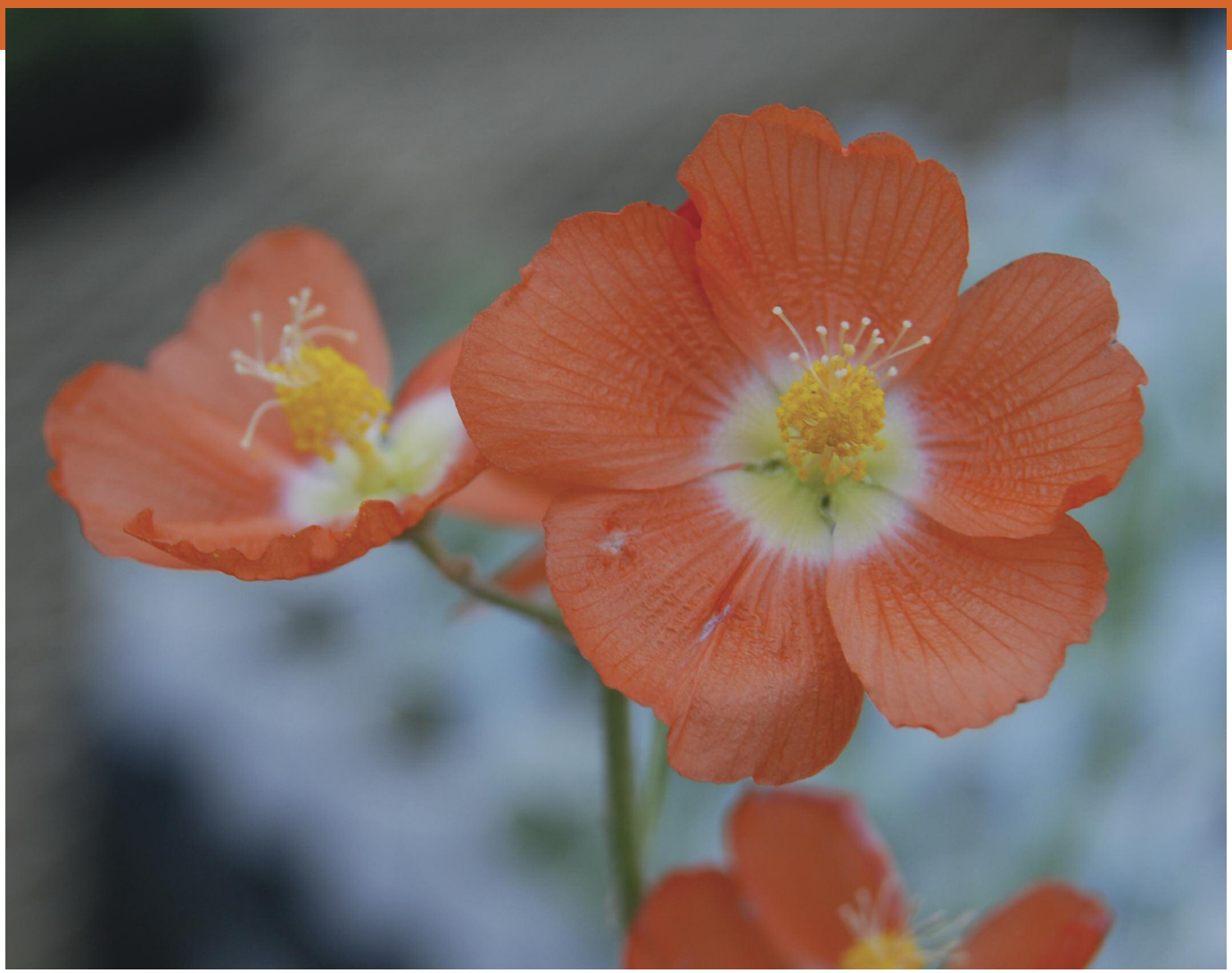

Figure 1. Inflorescence of Munro's globemallow (*Sphaeralcea munroana*). Photo by Olga A Kildisheva

Boiled, tumbled, burned, and heated: seed scarification techniques for Munro's globemallow appropriate for large-scale application

