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Germination pretreatments to break hard-seed dormancy in *Astragalus (Fabaceae)*

Joseph M Statwick

Conservationists often propagate rare species to improve their long-term population viability. However, seed dormancy can make propagation efforts challenging by substantially lowering seed germination. Here I statistically compare several pretreatment options for seeds of *Astraglus cicer*: unscarified controls and scarification via physical damage, hot water, fire, acid, and hydrogen peroxide. Although only 30% of unscarified seeds germinated, just physical scarification significantly improved germination, whereas two treatments, hot water and fire, resulted in no germination at all. I recommend that rare species of *Astragalus*, as well as other hard-seeded legumes, be pretreated using physical scarification. Other methods have the potential to be effective, but may require considerable optimization, wasting precious time and seeds.

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ABSTRACT

Conservationists often propagate rare species to improve their long-term population viability. However, seed dormancy can make propagation efforts challenging by substantially lowering seed germination. Here I statistically compare several pretreatment options for seeds of *Astraglus cicer*: unscarified controls and scarification via physical damage, hot water, fire, acid, and hydrogen peroxide. Although only 30% of unscarified seeds germinated, just physical scarification significantly improved germination, whereas two treatments, hot water and fire, resulted in no germination at all. I recommend that rare species of *Astragalus*, as well as other hard-seeded legumes, be pretreated using physical scarification. Other methods have the potential to be effective, but may require considerable optimization, wasting precious time and seeds.

Key words: Astragalus; Dormancy; Germination; Milkvetch; Propagation; Scarification

INTRODUCTION

Propagating wild species in greenhouses and common gardens for their restoration or reintroduction in native habitats can be an effective method of improving the size and viability of rare or threatened populations (Maunder, 1992; Menges, 2008). Such *in situ* and *ex situ* propagation techniques are beneficial, so long as these techniques are successful in establishing additional reproductive adults in novel, degraded, or extirpated sites (Maunder, 1992; Menges, 2008). If, however, reintroduction is unsuccessful (which it usually is (Godefroid *et al.*, 2011)), it accomplishes nothing more than wasting resources and even further threatening the species by removing seeds that would have become the future seed bank.

At ~3270 species, *Astragalus* (Fabaceae) is the largest genus of flowering plants in the world (Watrous and Kane, 2011). Though a few *Astragalus* are weedy, wide-ranging generalists, specialization on uncommon and infertile soils seems to be a hallmark of the genus (Barneby, 1964). Unfortunately, this specialization appears to restrict many species to small geographic ranges, making them more vulnerable to extinction. In the United States alone, the US Fish and Wildlife service (2014) has listed 3 *Astragalus* species as under review, 5 as candidate, 5 as threatened, and 16 as endangered. Although the IUCN database (2014) contains less than one half of one percent of known *Astragalus* species, nearly 40 percent of those with sufficient data are considered “vulnerable” or worse (9 vulnerable, 12 endangered, 18 critically endangered, and 1 extinct). NatureServe (2014), meanwhile, lists 100 vulnerable, 58 imperiled, and 31 critically imperiled species, which combine to nearly a third of the 616 *Astragalus* species in its database.

Astragalus species, like most temperate legumes, as well as species of as many as 15 different plant families, have hard seed coats and physical dormancy, which often require scarification or stratification to break (Baskin *et al.*, 2008; Long *et al.*, 2012). In particular, low germination rate is a known “weak point” in the life cycle of several rare species of *Astragalus*, including *A. nitidiflorus* (Vicente *et al.*, 2011), *A. bibullatus* (Albrecht & Penzagos, 2012), and *A. arpilobus* (Long *et al.*, 2012). Although prolonged dormancy of the seed bank may contribute to the maintenance of genetic diversity in rare *Astragalus* such as *A. albens* (Neel, 2007) in the wild, this dormancy is counterproductive for propagation efforts.

Many scarification treatments have been explored in the literature, including dry heat (Albrecht & Penzagos, 2012; Chou *et al.*, 2012; Long *et al.*, 2012), wet heat (Long *et al.*, 2012), stratification (Acharya *et al.*, 2006; Albrecht & Penzagos 2012; Long *et al.*, 2012), physical scarification (Acharya *et al.*, 2006; Albrecht & Penzagos, 2012), acid (Acharya *et al.*, 2006; Long *et al.*, 2012) smoke water (Chou *et al.*, 2012), etc., but it is rare that the results of more than one or two treatments have been compared in the same study. Because different species and even collections within species vary in germination rate, (Acharya *et al.*, 2006; Albrecht & Penzagos, 2012), the results of these studies are not directly comparable to one another in order to determine the most effective scarification treatment. I therefore explored six different pre-planting seed treatments (e.g. chemical and physical scarification) to determine which would best promote germination in the generalist forage crop, *Astragalus cicer* “Oxley”.

