A Range-Wide Seed Collection to Support the Genetic Resource Conservation of Atlantic White-Cedar

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Abstract

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.) is a wetland tree species native to Atlantic and Gulf coastal regions of the United States and has undergone an 80-percent reduction in its natural distribution during the past 200 years. Reasons for this decline include harvesting, habitat conversion, and stress related to catastrophic wildfires and major hurricanes. Over the past 20 years, growing interest in the preservation and restoration of Atlantic white-cedar ecosystems and the need for genetically diverse planting stock led to the development of a cooperative genetic resource conservation effort for the species between Camcore (an international tree breeding and conservation program in the Department of Forestry and Environmental Resources at North Carolina State University) and the U.S. Department of Agriculture (USDA), Forest Service, Southern Region National Forest System and Forest Health Protection. The objective of this project was to target seed collections across the entire geographic range of the species from Maine south to Florida and west to Mississippi that incorporate genetic material representative of four seed zones defined for the species. Between 2012 and 2016, collections were made from 255 mother trees in 33 populations with a total yield of 1,049,648 seeds. Seeds were distributed to the USDA Agricultural Research Service–National Center for Genetic Resources Preservation for long-term storage, the USDA Forest Service Ashe Nursery Facility for seed orchard and restoration activities, and the Camcore Seed Bank for research and field plantings. Collectively, the seed stored at these three facilities represents the largest genetic resource for Atlantic white-cedar that is known to exist outside of remnant natural stands.

Introduction

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.; hereafter referred to as AWC), a member of the cypress family (Cupressaceae), is a tidal forested wetland tree species that grows in small, dense stands along the margins of freshwater swamps and bogs. The species distribution is a narrow, 80- to 210-km (50- to 130-mile) wide coastal belt that extends from southern Maine south to northern Florida and west along the Gulf Coast into southern Mississippi (figure 1). Given its coastal distribution, the species typically occurs at elevations from 0 to 43 m (0 to 140 ft) above sea level, although it is occasionally found in upland bogs at elevations as high as 457 m (1,500 ft), most notably at High Point State Park in New Jersey (figure 2). Soils where the species occurs are mucky peats in the Spodosols and Histosols orders and can be as deep as 12 m (40 ft). AWC grows characteristically in pure stands (figure 3), but can also be found growing

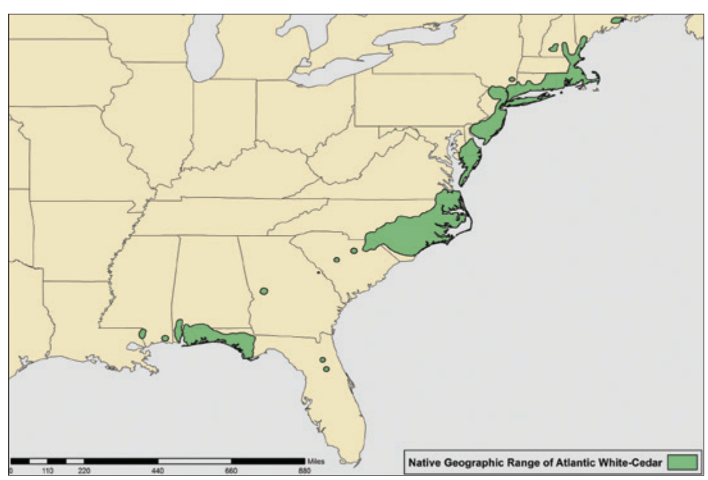

Figure 1. The geographic distribution of Atlantic white-cedar in the eastern United States.

Figure 2. Growing at an elevation above 457 m (1,500 ft), this mature Atlantic white-cedar stand in the Kuser Natural Area at High Point State Forest in New Jersey is the highest known elevation population of the species. (Photo courtesy of Camcore, North Carolina State University)

mixed with other tree species including pitch pine (*Pinus rigida* Mill.), slash pine (*P. elliottii* Engelm.), pond pine (*P. serotina* Michx.), eastern white pine (*P. strobus* L.), eastern hemlock (*Tsuga canadensis* [L.] Carr), baldcypress (*Taxodium distichum* [L.] Rich.), water tupelo (Nyssa aquatica L.), swamp tupelo/black gum (N. sylvatica Marsh.), red maple (*Acer rubrum* L.), and yellow birch (*Betula alleghaniensis* Britton) (Little and Garrett 1990).

