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Effect of stratification in polyethylene glycol solutions on germination of three North American shrub species

L.S. ROSNER[1] AND J.T. HARRINGTON[2]*

[1]Oregon State University Vegetation Management Research Cooperative, Corvallis, OR 97331-5752
[2]New Mexico State University Mora Research Center, Mora, NM 87732 (E-mail: joharrin@nmsu.edu)

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Summary

Cercocarpus montanus, *Amelanchier alnifolia* and *Shepherdia canadensis* are North American shrub species with seeds exhibiting physiological dormancy overcome by stratification (moist prechill). Germination of these species occurs during stratification, reducing the number of healthy non-dormant seeds available for sowing at the end of treatment. The effectiveness of incubating seeds in polyethylene glycol (PEG 8000, molecular weight 8000u) solutions to reduce germination during stratification was examined for each species using a factorial arrangement of PEG concentrations and 3°C stratification durations. PEG treatments reduced both moisture content and germination during stratification in a dose-dependent manner for each species. Incubation in PEG at concentrations necessary to suppress germination during stratification had differential effects on the total germination of each species, ranging from no reduction in total germination of *A. alnifolia* to a large reduction in the total germination of *S. canadensis*. A discussion of reduced oxygen availability in PEG stratification solutions and its effect on metabolic processes leading to dormancy release or secondary dormancy induction is presented. Species variability in response to stratification in PEG is discussed in terms of oxygen availability, minimum moisture content requirements and the removal of PEG after treatment.

Introduction

For many temperate woody species with physiological seed dormancy, germination occurs during stratification treatment, resulting in germinants that can become etiolated, weakened and susceptible to pathogens. Stratification at controlled moisture contents (from 2% to 10% below the moisture content at full imbibition) has been used to overcome this problem without reduction (and sometimes with improvement) in germination for numerous tree species (Edwards 1996, Jensen 1996, Poulsen 1996, Suszka *et al.*, 1996, Jones and Gosling 1994, Gosling and Rigg 1990). Moisture content is controlled by imbibing seeds (or re-drying fully imbibed seeds) to a pre-determined moisture content and stratifying seeds in polybags (permeable to air but not water) without media. This process requires careful monitoring of seed moisture content because moisture content can fall throughout the stratification period (Gosling and Rigg 1990). Moisture content can also be controlled during stratification by incubating seeds in a solution with the proper water potential. This technique has been implemented effectively with *Fagus sylvatica* L. (European beech) using 150-208 g/kg polyethylene glycol (PEG) 6000 solutions (-0.35

* Author for correspondence

to -0.8 megapascals [MPa]), which maintained moisture contents near 30%, about 10% below the moisture content at full imbibition (Muller and Bonnet-Massimbert 1983). Stratification in PEG (0 through -1.2 MPa) impaired germination of *Purshia tridentata* (Pursh) DC. (antelope bitterbrush) (Young and Evans 1976).

Cercocarpus montanus Raf. (mountain mahogany), *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roemer (western serviceberry) and *Shepherdia canadensis* [L.] Nutt. (russet buffaloberry) are North American shrub species with seeds exhibiting physiological embryo dormancy overcome by stratification. Germination of these species occurs during stratification treatment, reducing the number of healthy non-dormant seeds available for sowing at the end of treatment. The goal of this study was to examine the effectiveness of PEG treatments in reducing germination during stratification in order to maximize germination after stratification.