METHODS

Astragalus cicer (L.) (cicer milkvetch) is an old-world native that was introduced to North America as a hardy, palatable forage crop (Acharya *et al.*, 2006). “Oxley” is an ecotype that was first collected in the former USSR and introduced to the United States in 1971 (Acharya *et al.*, 2006). Although *A. cicer* is not rare, it is a suitable model for rare species because it is readily commercially available without threatening wild populations, and because it, like its rare congenerics, is well known for its slow stand establishment, largely due to low germination rates and prolonged seed dormancy (Acharya *et al.*, 2006).

I exposed 50 *A. cicer* seeds (Granite Seed, Denver, CO) to each of six different scarification treatments, starting March 15, 2013 at Denver Botanic Gardens (DBG) in Denver, Colorado. The scarification treatments were physical damage, hot water, hydrogen peroxide, acid, fire, and a control. Control seeds were planted in 1 cm^2 germination pots, without scarification, on the surface of a seed starter mix, and covered with approximately 3 mm of vermiculite. Treated seeds, except fire, were planted in the same manner, but after a scarification treatment. I physically scarified seeds by cracking the seed coat opposite the radicle with a pair of infant nail clippers, being careful to not damage the endosperm or embryo. For the hot water treatment, seeds were placed in a thermos and covered with boiling (~95 C) water. I closed the thermos and allowed the seeds to soak for 20 hours before planting. Peroxide seeds were soaked in pure ZeroTol (27% hydrogen peroxide) for one hour before planting. Acid treated seeds were soaked in lab grade sulfuric acid (98%) for five minutes. Fire treated seeds were scattered on the soil surface of two 10 cm clay pots, and then covered with ~2 cm of dry pine needles and grass. The dry material was lit with a butane torch and allowed to burn until naturally extinguished. Approximately 2 mm of ash remained, and the seeds were left undisturbed to germinate in the clay pots. The total number of seeds germinated in each treatment was recorded approximately twice per week for one month.

All seedlings were reared in a propagation greenhouse at DBG. The potting soil was checked daily and kept evenly moist by DBG horticulture staff. Plants were exposed only to natural sunlight, which, given the date and latitude, ranged between approximately 12 hours at the beginning of the trial and 13 hours and a half hours at the end of the trial.

Germination data were analyzed with a proportional hazards analysis using JMP v10. This analysis type is well suited to germination data in that it is intended for time series datasets composed of binary data in which each observation is a replicate (i.e. each seed has germinated or not germinated), and compares observed and expected frequencies with a χ distribution. Repeated measures ANOVA was not used because calculating the variance of proportions based on grouped binary data is inappropriate in that the proportions are both ordinal and bounded between 0 and 1.

RESULTS

Seed treatment was an exceptionally strong predictor of seed germination success (χ^2=101.4, P<0.0001, df=5, n=300). Physically scarified seeds germinated most quickly, and were more than twice as successful as any other treatment (Table 1), with a final germination rate of 74% over 33 days (Figure 1). Statistically similar percentages of unscarified, acid scarified, and peroxide scarified seeds germinated (30%, 34%, and 26%, respectively) (Table 1). No seeds from either hot water or fire scarification treatments germinated. Across all treatments, the bulk of germination occurred within the first 2 weeks, with virtually no germination after that point (Figure 1).

DISCUSSION

Although many scarification treatments have been attempted for *Astragalus* species, my data show that not all treatments are equal in efficacy. In fact, only one treatment, physical scarification, was significantly better than the control, and both the fire and hot water treatments were significantly worse than the control, effectively sterilizing all of the seeds.

Based on my data, I recommend that propagation efforts involving *Astragalus* species use physical scarification as the primary method for breaking seed dormancy. Whereas other scarification treatments have been effective in certain circumstances, physical scarification has generally been shown to be the most effective treatment in studies that have compared it to alternative methods (Acharya *et al.*, 2006; Albrecht & Penzagos, 2012). The only downside to physical scarification, the labor-intensive nature of damaging the seed coat with sandpaper, a razor blade, or nail clippers, can be overcome with commercial equipment, if necessary, although at the cost of slightly higher seed loss to excessive damage (Acharya *et al.*, 2006).