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ABSTRACT

Physically dormant seeds of Munro's globemallow (*Sphaeralcea munroana* (Douglas) Spach [Malvaceae]) were scarified by boiling, tumbling, burning, dry-heating, and burning + heating treatments in an attempt to find an effective, operational, large-scale treatment for nurseries and restoration activities. Results indicate that out of the tested treatments, seed germination was highest following boiling water scarification (49%). All other treatments did not achieve significant improvements in germination compared to the control. Findings should improve the use of this cool-season perennial for restoration in the Great Basin, where its effectiveness in soil stabilization; its tolerance of disturbance, drought, and extreme temperatures; and its importance as a food source for animals make it a suitable candidate. In addition, the tested treatments should serve as a foundation for further method refinement.

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KEY WORDS

Malvaceae, germination, physical dormancy, operational seed treatment, *Sphaeralcea munroana*

NOMENCLATURE

USDA NRCS (2011)

Munro's globemallow (*Sphaeralcea munroana* (Douglas) Spach [Malvaceae]) is a perennial, cool-season forb endemic to the Great Basin region of the western US (Figure 1). This species is an important contender for broadscale ecosystem restoration across its native range due to its environmental resilience and ecological importance. Munro's globemallow is a key host for numerous native pollinators and is a source of food for many mammals (Beale and Smith 1970; Pendery and Rumbaugh 1986; Rumbaugh and others 1993; Cane 2011). In addition, the species can establish on disturbed sites, serves as a soil stabilizer, and is tolerant of arid conditions (Pavek and others 2011). The current lack of successful large-scale techniques for breaking dormancy limits use of this species in restoration.

Several studies show that species in the *Sphaeralcea* genus are physically dormant (Page and others 1966; Roth and others 1987; Smith and Kratsch 2009; Dunn 2011; Kildisheva and others 2011). Physical dormancy characterizes seeds that possess a palisade layer of lignified cells that prevent water imbibition (Corner 1951; Vazquez-Yanes and Perez-Garcia 1976). In these species, a specialized structure (that is, a water gap) located within the seedcoat regulates water and oxygen uptake and is impermeable while dormant. *In situ*, physical dormancy is alleviated through temperature changes, rapid drying, or scarification through abrasion or animal digestion (Baskin and Baskin 1998; Baskin and others 2000; Baskin 2003). *Ex situ*, chemical and mechanical scarification have been traditionally used to improve germination of physically dormant seeds (Page and others 1966; Roth and others 1987; Hoffman and others 1989; Baskin and Baskin 1998). Although chemical treatments that consist of submergence in sulfuric acid or diethyl dioxide have proved to be successful for some *Sphaeralcea* species, only diethyl dioxide significantly improved germination (53%) of *S. munroana* compared to the control (2%) (Page and others 1966; Roth and others 1987). Chemicals, however, can be hazardous, be problematic to obtain, present serious health risks, be cumbersome to dispose, and are thus suboptimal for operational use (Mallinckrodt Baker 2008 a,b).

Mechanical scarification through clipping, filing, or piercing can enhance germination of physically dormant seeds and has been demonstrated to be effective for a number of Malvaceae species (Baskin and Baskin 1997; Dunn 2011; ISTA 2011), *S. munroana* in particular (Kildisheva and others 2011). Nonetheless, manual seed treatment techniques are time consuming and unrealistic for use on a large scale (Baskin and Baskin 1998). Mechanization of these techniques can result in embryo damage due to scarification severity, often overriding the benefits of the treatment. Page and others (1966) report decreases in germination of *S. grossulariifolia* (Hook. & Arn.) Rydb. with the duration of scarification time in a sandpaper-lined rotating drum, while Roth and others (1987) suggest that seeds of *S. grossulariifolia, S. coccinea* (Nutt.) Rydb., and *S. munroana* perished after mechanical scarification irrelevant of treatment duration.

As an alternative, less traditional techniques, such as scarification in boiling water, rock tumbling, fire, and heating have been effectively used to increase seedcoat permeability in some physically dormant species. Dreesen (2004) recommends the use of seed abrasion in a rotating rock tumbler to improve germination of physically dormant species. The effects of tumbling have not been evaluated for *S. munroana*, specifically. Smith and Kratsch (2009) suggest, however, that tumbling durations that exceed 24 h may benefit germination of *Sphaeralcea* species and should be further explored.

