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Seed Dormancy Mechanisms in Basalt Milkvetch and Western Prairie Clover☆

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ABSTRACT

A greater diversity of native legumes and forbs is desirable for rangeland restoration practice in the Intermountain Region of the western United States. But for such diversity to materialize in the seed marketplace and to be effective in restoration practice, seeds that germinate reliably in seed fields and on restoration sites are needed. We measured germination response of two native legumes, basalt milkvetch (*Astragalus filipes* Torr. ex A. Gray) and western prairie clover (*Dalea ornata* [Douglas] Eaton & Wright), after eight germination treatments. Treatments were a factorial combination of 1) seed scarification with sandpaper (or unscarified), 2) a substrate of moist sand (or blotter paper), and 3) a 3-wk prechill at 5° (or nonprechilled). Cumulative germination increased linearly throughout the 10-wk course of the experiment for all treatment combinations in both species. Scarification increased germination of western prairie clover, but prechilling and substrate had no effect. In contrast, prechilling, scarification, and a sand substrate all increased germination of basalt milkvetch. Hence, for this species the prechilled/scarified/sand treatment combination displayed the numerically highest germination for all 10 wk (30—43%), and the nonprechilled/unscarified/blotter paper treatment combination always germinated lowest (1—3%). Results were consistent with physical dormancy (hard-seededness) limiting germination of western prairie clover and combinational dormancy (i.e., co-occurrence of physical and physiological dormancy) limiting germination of basalt milkvetch. Of the two species, we have found basalt milkvetch to be the more difficult to establish from seed. By prechilling acid-scarified seed in moist sand, basalt milkvetch was successfully established in two field trials seeded in mid-April. Nonprechilled mechanically (sandpaper) scarified seed germinated as high as prechilled acid-scarified seed. By scarifying and prechilling basalt milkvetch seed to address physical and physiological dormancy mechanisms, respectively, this seed-treatment protocol may be "scaled up" to produce large quantities of germinable seed.

Introduction

Historically, the most important species for rangeland seedings in the Intermountain West have been introduced grasses, but more recently, native grasses have assumed increasing importance (Richards et al., 1998). Legumes have been used to a lesser extent, with introduced species such as alfalfa (*Medicago sativa* L.), sainfoin (*Onobrychis viciifolia* Scop.), and sweetclover (*Melilotus* spp.) dominating the seed trade, along with introduced forbs such as small burnet (*Sanguisorba minor* Scop. [Rosaceae]) and blue flax (*Linum perenne* L. [Linaceae]) (Lambert, 2005). The most widely used native legume in the Intermountain West has been Utah sweetvetch (*Hedysarum boreale* Nutt.), while frequently used native forbs include western yarrow (*Achillea lanulosa* Nutt. [Asteraceae]), Lewis flax (*Linum lewisii* Pursh [Linaceae]), Palmer penstemon (*Penstemon palmeri* A. Gray [Plantaginaceae]), Rocky Mountain beeplant (*Cleome serrulata* Pursh [Cleomaceae]), and globemallow (*Sphaeralcea* spp. [Malvaceae]) (Lambert, 2005).

Native species are typically preferred for restoration projects (Richards et al., 1998), and there is great interest in native legumes and forbs (Rowe, 2010). However, their use has been hindered by high seed costs and limited seed availability (Rowe, 2010). This may result from dependence on wildland seed collection, as cultivated seed production of most native legume and forb species is problematic (Wirth and Pyke, 2003). Due to these deficiencies, plant material development of native legumes and forbs has been assigned a higher priority than that of native grasses or shrubs by the Great Basin Native Plant Selection and Increase Project (Shaw et al., 2005, 2012).

