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Evaluation of Cold–Moist Stratification Treatments for Germinating Eastern and Carolina Hemlock Seeds for Ex Situ Gene Conservation

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Abstract - Populations of *Tsuga canadensis* (Eastern Hemlock) and *Tsuga caroliniana* (Carolina Hemlock) are declining due to infestation by *Adelges tsugae* (Hemlock Woolly Adelgid), an exotic insect pest. A better understanding of the environmental conditions required for seed germination is needed to more efficiently utilize the seeds collected for genetic-resource conservation and the establishment of seed orchards. This study examined the effect of cold–moist stratification treatments of varying duration (0, 1, 15, 30, 60, 90, and 120 days) on total germination (%) and the number of days to first and peak germination (germination speed) on seeds of both species in experiments conducted at 22 °C and 16 h:8 h, light:dark photoperiod. Overall total germination for Eastern Hemlock was 33.3% and increased with increasing duration of the stratification treatments. Carolina Hemlock total germination was 17.1% and varied little among the treatments, although fewer seeds tended to germinate following longer durations of stratification. Stratification increased germination speed of Eastern Hemlock but not Carolina Hemlock. The results indicate that Eastern Hemlock seeds should be cold–moist stratified at 4 °C for at least 30–60 days prior to sowing to promote higher total germination. Carolina Hemlock seeds can be sown directly following a 24-h soak with no additional cold–moist stratification.

Introduction

Tsuga canadensis (L.) Carr. (Eastern Hemlock) and *T. caroliniana* Englem. (Carolina Hemlock) are long-lived, slow-growing, shade-tolerant conifers native to eastern North America. Eastern Hemlock occurs across a broad elevation range from sea level to 1500 m and has a widespread geographic distribution that extends from Nova Scotia west to northern Minnesota and south through New England, the Middle Atlantic States, and the southern Appalachian Mountains into northern Georgia and the Cumberland Plateau of Alabama (Farjon 1990). The species is bimodal in habitat distribution, occurring in high abundance on moist, well-drained, nutrient-rich soils of mesic riparian zones and seasonally moist subxeric areas at the low and middle portions of its elevation range (Kessell 1979). At higher elevations, it is often more scattered along exposed xerophytic slopes and ridges. Carolina Hemlock has a much smaller distribution and is restricted to the southeastern United States where it is endemic to the southern Appalachian Mountain and Upper Piedmont regions of Virginia, Tennessee, Georgia, North Carolina, and South Carolina (Jetton et al. 2008). Unlike Eastern Hemlock, Carolina Hemlock is

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most abundant along dry, north-facing, rocky ridge-tops at elevations of 600–1500 m on soils that are dry, acidic, and nutrient-poor (Humphrey 1989).

Both Eastern and Carolina Hemlocks are considered to be at-risk species, and a number of factors threaten their long-term sustainability in eastern North America (Beardmore et al. 2006, Farjon et al. 1993). The most serious of these threats is the invasive *Adelges tsugae* Annand (Hemlock Woolly Adelgid [HWA]), an exotic, aphid-like insect introduced from Japan into the eastern United States in the early 1950s that currently infests approximately 50% of the Eastern Hemlock range and 100% of the Carolina Hemlock range (USDA Forest Service 2012). HWA feeds at the base of hemlock needles by inserting its piercing/sucking mouthparts and extracting stored nutrients from xylem ray-parenchyma, thereby disrupting vegetative and reproductive bud formation, causing needle desiccation and defoliation, and killing trees in 4–10 years (Young et al. 1995). Although estimates of the number of hemlocks killed by HWA in eastern North American are difficult to derive, widespread decline and morality have occurred among populations of both native hemlock species and continues unabated in most areas.

