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Emily M. Nielson Brigham Young University

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Use of Seed Coating Technologies to Improve *Cercocarpus ledifolius*

(Curl-Leaf Mountain Mahogany) Seed Germination and

Emergence to Reclaim Mine Lands

Emily M. Nielson

A thesis submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of

Master of Science

Bradley D. Geary, Chair Matthew D. Madsen April Hulet

Department of Plant and Wildlife Sciences

Brigham Young University

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ABSTRACT

Use of Seed Coating Technologies to Improve *Cercocarpus ledifolius* (Curl-Leaf Mountain Mahogany) Seed Germination and Emergence to Reclaim Mine Lands

Emily M. Nielson Department of Plant and Wildlife Sciences, BYU Master of Science

Globally, mining is vital to human interests, but its practice can cause landscape alteration which may look unnatural or engineered. The reintroduction of native plants to these areas is needed to restore the visual appeal and ecological function back into these altered mine lands. *Cercocarpus ledifolius* (curl-leaf mountain mahogany) is one desirable native species in the Intermountain West that is prized for its potential to grow on step and rocky hillsides and for the habitat it provides for wildlife. Unfortunately, *C. ledifolius* does not establish well from seed, which has been attributed to seed dormancy. The first objective of this study was to determine if scarification and gibberellic acid (GA₃) treatments improve germination by alleviating seed dormancy. We also aimed to determine if a combination of fungicide and hydrophobic seed coatings increased emergence and establishment of *C. ledifolius* seedlings in mine overburden by reducing loss from fungal pathogens and premature germination. We found that two treatments, GA₃ and GA₃ + hydrophobic coatings, improved emergence compared to untreated seed, producing 1.8 ($P = 0.0682$), and 2.2 ($P = 0.0751$) more seedlings per meter, respectively. The second objective of this study was to make improvements in the laboratory to treatments explored in the field trial. We found that *C. ledifolius* seed responded inconsistently to treatments applied in the lab. The 15-minute acid scarified seed in combination with various GA3 seed coatings had significantly higher germination than untreated seed in one trial but had no difference in a second trial. Overall, these results indicate that seed enhancement technologies have the potential to improve *C. ledifolius* emergence in reclaimed mine lands, but additional research is needed to understand the species' dormancy characteristics better and improve the efficacy of the applied seed treatments.

Keywords: restoration, seed enhancement technology, mine revegetation, seed dormancy

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CHAPTER 1

Use of Seed Coating Technologies to Improve *Cercocarpus ledifolius* (Curl-Leaf Mountain Mahogany) Seed Germination and Emergence to Reclaim Mine Lands

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ABSTRACT

Globally, mining is vital to human interests, but its practice can cause landscape alteration which may look unnatural or engineered. The reintroduction of native plants to these areas is needed to restore the visual appeal and ecological function back into these altered mine lands. *Cercocarpus ledifolius* (curl-leaf mountain mahogany) is one desirable native species in the Intermountain West that is prized for its potential to grow on step and rocky hillsides and for the habitat it provides for wildlife. Unfortunately, *C. ledifolius* does not establish well from seed, which has been attributed to seed dormancy. The first objective of this study was to determine if scarification and gibberellic acid (GA3) treatments improve germination by alleviating seed dormancy. We also aimed to determine if a combination of fungicide and hydrophobic seed coatings increased emergence and establishment of *C. ledifolius* seedlings in mine overburden by reducing loss from fungal pathogens and premature germination. We found that two treatments, GA₃ and GA₃ + hydrophobic coatings, improved emergence compared to untreated seed, producing 1.8 ($P = 0.0682$), and 2.2 ($P = 0.0751$) more seedlings per meter, respectively. The second objective of this study was to make improvements in the laboratory to treatments explored in the field trial. We found that *C. ledifolius* seed responded inconsistently to treatments applied in the lab. The 15-minute acid scarified seed in combination with various GA3 seed

coatings had significantly higher germination than untreated seed in one trial but had no difference in a second trial. Overall, these results indicate that seed enhancement technologies have the potential to improve *C. ledifolius* emergence in reclaimed mine lands, but additional research is needed to understand the species' dormancy characteristics better and improve the efficacy of the applied seed treatments.

INTRODUCTION

Drylands include arid, semi-arid, and dry-subhumid ecosystems, which account for 41% of the Earth's land surface and support upwards of two billion people (Adeel et al., 2005). Unfortunately, drylands are one of the most susceptible biomes to land degradation and climate change (James et al., 2013). It is estimated that 10-20% of global drylands suffer from one or more forms of degradation, with twelve million more hectares being degraded each year (Adeel et al., 2005). Several stressors contribute to the degradation of drylands, including invasive annual grasses (D'Antonio & Vitousek, 1992; Germino et al., 2016; Knapp, 1996), livestock grazing, urbanization (Lovich & Bainbridge, 1999), low to variable rainfall, low soil nutrient availability (James et al., 2013), and mining (Dentoni & Massacci, 2013).

Mining extracts valuable minerals, metals, and fuels from the earth, which create products needed globally. Some of the materials extracted in mining include copper, gold, platinum, boron, molybdenum, nickel, zinc, and many others that contribute to creating products from cell phones to hybrid cars, and solar panels (Levonas (n.d.); Muhovich (n.d.); Weichman (n.d.)). While mining is important to human interests and activity, it can pose risks to native flora and fauna and have a visual impact on surrounding areas (Amirshenava & Osanloo, 2019; Dudka & Adriano, 1997; Schwegler, 2006). Landscape alteration is a significant impact of mining as

native hillsides become covered with overburden substrate leftover from extracting valuable materials (Dentoni & Massacci, 2013). The establishment of plants in overburden substrate is challenging because it is often lacking in soil microbial diversity, and it frequently has reduced water and nutrient holding capacity (Bateman et al., 2018; Borůvka et al., 2012; Merino-Martín et al., 2017). Plant establishment on degraded sites is heavily influenced by the physical and chemical properties of the soil, which provide the water and nutrients required for sustaining plant growth (Bateman et al., 2018). Mining is vital to our global societies due to the importance of technology, so it is necessary to find methods to minimize the visual impact of mining and discover approaches to establish native flora and fauna on landscapes affected by mining (Menegaki et al., 2015).

