Conserved ex situ genetic resources of eastern and Carolina hemlock: Eastern North American conifers threatened by the hemlock woolly adelgid

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Conserved *Ex Situ* Genetic Resources of Eastern and Carolina Hemlock: Eastern North American Conifers Threatened by the Hemlock Woolly Adelgid

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Abstract

The long-term sustainability of the eastern North American conifers eastern hemlock (*Tsuga canadensis* [L.] Carriére) and Carolina hemlock (*T. caroliniana* Engelmann) is threatened by the exotic insect hemlock woolly adelgid (*Adelges tsugae* Annand; HWA). The integrated pest management strategy to mitigate HWA impacts on hemlock ecosystems includes a cooperative genetic resource conservation program being conducted by Camcore (International Tree Breeding and Conservation Program at North Carolina [NC] State University) and the U.S. Department of Agriculture (USDA) Forest Service Forest Health Protection. Through the first 10 years of this project (2003 to 2013), seeds have been collected from 60 populations of eastern hemlock and 19 populations of Carolina hemlock in the United States, representing 451 and 134 mother trees, respectively. Seeds have been distributed to the Camcore seed bank in Raleigh, NC, and the USDA Agricultural Research Service National Center for Genetic Resource Preservation in Fort Collins, CO, for long-term storage, and to forest nurseries in Brazil, Chile, and the United States, where seed orchards have been established.

Introduction

Hemlocks (*Tsuga* Carriére) are long-lived conifers that are among the most shade-tolerant and drought-susceptible species in the Pinaceae family, with some of the oldest recorded specimens surviving for 800 to 1,000 years. Worldwide distribution is restricted to three geographic regions (Farjon 1990) for the nine taxonomically accepted hemlock species. Five species occur in eastern Asia, distributed throughout mainland China, the Himalayan Mountains, and Taiwan (Chinese hemlock [*T. chinensis* (Franc.) Pritzel in Diels], Himalayan hemlock [*T. dumosa* (D. Don) Eichler], and Forrest's hemlock [*T. forrestii* Downie]) and in Japan (southern Japanese hemlock [*T. sieboldii* Carriére] and northern Japanese hemlock [*T. diversifolia* (Maxim.) Masters]). Four species occur in North America. Western hemlock (*T. heterophylla* [Raf.] Sargent) and mountain hemlock (*T. mertensiana* [Bong.] Carriére) occur in western North America in a range that extends from southern Alaska south into northern California. Eastern hemlock (*T. canadensis* [L.] Carriére) and Carolina hemlock (*T. caroliniana* Engelmann) are native to eastern North America; they are the subjects of this article.

Eastern hemlock is a widespread conifer species with a natural range that extends from Nova Scotia west to northern Minnesota, south throughout the New England and Middle Atlantic States, and down the southern Appalachian Mountains into northern Georgia and the Cumberland Plateau of Alabama (Farjon 1990). A number of disjunct populations occur to the west of the main distribution in Alabama, Tennessee, Kentucky, Indiana, Ohio, Wisconsin, and Minnesota and to the east in Maryland, Virginia, and North Carolina (figure 1). The species is found from sea level to 4,920 ft (1,500 m) elevation and

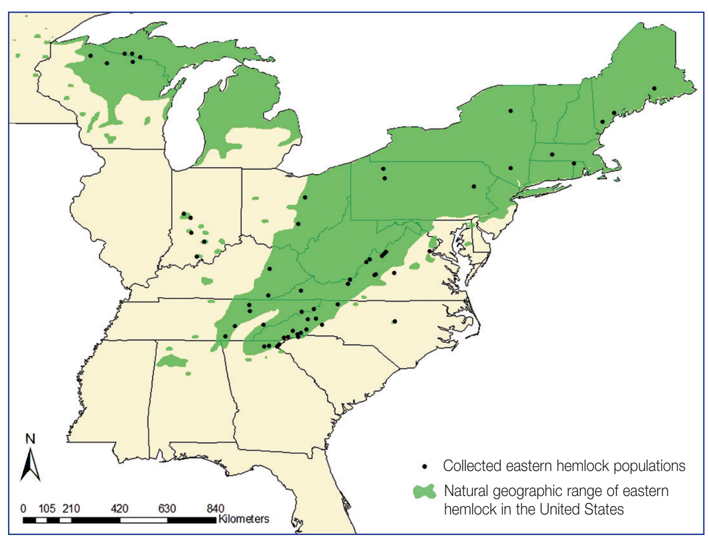

Figure 1. The geographic distribution of eastern hemlock (*Tsuga canadensis*) in the Eastern United States and the locations of provenance seed collections made by Camcore. (Shapefile based on Little 1971; downloaded from U.S. Geological Survey 2013)

is bimodal in habitat distribution (Kessell 1979). It occurs in high abundance on moist, well-drained, nutrient rich soils of mesic riparian zones and seasonably moist subxeric areas. At the higher end of its elevational range, eastern hemlock is often more scattered in occurrence along exposed xerophytic slopes and ridges. Where it occurs as a riparian species, the tree is important for soil stabilization and water quality and serves as a haven for associated aquatic species and as winter shelter for white-tailed deer (*Odocoileus virginianus* Zimm.), ruffed grouse (*Bonasa umbellus* L.), turkey (*Meleagris gallopavo* L.), and several other species (Ellison and others 2005).