The integrated strategy to manage the impacts of HWA on hemlock ecosystems in eastern North America includes a cooperative genetic-resource conservation program being conducted by Camcore (International Tree Breeding and Conservation Program) at NC State University, Raleigh, NC, and the USDA Forest Service Forest Health Protection, Asheville, NC. The objectives of this effort are to improve the general understanding of native hemlock genetic diversity, climatic and edaphic adaptability, reproduction and regeneration ecology, and silvicultural options for seed-orchard establishment, and to utilize this information to design and implement ex situ germplasm conservation strategies for both Eastern Hemlock and Carolina Hemlock (Jetton et al. 2013). The present study addresses one aspect of hemlock reproductive biology, the effect of cold–moist stratification treatments for alleviating seed dormancy and improving germination.

Hemlocks have male and female strobili that develop on the same branches (monoecious), and these are well distributed throughout the crown in open-canopy conditions and are more restricted to the upper crown under closed canopies (Barbour et al. 2008). Heavy cone crops in natural stands occur at 3–8-year intervals (Godman and Lancaster 1990, Means 1990, Packee 1990). Several studies have evaluated cold stratification treatments for improving germination of hemlock seeds collected from natural stands for a number of *Tsuga* species, including Eastern Hemlock (Baldwin 1930, 1934; Stearns and Olson 1958), *T. heterophylla* (Raf.) Sarg. (Western Hemlock) (Allen 1958, Edwards 1973, Edwards and Olsen 1973, Li and Burton 1994), and *T. mertensiana* (Bong.) Carr. (Mountain Hemlock) (Edwards and El-Kassaby 1996, El-Kassaby and Edwards 2001). Stratification treatments to improve seed germination in Carolina Hemlock have not been studied previously.

The objective of this study was to determine the effects of cold–moist stratification treatments of varying duration (0–120 d) on total germination and the number of days to reach first germination and peak germination for 6 natural-stand seed-sources of Eastern Hemlock and Carolina Hemlock (3 seed sources per species).

The data will be used to improve seed-management recommendations for foresters in Brazil, Chile, and the United States who are producing seedlings of both species in nurseries for ex situ conservation seed orchards as well as for land managers throughout eastern North America interested in growing native hemlocks for reforestation purposes.

Materials and Methods

We collected ripe seed cones in September (Carolina Hemlock) and October (Eastern Hemlock) 2009 from 6 natural stands (3 per species) located in the central and southern Appalachian Mountains of the eastern United States (Table 1).

We placed seed cones into cloth bags and stored them in a dry greenhouse ventilated to ambient conditions for 30 d to facilitate the opening of cones and release of seeds from the cone scales (Karrfalt 2008). After cone drying, we extracted seeds from the cones using a shaker box, de-winged them in a small tumbling drum, and removed empty seeds, loose wings, and chaff using a seed blower (Seedburo Model 757, Seedburo Equipment Company, Des Plaines, IL). We removed more empty seeds by conducting a float test in water. We determined the moisture content of a subset of seeds from each seedlot using a Mettler-Toledo moisture analyzer (Mettler-Toledo, Inc., Columbus, OH); average seed moisture content was 7.25% for Eastern Hemlock and 7.66% for Carolina Hemlock. We packaged the remaining seeds in small re-sealable plastic bags and placed them in dry–cold storage at 4 °C for 90 days prior to the beginning of the study.

Pre-germination treatments consisted of cold–moist stratification (hereafter stratification) of seeds for 0, 1, 15, 30, 60, 90, and 120 days at 4 °C in a dark walk-in cooler. For stratification, we soaked all seeds in distilled water for 24 h at room temperature and placed them in 9-cm Petri dishes with a substrate of white germination paper moistened to saturation with distilled water. Each Petri dish contained 50 seeds and there were 4 dishes per species/treatment/provenance combination for a total of 168 petri dishes and 8400 seeds in the study. We checked dishes daily, and

Table 1. Provenance location and Hemlock Woolly Adelgid infestation status for Eastern Hemlock and Carolina Hemlock seed sources utilized in the seed stratification study. Least square mean total germination (± SE) across all cold–moist stratification treatments is also reported for each seed source. Lat = latitude (ºN); long = longitude (ºW); elev = elevation (m); total germ = total germination (%).

