Effect of Seed Coat Sterilization and Photoperiod Treatments on the Germination of Atlantic White-Cedar Seeds

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Abstract

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.) (AWC) is an endangered, wetland tree species native to the coastal regions of the Eastern United States. Since 2012, AWC has been the target of *ex situ* gene conservation efforts by the Camcore Program at North Carolina State University and the U.S. Department of Agriculture, Forest Service. The gene conservation effort includes annual post-collection seed germination tests to evaluate seed quality and conservation value. Early germination trials were confounded by significant fungal growth that may have reduced overall seed germination. This study evaluated the effects of seed coat sterilization (no sterilization, bleach, hydrogen peroxide, and ethanol) and photoperiod (0:24, 8:16, 12:12, 16:8, and 24:0, light:dark) treatments on the germination of AWC seeds in the laboratory at 22 °C (71.6 °F) following cold-moist stratification at 4 °C (39.2 °F) for 30 days. Fungal growth in this study was minor and did not differ substantially between unsterilized and sterilized seeds. Treatment with hydrogen peroxide nearly doubled seed germination over the other sterilization treatments. There were no differences in seed germination among photoperiod treatments.

Introduction

Atlantic white-cedar (*Chamaecyparis thyoides* [L.] B.S. P.; hereafter referred to as AWC) is a wetland tree species that occurs on the margins of freshwater swamps and bogs in the coastal regions of the eastern United States (Little and Garrett 1990). AWC occupies a narrow distribution that extends from Maine south to northern Florida and west along the Gulf Coast to southeastern Mississippi (figure 1). These wetlands have important functions for coastal hydrology, including stream flow stabilization and water filtration and purification (Kuser and Zimmermann 1995). As a timber species (figure 2), AWC has long been prized for its decay-resistant wood that is harvested and sold for a variety of purposes, including siding, roofing shingles, fencing, decking, lawn furniture, boat planking, and duck decoys (Ward 1989). There were an estimated 202,343 ha (500,000 ac) of AWC-dominated swamps and bogs prior to European settlement, but due to subsequent harvesting, draining of coastal wetlands for agriculture and development, and catastrophic hurricanes and wildfires, today only about 40,469 ha (100,000 ac) remain. Because of this decline, AWC 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 programs. In response, Camcore (an international tree breeding and conservation program housed in the Department of Forestry and Environmental Resources at North Carolina State University) and the USDA Forest Service Southern Region National Forest System and Forest Health Protection, in 2012, initiated an *ex situ* gene conservation project for the species. Details on the objectives, protocols, and results of this project can be found in Jetton et al. 2019 (previous article in this issue).

The success of *ex situ* gene conservation depends on the collection of genetic material that is of high conservation value. This means that collections should capture not only representative genetic and adaptive variation of the species, but also sufficient amounts of viable seed (figure 3) to meet conservation objectives (Shaw and Hird 2014). The latter is challenging for tree species in the genus *Chamaecyparis*, where seed viability is variable and usually very low due to low