Carolina hemlock, first described in 1837 on Table Rock Mountain in South Carolina (James 1959), is a southern Appalachian endemic with a patchy distribution occurring throughout the mountain and upper Piedmont regions of Virginia, Tennessee, Georgia, North Carolina, and South Carolina (Farjon 1990; figure 2). The geographic range is small, approximately 289 mi by 102 mi (465 km by 165 km) with a latitudinal range from 37°40' N. in Rockbridge County, VA, south to 34°73' N. in Rabun County, GA (Jetton and others 2008a). Carolina hemlock populations have been reported as far north of the range as northeastern Ohio, where a small occurrence of the species is found at the Richie Ledges overlook in the Cuyahoga Valley National Park (Galehouse 2007). Although some researchers believe the occurrence to be natural, other researchers suggest the trees were likely planted by the Civilian Conservation Corps in the 1930s. Unlike eastern hemlock, Carolina hemlock is most abundant along dry, north-facing, rocky ridge tops at elevations of 1,960

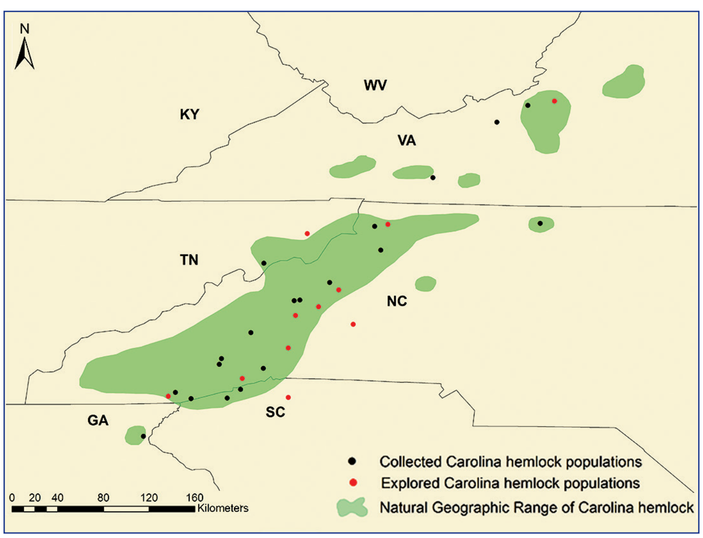

Figure 2. The geographic distribution of Carolina hemlock (*Tsuga caroliniana*) in the southern Appalachian Mountains and the locations of provenance seed collections made by Camcore indicated by black circles. Red circles indicate provenances where seed collections have not yet been made. (Shapefile based on Little 1971; downloaded from U.S. Geological Survey 2013)

to 4,900 ft (600 to 1,500 m) (Humphrey 1989). Preferred soils are dry, acidic, and nutrient poor, although more recent studies indicate the species to be more broadly adapted to a variety of soil types than initially thought (Jetton and others 2008a). Scattered populations occasionally are found growing in mesic or riparian settings more typical of eastern hemlock (James 1959). In its typical habitat, Carolina hemlock helps to reduce soil erosion while providing forage and shelter for white-tailed deer (Rentch and others 2000). Carolina hemlock is also highly regarded for its rugged aesthetic beauty and is commonly used in the ornamental industry (Swartley 1984).

Despite the differences in distribution and habitat between the two species, the long-term sustainability of both eastern and Carolina hemlock faces a significant threat because of the hemlock woolly adelgid (*Adelges tsugae* Annand; HWA). The HWA is an exotic insect introduced to eastern North America from Japan sometime before the mid-1950s and has caused widespread decline and mortality across 100 percent of the Carolina hemlock range and approximately 50 percent of the eastern hemlock range (McClure and others 2001, USDA Forest Service 2011). The integrated strategy to mitigate the impacts of HWA on hemlock ecosystems involves both *in situ* and *ex situ* approaches to hemlock conservation. The *in situ* approaches that have received the most attention are chemical control with systemic insecticides and biological control through the importation, rearing, and release of predators from the native range of HWA. Both show much promise, but the use of insecticides is limited in scope by logistical and ecological concerns, while biological control requires a decade or more of additional research and development before widespread effectiveness is realized. A complementary approach to these *in situ* efforts is *ex situ* genetic resource conservation, where genetically representative seed samples are collected from natural stands distributed across the range of eastern and Carolina hemlock. Seeds are placed either into seed banks for long-term storage or are used to establish seed orchards in areas where HWA is unlikely to occur or where the trees can be effectively protected from the insect with insecticides. After being established, these strategic seed reserves and seed orchards can serve as a source of highly diverse and broadly adaptable genetic material for restoration and reforestation when *in situ* HWA management strategies become more broadly effective.

This article reports on progress made through the first 10 years (2003 to 2013) of a cooperative hemlock genetic resource conservation program being conducted by Camcore (International Tree Breeding and Conservation Program at NC State University) and the USDA Forest Service, Forest

Health Protection program. The article also reviews the distribution, biology, and management of HWA. Progress at earlier stages of this project was documented by Tighe and others (2005), Jetton and others (2008a,b), and Jetton and others (2010).

Hemlock Woolly Adelgid

Worldwide Distribution

The worldwide distribution of HWA mirrors that of the hemlocks, with three primary concentrations found in eastern Asia, western North America, and eastern North America (Havill and others 2006). HWA is native to Asia, where it is widespread and can be found feeding on all five hemlock species native to the region but is largely innocuous because of a combination of evolved host resistance and predation by natural enemies (McClure and others 2001). The first scientific descriptions of the insect are from northwestern North America, where HWA occupies a range extending from southern Alaska to northern California. These initial reports were based on specimens collected from western hemlock in California and Oregon, where it was initially thought that HWA was exotic (Annand 1924) but is now known to be endemic (Havill and others 2011). HWA is an exotic pest in eastern North America, where little evidence exists of natural host resistance and where no native natural enemies are capable of limiting adelgid population growth (Wallace and Hain 2000; McClure and others 2001). It was introduced into Richmond, VA, sometime before the mid-1950s (Havill and others 2006), most likely on ornamental hemlock nursery stock imported from Japan. Although the initial spread of HWA from its point of origin was rather slow, its distribution in the Eastern United States has expanded rapidly since the mid-1980s and now covers a 19-State area, ranging from southern Maine south along the Appalachian Mountain chain to northern Georgia and west into Ohio (USDA Forest Service 2011).