The Bingham Canyon Mine is an open pit copper mine located in Salt Lake County, UT, west of Salt Lake City. It began operations in 1903 and has displaced more than 3 billion metric tons of waste rock, which now covers over 2,000 hectares of the native landscape (Borden & Black, 2005). The waste rock is a mixture of monzonite intrusive rock, quartzite, and lesser amounts of limestone (Borden, 2003). The hillsides surrounding the mine site are dominated by both *Cercocarpus ledifolius* Nutt. (curl-leaf mountain mahogany) and *Quercus gambelii* (gambel oak) communities, with *Pseudotsuga menziesii* (douglas fir) and *Acer grandidentatum* (bigtooth maple) found on the higher altitude slopes. In an analysis of the volunteer revegetation found at the Bingham Canyon Mine by Borden and Black (2005), curl-leaf mountain mahogany was found to be among the most successful woody species at the site, colonizing a wide variety of waste rock areas.

Cercocarpus ledifolius is an evergreen species that ranges in height from 2-9 m. It is often found on warm, dry, rocky slopes in the mountain brush zone of the Intermountain West in

North America, and frequently found colonizing habitats where few other desirable woody species grow (Brotherson, 1990; Davis & Brotherson, 1991; Ibanez & Schuup, 2002). *Cercocarpus ledifolius* is a browse species for large game such as mule deer, elk, and bighorn sheep, as well as an important species for smaller wildlife (Paschke et al., 2003; Scheldt & Tisdale, 1970; Wood et al., 1995). In addition to its value for wildlife, *C. ledifolius* is an actinorhizal plant species that influences the nitrogen cycling and status of the surrounding plant communities (Freund et al., 2018; Lepper & Fleschner, 1977).

Due to the many beneficial characteristics of *C. ledifolius*, this species is of value for mine land revegetation efforts; however, most attempts at establishing *C. ledifolius* by seed fail (Schultz, Tausch, & Tueller, 1996; Young et al., 1978). Failure of this species to establish has generally been attributed to seed dormancy inhibiting germination (Liacos & Nord, 1961; Young et al., 1978), but in addition to the challenges presented by seed dormancy, this species also has low seedling survival and slow growth rates (Ex et al., 2011; Ibanez & Schuup, 2002; Scheldt & Tisdale, 1970). Good seed crops of *C. ledifolius* may only occur a few times per decade, and of those seedlings that emerge, many are lost to summer drought or winter browsing (Ibanez $\&$ Schuup, 2002; Shaw et al., 2004). The slow natural regeneration of *Cercocarpus ledifolius* causes challenges when utilizing this species in revegetation projects (Ex et al., 2011).

To use *C. ledifolius* in restoration efforts, seeding projects should manage the dormancy and germination characteristics of the species. *Cercocarpus ledifolius* seeds exhibit traits of physiological dormancy, which can include several mechanisms by which germination is inhibited (Stidham et al., 1980; Young et al., 1978). Low growth potential of the embryo, restriction of radicle emergence by the structures covering the embryo, and chemical inhibitors are all aspects of physiological dormancy that may be limiting *C. ledifolius* seed from

germinating in a given season and contribute to its low success rates in seeding efforts (Baskin & Baskin, 2014).

Several chemical treatments have been attempted to help overcome *C. ledifolius* seed dormancy (Kitchen, 2008). Dormancy has been targeted with scarification methods such as soaking seed in sulfuric acid (Heit 1970; Liacos & Nord, 1961; Young et al., 1978). Liacos and Nord (1961) soaked seed in concentrated (\approx 98.0%) sulfuric acid for durations ranging from 5- to 20-min followed by a 4-hr soak in thiourea, resulting in a 62% increase in germination. Young et al. (1978) soaked seed in concentrated (≈98.0%) sulfuric acid for durations ranging from 1- to 30-min finding that those treatments reduced germination up to 15%. Acid scarification for 5- to 10-min, has also been used with a related species, *Cercocarpus montanus* (alder-leaf mountain mahogany), where it was successful in improving germination in 4 of 7 seed sources (Rosner et al., 2003). However, it was noted in this study that *C. montanus* does not exhibit typical traits of physical dormancy, for which acid scarification is generally used to treat, but instead contains germination inhibitors in the seed coat. The variable success of these previously tested acid scarification seed treatments is often attributed to ecotypic variation of the species and macroclimatic differences in the seed source (Rosner et al., 2003; Young et al., 1978), but may also be influenced by the variation in their research methods. For example, variations in the amount of time the seed is rinsed in water following treatment, use of a neutralizing bicarbonate solution, or other variations that may not have been specified could all influence the efficacy of an acid scarification treatment.

To target seed physiological dormancy, gibberellic acid (GA3) can be applied exogenously to stimulate germination. Gibberellic acid is a plant growth regulator that induces germination through mobilization of seed storage reserves and stimulation of cell expansion

(Lecat et al., 1992; Dewir et al., 2011; Gupta & Chakrabarty, 2013). Physiological dormancy, in some cases, is maintained through a high abscisic acid (ABA) to GA3 ratio. In those cases, the application of GA3 can induce a low ABA:GA ratio, which may release embryo dormancy (Finch-Savage & Leubner-Metzger, 2006). Young et al. (1978) tested levels of GA3 ranging from 0.35 mg to 97 mg⋅L⁻¹ of GA₃ added to the germination substrate. These treatments failed to enhance germination of *C. ledifolius*. Similar research on other shrub species has seen GA3 rates ranging from 50 to 500 mg‧L-1 for *Cercocarpus montanus* (alder-leaf mountain mahogany), which increased germination up to 29.4% in combination with two-month stratification, and 500 to 2000 mg‧L-1 for *Rhus coraria* (Sicilian sumac), which improved germination in unstratified seed, and 1-month stratified seed (Paudel et al., 2020; Pipinis et al., 2017). Further exploration of treatment procedures and rates of GA3 for this beneficial species is necessary to overcome establishment barriers presented by seed dormancy.

