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Author(s): Thomas A. Jones , Douglas A. Johnson , B. Shaun Bushman , Kevin J. Connors , Robert C. Smith

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## Seed Dormancy Mechanisms in Basalt Milkvetch and Western Prairie Clover<sup>☆</sup>



Thomas A. Jones<sup>\*</sup>, Douglas A. Johnson, B. Shaun Bushman, Kevin J. Connors, Robert C. Smith

U.S. Department of Agriculture (USDA) – Agricultural Research Service, Forage & Range Research Laboratory, 696 North 1100 East, Logan, UT 84322-6300, USA

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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.

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### 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 [Clemnaceae]), 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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<sup>\*</sup> Correspondence: Thomas A. Jones, Forage & Range Research Laboratory, USDA – Agricultural Research Service, Logan, UT 84322-6300, USA.

E-mail address: [thomas.jones@ars.usda.gov](mailto:thomas.jones@ars.usda.gov) (T.A. Jones).

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 tansyleaf 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 arpillobus* 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. arpillobus* displays physical seed dormancy (i.e., hard-seed) but not physiological dormancy. Exposure to  $\text{GA}_3$ ,  $\text{KNO}_3$ , 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  $\text{GA}_3$  (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<sup>-1</sup>  $\text{GA}_3$ , 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  $\text{GA}_3$  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. arpillobus* (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  $288 \times 10 = 2880$  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.

## Field Trials

Seeds of BMV were periodically harvested at Millville Farm as they ripened during late July to early August 2010. Seed was stored at 4°C until subjected to treatments to evaluate their efficacy for improving stand establishment in a field setting. Treatments applied in early April 2014 were 1) an untreated control, 2) scarification for 6 seconds with a Forsberg Line debearder (Fred Forsberg & Sons, Inc., Thief River Falls, MN) modified with four internal paddles and lined with medium-grit emery paper, 3) immersion in boiling water for 10 seconds, 4) scarification with 97% sulfuric acid for 5 minutes, and 5) acid scarification followed by a 14-d prechill (4°C) in the dark after blending into moist sand (250 mg sand + 60 mL tapwater).

Seeds of the five treatments were planted on 16 April 2014 with a Wintersteiger TRM seeder (Salt Lake City, UT) at a rate of 494 seeds in single-row 5-m-long plots at two locations in Cache County, Utah. The two locations were the Utah State University Millville Wildlife Research Center (41.657°N 111.814°W; 1425 m asl) south of Millville, Utah (Ricks gravelly loam [0–3% west-facing slopes]; coarse-loamy over sand or sandy-skeletal Calcic Haploxerolls) and North Park Farm (41.786°N 111.814°W; 1372 m asl) in Hyde Park, Utah (McMurdie silt loam [0–3% west-facing slopes]; fine, montmorillonitic, mesic Calcic Pachic Argixerolls). These two locations were chosen to be representative of agricultural sites in northern Utah where seed of *A. filipes* might be produced commercially. The nearest official weather station to both sites is at Utah State University, where precipitation for the water year (1 October 2013 through 30 September 2014) was 491 mm compared with the long-term average of 450 mm. Mean monthly temperatures for 2014 (and long-term averages in parentheses) were 7.5°C (8.3°C) in April, 14.1°C (13.2°C) in May, 17.6°C (18.0°C) in June, 24.2°C (22.9°C) in July, 20.8°C (22.1°C) in August, and 18.3°C (16.7°) in September. Seedling densities (numbers of seedlings per 5-m row) were counted 50 (5 June 2014), 77 (2 July), 161 (24 September), and 187 (20 October) days after planting (dates) at North Park and 50 (5 June), 77 (2 July), and 163 (26 September) days after planting at Millville.

At both locations, the five treatments were arranged in a randomized complete block design with six replications. Treatment, date, and treatment × date were regarded as fixed effects, while replication, replication × treatment, and replication × date were regarded as random effects. Seedling data were analyzed in PROC MIXED (SAS, 2011) using date as the unit of repeated measure, plot as the subject of repeated measure, and compound symmetry as the covariance structure, as a first-order autoregressive covariance structure did not display improved fit over compound symmetry. 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 dates are presented as opposed to a mean for the final date.