Life History and Host Impacts

HWA has a complex life cycle that includes multiple generations and life forms per year that alternate between hemlock and spruce (*Picea* spp.) hosts using a combination of sexual and parthenogenetic reproductive strategies. For additional details on HWA's life history and how its timing differs with latitude, readers are referred to McClure (1989), Gray and Salom (1996), Havill and Foottit (2007), and Joseph and others (2011). The following refers to those parts of the HWA life cycle that occur on hemlock in the Eastern United States. The two parthenogenetic generations of HWA that are damaging to hemlocks are known as the sistens and progrediens and are active on the tree from October through early July (McClure 1989). They are identified by the cottony white woolly masses from which the insect derives its common name. These woolly masses or "ovisacs" can be observed at the base of needles on the underside of infested hemlock branches (figure 3). Each ovisac contains a single adult female and her parthenogenetically produced eggs. HWA crawlers (first instar nymphs), the only mobile life stage, hatch from the eggs and settle at feeding sites at the base of hemlock needles, where the insects remain for the remainder of their life. Settlement occurs either on the natal tree or on a nearby tree after wind, bird, deer, or human mediated passive dispersal (McClure 1990). HWA feeds by inserting its piercing-sucking mouthparts into the

Figure 3. White woolly ovisacs of the hemlock woolly adelgid (HWA) on eastern hemlock (left). HWA caused eastern hemlock mortality in the Shenandoah National Park, VA (right). (Photos courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

needle cushion and extracting stored nutrients from xylem ray parenchyma cells (Young and others 1995). Under high HWA population densities, continued depletion of nutrient stores leads to needle desiccation and drop, abortion of vegetative and reproductive buds, and cessation of new growth. Severe infestations can kill trees in as little as 4 years, although some individual trees have persisted for 10 to 20 years before succumbing to HWA. A high lifetime fecundity rate of up to 300 viable eggs per female (McClure and others 2001) has likely attributed to HWA's rapid spread and widespread impacts despite its otherwise limiting life history strategies of parthenogenesis and passive dispersal.

Integrated Pest Management

The integrated pest management strategy for managing HWA damage to eastern North American hemlock forests is focused in the areas of chemical control, biological control, host resistance breeding, and gene conservation. The chemical insecticides imidacloprid (applied by soil drench, soil injection, or stem injection) and dinotefuran (applied as a basal trunk spray) are currently the only truly effective methods to control HWA and retain hemlock in a forest or landscape (Vose and others 2013). Single applications of each chemical can effectively control HWA for 3 or more years. However, due to logistic, economic, and ecological concerns, the use of these chemicals is limited to individual high-value trees or small groups of trees in ornamental and recreation settings or the more easily accessible woodland areas of forests and parks. Lower cost and less toxic high pressure foliar sprays of insecticidal soaps and horticultural oils are also available for HWA control, but lack extended efficacy and need to be reapplied annually to maintain control (Vose and others 2013).

Biological control through the importation and release of HWA predators from the native range of HWA is currently considered the best long-term solution to management of the pest in forest settings (Onken and Reardon 2011). Several species of predatory beetles (Coleoptera) in the genera *Sasajiscymnus*, *Scymnus*, and *Laricobius* and predatory flies (Diptera) in the genus *Leucopis* are currently in various stages of laboratory study, field efficacy trials, mass rearing, and widespread release. *Sasajiscymnus tsugae* (Sasaji and McClure) has been most widely distributed across the Eastern United States with close to 3 million adult beetles released to date. However, good estimates of establishment and field impact on HWA populations are generally lacking other than at intensively sampled release sites in Connecticut (Cheah 2011). *Laricobius nigrinus* Fender and *L. osakensis* Montgomery and Shiyake appear to be the most promising predators currently under evaluation, having demonstrated successful establishment and impact on HWA population density in the field (*L. nigrinus*; Mausel and others 2008) and the ability to respond in number and function to HWA density in laboratory evaluations (*L. osakensis*; Vieira and others 2012). The ultimate goal of this program is to establish a suite of natural enemies whose feeding and impact will combine to regulate HWA populations below damaging levels (Vose and others 2013). For more detailed information on the effort to implement HWA biological control in eastern North America, see Onken and Reardon (2011).

Host resistance breeding as a strategy to mitigate the impacts of HWA has received relatively little attention compared with other management options, but progress has been made in this field during the past 10 years. Most research has focused on understanding the host-insect interaction between hemlocks and HWA, and determining how this differs between resistant and susceptible hemlock genotypes. Plant characteristics evaluated include variation in foliar terpenoid, nutrient, amino acid, and wax chemistries (Lagalante and Montgomery 2003, Pontius and others 2006, Gomez and others 2012); changes in water conductivity and formation of false rings (Gonda-King and others 2012, Domec and others 2013); and the presence and severity of hemlock hypersensitive responses to HWA attack (Radville and others 2011). Emphasis has also been placed on the production of hybrids between HWA-susceptible eastern and Carolina hemlocks and resistant species from Asia and western North America. One goal of the hybridization program is to eventually establish a backcross breeding program similar to what has been accomplished for American chestnut (*Castanea dentate* [Marsh.] Borkh.) for reintroducing HWA-resistant hemlock genotypes into heavily impacted areas. Thus far, eastern hemlock has shown a high level of hybrid incompatibility with other hemlock species, but a number of successful crosses between Carolina hemlock and Chinese hemlock have been produced (Bentz and others 2002). Finally, although both eastern and Carolina hemlock were initially thought to be universally susceptible to HWA, some evidence suggests that natural HWA resistance might exist in both species (Caswell and others 2008; Jetton and others 2008c). More research is needed to verify the validity of these assertions, but, if verified and the level of genetic variation for such traits is adequate, it suggests the possibility of breeding and restoration programs based on the pure species rather than sole dependence on the use of genotypes that contain some proportion of genes from nonnative hemlocks.

