

Seed Germination of Selected Provenances of Atlantic White-Cedar as Influenced by Stratification, Temperature, and Light

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Abstract. Seeds of six provenances (Escambia Co., Ala.; Santa Rosa Co., Fla.; Wayne Co., N.C.; Burlington Co., N.J.; New London Co., Conn.; and Barnstable Co., Mass.) of Atlantic white-cedar [*Chamaecyparis thyoides* (L.) B.S.P.] were stratified (moist-prechilled) for 0, 30, 60, or 90 days at 4 °C. Following stratification, seeds were germinated at 25 °C or an 8/16-hour thermoperiod of 30/20 °C with daily photoperiods at each temperature of 0 (total darkness), 1, or 24 hours. The germination of nonstratified seed did not exceed 18%. Seeds germinated at 25 °C required 60 to 90 days stratification to maximize germination. In contrast, 30 days stratification maximized germination at 30/20 °C. Regardless of stratification duration, germination was generally lower at 25 °C than at 30/20 °C for each provenance. Averaged over all treatments, seeds of the Alabama provenance exhibited the greatest germination (61%), followed by those from Florida (45%), with the remaining provenances ranging from 20% to 38%. However, specific treatments for each provenance induced germination >50%. Germination of seeds not exposed to light was <8%, in contrast with 48% and 55% germination for daily photoperiods of 1 and 24 hours, respectively. Seeds from each of the provenances, except for Alabama, exhibited an obligate light requirement when germinated at 25 °C. At 30/20 °C, the North Carolina, New Jersey, Connecticut, and Massachusetts provenances required light for germination, whereas the Alabama and Florida provenances did not.

Atlantic white-cedar is a narrow, conical, evergreen tree reaching a height of 12 to 22 m and having horizontal to pendulous branches with soft foliage (Dirr, 1990; Taras, 1971; Welch, 1991). The species is distributed along an 80- to 209-km-wide belt from the southern coast of Maine to South Carolina, with isolated stands in Georgia and eastern Florida (Korstian and Brush, 1931). It also occurs from the panhandle of Florida to Mississippi, where it is regarded by some as *Chamaecyparis thyoides* var. *henryae* (Li) Little (Little, 1966).

Distribution of Atlantic white-cedar is scattered, owing to exacting site requirements, extreme sensitivity to fire, and the inability to compete on dry sites (Hinesley et al., 1994; Laderman, 1989). This tree is important both economically, for highly valuable wood products, and ecologically, as a habitat for many plant and animal species not common to other freshwater wetlands (Kantor, 1976; Laderman, 1989). Atlantic white-cedar also has potential for wetlands restoration and as an ornamental and Christmas tree (Hinesley et al., 1994).

Throughout its range, Atlantic white-cedar has been logged extensively, and its habitat has been so altered by human intervention that the species has lost much of its former abundance (Laderman, 1989). Seed production begins at 4 to 5 years of age in open stands and at 10 to 20 years in dense stands; fair to excellent crops of seed are usually produced each year (Korstian and Brush, 1931; Little, 1959). However, efforts to reestablish Atlantic white-cedar have been only partially successful because of excessive browsing by whitetail deer (*Odocoileus virginianus* Zimm.), competition from hardwoods, poor seed germination, and variable seedling development (growth rates and stem straightness) (Bianchetti et al., 1994; Little, 1950).

Seed germination of *Chamaecyparis* Spach sp. is inherently low, due to reduced seed quality, insect damage to the seeds, and embryo dormancy (Harris, 1974; Laderman,

1987). Laderman (1989) also reported that seed germination in Atlantic white-cedar varies greatly among seed lots from different swamps and provenances (geographic seed origin).

Currently, Atlantic white-cedar swamps are being restored along the Atlantic coast of the United States. Such restoration efforts require transplants produced either by stem cuttings or from seed. Although reports have appeared regarding protocols for rooting stem cuttings (Boyle and Kuser, 1994; Hinesley and Snelling, 1997; Hinesley et al., 1994), seed germination requirements of the species are not well established.

