Status of *Ex Situ* Conservation Efforts for Carolina and Eastern Hemlock in the Southeastern United States

Robert M. Jetton[1], W. Andrew Whittier[1], William S. Dvorak[1], and Kevin M. Potter[2]

[1]Camcore, Department of Forestry & Environmental Resources
North Carolina State University, Raleigh, NC 27695

[2]USDA Forest Service, Forest Health Monitoring Program
Department of Forestry & Environmental Resources
North Carolina State University, Research Triangle Park, NC 27709

ABSTRACT

Carolina hemlock (*Tsuga caroliniana* Engelmann) and eastern hemlock (*T. canadensis* Carriére) are threatened across their natural range in the southeastern United States by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand. Chemical and biological controls have much promise for limiting the spread and damage done by this exotic pest but are not yet effective on a widespread basis. Alternate conservation strategies are necessary to protect the dwindling gene pools of these import conifer species until such a time that adelgid management tools are available. Since 2003, Camcore (at North Carolina State University) and the USDA Forest Service have been collaborating to collect seeds from populations of both Carolina and eastern hemlock throughout the southern U.S. These seeds have been placed in cold storage or have been germinated to establish *ex situ* conservation plantings in Latin America and the Ozark Mountains of Arkansas. The goal is to conserve the genetic resource of each species in perpetuity until hemlock restoration is possible. To date, seeds have been sampled from 13 populations and 84 mother trees of Carolina hemlock and 18 populations and 110 mother trees of eastern hemlock. Here we discuss results of genetic diversity studies with both species, our conservation strategy, and progress on seed collections and *ex situ* conservation planting establishment.

KEYWORDS

gene conservation, *Tsuga caroliniana*, *Tsuga canadensis*, *Adelges tsugae*

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an exotic insect pest of Carolina hemlock (*T. caroliniana* Engelmann) and eastern hemlock (*Tsuga canadensis* Carriére) that threatens to eliminate these important tree species from their geographic ranges in the eastern United States (McClure et al. 2001). Efforts to manage HWA populations silviculturally, chemically, and via the introduction of natural enemies from the adelgid's native range (biological control) are ongoing and show much promise (Cheah et al. 2004; Ward et al. 2004, Cowles et al. 2006). However, considering the speed with which the adelgid spreads and kills trees, it is likely that some hemlock populations will be severely depleted or completely lost before widespread adelgid control becomes a reality. *Ex situ* conservation, the movement of germplasm (seeds) from its place of origin to other more protected areas, is one viable option for conserving these populations that face possible extinction.

Carolina hemlock is a rare conifer endemic to southern Appalachian Mountains. It is found in a relatively small number of isolated populations in Virginia, Tennessee, Georgia, and North and South Carolina, where it is typically found growing on mountain bluffs or exposed ridges between 600 and 1500 meters elevation (Farjon 1990). This species is most often described as occupying dry, coarse, nutrient-poor soils, although field research by Camcore has revealed that the species is much more broadly adapted to a greater variety of site types than originally thought (Jetton et al. 2008). Eastern hemlock has a much larger range, extending from Nova Scotia south to northern Georgia and as far west as northern Minnesota, with isolated populations occurring in Indiana, central Kentucky, and northern Alabama (Farjon 1990). It is typically a riparian species occupying moist, acidic soils and grows from sea level to about 730 meters elevation (Godman and Lancaster 1990).

The rapid progression of HWA makes urgent the need to protect dwindling genetic resources of both hemlock species. To that end, in 2003 Camcore (International Tree Conservation and Domestication, North Carolina State University) and the USDA Forest Service embarked on a joint, three-phase project to move representative seed samples of both Carolina and eastern hemlock to *ex situ* conservation banks in South America and the Ozark Mountains, where HWA is not present. The first phase, conducted from 2003 to 2006 with seed collections and the establishment of field *ex situ* conservation banks for Carolina hemlock, has been previously documented (Tighe et al. 2005; Jetton et al. 2008). The second phase began in 2005 and is a four-year project to conduct seed collections from eastern hemlock throughout its range in the southern U.S. and establish *ex situ* conservation banks. Phase 2 is currently ongoing. The third phase will sample seed from eastern hemlock populations in the northern and midwestern portions of the species' range and is tentatively set to begin in 2009. This effort will ensure that the gene pools of these important conifer species live on in perpetuity and that seeds will be available to repopulate lost hemlock stands once effective HWA management is available. These plantings will also provide a breeding population for adelgid resistant hybrids should HWA control remain elusive. This article reports on our progress with Phases 1 and 2 of the conservation effort.

