Germination of Carolina Silverbell Seed

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Seeds o f Carolina silverbell (Halesia tetraptera *Ellis var.* tetraptera) *from 4 seed lots were subjected to 7 pretreatment/stratification regimes. Germination rate and percentage differed significantly among seed lots, ranging from 0 to 80% at the end of 65 weeks. It appeared that seed lots varied in their requirements for cold stratification. An 8-hour sulfuric acid soak, followed by a warm-cold-warm stratification cycle significantly increased the rate and cumulative germination at the end of the 65 weeks for 3 of the 4 seed lots examined.* Tree Planters' Notes 46(4):134-137; 1995.

Carolina silverbell *(Halesia tetraptera* Ellis var. *tetraptera,* formerly *H. carolina* L.) is a native understory tree that provides a source of food for wildlife (Bonner and Mignery 1974). It also has potential for increased usage as an ornamental in the landscape. The species shows a high degree of pest tolerance and cold hardiness (Dirr 1990). Found in riparian and wooded areas, the species has a native range extending from Florida to West Virginia to eastern Oklahoma. The cultivated range is broader— USDA hardiness zones 5-8 (USDA 1990)— with limited possibilities in zone 4 (Dirr 1990).

Carolina silverbell can be propagated by seed or asexually by cuttings or micropropagation (Dirr and Heuser 1987). Seed propagation of this native tree would allow the nursery industry to preserve genetic diversity for reforestation and landscape uses. Seed propagation is usually done by dewinging the fruit, a 4-winged, reddish-brown, corky drupe with a 4-celled ellipsoid stone ovary (Bonner and Mignery 1974), and sowing the stone (seed).

Seed propagation has been reported to require either complex temperature regimes or a 2-year natural cycle for maximum uniform germination. Seed requires warm moist stratification, followed by a cold moist stratification period for germination (Bonner and Mignery 1974, Dirr and Heuser 1987, Giersbach and Barton 1932). Moist stratification at 13.3 °C (56 °F) for 60 to 120 days, followed by 60 to 90 days at 1 to 5 °C (33 to 41 °F) was effective in one study (Bonner and Mignery 1974); however, a second cold stratification period was required in another study (Dirr and Heuser 1987).

Deno (1993) reported that Barton and Crocker (1948) increased germination with sulfuric acid but did not give details of the treatment. Cutting both tips of freshly ripened fruits may also increase rate of germination (Dr. W. Witte. Personal communication. Orono: University of Maine); however, Bir (1987) was not able to overcome fruit dormancy by cutting a notch in the seed coat.

The reported discrepancies suggests that other factors, such as seed source, may affect germination requirements. Seeds lots have been reported to differ in their degree of dormancy (Young and Young 1992).

The objectives of this study were to (1) examine fruit pretreatments and stratification procedures to determine if accelerated uniform germination could be improved over the standard procedure of no pretreatment followed by warm-cold-warm stratification and (2) examine the role of different seed lots in optimizing germination.

Materials and Methods

In November 1992, mature fruits were obtained from 4 wild-collected sources (table 1). These collections were part of a larger provenance trial being conducted and were chosen because they had large numbers of fruit. They are not meant to represent the geographic range from which they were sampled.

Immediately following collection, fruits were stored in sealed plastic bags and placed in a refrigerator (4 °C). Wings were removed from each fruit by hand in February/March 1993. Fruits from each of the 4 seed lots were mixed and divided into 7 groups of 100, or 7 equal quantities for seed lots with less than 700 fruit. The number of fruit per subgroup for each seed lot was West Virginia, 100; Tennessee, 33; Georgia, 100; and North Carolina, 98. Fifty surplus fruit from the West

Table 1-*Carolina silverbell seed lots examined for germination*

Location	No. of trees	Collector
Kanawha Co., West Virginia	12	Dr. Edward Garvey U.S. National Arboretum
Macon Co., Tennessee	1	Dr. Scott Schlarbaum University of Tennessee
Polk Co., North Carolina	1	Dr. Scott Schlarbaum University of Tennessee
Richmond Co., Georgia	6	Mr. George Barrett

Virginia lot and 100 from the Georgia lot were removed from cold storage in the spring of 1995 and examined with a cut test to determine the percentage of sound seed.