Hemlock Genetic Resource Conservation

Genetic Resource Conservation Rationale and Objectives

Because chemical insecticides are limited in use, biological control requires additional years of research and development before reaching anticipated levels of efficacy, and hemlock decline and mortality in the Eastern United States continues unabated, an effort to conserve gene pools of eastern and Carolina hemlock being lost to HWA is critical to the long-term sustainability of these ecologically vital species. The primary objective of the Camcore/USDA Forest Service cooperative hemlock genetic resource conservation program is to maintain, in perpetuity, viable *ex situ* seed reserves and seedling seed orchards of both species that will serve as a source of genetic material for breeding and restoration activities once effective *in situ* hemlock conservation strategies are in place. Another way to view this effort is as an insurance policy against the "worst case scenario," where both eastern and Carolina hemlocks are functionally eliminated by HWA from the forest ecosystems of eastern North America.

The conservation program was initiated in 2003 and was designed to include four phases. Phase 1 (2003 to 2005) focused on seed collections from stands of Carolina hemlock throughout its southern Appalachian range in Georgia, North Carolina, South Carolina, Tennessee, and Virginia. Phase 2 (2005 to 2009) focused on seed collections from stands of eastern hemlock in the southern portion of its range. The southern range was defined as Alabama, Georgia, Kentucky, North Carolina, South Carolina, Tennessee, and Virginia, where eastern hemlock occurs. Phase 3 (2009 to 2012) focused on seed collections across eastern hemlock's northern range and included Connecticut, Delaware, Indiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, West Virginia, and Wisconsin. Phase 4 is currently under way and is focused on establishing conservation seed orchards inside and outside the United States and additional seed collections, where needed, in the three regions described previously.

Given the much larger geographic distribution of eastern hemlock compared with Carolina hemlock, the seed collection effort for the species was split among the USDA Forest Service Southern and Eastern Regions to make collection planning and implementation logistically easier. The initial focus was on the Southern Region because of the much higher rates of HWA-related decline and mortality that have occurred among hemlock populations in the region. HWA impacts have been less severe in the Eastern Region (northern range of eastern hemlock), and, even now, large areas remain HWA free (USDA Forest Service 2011).

Seed Collection Strategy and Protocols

Common questions associated with the beginning of a new gene conservation program are how many populations and mother trees per population to sample, and how to distribute seed collections across the range of a species to capture maximum levels of diversity and broad adaptability. A good understanding of species population genetic structure and environmental adaptability are key to answering these questions and designing gene conservation strategies that are effective at capturing a representative number of alleles. From lessons learned during 32 years of research focused on the conservation and testing of pine species native to the fragmented tropical and subtropical forests of Mexico and Central America (Dvorak and others 2000), Camcore has determined that seed collections from 10 to 20 trees per population, depending on population size, will capture most alleles that occur at frequencies of 5 percent or greater, assuming low to moderate levels of genetic diversity (Dvorak and others 1999). Sampling 6 to 10 populations distributed across the geographic range of a species is necessary to also capture broad environmental adaptability.

Before the beginning of this project, no research on the population genetic structure of Carolina hemlock had been conducted. Therefore, as part of the effort to design the seed sampling strategy for the species, Camcore conducted an amplified fragment length polymorphism (AFLP) molecular marker analysis of 15 Carolina hemlock populations (Potter and others 2010). This study indicated that the species has a moderate level of genetic diversity (He = 0.302) for a conifer, and, because most Carolina hemlock populations are relatively small (Jetton and others 2008a), indicates that a sample of up to 10 trees per population should be sufficient to obtain a genetically representative seed sample. However, the results also indicated a high level of genetic differentiation exists among the populations, likely because of their isolated nature. Furthermore, Carolina hemlock is adapted to a number of soil types (Jetton and others 2008a) and appears to have moderately broad climatic adaptability in a range that extends across five (5b to 7b) plant hardiness zones (USDA Agricultural Research Service 2012). These factors, together with the fact that Carolina hemlock has been identified as the tree species most at risk for genetic degradation because of climate change (Erickson and others 2012), suggest that seed should be sampled from a larger than typical number of populations to adequately capture the diversity and adaptability present in the species. Therefore, to protect against the loss of the species to both HWA and climate change, the seed collection strategy that has been adopted for Carolina hemlock is to sample up to 10 mother trees per population in as many populations as can be identified. This strategy includes an effort to sample locations within each of the five plant hardiness zones occupied by the species.

Similar to the situation with Carolina hemlock, at the outset relatively little data existed on population genetic structure and diversity in eastern hemlock that was useful for designing a gene conservation strategy focused on the entire range of the species. One small study, an isozyme diversity analysis, had been completed and found that eastern hemlock has an extremely low level of genetic diversity (Zabinski 1992). The results suggest that seed collection intensity could be relatively low (i.e., few populations and few trees per population) and still be genetically representative. Zabinski's study focused primarily on eastern hemlock populations in the Lake States region, however, and thus was not sufficient for basing sampling decisions in other parts of the species' range.