One method to address the barrier presented by seed dormancy not previously explored with *C. ledifolius* is seed coating methods. Such technologies are commonly used in agriculture, and more recently have begun adaptation for use in dryland restoration (Archer & Gesch, 2003; Johnson et al., 2004; Madsen et al., 2016; Pedrini et al., 2020). Polymer seed coatings involve applying mineral products and binding agents such as polyvinyl polymers and glue in alternating steps to create a coat of material around the seed (Erickson et al., 2019). These polymers are applied in liquid form, which when dried forms a film around the seed, binding the various products used during coating (Pedrini et al., 2017). Coatings can be formulated to contain growth hormones such as GA₃ to target physiological dormancy and influence germination timing (Pedrini et al., 2020; Richardson et al., 2019; Scott, 1989).

One potential drawback of utilizing treatments to break physiological dormancy of *C. ledifolius*, is the potential for premature germination in the field. Seed that germinates too early in the season will be susceptible to freeze-thaw events that often continue occurring through early spring (Boyd & Lemos, 2013; Roundy & Madsen, 2016). Freezing events can reduce vigor of emerging seedlings and may also be an important source of pre-emergent mortality (Boyd & Lemos, 2013; Monsen & Stevens, 2004; Roundy & Madsen, 2016). These seed coating techniques can be used to protect seed from the potential effects of premature germination. A hydrophobic polymer seed coating, for example, can potentially delay water imbibition of seed, therefore delaying germination. Hydrophobic seed coatings have been applied in studies with other native species including *Pseudoroegneria spicata* (bluebunch wheatgrass) (Madsen et al., 2016), and *Astragalus filipes* (basalt milkvetch) (Fund et al., 2019), where it had positive results in delaying germination and increasing emergence.

Another application for these seed coatings is in protecting seed from pathogens. Evidence suggests that pathogens, particularly fungal pathogens are limiting to the survival of both seed and seedlings of native plants in restoration settings (Blaney & Kotanen, 2001; Dalling et al., 2011; Fawke et al., 2015; Gilbert, 2002). The effect of pathogens increases when seeds are planted in the fall, which is common in dryland restoration projects in North America, where the seeds are exposed to pathogens that are promoted by long cool, moist soil conditions during winter periods (Baskin & Baskin, 2014; Dalling et al., 2011; Kildisheva et al., 2020). Scarification may also exacerbate *C. ledifolius* seed susceptibility to pathogens (Gornish et al., 2015; Madsen et al., 2016). A fungicide seed coating formulated to target these seed and soilborne pathogens may improve the emergence and establishment from seed (Madsen et al., 2016; Munkvold 2009; Nuyttens et al., 2013). The use of a fungicide seed coating has been tested with

P. spicata where it has had success in lowering fungal abundance compared with untreated seed (Gornish et al., 2015; Hoose et al., 2022). Seed coatings containing both a hydrophobic and a fungicide layer may have increased strength in protecting from fungal pathogens and decreasing premature germination, particularly for *C. ledifolius* seed that has been treated to overcome seed dormancy.

The primary purpose of our research was to determine if seed scarification, GA3, hydrophobic, and fungicide seed coatings could improve seed germination and plant establishment under laboratory trials and field evaluations at the Bingham Canyon Mine. Our objectives were 1) determine if scarification and GA3 treatments improved germination in the field; 2) identify if hydrophobic seed coating materials and fungicides increased emergence in the field; and 3) identify optimal seed scarification times and improve the formulation of the GA3 coating in the laboratory. We hypothesized that a combination of seed scarification and seed coating technologies will improve the establishment of *C. ledifolius* in the field across a variety of soil conditions, and that testing additional formulations of seed coatings in combination with scarification methods will increase the germination of *C. ledifolius* in a laboratory setting.

MATERIALS & METHODS

Field Trial

Site Description. This trial was conducted at the Bingham Canyon Mine located at a site previously prepared for seeding experiments on mine overburden with and without topsoil amendments. This site (40.541205, -112.129868) has an approximate elevation of 2175 m, a 30 year historic mean annual temperature of 7.1ºC, and 30-year historic mean annual precipitation of approximately 577 mm (Figure 1-1) (Prism Climate Group at Oregon State University. (n.d.)).

Study Design. The field trial was organized in a randomized complete block design with 18 treatments (Table 1-1), repeated on two different sites. The two sites were 1) overburden amended with topsoil, and 2) overburden without a topsoil amendment. In November 2020, treated seeds were planted in 3 m rows and buried at a depth of approximately 5 mm. Each treatment row was planted with 500 pure live seed (PLS) seeds. Seed was purchased from Granite Seed and Erosion Control (Lehi, UT, USA), and was collected in Utah County in 2019. One mesh germination bag containing 50 seeds of the respective treatment and soil from the respective site were buried at the end of each treatment row (Abbott & Roundy, 2003). Germination bags were collected on 29 April 2021, transported to a laboratory where seeds were sieved from the soil and germinated seeds were counted over a 2-d period quantify germination of each treatment. Emergence of seedlings was counted on 29 April 2021, and the number of established plants was counted on 15 July 2021, and 15 October 2021.

We evaluated the effect of our treatments on the proportion of germinated and emerged seedlings using generalized linear mixed-effect models (Sileshi, 2012) in the R programming environment using the 'glmer' function of the 'lme4' package (Bates et al., 2015; R Core Team, 2022). These models included treatment as a fixed effect and block as a random effect and were pooled across sites. For each model, we first checked that the treatment factor was significant before proceeding with any pairwise comparisons of treatments. Pairwise comparisons between treatments for germination were performed using the 'emmeans' function from the 'emmeans' package in the R programming environment (Lenth, 2022; R Core Team, 2022) and P-values were adjusted using the Tukey method for comparing a family of 9 estimates ($\alpha = 0.10$). For emergence, pairwise comparisons between treatments were performed using the 'difflsmeans' function from the 'lmerTest' package in the R programming environment (Kuznetsova et al.,

2017; R Core Team, 2022) using Kenward-Roger's approximation to degrees of freedom (α = 0.10).