The 7 subgroups were subjected to four pretreatments and three stratification cycles (table 2). Insufficient fruit did not allow for all 12 possible combinations. The fruit pretreatments included (1) nontreated, (2) tip cutting, (3) hot water, and (4) sulfuric acid. For the second pretreatment, the pedicel end of each fruit was excised with pruners carefully, so that the embryo was not cut into. The process was time consuming because embryo chamber location varied with fruit size. The purpose of this treatment was to provide an easy imbibition point for moisture and an "exit" point for embryos. For the third pretreatment, hot water (exact temperature unknown, >85 ºC) was poured over the fruit and left to soak for 24 hours. For the fourth pretreatment, fruits were soaked for 8 hours in concentrated sulfuric acid, followed by a 21-hour soak in water to thoroughly rinse the fruits. The water was changed after 16 hours and the fruits were rubbed to remove any of the outer deteriorated fruit/stone. The 8-hour acid soak was selected after a preliminary trial with a local seed source indicated that embryos were not damaged in that time (visual observation only).

On March 11 and 12, 1993, after pretreatments were performed, fruits for each of the seven treatments were sown in plastic seed flats (24 x 58 x 9 cm) with drainage holes. The medium consisted of half milled sphagnum and half Q-roc #4 sand. Flats were filled with 8 cm of medium and fruits planted approximately 2 cm deep.

To reduce the length of the study, stratification cycles lasted for approximately 12 weeks. These are shorter than natural cycles, but a 2 to 2 ½-year natural time period was simulated in 65 weeks. If germination occurred while a tray was undergoing cold stratification it was taken into the greenhouse the week germination began. This reduced the cold stratification for some flats (noted in table 2). Three stratification regimes were examined: no cold stratification, warm-cold-warm-cold-warm (W-C-W-C-W), and cold-warm-cold-warm-warm (C-W-C-W-W). Cold stratification was accomplished by placing the flats in a dark walk-in refrigerator (4 ºC). During warm periods, flats were placed in a greenhouse where temperatures were maintained between 24 and 35 ºC. Seed flats were kept moist, but not saturated.

The flats were examined weekly for shoot emergence, that is, germination. Cumulative germination, the total number of germinants divided by the number of fruit sown, was determined for each seed lot by treatment combination at the end of each stratification cycle.

Differences in germination were tested using a linear model with the SAS CATMOD procedure (SAS 1988). The first model examined the effect of seed lot, treatment, and their interaction (independent variables) on germination at the end of each cycle (dependent variable). This procedure is similar to linear regression/ ANOVA for continuous dependent variables, except that the dependent variable is categorical instead of continuous. The categorical model allowed for the testing of the treatment-by-seed lot interaction term; this would not have been possible using normal linear regression.

Table *2-Cumulative germination percentage by treatment at 14 to 65 weeks after sowing averaged over 3 seed lots of Halesia carolina (NC,GA, and WV); dates represent the end of each stratification cycle*

Pretreatment	Stratification[z]	Percent germination				
		Week 14 (6/18/93)	Week 26 (9/17/93)	Week 39 (12/10/93)	Week 51 (3/11/94)	Week 65 (6/17/94)
Untreated	W-C-W-C-W	1	2[y]	32	35[x]	54
Cut tip[w]	W-C-W-C-W	3	3	41	44[v]	52
Hot H20[u]	W-C-W-C-W	3	4[t]	32	41[s]	53
Acid[r]	W-C-W-C-W	15	17[y]	72	73[q]	88
Untreated	C-W-C-W-W	0	5	6	43	51
Cut tip	C-W-C-W-W	0	4	4	29	45
Cut tip	W-W-W-W-W	6	17	25	34	47