AWC swamps are recognized as ecologically significant due to the ecosystem services they provide, particularly with respect to their role in hydrological processes (Kuser and Zimmermann 1995). AWC swamps stabilize stream flows, store flood waters, help to mitigate the effects of drought, and filter and purify water as it flows through them. They are also home to a great diversity of plant, mammal, amphibian, and bird species, many of them rare and/or threatened and some that are obligates to AWC habitats.

Prior to European settlement, there were an estimated 202,343 ha (500,000 ac) of AWC swamps and wetlands in the eastern United States, but today only about 40,469 ha (100,000 ac) remain (Kuser and Zimmermann 1995). The primary cause of this decline was over-harvesting. Across much of its distribution, AWC was historically of minor importance because the scarcity of suitable habitat made distribution of the species within its narrow range exceedingly patchy. It became an important commercial species during the 19th and early 20th centuries in areas where it was more widespread, such as eastern North Carolina, southeastern New Jersey, and the western Florida panhandle, where it was heavily harvested for its lightweight, decay-resistant wood (Laderman 1989). Annual harvests yielded up to 44,835 m^3 (19 million board feet) sold for a variety of uses including siding, fencing, decking, lawn furniture, boat planking, and small specialty products like roofing shingles and duck decoys (Ward 1989). Harvesting is not the only culprit behind the decline of AWC. Across most of its distribution, the draining of coastal wetlands for agriculture and the development of desirable coastal areas, catastrophic wildfires (figure 4), and shifting fire regimes that promote hardwood regeneration have all fragmented what remains of the species' natural habitat, contributing substantially to its continued decline (Kuser and Zimmermann 1995). Although listed as a species of least concern (LC) on the IUCN Red List of Threatened Species (Farjon 2013), AWC is considered rare in Georgia, Maryland, Mississippi, New Hampshire, and New York, of special concern in Maine, and extirpated in Pennsylvania (Nesom 2006).

With growing public awareness of the importance of these unique wetland ecosystems, efforts to protect, regenerate, or restore AWC swamps have increased during the past 20 years. Today, a relatively small number of protected and managed AWC wetlands survive within Federal, State, and private land holdings along the Atlantic and Gulf Coasts. The long-term outlook for these areas is mixed. In some areas, seed availability and moisture, light, temperature, and soil substrate conditions are favorable to natural regeneration following stand disturbance. In many instances, however, disturbance is so severe that stand conditions become suboptimal and, when combined with severe browsing from deer and other animals, natural regeneration failures are common. Over the past two decades, research and management activities have shifted to artificial regeneration of AWC ecosystems (Pickens 2009). These activities require that sufficient genetic resources be

Figure 3. This pure stand of Atlantic white-cedar at Appleton Bog Preserve in Maine is the northernmost known population of the species. (Photo courtesy of Camcore, North Carolina State University)

available in the form of seed stores or seed orchards to support nursery production of genetically diverse and broadly adaptable planting stock.

Given AWC's historic decline, its patchiness across its distribution, its exacting site requirements, and growing public awareness of its importance, the species was recognized by the U.S. Department of Agriculture (USDA), Forest Service as a good candidate for genetic resource conservation efforts to support ongoing ecosystem restoration and regeneration efforts. In 2012, a gene conservation effort was initiated as a collaborative effort between Camcore (an international tree breeding and conservation program housed in the Department of Forestry and Environmental Resources at North Carolina State University; NCSU) and the USDA Forest Service Southern Region National Forest System and Forest Health Protection Program. This article summarizes project objectives, seed collection strategy and protocols, and project results following 5 years of field work.