HEMLOCK GENETIC DIVERSITY AND CONSERVATION STRATEGY

The goal of any *ex situ* conservation program is, by collecteding seed from an appropriate number of trees and populations, to capture a representative number of alleles to protect dwindling gene pools (FAO et al. 2004). To do this, one must understand the population genetic structure and the environmental factors that influence genetic diversity (Eriksson et al. 1993). Researchers at Camcore and the USDA Forest Service are addressing these population genetics issues for Carolina and eastern hemlocks with several recent and ongoing molecular marker studies.

An amplified fragment length polymorphism (AFLP) molecular marker study of Carolina hemlock indicates that this species has a moderate amount of overall genetic variation and a fairly high amount of genetic differentiation among populations. Additionally, the results suggest that a recent *ex situ* seed collection of nine populations has more than adequately conserved the genetic variation present throughout the range of the species (Camcore 2006).

Population genetic studies of eastern hemlock have focused on the southern portion of the species' range. An allozyme investigation of genetic variation across 20 eastern hemlock populations in the southeastern U.S. suggests that the species has a low level of diversity in the region compared to most other conifers but greater population differentiation (Potter et al., in press). Populations along the eastern periphery and in the Appalachian interior exhibited greater diversity than those to the west of the mountain chain, indicating that the glacial refuge of the species was located east of the southern Appalachian Mountains, the area in which *ex situ* conservation seed collections should be concentrated to capture greater genetic variation.

An ongoing microsatellite marker study using primer combinations recently isolated from both hemlock species should further elucidate the relationships among eastern hemlock populations in the Southeast. Microsatellite molecular markers, also known as simple sequence repeats (SSRs), generally exhibit much higher variability than enzyme markers such as allozymes, and should therefore allow for improved testing of hypotheses about how post-Pleistocene migration and isolation have affected the genetic composition of eastern hemlock populations.

Previous conservation efforts by Camcore with Central American and Mexican pines indicate that, for species with low to moderate levels of genetic diversity, a sample size of six to eight populations throughout a species' geographic range and 10 to 20 trees per population will conserve most alleles with frequencies of 5% or greater (Dvorak et al. 1999). Thus far, our molecular studies with Carolina and eastern hemlocks have revealed that these species have low to moderate genetic diversity in the southern U.S. Based on these results, our conservation strategy for hemlocks is to sample 10 mother trees in 1) as many populations of Carolina hemlock as we can identify and 2) 60 populations of eastern hemlock distributed throughout the seven states in the southern region in which it occurs.

PROVENANCE SEED COLLECTIONS

Carolina Hemlock

Since 2003, Camcore has explored 21 Carolina hemlock populations distributed across the species' range (Figure 1). Due to its rarity and small geographic range, our conservation goal for this species is to sample 10 trees per population in as many populations as we can locate. We have collected seed from 13 provenances yielding a total of 84 mother trees that are represented in the hemlock seed bank at North Carolina State University (Table 1). Collections at three sites in North Carolina (Linville Falls, Carolina Hemlocks Campground, and Wildcat) provided the full complement of 10 mother trees sampled. The Tallulah Gorge collection is also complete as this sample contains three of the four known trees at this site. Despite yearly evaluations at the remaining nine sites where we have made partial seed collections and eight others we continue to explore, we have been unable to locate the additional cone bearing trees necessary to complete the sampling. We surmise this could be due to a number of factors, including but not limited to variation in cone production cycles among trees within populations, lack of proper exposure to sunlight for less dominant trees, or a combination of stress from drought and HWA infestation.