Seed Coating. Seed was coated using a 30-cm diameter rotary drum seed coater (Universal Coating Systems, Independence, OR, USA). We used Agrimer SCPII (Ashland Inc., Covington, KY , USA) as a binder, and limestone powder $(CaCO₃)$ as a filler material. Seed coating was performed on 200 g batches of seed, with the drum rotating at 20% of its maximum velocity for all coatings excluding the hydrophobic coating which was rotated at 15% of its maximum velocity.

The GA3 seed coating was applied by first adding 0.382 g of GA3 in a solution of ethyl cellulose polymer dissolved in acetone to the 200 g batches of seed. This solution was added directly to the seed through a syringe as a liquid. Once the GA3 solution was dried on the seed, 20 ml of binder was applied to the seed through a syringe, followed by the application of limestone powder. Small amounts of limestone and binder were added gradually in alternating steps for the remainder of the coating process using standard coating techniques, until a total of 350 g of limestone powder and 128 ml of binder were applied to the 200 g batch of seed. Limestone powder was applied directly over the seed during the coating process, and the binder was applied to the spinning disk using a syringe. This technique encrusted the seed in a durable layer, maintaining the treatment near the seed.

The fungicide coating was applied in a similar manner, first being coated with 20 ml of a fungicide and binder dilution, applied fungicides are listed in Table 1-2. Directly following the fungicide-binder mixture application, the seed was coated with binder and limestone using the same methods used in the GA_3 coating. In addition to the GA_3 and fungicide coatings, our trial included a treatment consisting of seeds coated with only binder and limestone powder (blank).

The blank coating served as a procedural control to observe the effects of the coating alone without the effects of the GA3 or the fungicide.

The hydrophobic coating contained 10% ethyl cellulose polymer prepared in 1500 g of acetone, and a dye was added to ensure seeds were fully coated. Seeds were first coated with 40 ml of the ethyl cellulose solution through a syringe, the remainder of the solution being pumped onto the seeds at a steady rate for a total of 350 ml applied to 200 g of seed. During the application of the solution, the temperature was kept at ≈ 138 °F and a blower (Metropolitan Vacuum Cleaner Co., Inc, Oakland, NJ) was used to dry the solution onto the seed during the application of the hydrophobic coating. For the coating treatments with multiple products being applied to the seed, coating layers were applied in the following order: GA3, fungicide, and hydrophobic. Following each seed coating, the seed was dried using a forced-air dryer (Braceworks Automation and Electric, Lloydminster, SK, CAN) at 43°C for approximately 12 minutes.

Laboratory Trials

Cercocarpus ledifolius seed used in the laboratory trials was from the same seed lot as in the field trial. In all trials, seeds were counted as germinated when the radicle had emerged at least 2 mm. Seeds that had germinated were removed from the Petri dish at the time of counting. Using germination data, we estimated the time to reach 50% germination (T_{50}) , and final germination percentage (FGP) using a non-linear, three-parameter log-logistic time-to-event model (Ritz et al., 2013). Time-to-event models were fitted using the 'drm' function of the 'drc' package in the R programming environment (R Core Team, 2022; Ritz et al., 2015). We compared treatment effects by performing pairwise comparisons between treatments using the

'compParm' function in the 'drc' package, adjusting P-values with the Bonferroni method (α = 0.10) (Ritz et al., 2015).

For the trials testing seed coatings, seed was scarified in batches of 25 grams, soaking in concentrated (\approx 98%) sulfuric acid for the designated treatment time. Then seed was removed from sulfuric acid, rinsed for 3-5 minutes under running water and air dried. Scarified and unscarified seed was coated using a 30-cm diameter rotary drum seed coater (Universal Coating Systems, Independence, OR, USA). We used Agrimer 15 (Ashland Inc., Covington, KY, USA) as a binder at 45% solids and limestone powder (CaCO₃) as a filler material. Seed coating was performed on 100 g of seed, with the drum rotating at 20% of its maximum velocity. Seed was first coated with 50 ml of the designated GA3 solution of ethyl cellulose in acetone or DCM (dichloromethane) being added directly to the seed as a liquid binder. This solution was dried on the seed. Following the GA3 application, seed was coated with 10 ml of the binder which was directly followed by the gradual addition of limestone and binder in alternating steps, using standard seed coating techniques until a total of 175 g of limestone powder and 44 ml of binder was applied to the 100 g of seed. Limestone powder was applied directly over the seed during the coating process, and the binder and GA3 were applied to the spinning disk using a syringe. This technique encrusts the seed in a durable layer, maintaining the treatment near the seed. The blank coating was applied in the same manner except for the GA3 solution application. Following the seed coating process, the seed was dried using a forced-air dryer (Braceworks Automation and Electric, Lloydminster, SK, CAN) at 43°C for approximately seven minutes.

Gibberellic Acid trial

Breaking of physiological dormancy was tested with six solutions, 0, 250, 500, 1000, 2500, and 5000 mg·L⁻¹ of GA₃. Each treatment group contained 50 seeds from the granite seed lot and was replicated 8 times. Seeds were placed in Petri dishes containing two sheets of blotter paper, which were moistened with 7 ml of the designated treatment solution. These dishes were incubated for 30 d at 4ºC with no light before being exposed to a constant temperature of 10ºC with a 12-hr light cycle for germination. Germination was recorded weekly up to one month, with distilled water being added to Petri dishes as needed to maintain moisture levels.

Seed Coating Concentrations trial

This trial combined several durations of acid scarification and concentrations of GA3 in seed coatings to explore the collective effects of scarification and GA3. Scarification treatments included 0-, 2.5-, 8-, and 15-minute acid scarification, and GA3 treatments included 0, 328 (1x), 656 (2x), and 1312 (4x) mg treatments. The GA3 treatments were applied as a seed coating as they were applied in our field trial. All treatments were combined in a full factorial design with the inclusion of a "blank" treatment consisting of the seed coating with no GA3. Each treatment group contained 25 seeds and was replicated 8 times.

