small hemlock seed orchards within the native range of both eastern and Carolina hemlock. The first of these was planted in 2007 at the N.C. State University Upper Mountain Research Station in Ashe Co., NC and contains 400 seedlings representing 53 open-pollinated families of Carolina hemlock. Although such plantings will eventually require insecticide protection from HWA, they are important tools for research on hemlock establishment requirements in seed orchard settings and breeding strategies that will be needed for restoration efforts.

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