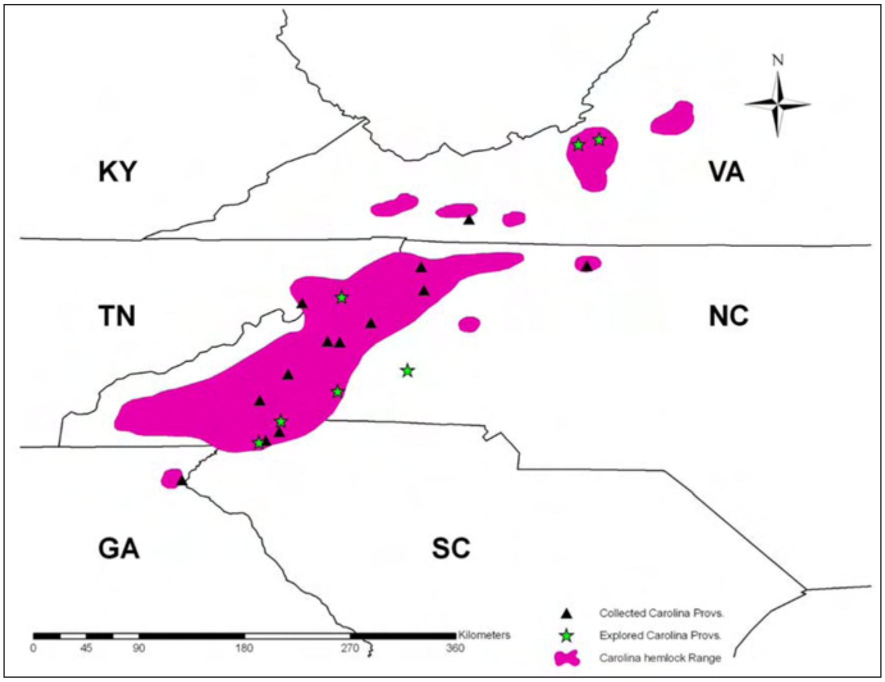

Figure 1. Camcore Carolina hemlock site explorations (stars) and seed collections (triangles), 2003-2007.

Eastern Hemlock

We have had greater success locating cone producing populations of eastern hemlock. Between 2005 and 2007, Camcore explored 56 eastern hemlock provenances across the southeastern U.S. (Figure 2). Seed collections were made in 18 of these, yielding a total of 110 mother trees currently represented in the Camcore seed bank (Table 1). This is a little more than one-sixth our conservation goal of 600 mother trees (60 populations, 10 trees per population) for this species. However, similar to our experience with Carolina hemlock, most eastern hemlock populations have yielded small amounts of seed or no seed at all during collection efforts. Again, we feel this could be due to a number of factors, including within-population cone-cycle variation and lack of exposure to sunlight. In the case of eastern hemlock, we feel that much of the difficulty in finding cone bearing trees is due to severe range-wide drought and HWA-related decline. Field observations suggest that Eastern hemlocks decline much more rapidly and succumb more quickly to adelgid infestation than do Carolina hemlocks, a pattern likely to be magnified under current drought conditions in the southeastern U.S. Among the many symptoms of adelgid infestation that may be more acute in eastern hemlock is the abortion of vegetative and reproductive buds leading to reduced cone production (McClure et al. 2001). Furthermore, we have explored several sites in Kentucky, Tennessee, and Alabama in the western portion of the eastern hemlock range. These sites are experiencing severe drought but are far removed from the adelgid infestation, suggesting that low water availability may also be a significant limiting factor to successful hemlock reproduction.

