The *Ex Situ* Conservation of Carolina Hemlock

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ABSTRACT

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

KEYWORDS

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

INTRODUCTION

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

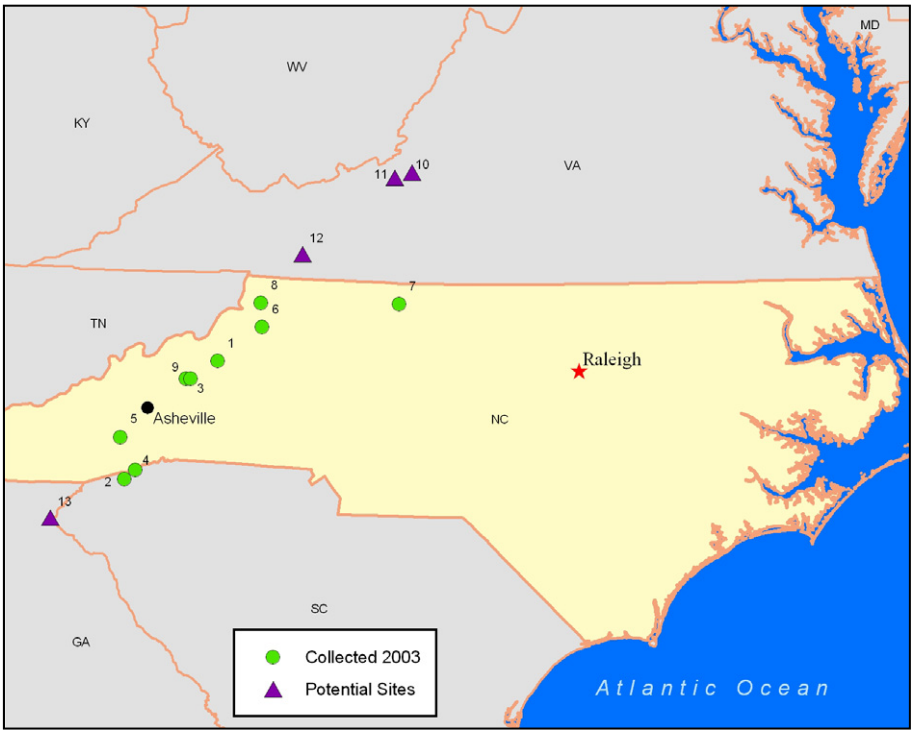

Figure 1. Carolina hemlock collection sites.

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

EXPERIMENT 1. STRATIFICATION/GERMINATION

Most references for stratification and germination methods for hemlock are based on experiments with *T. canadensis* (Godman and Lancaster 1990, U.S. Forest Service 1948), *T. mertensiana* (Ruth 1974, U.S. Forest Service, 1948) and *T. heterophylla* (Edwards 1995, U.S. Forest Service 1948, Packee 1990). The only reference to Carolina hemlock seed stratification

found for this study suggests that treating seed for 30 days @ 4°C on a moist paper towel, then sowing on a 1:1 coarse sand-potting soil medium, and covering pots with plastic film improves germination (Brown 2002). Recommendations from the past research listed above guided us in the development of seed treatments for Carolina hemlock.

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

Replication 1—8 packets

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

Replication 2—8 packets

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

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

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

EXPERIMENT 2. FUMIGATION

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

tion treatments required for shipment to Latin America and to evaluate their effect on seed germination.