## Results

### Laboratory Germination Trial

Seed viability for BMV (94.5%) was greater than for WPC (87.7%) ( $P < 0.05$ ), but neither scarification nor species × scarification terms affected viability ( $P > 0.10$ ) (Table 1). Seed that did not imbibe (i.e., hard seed) but tested positive with tetrazolium stain ( $Tz^+$ ) was reduced by scarification (75.2%) relative to the unscarified control (83.5%), while imbibed  $Tz^+$  seed increased with scarification (8.7–14.8%). However, hard seed did not differ between species or exhibit a species × scarification interaction ( $P > 0.10$ ). In summary, the scarification protocol that we used reduced hard-seed percentage similarly for the two species (about 8.3%) without reducing viability percentage.

In the germination trial, the number of germinated seeds of both species increased linearly through time for all treatment combinations (Figs. 1–3). During the 10 wk that germination was monitored,

**Table 1**

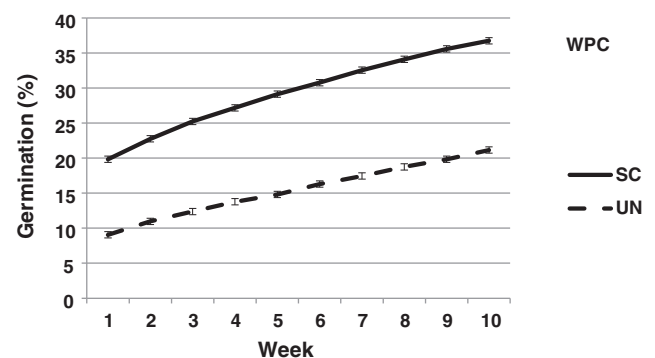
Percentages of imbibed viable ( $Tz^+$ ) seed, hard viable ( $Tz^+$ ) seed, total viable ( $Tz^+$ ) seed, and inviable ( $Tz^-$ ) seed in seed lots of Spectrum Germplasm of western prairie clover and NBR-1 Germplasm of basalt milkvetch that were either mechanically scarified or unscarified.

	Imbibed ( $Tz^+$ ) %	Hard ( $Tz^+$ ) %	Total viable ( $Tz^+$ ) %	Inviable ( $Tz^-$ ) %
<b>Spectrum Germplasm</b>				
Scarified	10.7	75.7	86.3	13.7
Unscarified	5.3	83.7	89.0	11.0
<b>NBR-1 Germplasm</b>				
Scarified	19.0	74.7	93.7	6.3
Unscarified	12.0	83.3	95.3	4.7
<b>Mean</b>				
Scarified	14.8 <sup>+</sup>	75.2 <sup>*</sup>	90.0 ns	10.0 ns
Unscarified	8.7	83.5	92.2	7.9

+, \* Significant at  $P < 0.10$  and 0.05, respectively.

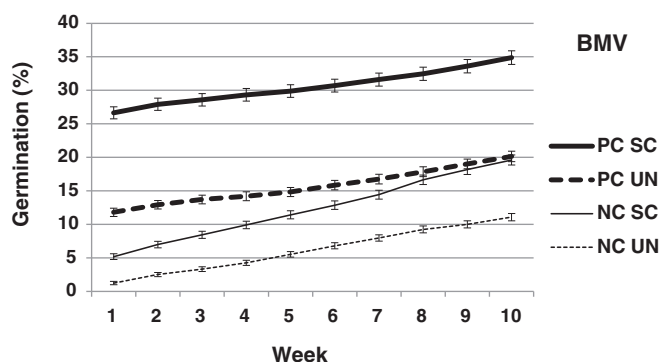
seedling numbers did not plateau. In the initial statistical analysis, the species main effect interacted with several other main effects and combinations of main effects (e.g., prechill, substrate, prechill × substrate, day, prechill × day, substrate × day, and prechill × substrate × day ( $P < 0.05$ )). For this reason, the two species were analyzed separately and are presented separately herein. Overall, germination of WPC was considerably less complex than that of BMV. Scarification increased WPC germination ( $P < 0.0001$ ) (Table 2), with least squares means increasing from  $15.4 \pm 0.4\%$  without scarification to  $29.4 \pm 0.4\%$  with scarification. However, neither prechilling nor substrate main effects were significant ( $P > 0.05$ ). The benefit of scarification increased across the 10-wk course of germination (see Fig. 1), as evidenced by a significant ( $P < 0.0001$ ) scarification × week interaction (see Table 2). Despite the absence of a significant prechilling main effect, prechilling increased cumulative germination as the trial progressed, as evidenced by a significant ( $P < 0.01$ ), though small ( $F = 2.84$ ), prechill × week interaction. Substrate also interacted with week ( $P < 0.05$ ), but no trend was observed through time.