Species/provenance	County, State	Lat	Long	Elev	HWA status	Total germ
Eastern Hemlock						
Cook Forest	Forest, PA	41.33	79.20	387	None	43.4 (± 5.3)
Kentland Farm	Montgomery, VA	37.21	80.60	556	Infested	8.3 (± 1.6)
Lake Toxaway	Transylvania, NC	35.12	82.95	922	Treated	48.1 (± 6.3)
Carolina Hemlock						
Carl Sandburg	Henderson, NC	35.27	82.44	682	Treated	31.0 (± 2.2)
Hanging Rock	Stokes, NC	36.41	80.26	662	Infested	10.2 (± 1.1)
New River	Montgomery, VA	37.39	80.74	606	Infested	9.9 (± 1.1)

remoistened the germination paper as needed. We began the seed stratification with the 120-d treatment and worked backwards so that all Petri dishes were ready to start the germination period of the experiment on the same day. All seeds remained packaged in the resealable plastic bags at 4 °C until placed into their particular stratification treatments. Therefore, seeds for each treatment experienced different lengths of dry–cold storage, for example, 90 days for the 120-d stratification treatment and 210 days for the 0-day treatment.

We conducted the seed-germination experiment over a 30-day period in an environmental chamber at the North Carolina State University Southeastern Plant Environment Laboratory (Phytotron). Environmental conditions in the chamber were a constant temperature of 22 °C, 20–50% relative humidity, and 16:8 light–dark photoperiod, lit by a combination of fluorescent and incandescent bulbs that provided approximately 31.6 klx of illumination. We placed Petri dishes on two shelves inside the chamber with two blocks of dishes per shelf. Each block contained 1 Petri dish per species/provenance/treatment combination. We remoistened the dishes with distilled water daily during the germination period of the experiment. During daily inspection, we recorded the number of newly germinated seeds in each dish. We classified seeds as germinated when the emerging radicle was 5 mm long. At the end of the 30-d germination period, we calculated the total number of seeds germinated and the number of days to first and peak germination for each Petri dish in the experiment.

Statistical analysis

We analyzed the germination data for Eastern Hemlock and Carolina Hemlock seeds using a logistic regression model with a binomial distribution and logit link function in the GLIMMIX procedure of SAS 9.2 (SAS 2008) to evaluate the probability of seed germination after 30 days. The response variable was total percent germination (hereafter referred to as total germination), defined in the model statement by the events/trials syntax or the number of germinated seeds per petri dish/total seeds per petri dish. The model tested the main effects of block, species, provenance (species), and stratification and all two-way interactions on total germination. To investigate within-species variation, we used similar logistic regression models to test the main effects of block, provenance, and stratification, and all two-way interactions on the probability of seed germination after 30 days for each species individually. We conducted analysis of variance using the GLM procedure of SAS 9.2 to analyze the number of days to first germination and peak germination for the species individually, testing the main effects of block, provenance, stratification, and all two-way interactions. All means reported are least square means. All variances reported are standard errors. Only significant effects from the analysis are reported in the results.

Results

Of the 8400 Eastern Hemlock and Carolina Hemlock seeds assessed in this study, 2114 or 25% germinated successfully by the end of the 30-d experiment. The logistic regression analysis including species as a main effect indicated that stratification ($P = 0.0039$), species ($P < 0.0001$), provenance (species) ($P < 0.0001$), and the interaction of species*stratification ($P < 0.0001$) significantly affected the probability of seed germination. Total germination was higher for Eastern Hemlock (33.3 ± 3.4%) compared to Carolina Hemlock (17.1 ± 1.4%), and ranged from 8.3–48.1% among the provenances (Table 1). The 3 provenances with the highest total germination were either uninfested or had received insecticide treatments in the field to control HWA. The 3 provenances with the lowest germination were HWA-infested at the time of seed collection.