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

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

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

FLORAMAP® TOOL AND SITE SELECTION PROCESS

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

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

RESULTS AND DISCUSSIONS

STRATIFICATION AND GERMINATION EXPERIMENT

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

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

FUMIGATION EXPERIMENT

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

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

FLORAMAP® SITE MATCHING

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

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

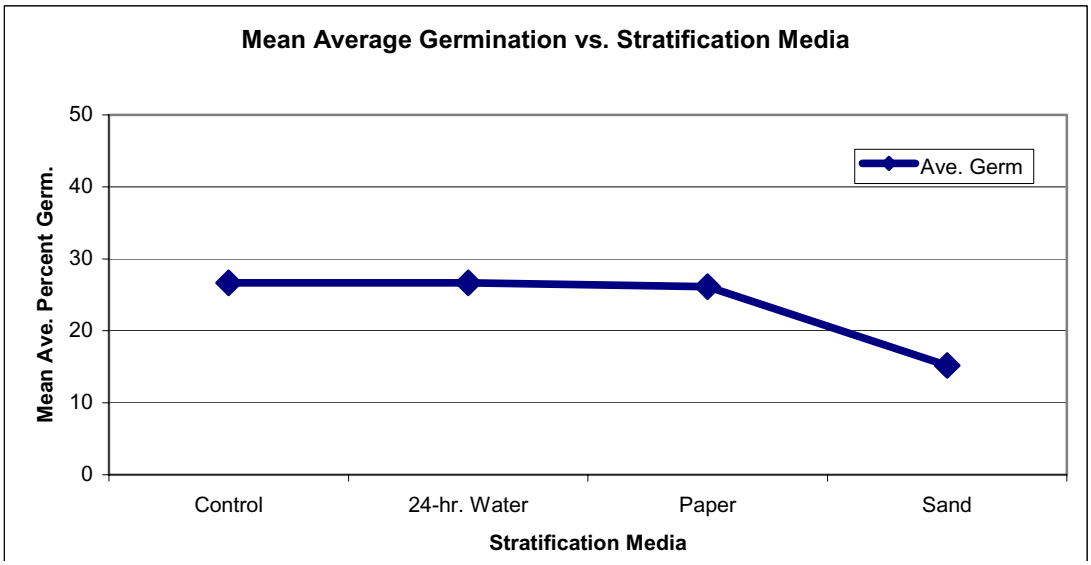

Figure 2. Assessment of stratification media for germination.

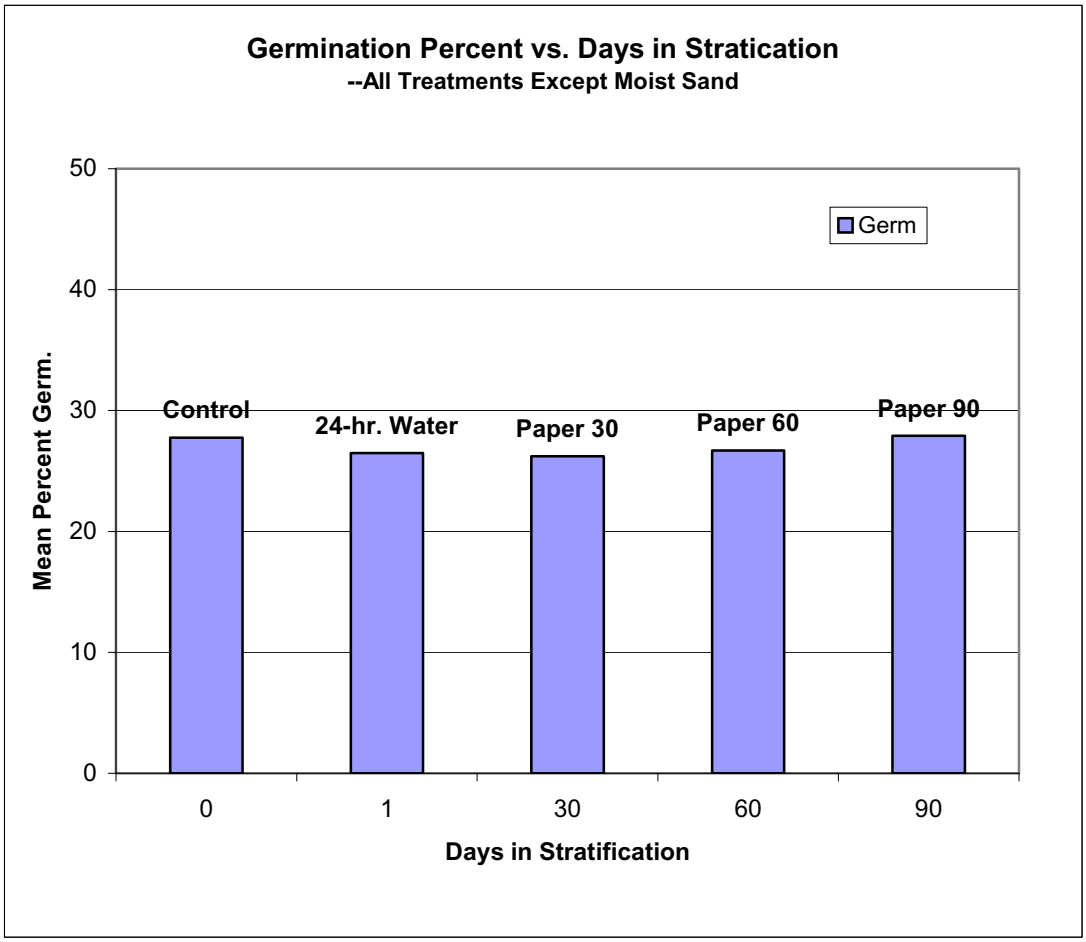

Figure 3. Comparison of duration of stratification time, excluding sand.

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

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

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

EX SITU CONSERVATION EFFORTS

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

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

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Preserving Eastern Hemlock Gene Pools through *Ex Situ* Plantings

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ABSTRACT

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

KEYWORDS

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