In contrast to WPC, all three treatments impacted germination of BMV. Scarification increased germination ( $P < 0.0001$ ) (see Table 2), though for BMV the effect was smaller than in WPC, increasing the least squares mean ( $\pm$  s.e.) for germination from  $9.4 \pm 0.4\%$  to  $19.5 \pm$



**Figure 1.** Mean cumulative germination and standard errors of scarified (SC) seed and unscarified (UN) seed of Spectrum Germplasm of western prairie clover (WPC) across 10 wk. Data for prechill and substrate treatments are not presented because their main effects and interactions with each other and with scarification treatments were not significant ( $P > 0.10$ ).

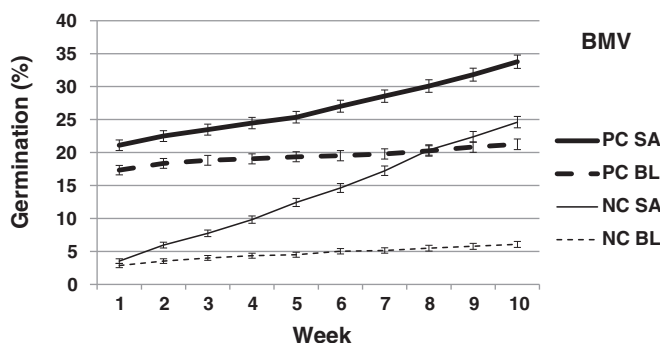




**Figure 2.** A description of the significant ( $P < 0.01$ ) interaction between two prechill and two scarification treatments for cumulative germination of seed of NBR-1 Germplasm of basalt milkvetch (BMV) across 10 wk. Presented here are least squares means and standard errors for cumulative germination of prechilled scarified (PC SC) seed, prechilled unscarified (PC UN) seed, nonchilled scarified (NC SC) seed, and nonchilled unscarified (NC UN) seed.

0.5%. The effect of prechill was greater than that of scarification, increasing germination from  $7.8 \pm 0.3\%$  to  $22.0 \pm 0.5\%$ . Basalt milkvetch germination was also greater ( $P < 0.0001$ ) when sand was used as a substrate (averaging  $18.5 \pm 0.5\%$ ) compared with blotter paper ( $10.2 \pm 0.4\%$ ). Suppression of germination by blotter paper was minimal at the first-week count, but blotter paper hindered germination at later weeks. The effects of prechilling and scarification interacted ( $P < 0.01$ ; see Table 2) and were synergistic (see Fig. 2)—prechilling increased germination of scarified seed (averaging  $18.7 \pm 0.4\%$ ) more than unscarified seed ( $10.3 \pm 0.4\%$ ). Prechilling interacted with substrate ( $P < 0.01$ ; see Table 2), as the lack of prechilling was less of a disadvantage on sand than on blotter paper, an effect that became more pronounced through time (Fig. 3). Though the prechill  $\times$  scarification and prechill  $\times$  substrate interactions were significant ( $P < 0.01$ ), they were much smaller in magnitude than the main effects of scarification, prechilling, and substrate (see Table 2). Neither the scarification  $\times$  substrate interaction nor the prechill  $\times$  scarification  $\times$  substrate interaction was significant ( $P > 0.10$ ). In summary, germination of the prechilled/scarified/sand treatment combination was numerically highest at all 10 weeks ( $42.3 \pm 1.6\%$  at wk 10), while the nonchilled/unscarified/blotter paper treatment combination was always numerically lowest ( $3.0 \pm 0.5\%$  at wk 10).

In some treatment combinations, nonprechilled BMV seed was able to partially “catch up” to prechilled seed across the 10-wk germination period. This occurred when seed was either scarified (see Fig. 2) or germinated on sand (see Fig. 3) but not if it was unscarified (see Fig. 2) and germinated on blotter paper (see Fig. 3). This effect resulted in



**Figure 3.** A description of the significant interaction ( $P < 0.01$ ) between two prechill and two substrate treatments for cumulative germination of seed of NBR-1 Germplasm of basalt milkvetch (BMV) across 10 wk. Presented here are least squares means and standard errors for cumulative germination of prechilled seed on sand (PC SA), prechilled seed on blotter paper (PC BL), nonchilled seed on sand (NC SA), and nonchilled seed on blotter paper (NC BL).