Seeds were placed in 9 mm Petri dishes containing two sheets of blotter paper, which were moistened with 7 ml of distilled water. This trial was repeated with and without cold stratification, those that were cold stratified were incubated at 30 d at 4ºC with no light before being exposed to a constant temperature of 15ºC with a 12-hr light cycle for germination. Germination was recorded three times in the first week, twice in the second week, and once a

week in all following weeks until germination had stopped, up to 3 months, distilled water being added to Petri dishes as needed to maintain moisture.

Seed Coating Formulations trial

This trial included both acid and mechanical scarification, as well as GA₃ treatments. Scarification treatments included unscarified seed, 15-minute acid scarification, and 5- and 10 second mechanical scarification and GA₃ treatments included 0 mg, 328 mg GA₃ with acetone as the solvent, and 328 mg GA³ with DCM as the solvent. The GA³ treatments were applied as a seed coating as they were applied in our field trial. These treatments were combined in a full factorial design with the inclusion of a "blank" treatment consisting of the seed coating with no GA3. Each treatment group contained 25 seeds and was replicated 8 times.

Seeds were placed in 9 mm Petri dishes containing two sheets of blotter paper, which was moistened with 7 ml of distilled water. This trial was repeated with and without cold stratification, those that were cold stratified were incubated at 30 d at 4ºC with no light before being exposed to a constant temperature of 15ºC with a 12-hr light cycle for germination. Germination was recorded three times in the first week, twice in the second week, and once a week in all following weeks until germination had stopped, up to 3 months, with distilled water being added to Petri dishes as needed to maintain moisture levels.

RESULTS

Field trial

Average germination percentages for all our treatments (Table 1-3) were similar, except for acid scarified treatments, which had < 2.5% germination overall (Figure 1-2). Since acid

scarified germination was so low, it was removed from analysis because the acid damaged the seed. For unscarified treatments, there were no significant differences ($P > 0.10$) between any treatment and the control (Table 1-4).

Acid scarified treatments were again removed from emergence analysis due to having < 0.45 plants per meter overall (Table 1-5). For our unscarified treatments, we found the GA_3 and GA_3 + hydrophobic coatings were significantly different from the control seed, having 1.8 (P = 0.0682) and 2.2 ($P = 0.0751$) more seedlings emerge per meter, respectively. The GA₃ and GA₃ + hydrophobic coatings also had 0.63 (P = 0.0159) and 0.62 (P = 0.0178) more seedlings emerge per meter, respectively, than the blank coating (Figure 1-3, Table 1-6). Seedling density was evaluated on 15 July and 15 October 2021. Acid scarified treatments were again removed from the analysis (Tables 1-7, 1-9). Each date was analyzed separately, and analysis for survival at both dates had no differences ($P > 0.10$) among treatments (Tables 1-8, 1-10).

Gibberellic Acid trial

The FGP of the 0, 250, 500, 1000, 2500, and 5000 mg GA³ treatments were 49.6, 50.9, 58.6, 67.2, 66.3, and 53.5%, respectively. The T⁵⁰ germination estimates for each treatment were 27.9, 22.1, 22.3, 24.5, 23.7, and 25.7 d, respectively. Treatment pairwise comparisons indicated that both FGP and T₅₀ were not significantly different from the control across all GA₃ treatment concentrations ($P > 0.10$).

Seed Coating Concentrations trial

Unstratified. The FGP pairwise comparisons had some significant differences $(P < 0.10)$ among treatments and the control (Table 1-13). Fifteen minutes of acid scarification with 1x

GA₃, 15-minute with $2x$ GA₃, and 15-minute with $4x$ GA₃ coating had 20.9 (P = 0.0561), 21.9 (P $= 0.0400$), and 18.1% (P = 0.0940) higher germination than control seed, respectively (Table 1-11, Figure 1-4). Fifteen minutes of acid scarification, 15-minute with 1x GA3, 15-minute with 2x GA₃, and 15-minute with 4x GA₃ coating had 16.9 (P = 0.0702), 22.1 (P = 0.0302), 23.0 (P = 0.0195), and 19.2% ($P = 0.0550$) higher germination than seed with the blank coating, respectively (Table 1-11, Figure 1-4). Pairwise comparisons for T_{50} germination had no significant differences ($P > 0.10$) among treatments for the granite seed lot (Table 1-14).

Stratified. There were no differences ($P > 0.10$) between control seed and any combination of scarification and GA₃ coating for FGP and T_{50} when stratified for 30 d at 4° C (Table 1-15, 1-16).

Seed Coating Formulations trial

Unstratified. There were no differences ($P < 0.10$) between control seed and any combination of scarification and GA_3 coating for FGP (Table 1-17). For T₅₀ there was a significant difference ($P < 0.10$) between 15-minute acid scarification with acetone coating and control seed (Table 1-18). The 15-minute scarification with acetone coating reached 50% germination 2.4 d earlier than control seed $(P = 0.0824)$ (Table 1-12).

Stratified. There were no differences ($P > 0.10$) between control seed and treatments for FGP or T₅₀ when seeds were stratified for 30 d at 4^oC (Table 1-19, 1-20).

DISCUSSION

Dryland ecosystems support of billions of people, and when these ecosystems are disturbed through anthropogenic activities it is important to implement reclamation of disturbed areas before invasive species cause further degradation. *Cercocarpus ledifolius* is a native plant with substantial beneficial characteristics supporting wildlife and the surrounding soils and plants. Unfortunately, *C. ledifolius* does not establish well from seed, which is likely due to seed dormancy inhibiting germination (Liacos & Nord, 1961; Young et al., 1978). Our research targeted *C. ledifolius* dormancy by treating seeds to alleviate dormancy and using seed coatings to minimize losses from fungal pathogens and premature germination. By combining seed scarification treatments and seed coatings, it was hypothesized that *C. ledifolius* seed germination, emergence, and establishment would improve at the Bingham Canyon Mine where revegetation efforts were underway. Our research efforts also focused on testing additional concentrations and formulations of seed coatings in combination with scarification methods, hypothesizing that these would increase the germination of *C. ledifolius* in a laboratory setting.