The probability of seed germination for Eastern Hemlock was significantly affected by stratification ($P < 0.0001$), provenance ($P < 0.0237$), and the provenance*stratification interaction ($P < 0.0001$). The overall species trend was for increasing total germination with increasing duration of the stratification treatments (Fig. 1). We also observed this trend for the individual provenances, although the slope of increase for the Kentland Farm seed-source was much lower than that for the other 2 populations. Total germination of seeds from the Kentland Farm population was 8%, which was 35 and 40% lower than seeds from the Cook Forest and Lake Toxaway populations, respectively (Table 1).

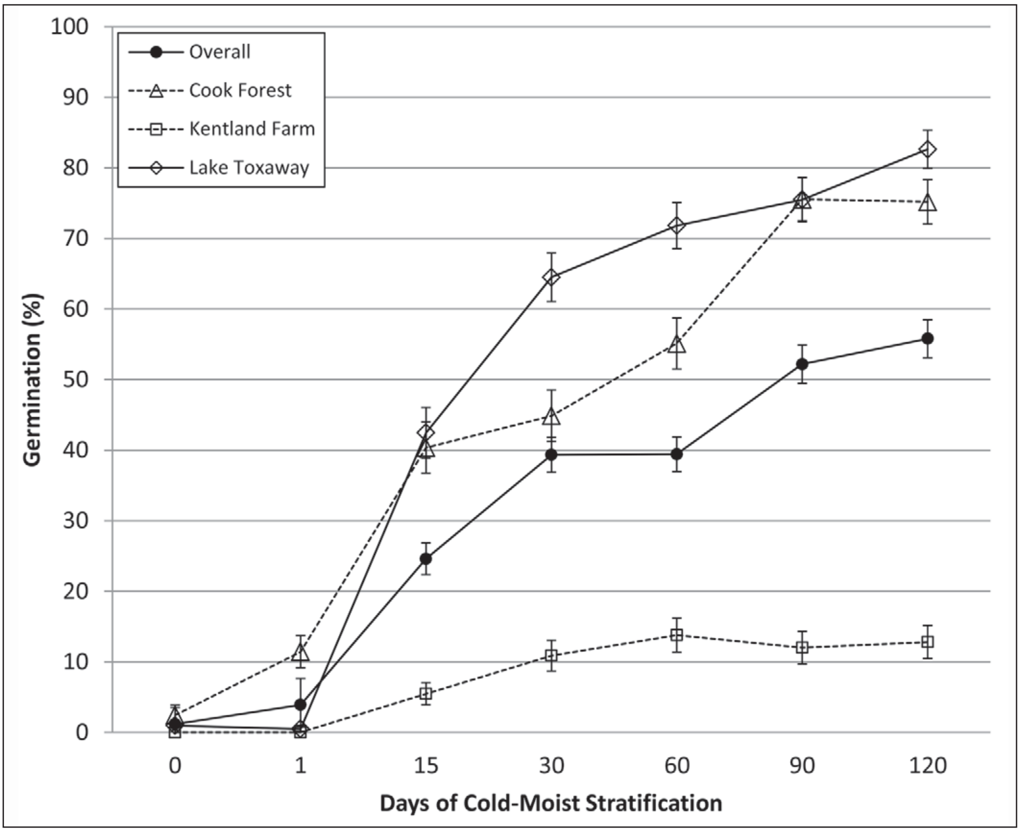

Figure. 1. Least square mean (± SE) total-germination response of Eastern Hemlock seed to cold–moist stratification treatments at the species (overall) and individual provenance levels.