Figure 4. Severe wildfire damage to a population of Atlantic white-cedar at the Great Dismal Swamp National Wildlife Refuge. (Photo courtesy of Camcore, North Carolina State University)

Project Objectives

The overall goal of this project was to obtain a seed collection that is representative of the genetic and adaptive variation present across the range of AWC and to distribute that seed to facilities where it can be utilized for the conservation and restoration of the species. Specific project objectives were to: (1) collect seed from up to 40 populations and 400 mother trees (10 per population) distributed across four seed zones (10 populations and 100 mother trees per zone) that have been defined for the species; (2) place seeds into cold storage at the USDA Forest Service Ashe Nursery Facility in Brooklyn, MS, and the Camcore Seed Bank at NCSU in Raleigh, NC to support the establishment of seed orchards and subsequent restoration activities; and (3) submit 100 to 500 seeds per mother tree to the USDA National Center for Genetic Resources Preservation in Fort Collins, CO, for long-term preservation.

Seed Collection Strategy and Protocol

Locating AWC populations for seed collection was the first step in developing the seed collection strategy. This step was accomplished through survey of the available scientific and technical literature and analysis of species occurrence data available from Federal, State, local, and private land management agencies and conservancies. A total of 65 potential collection sites well distributed across the range of AWC were identified and visually assessed through field explorations. Of those assessed sites, 56 were found to contain intact AWC populations of varying size and became the focus of this gene conservation effort. Presence of trees could not be confirmed at the remaining nine locations.

The next step in the seed collection process was to determine how to distribute collections across the range of AWC to maximize the amount of genetic and adaptive variation captured in the seed collections. Mylecraine et al. (2004) assessed allozyme variation across the AWC range. Their results showed that AWC has an overall moderate level of genetic diversity compared to other conifers, and a weak trend for increasing mean number of alleles per locus and percent polymorphic loci with decreasing latitude (r^2 0.29 and 0.23, respectively), indicating slightly higher levels of genetic diversity towards the southern portion of the species' range. All other diversity measures, however, showed no trends with latitude suggesting that overall genetic diversity in AWC is evenly distributed across the range and that seed collections should be similarly distributed to capture representative variation. Mylecraine et al. (2004) also showed significant genetic clustering within AWC with three distinct clusters (figure 5), one extending along the entire Atlantic coast from Maine to South Carolina, a second within peninsular Florida encompassing AWC occurrences on the Ocala National Forest, and a third that includes populations in Georgia and along the Gulf coasts of Florida, Alabama, and Mississippi. These genetic clusters in effect represent three distinct AWC gene pools that should also be considered when selecting seed collection locations.

Adaptive variation across the AWC geographic range was assessed by developing generalized seed zones using climate data. Seed zones were defined using the ecological niche model FloraMap™ (Jones and Gladkov 1999) and geographic coordinates and elevation for each of 34 AWC populations reported by Mylecraine et al. (2005). FloraMap™ was used to predict mean monthly minimum and maximum temperature and total precipitation for each population site by calculating average values from the five nearest meteo-