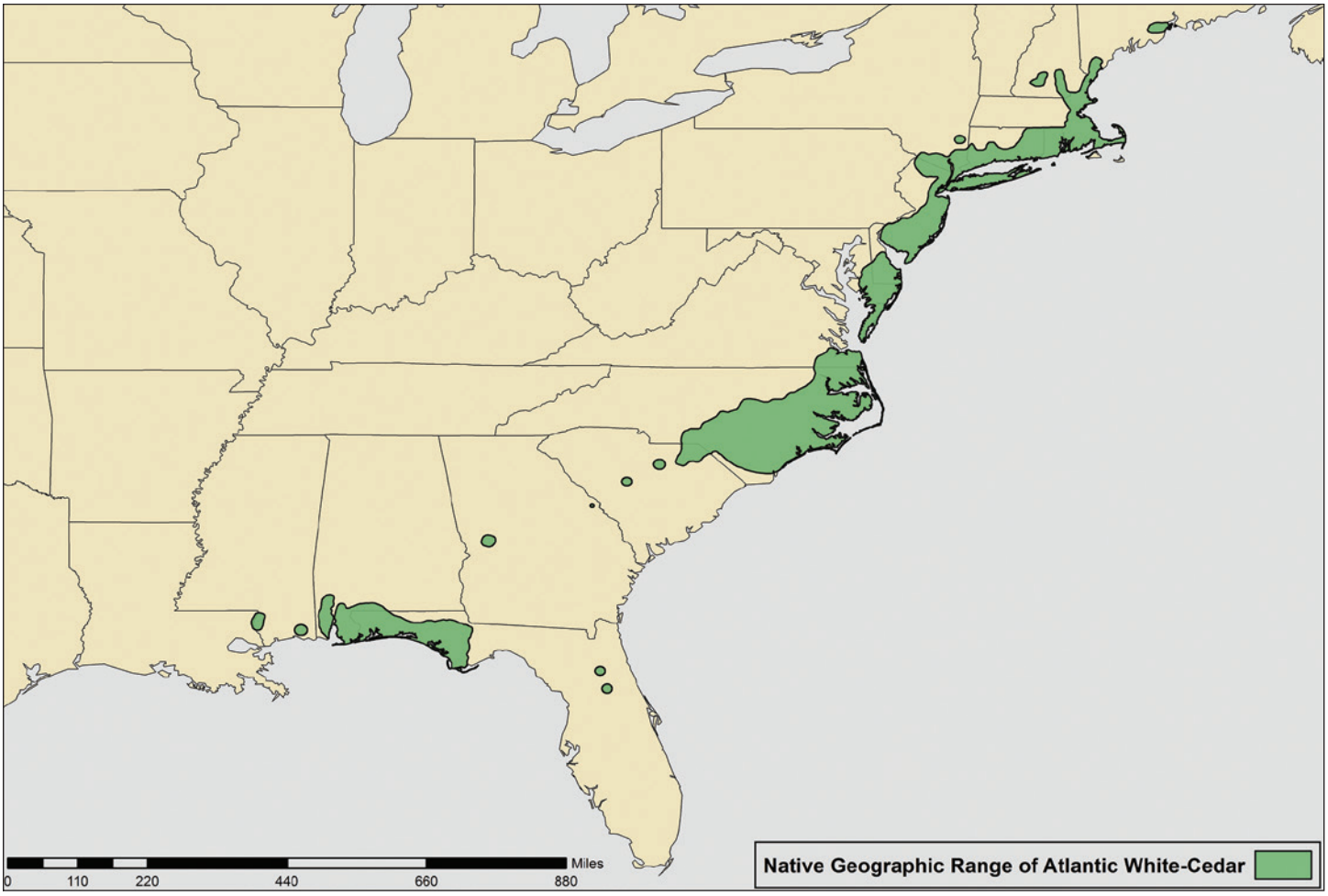

Figure 1. The geographic distribution of Atlantic white-cedar in the Eastern United States (Map courtesy of Camcore, North Carolina State University).

percentages of filled seeds (Bonner 2008a). This is particularly true for AWC, which produces on average 8 seeds per cone (figure 4) with one-third of the seeds expected to be filled, resulting in 3- to 25-percent germination under field conditions (figure 5), depending on location (Bonner 2008a, Little and Garrett 1990).

The potential for collecting large amounts of non-viable seeds requires regular post-collection germination testing of seeds to determine if conservation objectives are being met. Through the first two AWC seed-collection seasons (2012 and 2013), seed was collected from 15 populations and 120 mother trees distributed across the southern Atlantic and Gulf Coast seed zones (Jetton et al. 2019). Following the 2012 collections, Petri dish assays were conducted in Camcore's seed laboratory to test provenance-level germination (Jetton and Whittier, unpublished data). Seeds were first cold-moist stratified at 4 °C (39.2 °F) for 30 days, then germination assays were carried out in an environmental chamber at alternating temperature (30 °C:20 °C [86 °F:68 °F], day:night) and photoperiod regimes (8:16 light:dark). Although some seedlots had 20- to 28-percent germination, overall germination was low at only 7 percent. Significant fungal growth was noted in most of the Petri dishes that showed little or no germination and may have interfered with germination

Figure 2. A mature stand of Atlantic white-cedar at the Great Dismal Swamp along the North Carolina-Virginia border. The species is prized for its decay-resistant wood that is used for a number of wood products (Photo courtesy of Camcore, North Carolina State University).