Seed germination for Carolina Hemlock was significantly affected by stratification ($P = 0.0040$) and provenance ($P < 0.0001$) and there was much less variation in total germination among the stratification treatments compared to Eastern Hemlock. Germination varied little from 0–30 days, and then decreased following the 60, 90, and 120-d treatments (Fig. 2). The tendency of total germination to decrease following the longer-duration stratification treatments was apparent for the individual provenances as well. Total germination among seeds from the Carl Sandburg population was 21% higher than for seeds from Hanging Rock and New River (Table 1).

The speed of Eastern Hemlock and Carolina Hemlock seed germination was significantly influenced by the stratification treatment and provenance as well. Overall, Carolina Hemlock seeds germinated first and reached peak germination sooner (6.7 ± 0.2 d and 14.2 ± 0.5 d, respectively) compared to Eastern Hemlock (8.4 ± 0.7 d and 18.2 ± 0.7 d, respectively). Although overall germination speed in Eastern Hemlock was slower, stratification improved the pace of germination in this species. Among Eastern Hemlock seeds, the number of days to first germination was significantly affected by both stratification and provenance ($P < 0.0001$), while the number of days to peak germination was affected by stratification alone ($P = 0.0029$). As the duration of the stratification treatments increased, it took fewer days to attain both first and peak germination (Table 2). Among the provenances, the Kentland Farm seed source took longer to reach first and peak germination compared to Cook Forest and Lake Toxaway.

Like Eastern Hemlock, the number of days to first germination in Carolina Hemlock was also significantly affected by stratification ($P = 0.0021$) and provenance ($P = 0.0075$). However, we observed the opposite response to stratification: the number of days to first germination increased slightly with increasing duration of stratification (Table 2). The number of days to peak germination was significantly

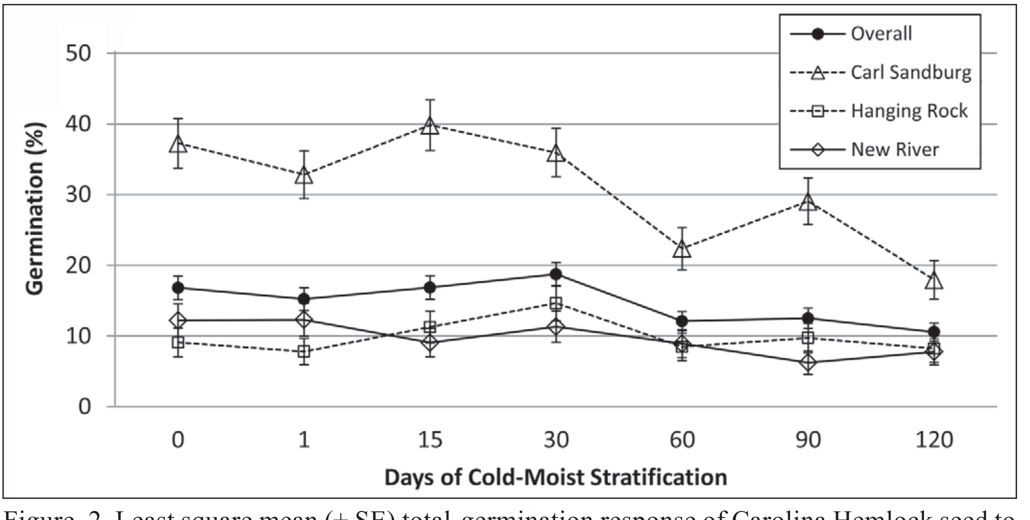

Figure. 2. Least square mean (± SE) total-germination response of Carolina Hemlock seed to cold–moist stratification treatments at the species (overall) and individual provenance levels.

affected by provenance ($P < 0.0001$), with the Carl Sandburg seed source taking the fewest days to reach first germination and the most days to reach peak germination (Table 2).