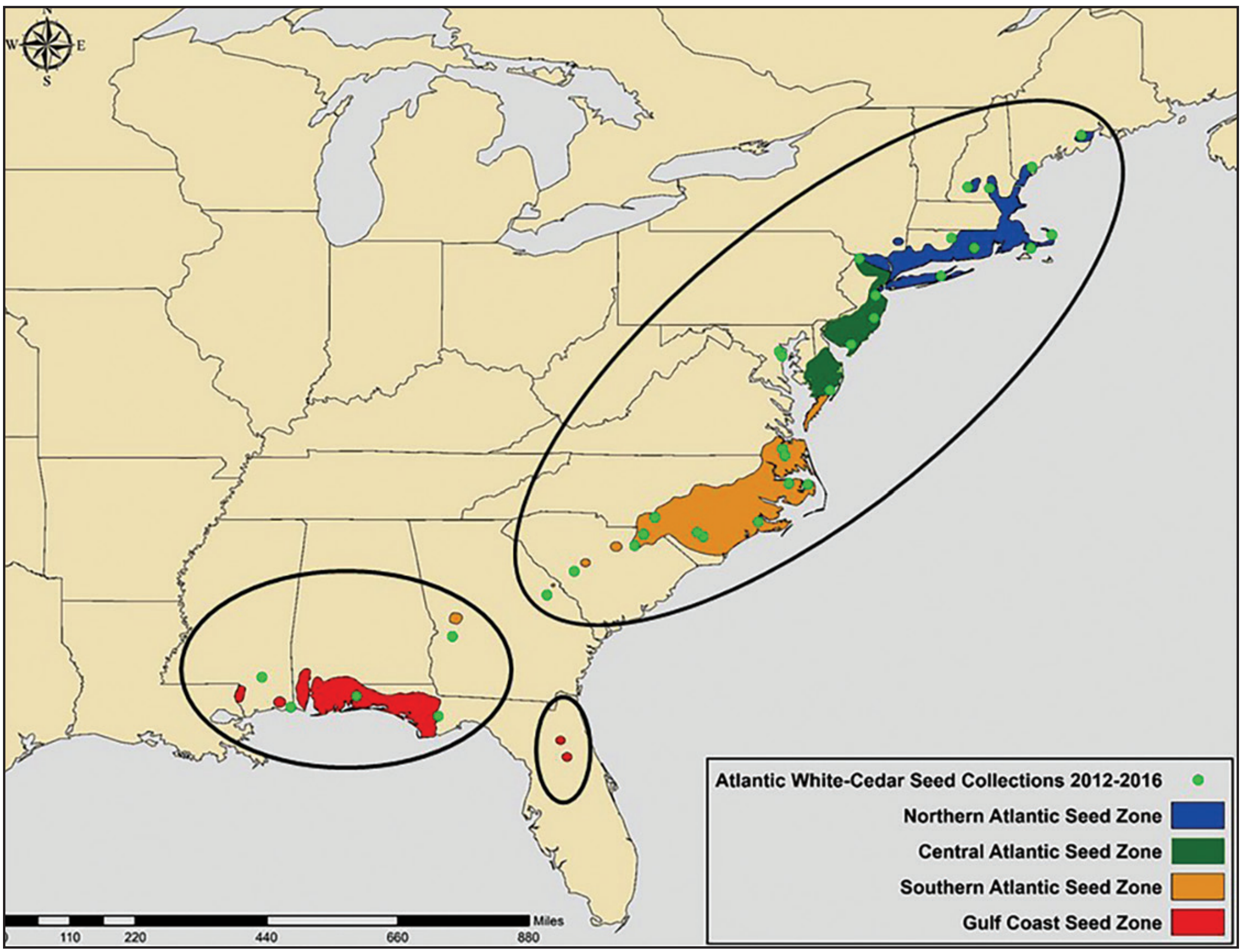

Figure 5. Atlantic white-cedar seed zones and locations of the 33 populations where seed collections were made. Black ovals represent the three genetic clusters identified by Mylecraine et al. (2004).

rological stations in its database, using a lapse rate correction to adjust temperature for elevation. In total, 36 climate variables (3 variables per month for 12 months) were derived. These variables were used to conduct a weighted paired group method (WPGMA) cluster analysis to group the 34 populations into clusters based on climate similarity. This analysis indicated that 85 percent of the variation in climate among the populations was explained by four clusters indicating four seed zones for AWC. These are: a Northern Atlantic zone extending from Maine south to New York; a Central Atlantic zone extending from New Jersey south to Maryland; a Southern Atlantic zone extending from Virginia south to Georgia; and a Gulf Coast zone extending from Florida west to Mississippi (figure 5). The seed zones are characterized by trends in increasing average annual minimum and maximum temperature moving from north to south across the geographic range of AWC (table 1).

Using these genetic parameters and seed zones, a seed collection protocol was developed. The collection target was to sample seeds from 40 populations and 400 mother trees (10 trees per population) distributed across the range of AWC. Collections were divided among the four seed zones with a target of 10 populations and 100 mother trees within each zone. These targets are actually higher than typically recommended for conifers of low to moderate levels of genetic diversity. Previous work by the Camcore program with tropical pine species demonstrated that 95 percent of genes occurring in a population at frequencies of 5 percent or higher can be captured with a seed sample from 6 to 10 populations and 10 to 20 trees per population distributed across the range of a species (Dvorak et al. 1999). Camcore has applied this approach successfully to genetic resource conservation efforts with eastern hemlock (*Tsuga canadensis* [L.] Carr.), Carolina hemlock (*T. caroliniana* Engelm.), and Table Mountain pine (*Pinus pungens* Lamb.) (Jetton et al. 2013, 2015). Details on how seed collections were carried out at a population level and the post-harvest handling of seeds are reported in Jetton et al. (2012) and summarized in figures 6 and 7.

