Pre-chilling and Gibberellic Acid Improve Seed Germination in *Lewisia* Species and *Meconopsis betonicifolia*

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Germination of naked embryos in *Lewisia tweedyi* was almost 90% within 4 weeks of decoating, suggesting that the species has a seed-coat-imposed dormancy. Maximum germination of whole seeds after 32 weeks under axenic conditions was 66.7% in *L. tweedyi* and 87.8% in *L. cotyledon*. Moist pre-chilling in combination with gibberellic acid (GA_3) treatments improved germination in *L. cotyledon* and *Meconopsis betonicifolia*. Low percent germination over extended periods of time limits the commercial production of *L. tweedyi*.

INTRODUCTION

Seed dormancies of many herbaceous garden perennials including *Meconopsis betonicifolia* Franch and native species, such as montane *Lewisia tweedyi* (A. Gray) Robinson and *L. cotyledon* (S. Watson) Robinson, limit their production in highly controlled environments.

Lewisia tweedyi is highly valued as an ornamental (Straley, 1988), but has been cultivated only by alpine specialists who report very low percent germination over long periods of time (Colley and Mineo, 1985). Lewisias (Portulacaceae) are members of the Centrospermae; a linear peripheral embryo surrounds centrally located perisperm (Bittrich, 1993). Atwater (1980) reported seed-coat-imposed dormancies in the Centrospermae. In this study, we determined if dormancy in *L. tweedyi* is imposed by the seed coat by observing the germination of decoated seeds (Côme and Corbineau, 1992).

Lewisia cotyledon and *M. betonicifolia*, the blue poppy, are minor commercial crops in some areas of Europe and western North America. Aelbrecht (1989) found that pre-chilling increased *L. cotyledon* germination. However, germination recommendations are inconsistent and imprecise. Methods are based on stratification for unspecified periods in a garden cold frame. The objective of this study was to improve germination by testing the effects of moist pre-chilling and gibberellic acid on *L. tweedyi*, *L. cotyledon*, and *M. betonicifolia*.

MATERIALS AND METHODS

Germination of Naked Embryos. *Lewisia tweedyi* seed was surface sterilized in 15% NaOCl for 15 min before carefully removing the entire testa with a dissecting needle. Three, 25-seed replicates of decoated and whole seeds were sown in 10-cm petri dishes on 3 layers of Whatman No.1 filter paper moistened with 5 ml distilled water. Dishes were incubated in the dark at 22C for 4 weeks. Germinated seeds (radicle > 1 mm) were counted and percent germination (PG) was calculated. A t-test was used to compare the difference between the two treatment means.

Axenic Germination of *Lewisia* Taxa. *Lewisia cotyledon*, *L. tweedyi*, *L. tweedyi* 'Alba', *L. tweedyi* 'Rosea', and *L. tweedyi* 'Elliot's Variety' seeds were obtained from Ashwood Nurseries (West Midlands, U.K.). One hundred seeds of each species and cultivar were surface sterilized and sown individually in 30-ml test tubes filled with 15 ml of one-tenth MS (Murashige and Skoog, 1962) agar medium, pH 5.5; sugar was not added to simulate in vivo germination conditions. Test tubes were placed in a growth room set at constant 19C with a 16-h photoperiod or pre-chilled at 4C in the dark for 4 weeks before transfer to the growth room. Number of germinated seedlings was recorded weekly and overall PG calculated after 32 weeks.

***Lewisia* Seed Treatments.** Gibberellic acid (Sigma, St. Louis, MO) at concentrations of 0, 1.0, or 10 mM was dissolved in pure acetone to volume based on improved germination in some perennial species after organic infusion of GA_3 (Persson, 1993). *Lewisia tweedyi* and *L. cotyledon* seeds were immersed for 24 h in covered beakers containing the GA_3 solutions at room temperature. Decanted seeds were sown in petri dishes. *Lewisia tweedyi* seed was pre-chilled (3±0.5C) for 2, 4, 8, or 12 weeks and *L. cotyledon* for 2, 4, or 8 weeks. After pre-chilling, seeds were removed to a growth chamber set at 18±2C with a 16-h photoperiod.