Native legumes and forbs may contribute greatly to species richness and diversity in Intermountain rangelands, which in turn may increase community stability and productivity and decrease the risk posed by invasive species (Sims et al., 1978; Pokorny et al., 2004). In a growth chamber study of forbs, Roberts et al. (2010) related 11 functional traits (i.e., traits that impact ecological fitness) to restoration performance. Ecological fitness, usually expressed as the product of fecundity and

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survivorship, is a measure of the ability of a plant population to perpetuate its genotypes from one generation to the next. Thus, any trait that impacts fecundity, survivorship, or both can be considered a functional trait. Sets of traits, particularly those relating to above-ground biomass and establishment frequency in monoculture, explained much of the variation in plant cover and establishment achieved with restoration seed mixes (Roberts et al., 2010). Barak et al. (2015) identified three "native winner" forb species for the Colorado Plateau Region, namely foothill deervetch (*Acmispon humistratus* [Benth.] D.D. Sokoloff), sanddune crytantha (*Cryptantha fendleri* [A. Gray] Greene), and tanseyleaf tansyaster (*Machaeranthera tamacetifolia* [Kunth] Nees). These species germinated at high percentages across a variety of environmental conditions and competed effectively against the invasive annual, downy brome (*Bromus tectorum* L.).

A need exists to expand the diversity of native legume and forb species that are amenable to cultivated seed production, as well as to find ways to lower seed costs so that they may become more widely used (Broadhurst et al., 2016). Basalt milkvetch (BMV; *Astragalus filipes* Torr. ex A. Gray) and western prairie clover (WPC; *Dalea ornata* [Douglas] Eaton & Wright = *Petalostemon ornatus* Douglas) are two native legumes (Fabaceae) that have the potential to augment the few existing native forb species that are currently available for commercial use (Bhattarai et al., 2008, 2010). For this to occur, plant materials must 1) germinate and establish and 2) produce sufficient quantities of seed that can be mechanically harvested. While western prairie clover seed can be germinated successfully following acid or mechanical scarification (60−85%), reliable germination of BMV seed remains elusive (4%) despite scarification (Bushman et al., 2015).

The search for treatments to break seed dormancy in *Astragalus* spp. has achieved mixed success. Most seeds of *Astragalus arpilobus* Kar. et Kir., a cold-desert annual native to northwestern China, have water-impermeable seed coats that become permeable and germinate over a wide range of temperatures following acid or mechanical scarification (Long et al., 2012). On the other hand, dormancy was not broken by hot or boiling water, alternating hot (60−80°C) and cold (4°C) water, light or darkness, or dry storage up to 12 months. These results suggest that *A. arpilobus* displays physical seed dormancy (i.e., hard-seed) but not physiological dormancy. Exposure to GA_3, KNO_3, and thiourea did not improve germination of seed of an unidentified *Astragalus* species from southern Pakistan, but no treatments to break mechanical scarification were applied (Ikram et al., 2014). Treatment with GA_3 (100−500 ppm) or sulfuric acid (50%, 98%) improved germination of *A. cyclophyllon* Beck ex Stapf, a species native to high-elevation deserts in Iran, while hot water (60−100° for 5−10 minutes) had no effect (Keshtkar et al., 2008). Greatest germination was reached with 500 mg kg^{-1} GA_3, which increased germination from 51% (untreated) to 81%. These data suggest the possibility of combinational dormancy (i.e., both physical and physiological dormancy). However, the tandem treatment of sulfuric acid to break physical dormancy and GA_3 to break physiological dormancy was not attempted.

In our experience, establishment of WPC has been relatively successful, but success with BMV has been minimal. Because of our prior success in breaking seed dormancy of WPC by mechanical scarification (Bushman et al., 2015), we compared this species to BMV in a laboratory germination trial. Our objective was to determine the efficacy of prechilling (stratification), mechanical scarification, and the two treatments together. Because germination substrate may also impact germination for some species (Baskin and Baskin, 2001), we also examined the effect of substrate in our germination trial, using either blotter paper or moist sand. If effective, scarification and/or prechilling treatments could be applied before planting to enhance field establishment (Wirth and Pyke, 2003), either in a seed field or on a restoration site. We conducted a field study at two representative seed-production locations in northern Utah to compare the effectiveness of these and other seed treatments for germination and establishment of basalt milkvetch.

