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Review

Ecological and genetic factors that define the natural distribution of Carolina hemlock in the southeastern United States and their role in *ex situ* conservation

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Abstract

Carolina hemlock (*Tsuga caroliniana* Engelm.) is a rare endemic found in the Appalachian Mountains and Upper Piedmont of the southeastern United States. It is being decimated by the hemlock woolly adelgid (*Adelges tsugae* Annand), an exotic pest introduced into the region from Japan several decades ago. We examine ecological, genetic and climatic factors in natural stands that characterize the species' occurrence to better determine where *ex situ* conservation plantings should be established. To facilitate species/site matching, we use FloraMap^{TM} software that quantifies climatic variables at provenance collection sites to predict other areas where Carolina hemlock could be planted in the U.S. and Latin America. Results indicate that based on analysis of 15 populations, Carolina hemlock is found on predominantly sandy-clay loam soils but occurs on a wider range of soil textural groups than previously thought. Its natural occurrence represents two different climatic groups, with the Cradle of Forestry, NC site most different than the rest. FloraMap^{TM} predicts with high probability that Carolina hemlock populations can be successfully moved to central Chile, and with lower probability to the Ozark region of Arkansas and southern Brazil. Camcore, North Carolina State University, has now collected seeds from 12 provenances and 77 mother trees in natural populations of Carolina hemlock and distributed these to the three regions listed above. Our goal is to sample 150 trees from the 15 provenances which will sample most of the species' genetic diversity for *ex situ* conservation. The *ex situ* approach offers an alternate means of protecting the species if efforts to control the adelgid fail in the southeastern US.

Keywords: *Tsuga caroliniana*; Gene conservation; *Adelges tsugae*

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1. Introduction

Carolina hemlock (*Tsuga caroliniana* Engelm.) is a rare conifer that is endemic to the southern Appalachian and upper Piedmont regions of the southeastern United States (Fig. 1). Its geographic range is small, approximately 465 km × 165 km. The tree reaches 20 m height with diameter of 80 cm (Harlow et al., 1996) and occurs in small disjunct populations that are usually located on mountain bluffs and dry ridges but occasionally are found in cool, moist valleys and ravines (Rentch et al., 2000). Although descriptions of Carolina hemlock can be found dating as far back as the early 1900s (Mathews, 1915), it remained relatively unknown until the last two decades when the first thorough ecological studies of the species appeared in the literature (Humphrey, 1989; Rentch et al., 2000). This interest coincides with the rapid population growth and spread of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, an exotic insect from Asia that threatens to eliminate Carolina hemlock in its native range throughout the southern Appalachian region (McClure et al., 2003). Despite the renewed scientific interest, recent accounts of the ecology, biology, and conservation of Carolina hemlock are limited in scope (Harlow et al., 1996; Preston and Braham, 2002).

To design effective gene conservation programs, one must first understand present population structures and the factors that influence them (Eriksson et al., 1993). Protection of threatened Carolina hemlock populations in their native environment (*in situ* conservation) is difficult because HWA infestations are now found in most stands throughout its range.

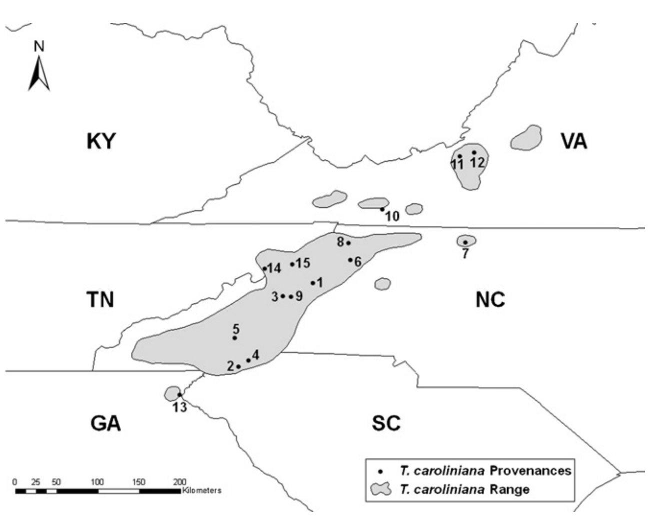

Fig. 1. Carolina hemlock native range in the southeastern United States and provenances visited by Camcore for the collection of seed, soil samples, and foliage for genetic diversity analysis. Numbers correspond to site descriptions in Table 1.