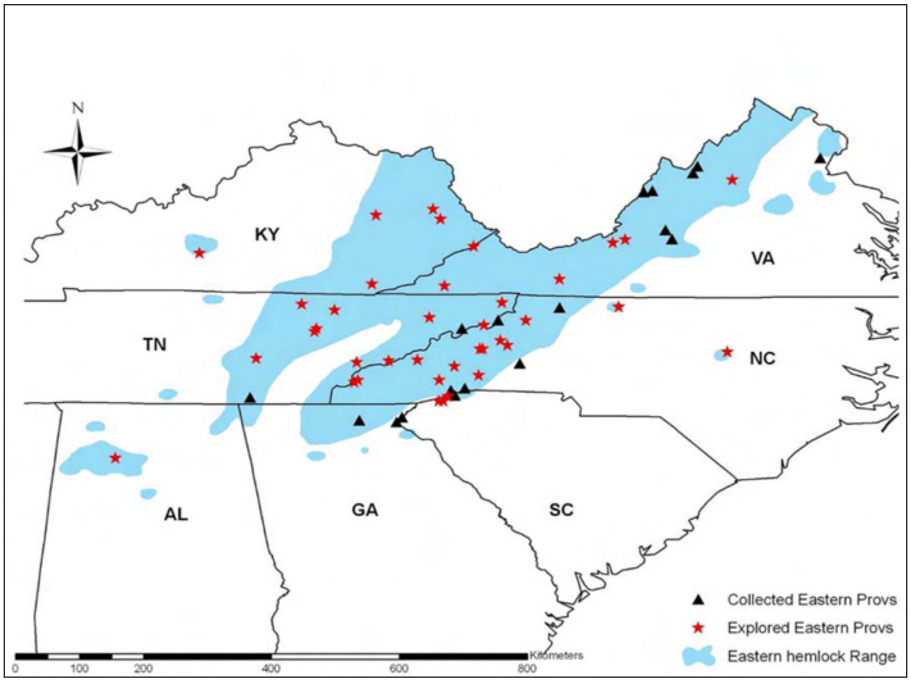

Figure 2. Camcore eastern hemlock site explorations (stars) and seed collections (triangles) 2005–2007.

Table 1. Seed Collections of Carolina and eastern hemlock in the southeastern U.S. by Camcore, 2003–2007.

Prov. #	Provenance	County/State	Latitude & Longitude (Dec. Deg.)	Elevation (m)	Collection Year(s)	# Mother Trees
Carolina Hemlock						
1	Linville Falls	McDowell, NC	35.94 N 81.92 W	995	2003	10
2	Table Rock	Pickens, SC	35.04 N 82.73 W	956	2003	3
3	C. Hemlocks Campground	Yancey, NC	35.80 N 82.20 W	823	2003	10
4	Caesar's Head	Greenville, SC	35.11 N 82.63 W	933	2003, 2006	5
5	Cradle of Forestry	Transylvania, NC	35.35 N 82.78 W	1017	2003	8
6	Wildcat	Watauga, NC	36.20 N 81.52 W	297	2003	10
7	Hanging, Rock	Stokes, NC	36.39 N 80.27 W	146	2003	5
8	Bluff Mtn.	Ashe, NC	36.38 N 81.54 W	1375	2003	8
9	Crabtree	Yancey, NC	35.80 N 82.20 W	1132	2003	6
10	Cripple Creek	Wythe, VA	36.75 N 81.17 W	766	2006	4
13	Tallulah Gorge	Rabun, GA	34.73 N 83.38 W	576	2005	3
14	Cliff Ridge	Unicoi, TN	36.10 N 82.45 W	671	2006	6
21	Biltmore Estate	Buncombe, NC	35.33 N 82.32 W	650	2007	6
Eastern Hemlock						
13	Tallulah Gorge	Rabun, GA	34.73 N 83.38 W	576	2005	13
14	Cliff Ridge	Unicoi, TN	36.10 N 82.45 W	671	2006	2
16	Stone Mtn.	Alleghany, NC	36.38 N 81.02 W	470	2006, 2007	7
17	South Mtn.	Burke, NC	35.59 N 81.60 W	400	2007	10
20	Quantico	Prince Wm., VA	38.53 N 77.38 W	53	2006	7
25	North Creek	Botetourt, VA	37.54 N 79.58 W	345	2006, 2007	10
26	Hone Quarry	Rockingham, VA	38.46 N 79.13 W	600	2006, 2007	7
27	Beech Mtn.	Avery, NC	36.22 N 81.94 W	959	2006	5
33	Helton Creek	Union, GA	34.75 N 83.89 W	727	2007	4
37	Todd Lake	Augusta, GA	38.36 N 79.20 W	612	2006, 2007	5
38	DuPont State Forest	Transylvania, NC	35.18 N 82.60 W	766	2006, 2007	10
39	Blowing Springs	Bath, VA	38.06 N 79.89 W	526	2007	3
40	Hidden Valley	Bath, VA	38.15 N 79.76 W	580	2007	4
41	Cave Mtn. Lake	Rockbridge, VA	37.57 N 79.53 W	371	2007	3
43	Jones Gap	Greenville, SC	35.12 N 82.58 W	470	2007	2
46	Prentice Cooper	Marion, TN	35.13 N 85.42 W	536	2007	3
50	Carl Sandburg Home	Henderson, NC	35.27 N 82.44 W	745	2007	5
51	Chattooga River	Oconee, SC	34.79 N 83.31 W	377	2007	10