Boiling water scarification has been shown to promote seed permeability and subsequent germination of several Malvaceae species (Christiansen and Moore 1959; Baskin and Baskin 1997; Himanen and others 2012). For example, seeds of *Iliamna corei* Sherff germinated to 93% (as compared to 0% germination of the control), following 5-s submergence in boiling water (Baskin and Baskin 1997). Boiling water scarification causes the opening of the water gap through the separation of the palisade and subpalisade layers of cells in the chalazal region of the seedcoat, which allows for imbibition to occur (Egley and Paul 1981, 1982; Egley and others 1986; Serrato-Valenti and others 1992; Gama-Arachchige and others 2010; Kildisheva and others 2011).

Abrasion through fire has also been observed to benefit germination. For example, *I. corei* demonstrated increased germination following simulated annual summer burning (1 to 2 min duration), with the highest germination achieved after 6 y of consecutive treatment (39 ± 6%) compared to the control (0%) (Baskin and Baskin 1997). Moreover, germination of physically dormant seeds of 8 Fabaceae species was substantially amplified after ignition with a torch (Sugii 2003). Dry heat may be a substitute for fire, often achieving superior results. Baskin and Baskin (1997) found that several dry heat temperatures and exposure durations optimized *I. corei* germination.

The use of native species for restoration is limited by high seed procurement cost and low establishment rates (when compared with the use of nonnative cultivars). Thus, economically feasible use of *S. munroana* is dependent on the development of efficient, large-scale seed treatments that break dormancy. To address this issue, we evaluated the effectiveness of 5 techniques (boiling water, tumbling, burning, heating, and burning + heating scarification) as potential treatments for large-scale use.

MATERIALS AND METHODS

Seeds were collected from native stands throughout the Wasatch Mountains of northern Utah (Great Basin Seeds, Ephraim, Utah) and stored at 1.5 ± 0.5 °C (35 ± 0.9 °F) for 6 mo. All treatments included five 50-seed replicates. The experiment was conducted at the University of Idaho, Center for

Forest Nursery and Seedling Research, Moscow. Prior to the start of the experiment, seeds were sterilized for 15 min with a 0.5% NaOCl solution and double-rinsed with deionized (DI) water.

Seeds were given one of 6 treatments: 1) control (no scarification); 2) boiling water; 3) tumbling; 4) burning; 5) dry-heating; and 6) burning + heating. Boiling water scarification was achieved by 10-s submergence in 100 °C (212 °F) water. Seeds were tumble-scarified in a rotary rock tumbler (Model AR-1, Tru-Square Metal Products, Auburn, Washington) with dry aluminum oxide grit (12 Mesh, Kramer Industries, Piscataway, New Jersey) for 72 h. Following tumbling, seeds were separated from grit using a series of sieves. For burning scarification, seeds were placed in single layer onto a metal mesh screen and submerged uniformly in 95% ethyl alcohol for 1 min. Seeds were removed from the alcohol, ignited with a hand-held butane torch, and allowed to burn for 10 s before being extinguished with DI water (Sugii 2003). For dry-heat scarification, seeds were placed into a laboratory oven at 80 °C (176 °F) for 60 min (Baskin and Baskin 1997). Seeds subject to burning + heating scarification were burned first.

Seeds were placed onto moistened blotter paper (Steel Blue germination blotter, Anchor Paper Company, St Paul, Minnesota) inside sealed, randomly arranged, plastic Petri plates (Fisher Scientific Company, Pittsburgh, Pennsylvania). Blotters were remoistened with 5 ml of DI water every 3 d. Seeds were incubated in a growth chamber at 24 °C day/17 °C night (75.2 °F/62.6 °F) temperature regime (Sabo and others 1979). Germination, defined as radicle protrusion to ≥5 mm (0.2 in), was monitored daily for 21 d.