Once infested, it takes only 2–4 years for Carolina hemlock trees to die (McClure et al., 2003). Insecticides are highly effective at controlling HWA (Cowles et al., 2006), but their use is limited to trees of high aesthetic or recreational value in areas of high tourist impact (Ward et al., 2004). Range-wide insecticide treatment across all populations in the five state area where Carolina hemlock occurs is economically impractical and environmentally unsound.

Classical biological control, the release in the eastern U.S. of exotic natural enemies from the native range of HWA, has shown the most promise as a long-term solution to *in situ* conservation of Carolina hemlock. A number of predators and pathogenic fungi of HWA have been identified and shown effective for controlling the adelgid in laboratory and confined-release field studies (Cheah et al., 2004). However, only one of these, the adelgid predator *Sasajiscymnus tsugae* Sasaji & McClure has been released on a large-scale basis in the eastern U.S. and has yet to demonstrate effective HWA control.

A complimentary approach to *in situ* conservation is to plant Carolina hemlock seeds outside the geographic range (*ex situ* conservation) of the species, in areas where HWA does not occur. *Ex situ* conservation programs have been successful for a number of tropical and subtropical tree species that are threatened by fuel-wood harvesting and agricultural expansion in Central America, Mexico and Southeast Asia (Dvorak et al., 1996). However, the efficacy of these *ex situ* conservation programs rests on researchers understanding the ecology, biology and genetics of the species in its native environment so that the ‘‘rescued’’ genetic material can be established in climatic and environmental niches that match those where the species originated. Insufficient knowledge of simple things like how to store seeds after collection, how to grow seedlings in the nursery, and how best to match species to site have caused some *ex situ* conservation efforts to fail.

Often, there is much debate on how many populations and trees to sample in *ex situ* conservation programs to capture a representative number of alleles to protect dwindling gene pools (FAO et al., 2004). However, in the case of Carolina hemlock, with its restricted range and HWA infestation problems both occurring during a period of global climatic change, the answer is to sample as much germplasm as one reasonably can and move it to other locations as quickly as possible. Despite the potential challenges, *ex situ* conservation of Carolina hemlock remains a practical approach to protect gene pools in the event of the worst case scenario where tree populations are destroyed locally because reliable and effective management of HWA has failed in the southeastern US.

In 2003, Camcore (International Tree Conservation & Domestication Program), North Carolina State University, and the U.S. Forest Service began a collaborative effort to conserve populations of Carolina hemlock by moving seeds and establishing field conservation plantings in areas of the U.S. outside its natural range (Ozark Mountains of Arkansas) and to Latin America where HWA is not present. Our paper reviews the current knowledge of the evolutionary history, ecology, and genetics of the Carolina hemlock including the recent experiences gained by Camcore through field reconnaissance in the southeastern US. We provide updated information on the species distribution in the Southeast and better define climatic patterns and soil characteristics where it naturally occurs. We utilize the software model FloraMap™ to predict where *ex situ* plantings should be established based on climatic matches. Based on these findings, we summarize our conservation strategy for Carolina hemlock in the future.

2. The ecology of Carolina hemlock

2.1. Description of the genus

Carolina hemlock belongs to a genus that is among the most shade tolerant and drought susceptible in the Pinaceae (Farjon, 1990). Although as many as 18 individual hemlock species are described by various authors (Krussman, 1985; Gelderen and Smith, 1986), only nine are known to naturally occur while the others are now recognized as subspecies and cultivars or have been placed in sister genera (Farjon, 1990). The world-wide distribution of *Tsuga* species is discontinuous with species concentrated in the temperate regions of North America and eastern Asia. Two concentrations are found in North America. One is in the coastal mountains and islands of the Pacific Northwest extending into the northern Rocky and Sierra Nevada Mountains where *T. heterophylla* Sargent, and *T. mertensiana* (Bong.) Carr. are found. The second is in eastern North America, extending from Nova Scotia south to Alabama and west into Minnesota, where Eastern (*T. canadensis* Carriére) and Carolina hemlock occur, although the latter is restricted to the southeastern US. A third concentration of *Tsuga* is in eastern Asia, with species occurring in Japan, *T. sieboldii* Carriére and *T. diversifolia* Masters, China, *T. chinensis* (Franchet) Pritzel in Diels and *T. forrestii* Downie, and the Himalayan Range, *T. dumosa* (D Don) Eichler (Farjon, 1990). Although HWA is now found in all three regions worldwide where hemlocks occur, it causes widespread tree mortality only in the eastern United States where it is exotic.

Taxonomists place Carolina hemlock, and seven other naturally occurring hemlock species, in the Tsuga (formerly Micropeuce) section (Krussman, 1985; Gelderen and Smith, 1986). The ninth species, *T. mertensiana*, is classified alone in the Hesperopeuce section, a separation based largely on morphological differences among the species and is not universally accepted (Farjon, 1990).