Figure 3. Seeds of Atlantic white-cedar collected by Camcore for genetic resource conservation (Photo courtesy of Camcore, North Carolina State University).

of those seedlots. Seed coat sterilization with fungicides, bleach, hydrogen peroxide, and other sterilants to reduce infections of saprophytic and pathogenic fungi has been shown to improve seed germination in a number of conifer species (Barnett 1976, Barnett and Varela 2004, Wenny and Dumroese 1987). The objective of the study reported here was to test seed coat sterilization and photoperiod treatments on the germination of AWC seeds to optimize the laboratory seed germination protocol and reduce fungal growth.

Methods

In March 2014, a total of fifty, 25-seed bulks (1,250 seeds total) were prepared from the November 2012 (16 months post-collection) and November 2013 (4 months post-collection) AWC seed stocks stored at 4 °C in the Camcore seed bank. The individual seed bulks were prepared from a large, 2,000 seed bulk containing seeds from all 15 populations and 120 families collected during those years (Jetton et al. 2019). The seed bulks were cold-moist stratified on filter paper in Petri dishes at 4 °C (39.2 °F) for 30 days in a walk-in cooler following the protocol of Jetton et al. (2014). Following stratification, the 50 seed bulks were randomly assigned to one of 25 treatment combinations (5 seed-coat sterilizations by 5 photoperiods). The five sterilization treatments were: (1) unsterilized seeds on germination paper; (2) unsterilized seeds sown in growing medium (Fafard® Germination Mix, Sungrow Horticulture, Agawam, MA); (3) seeds sterilized by soaking in a 10-percent bleach solution for 10 minutes; (4) seeds sterilized by soaking in a 3-percent hydrogen peroxide solution for 1 hour; and (5) seeds sterilized by soaking in 10-percent ethanol for 10 minutes. The five photoperiods (light:dark) were: 0:24, 8:16, 12:12, 16:8, and 24:0. For those sown into growing medium, seeds were sown 1 cm deep in small garden pots after stratification. For all other seed treatments, seeds were placed into Petri dishes on moist germination paper.

Figure 4. Cones of Atlantic white-cedar at the Croatan National Forest in eastern North Carolina. Pictured in early summer, these cones will ripen by the fall and yield an average of eight seeds each (Photo courtesy of Camcore, North Carolina State University).

Two Petri dishes/garden pots (reps) per seed treatment were placed into each of the five photoperiod chambers. The germination experiment was conducted at 22 °C (71.6 °F) for 30 days. Each Petri dish or pot was checked daily for newly germinated seeds and for fungal growth on seed coats. Germination paper and garden pots were remoistened as needed with filtered, deionized water.

The probability of seed germination after 30 days was determined using a logistic regression model assuming a binomial distribution and logit link function in the GLIMMIX procedure of SAS 9.4 (SAS Institute 2013). The response variable was total percent germination, defined in the model statement by the events/trials syntax or the number of germinated seeds per Petri dish (or pot)/total seeds per Petri dish (or pot). The model tested the main effects of rep, sterilization treatment, photoperiod, and the sterilization treatment by photoperiod interaction. Where significant differences were found, means were compared using the Tukey-Kramer Multiple Comparison Test at $\alpha = 0.05$. All means reported are least square means, and all variances reported are standard errors.

Figure 5. Germinating seedling of Atlantic white-cedar at the Great Dismal Swamp along the North Carolina-Virginia border (Photo courtesy of Camcore, North Carolina State University).