FLORAMAP™ CLIMATIC MODEL AND SOIL SAMPLING

Two important factors to consider when selecting sites for the establishment of *ex situ* conservation stands are that 1) areas must have suitable climate and weather for the species of concern, and 2) soil conditions must be suitable to meet the species' resource needs (FAO et al. 2004). To address these issues for hemlock, Camcore is utilizing a computer climate model and range-wide analyses of soil cores from hemlock stands.

We used the FloraMap™ climate model (Jones and Gladkov 1999) to select areas of the world with climates suitable for growing hemlocks. Based on the geographic coordinates and elevations of the hemlock populations in Figure 1, FloraMap™ predicted with high probability (>90%) that Carolina hemlock will survive when planted along the coastal areas of Washington and Oregon, in isolated areas of the Himalayan Mountains, and at similar southern hemisphere latitudes in Chile, from the city of Concepción south towards Valdivia. Predictions of survival in the Pacific Northwest and Asia are not surprising since other hemlock species occur naturally in these regions. However, because adelgids also occur on the hemlocks in these regions (Havill et al. 2006), they are not suitable for hemlock conservation plantings. FloraMap™ also predicted that Carolina hemlock can be grown in the Ozark Mountains of Arkansas and small areas of southern Brazil near the city of Lages, although with a lower probability (40%) of suitable climatic matches. Similar results were found for a model based on eastern hemlock populations in North Carolina and Virginia (Figure 2). Based on this climatic modeling, Camcore has targeted Chile, Brazil, and the Ozark Mountains for *ex situ* conservation of Carolina hemlock. We are cooperating with forest industries in Chile and Brazil, the University of Arkansas, and USDA Forest Service in the Ozarks to grow seedlings and acquire land for planting. Our progress here is outlined in the following section. The methodology and analyses conducted with the FloraMap™ model have been previously documented by Tighe et al. (2005) and Jetton et al. (2008), and readers are referred to these for specific details on model parameters and outputs.

Unfortunately, the FloraMap™ program does not contain a soils component. Therefore, to further refine our site selections within the regions selected by the climate model for hemlock planting, we have conducted a range-wide analysis of soil conditions in eastern and Carolina hemlock stands in the southern U.S. (Jetton et al., in press). We are using this data to pin-point the best possible sites in Chile, Brazil, and the Ozarks for *ex situ* conservation bank establishment.

PROGRESS ON *EX SITU* CONSERVATION BANK ESTABLISHMENT

Carolina Hemlock

Camcore currently has Carolina hemlock seedlings growing at forest nurseries in Chile, Brazil, and at the University of Arkansas. In Chile, Camcore cooperative member Bioforest-Arauco is coordinating the nursery production, field establishment, and subsequent management of the trees for seed production. This planting will represent all Carolina hemlock populations and open-pollinated families (64 families total) collected in 2003 (Provenances 1 – 9; Table 1). Seedlings will be established on a plantation site in the Los Alamos Zone as 3-0 stock in September 2008. In Brazil, Camcore members Klabin SA and Rigesa-MeadWestvaco are coordinating seedling production, establishment, and management. At their Tres Barras nursery facility, Rigesa is growing seedlings for all Carolina hemlock populations and families listed in Table 1 with the exception of the Biltmore Estate provenance. Rigesa will grow the seedlings up to 3-0 stock plants, at which time half of the Carolina hemlocks will be given to Klabin. Both companies will establish *ex situ* conservation banks on plantation sites in Santa Catarina State in 2010.