2.2. Distribution

Carolina hemlock is endemic to the southeastern United States (Fig. 1) where it grows in a small number of isolated populations in Virginia, Tennessee, Georgia, and North and South Carolina (Farjon, 1990). Its typical habitat is along exposed ridges of the southern Appalachian Mountains in pure and mixed stands at elevations between 600 and 1500 m, although, it is sometimes found stream-side in moist, cool ravines (Humphrey, 1989). A few remnant populations are also found in the Piedmont of North Carolina and Virginia at elevations between 100 and 600 m (Stevens, 1976).

Based on Camcore field reconnaissance, the Carolina hemlock range extends from 37°40′ N latitude in Rockbridge County, Virginia south to 34°73′ North latitude in Rabun County, Georgia (Table 1). Anecdotal accounts (J.D. Brown, personal communication) suggest that this distributional range has been limited to the south by high summer temperature, historically frequent fires, and the occurrence of cliffs and rock outcroppings on which the species finds a competitive advantage (see Section 2.6). Conversely, its range to the north is likely restricted by lower summer precipitation and less frequent fires that lead to increased hardwood competition.

To date, Camcore's conservation work and research on Carolina hemlock has focused on 15 populations distributed throughout its range (Fig. 1, Table 1). Most of these are of moderate size while one, the Table Rock, South Carolina site where the species was first observed (James, 1959), are quite large. The smallest known population is the Tallulah Gorge, Georgia site at the species southern extreme where only four cone bearing trees have been found.

2.3. Evolution and genetic diversity

The earliest fossil records of species described as Carolina hemlock ancestors are from Oligocene and Miocene sediments in North America, Europe, and Asia that contained *Tsuga* cones, cones scales, seeds, twigs, leaves, and pollen. Fossils of extant Carolina hemlock cones, pollen, and leaves first appear in Pliocene sediments from Europe. These data suggest that the species likely evolved during the Miocene when hemlock diversification was underway and numerous *Tsuga* species begin appearing in the fossil record (LePage, 2003 and references therein). The fossil record also indicates that Carolina hemlock and its ancestors had a much wider range that extended across much of the northern hemisphere in the geologic past. Its current range is likely explained by Pleistocene glaciations that isolated hemlocks in their current refugia in North America and Asia (LePage, 2003).

Early research concluded that Carolina hemlock must be closely related genetically to Eastern hemlock (Szafer, 1949) because the two species' geographic ranges overlap and they occasionally occur sympatrically. However, subsequent biogeographical and morphological studies indicated that Carolina hemlock is more closely related to *Tsuga* species occurring in Asia than to either Eastern hemlock or *Tsuga* species in western North America (Little, 1970; LePage, 2003). These findings are supported by molecular analyses of ribosomal DNA among the members of the genus that identified two clades within *Tsuga*: (1) a Western North American grouping containing *T. heterophylla* and *T. mertensiana* and (2) an Asian grouping containing the five Asian species and Carolina hemlock. Eastern hemlock was supported as a sister group to the Asian

Table 1
Site descriptions for 15 Carolina hemlock provenances visited by Camcore for the collection of seed, soil samples, and foliage for genetic diversity analysis

No.	Provenance name	State	Latitude	Longitude	Elevation (m)	Rainfall (mm)	Seed	No. of trees collected
1	Linville Falls	NC	35.94 N	81.92 W	995	1376	Yes	10
2	Table Rock	SC	35.04 N	82.73 W	956	1651	Yes	3
3	Carolina Hemlocks Campground	NC	35.80 N	83.20 W	823	1340	Yes	10
4	Caesar's Head	SC	35.11 N	82.63 W	933	1797	Yes	4
5	Cradle of Forestry	NC	35.35 N	82.78 W	1017	2039	Yes	8
6	Wildcat	NC	36.20 N	81.52 W	297	1265	Yes	10
7	Hanging Rock	NC	36.39 N	80.27 W	146	1199	Yes	5
8	Bluff Mountain	NC	36.38 N	81.54 W	1375	1299	Yes	8
9	Crabtree	NC	35.80 N	82.16 W	1132	1337	Yes	6
10	Cripple Creek	VA	36.75 N	81.17 W	766	1058	Yes	4
11	Sinking Creek	VA	37.33 N	80.33 W	1009	954	No	n/a
12	Dragon's Tooth	VA	37.37 N	80.17 W	852	994	No	n/a
13	Tallulah Gorge	GA	34.73 N	83.38 W	410	1584	Yes	3
14	Cliff Ridge	TN	36.10 N	82.45 W	671	1228	Yes	6
15	Iron Mountain	TN	36.15 N	82.15 W	1503	1198	No	n/a