Jull (1998) investigated the effects of stratification (moist-prechilling), temperature, and light on seed germination in provenances of Atlantic white-cedar from Alabama and North Carolina. Germination requirements of seeds from Alabama were different from those from North Carolina (Jull, 1998). Regardless of the duration of stratification, germination was lowest at 25 °C for both provenances. Alabama seeds required only 30 d of stratification, followed by germination at 8/16-h thermoperiods of 30/20 °C or 25/15 °C with daily photoperiods ≥1/2 h, to maximize germination. In contrast, when germinated under the same temperature and light conditions, the North Carolina provenance required 60 to 90 d of stratification. The species occurs over a wider geographic range than that studied by Jull (1998). Therefore, the objectives of this research were to examine the influence of stratification, temperature, and light on seed germination of six provenances of Atlantic white-cedar that represent its extensive geographical distribution.

Materials and Methods

Mature cones of Atlantic white-cedar from Escambia Co., Ala.; Santa Rosa Co., Fla.; Wayne Co., N.C.; Burlington Co., N.J.; New London Co., Conn.; and Barnstable Co., Mass., were harvested from open-pollinated trees during Fall 1994 (Ala., N.C., N.J., and Conn.), Winter 1995 (Mass.), or Fall 1995 (Fla.). Trees in Alabama and Florida, from which cones were collected, were 40 to 45 and 25 to 30 years old, respectively; those from North Carolina were 3 to 4 years old; those from New Jersey, Connecticut, and Massachusetts were 40 to 50 years old.

Cones were collected from representative trees within each provenance, and dried on wire mesh racks at 21 °C for 2 months. Many cone scales closed and hardened, preventing seed extraction. To enhance seed release, cones were soaked overnight in water, drained, and allowed to dry for 4 d at 30 °C. Extracted seeds were stored in sealed glass bottles at 4 °C for 6 months at moisture contents of 5.3%, 6.3%, 5.4%, 9.1%, 7.1%, and 7.5% for the Alabama, Florida, North Carolina, New Jersey, Connecticut, and Massachusetts provenances, respectively. Moisture contents were determined by calculating the mean moisture contents of six 100-seed samples of graded seeds following drying at 105 °C for 24 h.

In June 1996, seeds were removed from

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storage and cleaned initially with an air column (General Seed Blower-Model ER; Seedbuo International Equipment Co., Chicago). The column was operated for 10 min using a no. 2 (60-mesh) screen and a setting of 21 (North Carolina, Connecticut, and Massachusetts provenances) or 20 (Alabama, Florida, and New Jersey provenances) to remove chaff and empty seeds. During seed grading, the ports of the column remained closed. Seeds retained on the pan screen were then subjected to additional grading. Abnormal, damaged, undersized, or discolored seeds, and other large debris not eliminated by the air column, were removed manually. Graded seeds were firm and dark brown.

The seeds were stratified (moist-prechilled) for 0, 30, 60, or 90 d at 4 °C, as follows: dry sand was sieved through an 18-mesh (1.0 mm) screen and the fine particles retained. One-hundred seeds were mixed with 20 mL moist sand [10 dry sand : 1 water (by volume)] and were placed in 476-mL nonvented, polyethylene freezer bags. After the designated stratification interval, 24 randomly selected bags from each provenance were removed from stratification. Seeds were separated from sand by flushing with tap water in a colander, and sown in covered, 9-cm glass petri dishes containing two prewashed (rinsed) germination blotters (Filtration Sciences Corp., Mt. Holly Springs, Pa.) moistened with tap water. Four dishes per replicate were placed in black sa-teen cloth bags. The seeds imbibed water overnight at 21 °C. On the following day, bags were placed randomly within two growth chambers [C-chambers (Downs and Thomas, 1991)] at the Southeastern Plant Environment Laboratory (Phytotron). The chambers were maintained at 25 ± 0.5 °C or at an 8/16-h thermoperiod of 30/20 ± 0.5 °C.