From the field trial, we found that GA_3 and GA_3 + hydrophobic coatings were the only treatments to improve *C. ledifolius* emergence density (Figure 1-3). These two seed coatings were able to produce 1.8 and 2.2 more seedlings per meter than the control seed (Figure 2-2). Seed coatings have been used to successfully influence germination timing in other species as well, such as *Artemisia tridentata* (big sagebrush), where GA3 seed coatings decreased the time to seed germination to between 9 and 11 days (Keefer et al., 2021). Our results add to this finding, showing that seed coatings containing GA3 can influence the germination timing of different plant species used in revegetation efforts. The ability to influence germination timing with seed coatings can improve the outcomes of revegetation by increasing the number of seedlings that emerge successfully. Unfortunately, while emergence was improved by the GA3 and GA_3 + hydrophobic coatings, these coatings did not significantly improve germination or survival of *C. ledifolius* seedlings when compared with control seed. Other treatments in the field

trial did not improve germination, emergence, or survival, and some reduced seed viability compared to control seed.

Treatments that did not improve *C. ledifolius* germination, emergence, or survival included seed scarified with sulfuric acid. In fact, this treatment significantly reduced seed viability to nearly zero (Figure 1-2). Germination of the rest of the seed treatments was not statistically different from the control seed and did not kill the seed like the sulfuric acid treatment. Interestingly, acid scarified seed had improved germination in laboratory studies with *C. ledifolius* but was unsuccessful at the mine site (Figure 1-2) (Heit, 1970; Liacos & Nord, 1961). The detrimental effect of acid scarification in our field trial was likely due to the scale at which seed was scarified for the field trial. In laboratory studies, seed was scarified in small batches of 50 seeds, but for the field trial considerably more seed, up to 100 grams, needed to be scarified as a batch at once (Appendix). This larger mass of seed likely generated substantially more heat when rinsed following soaking in sulfuric acid, and the heat likely killed the seed. Seed that was not acid scarified was viable and germinated. But no seed coating treatments improved germination (Figure 1-2) which does not support our hypothesis that a combination of seed treatments and seed coatings would improve germination on mine overburden sites.

The lack of treatment response of GA_3 coatings to improve germination in germination bags agrees with findings from Young et al., (1978), where the addition of GA3 to the germination substrate did not significantly influence germination of *C. ledifolius*. We found this to be true even with increased rates of GA3 and when applying it as a seed coating (Figure 1-2). Fungicide seed coatings also did not improve germination, which is similar to a study by Hoose et al. (2022) where they reported that a fungicide seed coating on *P. spicata* (bluebunch wheatgrass) improved emergence but did not have a significant effect on germination. This may

indicate that pathogen pressure is not high during germination, or that pathogen pressure was low at the mine overburden site; therefore, the coating did not have a noticeable effect on *C. ledifolius*. Hydrophobic seed coating also did not have any significant effect on germination percentage in the field, either alone or combined with other coatings (Figure 1-2). These results agree with a study by Fund et al. (2019) on *Astragalus filipes* (basalt milkvetch) where a hydrophobic seed coating had minimal effects on germination.

As with many species used in restoration, one of the main bottlenecks of successful establishment is the period between germination and emergence (Ex, DeRose, & Long, 2011; James, Svejcar, & Rinella, 2011). Control seed in our field trial had 33.3% germination from the germination bags, but only 2.3 plants per meter (1.4%) of control seedlings emerged in field rows (Table 1-5). GA₃ and GA₃ + hydrophobic seed coatings improved upon this emergence density, but still only had 4.1 and 4.5 plants per meter (2.46 and 2.72%) emerge, respectively (Table 1-5). This may mean the GA_3 and GA_3 + hydrophobic seed coatings are successfully influencing germination timing and enhancing potential for *C. ledifolius* establishment on mine overburden, but it also indicates that other factors are likely influencing establishment, such as temperature fluctuations, water availability, nutrient availability, and herbivory. These other factors may be more influential than seed dormancy for field establishment of *C. ledifolius*. The seed coating combinations, including fungicides, and the hydrophobic coating by itself were not successful in improving emergence, unlike studies by Hoose et al., (2022) with *P. spicata* who found fungicide coatings improved emergence in five out of six sites and years, and Fund et al., (2019) with *A. filipes* who found that combinations of fungicide and hydrophobic coatings increased seedling emergence 37-112% on average. Our results indicate that fungal pathogens may not be a limiting factor in *C. ledifolius* emergence at the mine overburden site, and that the

influence of a hydrophobic coating on germination timing in a field setting may not be substantial.

 Seedling density was recorded in summer 2021 and fall 2021. The number of seedlings for each treatment decreased over the course of the growing season with no treatments significantly different from the control. Even though our GA_3 and GA_3 + hydrophobic coating treatments were successful in promoting higher emergence, this higher emergence did not result in a higher number of established seedlings over time. This low seedling survival has been recorded in other research on *C. ledifolius*, with large numbers of seedlings germinating in good years, but low numbers of those seedlings surviving. Seedling mortality has been attributed to several factors including summer moisture, seasonal temperatures, and herbivory (Ex, DeRose, & Long, 2011; Ibanez & Schuup, 2002). The year of our observation, 2021, there was lower precipitation during the winter and spring months than has been the case historically, which likely impacted the initial emergence and establishment of our seedlings (Figure 1-1). From our results, seed coatings appear to have the greatest impact during the emergence stage, and less effect on the germination and ultimately on seedling establishment and survival in the first year. Our results support the hypothesis that seed coatings improve the emergence of *C. ledifolius* in mine overburden and could be used in revegetation efforts. Survival of the seedlings in the overburden may improve with increased soil moisture and when seeded over larger areas of land where potential herbivory would not be as influential.