Annual rainfall data generated by FloraMap^{TM}. "No. of trees collected" indicates the number of mother trees from which seed was collected at each site.

clade (LePage, 1997; Vining, 1999). Further support is found in the success of *T. caroliniana* x *T. chinensis* hybridization on arboretum specimens that could not be replicated in *T. canadensis* x *T. chinensis* crosses (Bentz et al., 2002; Pooler et al., 2002). One evolutionary scenario to explain the biological and morphological differences between Carolina and Eastern hemlock, in concordance with fossil records, suggests that *T. caroliniana* evolved and migrated into the region millions of years before *T. canadensis* and all that remains of Carolina hemlock are remnant populations of what was once a much more widely distributed ancient race.

Assessment of genetic diversity in Carolina hemlock populations appears to be in agreement with a larger historic distribution. Using amplified fragment length polymorphism (AFLP) molecular markers, Camcore investigated the genetic relationships among *T. caroliniana* populations in the southeastern US. (Fig. 1, Table 1). Despite its restricted occurrence, our results indicate that Carolina hemlock exhibits a moderate amount of genetic variation (Camcore, 2006). At the same time, eastern hemlock, which is found throughout eastern North America, appears to have relatively low genetic variation in the southeastern states (Potter et al., 2008).

Our results also indicate that, among the populations we sampled, Carolina hemlock may have greater genetic diversity towards the southern portion of its range, suggesting a potential Pleistocene glacial refuge in or around those populations located in northwestern South Carolina (Camcore, 2006). However, this refuge may also have been located in the Piedmont regions of North Carolina and Virginia where several small, remnant populations exist today, far removed from the species' typical distribution.

2.4. Soils

Most sources indicate that Carolina hemlock typically occupies and dominates forest stands at sites with dry, coarse sandy to sandy loam soils that are nutrient poor, well-drained, and highly acidic, although, these soil conditions are not exacting for the species (Sargent, 1933; James, 1959; Radford et al., 1968; Farjon, 1990; Harlow et al., 1996; Preston and Braham, 2002). For example, Carolina hemlock dominated stands on Bluff Mountain in North Carolina all have the above characteristics but are described as mesic rather than dry (Humphrey, 1989). Likewise, the species can also occur in moist, well-drained nutrient rich mountain coves and valleys but rarely becomes a forest dominant under these conditions (Farjon, 1990; Rentch et al., 2000). Other anecdotal accounts suggest that Carolina hemlock is restricted to rock outcroppings, talus slopes, and dry, rocky ridges and does not tolerate saturated soils, but that abundant soil moisture is not limiting once seedlings become established (J.D. Brown, personal communication).

Given that there is some variability in the type of site on which Carolina hemlock can successfully grow, Camcore has quantitatively described the soil texture and nutrient conditions expected in a typical Carolina hemlock stand on a range-wide basis by assessing soil samples. Forty-eight soil cores (2–4 cores per site) were taken from the 15 forest stands throughout the southern Appalachian Mountains that support *T. caroliniana* populations (Fig. 1, Table 1). All samples were sent to A&L Analytical Laboratories, Inc. (Memphis, TN) for texture and nutrient analysis. The goal was to develop a set of soil characteristics that can be used to select sites for the establishment of *ex situ* conservation plantings in the Ozark Mountains and Latin America described in the following section of this paper.

The difficulty with which soil cores were collected, due to high concentrations of rocks and rock fragments at most sites, confirms the coarse nature of soils in a typical Carolina hemlock stand. The soil analysis revealed that while some stands do occur on sandy and sandy loam soils, the majority of sites sampled in this study had sandy clay loam textures (Fig. 2). It appears also that Carolina hemlock is more broadly adapted to a wide range of texture classes than previously reported and can survive on soils that are very sandy or clayey but does not typically occur on sites with high silt content.

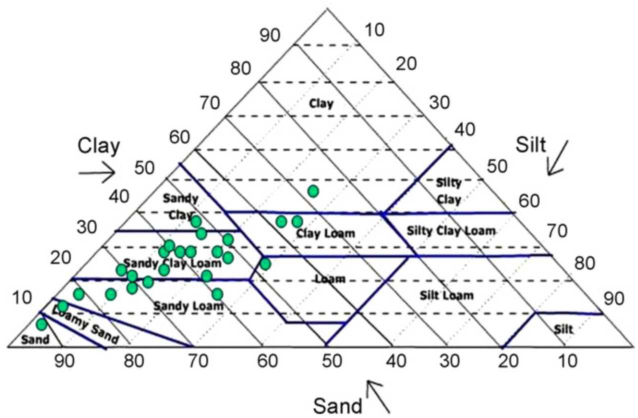

Fig. 2. Soil texture classes for soil cores collected from 15 Carolina hemlock populations in the southeastern United States. Data represents a subset of 24 samples from a total of 48 mineral soil samples collected.