To expand on the available data, Camcore conducted two studies on the population genetics of eastern hemlock: one that used isozymes to evaluate 20 populations in the southern Appalachian region (Potter and others 2008), and a second that used microsatellite molecular markers to assess genetic structure in 60 populations distributed across the entire range of the species (Potter and others 2012). As the gene conservation program for eastern hemlock has developed, the results of the latter study have been the most useful to the design of the seed collection strategy and fit nicely with the two-region approach to sampling. The microsatellite data revealed two pockets of high genetic diversity for eastern hemlock where higher seed sampling intensity is necessary. One pocket is located in the Blue Ridge Mountains of the southern Appalachian region, and the second is in New York and southern New England. Diversity was low to moderate outside of these pockets, especially in the disjunct populations that occur to the east and west of the main eastern hemlock distribution. Although disjunct populations are expected to have low diversity, Potter and others (2012) found that many of these harbor a high number of rare alleles that do not occur elsewhere in the range, making them important targets for seed collection. Eastern hemlock also has broad climatic adaptability across its large geographic distribution in the United States that extends across 10 (3a to 7b) plant hardiness zones (USDA Agricultural Research Service 2012).

Based on the patterns of genetic structure and climatic adaptability described previously, it was determined that seed collections targeting 10 mother trees per population across 30 populations in both the southern and northern ranges would be sufficient to obtain representative seed samples of eastern hemlock. Emphasis in the collection work is being placed in the pockets of high genetic diversity and disjunct populations that occur in each region, with a lower sampling intensity in lower diversity portions of the main species distribution. As with Carolina hemlock, an effort to sample all plant hardiness zones occupied by eastern hemlock is being made. This strategy should yield collections from up to 600 mother trees and 60 populations.

During seed collections for both species, a distance of 160 to 320 ft (50 to 100 m) is maintained between selected trees within individual populations as a buffer against relatedness. All trees sampled are tagged with a unique pedigree number, and height (m), diameter (cm), elevation (m), geographic coordinates, and presence/absence of HWA is recorded. A detailed description of the site selection and seed collection protocols for this project is available in Jetton and others (2007).

Provenance Seed Collections

Between 2003 and 2010, Camcore collected seed from 134 mother trees in 19 populations that were well distributed across the range of Carolina hemlock (table 1, figures 2 and 4). Although 11 additional populations have been identified and explored, seed collections have not yet been completed in those locations because of recurrence of poor cone crops. Where collections were conducted, an average of 7 mother trees per population were sampled, ranging from as few as 1 (Upper Whitewater Falls and Whiteside Mountain) to as many as 12 (Cliff Ridge and Hanging Rock). Total seed yield from these 19 populations was 1,515 g (53 oz) (table 1). At an estimated 360 seeds per gram (Barbour and others 2008), this totals more than 500,000 Carolina hemlock seeds placed into conservation. An average of five viable seeds per cone were obtained. Based on an estimated seed potential per cone of 24 (Farjon 1990), seed efficiency in the Carolina hemlock populations sampled was 21 percent (seed efficiency = number of filled seeds/seed potential). Collections represent four of the five plant hardiness zones where the species occurs (table 1; zone 5b not yet sampled).

Table 1. Location, climate, seed collection, and viability data for Carolina hemlock (*Tsuga caroliniana*) provenances sampled for *ex situ* gene conservation.

Provenance	County, State	Lat. (D.d)	Long. (D.d)	Elev. (m)	Plant[a] hardiness zone	Seed collection year	Mother[b] trees (#)	Total seeds (g)	Total[c] seed (#)	Seed[d] viability (%)
Biltmore Estate	Buncombe, NC	35.55	– 82.54	573	7a	2007	6	66.4	24,435	25.0
Bluff Mountain	Ashe, NC	36.39	– 80.25	1,420	6a	2003	8	23.9	8,795	11.3
Caesar's Head	Greenville, SC	35.10	– 82.62	920	7a	2003/2006	7	56.6	20,821	12.5
Carl Sandburg Home	Henderson, NC	35.27	– 82.44	682	7a	2009	6	83.5	30,728	25.5
Carolina Hemlocks Campground	Yancey, NC	35.80	– 82.20	880	6b	2003/2008	11	234.8	86,406	43.7
Cliff Ridge	Unicoi, TN	36.10	– 82.44	550	6b	2006/2008	12	151.1	55,605	29.3
Crabtree	Yancey, NC	35.81	– 82.16	1,170	6b	2003	6	43.8	16,100	11.2
Cradle of Forestry	Transylvania, NC	35.34	– 82.77	990	6b	2003/2008	11	89.2	32,826	26.2
Cripple Creek	Wythe, VA	36.77	– 81.11	740	6a	2006/2008	7	99.8	36,726	3.5
Hanging Rock	Stokes, NC	36.41	– 80.26	480	6b	2003/2009	12	128.8	47,398	1.3
Linville Falls	McDowell, NC	35.94	– 81.92	970	6a	2003	10	247.8	91,190	51.2
Looking Glass Rock	Transylvania, NC	35.30	– 82.79	1,179	7a	2010	8	36.2	13,322	0.5
New River	Montgomery, VA	37.21	– 80.60	556	6b	2009	6	113.6	41,805	5.5
Sinking Creek	Craig, VA	37.34	– 80.36	880	6a	2009	6	38.9	14,315	16.0
Table Rock	Pickens, SC	35.03	– 82.73	992	7a	2003	3	2.8	1,045	16.3
Tallulah Gorge	Rabun, GA	34.73	– 83.39	430	7b	2005	3	27.6	10,157	21.5
Upper Whitewater Falls	Jackson, NC	35.03	– 83.01	790	7a	2009	1	16.5	6,083	2.0
Whiteside Mountain	Jackson, NC	35.08	– 83.13	1,407	6b	2009	1	9.9	3,647	25.0
Wildcat	Watauga, NC	36.20	– 81.52	850	6b	2003	10	43.8	16,100	3.8

[a] Determined using interactive plant hardiness zone map available online: http://planthardiness.ars.usda.gov/PHZMWeb/InteractiveMap.aspx.

[b] Total number of mother trees per provenance from which seed was collected.

[c] Based on an average of 360 seeds/g (Barbour and others 2008).