Within each temperature regime, seeds were subjected daily to the following photoperiods: 0 (total darkness), 1, or 24 h. The 1-h photoperiod was administered the same time each day regardless of stratification and germination temperature. For the alternating temperature of 30/20 °C, the photoperiod began with

the transition to the high temperature portion of the cycle. Growth chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400 to 700 nm) of 26 to 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ as measured at dish level with a cosine corrected LI-COR LI-185 quantum/radiometer/photometer (LICOR, Lincoln, Nebr.). The 1-h photoperiod treatment was regulated by removal and placement of the petri dishes in black sa-teen cloth bags. For the 24-h photoperiod treatment, the petri dishes remained continuously unbagged in open chamber conditions. Regardless of the photoperiod, temperatures within the petri dishes did not exceed the ambient temperature by >1 °C from the set point. For constant darkness, the petri dishes were kept in the black cloth bags throughout the experiment. Watering and germination counts were performed in a darkroom under a fluorescent lamp equipped with a green acetate filter (Rosco Laboratories, Port Chester, N.Y.). Germination blotters were kept moist with tap water throughout the experiment. Seeds showing signs of decay were removed from the dishes.

For the Massachusetts provenance, each replicate petri dish contained 40 seeds; replicate dishes for the other provenances contained 100 seeds. Germination counts were recorded every 3 d for 30 d. A seed was considered germinated when radicle emergence was ≥ 1 mm. Mean percent germination was calculated from four replicates per treatment.

The experimental design was a split-split plot with temperatures as the main plots, stratification treatments as the subplots, and provenances and photoperiods as the sub-sub plots. Data were subjected to analysis of variance procedures and regression analyses (SAS Institute, 1990). Data were also analyzed following arcsin transformation, and the calculated significant differences were similar to those for the nontransformed data. Hence, the data presented are not transformed. Mean separa-

tions were performed by least significant difference (LSD) procedures at $P \leq 0.05$.

Results and Discussion

Total percentage of germination (total germination at the end of each 30-d germination period). Stratification, temperature, and light significantly affected germination of Atlantic white-cedar, but response to these factors varied among provenances. Similar variation has been reported for other species of *Chamaecyparis*, including *Chamaecyparis thyoides* (Jull, 1998; Li, 1977).

Regardless of stratification duration, germination was lower at 25 °C than at 30/20 °C for seeds of all provenances (Table 1, Fig. 1), confirming results of earlier experiments (Bianchetti et al., 1994; Jull, 1998). The Alabama provenance exhibited the greatest germination at both temperatures and all photoperiods, confirming the observations of Jull (1998). However, this may be due to the increased seed vigor of this particular provenance. Seeds of the New Jersey provenance had the next highest germination at 30/20 °C, followed by the Florida, North Carolina, Connecticut, and Massachusetts provenances. In contrast, germination of the Florida provenance exceeded the New Jersey provenance when germinated at 25 °C with a daily 1-h photoperiod.

Fowells (1965) reported that "a fair amount of light is necessary for good seed germination of Atlantic white-cedar." With the exception of the Alabama provenance, seeds exhibited an obligate light requirement (<5% germination) when germinated at 25 °C (Table 1). At 30/20 °C, Alabama and Florida seeds did not exhibit an obligate light requirement, but the North Carolina, New Jersey, Connecticut, and Massachusetts provenances did. In a previous study by Jull (1998), seeds from North Carolina exhibited an obligate light requirement but seeds from Alabama did not. Similarly,

Table 1. Effects of photoperiod and temperature on total percentage of seed germination of six provenances of Atlantic white-cedar.