In our laboratory trials, we aimed to improve upon seed treatments tested in our field trial. We first tested concentrations of GA3 applied to the germination substrate in concentrations ranging from 250 to 5000 mg and found this did not have a significant positive effect on germination. This is consistent with and adds to results from Young et al. (1978), who tested

concentrations ranging from 0.35 to 97 mg, which had no impact on germination. We had hypothesized that higher concentrations of GA3 may produce a positive effect on germination but, according to our trials, this was not the case. The lack of GA3 influence may be due to not targeting the proper mechanism by which physiological dormancy inhibits germination in *C. ledifolius*. Liacos and Nord (1961) suggested that perhaps a saponin was present in the seed coat which, in high enough quantities, could reduce germination of *C. ledifolius*. In looking at a related species, Moore (1963) also reported that *Cercocarpus montanus* (alder-leaf mountain mahogany) seeds were found to have a water extractable chemical which inhibited seed germination, likely to be a cyanogenic compound. However, it was noted that with adequate soil moisture the chemical should not cause great limitations to seed germination in the field (Moore, 1963). It is possible that *C. ledifolius* dormancy is caused by a chemical inhibitor in the seed coat, and so the addition of GA3 alone cannot break dormancy.

Cercocarpus ledifolius seed dormancy may be caused by a combination of physiological dormancy mechanisms and by treating seed with physical treatments and plant hormones we may be able to consistently break dormancy. Therefore, in our seed coating concentration and seed coating formulation trials, we looked at treatments combining various scarification methods and GA3 seed coatings. We tested seed in both stratified and unstratified conditions. The seed coating concentrations trial tested combining acid scarification with GA3 concentrations as a seed coating. With unstratified seed, treatments of 15-minute acid scarification with any GA3 concentration $(1x, 2x, or 4x)$ had significantly higher germination than control seed (Figure 1-4). For stratified seed, however, these treatments were not significant. These results suggest that stratification itself may be more successful in improving the germination of *C. ledifolius* than

seed treatments and seed coatings, but that seed treatments may be beneficial in cases where seed will not undergo stratification.

For the seed coating formulations trial where both acid and mechanical scarification were combined with GA3 coating formulations, it was found that for unstratified and stratified seed there were no significant differences for any combination of scarification and GA3 coating. Results for both the seed coating concentrations and seed coating formulations trials emphasize that scarification treatments are highly variable in their success. The acid scarification combination treatments were not consistent between trials. In the seed coating concentrations trial, the 15-minute with a 1x GA³ coating had 20.9% higher germination than the control for the unstratified seed, but that same coating $(15 \text{ min} + \text{Acetone})$ in the seed coating formulations trial was not significantly different from the control. Seed germination in general was higher when seed was stratified, with germination percentages in the 40 -50% range, compared with unstratified in the 10 - 20% range (Table 1-11, 1-12). The higher germination rate in seed that was stratified was consistent across both the seed coating concentration and seed coating formulations trials and agrees with conclusions from Stidham et al. (1980) stating that *C. ledifolius* would not be likely to produce suitable stands if planted in the spring as opposed to fall planting where it would receive cold stratification over the winter months.

Our laboratory trials of seed treatments and seed coatings reiterate the difficulty of pinpointing one treatment that will consistently overcome *C. ledifolius* dormancy and improve germination. Results of treatments tested were variable between different trials. It will be important for future research on *C. ledifolius* seed to determine more precisely the mechanism of physiological dormancy. From our findings, one potential mechanism of physiological dormancy in *C. ledifolius* may be a chemical inhibitor as suggested by Liacos and Nord (1961) and

suggested by Moore (1963) with *C. montanus*. Finding if an inhibitor exists in *C. ledifolius* seeds would allow use of more accurately targeted treatments to leach or nullify effects of the inhibitor in some way. Another possible dormancy mechanism could be structures surrounding the embryo restricting the radicle emergence (Baskin & Baskin, 2014; Heit, 1970). If structures such as the seed coat were restricting radicle emergence, this could explain why seed benefitted from acid scarification in the seed coating concentrations trial, even though it has been noted by Heit (1970), that *C. ledifolius* seed is not water impermeable, which is what acid scarification is generally used to treat. Overall, we recommend testing several time durations, ranging from 2.5- 15 minutes of sulfuric acid scarification on a sample of each seed lot since this treatment had the most success and testing a range of sulfuric acid treatments will identify the best treatment for a particular seed lot.

CONCLUSION

This study demonstrates that GA_3 and GA_3 + hydrophobic coatings have potential to improve the emergence density of *C. ledifolius* in restoration settings on mine overburden at the Bingham Canyon Mine. The success of these coatings provides evidence that GA3 seed coatings can be used to influence germination timing to promote higher emergence density. This study also demonstrates that *C. ledifolius* seed has complicated dormancy mechanisms limiting revegetation efforts. For native shrub species, dormancy can pose significant barriers to germination and potential use in land restoration efforts. The inconsistent results from treatments of acid scarification and the GA3 seed coatings indicate a need to understand more precisely the mechanism by which *C. ledifolius* seed is physiologically dormant.

Future research should explore the potential of chemical inhibitors and restrictive structures limiting radicle emergence as mechanisms of dormancy. This information may allow for more targeted treatment selection. Another consideration is the genetic variation of the species. Any treatment which may be successful with one seed source may not be successful with another. This means that for revegetation efforts, it will be vital to test several potential seed treatments on samples of each seed lot before treating seed on a large scale. Future research should also explore other limiting factors in *C. ledifolius* establishment outside of dormancy and fungal pathogens to determine if there are any cost-effective ways to improve the survival of this species in restoration settings. Additionally, GA3 seed coatings should be explored with other species whose emergence may be limited by physiological dormancy. Exploring the effects these seed coatings have on other species and at other sites would allow for higher success overall in restoration seeding efforts.
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FIGURES

Figure 1-1. Mean precipitation and temperature data for field site. Includes 30-year normals, and annual values for 2021 (Prism Climate Group at Oregon State University. (n.d.)).

Treatment

Figure 1-2. Percent germination of *Cercocarpus ledifolius* seed planted at Bingham Canyon Mine in 2020. Germination data was collected 29 April 2021 and pooled by unamended and amended overburden sites. Error bars represent standard error.

Treatment

Figure 1-3. *Cercocarpus ledifolius* seedling density for seed planted at Bingham Canyon Mine in 2020. Plant emergence density was collected 29 April 2021 and pooled by unamended and amended overburden sites. Error bars represent standard error.