Materials and methods

Commercial seeds (Western Native Seeds, Coaldale, CO) of *C. montanus* (Chaffee county Colorado–elevation 2,591 m), *A. alnifolia* (Idaho–county and elevation unknown) and *S. canadensis* (Montana–county and elevation unknown) were used in these studies. For each species, the combined effects on germination of PEG concentration and stratification duration were tested using a completely randomized design with a factorial treatment structure. Factors were concentration of PEG 8000 (0, 100, 150, 200 and 250 g/L) and duration of stratification (6, 9 or 12 weeks – *C. montanus*; 9, 14 or 18 weeks – *A. alnifolia*; 9 or 14 weeks – *S. canadensis*). Stratification durations were selected to frame the suspected stratification requirement of each seedlot. At the stratification temperature (3°C), these PEG concentrations result in water potentials of 0, -0.18, -0.37, -0.63 and -0.95 MPa, respectively (Michel 1983). These calculated water potentials are used in place of the corresponding PEG 8000 concentrations in the remainder of this text. Four replications of 100 seeds were used to test each treatment combination. In addition, four 100-seed replications were used to test control seeds (no PEG, no stratification) for each species. To determine the baseline stratification requirement of each seed lot, data for the control samples were compared with seeds undergoing stratification in combination with the 0 MPa treatment.

Prior to other treatments *C. montanus* and *A. alnifolia* seeds were surface sterilized with two-hour soaks in 3% hydrogen peroxide followed by a thorough rinse in running tap water and brief soaks in several changes of fresh water. In order to break physical seed dormancy, *S. canadensis* seeds were scarified for five minutes in concentrated sulfuric acid (Reagent ACS, 95.0-98.0%, VWR) followed by a thorough rinse in running tap water and brief soaks in several changes of fresh water. After surface sterilization and acid scarification treatments, seeds were allowed to dry overnight. The following day, seeds of each species were randomly assigned into experimental samples for germination testing and moisture content evaluation and representative samples were sent to the National Tree Seed Lab (Dry Branch, Georgia) for viability testing via tetrazolium staining of 400 seeds. Those tests indicated that seedlot viability was 83% for *A. alnifolia*, 73% for *C. montanus* and 44% for *S. canadensis*.

Stratification solutions were mixed by adding PEG (Carbowax PEG 8000, Fisher Scientific) to deionized water. Seeds were stratified on filter papers (VWR Qualitative 413) within 100 mm petri dishes wetted with 4 mL of PEG solution. Petri dishes were placed in a low temperature incubator (Fisher Scientific) at 3°C for stratification. For each species, stratification start dates were staggered so that all samples completed stratification at the same time. Seeds were checked weekly during stratification to monitor germination and to add PEG solution if necessary.

Upon the completion of stratification treatments, each sample (including controls) was rinsed for one minute under tap water, soaked two hours in water and again rinsed under tap water for one minute. Seeds were germinated on sterilized sand (ISTA 1999) within petri dishes in a greenhouse, with the thermostat set to achieve daytime high temperatures of 30°C and nighttime low temperatures of 15°C. Mean daily high/low temperatures during germination testing were 30/11°C (86/51°F) for *C. montanus*, 29/15°C (84/59°F) for *A. alnifolia* and 29/17°C (84/62°F) for *S. canadensis*. For each species, petri dishes were arranged on a single greenhouse bench using a completely randomized design. Germination was monitored daily for two weeks and weekly for two additional weeks.

Germination data were analyzed on the basis of 100 seeds per sample and germination percentages presented are also based on 100-seed samples. Germination percentages based on sample viability percentages (assumed to be equivalent to the measured seedlot viability) are presented in parentheses for reference. Categorical analysis (SAS Proc Catmod, SAS Institute 1989) was used to determine germination differences using α=0.05. Approximate pairwise Z-statistics were used to conduct pairwise comparisons of stratification simple effects, using an alpha value of 0.05 divided by the total number of comparisons.

Germination rate, measured for seeds germinating after stratification, was expressed as mean germination time (MGT), the average number of days required for seeds to germinate. MGT was calculated by the formula:

Formula 1. $$MGT = \frac{\sum_{1}^{i} g_i n_i}{G}$$

where g_i is the number of seeds germinating on the n_i^{th} day of germination testing and G is the total number of seeds germinating during the 28-day test. Because germination counts were conducted weekly after day 14, n_{21} was considered to be (15+21)/2 and n_{28} was considered to be (22+28)/2. Some treatment combinations were dropped due to low after-stratification germination and one-way ANOVA was used to test for differences among MGT means. For the *A. alnifolia* data set only, non-normality of MGTs required that data be rank-transformed prior to analysis. Observed significance levels for F-tests were identical for non-transformed and rank-transformed data and general patterns of significance for pairwise comparisons were similar. Therefore, means and pairwise comparisons performed on the non-transformed data are presented.