Statistical Analysis

Germination capacity, or the extent to which seeds germinate within a given duration of time, is conventionally used to evaluate seed performance; however, other factors such as germination rate and uniformity are important in describing germination behavior and must be considered (Ching 1959; Thomson and El-Kassaby 1993). Although efforts to incorporate several parameters into one (for example, Czabator 1962) have been made, they do not provide an accurate representation of germination over time. To circumvent this, we fit daily germination data to a mathematical model, the parameters of which provide a comprehensive portrayal of germination behavior (Equation 1); where $G(t)$ is the cumulative germination (%) at time (t) expressed in days (d), G_c is cumulative germination or the germination capacity (%), GC_{50} is the time (d) necessary to achieve 50% germination, and G_d is the rate (%/d) of germination (Kildisheva and others 2011). For each treatment replicate, parameter estimates (G_c, GC_{50}, G_d) were generated through curve-fitting from which expected mean squares, components of variance, and R^2 values were obtained. Specific differences between treatments were determined with one-way ANOVA and Tukey's HSD ($\alpha = 0.05$) (SAS Institute, Cary, North Carolina).

$$G(t) = \frac{G_c}{1+e^{\left[\frac{GC_{50}-t}{G_d}\right]}} \qquad \text{(Equation 1)}$$

RESULTS

Results indicate that G_c, GC_{50}, and G_d varied significantly ($P < 0.0001$, $P < 0.0001$, and $P = 0.0115$, respectively) among treatments (Table 1). Most variation in GC_{50} and G_c ($R^2 = 0.75$, $R^2 = 0.64$) could be explained by differences in treatment, with a weaker correlation for G_d ($R^2 = 0.44$). The G_c was highest (49%) after boiling water scarification compared with all other treatments ($P < 0.0001$) (Table 1; Figure 2). The remaining treatments did not enhance germination (G_c <20%). Boiling water scarification produced germination behavior that was slightly different from the other treatments. Primarily, following day 7 (when the G_d of the remaining treatments began to slow), the G_d of boiling water scarified seeds continued to increase ($P = 0.0165$) without reaching an asymptote within the test duration. The regression model used to describe seed behavior is designed to estimate germination capacity (germination percentage at the asymptote). Because this point was not reached by seeds treated with boiling water, the cumulative germination by day 21 reached 43% but was projected to have reached its asymptote (49%) after 52 d. For this reason, the G_c parameter estimate for the boiling water treatment differs from the cumulative germination; this difference is unique only to the boiling water treatment.

DISCUSSION

Although in our previous work mechanical scarification of individual seeds using a blade achieved high germination (93%) (Kildisheva and others 2011), that method is impractical when

TABLE 1

Effects of scarification techniques suitable for large-scale use on the germination behavior of Sphaeralcea munroana.

Treatment	G_c (%)	GC_{50} (d)	G_d (%/d)
Control	10.7 ± 1.1 b	1.6 ± 0.2 b	1.4 ± 0.5 b
Boiling water	49.0 ± 12.9 a	8.2 ± 1.2 a	4.2 ± 0.8 a
Tumbling	20.3 ± 1.3 b	2.1 ± 0.1 b	2.3 ± 0.3 b
Burning	17.0 ± 1.6 b	2.0 ± 0.3 b	2.0 ± 0.6 b
Heating	10.7 ± 1.3 b	4.4 ± 0.2 b	2.1 ± 0.5 b
Burning + Heating	4.2 ± 1.8 b	4.2 ± 1.0 b	1.2 ± 0.5 b
P values	0.0001	0.0001	0.0115

Notes: G_c = germination capacity (%), GC_{50} = the time (d) necessary to achieve 50% germination, and G_d = the rate (%/d) of germination. Multiple comparisons were obtained using Tukey's HSD ($\alpha = 0.05$). Different letters indicate significant differences among treatments.