**Table 2**

Sources of variation, their associated degrees of freedom, F-values, and significance levels for cumulative germination of Spectrum Germplasm of western prairie clover (WPC) and NBR-1 Germplasm of basalt milkvetch (BMV) in a repeated measures design conducted across 10 wk.

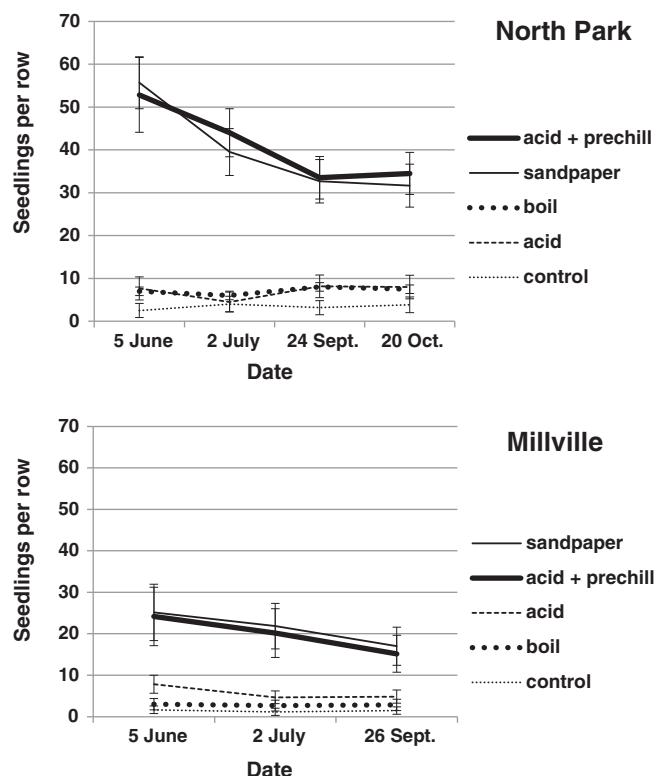
Source	Spectrum WPC			NBR-1 BMV		
	df	F-value	Sig. level	df	F-value	Sig. level
Prechill (PC)	1, 120	0.30	ns	1, 120	538.69	***
Scarification (SC)	1, 120	584.69	***	1, 120	273.04	***
PC $\times$ SC	1, 120	0.27	ns	1, 120	9.07	**
Substrate (SUB)	1, 120	0.20	ns	1, 120	185.81	***
PC $\times$ SUB	1, 120	0.14	ns	1, 120	14.70	**
SC $\times$ SUB	1, 120	1.57	ns	1, 120	0.68	ns
PC $\times$ SC $\times$ SUB	1, 120	0.03	ns	1, 120	1.01	ns
Week (WK)	9, 1 080	1 106.97	***	9, 1 072	156.46	***
PC $\times$ WK	9, 1 080	2.84	**	9, 1 072	24.11	***
SC $\times$ WK	9, 1 080	34.76	***	9, 1 072	0.49	ns
PC $\times$ SC $\times$ WK	9, 1 080	0.30	ns	9, 1 072	1.77	+
SUB $\times$ WK	9, 1 080	2.08	*	9, 1 072	47.40	***
PC $\times$ SUB $\times$ WK	9, 1 080	0.26	ns	9, 1 072	14.28	***
SC $\times$ SUB $\times$ WK	9, 1 080	1.04	ns	9, 1 072	1.05	ns
PC $\times$ SC $\times$ SUB $\times$ WK	9, 1 080	0.27	ns	9, 1 072	0.56	ns

+, \*\*, \*\*\*, \*\*\*\*  $P < 0.10, 0.05, 0.01, \text{ and } 0.0001$ , respectively.

significant prechill  $\times$  scarification  $\times$  week ( $P < 0.10$ ), prechill  $\times$  week ( $P < 0.0001$ ) (see Fig. 2), substrate  $\times$  week ( $P < 0.0001$ ) (see Fig. 3), and prechill  $\times$  substrate  $\times$  week ( $P < 0.0001$ ) (see Fig. 3) interactions (see Table 2).