The effect of PEG treatments on seed moisture content was measured directly using two samples per treatment combination with samples weighing 0.75-gram for *C. montanus* (67 ± 1.8 seeds per sample), 1.0-gram for *A. alnifolia* (172 ± 1.7 seeds per sample) and 0.73-gram for *S. canadensis* (106 ± 1.32 seeds per sample). Following weighing, seeds

underwent stratification at 3°C incubated in 0, -0.18, -0.37, -0.63 or -0.95 MPa PEG solutions in petri dishes for 0, 1, 2 or 3 weeks (*C. montanus* and *S. canadensis*) or 0, 2, 4 or 6 weeks (*A. alnifolia*). Following stratification, seeds were surface dried, weighed and oven dried using the low constant temperature oven method (ISTA 1999). Moisture content was calculated on a fresh weight basis.

Results

Moisture content

Incubating seeds in PEG during stratification reduced seed moisture content for all species and the effect was dose-dependent (figure 1). Equilibrium moisture contents were achieved, more or less, by the first measurement after the start of stratification (one week for *C. montanus* and *S. canadensis*; two weeks for *A. alnifolia*).

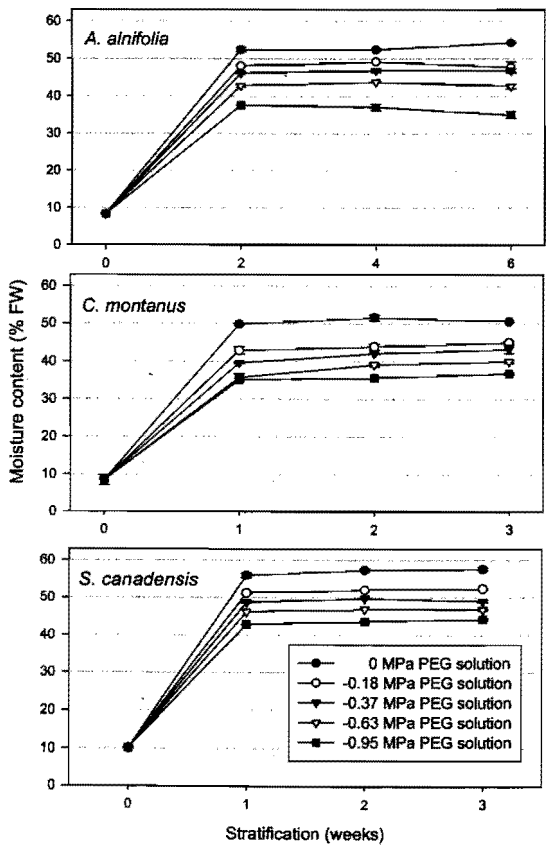

Figure 1. Change in *A. alnifolia*, *C. montanus* and *S. canadensis* seed moisture content during the initial six weeks (*A. alnifolia*) or three weeks (*C. montanus, S. canadensis*) of incubation at 3°C in 0, –0.18, –0.37, –0.63 or –0.95 MPa PEG 8000 solutions.

Simple effect of stratification

Germination of *A. alnifolia* incubated in water increased with increasing duration of stratification ($p<0.0001$), although mean germination percentages for 14- and 18-week stratification treatments were not significantly different (figure 2). All three *C. montanus* stratification-only treatments improved germination relative to non-stratified seeds ($p<0.0001$). Mean germination percentages for 6-, 9- and 12-week stratification treatments were not significantly different (figure 2). *Shepherdia canadensis* germination improved with each increase in stratification duration ($p<0.0001$) (figure 2).