Discussion

The overall total germination of 25% reported in this study is within the expected range (20–35%) for seeds from natural stands of Eastern Hemlock and Carolina Hemlock (Godman and Lancaster 1990, Jetton et al. 2008). Total species-level germination of 33% and 17% is consistent with the results of germination tests conducted on 451 and 134 natural-stand seed sources of Eastern Hemlock and Carolina Hemlock, respectively, that have been collected for genetic-resource conservation (Jetton et al. 2013). These levels of seed germination are lower than those expected for the 2 other hemlock species native to North America, Western Hemlock and Mountain Hemlock, where natural-stand seed germination of 50–75% is typical (Means 1990, Packee 1990).

Eastern Hemlock and Carolina Hemlock are sympatric species in the southern Appalachian Mountains, and many silvicultural descriptions have historically lumped the two species together, leaving the reader to assume them to be similar in most aspects of their biology (e.g., Godman and Lancaster 1990). However, under the experimental conditions tested here, in terms of total germination and germination speed, seeds of these 2 hemlock species demonstrated substantially different responses to the cold–moist stratification treatments. Stratification generally improved the germination of Eastern Hemlock seeds, and the amount of improvement

Table 2. Least square mean (± SE) number of days to first germination and peak germination for Eastern Hemlock and Carolina Hemlock seeds among the cold–moist stratification (CMS) treatments (in days of stratification) and provenances.

	Days to first germination		Days to peak germination	
	Eastern Hemlock	Carolina Hemlock	Eastern Hemlock	Carolina Hemlock
CMS treatment				
0	17.7 (± 1.8)	5.6 (± 0.1)	27.0 (± 1.1)	13.4 (± 1.2)
1	17.6 (± 1.1)	6.2 (± 0.4)	20.0 (± 1.5)	16.0 (± 1.2)
15	12.1 (± 0.9)	6.6 (± 0.4)	20.4 (± 0.47)	13.0 (± 1.5)
30	10.6 (± 1.1)	6.8 (± 0.4)	20.3 (± 1.4)	13.6 (± 1.1)
60	6.6 (± 0.9)	6.6 (± 0.5)	16.2 (± 1.1)	15.3 (± 1.6)
90	4.6 (± 1.7)	7.6 (± 0.4)	18.0 (± 1.8)	14.5 (± 1.7)
120	1.0 (± 0.1)	7.6 (± 0.2)	12.1 (± 2.1)	14.6 (± 1.3)
Provenance				
Cook Forest	7.6 (± 1.1)	-	17.9 (± 1.2)	-
Kentland Farm	10.0 (± 1.5)	-	19.4 (± 1.3)	-
Lake Toxaway	7.8 (± 1.4)	-	17.5 (± 1.3)	-
Carl Sandburg	-	6.1 (± 0.2)	-	18.4 (± 0.9)
Hanging Rock	-	7.1 (± 0.3)	-	13.0 (± 0.6)
New River	-	6.9 (± 0.2)	-	11.3 (± 0.7)

in both total germination and germination speed increased with increasing duration of the stratification treatments. By comparison, total germination of Carolina Hemlock seed varied less among the stratification treatments and tended to decrease slightly following the longer durations of stratification (60, 90, and 120 d).

The favorable response of Eastern Hemlock seed germination to stratification is consistent with previous studies on this species as well as those with Western Hemlock. Stratification of Eastern Hemlock seeds in cold–moist peat for 30–70 days improved both total germination (%) and germination rate (days to 50% germination) (Baldwin 1930, 1934; Stearns and Olson 1958). In studies with Western Hemlock, cold stratification periods of 7–40 days improved both total germination and germination rate (Allen 1958, Li and Burton 1994) or germination rate alone (Edwards 1973, Edwards and Olsen 1973). The response of Carolina Hemlock seeds to stratification treatments is similar to what is known from studies with Mountain Hemlock where seed stratification had no meaningful effect on total germination (Edwards and El-Kassaby 1996, El-Kassaby and Edwards 2001). However, Edwards and El-Kassaby (1996) found that stratification improved the germination rate of Mountain Hemlock seeds, while in the present study Carolina Hemlock seed germination speed was relatively unaffected by stratification and even slightly slower in days to first germination.

