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Seed Stratification of an Intermountain West Chokecherry Ecotype

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Abstract

An experiment was carried out to evaluate the effects of stratification treatments on the germination of an Intermountain West ecotype of chokecherry (*Prunus virginiana* L.). Seeds from a single wild stand were cleaned and subjected to one of 12 treatments. Treatments included stratification at 3ºC for durations ranging from 0 to 24 weeks. Additional treatments included exposure to GA_3 during a 12-week stratification, or two stratification cycles interrupted by 4 weeks at 20ºC. Each treatment consisted of four replicates of 50 seeds each. None of the seeds stratified for 4 weeks or less germinated after 12 weeks at 20ºC. Germination percentage increased with stratification time from 8 to 16 weeks. With the 20 and 24 week stratification treatments, much of the germination occurred during the 3ºC stratification period. Interrupted stratification cycles did not increase germination percentages. Treatment with GA_3 had no significant effect on germination percentage.

Chokecherries (*Prunus virginiana* L.) are native to North America and widely distributed. Plants are commonly grown for revegetation projects and are increasingly being utilized as ornamentals in native landscaping. The fruit is commonly collected from the wild for home and small-scale commercial processing, but there is no commercial fruit production in the U.S. A rising interest in breeding efforts for more efficient chokecherry fruit production demands a deeper understanding of the germination and growth traits in this species. Published literature dealing with chokecherry propagation is lacking, with the few available studies focusing on northeastern U.S. and Canadian ecotypes (5, 9). Little is known about optimum conditions for seed propagation of the high-elevation ecotypes of the Intermountain West.

In evaluating germination behavior, it is prudent to consider the environmental conditions existent during the seed phase of the life cycle (2). Understanding that germination strategies are often habitat-correlated is useful in designing meaningful propagation experiments. Physiological seed dormancy is an example of a regional adaptation to climate that is common in species occupying predictable seasonal patterns (2). Our seed collection site in southeastern Idaho has predictable patterns of continuous snow cover in the winter providing an environment in which it is advantageous for seed germination to be delayed until favorable spring conditions. Many mountain species have been shown to require stratification temperatures comparable to the relatively constant soil temperatures under the snowpack (2, 4, 6, 7, 8, 10). It is generally accepted that stratification temperatures ranging from 0 to 5°C are appropriate for breaking seed dormancy. Lockley (5) reported that seeds of a northeastern ecotype of chokecherry that did not germinate at room temperature after a 16-week stratification treatment, did germinate after an additional 9 weeks of stratification, suggesting that two interrupted stratification periods may improve chokecherry germination. Gibberellic acid also has been shown to

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promote seed germination in related species including *Prunus persica* and *Malus arnoldiana* (1, 2, 3).

Our study was designed to examine the effect of cold moist stratification, both continuous and interrupted, and the addition of GA_3 on germination of an intermountain chokecherry ecotype.

Materials and Methods

Ripe fruit were collected from a wild stand in southeastern Idaho (N 42°19.501', W 111° 14.656'; 1886 m elevation) on 18 Aug. 2006, and stored at 3°C for approximately 2 weeks. Seeds were separated from the fruit and cleaned using a low-speed blender. Cleaned seeds were placed on paper towels and dried at room temperature for 4 days. Dried seeds were subjected to one of 12 treatments. Each treatment consisted of four replicate 100 mm ×15 mm Petri plates with 50 seeds per plate. Seeds were placed on sterile filter paper moistened with 6 mL of a 0.30% a.i. Captan solution (Captan 50 WP, Stauffer Chemical, Westport, CT). Plates were sealed and stratified at 3°C for durations of 0, 4, 8, 12, 16, 20, or 24 weeks or for various interrupted stratification cycles. The interrupted stratification treatments consisted of stratification for 4, 8, or 12 weeks interrupted by 4 weeks at room temperature before being returned to the 3°C stratification environment for 4 or 8 weeks (Table 1). Two additional treatments contained 0.03 or 0.30 mM GA_3 (ProGibb®, Valent BioSciences Corporation, Libertyville, Ill.) added to the stratification solution, and were stratified for 12 weeks. After stratification, plates were incubated at 20ºC in the dark, and seed germination was rated at weekly intervals for 4 weeks. Seeds were classified as germinated when the combined length of the taproot and hypocotyl reached approximately 2.5 cm in length.

Mean separation of the total percent germination in each treatment was carried out on arcsine square root transformed data using the LSMEANS PDIFF option in the GLM procedure of SAS© (SAS Institute, Cary NC). Untransformed means are shown in the tables.

Results and Discussion

Seeds stratified for 4 weeks or less did not germinate during stratification or after being

Table 1. Treatments of chokecherry (*Prunus virginiana* L.) seeds carried out during the winter of 2006-07, comparing a single stratification period of varying lengths (treatments 2 to 7) to multiple stratification periods interrupted by 4 weeks at room temperature (treatments 8 to 10) or GA_3 treatments (treatments 11 and 12).