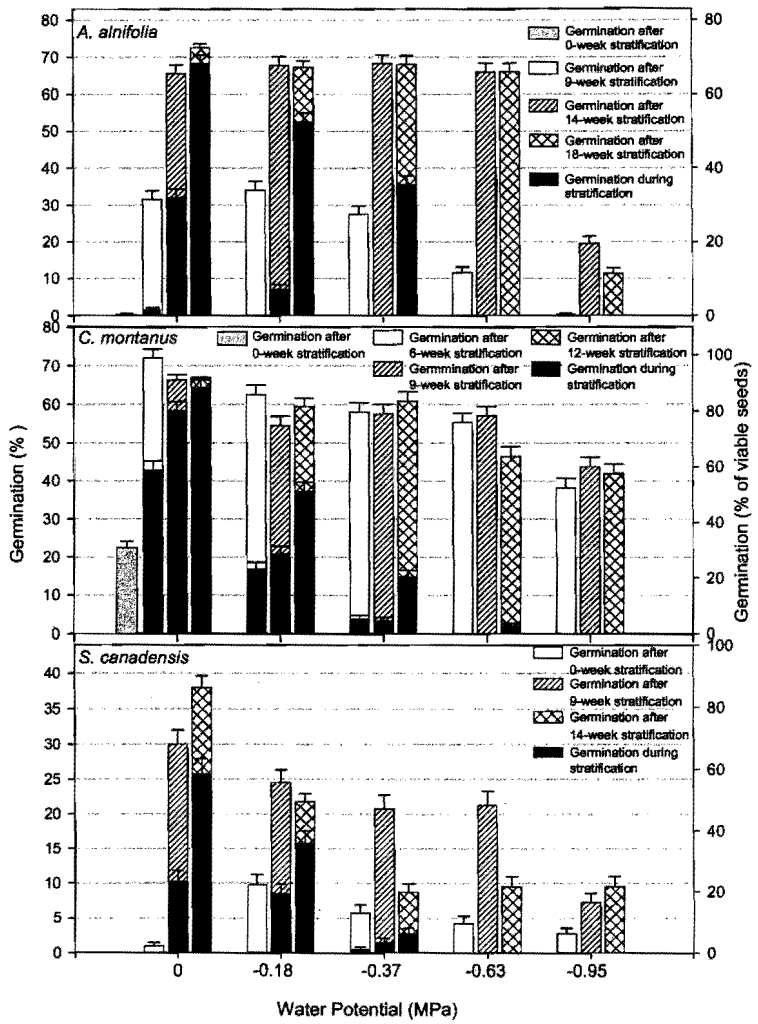

Figure 2. Total germination of *A. alnifolia*, *C. montanus* and *S. canadensis* seeds as the sum of germination during stratification and germination after stratification for each treatment combination. Within bars, upper and lower error bars are shown for germination after stratification and germination during stratification, respectively.

Combined effects of stratification and PEG treatments

All main effects and two-factor interactions were significant ($p<0.0001$ for all tests) for germination during stratification, germination after stratification and total germination of *A. alnifolia*. For seeds not treated in PEG, germination during stratification was problematic for all stratification durations except the shortest (least effective) (figure 2). Incubation in -0.63 and -0.95 MPa solutions completely suppressed *A. alnifolia* germination during stratification for all durations, while the -0.37 MPa solution completely suppressed germination during stratification except in combination with the longest duration (18 weeks). Total germination was nearly equal (66% to 72%) for all treatments combining either the 14- or 18-week stratification duration with any water potential except -0.95 MPa. When 0 or -0.18 MPa solutions were used, however, germination after stratification was considerably less than total germination because of germination during stratification. Thus, germination after stratification was maximized when seeds were stratified in either -0.37 or -0.63 MPa solutions for 14 weeks or in the -0.63 MPa solution for 18 weeks. Stratification for 14 weeks at -0.18 MPa resulted in near maximal germination after stratification.