Results

Overall, mean seed germination was 10 percent, or 125 of the 1,250 seeds. The hydrogen peroxide sterilization treatment, however, had about twice as much germination as all other seed treatments, regardless of photoperiod (table 1, figure 6). Total germination varied little among the photoperiod treatments and was lowest for seeds in the 24:0 photoperiod.

The overall number of seeds with fungal growth was 14, or approximately 1 percent of all seeds in the experiment. Among the sterilization treatments, the highest number of moldy seeds occurred in the control (6), followed by bleach (4), hydrogen peroxide (3), and ethanol (1). In the photoperiod treatments, the moldiest seeds occurred in 0 hours light (8), followed by 8:16 (2), 12:12 (2), 16:8 (1), and 24:0 (1).

Discussion

The bleach, ethanol, and hydrogen peroxide treatments reduced fungal growth relative to the unsterilized control, but this difference was minor and the overall occurrence of fungal growth was very low. This growth was much lower than that in the 2012 seed germination test, where more than 50 percent of seed coats had fungal growth (Jetton and Whittier, unpublished data). The 2012 result is likely related to the fact that tap water was used to maintain germination paper moisture, whereas filtered, deionized water was used in the current study.

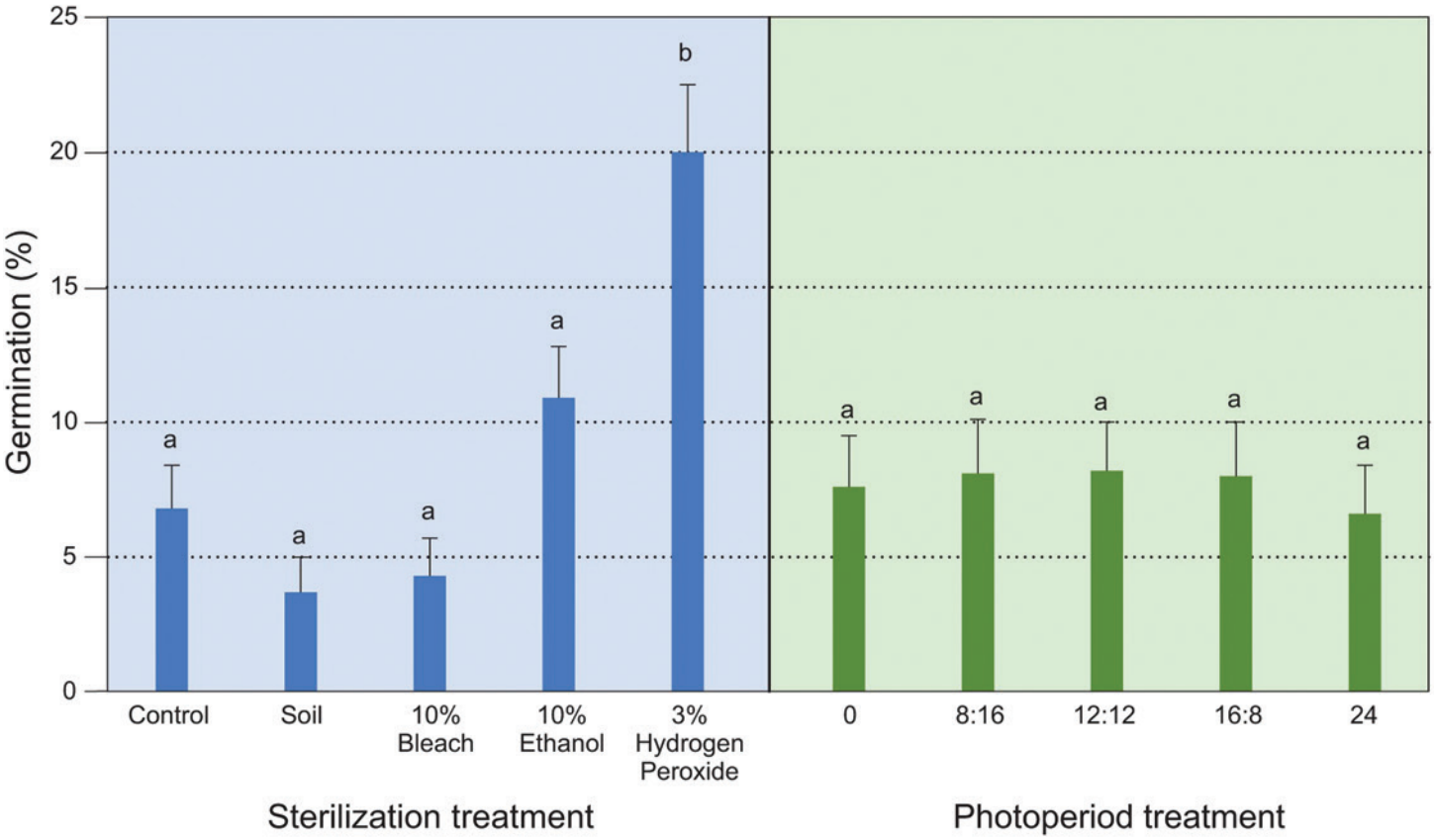

Figure 6. Least square mean (±SE) total germination of Atlantic white-cedar seed in response to sterilization treatments and photoperiod. There were no significant interactions. Within treatments, bars with the same letter did not differ signifcantly.

Table 1. Type III tests of fixed main effects in the logistic regression model for probability of seed germination after 30 days.

Effect	*DF*	*F*	*P*
Rep	1	1.16	0.2922
Sterilization treatment	4	10.58	<0.0001
Photoperiod	4	0.12	0.9748
Sterilization*Photoperiod	16	0.68	0.7893

The bleach and ethanol treatments did not significantly increase germination of AWC seeds relative to the control and soil treatments, but, not surprisingly, germination of seeds soaked in hydrogen peroxide was significantly higher than the other four sterilization treatments. Soaking seeds in hydrogen peroxide is known to be effective for sterilizing seed coats infected with saprophytic and pathogenic fungi (Barnett 1976, Barnett and Varela 2004) and can stimulate germination in seeds with scarified, nicked, cracked, or intact seed coats (Bonner 2008b). While not common in commercial nursery practice, this method is commonly used to initiate tissue culture in a number of conifer species (Amerson et al. 1985).