z Refers to warm and cold periods, each lasting about 12 weeks.
y North Carolina and Georgia lots moved to greenhouse 2 weeks early because of active germination; cumulative germination reflects 24 weeks' cumulative germir.ation.
x North Carolina lot moved to greenhouse 7 weeks early because of active germination.
w The pedicel tip of seed was cut, with care taken to avoid the embryos.
v Georgia lot moved 6 weeks early and North Carolina lot moved 4 weeks early to green house because of active germination.
u Twenty-four-hour water soak pretreatment.
t West Virginia and Georgia lots moved to greenhouse 6 weeks early because of active germination.
s Georgia lot moved to greenhouse 7 weeks early because of active germination.
r Eight-hour soak in concentrated sulfuric acid, followed by a 24-hr rinse/soak in water.
q North Carolina lot moved to greenhouse 3 weeks early because of active germination.

Chi-squares (χ^2) are produced for each dependent variable and used to test the significance level. For a more detailed explanation see Grizzle and others (1969) and SAS (1988). A second model tested for differences in seed lot germination patterns over time. Cumulative germination was the dependent variable and the independent variables were: week, treatment, lot, treatment-by-lot, week-by-lot, and week-by-treatment. Week (time) was modelled as a categorical variable and represented the end of each cycle. This was done because the cold stratification periods resulted in uneven germination patterns, and a complex function would be required to model time as a continuous variable.

Results and Discussion

Seed lots differed significantly in cumulative germination at the end of each cycle. Ignoring the Tennessee seed lot, which did not germinate in these accelerated treatments (7% germination was found in the accompanying provenance study), significance levels (α) for seed lots ranged from 0.034 to 0.000 (table 3). An examination of 6 fruits from the Tennessee lot halfway through the study found that half had embryos. The cut tests of 50 and 100 fruits showed the West Virginia seed lot had 82% filled fruit (1.18 embryos/fruit) and the Georgia lot had 86% filled fruit (1.09 embryos/fruit). These statistically nonsignificant (α=0.27) differences in filled fruit did not reflect the substantial and statistically significant germination differences (43% germination for West Virginia, 80% germination for Georgia). Rankings of total germination by lot remained constant throughout the study; however, the rate of germination differed among lots as indicated by a significant lot-by-week interaction in the second model (χ^2=84.21, df=8, α=0.000). The Georgia and North Carolina seed lots germinated more quickly than the other two lots (table 4).

Treatment effects were examined using only the 3 seed lots that germinated (Georgia, North Carolina, and West Virginia). The acid-treated group had the highest germination percentage of any treatment group at the end of every cycle, with one exception (table 2). After 2 cycles (26 weeks), fruit with cut tips that had not undergone cold stratification had the same germination (statistically) as acid-treated fruit. After 3 cycles (39 weeks), the acid-treated fruit had almost twice the germination of the next best treatment group. The tip cutting and hot water pretreatments did not statistically increase germination on any date as compared to the standard warm–cold–warm stratification with no pretreatment.

After 65 weeks, total germination ranged from 45 to 88%. Except for the acid-treated group, germination was between 45 and 54%. A χ-square test indicated that these treatments were statistically equal (α=0.22). The acid treatment produced significantly more germination than any other treatment.

The 2 treatment groups that started out with cold stratification had minimal germination after their first warm period (table 2). This agrees with other studies that have shown the need for an initial warm stratification period before cold stratification (Bonner and Mignery 1974, Giersbach and Barton 1932).

Table 3—*"Analysis of variance" table examining the effect of the 7 accelerated treatments, 3 seed lots, and their interaction on germination at the end of each stratification cycle. The chi-square (χ^2) and significance level (α) are shown for the end of each cycle.*

		Week 14		Week 26		Week 39		Week 51		Week 65	
Lot	DF	χ^2	α	χ^2	α	χ^2	α	χ^2	α	χ^2	α
Intercept	1	278.9	0.000	315.9	0.000	139.9	0.000	41.3	0.000	38.4	0.000
Treatment	6	18.0	0.006	27.0	0.000	225.4	0.000	143.9	0.000	115.3	0.000
Lot	2	6.8	0.034	24.6	0.000	94.1	0.000	323.1	0.000	167.7	0.000
Treatment × lot	12	12.5	0.408	0.3	0.594	26.2	0.010	57.0	0.000	110.3	0.000
Residual	0										