#### Field Trials

Differences between BMV seed treatments for seedling density were dramatic at both North Park and Millville (Fig. 4). At North Park, least



**Figure 4.** Mean seedling densities (seedlings per 5-m row) of NBR-1 Germplasm of basalt milkvetch (BMV) and their standard errors for four seed treatments and an untreated control at four dates at North Park Farm and three dates at Millville Farm. For both locations, the acid + prechill and sandpaper treatments displayed similar seedling densities ( $P > 0.05$ ), but both of these treatments displayed greater seedling density than 10-second boil, 5-minute 97% sulfuric acid, and control treatments ( $P < 0.05$ ).

squares means ( $\pm$  s.e.) for acid ( $6.3 \pm 2.2$  seedlings per row) and boil ( $6.6 \pm 2.3$ ) treatments were not significantly different ( $P > 0.05$ ) from the untreated control ( $2.5 \pm 1.4$ ). Results were similar at Millville, with the acid ( $5.6 \pm 1.7$ ) and boil ( $4.0 \pm 1.4$ ) treatments again being similar to the control ( $1.9 \pm 0.9$ ) ( $P > 0.05$ ). However, the acid + prechill and sandpaper treatments were more effective ( $P < 0.01$ ) than the control treatment at both North Park ( $36.7 \pm 6.9$  and  $38.0 \pm 7.1$  vs.  $2.5 \pm 1.4$ , respectively) and at Millville ( $22.9 \pm 5.5$  and  $22.3 \pm 5.4$  vs.  $1.9 \pm 0.9$ ). However, while seedling density for the acid, boil, and control treatments remained stable across the evaluation period, we found seedling attrition for acid + prechill and sandpaper treatments at both locations at July and September dates (see Fig. 4). These trials were primarily meant to draw inferences regarding establishment of seed fields, so any inferences regarding rangeland restoration efforts must be tentative.

## Discussion

Bushman et al. (2015) measured emergence of unscarified, mechanically scarified, and acid-scarified seed from sandy soil in a greenhouse using seed lots of NBR-1 BMV and Spectrum WPC Germplasms different from those used in our study. Their results showed a larger response to mechanical scarification for WPC compared with the control at 4 weeks (61 vs. 22% emergence, which may underestimate germination) than what we observed herein (27 vs. 14%). However, Bushman et al. (2015) found no response to mechanical scarification for BMV emergence (4 vs. 4%), while we observed an increase in germination (5 vs. 10%). We cannot speculate as to why Bushman et al.'s (2015) study found a greater response to mechanical scarification than we did for WPC but a lesser response for BMV.

The finding for WPC that dormancy is alleviated by scarification, both here and by Bushman et al. (2015), is consistent with germination being controlled by physical dormancy. Physical dormancy (i.e., hard-seededness) is common in the Fabaceae (Baskin and Baskin, 2001) and confers seed longevity (Long et al., 2012). The case for physical dormancy in WPC is also supported by a 5.4% increase in imbibition upon scarification (see Table 1). Relative to physical dormancy, however, the case for physiological dormancy in this species is less convincing. We found no response to prechill, but we did find two pieces of supporting evidence for a small degree of physiological dormancy in WPC. First, the benefit of mechanical scarification was slightly but consistently accentuated through time, as evidenced by a scarification  $\times$  week interaction. Second, a similar pattern was seen for increased response to prechilling through time. However, physical dormancy, rather than physiological dormancy, clearly limits germination of WPC.

For BMV, in contrast to WPC, both scarification and prechilling conspicuously increased germination (see Table 2, Figs. 2 and 4). Thus, a combination of physical dormancy and nondeep physiological dormancy (Baskin and Baskin, 2001) (i.e., combinational dormancy) appears to be involved. Physical dormancy usually results from the impermeability of the seed to water (Baskin and Baskin, 2001). The causes of physiological dormancy are unclear, but it may be caused by embryo-covering structures that limit  $O_2$  permeability to the embryo, prevent leaching of germination inhibitors from the embryo, or physically restrict the embryo (Nikolaeva, 1969; Baskin and Baskin, 2001). Physical dormancy has been reported in 33 perennial species of *Astragalus* (Long et al., 2012), but far less common is combinational dormancy (Baskin and Baskin, 2001), having been found only in the Fabaceae and seven other angiosperm families (Long et al., 2012). Another *Astragalus* example of combinational dormancy is *A. siliquosus* Boiss., for which mechanical scarification followed by prechill led to nearly complete germination (Eisvand et al., 2006).