This experiment was designed to understand how differing lengths of cold–moist stratification affect Eastern and Carolina Hemlock seed germination rates. We did not address why the species and provenances responded to the treatments in the manner they did, but the data do suggest several interesting questions for future studies. First, are the species-level seed-germination responses to moist stratification related to the soil moisture conditions experienced by the species in their typical habitats? Eastern Hemlock typically inhabits moist soils (Kessell 1979) and it responded favorably to stratification, while Carolina Hemlock typically inhabits dry soils and it showed little response to cold–moist stratification (Humphrey 1989). Second, are seed germination responses controlled at the population level and determined by the climate, soil-moisture conditions, effective population sizes, and pollen loads experienced by trees in the individual provenances? Any one of these factors might explain why the Kentland Farm population had a much more muted response to cold–moist stratification compared to the other Eastern Hemlock seed sources. Seed-source differences have been shown to have significant influence on seed germination in Mountain Hemlock (El-Kassaby and Edwards 2001). Finally, how might HWA infestation and soil insecticide injections influence flowering, seed set, and subsequent seed germination in Eastern Hemlock and Carolina Hemlock? Both are likely to have significant implications for the quality of seeds collected for genetic-resource conservation programs, the development of soil seedbanks, and the potential for natural stand regeneration following large-scale HWA-related decline and mortality.

Further research is also needed to better understand how the germination of Eastern Hemlock and Carolina Hemlock seeds might differ under varying temperature and photoperiod regimes. For example, the germination rate of Western Hemlock seeds benefits from photoperiod regimes with relatively short 4-hour light periods (Edwards and Olsen 1973), and total germination and germination rate of Mountain Hemlock seeds is best under alternating temperature regimes of 25:15 and 20:15 °C on a complementary 8:16 L:D photoperiod (El-Kassaby and Edwards 2001). Stearns and Olson (1958) found that the germination of Eastern Hemlock seed varies considerably with both temperature and photoperiod, and that the most favorable photoperiod depends on the temperature under which germination is carried out.

Based on the data available from this study, we suggest the following for stratifying seeds of Eastern Hemlock and Carolina Hemlock prior to sowing and germinating seeds at 22 °C under a 16:8 L:D photoperiod. Following a 24-hour water soak, Eastern Hemlock seeds should be cold–moist stratified at 4 °C for at least 30–60 d prior to sowing to promote higher total germination, recognizing that, although it may not be operationally efficient, an additional 10–15% improvement in germination may be achieved with stratification periods of 90–120 d. Carolina Hemlock seeds can be sown directly following a 24-hour soak with no additional cold–moist stratification, although total germination of some seedlots may improve slightly following 30 days of stratification. Longer stratification periods appear to decrease germination in Carolina Hemlock and should be avoided.

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Literature Cited

Allen, G.S. 1958. Factors affecting the viability and germination behavior of coniferous seed: Part 1. Cone and seed maturity, *Tsuga heterophylla* (Rafn.) Sarg. The Forestry Chronicle 34:266–274.

Baldwin, H.I. 1930. The effect of after-ripening treatment on the germination of Eastern Hemlock seed. Journal of Forestry 28:853–857.

Baldwin, H.I. 1934. Further notes on the germination of hemlock seed. Journal of Forestry 32:99–100.

Barbour, J.R., R.H. Ruth, and R.P. Karrfalt. 2008. *Tsuga* Carr., hemlock. Pp. 1127–1139, *In* F.T. Bonner and R.P. Karrfalt (Eds.). The Woody Plant Seed Manual. USDA Forest Service Agriculture Handbook 727. Washington, DC.

Beardmore, T., J. Loo, B. McAfee, C. Malouin, and D. Simpson. 2006. A survey of tree species of concern in Canada: The role for genetic conservation. The Forestry Chronicle 82:351–363.