For *C. montanus* the two-factor interaction between water potential and stratification duration was significant for total germination ($p<0.007$), germination during stratification ($p<0.016$) and germination after stratification ($p<0.0001$). Germination during stratification was high for seeds not treated in PEG and was reduced in a dose-dependent manner by incubation in PEG (figure 2). The -0.95 MPa solution completely suppressed *C. montanus* germination during stratification for all stratification durations and the -0.63 MPa solution nearly did so also. Incubation in the -0.37 MPa solution suppressed germination during stratification to below 10% for all durations except 12 weeks. Total germination tended to fall with decreasing water potential, regardless of stratification duration. Germination after stratification was maximized (>50%) when seeds were stratified either six or nine weeks in -0.37 or -0.63 MPa solutions.

For *S. canadensis* the two-factor interaction between water potential and stratification duration was significant for total germination ($p<0.0001$) and germination after stratification ($p<0.0001$), but not germination during stratification ($p=0.0611$). For seeds not treated in PEG, about one third of all germination took place during the nine-week stratification treatment and about two thirds of germination occurred during the 14-week stratification treatment. Decreasing water potentials reduced germination during stratification and water potentials below -0.37 MPa completely suppressed *S. canadensis* germination during stratification. However, incubation in PEG reduced total germination and no PEG treatment improved germination after stratification compared to seeds stratified nine weeks in pure water.

Onset of germination during stratification

Non-PEG-treated *A. alnifolia* seeds began germination by week nine of stratification and by week 18 nearly all germination had occurred (figure 3). Solutions with water potentials of -0.18 and -0.37 MPa delayed the onset of germination during stratification only slightly (0-1 week) compared to non-PEG-treated controls, but reduced the rate of germination during stratification in a dose-dependent manner. For *C. montanus*, non-PEG-

treated seeds began germination by week three of stratification and by week 12 nearly all germinable seeds had germinated. With each reduction in water potential, the onset of germination during stratification was delayed and the rate of germination substantially reduced. *Shepherdia canadensis* seeds not treated in PEG began germination by week four of stratification and by week 14 almost two thirds of germinable seeds had germinated. Stratification in solutions with water potentials of -0.18 and -0.37 MPa delayed germination by one and three weeks, respectively and reduced the rate of germination during stratification, although it should be noted that these treatments reduced total germination substantially.