Increased germination percentage and rate is an obvious advantage for fall establishment of a seed field, but for rangeland applications any potential advantages of seed treatment are more nuanced. The delay of germination by seed dormancy is regarded as a bet-hedging (risk

avoidance) strategy (Childs et al., 2010) or a strategy to avoid environments that favor germination but are unfavorable for seedling survival (Vleeshouwers et al., 1995). However, advantages of germination in the spring following rangeland planting, such as reduced competition and increased nutrient availability, can be lost if germination is delayed (Wirth and Pyke, 2003). While we found that WPC seeds continued to germinate through the course of 10 weeks, seedlings that are late to germinate will be much less likely to establish, particularly on restoration sites where competition and abiotic stress are typically severe (Wirth and Pyke, 2003). Consequently, in biological terms, germination during the first 2 wk is probably most relevant. Thus, a certain degree of compromise may be necessary as far as evolutionary strategy is concerned, namely by applying the seed treatment to increase the speed of germination. For this reason, dormancy-breaking protocols are desirable for species that display seed dormancy and are seeded on rangelands.

In retrospect, our mechanical scarification treatment applied before the germination trial appears to have been mild for both species. Of about 83% hard seed in the western prairie clover and basalt milkvetch seed lots, about 75% of the seed remained hard (viable, yet failed to imbibe) following scarification with no loss of viability (see Table 1). Thus, it appears likely that a more aggressive scarification protocol would have resulted in a considerably greater dormancy loss, and consequently greater germination percentages, than reported herein.

## Management Implications

Until now, the success of seed-field establishment for BMV has been sporadic. Only a few seed fields of BMV have been established from seed, with most fields having been established with greenhouse-grown transplants, which is a laborious and expensive process. An additional problem is that BMV transplanting success is highly variable, sometimes exhibiting a high rate of mortality. On the other hand, we have had much greater success transplanting WPC and Searls' prairie clover (*D. searlsiae* A. Gray). Consequently, for practical purposes, the most important new finding reported here is the favorable germination response of BMV seed to prechilling. The utility of prechilling was demonstrated both in the laboratory and at two field locations. In all three environments, scarification + prechilling was substantially more effective than scarification alone, either when scarification was done mechanically (laboratory trial) or with acid (field trials). This knowledge is critical to the production of large quantities of seed that can be used for rangeland seeding operations. This increases the likelihood that NBR-1 and future releases of BMV will be successful in the commercial seed trade. This will augment the suite of native species available for ecological restoration, enhance the provision of ecosystem services such as nitrogen fixation and pollinator benefits, and increase the biodiversity of restored plant communities. Basalt milkvetch has been observed to be an early colonizer in postfire situations (R.F. Miller, personal communication July 2004, Burns, OR). This suggests that BMV may be of particular value in rangeland restoration efforts, as these commonly entail postfire seedings in the Intermountain West.

The establishment of low-elevation seed fields in arid eastern Washington, where the Intermountain native seed production industry is centered, typically takes place in late summer in irrigated and fertilized fields. Seedlings are able to establish in the fall and become large enough to survive the region's relatively mild winters. Once temperatures increase in the spring, young plants are poised to grow rapidly, generating inflorescences and ultimately a seed crop the following summer.

Seeds for late-summer seed-field establishment of BMV may be treated in batches to improve germination by "scaling up" the experimental procedure described here (i.e., scarification followed by prechilling with moist sand in a refrigerator). After the prechilling treatment is completed, the sand/seed mixture may then be sifted to remove the sand, leaving prechilled seed ready for planting. We recommend keeping the seed cool during this process and refrigerating until the seed is to be planted in order to reduce the potential for generating

secondary seed dormancy (Vleeshouwers et al., 1995) that may result when seed is exposed to higher temperatures before planting.

In the Intermountain West, most rangeland restoration efforts involve “dormant” seedlings that are planted in late fall to intentionally delay germination until the following spring, when soil moisture is greatest. Dormant seedlings obviate the need for prechilling before planting when the seed overwinters in moist soil. However, our research suggests the importance of scarifying seed of both WPC and BMV before seedings of rangelands. However, scarified seed that is planted in the fall may germinate prematurely or succumb to pathogens. For these reasons, further research is required to substantiate the utility of scarification for fall-dormant rangeland seedings of these legumes.

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