The soil nutrient analysis indicates that descriptions of Carolina hemlock growing on acidic, nutrient poor soils are largely accurate. We have found that the soils have an average pH of 4.27 ($\pm$0.05); have low concentrations of phosphorus (17.66 $\pm$ 3.14 kg/ha), potassium (118.14 $\pm$ 7.47 kg/ha), calcium (302.76 $\pm$ 50.05 kg/ha), magnesium (80.80 $\pm$ 15.45 kg/ha), copper (3.01 $\pm$ 0.22 kg/ha), and sodium (41.49 $\pm$ 0.71 kg/ha); moderate concentrations of manganese (58.33 $\pm$ 14.02 kg/ha) and zinc (4.75 $\pm$ 0.53 kg/ha); high concentrations of iron (591.05 $\pm$ 48.91 kg/ha); and optimum levels of boron (1.77 $\pm$ 0.12 kg/ha) and sulfur (77.37 $\pm$ 4.72 kg/ha). The high average soil iron content is the result of above normal readings (900–1000 kg/ha) for this element in the soils at three sites; Carolina Hemlocks Campground, Bluff Mountain, and Hanging Rock. All other sites had iron concentrations in the optimum range (200–500 kg/ha).

2.5. Reproductive biology

The reproductive biology of Carolina hemlock has not been studied in detail, therefore, the following paragraph describes reproduction for *Tsuga* species in general, and it should be recognized that individual species are likely to demonstrate slight variations in these characteristics. Hemlocks are monoecious, male and female flowers develop on the same branches and are common throughout the outer crown in good cone years, and have a two-year reproductive cycle (Ruth, 1974). Reproductive buds are formed late in the growing season of year 1. Male strobili are solitary, arising from axillary buds on shoots in spring of the second year. Female strobili are also solitary on second year shoots but arise from terminal buds (Farjon, 1990). Flowering occurs April though June of each year, depending on elevation, and fertilization of the ovules lasts for approximately 6 weeks (Ruth, 1974). The cones ripen 4 months later, progressing from green to yellowish-green to purplish-brown when cone scales open and seeds are shed between mid-August and mid-September (Farjon, 1990). Carolina hemlock cones are the second largest in the genus (*T. mertensiana* having cones that are only slightly larger) and its seeds are the largest among all *Tsuga* species (Ruth, 1974).

Based on our experience, cone production in natural stands of Carolina hemlock begins when trees are between 20 and 30 years of age. However, the exact age of first flowering depends on light availability in the crown (Ruth, 1974). Most healthy trees produce some cones each year, although, heavy cone crops are sporadic, affected by local weather patterns, and highly variable among provenances (Farjon, 1990). Seed viability for *T. canadensis* is reported at 25 to 30% filled seed per cone (Godman and Lancaster, 1990) and similar rates have been reported for Carolina hemlock in germination tests conducted by Camcore (Tighe et al., 2005).

2.6. Ecology and associated species

The distribution and ecology of Carolina hemlock is defined by its adaptation to a stress-tolerant life history strategy (Humphrey, 1989; Rentch et al., 2000). It possesses many characteristics that allow it to survive and thrive in multiple types of marginal habitats (Grime, 1977). Along nutrient poor, dry, exposed bluffs and ridges its slow growth and long-lived foliage reduce demand for and turnover of nutrients to resource limited soils, and its small, evergreen leaves protect it against desiccating wind (Grime, 1977; Humphrey, 1989). Likewise, these same characteristics allow it to survive in the dense shade of the mixed deciduous forest where soil resources are more abundant but light is limiting. Its retention of multiple leaf cohorts and lack of flowering in shaded environments reduces the need for above ground resource use (Grime, 1977), allowing Carolina hemlock to direct resources to the production of root biomass. Well established root systems allow the species to react quickly when light availability increases in new canopy openings. Additionally, its broad, domed canopy growth form ensures maximum light interception in the shade and a competitive advantage over other under-story species when light becomes available (Farjon, 1990).