The lack of photoperiod effect on seed germination was surprising, given that AWC has demonstrated an obligate light requirement in previous research, although the duration of the light period that provides the best and worst germination varies among studies. Jull and Blazich (2000) reported 8-percent germination under zero light, but 48 percent and 55 percent under 1- and 24-hour photoperiods, respectively. Boyle and Kuser (1994) found that AWC seeds germinated at a higher rate under a 16-hour photoperiod (31.9 percent) compared with a 10-hour photoperiod (0.7 percent). Testing under an 8-hour photoperiod, Bianchetti et al. (1994) found that seed germination varied if temperature conditions were set to a constant or variable thermo-period.

Further research is needed to improve and optimize the laboratory seed germination protocols for AWC. Specific topics we plan to address are: (1) improving the seed cleaning process to remove more empty seeds and increase the number of filled seeds in each seedlot; (2) determine if 30 days at 4 °C (39.2 °F) or other combinations of duration and temperature are best for cold-moist stratification; and (3) further investigate the effect of alternating thermo-periods on AWC seed germination. In the meantime, based on the results of this study, the following protocol is recommended for germination testing of AWC seeds at Camcore. Following collection, extraction, cleaning, and storage, seeds should be cold-moist stratified at 4 °C (39.2 °F) for 30 days prior to testing. Following stratification, seeds should be surface sterilized by soaking in a 3-percent hydrogen peroxide solution for 1 hour, then sown on moist germination paper in Petri dishes. Germination should proceed under a 12:12 photoperiod at 22 °C (71.6 °F) for 30 days. Moistening of the germination paper should be done with filtered, deionized water to limit fungal growth on seed coats.

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