Table 4 - *Cumulative germination percentage of 4 Carolina silverbell seed lots at 14 to 65 weeks from sowing averaged over 7 treatments. Dates represent the end of each stratification cycle*

	Percent germination				
Seed Lot	Week 14 (6/18/93)	Week 26 (9/17/93)	Week 39 (12/17/93)	Week 51 (3/11/94)	Week 65 (6/17/94)
West Virginia	0	0	15	20	43
Tennessee	0	0	0	0	0
Georgia	7	12	49	71	80
North Carolina	6	10	27	36	44

The tip cutting pretreatment was an unsuitable substitute for an initial warm stratification period. At the end of the second stratification cycle, the group receiving the tip- cutting pretreatment with no cold stratification had better germination than the treatment groups that began started with cold stratification. However this temporary improvement appeared to be a result of not having a cold period to slow germination, because by the end of the fourth cycle it had lost its superiority. When comparing within stratification regimes, the tip-cutting treatment group never had significantly more germination than the untreated groups. This is in agree ment with Bir (1987).

Treatment effects differed by seed lot; the treatment-lot interaction was significant at the end of the last 3 stratification cycles (table 3). The Tennessee seed lot did not germinate with any accelerated treatment (table 4). The acid-treated Georgia and North Carolina lots began to germinate without any cold stratification in the first cycle, whereas the West Virginia lot appeared to require a cold stratification period (figure 1). It is possible that the acid pretreatment overcame the need for a cold stratification period for the Georgia and North Carolina seed lots. Unfortunately it was impossible to tell whether these 2 lots would have continued to germinate without a cold stratification period because the study did not include an acid pretreatment without a cold stratification period. Examination of the non-pretreated W-C-W-C-W germination showed that the Georgia lot needed only one cold stratification period to achieve most of its germination potential, whereas the North Carolina and West Virginia lots benefited greatly from a second cold stratification (figure 1).

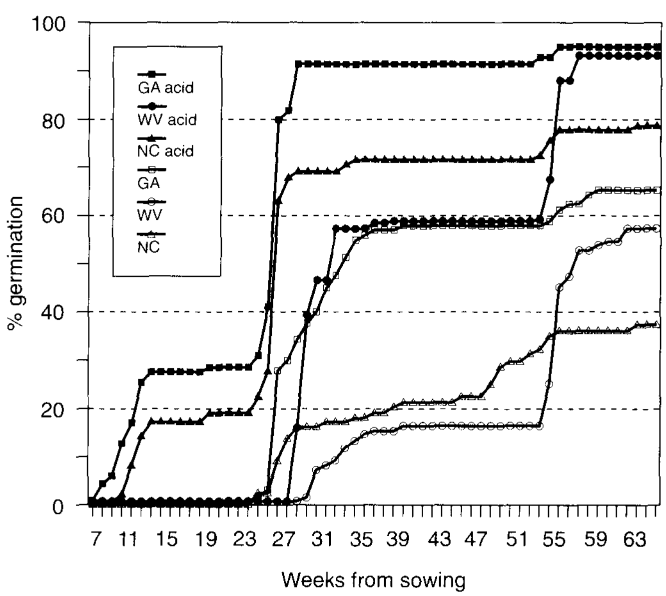

Figure 1— *Cumulative germination of 3 seed lots comparing no pretreatment and an 8-hour acid soak. Stratification cycle is weeks 1–4, warm; weeks 15–26, cold; weeks 27–39, warm; weeks 40–51, cold; and weeks 52–65, warm*

Conclusions

To achieve adequate germination a reliable source of seed must be used. Germination at 65 weeks for the four seed lots examined in this study ranged from 0 to 83%. The 8-hour soak in concentrated sulfuric acid increased germination rate and total germination for 3 of 4 seed lots. An acid soak before beginning a warm-cold-warm stratification cycle should increase overall germination and germination speed.

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