Because of its slow growth and tolerance to nutrient, water, and light stress, Carolina hemlock will come to dominate late successional stands in both the ridge top and mixed deciduous forest habitats. Carolina hemlock dominated stands are characteristically low in species diversity, with under-stories dominated by evergreen shrubs that suppress the regeneration of oaks and other broad leaf species but not shade tolerant *T. caroliniana* seedlings and saplings. The species also has a tendency to modify the environment under its own canopy through dense shade and the creation of highly acidic soil conditions that limit the recruitment of early successional species into the stand. These characteristics allow Carolina hemlock to sustain itself on a site until the next large-scale natural or man-made disturbance (Humphrey, 1989).

Carolina hemlock can sometimes be found growing in the riparian zones and rich soils along mountain streams where it is associated with Eastern hemlock (Farjon, 1990). Such occurrences are considered off-site for Carolina hemlock and the accompanying plant communities more closely resemble those typically associated with *T. canadensis* (Rentch et al., 2000). In its natural habitat of high mountain ridges or the mixed deciduous forest, it grows alongside *Pinus strobus* L., *Quercus montana* Willdenow, *Q. alba* L., *Q. coccinea* Muenchh., *Nyssa sylvatica* March., *Liriodendron tulipifera* L., and *Acer rubrum* L. with under-stories dominated by ericaceous shrubs such as *Kalmia latifolia* L., *Rhododendron catawbiense* Michaux, and *R. minus* Michaux (Humphrey, 1989; Farjon, 1990; Taylor, 1993).

2.7. Economic uses

Although the wood of Carolina hemlock is of moderate quality, it is suitable for construction and its bark is rich in tannins that can be used to cure leather (Taylor, 1993). Most likely it was used with Eastern hemlock in the construction of barns that can still be seen in the Appalachian Mountains today. However, due to its rarity and the isolation of most natural stands, it has seldom been harvested (Farjon, 1990; Taylor, 1993). It has been planted widely as an ornamental and for hedgerows and a number of attractive cultivars have been developed (Swartley, 1984).

3. The *ex situ* conservation of Carolina hemlock

The goal of the collaborative effort between Camcore and the U.S. Forest Service is to sample as many populations of Carolina hemlock as possible, distribute the seeds to areas of the world where HWA does not occur, and establish *ex situ* conservation areas for the long-term protection of the species. There are two challenges to the success of this effort. The first is to collect a sufficient number of seeds from a reasonable number of trees and populations before they succumb to HWA attack. The second is to understand the climatic variables and soil conditions that determine the current distribution of the species in its native range, and to match these characteristics to areas of the world where the species will have a reasonable probability of survival in conservation plantings. The following sections describe Camcore's efforts to address these challenges.

3.1. Provenance seed collections

Previous conservation efforts by Camcore with the Central American and Mexican pines indicate that a sample size of 6 to 8 populations from throughout the geographic range of the species and 10–20 trees per population will often conserve most alleles at frequencies of 5% or greater for those species with moderate levels of genetic diversity (Dvorak et al., 1999). Our initial strategy for Carolina hemlock was to sample 10 trees per population from as many populations as possible. This sample size takes into account the physical structure of Carolina hemlock populations (often few trees at low density), poor seed years, the species' limited natural range and moderate levels of genetic diversity (Camcore, 2006), and the continued advance of HWA infestations. Using this approach, Camcore has visited 15 Carolina hemlock provenances in Georgia, Tennessee, Virginia, and North and South Carolina (Fig. 1; Table 1). Seed collections have been made from 12 provenances and 77 mother trees since the first collections of the species in 2003 (Table 1). Each tree sampled is tagged and its geographic coordinates recorded, maintaining a distance of 50–100 m between selected trees. Because hemlock seed is dispersed within a distance equivalent to tree height (Godman and Lancaster, 1990), preserving this buffer reduces the chance of high levels of relatedness among mother trees (i.e. at a distance of 50 m we are unlikely to sample seed from a mother tree and one of its offspring).

At this stage, our gene conservation efforts for Carolina hemlock have been hampered by difficulties in finding 10 cone bearing trees per population due to the cyclic and unpredictable nature of hemlock cone production (Table 1). Our current strategy is to return to these past collection sites each year in order to increase the number of mother trees sampled in each population, although, we do continue exploration for new populations throughout the range to increase the likelihood of broadening adaptability and capturing the rare alleles within the species.