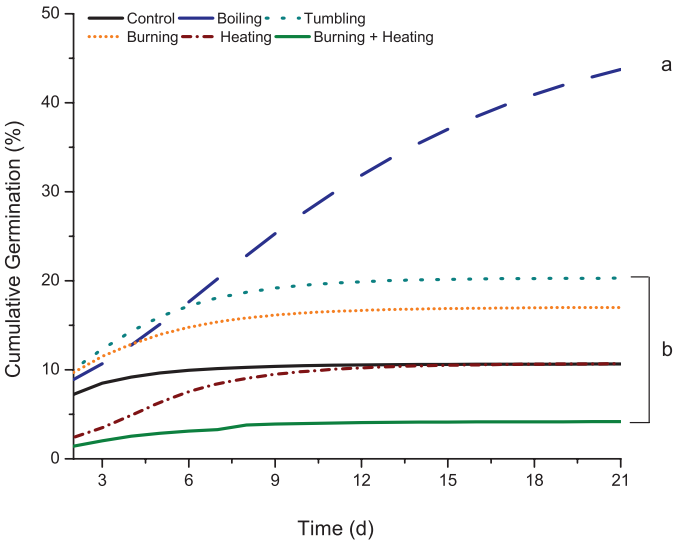

Figure 2. Cumulative germination percentage of *Sphaeralcea munroana* seeds subject to 5 experimental treatments during a 21-d observation period. Each line represents the mean of 5 replicates. Different letters indicate significant differences (P <0.05) between treatments.

large quantities of seeds must be treated. Our results suggest that boiling water scarification provides an operationally suitable technique that can be used on a large scale. Furthermore, while not directly tested in this study, results suggest that this technique can be a safe alternative to the use of chemical scarification treatments, including sulfuric acid and diethyl dioxide. For boiling water scarification, the germination curve never reached its asymptote within the duration of the study, and although the model estimates that germination would reach its asymptote at 49%, further work should evaluate the extent of germination given a longer testing period. In addition, the relatively large standard error associated with germination capacity may indicate an incomplete opening of the water gap, resulting in lower total water uptake. In our previous work (Kildisheva and others 2011) we suggest that 10-s submergence of *S. munroana* seeds in boiling water achieved only partial water gap opening. Modifications to the tested boiling water procedures that include an extended period of submergence, the use of continuously running hot water, or post-scarification submergence in cool water may maximize the surface area and number of affected seeds, allowing for a more uniform water gap opening and increased imbibition.

Neither heating nor burning yielded significant germination improvements, which is inconsistent with the optimized germination of *I. corei* following a 60-min dry heat (80 °C [176 °F]) application (Baskin and Baskin 1997). Similarly, tumbling for 72 h was ineffective at breaking dormancy. Modifications such as further extending the tumbling duration, using alternative abrasive media, or adding water to the media to allow for partial seed hydration in the tumbling process may improve results. Thus, although general patterns of dormancy may be similar across members of the same genus, explicit temperature and moisture requirements can be species specific and should be further examined.

MANAGEMENT IMPLICATIONS

Although mechanical scarification of individual seeds yields the highest germination of *Sphaeralcea munroana* seeds (Kildisheva and others 2011), such a method is practical only when few plants are required or a limited number of seeds is available. For large-scale applications, our finding that boiling water scarification would achieve nearly 50% germination makes it an appealing treatment, considering the difficulty, labor requirements, and potential hazards of mechanical and (or) chemical scarification. Moreover, the lack of uniformity in dormancy break following boiling water scarification may offer a benefit under some restoration scenarios, as the dormant portion of the seedlot will become part of the seedbank, ensuring recruitment at a later date. *In situ*, *Sphaeralcea* plants generally do not occur in high densities and rely heavily on recruitment from the seedbank, where viable seeds can persist for years (Pendery and Rumbaugh 1993). For example, *S. ambigua* A. Gray recruitment is strongly correlated to moisture availability and restricted to establishment between perennial shrubs, with no strong associations with other species, pointing to the harsh climatic factors being the primary limitation to plant density (Wright and Howe 1987; Henderson and others 1988). Thus, the addition of a dormant portion of the seedlot to the soil bank may benefit the long-term persistence of the *Sphaeralcea* community.

Further investigation of potential treatment improvements, such as alternative scarification techniques and an evaluation of treatment durations, are necessary for a more complete and uniform dormancy break. Our findings should aid in broader use of this drought-tolerant perennial in plant production programs investigating landscaping and restoration using native plants of the Great Basin in the US.

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