Provenance	Photoperiod (h)		
	0	1	24
	25 °C		
Alabama	5.9 a ^c	69.1 a	74.1 a
Florida	2.2 ab	54.4 b	59.6 b
North Carolina	0.8 b	39.3 c	41.6 c
New Jersey	0.1 b	33.4 d	57.8 b
Connecticut	0.1 b	30.4 d	41.2 c
Massachusetts	0.2 b	19.1 e	23.6 d
	30/20 °C		
Alabama	50.0 a ^c	82.9 a	84.8 a
Florida	32.7 b	57.5 c	63.2 c
North Carolina	4.4 c	51.8 d	55.1 d
New Jersey	0.8 c	65.7 b	69.1 b
Connecticut	0.4 c	39.5 e	47.0 e
Massachusetts	1.0 c	32.8 f	43.4 f

^cMean separation within columns and temperatures by LSD, $P \leq 0.05$.

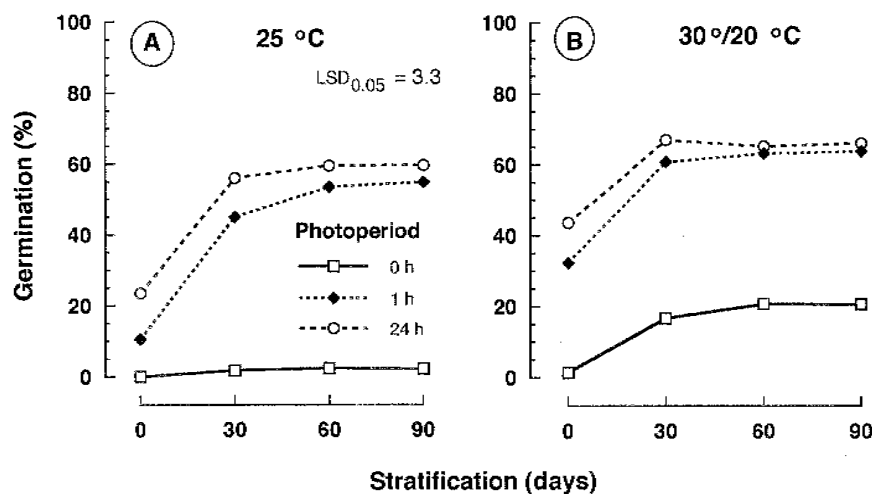


Fig. 1. Influence of stratification, temperature, and photoperiod on total percentage of seed germination of Atlantic white-cedar combined over all provenances. $\text{LSD}_{0.05} = 3.3$ for comparisons among temperatures when two factors (duration of stratification and photoperiod) are held constant. (A) Germination at 25 °C, with daily photoperiods of 0, 1, or 24 h following stratification for 0, 30, 60, or 90 d. (B) Germination at 30/20 °C, utilizing the same treatments as in A.

Bewley and Black (1985) reported that seeds of several conifers require light to germinate.

When germination data were combined over all provenances, seed stratification was required to maximize germination, but its optimum duration varied with germination temperature (Fig. 1). When seeds exposed to light were germinated at 25 °C, 60 to 90 d stratification were needed to maximize germination. In contrast, only 30 d stratification optimized germination at 30/20 °C. Similar results were reported by Jull (1998). Adkins et al. (1984) also reported for Fraser fir [*Abies fraseri* (Pursh) Poir.] that the duration of stratification needed to maximize germination was shorter in seeds germinated at 30/20 °C than in those germinated at 25 °C. Regardless of the germination temperature, germination of Atlantic white-cedar did not exceed 1.4% for nonstratified seeds germinated in darkness. At 25 °C, a 24-h photoperiod maximized germination regardless of the duration of stratification. When seeds were stratified for 60 to 90 d and germinated at 30/20 °C, only 1 h of light was required. However, a longer photoperiod was needed if seeds were stratified for only 30 d. For seed germination of many conifer species, the effect of stratification is more important than that of light (Li et al., 1994).

Stratification requirements varied according to provenance (Fig. 2). The Alabama, Florida, and New Jersey provenances required only 30 d stratification to maximize germination, whereas the North Carolina, Connecticut, and Massachusetts provenances required >30 d. Similarly, Jull et al. (1998) reported that seeds from the North Carolina provenance required longer durations of stratification than those from Alabama. Boyle and Kuser (1994) reported no significant effect on seed germination of several New Jersey provenances of Atlantic white-cedar when stratification was extended from 30 to 60 d.