[d] Based on 30-day Petri dish germination assays conducted at 22 °C, 16:8 L:D, and with two 50-seed replications per provenance.

Figure 4. Andy Whittier (Camcore) collecting seed cones from Carolina hemlock on Looking Glass Mountain, Pisgah National Forest, NC. (Photo courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

For eastern hemlock, Camcore collected seed from 451 mother trees in 60 populations distributed across the species' southern and northern ranges between 2005 and 2012 (table 2, figure 1). Most of these collections occurred in the southern range, where 270 trees in 37 populations were sampled. In the northern range, 181 mother trees in 23 populations have been sampled. Total seed yield across all 60 populations was 5,544 g (196 oz) (figure 5). At an estimated 412 seeds per gram (Barbour and others 2008), 5,544 g equates to more than 2 million seeds conserved. The number of mother trees sampled per population ranged from 1 (Whiteside Mountain) to 24 (Great Smoky Mountains National Park), with an average of 8. Seed efficiency in eastern hemlock was also 21 percent, based on an average of 6 viable seeds per cone and an estimated seed potential of 28 (Farjon 1990). Collections represent 7 of the 10 plant hardiness zones where the species occurs (table 2; zones 3a, 3b, and 4b not yet sampled).

Table 2. Location, climate, seed collection, and viability data for eastern hemlock (*Tsuga canadensis*) provenances sampled for *ex situ* gene conservation.

Provenance	County, State	Range[a]	Lat. (D.d)	Long. (D.d)	Elev. (m)	Plant[b] hardiness zone	Seed collection year	Mother[c] trees (#)	Total seeds (g)	Total[d] seeds (#)	Seed[e] viability (%)
Arbutus Pond	Essex, NY	N	43.97	– 74.23	497	4a	2011	10	118.1	48,657	22.8
Big Walnut Nature Preserve	Putnam, IN	N	39.77	– 86.78	232	5b	2011	5	56.7	23,360	16.0
Bradbury State Park	Cumberland, ME	N	43.89	– 70.18	84	5b	2011	7	178.7	73,624	7.1
Cook Forest State Park	Forest, PA	N	41.32	– 79.18	357	5b	2009/2011	8	317.8	130,917	28.7
Echo Lake	Vilas, WI	N	45.91	– 89.04	525	4a	2010	10	43.6	17,963	37.0
George Washington National Forest	Providence, RI	N	41.93	– 71.75	207	6a	2011	9	24.8	10,218	9.1

Table 2. Location, climate, seed collection, and viability data for eastern hemlock (*Tsuga canadensis*) provenances sampled for *ex situ* gene conservation (continued).