Table 1. Climate attributes for four Atlantic white-cedar seed zones defined by FloraMap™.

Seed zone	Average annual minimum temperature (˚C)	Average annual maximum temperature (˚C)	Average annual total precipitation (mm)
Northern Atlantic	3.64	14.35	98.25
Central Atlantic	7.15	18.16	91.11
Southern Atlantic	10.22	22.90	102.11
Gulf Coast	13.90	25.62	126.54

T(°F) = T(°C) × 1.8 + 32

Seed Collections and Distribution

The AWC gene conservation effort was conducted over five field seasons from 2012 to 2016. Collection efforts acquired seed from 255 mother trees and 33 populations well distributed across the entire geographic range of the species with multiple populations sampled within each of the four seed zones (table 2, figure 5). Best represented are the Northern and Southern Atlantic seed zones, with 78 mother trees in 9 populations and 108 mother trees in 13 populations sampled, respectively. Less represented are the Central Atlantic and Gulf Coast seed zones, where sampling was limited to 32 mother trees in 7 populations and 37 mother trees in 4 populations, respectively. Also represented in these

Figure 6. Andy Whittier of Camcore climbs an Atlantic white-cedar tree for seed collection at Jones Lake State Park in North Carolina. (Photo courtesy of Camcore, North Carolina State University)

Table 2. Population, tree, and seed attributes for 33 Atlantic white-cedar populations from which seed was collected for genetic resource conservation.