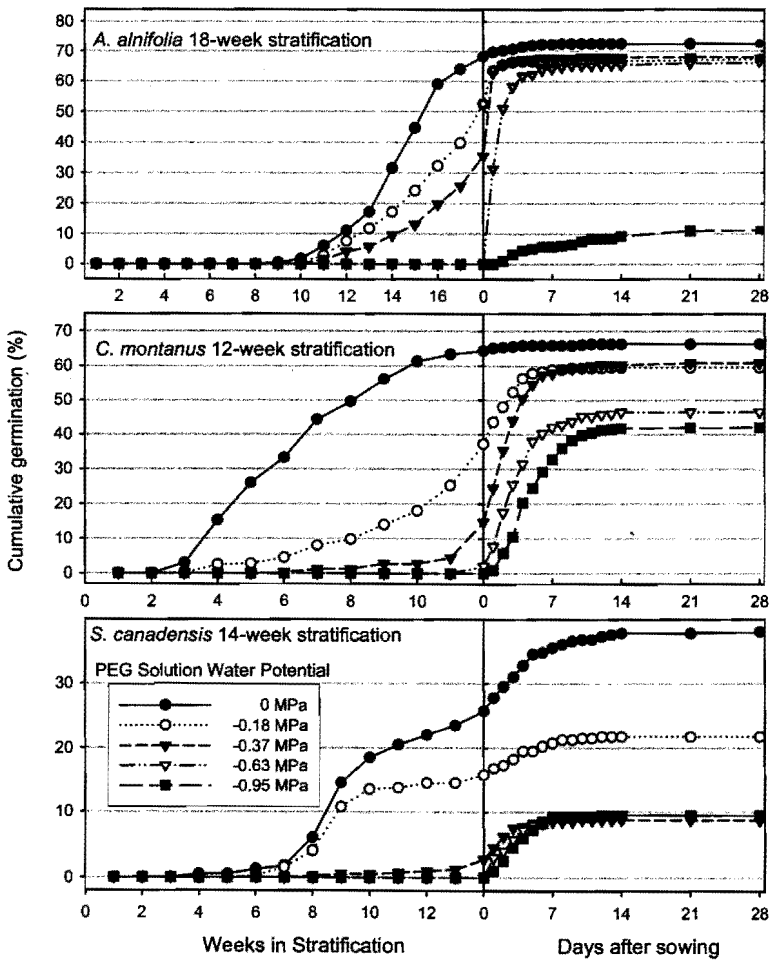

Figure 3. Timecourse of *A. alnifolia*, *C. montanus* and *S. canadensis* germination during and after the longest stratification treatment (18, 12 and 14 weeks, respectively) in combination with incubation in 0, –0.18, –0.37, –0.63 or –0.95 MPa PEG 8000 solutions.

Mean germination time

Amelanchier alnifolia seeds germinated most rapidly when stratified 14 weeks in -0.18 or -0.37 MPa PEG solutions or 18 weeks in -0.18, -0.37 or -0.63 MPa PEG solutions (table 1). All treatments involving nine weeks of stratification or the lowest water potential (-0.95 MPa) resulted in low germination and those seeds that did germinate took between 5.1 and 10.0 days to do so. In contrast, seeds stratified 14 or 18 weeks in -0.18, -0.37 or -0.63 MPa solutions germinated in the first 1.4 to 3.2 days on average.

Stratification had no effect on *C. montanus* germination speed, except when PEG was not used, in which case MGT was 1.6 days less for seeds stratified six weeks compared to those stratified nine weeks. Generally, seeds germinated more slowly when incubated in solutions with lower water potentials, but there was no difference in MGT for seeds stratified in -0.18 or -0.37 MPa solutions.

When *S. canadensis* seeds were stratified for nine weeks, PEG incubation at most concentrations had no effect on MGT, but the lowest water potential (-0.95 MPa) reduced MGT. Incubation in the -0.37 MPa PEG solution increased germination speed relative to seeds incubated in 0, -0.18, or -0.95 MPa solutions for *S. canadensis* seeds stratified 14 weeks.

Table 1. Mean germination time (days) for *A. alnifolia*, *C. montanus* and *S. canadensis* seeds germinating in water following stratification in polyethylene glycol (PEG 8000) solutions. Missing cells were dropped due to low after-stratification germination.

	PEG 8000 Solution Water Potential (MPa)				
Stratification Duration (weeks)	0	-0.18	-0.37	-0.63	-0.95
Amelanchier alnifolia					
9	*6.09 d	5.10 d	5.33 d	10.03 f	
14	3.49 c	2.82 abc	2.50 abc	3.17 bc	6.38 d
18		2.02 abc	1.35 a	1.65 ab	8.25 e
Cercocarpus montanus					
6	2.15 a	3.08 bc	3.41 bcd	4.39 ef	5.96 g
9	3.77 cde	3.28 bcd	3.24 bc	4.53 f	5.48 g
12		2.88 ab	3.36 bcd	3.99 def	5.39 g
Shepherdia canadensis					
9	3.85 abc	3.65 ab	3.38 ab	3.71 abc	4.91 cd
14	5.44 d	4.39 bcd	2.69 a	3.39 ab	4.48 bcd

*Mean germination times labelled with the same letter within each species are not different at the 0.05 level of significance.