Provenance	County, State	Range[a]	Lat. (D.d)	Long. (D.d)	Elev. (m)	Plant[b] hardiness zone	Seed collection year	Mother[c] trees (#)	Total seeds (g)	Total[d] seeds (#)	Seed[e] viability (%)
Green's Bluff Nature Preserve	Owen, IN	N	39.20	– 86.76	175	6a	2011	3	10.9	4,491	0.0
Hearts Content Recreation Area	Carbon, PA	N	41.71	– 79.24	527	5b	2011	10	196.1	80,793	19.8
Hemlock Bluff Nature Preserve	Jackson, IN	N	38.84	– 86.26	190	6a	2011	5	7.6	3,131	0.5
Hemlock Cliffs	Crawford, IN	N	38.27	– 86.53	192	6a	2011	4	3.8	1,566	2.0
Hickory Run State Park	Carbon, PA	N	41.02	– 75.68	461	5b	2011	10	62.4	25,709	14.0
Hocking Hills State Park	Hocking, OH	N	39.55	– 82.57	324	5b	2011	6	24.1	9,929	17.5
Imp Lake	Gogebic, MI	N	46.22	– 89.07	537	4a	2011	10	49.3	20,312	25.9
Lake Ottawa	Iron, MI	N	46.08	– 88.75	500	4a	2011	8	47.8	19,694	22.5
Massabesic Experimental Forest	York, ME	N	43.56	– 70.64	64	5b	2011	7	279.5	115,154	16.5
Minnewaska State Park	Ulster, NY	N	41.73	– 74.23	426	6a	2011	10	152.2	62,706	14.2
Mohican Forest State Park	Ashland, OH	N	40.59	– 82.30	424	5b	2011	5	4.1	1,689	3.0
Mount Tom State Reservation	Hampden, MA	N	42.26	– 72.61	350	6a	2011	9	257.1	105,925	4.2
Muskellunge Creek	Ashland, WI	N	46.14	– 90.70	483	4a	2010	10	40.7	16,768	46.0
Penobscot Experimental Forest	Penobscot, ME	N	44.85	– 68.62	51	5a	2011	10	263.6	108,603	7.7
Pine Hills Nature Preserve	Parke, IN	N	39.94	– 87.05	176	5b	2011	5	35.9	14,791	0.5
Round Lake	Price, WI	N	45.84	– 90.07	501	4a	2010	10	75.2	30,987	59.0
Sylvania Wilderness	Gogebic, MI	N	46.23	– 89.36	542	4a	2011	10	6.8	2,802	31.0
Anna Ruby Falls	White, GA	S	34.76	– 83.71	708	7b	2010	2	11.3	4,651	13.0
Back Creek	Burke, NC	S	35.83	– 81.86	412	7b	2008	6	50.7	20,884	19.6
Beech Mountain	Avery, NC	S	36.22	– 81.94	987	6a	2006	5	35.4	14,577	12.9
Blowing Springs	Bath, VA	S	38.06	– 79.89	522	6a	2007	3	4.9	2,019	4.5
Braley Pond	Augusta, VA	S	38.28	– 79.29	614	6a	2009	3	7.9	3,255	6.5
Carl Sandburg Home	Henderson, NC	S	35.27	– 82.44	689	7a	2007	5	8.8	3,626	13.5
Carolina Hemlocks Campground	Yancey, NC	S	35.80	– 82.20	880	6b	2008	7	102.1	42,045	22.8
Cave Mountain Lake	Rockbridge, VA	S	37.57	– 79.53	370	7a	2007	3	3.8	1,566	7.0
Chattooga River	Oconee, SC	S	34.81	– 83.30	344	7b	2007	10	116.2	47,874	50.5
Cliff Ridge	Unicoi, TN	S	36.10	– 82.44	522	6b	2006/2008	10	113.7	46,832	21.5
Cradle of Forestry	Transylvania, NC	S	35.34	– 82.77	990	6b	2008	10	144.6	59,588	23.0
DuPont State Forest	Transylvania, NC	S	35.21	– 82.58	820	7a	2006/2007	10	19.1	7,869	11.4
Frozen Head State Park	Morgan, TN	S	36.14	– 84.47	557	6a	2008/2012	15	90.2	37,162	44.3
Great Smoky Mountains National Park	Blount, TN	S	35.61	– 83.93	427	6a	2008	24	1399.5	576,578	56.7
Guest River Gorge	Wise, VA	S	36.92	– 82.45	606	6b	2009	3	3.8	1,578	22.5
Helton Creek	Rabun, GA	S	34.75	– 83.89	731	6b	2007	4	54.1	22,297	21.5
Hemlock Bluffs Nature Preserve	Wake, NC	S	35.72	– 78.78	89	7b	2009	3	10.5	4,334	5.5
Hidden Valley	Bath, VA	S	38.15	– 79.76	580	6a	2007	4	2.8	1,154	5.5
Hone Quarry	Rockingham, VA	S	38.45	– 79.13	560	6a	2006/2007	7	4.9	2,019	14.0
James River State Park	Buckingham, VA	S	37.63	– 78.80	179	7a	2008	4	15.7	6,464	7.0
Jones Gap State Park	Greenville, SC	S	35.12	– 82.58	449	7b	2007/2009	6	27.9	11,495	14.0
Kentland Farm	Montgomery, VA	S	37.21	– 80.60	561	6b	2009	4	43.5	17,906	2.0
Lake Toxaway	Transylvania, NC	S	35.12	– 82.95	922	7a	2009	5	142.6	58,751	47.5
Laurel Snow	Rhea, TN	S	35.55	– 85.03	116	6b	2012	10	63.4	26,121	NA
Mountain Lake Conservancy	Giles, VA	S	37.35	– 80.53	1,192	5b	2009	10	62.8	25,874	6.0
Natural Bridge State Park	Powell, KY	S	37.77	– 83.68	336	6b	2008	10	215.8	88,905	29.8
North Creek	Botetourt, VA	S	37.54	– 79.58	354	7a	2006	10	22.8	9,394	18.1
Pickett State Park	Fentress, TN	S	36.36	– 84.48	476	6b	2012	13	13.9	5,706	NA
Pine Mountain State Park	Bell, KY	S	36.73	– 83.73	457	6b	2008	7	103.8	42,749	45.7
Pooles Creek	Rutherford, NC	S	35.41	– 82.23	403	7a	2010	5	8.0	3,284	47.0
Prentice Cooper State Forest	Marion, TN	S	35.13	– 85.42	534	7a	2007	3	11.4	4,697	7.0
Quantico	Stafford, VA	S	38.48	– 77.43	18	7a	2006/2008	10	77.7	32,017	18.6
South Mountains State Park	Burke, NC	S	35.60	– 81.63	411	7a	2007	10	98.6	40,623	45.5
Stone Mountain State Park	Wilkes, NC	S	36.39	– 81.02	536	6b	2006/2007	7	14.1	5,809	36.8
Tallulah Gorge State Park	Rabun, GA	S	34.73	– 83.39	435	7b	2005	14	129.2	53,230	50.6
Todd Lake	Augusta, VA	S	38.36	– 79.20	601	6a	2006/2007	7	50.7	20,905	13.8
Whiteside Mountain	Jackson, NC	S	35.08	– 83.13	1,441	6b	2009	1	1.5	610	0.0

[a] Range: N = Northern. S = Southern.

[b] Determined using interactive plant hardiness zone map available online: http://planthardiness.ars.usda.gov/PHZMWeb/InteractiveMap.aspx.

[c] Total number of mother trees per provenance from which seed was collected.

[d] Based on an average of 412 seeds/g (Barbour and others 2008).

[e] Based on 30-day Petri dish germination assays conducted at 22 °C, 16:8 L:D, and with two 50-seed replications per provenance.

NA = germination testing not completed at the time this article was written.

Figure 5. Freshly collected ripe seed cones of eastern hemlock from Minnewaska State Park, NY (left). Seeds of eastern hemlock collected from the Great Smoky Mountains National Park, packaged and ready for cold storage (right). Each packet represents seed from an individual mother tree. (Photos courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

So far, the eastern and Carolina hemlock gene conservation project has collected an estimated 2.5 million seeds. This number is impressive, but it is important to point out that only a small portion of this seed is actually usable to meet the conservation objectives outlined previously. At the end of each seed collection year, Camcore conducts provenance level seed viability tests using Petri dish germination assays conducted at 22°C (71.6 °F), 16:8 hours light:dark, and with two 50-seed replications per population. Based on these tests, average seed viability was low and highly variable for both species; averaging 20 percent (range 0 to 59 percent) and 17 percent (range 0.5 to 44 percent) for the eastern and Carolina hemlock seed reserves, respectively. These germination values are lower than expected for seed from natural stands of these species (reported at 25 to 35 percent by Godman and Lancaster 1990 and Tighe and others 2005) and can be expected to continue decreasing over time in cold storage. This condition highlights the importance of establishing both germplasm reserves in seeds banks and conservation seed orchards to ensure the long-term survival of both hemlock species.