Whereas other studies have demonstrated that methods involving cold, heat, acid, etc., can improve germination over controls, I recommend against their use in *Astragalus,* as the studies comparing different durations and intensities (temperature, concentration) of these treatments have found a relatively narrow range of optimal conditions (Albrecht & Penzagos, 2012; Chou *et al.*, 2012; Long *et al.*, 2012). Treatments of insufficient duration or intensity appear to be incapable of breaking seed dormancy, whereas treatments of excessive duration or intensity damage not only the seed coat, but the embryo as well, causing a loss of viability (Albrecht & Penzagos, 2012; Chou *et al.*, 2012; Long *et al.*, 2012). This is evidenced in our own study by the apparently insufficient acid and peroxide treatments compared to the apparently excessive fire and hot water treatments.

CONCLUSIONS

Physical scarification is a simple and reliable way to improve germination rates in *Astragalus* species with hard seed dormancy. I advise that, particularly for rare species for which seeds are limited, attempting to optimize other techniques is an unnecessary waste of resources when physical scarification is equally if not more effective.

Acknowledgements. - I would like to thank Anna Sher for her help with data analysis and Jennifer Neale for her comments on the manuscript and facilitation of use of the DBG greenhouse. I also thank the DBG horticulture staff, particularly Mike Bone and Katy Wilcox, for permission to use valuable greenhouse space and planting materials, and for their invaluable aid and expertise. I thank Elizabeth Pilon-Smits for advice on cultivating *Astragalus* species.

References

Acharya, S.N., Kastelic, J.P., Beauchemin, K.A. & Messenger, D.F. (2006) A review of research progress on cicer milkvetch (Astragalus cicer L .). *Can J Plant Sci*, 1, 50–62.

Albrecht, M.A. & Penzagos Z, J.C. (2012) Seed germination ecology of three imperiled plants of rock outcrops in the southeastern United States. *J Torrey Bot Soc*, 139, 86–95.

Barneby, R.C. (1964) Atlas of North American Astragalus. The New York Botanical Garden, Bronx, NY.

Baskin, J.M., Baskin, C.C. & Li, X. (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Spec Biol*, 15, 139–152.

Chou, Y.-F., Cox, R.D. & Wester, D.B. (2012) Smoke Water and Heat Shock Influence Germination of Shortgrass Prairie Species. *Rangeland Ecol Manag*, 65, 260–267.

Godefroid, S., Piazza, C., Rossi, G., Buord, S., Stevens, A.-D., Aguraiuja, R., Cowell, C., Weekley, C.W., Vogg, G., Iriondo, J.M., Johnson, I., Dixon, B., Gordon, D., Magnanon, S., Valentin, B., Bjureke, K., Koopman, R., Vicens, M., Virevaire, M. & Vanderborght, T. (2011) How successful are plant species reintroductions*? Biol Conserv*, 144, 672–682.

Long, Y., Tan, D.Y., Baskin, C.C. & Baskin, J.M. (2012) Seed dormancy and germination characteristics of Astragalus arpilobus (Fabaceae, subfamily Papilionoideae), a central Asian desert annual ephemeral. *S Afr J Bot*, 83, 68–77.

Maunder, M. (1992) Plant reintroduction: an overview. Biodiversity and Conservation, 1, 51–61.

Menges, E.S. (2008) Restoration demography and genetics of plants: when is a translocation successful? *Aust J Bot*, 56, 187.

Neel, M. (2008) Patch connectivity and genetic diversity conservation in the federally endangered and narrowly endemic plant species Astragalus albens (Fabaceae). *Biol Conserv*, 141, 938–955.

Vicente, M.J., Segura, F., Aguado, M., Migliaro, D., Franco, J. a. & Martínez-Sánchez, J.J. (2011) Genetic diversity of Astragalus nitidiflorus, a critically endangered endemic of SE Spain, and implications for its conservation. *Biochem Syst Ecol*, 39, 175–182.

Watrous, K.M. & Cane, J.H. (2011) Breeding Biology of the Threadstalk Milkvetch, Astragalus filipes (Fabaceae), with a Review of the Genus. *Am Midl Nat*, 165, 225–240.