Dr. Brad Murphy in the Department of Horticulture at the University of Arkansas (Fayetteville) is growing Carolina hemlock seedlings for the Ozarks-based *ex situ* conservation plantings. He has recently germinated seedlings for all populations and families listed in Table 1 (excluding the Biltmore Estate) and should have 3-0 seedlings available for planting in late 2010 or early 2011. These seedlings will be established and managed by the USDA Forest Service at seed orchard sites on the Ozark and Ouachita National Forests.

Eastern Hemlock

Rigesa-MeadWestvaco and Dr. Murphy have agreed to also coordinate *ex situ* conservation of eastern hemlock in Brazil and Arkansas, respectively. Rigesa received seed for all eastern hemlock provenances and families collected in 2005 and 2006 (Table 2) with its Carolina hemlock seed shipment. Seedlings have been germinated and should be ready for field establishment in 2010. Dr. Murphy will receive eastern hemlock seeds from all collections listed in Table 1 in 2008. As with Carolina hemlock, the USDA Forest Service will coordinate the establishment and management of eastern hemlocks in Arkansas.

Camcore member CMPC Forestal Mininco has agreed to coordinate the *ex situ* conservation of eastern hemlock in Chile. Unfortunately, since our initial shipment of Carolina hemlock seeds to Bioforest-Arauco, the Chilean Servicio Agrícola Ganadero – SAG (Ministry of Agriculture) has changed its regulations regarding the shipment of exotic plant seeds into the country. Therefore, we cannot ship seeds to CMPC until they have completed construction and received SAG certification of a new quarantine greenhouse at their Centro Experimental Escuadrón nursery facility. The company has made excellent progress with the greenhouse and it should be ready to receive its their first shipments of eastern hemlock seed in late 2008.

CLOSING REMARKS

Camcore and the USDA Forest Service continue to make good progress with the conservation of Carolina and Eastern hemlocks in the southern U.S. Although we continue explorations and seed collections for Carolina hemlock, our conservation effort with this species is largely complete ,and AFLP analysis indicates that our work has adequately conserved its genetic diversity across the range (Camcore 2006). Our efforts with Carolina hemlock are now focused on conservation bank establishment, breeding, and seed production for eventual reintroduction purposes. With respect to breeding, we have established a small seedling seed orchard of Carolina hemlock in the mountains of North Carolina and plan to establish more of these in the future.

Gene conservation collections for eastern hemlock are still in progress. Through two years of field work we have catalogued approximately one-sixth of our seed goal for the species. Hemlock decline associated with HWA infestation and the severe range-wide drought is having a significant impact on the availability of seeds across the southeast. This has certainly slowed our progress and may effectively eliminate some populations before trees are able to recover and produce seed. One option to increase seed availability may be to apply soil or trunk injected imidacloprid treatments to mother trees we have targeted for seed collection in eastern hemlock populations identified as critical to the conservation effort. Of course, the success of such an effort will depend on the duration of current drought and its effect on tree uptake and mobilization of the insecticide. In the meantime, we will continue to collect what seed is available each year and send that seed to our cooperators for inclusion in *ex situ* conservation plantings.

ACKNOWLEDGEMENTS

James "Rusty" Rhea and Barbara Crane: USDA Forest Service.

Claudio Balocchi, Jaime Zapata, and Pepe Ordoñez: Bioforest-Arauco.

Gleison dos Santos, Mauricio Moreira, and Ivone Fier: Klabin SA.

Ricardo Paím, Laercio Duda, and Gisela Andrejow: Rigesa-MeadWestvaco.

Dr. Brad Murphy and Matthew Pelto: University of Arkansas-Fayetteville.

Carlos Gantz and Marcela Labra: CMPC Forestal Mininco.