Petri dishes containing 25 seeds each were randomly placed in 2 plastic containers during pre-chilling and re-randomized during germination in all combinations of factors and levels (2 plant species × 3 levels of GA_3 × 4 or 5 levels of pre-chilling × 2 blocks × 2 replicate dishes). Number of germinated seeds (radicle > 1 mm) was recorded at the time of removal from the pre-chill and then weekly for 10 weeks. Percent germination was calculated and the data analyzed using the PC-SAS software package (SAS Institute, 1985).

Blue Poppy Seed Treatments. *Meconopsis betonicifolia* seed was obtained from two commercial sources, Jelitto Seeds and Thompson & Morgan, and from the University Botanical Garden. They were immersed in an aqueous solution of GA_3 at concentrations of 0 or 10 mM for 24 h. Decanted seeds were sown in petri dishes (2 replicates of 50 seeds per treatment) to test seed quality from different sources or directly in styrofoam plug trays containing a commercial peat-based medium. Petri dishes were incubated at 5C for 6 weeks and then moved to a 12/10C (day/night) greenhouse for germination. Plug trays were divided into three 60-cell sections and seeded sequentially for different pre-chilling periods. Plastic-wrapped trays were pre-chilled for 4, 6, or 8 weeks at 5C before transfer to a cool (12/10C) or warm (24/22C) greenhouse for germination. Number of live seedlings was counted weekly.

RESULTS AND DISCUSSION

Seed-Coat-imposed Dormancy in Lewisias. Impenetrable seed coats impose dormancy on many herbaceous plants and unselected wild species (Kelly et al., 1992). Germination within 4 weeks of decoating suggests that the covering structure imposes dormancy in *L. tweedyi*. Mean PG (3 × 25-seed replicates) was 86.9% for naked embryos and 0% in the control ($P = 0.001$).

Low PG over extended periods of time is characteristic of plants growing in extreme habitats (Gutterman, 1993), such as montane environments. Individual seeds of *L. cotyledon* and *L. tweedyi* sown under sterile conditions germinated throughout the entire 32-week test period. Maximum overall germination after 32 weeks was 77.9% in *L. cotyledon* and 61.4% in *L. tweedyi*.

Seed Treatments to Improve Germination. Seed dormancies are an advantage in nature, but they must be overcome in cultivation. Germination performance of wild and other perennial species has been improved by pre-chilling (Bratcher el al., 1993) and gibberellic acid treatments (Sulaiman, 1993).

Pre-chilling or GA_3 increased PG in both *Lewisia* species (Table 1), but maximum germination was 59.0% in *L. cotyledon* ($P = 0.05$) and 28% in *L. tweedyi* ($P = 0.01$) after pre-chilling in combination with 10mM GA_3. Percent germination was different (P=0.001) between *L. tweedyi* and *L. cotyledon*.

Table 1. Percent germination in *Lewisia tweedyi* and *L. cotyledon* soaked for 24 h in 0, 1.0, or 10.0 mM gibberellic acid (GA_3) in acetone after pre-chilling at 3±0.5C for 0, 2, 4, 8, or 12 weeks. Seeds were evaluated over 10 weeks in a growth chamber at 18±2C with a 16-h photoperiod.

	Plant species	
Treatments	*L. tweedyi*	*L. cotyledon*
Pre-chilling (weeks)		
0	4.7	34.2
2	4.2	35.3
4	9.3	37.7
8	13.8	48.3
12	19.3	—
Significance	***	***
GA_3 in acetone (mM)		
0	6.6	36.8
1.0	9.4	38.0
10.0	14.8	41.9
Significance	***	***

*** Significant at P=0.001.

Maximum live seedlings in *M. betonicifolia* was 88% after 4 weeks in a cool greenhouse with seed obtained from Jelitto, soaked in a 10 mM GA_3 aqueous solution and sown in plug trays, in combination with a 4-week prechill ($P = 0.01$).

Khan et al. (1992), Bradbeer (1988), and Corbineau and Côme (1995) discuss the role of GA and stratification in seed dormancy and germination. Gibberellic acid mimics moist chilling and moist chilling induces the production of GA. When the seed coat is impermeable to oxygen, chilling increases the oxygen supply to the embryo. When secondary dormancy is induced at warm temperatures, GA stimulates metabolic activity in the embryo.

Implications for Growers. Germination of *L. tweedyi* would be improved by scarification of the testa to overcome the coat-imposed dormancy. Soaking seeds in a 10 mM aqueous solution of GA_3 followed by moist pre-chilling for 4 weeks is effective for *L. cotyledon* and *M. betonicifolia*. Growers should also be advised that seed quality is variable (Fig. 1).