Population Name	County	State	Lat.	Long.	Elev. (m)	Trees (#)	Height (m)	DBH (cm)	Seeds (#)	Seeds (g)	Seed Year
Northern Atlantic Seed Zone											
Appleton Bog (TNC)	Knox	ME	44.33	-69.26	90	10	8.70 (± 4.42)	28.94 (± 9.38)	32,579	26.93	2016
Bolton Lakes	Tolland	CT	41.82	-72.41	215	10	14.30 (± 7.78)	25.71 (± 12.68)	133,235	110.11	2016
Cranberry Bog Preserve	Suffolk	NY	40.90	-72.67	5	8	6.81 (± 3.03)	10.36 (± 4.91)	24,163	19.97	2015
Lovernens Mill (TNC)	Hillsborough	NH	43.07	-72.02	348	10	6.11 (± 3.55)	11.18 (± 9.64)	32,740	27.06	2016
Manchester Cedar Swamp (TNC)	Hillsborough	NH	43.04	-71.49	125	6	6.60 (± 6.05)	14.36 (± 13.48)	12,112	10.1	2015
Marconi	Barnstable	MA	41.91	- 9.97	10	10	8.80 (± 5.47)	19.47 (± 8.58)	70,000	57.85	2016
Mashpee	Barnstable	MA	41.59	-70.48	12	10	7.20 (± 2.34)	15.59 (± 5.48)	30,671	25.35	2016
Pachaug State Forest	Voluntown	CT	41.59	-71.86	90	4	9.52 (± 4.55)	23.70 (± 12.31)	7,441	6.15	2015
Saco Heath Preserve (TNC)	York	ME	43.55	-70.46	51	10	7.31 (± 3.19)	21.63 (± 6.75)	41,648	34.42	2015
Central Atlantic Seed Zone											
Arlington Echo 1	Anne Arundel	MD	39.07	-76.6	0	8	3.78 (± 1.69)	6.81 (± 3.54)	24,576	20.31	2014
Arlington Echo 2	Anne Arundel	MD	38.96	- 6.54	5	10	4.75 (± 1.53)	11.52 (± 4.01)	12,922	10.68	2014
Belleplain State Forest	Cape May	NJ	39.24	-74.85	24	1	11.58	35.00	87	0.07	2014
Brendan Byrne State Forest	Burlington	NJ	39.89	-74.3	33	2	9.44 (± 2.15)	29.15 (± 14.63)	6,545	5.41	2014
Cheesequake State Park	Middlesex	NJ	40.43	-74.26	5	3	13.71 (± 4.03)	29.15 (± 9.94)	14,293	11.81	2014
High Point State Park	Sussex	NJ	41.33	-74.65	453	1	N/A	N/A	24	0.02	2014
Ponders Tract (TNC)	Sussex	DE	38.13	-75.37	13	7	8.92 (± 4.06)	29.15 (± 3.68)	1,996	1.65	2015
Southern Atlantic Seed Zone											
Alligator River NWR	Dare	NC	35.83	-75.91	1	10	8.22 (± 0.78)	15.62 (± 6.37)	18,276	13.77	2012
Catfish Lake (Croatan NF)	Craven	NC	34.94	-77.11	12	10	8.56 (± 2.82)	30.96 (± 12.37)	51,708	44.38	2012
Cheraw State Park	Chesterfield	SC	34.64	-79.89	41	10	10.72 (± 4.77)	19.51 (± 8.63)	10,920	9.21	2012
Fort Gordon	Richmond	GA	33.16	-82.24	98	13	7.69 (± 6.99)	11.10 (± 11.72)	35,903	29.67	2015
Fort Perry	Marion	GA	32.15	-84.54	150	12	7.54 (6.54)	13.13 (± 16.31)	59,786	49.41	2015
Gravatt Center	Aiken	SC	33.73	-81.58	117	10	15.30 (± 9.92)	25.76 (± 13.21)	25,393	20.59	2012
Great Dismal Swamp NWR NC	Camden	NC	36.54	-76.46	5	5	14.93 (± 7.72)	25.90 (± 14.89)	17,169	16.13	2012
Great Dismal Swamp NWR VA	Suffolk	VA	36.70	-76.52	7	6	10.56 (± 6.82)	22.41 (± 8.71)	13,668	11.48	2012
Jones Lake State Park	Bladen	NC	34.68	-78.59	25	8	12.26 (± 3.77)	28.80 (± 14.27)	9,972	6.2	2012
Kalmia Gardens	Darlington	SC	34.36	-80.11	56	1	18.00	41.10	1,272	1.05	2012
Pettigrew State Park	Tyrrell	NC	35.86	-76.37	11	5	10.51 (± 1.13)	15.95 (± 3.18)	5,286	3.5	2012
Sandhills Gameland	Richmond	NC	35.05	-79.62	109	10	10.88 (± 3.71)	23.48 (± 12.04)	6,960	7.04	2012
Singletary Lake State Park	Bladen	NC	34.58	-78.44	10	8	13.25 (± 5.40)	28.71 (± 10.77)	21,793	18.06	2012
Gulf Coast Seed Zone											
Apalachicola NF	Liberty	FL	30.21	-84.89	25	12	14.98 (± 5.31)	28.35 (± 14.32)	170,741	193.4	2013
Blackwater River State Park	Santa Rosa	FL	30.70	-86.87	3	10	11.88 (± 5.31)	30.85 (± 14.51)	74,698	77.2	2013
CaBlackwater River State Parkmp Shelby	Forest	MS	31.16	-89.17	59	5	17.67 (± 5.55)	23.06 (± 10.56)	13,821	18.2	2013
Escatawpa River NWR	Jackson	MS	30.43	-88.47	0	10	7.04 (± 2.56)	16.19 (± 7.14)	67,247	64.1	2013

DBH = diameter at breast height; 1 m = 3.28 ft; 1 cm = 0.393 in.

collections are two of the three AWC gene pools identified by Mylecraine et al. (2004). Missing are populations from the peninsular Florida genetic cluster in the Juniper Springs area of the Ocala National Forest. Two separate explorations of that area by the authors during the 2013 field season failed to locate populations for seed collection. Following those explorations, the authors inquired with local foresters about these stands and were told that they were likely extirpated by severe wildfires that heavily impacted the area in 2006 and 2009.