Establishment of Seed Orchards and Reserves

Since 2008, Camcore and its associates have planted more than 2,000 hemlock seedlings into 5 hemlock seed orchards at locations inside and outside of the United States (table 3). The two Carolina hemlock plantings inside the United States are within the native range of the species in North Carolina and were established by Camcore at the North Carolina Department of Agriculture/NC State University Upper Mountain Research Station in Ashe County. Because these two orchards are within the generally infested range of HWA in the southern Appalachian region, they are being monitored regularly for HWA infestation and will be protected with insecticides when necessary.

Three conservation seed orchards were planted outside of the United States in Brazil and Chile (table 3, figure 6). This region was chosen because no native hemlock species are found in South America and chances are low that HWA would ever find its way into the plantings. The particular areas within each country where the hemlocks are planted were chosen based on the results of species habitat distribution modeling software programs using FloraMap™ (Jones and Gladkov 1999) and MaxEnt (Phillips and others 2006). These

Table 3. Location and establishment data for eastern and Carolina hemlock seed orchard conservation banks that were planted inside and outside the United States.

Species	State/department, country	Latitude (D.d)	Longitude (D.d)	Year planted	Provenances (#)	Families (#)	Seedlings (#)
Carolina hemlock	North Carolina, USA	36.41	– 81.31	2008	9	53	400
Carolina hemlock	Bio Bio, Chile	– 37.70	– 73.39	2008	9	56	1,140
Eastern hemlock	Santa Catarina, Brazil	– 26.09	– 50.26	2010	7	25	167
Carolina hemlock	Parana, Brazil	– 26.01	– 50.38	2010	9	37	182
Carolina hemlock	North Carolina, USA	36.40	– 81.32	2012	10	33	315

Figure 6. Ricardo Paim and Laercio Duda with a newly planted eastern hemlock seedling in the conservation seed orchard in Santa Catarina, Brazil (left). Saplings in the Carolina hemlock conservation seed orchard at the North Carolina Department of Agriculture /North Carolina State University Upper Mountain Research Station in Ashe County, NC (right). (Photos courtesy of Camcore, Department of Forestry and Environmental Resources, North Carolina State University)

programs use geographic coordinate and elevational data of known natural populations within the geographic range of a species to predict the most suitable climatic matches in areas outside of the species' natural range. FloraMap™ uses mean monthly precipitation, mean monthly temperature, and mean monthly diurnal temperature for all 12 months of the year (36 variables total). MaxEnt is programed to use a number of different bioclimatic variables found on the WorldClim Global Climate Database (WorldClim 2013). After the programs have determined the climatic conditions across the range of input sites (provenances), they then predict other regions of the world with similar climates where the species has a reasonable probability of occurrence/survival. In the case of eastern and Carolina hemlock, the programs indicated the areas in southern Brazil and south-central Chile where the seed orchards have been planted. The eastern and Carolina hemlock sites in Brazil are being maintained by Rigesa MeadWestvaco, and the Carolina hemlock site in Chile is managed by Arauco Bioforest. Both companies are members of the Camcore program and have donated their time, effort, and land for this gene conservation effort.

Table 4. Summary of eastern and Carolina hemlock seed submissions made to the USDA Agriculture Research Service National Center for Genetic Resource Preservation (Fort Collins, CO) via the USDA Forest Service National Seed Laboratory (Dry Branch, GA).

Species	Year submitted	Provenances (#)	Families (#)	Seeds (g)
Carolina hemlock	2003	3	Bulks	60
Carolina hemlock	2011	13	47	235
Eastern hemlock	2011	19	83	415
Carolina hemlock	2012	3	5	10
Eastern hemlock	2012	25	83	362

Camcore has also established seed reserves of both hemlock species at two germplasm repositories inside the United States (table 4). The first is at the USDA Agricultural Research Service National Center for Genetic Resource Preservation (NCGRP) located in Fort Collins, CO. In total, 1,082 g (38 oz) of seed (777 g [27 oz] and 305 g [11 oz] for eastern and Carolina hemlock, respectively) have been submitted to this facility for long-term preservation. The USDA Forest Service National Seed Laboratory in Dry Branch, GA facilitated this submission. The second repository is the Camcore seed bank at NC State University in Raleigh, NC, where all seed that has not been distributed for seed orchard establishment, submitted to the NCGRP, or used for germination testing currently resides.

Conclusions and Future Objectives

Ongoing work in hemlock genetic resource conservation is focused on two main objectives. The first is the expansion of the seed orchard program to include additional plantings of both species inside and outside the United States. Inside the country, orchards will be established both within the native range of the hemlocks (monitored and insecticide treated when needed) and on USDA Forest Service sites in the Ozark Mountains of Arkansas. With increasing restrictions on the international movement of tree seeds for research and planting, locations for plantings outside the United States are more uncertain, but plans are in place for additional orchards in Brazil and Chile.

The second objective for the ongoing work is additional seed collections. For Carolina hemlock, emphasis will be on the 11 populations in which seed has not yet been collected. Collections of eastern hemlock will focus on locations not yet well represented in seed stocks, including the high-diversity pocket in New York and southern New England and the disjunct populations in Alabama, Kentucky, and Tennessee.

Although much remains to be accomplished, the first 10 years of the Camcore/USDA Forest Service cooperative hemlock gene conservation project has been a success. It has amassed the largest genetic base of eastern and Carolina hemlock that exists outside of natural stands, established strategic seed reserves in seed banks for both species, and initiated the process of conservation seed orchard establishment. These valuable resources will be used to address a variety of research and management objectives related to HWA control, breeding of HWA-resistant genotypes, and restoring devastated hemlock ecosystems in eastern North America.

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