FIGURES

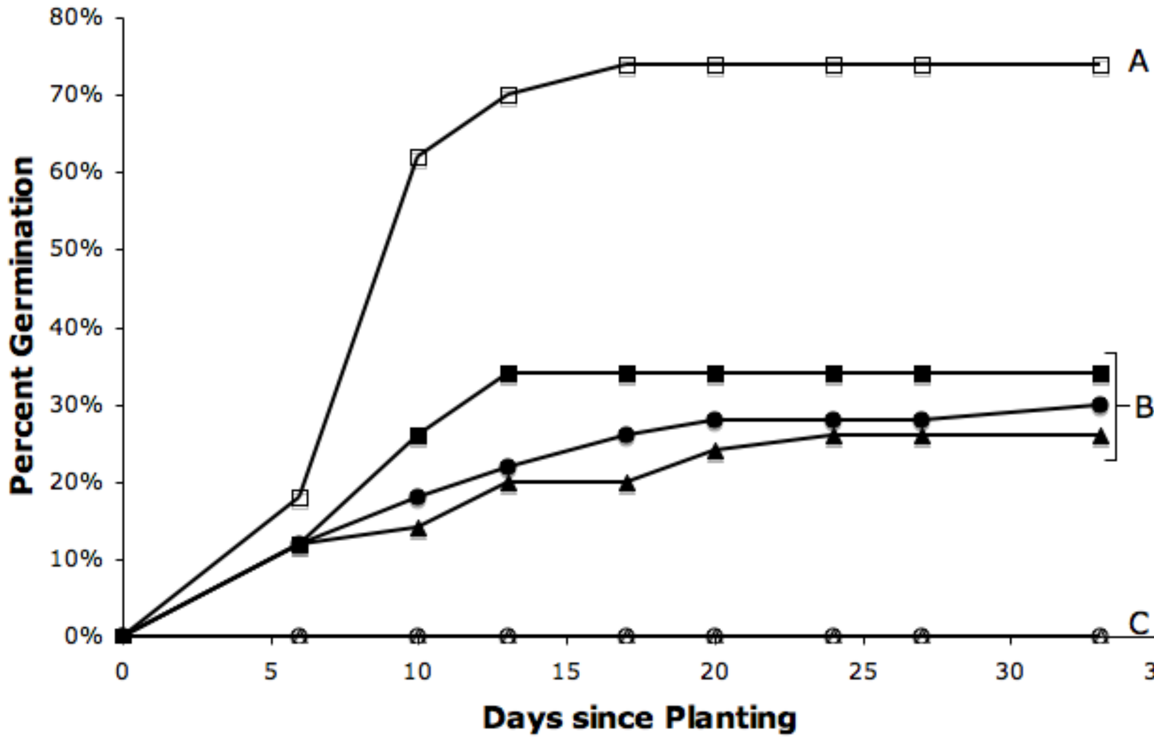

Figure 1: Germination rates over time for different scarification treatments for *Astragalus cicer.* The treatments include an unscarified control (closed circles) and seeds scarified with hot water (open circles), sulfuric acid (closed squares), nail clippers (open squares), hydrogen peroxide (closed triangles), and fire (open triangles). Letters indicate statistically different treatments via proportional hazards analysis.

Table 1: Pairwise risk ratios for treatments, expressed as the ratio of the germination success of the row relative to the column. n=50 for each treatment. * represents statistical significance at the P<0.001 level.

Treatments	Control	Hot Water	Sulfuric Acid	Nail Clippers	Hydrogen Peroxide	Fire
Control	1	>100*	0.85	0.32*	1.17	>100*
Hot Water	<0.01*	1	<0.01*	<0.01*	<0.01*	1
Sulfuric Acid	1.17	>100*	1	0.37*	1.38	>100*
Nail Clippers	3.17*	>100*	2.69*	1	3.72*	>100*
Hydrogen Peroxide	0.85	>100*	0.72	0.27*	1	>100*
Fire	<0.01*	1	<0.01*	<0.01*	<0.01*	1

Table of raw data: Number of germinated seeds (out of 50) for each pretreatment, on a given day of the experiment.

Treatment	**0**	**6**	**10**	**13**	**17**	**20**	**24**	**27**	**33**
Control	0	6	9	11	13	14	14	14	15
Hot water	0	0	0	0	0	0	0	0	0
Acid	0	6	13	17	17	17	17	17	17
Physical	0	9	31	35	37	37	37	37	37
Peroxide	0	6	7	10	10	12	13	13	13
Fire	0	0	0	0	0	0	0	0	0