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Do Abrasion- or Temperature-Based Techniques More Effectively Relieve Physical Dormancy in Seeds of Cold Desert Perennials?

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ABSTRACT

Seed dormancy can present a significant barrier to restoration outcomes in dryland systems. Physical and combinational (physical + physiological) dormancy are prevalent among seeds of many herbaceous perennials used in restoration of drylands throughout the western United States. Although many techniques designed to alleviate these dormancy traits exist, their efficacy is species specific, may result in embryo damage, and may have limited large-scale application. To identify the most effective means of dormancy alleviation with the potential to be used on an operational scale, we examined the effects of 16 temperature-based techniques (altering temperature and duration of wet heat, freezing and wet heat, and freeze-thaw cycles) and 6 abrasion-based techniques (altering pneumatic scarification length or using a single duration of manual scarification) on the enhancement of seed permeability among two physically dormant (western prairie clover [*Dalea ornata* {Douglas} Eaton & Wright] and Munro's globemallow [*Sphaeralcea munroana* {Douglas} Spach]) and two combinationally dormant species (basalt milkvetch [*Astragalus filipes* Torr. ex A. Gray] and longspur lupine [*Lupinus arbustus* {Douglas} ex Lindl.]). We first assessed seed imbibition following exposure to all temperature- and abrasion-based techniques to identify those most successful at promoting seed permeability and then evaluated the effectiveness of those techniques through a series of germination experiments. For combinationally dormant species, we also tested whether exposure to GA_3 enhanced germination. Abrasion-based techniques were more effective than temperature-based techniques at improving water uptake across all species. Pneumatic scarification significantly improved germination, but optimal treatment durations were species specific. GA_3 did not enhance germination under the tested conditions. We conclude that pneumatic scarification is a fast, safe, and effective method for alleviating physical seed dormancy with a potential to be scaled up for operational use in restoration.

Introduction

Physical seed dormancy occurs in 18 angiosperm families and is common among many forbs native to arid and semiarid regions worldwide (Gama-Arachchige et al., 2013). This dormancy is imposed by one or more layers of palisade cells that prevent water uptake in mature seeds (Rolston, 1978). In situ, seeds become permeable when a specialized structure known as the "water gap" opens in response to specific environmental conditions, creating a passage for water entry (Baskin, 2003; Van Assche et al., 2003) through numerous modes (e.g., abrasion, temperature fluctuations, fire-induced heat) (Baskin and Baskin, 2014; Ooi et al., 2014; Erickson et al., 2016b). Some species can also be classified as combinationally dormant and produce seeds with impermeable seed coats (i.e. physical dormancy) and embryos with an initially inhibited growth potential (i.e. physiological dormancy). In combinationally dormant species, physiological dormancy must be alleviated through stratification, after-ripening, or the use of chemical stimulants to initiate germination once seeds are permeable (Baskin and Baskin, 2014; Turner et al., 2006a).

Under natural recruitment conditions, seed dormancy enables seeds to remain in the soil for many years, or even decades, and is a means of

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bet-hedging against unfavorable environmental conditions for seedling establishment (Baskin and Baskin, 2014). However, in the restoration context, where predictable and synchronous plant establishment from seeds is critical, seed dormancy can be a barrier to plant recruitment, leading to excessive seed wastage (Merritt and Dixon, 2014) and thwarting efforts to increase commercial seed availability through field propagation (Shaw et al., 2012). To address this, the development of reliable dormancy alleviation techniques in a scalable, affordable, and safe manner is needed. Some of the most effective means for increasing seed permeability of physically dormant seeds rely on the use of harsh chemicals that can cause embryo damage (i.e., sulphuric acid, diethyl dioxide) or are exceedingly labor intensive to employ (e.g., manual scarification) (Kimura and Islam, 2012), inhibiting large-scale use. Other approaches to increase seed permeability include the use of temperature treatments (e.g., freeze-thaw cycles [Stout, 1990; Tiryaki and Topu, 2014] and wet or dry heat [Kildisheva et al., 2011; Pound et al., 2015; Erickson et al., 2016a]), which can trigger the opening of the water gap, or mechanized scarification techniques, which facilitate the physical abrasion of the seed coat (Khadduri and Harrington, 2002; Kimura and Islam, 2012; Bushman et al., 2015; Jones et al., 2016a, 2016b). However, the effectiveness of these techniques are species or accession specific and require extensive empirical evaluation to avoid treatment failure or embryo damage due to overgeneralized treatment prescriptions (Kimura and Islam, 2012).

This study identified the most effective technique for improving seed permeability and subsequent germination among two physically dormant (*Dalea ornata* [Fabaceae], *Sphaeralcea munroana* [Malvaceae]) and two combinationally dormant (*A. filipes* and *L. arbustus* [Fabaceae]) herbaceous perennials that are widely distributed and highly desirable for restoration use in the Great Basin region of North America (Bushman et al., 2015; Dumroese et al., 2015; Jones et al., 2016a, 2016b).