Time course of germination. For all treatment combinations, germination was essentially complete by day 18 (Fig. 3). Analysis of variance showed that temperature, stratification, provenance, and photoperiod interactions with time (days) were highly significant. Therefore, regression analysis was conducted on time course of germination.

There were highly significant quadratic responses ($P \leq 0.0001$) in time course of germination for both temperatures, all durations of stratification, and all six provenances (Fig. 3). Averaged over all treatments, germination was greater at 30/20 °C (43%) than at 25 °C (31%) (Fig. 3A). Germination of nonstratified seeds did not exceed 18% (Fig. 3B). The time course of germination was similar whether seeds were stratified for 30, 60, or 90 d. However, the rate of germination increased as stratification was prolonged. In contrast, Bianchetti et al. (1994) reported that the effect of stratification on stimulating germination of Atlantic white-cedar was marginal.

When averaged over all treatments, germination was highest in the Alabama seeds (61%), followed by Florida (45%), with values for the remaining sources ranging from 20% to 38% (Fig. 3C). However, specific treatments for

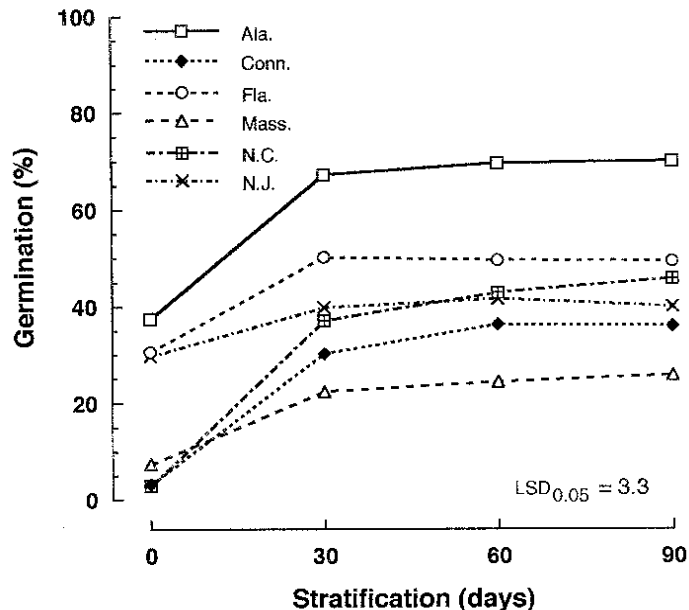


Fig. 2. Influence of stratification and provenance on total percentage of seed germination of Atlantic white-cedar combined over all temperatures and photoperiods. $LSD_{0.05} = 3.3$ for comparisons among provenances when duration of stratification is held constant.