Seed collections were from as few as one mother tree per population (3 populations) to as many as 12 (2 populations) and 13 (1 population). The majority of populations are represented by 5 to 10 mother trees (24 populations). Average harvest per mother tree was 1,309 cones with an average of 4.47 seeds per cone. This is lower than the expected 8 seeds per cone reported for the species (Bonner 2008). Overall, an average 4,116 seeds per mother tree and 1,049,648 total seeds were collected to support the genetic resource conservation of AWC.

Germination tests have not been completed for all seedlots, but trials for the 2012 and 2013 collections resulted in 7 percent seed germination. These tests also included an evaluation of seed coat sterilization and photoperiod treatments for improving AWC seed germination (see Jetton and Whittier in this issue for details). The USDA Forest Service National Seed Laboratory (Dry Branch, GA) has completed x-ray tests on seed samples of 120 mother trees from 15 populations collected during the 2012 and 2013 field seasons. These tests indicated an average of 13.25 percent filled seeds per seedlot, ranging from a low of 1 percent to a high of 59 percent filled seed. Although this may seem low, previous reports indicate less than one-third of AWC seeds are expected to be filled (Bonner 2008) with a viability (based on actual seed germination) range of 3 to 25 percent (Little and Garrett 1990).

Of the 1,049,648 seeds collected, 278,938 representing 236 mother trees and 33 populations are stored at the USDA Forest Service Ashe Nursery Facility for future use in seed orchard and restoration activities. An additional 85,872 seeds representing the same 236 mother trees and 33 populations reside at the USDA Agricultural Research Service

Figure 7. Mature seed cones of Atlantic white-cedar were (a) collected and returned to the Camcore lab in Raleigh, NC where they were (b) dried and allowed to open. Seeds were (c) separated from foliage and other chaff prior to (d) packaging for placement in cold storage. (Photos courtesy of Camcore, North Carolina State University)

National Center for Genetic Resources Preservation for long-term preservation. Information on seed longevity specific to AWC is lacking, but seeds of Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.) have survived storage at -15 °C (5 °F) for more than 11 years with no loss in germination capacity (Zobel 1990). The Camcore Seed Bank at NCSU retained 683,588 seeds representing all 255 mother trees and 33 populations as a backup collection for conservation, restoration, and associated research. An additional 1,250 seed were used for seed testing.

Summary and Conclusions

The 5-year cooperative effort between Camcore and the USDA Forest Service to conserve the genetic resources of AWC captured more than 1 million seeds for conservation. Seeds were acquired from 255 mother trees and 33 populations distributed across the full geographic extent of the species, representing the largest genetic base for the species known to exist outside of remnant natural stands. This material has been distributed to three seed repositories where it is maintained for long-term preservation and eventual use in research and restoration activities.

Although the number of populations and mother trees sampled fell short of the collection targets defined for this project, this project should be considered successful given recent disturbances that have had significant impacts on AWC populations across much of the species' range during the past 15 years. Already mentioned are the wildfire impacts on the Ocala National Forest. Additionally, Hurricane Isabel in 2003 and subsequent wildfires in 2008 (South One Fire) and 2011 (Lateral West Fire) destroyed or severely damaged 90 percent of AWC stems in the Great Dismal Swamp (Belcher and Poovey 2009, Mitchell 2013). Populations along portions of the Gulf Coast were also damaged by Hurricane Katrina in 2005. Most notable was the region's largest population on the Grand Bay National Wildlife Refuge where winds snapped or uprooted 32 percent of trees in mature, reproductive age classes (McCoy and Keeland 2009). Similarly, the majority of AWC stands in New Jersey suffered significant mortality and stress from wind and seawater inundation associated with Hurricane Sandy in 2012 (New Jersey Department of Environmental Protection 2015). These disturbance events certainly impacted the number and quality of mother trees sampled during this project and highlight the importance of gene conservation efforts. The availability of genetically diverse and broadly adaptable seed resources for breeding and restoration is important for the resilience of tree species such as AWC whose already reduced abundance is further threatened by environmental stress (e.g., storms, drought, wildfire) and an uncertain climate future when sea level rise and saltwater inundation are expected to impact the health and sustainability of tidal forested wetlands in the eastern United States (Day et al. 2007).

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