Discussion

Success of PEG treatments in controlling germination during stratification and maintaining high total germination appears to be highly species-dependent. This technique has shown promise in *F. sylvatica* (Muller and Bonnet-Massimbert 1983) but was unsuccessful in *P. tridentata* (Young and Evans 1976) and *Picea sitchensis* (Bong.) Carr. (Sitka spruce)

(Gosling and Dodwell, unpublished-cited in Jones and Gosling 1994). In the present study, there was consistency among species in the moisture content at full imbibition (51-58%), the reduction in moisture content after incubation in -0.18, -0.37, -0.63 and -0.95 MPa solutions (3-6%, 5-9%, 8-11% and 14-15%, respectively) and the water potential necessary to completely (or nearly so) suppress germination during stratification (-0.63 MPa). There was inconsistency, however, among these species in the effect of PEG treatments on overall germination. When seeds were incubated in PEG concentrations high enough to suppress germination during stratification, there was no reduction in overall germination of *A. alnifolia*, some reduction in germination of *C. montanus* and a large reduction in germination of *S. canadensis*.

PEG treatments also had inconsistent effects on germination speed (MGT). For *A. alnifolia*, both stratification longer than nine weeks and incubation in -0.18 to -0.63 MPa solutions improved germination speed. PEG incubation also improved germination speed for *S. canadensis*, but only for seeds stratified 14 weeks. Both stratification beyond six weeks and incubation in increased concentrations of PEG tended to slow germination of *C. montanus*.

Results for *A. alnifolia* indicate that incubation in PEG at the proper concentration inhibited germination during stratification, but allowed dormancy-breaking processes to continue. As stratification proceeded, the pool of non-dormant seeds increased and with the removal of osmotic stress at the end of stratification treatment, these seeds germinated rapidly and uniformly. At the lowest water potential (-0.95 MPa), PEG incubation prevented dormancy-breaking processes from taking place during stratification, resulting in slow, incomplete germination.

It is surprising that *A. alnifolia* was the only species in this study with no loss in total germination as a result of stratification in PEG, considering it had the longest stratification requirement and seed exposure to PEG. Variability among species in their oxygen and moisture content requirements during stratification and ease of PEG removal after stratification may explain the differential results found here and elsewhere. Young and Evans (1976) theorized that reduced oxygen availability in the PEG stratification solution might have accounted for detrimental effects on *P. tridentata* germination. Mexal *et al.* (1975) found reduced oxygen solubility in solutions of PEG 4000 and 6000 and the effect was concentration-dependent. In that study, a PEG 6000 solution with a water potential of -0.67 MPa was found to reduce relative oxygen solubility by nearly 20%. Availability of oxygen is thought to be important in the maintenance of metabolic functions necessary for dormancy release during stratification and prevention of secondary dormancy induced by oxygen deficiency (Hartmann and Kester 1990, Murdock and Ellis 1992).

The negative effects of PEG incubation could also be related directly to reduced seed moisture content during stratification. Successful stratification in PEG solutions is premised on the hypothesis that there is some overlap between moisture contents low enough to suppress germination and those high enough to allow dormancy-breaking processes to occur. This hypothesis may not hold for all species. For *S. Canadensis*, reduced total germination resulted from stratification in -0.18 and -0.37 MPa solutions, yet at these low PEG concentrations, hypoxia was probably not an issue. Other explanations for the negative effect of PEG treatment on *S. Canadensis* seeds include the possibility

that scarification may have allowed PEG to penetrate the seed coat, preventing complete removal at the end of treatment. As a result, residual PEG may have limited oxygen or water availability to the embryo during germination. In addition, the *S. Canadensis* seed lot used in this study had poor viability (44%) and possibly low vigour. This characteristic may have interacted with oxygen availability, moisture content or other factors to exacerbate negative effects of PEG treatment.

The technique of stratification in PEG solutions is simple and requires little vigilance during the stratification period, but seeds must be thoroughly washed after treatment. Further research is needed to compare the effectiveness of PEG incubation and other controlled-moisture-content stratification techniques across a range of species and to determine the physiological characteristics that influence species' suitability to these treatments.

Acknowledgements

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