Valerie Hipkins, Jennifer DeWoody, and Ricardo Hernandez: National Forest Gel Electrophoresis Laboratory, USDA Forest Service.

Dana Nelson, Sedley Josserand, and Craig Echt: Southern Institute of Forest Genetics, USDA Forest Service.

John Frampton and Jianfeng Li: Christmas Tree Genetics program, North Carolina State University.

Gary Hodge, Juan Luis Lopez, Robert McGee, Michael Tighe, and Willi Woodbridge, Camcore, North Carolina State University.

REFERENCES

Camcore. 2006. Camcore 2006 Annual Report. North Carolina State University, Raleigh, NC. 34 p.

Cheah, C., M.E. Montgomery, S. Salom, B.L. Parker, S. Costa, and M. Skinner. 2004. Biological control of hemlock woolly adelgid. USDA Forest Service, FHTET-2004-04. Morgantown, WV.

Cowles, R.S., M.E. Montgomery, and C.A.S.-J. Cheah. 2006. Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *Journal of Economic Entomology* 99:1258-1267.

Dvorak, W.S., J. Hamrick, and G.R. Hodge. 1999. Assessing the sampling efficiency of *ex situ* gene conservation in natural pine populations in Central America. *Forest Genetics* 6:21-28.

Eriksson, G., G. Namkoong, and J.H. Roberds. 1993. Dynamic gene conservation for uncertain futures. *Forest Ecology and Management* 62:15-37.

Food and Agriculture Organization (FAO) of the United Nations, Forest Genetic Resources (FGR), Forest & Landscape Denmark (FLD), and International Plant Genetic Resources Institute (IPGRI). 2004. Forest Genetic Resources Conservation and Managment, Vol. 3 of Plantations and Genebanks (*ex situ*). International Plant Genetic Resources Institute, Rome, Italy. 86 p.

Farjon, A. 1990. Pinaceae: drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nototsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*. Koeltz Scientific Books, Konigstein, Germany. 330 p.

Godman, R.M., and K. Lancaster. 1990. *Tsuga canadensis* (L.) Carr. Eastern Hemlock. *In*: R.M. Burns and B.H. Honkala (eds.), Silvics of North America Vol. 1. Conifers. USDA Forest Service Agricultural Handbook 654. Washington, DC. 675 p.

Havill, N. P., M.E. Montgomery, G. Yu, and S. Shiyake. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Ann. Entomol. Soc. Am*. 99:196-203.

Jetton, R.M., W.S. Dvorak, and W.A. Whittier. In press (2008). Ecological and genetic factors that define the natural distribution of Carolina hemlock in the southeastern United States and their role in *ex situ* conservation. *Forest Ecology and Management*.

Jones, P.G. and A. Gladkov. 1999. FloraMap™: A computer tool for predicting the distribution of plants and other organisms in the wild. International Center for Tropical Agriculture. Cali, Colombia.

McClure, M.S., S.M. Salom, and K.S. Shields. 2001. Hemlock woolly adelgid. USDA Forest Service, FHTET-2001-03. Morgantown, WV.

Potter, K.M., W.S. Dvorak, B.S. Crane, V.D. Hipkins, R.M. Jetton, W.A Whittier, and R.R. Rhea. In Press (2008). Allozyme variation and recent evolutionary history of eastern hemlock (*Tsuga canadensis*) in the southeastern United States. *New Forests*.

Tighe, M.E., W.S. Dvorak, W.A. Whittier, J.L. Romero, and J.R. Rhea. 2005. The *ex situ* conservation of Carolina hemlock. Pp. 180-190 *in* Onken, B., and R. Reardon (comps.), Third Symposium on Hemlock Woolly Adelgid in the Eastern United States, Asheville, North Carolina, February 1-3, 2005. USDA Forest Service, FHTET-2005-01. Morgantown, WV.

Ward, J.S., M.E. Montgomery, C.A.S.-J. Cheah, B.P. Onken, and R.S. Cowles. 2004. Eastern hemlock forests: guidelines to minimize the impacts of hemlock woolly adelgid. USDA Forest Service. NA-TP-03-04. Morgantown, WV. 27 p.