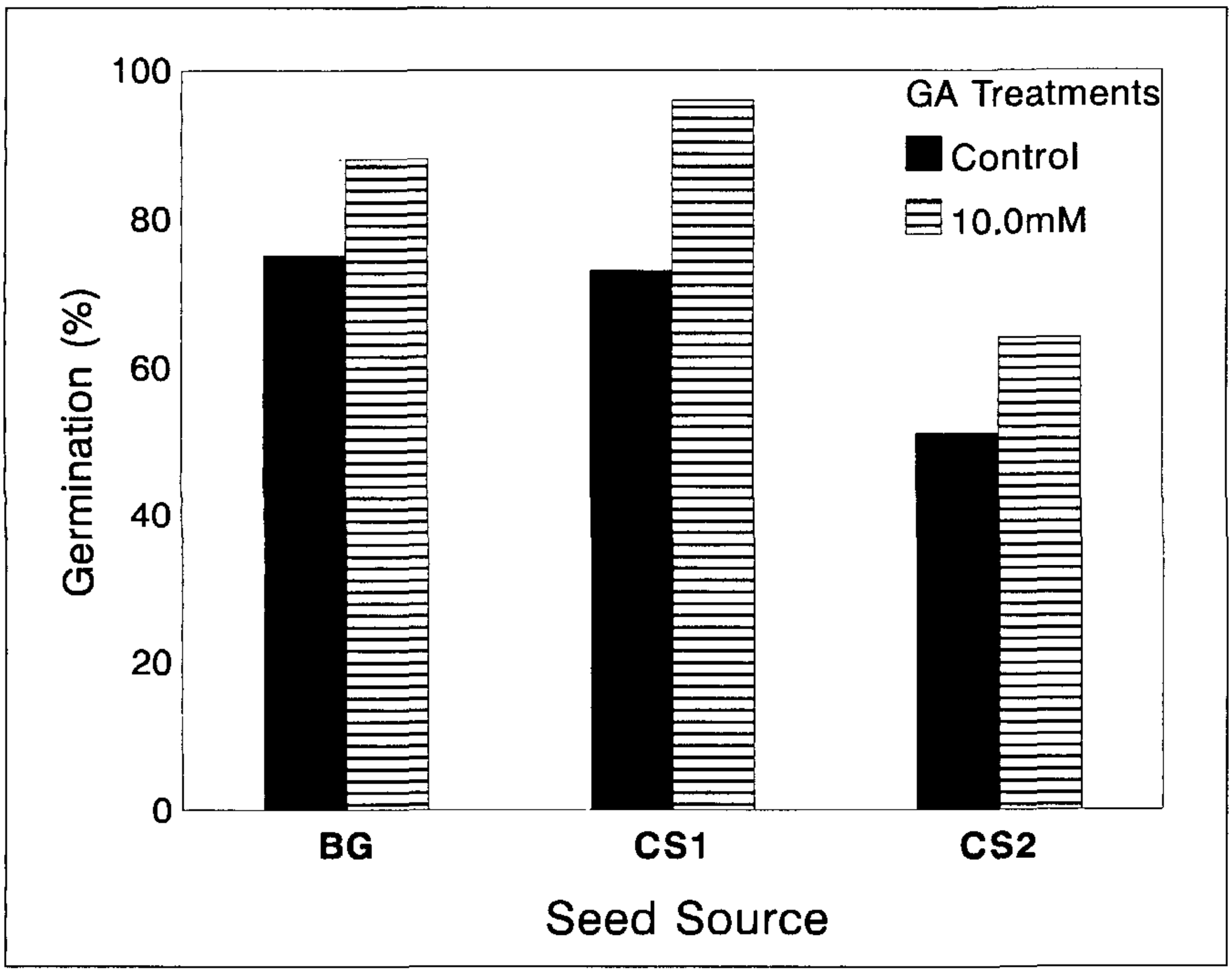

Figure 1. Percent germination in *Meconopsis betonicifolia* soaked for 24 h in 0 or 10.0 mM gibberellic acid (GA_3) in water after pre-chilling at 5C for 6 weeks. Seeds were evaluated from three different sources: a botanical garden (BG), and two commercial suppliers (CS1) or (CS2).

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