Edwards, D.G.W. 1973. Effects of stratification on Western Hemlock germination. Canadian Journal of Forest Research 3:522–527.

Edwards, D.G.W., and Y.A. El-Kassaby. 1996. The effect of stratification and artificial light on the germination of Mountain Hemlock seeds. Seed Science and Technology 24:225–235.

Edwards, D.G.W., and P.E. Olsen. 1973. A photoperiod response in germination of Western Hemlock seeds. Canadian Journal of Forest Research 3:146–148.

El-Kassaby, Y.A., and D.G.W. Edwards. 2001. Germination ecology in Mountain Hemlock (*Tsuga mertensiana* (Bong.) Carr.). Forest Ecology and Management 144:183–188.

Farjon, A. 1990. Pinaceae. Drawings and descriptions of the genera *Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix, and Picea*. Koeltz Scientific Books, Konigstein, Germany.

Farjon, A., C.N. Page, and N. Schellevis. 1993. A preliminary world list of threatened conifer taxa. Biodiversity and Conservation 2:304–326.

Godman, R.M., and K. Lancaster. 1990. *Tsuga canadensis* (L.) Carr., Eastern Hemlock. Pp. 604–612, *In* R.M. Burns and B.H. Honkala (Eds.). Silvics of North America Vol. 1. Conifers. USDA Forest Service Agriculture Handbook 654. Washington, DC.

Humphrey, L.D. 1989. Life-history traits of *Tsuga caroliniana* Engelm. (Carolina Hemlock) and its role in community dynamics. Castanea 54:172–190.

Jetton, R.M., W.S. Dvorak, and W.A. Whittier. 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 255:3212–3221.

Jetton, R.M., W.A. Whittier, W.S. Dvorak, and J. Rhea. 2013. Conserved ex situ genetic resources of Eastern and Carolina Hemlock: Eastern North American conifers threatened by the Hemlock Woolly Adelgid. Tree Planters' Notes 56:59–71.

Karrfalt, R.P. 2008. Seed harvesting and conditioning. Pp. 57–83, *In* F.T. Bonner and R.P. Karrfalt (Eds.). The Woody Plant Seed Manual. USDA Forest Service Agriculture Handbook 727. Washington, DC.

Kessell, S.R. 1979. Adaptation and dimorphism in Eastern Hemlock, *Tsuga canadensis* (L.) Carr. American Naturalist 113:333–350.

Li, X.J., and P.J. Burton. 1994. Interactive effects of light and stratification on the germination of some British Columbia conifers. Canadian Journal of Botany 72:1635–1646.

Means, J.E. 1990. *Tsuga mertensiana* (Bong.) Carr. Mountain Hemlock. Pp. 623–634, *In* R.M. Burns and B.H. Honkala (Eds.). Silvics of North America Vol. 1. Conifers. USDA Forest Service Agriculture Handbook 654. Washington, DC.

Packee, E.C. 1990. *Tsuga heterophylla* (Raf.) Sarg. Western Hemlock. Pp. 613–622, *In* R.M. Burns and B.H. Honkala (Eds.). Silvics of North America Vol. 1. Conifers. USDA Forest Service Agriculture Handbook 654. Washington, DC.

SAS Institute. 2008. The SAS System for Windows, Version 9.2. Cary, NC.

Stearns, F., and J. Olson. 1958. Interactions of photoperiod and temperature affecting seed germination in *Tsuga canadensis*. American Journal of Botany 45:53–58.

USDA Forest Service. 2012. Counties with established HWA Populations 2012. Available online at http://na.fs.fed.us/fhp/hwa/maps/2012.pdf. Accessed 22 November 2013.

Young, R.F., K.S. Shields, and G.P. Berlyn. 1995. Hemlock Woolly Adelgid (Homoptera: Adelgidae): Stylet bundle insertion and feeding sites. Annals of the Entomological Society of America 88:827–835.