We are also trying to better understand the factors that influence flowering in Carolina hemlock to improve our ability to predict which populations are likely to have good cone crops each year. There are a number of environmental and physiological factors that interact to control the nature of flowering in trees (Krugman et al., 1974), and, as mentioned previously, these remain relatively unstudied for Carolina hemlock. We examined one of these, annual variation in rainfall, in relation to years reported to have had abundant cone crops for the species. Excellent cone production was reported in eastern Tennessee in 1957 (James, 1959) and throughout western North Carolina in 2003 (Camcore, 2003). Using climate data obtained from the Southeast Regional Climate Center website (http://radar.meas.ncsu.edu/climateinfo/historical/historical.html) for both regions, we discovered that in both 1957 and 2003 the total annual rainfall in the mountains of eastern Tennessee and western North Carolina was at least 250 mm greater than the preceding year. Although drawing any conclusions at this stage is speculative, it is possible that one factor controlling the size and extent of Carolina hemlock cone crops in the southern Appalachian Mountains is the relationship between years with dry to normal rainfall and those with excessive rainfall. However, additional research is needed to more completely understand flowering periodicity in Carolina hemlock.

3.2. Climatic modeling and selection of locations for ex situ conservation

3.2.1. Climatic model

The range of Carolina hemlock falls within a temperate climate zone where rainfall is plentiful throughout the growing season. Atmospheric conditions tend to be humid and cool at the higher elevations in the southern Appalachian Mountains and slightly warmer where the species grows in the Piedmont (Farjon, 1990). Average annual temperature ranges from 0 °C in winter to 20 °C in summer with rainfall in excess of 1000 mm annually. The growing season (frost free period) is approximately 200 days long (Thompson et al., 2006).

While this climate description is sufficient as a general account of weather patterns in a typical Carolina hemlock stand in the southern Appalachian Mountains, it lacks the precision necessary to predict where the species can be planted and expected to survive outside of its native range. To improve precision, we used the FloraMap™ climatic model developed at the International Center for Tropical Agriculture (CIAT) by Jones and Gladkov (1999). Using the geographic coordinates and elevation of each of the 15 Carolina hemlock populations that Camcore has visited (Table 1), FloraMap™ predicts mean monthly precipitation, mean monthly temperature, and mean monthly diurnal temperature at each site by calculating the average value from the five nearest meteorological stations in its database, using a lapse rate correction to adjust temperature by elevation. In total, the program calculates 36 variables (3 variables per month $\times$ 12 months) that can be used to describe climatic patterns and predict species distributions.

We used FloraMap™ to conduct two analyses on these 36 climatic variables for Carolina hemlock. The first was a weighted paired group method (WPGMA) cluster analysis to determine if the 15 Carolina hemlock sites occupy different climates types. Other clustering methods, such as unweighted group average (UPGMA) or weighted centroid (WPGMC), are available in FloraMap™, but all provided results similar to WPGMA for the hemlock populations included in this analysis. The second analysis performed with FloraMap™ was a principle components (PCA) probability analysis to predict areas of the world with a high probability of having climates similar to the 15 Carolina hemlock collection points in the southeastern US, and where the species can be planted and expect to survive for *ex situ* conservation. We conducted separate PCA analyses for the various climatic clusters produced in the WPGMA analysis, weighted the importance of annual precipitation slightly higher (1.10) than monthly temperature (0.90), and limited the analyses to exclude regions with $<$0.40 probability of where Carolina hemlock can be planted for conservation. Camcore has successfully used the FloraMap™ model to improve climatic information for pine species that naturally occur in isolated areas in Central America and Mexico and to predict where they could be planted in other regions of the world (Dvorak et al., 2005, 2007).

3.2.2. Results from the climatic model

FloraMap™ WPGMA analysis indicated that although all 15 Carolina hemlock populations sampled have precipitation well distributed throughout the year, they separate into two main climatic clusters based on rainfall (Table 1). The first includes the Cradle of Forestry, North Carolina site that receives at total of 2040 mm of rain per year, and the second includes the remaining 14 populations which receive an average total of 1305 mm rainfall per year. Based on these results, we determined that the PCA probability analysis that best describes where Carolina hemlock can be planted outside its range with a high likelihood of survival is one that includes the 14 population cluster and excludes the Cradle of Forestry site due to its atypical total annual rainfall.

FloraMap™ predicted that Carolina hemlock could be planted with a high likelihood of success along the west coast of Washington and Oregon (probability 90%) and in small areas of the Himalayan Mountains (probability 90%). This result is not surprising since other *Tsuga* species occur naturally in these regions. However, HWA is also found in these areas (Havill et al., 2006) and would quickly infest Carolina hemlocks if planted, therefore these regions are not considered appropriate for the establishment of *ex situ* conservation plantings. Likewise, FloraMap™ also predicted a suitable climatic match for the species at similar latitudes in the southern hemisphere (probability 90%), in areas in central Chile near the city of Concepción (Fig. 3). The Ozark Mountains of Arkansas and

Fig. 3. FloraMap™ predictions ($P > 40\%$) of where Carolina hemlock can be grown in South America using climatic data from 14 natural populations in the Southern Appalachian Mountains. Each pixel is approximately 18 km on a side.

small areas in southern Brazil near the city of Lages (Fig. 3) were also indicated by FloraMap™ to be reasonably suitable for growing Carolina hemlock, although with a lower probability (40%) of success. Interestingly, FloraMap™ predicted that Carolina hemlock should occur in large areas of West Virginia, Tennessee, and Kentucky on the Allegheny and Cumberland Plateaus where it is not found today.