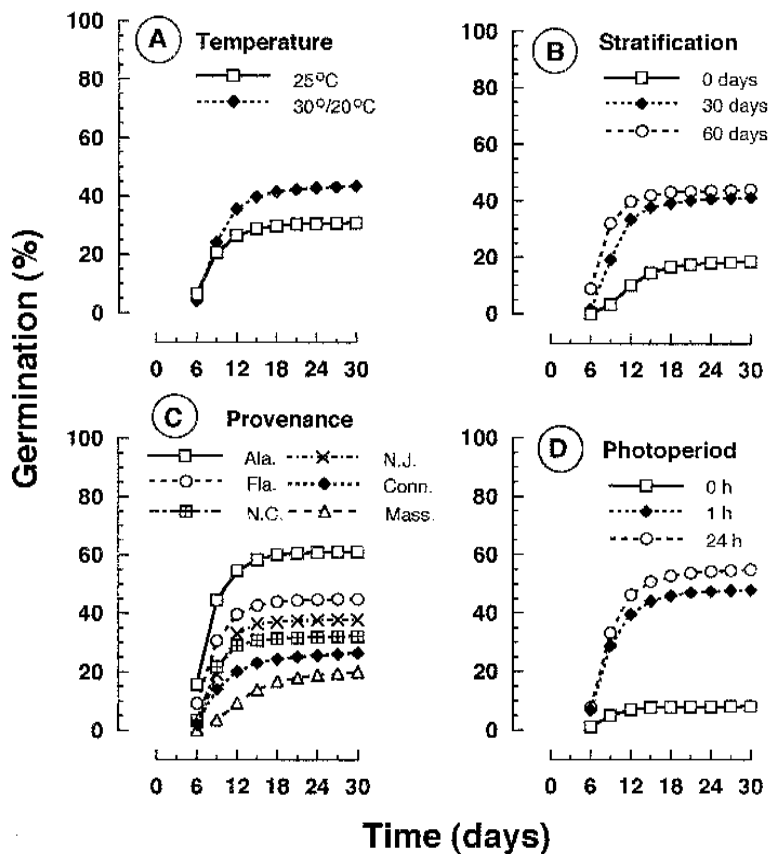


Fig. 3. Influence of stratification, temperature, light (photoperiod), and provenance on time course of germination of Atlantic white-cedar. No germination was observed until day 6. (A) Mean germination at 25 °C or an 8/16-h thermoperiod of 30/20 °C over all durations of stratification, provenances, and photoperiods; (B) mean germination following stratification for 0, 30, or 60 d over all temperatures, provenances, and photoperiods. Data for the 90-d stratification treatment were omitted since they were similar to those for the 60-d treatment; (C) mean germination of the Alabama, Florida, North Carolina, New Jersey, Connecticut, and Massachusetts provenances over all temperatures, durations of stratification, and photoperiods; (D) mean germination at photoperiods of 0, 1, or 24 h over all temperatures, durations of stratification, and provenances.

each provenance resulted in germination >50% (Table 1). The basic pattern of germination was the same for all provenances (Fig. 3C).

Light is an absolute requirement for germination of many species that inhabit swamps (Deno, 1994). The germination of Atlantic white-cedar seeds kept in darkness was <8% (Fig. 3D). Germination increased with photoperiod; 1- and 24-h photoperiods yielded 48% and 55% germination, respectively. This confirms the data of Boyle and Kuser (1994), who found that germination of seeds of Atlantic white-cedar from New Jersey provenances was greater under a 16-h photoperiod (32%), than under a 10-h photoperiod (0.5%) over combined durations of stratification. The quadratic responses for time course of germination for 0-h photoperiod ($P = 0.0014$) and for 1- and 24-h photoperiods ($P \leq 0.0001$) were highly significant.

Prior to initiation of this research, the germinative capacity of graded seeds of the Alabama, Florida, North Carolina, New Jersey, Connecticut, and Massachusetts provenances was estimated by cutting tests to be 85%, 81%, 90%, 61%, 84%, and 62%, respectively. For some provenances (e.g., Alabama and New Jersey), these estimates agreed with maximum germination as presented in Table 1; for other provenances (e.g., Florida, North Carolina, Connecticut, and Massachusetts), they did not. This suggests that data from cutting tests of Atlantic white-cedar should be interpreted conservatively. The germination conditions (e.g., temperature) may not have been optimum. Seed decay during germination, especially for the Florida provenance, reduced total percentage of germination. Tetrazolium tests were considered as an alternative to estimating viability, but were rejected because of difficulties in interpretation. Boyle and Kuser (1994) reported that such tests were inconclusive for Atlantic white-cedar.

In summary, our data indicate that the

stratification, temperature, and light requirements necessary to maximize seed germination of Atlantic white-cedar vary according to provenance. Although many reports mention that seed germination of this species is inherently poor, our data refute this. Germination can be greatly enhanced by rigorous seed cleaning followed by stratification for particular durations, and then subjecting seeds to an 8/16-h thermoperiod of 30/20 °C with daily photoperiods ≥ 1 h.

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