Treatment	Description	Dates of stratification				Date germination observed
		Start	Finish	Start	Finish	
1	Control					--[z]
2	4 weeks	19 Oct	16 Nov			14 Dec
3	8 weeks	19 Oct	14 Dec			11 Jan
4	12 weeks	19 Oct	11 Jan			8 Feb
5	16 weeks	19 Oct	8 Feb			8 Mar
6	20 weeks	19 Oct	8 Mar			5 Apr
7	24 weeks	19 Oct	5 Apr			3 May
8	4 wk - 4 wk - 4 wk	19 Oct	16 Nov	14 Dec	11 Jan	8 Feb
9	8 wk - 4 wk - 8 wk	19 Oct	14 Dec	11 Jan	8 Mar	5 Apr
10	12 wk - 4 wk -8 wk	19 Oct	11 Jan	8 Feb	5 Apr	3 May
11	12 wk + 0.03 mM GA_3	19 Oct	11 Jan			8 Feb
12	12 wk + 0.30 mM GA_3	19 Oct	11 Jan			8 Feb

[z]no germination was observed in this treatment

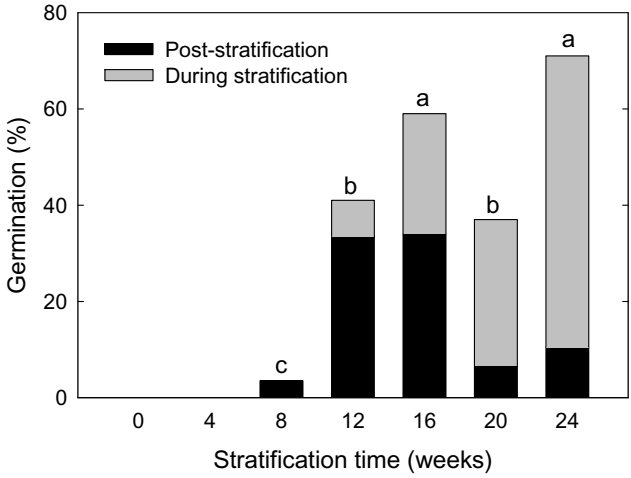

Figure 1. Mean total germination percentages for chokecherry (*Prunus virginiana* L.) seeds exposed to stratification at 3°C showing germination during stratification and germination in the ensuing 4 week incubation at 20°C. Letters indicate significant differences in total germination after four weeks at 20°C. Mean separation was carried out on arcsine transformed data using the LSMEANS PDIFF option in the GLM procedure of SAS©. Untransformed data are presented here.

transferred to the 20ºC incubation environment, indicating physiological seed dormancy (Figure 1). Seeds stratified for only 8 weeks had a mean germination of 4%, compared to 41% and 59% germination of seeds stratified for 12 and 16 weeks, respectively. Seeds exposed to 3ºC for 12 weeks began to germinate in the stratification environment, with the proportion of seeds germinating during stratification increasing with stratification duration (Figure 1). After 20 weeks of stratification, the majority of germination occurred before removal from the stratification environment (Figure 1). For the purposes of this study, germinated seeds were observed and removed to the greenhouse at weekly intervals. However, this would be impractical for large-scale germination. Therefore, the optimum stratification treatment (maximum post-stratification germination with minimum germination during stratification) was 16 weeks of continuous stratification.

Interrupted stratification regimes did not appear to have a significant effect on germination percentages. Seeds stratified for 8 weeks, whether continuously or interrupted by a 4 week warm cycle, exhibited similarly low germination percentages (treatments 3 and 8, Figure 1 and Table 2). The same phenomenon was observed in seeds stratified for 20 weeks, with the continuous stratification (37%, Fig. 1) and the interrupted stratification (27%, Table 2) being statistically similar. However, seeds stratified continuously for 16 weeks had significantly higher germination percentages (59%, Fig. 1) than those seeds whose 16 weeks of stratification was interrupted by 4 weeks of warm temperatures (17.5%, Table 2).

Table 2. Germination of chokecherry seeds treated with multiple stratification periods interrupted by 4 weeks at room temperature (RT).

3°C - RT - 3°C	Germination[z] (%)
4 wk - 4 wk - 4 wk	6.0 a
8 wk - 4 wk - 8 wk	17.5 b
12 wk - 4 wk -8 wk	26.5 b

[z] Means followed by the same letter were not significantly different at α = 0.05, as determined by using the LSMEANS PDIFF option in the GLM procedure of SAS.

Seeds exposed to 0.03 and 0.30 mM GA_3 during 12 weeks of stratification did not differ significantly in germination percentage compared to seeds stratified for 12 weeks without GA_3, where germination was 41%. However, germinated seeds from the 0.30 mM GA_3 stratification treatment produced seedlings with >2-fold increase in hypocotyl and internode lengths (data not shown).

For the ecotype in our study, optimum germination (59%) occurred with 16 weeks of uninterrupted cold moist stratification. Lockley (5) tested stratification periods of 10, 16, and 24 weeks, and then re-stratified ungerminated seeds for an additional 9 weeks. For seeds treated with a single stratification period, they also found 16 weeks to be optimal (77%). However, maximum germination occurred when seeds were re-stratified for an additional 9 weeks (5). We did not find improved germination with re-stratification, when compared to a single stratification period, which may reflect ecotypic differences in germination behavior. It is clear from our results that a single 16-week stratification at 3°C yields the highest germination percentage, with the lowest proportion of seeds germinating while still in the stratification environment, thus providing the uniformity in germination timing desired by propagators.

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