Materials and Methods

Laboratory Germination Trial

Seed lots of NBR-1 Germplasm of BMV (Johnson et al., 2008) and Spectrum Germplasm of WPC (Johnson et al., 2011) were harvested at Utah State University's Millville Farm near Millville (Cache County), Utah on multiple dates as they ripened from late July to early August 2011. Thus, the two seed lots were produced under similar environmental conditions. Seed was stored at 22°C until germination testing began in late March (repetition 1), late July (repetition 2), and late November (repetition 3) 2012. Seeds of each species were germinated in plastic boxes (110 × 110 × 35 mm) with pressure-fitted lids.

Substrates were either a moistened, nontoxic, steel blue blotter paper (Anchor Paper, St. Paul, MN) or 250 g of sand. One hundred seeds were planted in each box on top of the substrate using a vacuum seed head (Hoffman Manufacturing, Albany, OR). For the sand substrate, seeds were covered with a blotter paper and then watered over the blotter with 60 mL of tapwater so that seed disturbance was minimal. Soil matric potential of sand-filled boxes was approximately −0.17 MPa, determined by a combination of three methods, namely a soil pressure plate apparatus, a psychrometric sample chamber (Model C-52, Wescor, Logan, UT), and a psychrometer fitted to a stainless steel cap (Brown, 1976). This matric potential is within the ideal range for germination of *A. arpilobus* (Long et al., 2012).

Germination boxes contained either mechanically scarified or unscarified seeds. Scarification was performed using a wooden block covered with sandpaper (100 grit aluminum oxide, 3M Corporation, St. Paul, MN) over a flat surface, also covered with the sandpaper. Two-g batches of seed were scarified at a time by rubbing with the wooden block in a 180° twisting motion for 10 repetitions (five clockwise and five counterclockwise) while applying slight downward pressure. Batches of scarified seed were then combined and thoroughly mixed to generate scarified seed lots for each species. The four seed lots, BMV scarified and unscarified and WPC scarified and unscarified, were tested for viability by tetrazolium staining of 200 seeds at the Utah State Seed Laboratory (Salt Lake City, UT). These tests were repeated two additional times.

Boxes of seeds were either prechilled in the dark at 5°C for 3 weeks before the 10-week germination period or not prechilled. For replication, six germination boxes were employed for each combination of treatments. Thus, 96 boxes of seeds were prepared (2 species × 2 substrates × 2 scarification treatments × 2 prechilling treatments × 6 replicate boxes) for each of three separate repetitions totaling 288 boxes.

Germination was conducted at 22°C under ambient daylength. Boxes were randomized across all combinations of seed treatments before the beginning of the germination period and then randomized again after each weekly germination count. Week-1 counts were made on 5 and 26 April (repetition 1), 2 and 23 August (repetition 2), and 6 and 27 December (repetition 3) 2012 for nonprechilled boxes and prechilled boxes, respectively. Counts were made weekly for a 10-week period for a total of 288 × 10 = 2 880 observations.

The experiment was analyzed as a completely randomized, repeated measures design with week as the unit of repeated measure and germination box as the subject of repeated measure, as the same box was counted each week for 10 wk. To implement the repeated measures design, a first-order autoregressive covariance structure was fit using the REPEATED statement in PROC MIXED (SAS, 2011). According to Akaike's Information Criterion (Akaike, 1983), this covariance structure showed improved fit compared with a compound symmetry covariance structure. Scarification, substrate, and prechill treatments, as well as repetitions, were regarded as fixed effects, while replicate boxes were regarded as random effects. Transformations suggested by Box-Cox analysis using PROC TRANSREG (SAS, 2011) were applied as needed to normalize the data before analysis. Unless otherwise stated, least squares means across all weeks are presented as opposed to a mean for wk 10.