Materials and Methods

Seeds were collected throughout the Great Basin floristic region in 2015 (Table S1; available online at https://doi.org/10.1016/j.rama.2018.02.004), cleaned, and stored in dry, cool conditions until treatments were applied in summer 2016. First, we assessed seed imbibition following exposure to all 22 scarification treatments relative to untreated seeds. Second, we selected techniques that significantly increased seed permeability and conducted a germination experiment to identify the optimal treatment duration to improve germination capacity without causing embryo damage. Finally, for species with combinational dormancy, we tested whether exposure to gibberellic acid (GA_3) provided additional germination benefits.

Seed Imbibition

We first assessed the impact of 22 individual treatments on seed permeability of each species using three replicates of 30 seeds. Treatments consisted of various applications of wet heat (5-, 30-, 120-, or 300-s immersion in 90°C deionized water), freezing + wet heat (2 h at −80°C or −20°C followed by 5- or 30-s immersion in 90°C deionized water), freeze-thaw cycles (2 h at −80°C or −20°C followed by 2 h at 23°C ± 2°C, repeated 1, 2, 3, or 6 times), pneumatic scarification (for 10, 20, 40, 80, or 160 s at 138 KPa applied using a Mater seed scarifier [PSS2000, OEM, Inc., Corvallis, OR], and manual sandpaper scarification (120 grit), compared against an untreated seeds (Table 1). Following treatment, each replicate was weighed, placed onto a square of organza mesh on filter paper (moistened with deionized water) inside Petri dishes, and kept at ambient laboratory conditions (23°C ± 2°C). After 48 h, replicates were blotted dry and reweighed to calculate water uptake: Mass Increase (%) = $[(W_i - W_d)/W_d] \times 100$, where W_i and W_d equal the mass of imbibed and dry seed, respectively. The proportion of permeable seeds (% permeable) per sample was calculated on the basis of the number of seeds that increased in volume after 48 h of imbibition (Table 2). Of all tested techniques, pneumatic and manual scarification achieved the highest mass increase and permeability percentages and were subsequently chosen for examination in the germination experiment.

Seed Germination

Selecting the most effective treatments from the imbibition experiment, we evaluated the capacity of pneumatic scarification (for 10, 20, 40, 80, or 160 s) and manual sandpaper scarification (120 grit) to alleviate physical dormancy. Each treatment was replicated four times, using 25 "seeds per replicate". Following treatment, seeds were sterilized in 2% (w/v) calcium hypochlorite for 30 min and triple-rinsed with deionized water. For the two physically dormant species (*D. ornata* and *S. munroana*), seeds were plated onto water agar (0.7% w/v) in sealed Petri dishes. Seeds of the two species with combinational dormancy

Table 1
Description of the "five" dormancy alleviation techniques and 22 treatments evaluated for their ability to increase permeability in physically and combinationally (physically + physiologically) dormant seeds.

Technique	Treatment	Method
Control	Control	No treatment
Wet heat	90°C (5 s) 90°C (30 s) 90°C (120 s) 90°C (300 s)	Seeds were placed into mesh satchels and immersed in deionized water heated to 90°C for 5, 30, 120, or 300 s.
Freeze + wet heat	−20°C (2 h) + 90°C (5 s) −80°C (2 h) + 90°C (5 s) −80°C (2 h) + 90°C (30 s)	Seeds placed into mesh satchels inside Ziploc bags and exposed to −20°C or −80°C or for 2 h inside laboratory freezers followed by immediate submergence in deionized water heated to 90°C for 5 or 30 s.
Freeze-thaw cycle	−20°C (2 h) + 23°C (2 h) × 1 −20°C (2 h) + 23°C (2 h) × 2 −20°C (2 h) + 23°C (2 h) × 3 −20°C (2 h) + 23°C (2 h) × 6 −80°C (2 h) + 23°C (2 h) × 1 −80°C (2 h) + 23°C (2 h) × 2 −80°C (2 h) + 23°C (2 h) × 3 −80°C (2 h) + 23°C (2 h) × 6	Seeds placed into mesh satchels inside Ziploc bags and exposed to −20°C or −80°C or for 2 h inside laboratory freezers and followed by a 2-h thaw at (23°C) after removal from the freezers, with the entire 4-h cycle repeated 1, 2, 3, or 6 times for both of the freezing temperatures.
Pneumatic	10 s 20 s 40 s 80 s 160 s	Samples of seeds (5 g sample^{-1}) were treated for 10, 20, 40, 80, or 160 s using a Mater seed scarifier [PSS2000, OEM, Inc., Corvallis, OR] attached to an air compressor and set to 138 KPa with a 180-grit abrasive liner.
Manual scarification	Manual	Treatment applied by rubbing both lateral surfaces of each seed against 120-grit sandpaper until seed coat abrasion was observed.

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