The Arkansas Ozarks, central Chile and southern Brazil all have been targeted as prospective locations for the *ex situ* conservation of Carolina hemlock. Results from soil analyses described previously in Section 2.4 of this paper are being used to further refine our site selections in these regions. Seeds have been sent by Camcore to all three locations. The Ozark planting is being managed by the U.S. Forest Service and the University of Arkansas, Fayetteville. The Latin American plantings are being sponsored by private companies that are members of the Camcore program. Seeds were sown in the Chilean nursery in 2005 and the seedlings are healthy and growing well. We are currently in the process of locating sites for their field establishment in 2008. Sponsors in Arkansas and Brazil have received seed and began nursery production in 2007. As trees in these exotic environments develop, we hope that seeds or scion material will one day be available for shipment to the US if ever needed. In the interim, we have sent small samples of some of our collections to the National Seed Laboratory in Fort Collins, Colorado for long term storage (Camcore, 2005).

3.3. The future

Once we move beyond the limitations of poor seed years and phytosanitary restriction in transport of genetic material, there are many other challenges still to be faced for the successful *ex situ* conservation of Carolina hemlock. Silvicultural techniques used for hemlocks in nurseries and ornamental production in the southeastern US will need to be altered for use in operational forestry settings and translated to the new environments. Additionally, we will have to train local foresters who are not familiar with hemlock or the cultural requirements of the species. Patterns of flowering and seed potential are likely to vary in new environment as well and will need to be evaluated.

One of our greatest needs is to develop technology to induce early flowering and seed production in Carolina hemlock to shorten the breeding cycle and make planting stock for restoration efforts available more quickly. These techniques will have utility in *ex situ* field trials and for breeding facilities located in the U.S. Simple techniques like ‘‘top-working’’, a process where vegetative material from young trees is grafted into the crowns of older individuals, might be effective to promote early flowering and seed production in *ex situ* plantings like it does for *Pinus taeda* (Bramlett and Burris, 1998). Likewise, the use of plant hormone (gibberellins) treatments, in combination with light or water stress, that have been used with success in accelerated *T. heterophylla* breeding (Brix and Portlock, 1982; Pollard and Portlock, 1984; Owens and Colangeli, 1989), might also promote flowering on young potted Carolina hemlock seedlings in breeding greenhouses located in the U.S. The small amounts of seed produced in these operations could be sown and seedlings multiplied by rooted cuttings (Jetton et al., 2005) to make planting stock available for reforestation efforts. In the event that HWA management remains elusive, these indoor breeding facilities could also be used to produce adelgid resistant hemlocks through full-sib crosses and inter-specific hybridization. There are numerous hemlock species believe to be putatively resistant to HWA, and at least one of these has been successfully crossed with Carolina hemlock (Bentz et al., 2002). Additionally, there is evidence that some Carolina hemlocks harbor tolerance to HWA, and that this trait may vary among provenances (Camcore, 2006). Currently, Camcore is planting small field trials in HWA infested areas in the Southeast to determine if these resistance traits can be confirmed and, if so, the level of genetic control under which they operate.

4. Conclusions

Had forest resource managers had the opportunity 120 years ago to establish an *ex situ* conservation program during the demise of the American chestnut (*Castanea dentata*), we possibly would have a much larger genetic base for resistance breeding and be further along in the re-establishment of native chestnut populations throughout the Appalachian region. We face a similar dilemma with the Carolina hemlock in the southern Appalachian Mountains where the hemlock woolly adelgid might cause its extinction. Global climate change most likely will exacerbate this situation as the amount of suitable hemlock habitat is forecast to decline by as much as 40 percent (Iverson et al., 1999; Iverson and Prasad, 2002). Successful *ex situ* gene conservation depends on a coordinated plan to move germplasm quickly to protected locations and sound knowledge on how to silviculturally and genetically manage gene pools. The more time, effort, and resources dedicated to the study of Carolina hemlock ecology, genetics and silviculture in the Southern Appalachian region, the greater are our chances for success.

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