Figure 1-4. Time to event model for germination in the seed coating concentrations trial. Error bars represent standard error. For clarity, only the treatments that were significantly different (P < 0.10) than the control or control with blank coating are shown.

TABLES

Table 1-1. List of treatments applied to *Cercocarpus ledifolius* for the field trial planted at the Bingham Canyon Mine, including justification for each.

PD - Physiological Dormancy

Table 1-2. Fungicides applied to *Cercocarpus ledifolius* via seed coating for the field trial, including the corresponding active ingredients, and the pathogens they influence.

¹Syngenta, Basel, Switzerland, Mefenoxam
²Syngenta, Basel, Switzerland, Fludioxonil
³Syngenta, Basel, Switzerland, Azoxystrobin
⁴Syngenta, Basel, Switzerland, Difenoconazole

Table 1-3. Percent germination for *Cercocarpus ledifolius* seed planted at Bingham Canyon Mine in November 2020. Germination data was collected 29 April 2021, unamended and amended overburden sites were pooled for analysis.

Treatment	Germination $(\%)$	Lower conf. interval	Upper conf. interval
Control	33.3	24.7	41.9
Seed Coating (Blank)	28.5	19.9	37.2
Gibberellic Acid (GA3)	30.3	21.7	38.9
Fungicide (Fung)	17.7	9.1	26.3
Hydrophobic (Phobic)	19.9	11.3	28.5
$GA_3 + Fung$	32.1	23.5	40.7
$GA3 + Phobic$	35.4	26.8	44.1
Fung + Phobic	22.8	14.2	31.5
GA_3 + Fung + Phobic	23.8	15.1	32.4
Scarified (Scar)	0.20	$\boldsymbol{0}$	8.8
$Scar + Blank$	2.4	θ	11.0
$Scar + GA3$	0.78	$\boldsymbol{0}$	9.4
$Scar + Fung$	0.89	$\overline{0}$	9.5
$Scar + Phobic$	1.8	$\mathbf{0}$	10.5
$Scar + GA3 + Fung$	0.66	$\overline{0}$	9.3
$Scar + GA3 + Phobic$	0.0	$\overline{0}$	8.6
$Scar + Fung + Phobic$	0.56	$\mathbf{0}$	9.2
$Scar + GA3 + Fung + Phobic$	0.20	$\boldsymbol{0}$	8.8

Table 1-4. Statistical comparisons for *Cercocarpus ledifolius* germination bag counts from the field trial planted at the Bingham Canyon Mine in November 2020. Acid scarification treatments were removed for clarity due to negligible (< 2.5%) germination.

`. ` - 0.10

Table 1-5. *Cercocarpus ledifolius* seedling emergence density for seed planted at Bingham Canyon Mine in November 2020. Emergence data was collected 29 April 2021, unamended and amended overburden sites were pooled for analysis.

Treatment	Germination $(\%)$	Lower conf. interval	Upper conf. interval
Control	2.3	1.2	3.5
Seed Coating (Blank)	1.9	0.78	3.0
Gibberellic Acid (GA3)	4.1	3.0	5.2
Fungicide (Fung)	1.6	0.45	2.7
Hydrophobic (Phobic)	1.4	0.28	2.5
$GA_3 + Fung$	2.9	1.8	4.0
$GA3 + Phobic$	4.5	3.4	5.7
$Fung + Phobic$	1.5	0.42	2.7
GA_3 + Fung + Phobic	2.5	1.4	3.7
Scarified (Scar)	0.43	θ	1.6
$Scar + Blank$	0.13	$\boldsymbol{0}$	1.3
$Scar + GA3$	0.27	$\boldsymbol{0}$	1.4
$Scar + Fung$	0.10	θ	1.2
$Scar + Phobic$	0.37	$\boldsymbol{0}$	1.5
$Scar + GA3 + Fung$	0.33	$\boldsymbol{0}$	1.5
$Scar + GA3 + Phobic$	0.20	$\boldsymbol{0}$	1.3
$Scar + Fung + Phobic$	0.17	$\boldsymbol{0}$	1.3
$Scar + GA3 + Fung + Phobic$	0.37	$\boldsymbol{0}$	1.5

Table 1-6. Statistical comparisons for *Cercocarpus ledifolius* emergence density, collected 29 April 2021, from the field trial planted at the Bingham Canyon Mine in November 2020. Acid scarification treatments were removed for clarity due to negligible germination.

	Control	Blank	GA ₃	Fung	Phobic	$GA_3 +$ Fung	$GA_3 +$ Phobic	Fung $+$ Phobic	$GA_3 + Fung +$ Phobic
Control	.0000								
Blank	0.5376	0000.1							
GA ₃	0.06822	$*0.01585$	1.0000						
Fung	0.6742	0.8442	$*0.02598$	1.0000					
Phobic	0.6470	0.8738	$*0.02368$	0.9700	1.0000				
$GA_3 + Fung$	0.4200	0.1569	0.3017	0.2215	0.2078	1.0000			
$GA_3 + Phobic$	0.07507	$*0.01779$	0.9641	$*0.02898$	$*0.02645$	0.3231	1.0000		
$Fung + Phobic$	0.5328	0.9942	$*0.01555$	0.8385	0.8680	0.1548	$*0.01746$	0000.1	
$GA_3 + Fung + Phobic$	0.6931	0.3132	0.1500	0.4158	0.3947	0.6795	0.1629	0.3097	.0000

Table 1-7. *Cercocarpus ledifolius* seedling summer survival density, collected 15 July 2021, for seed planted at Bingham Canyon Mine in November 2020, unamended and amended overburden sites were pooled for analysis.

Treatment	Density (plants/m)	Lower conf. interval	Upper conf. interval
Control	0.90	0.457	1.343
Seed Coating (Blank)	0.92	0.477	1.363
Gibberellic Acid (GA3)	1.18	0.737	1.623
Fungicide (Fung)	0.84	0.397	1.283
Hydrophobic (Phobic)	0.82	0.377	1.263
$GA_3 + Fung$	0.58	0.137	1.023
$GA3 + Phobic$	1.14	0.697	1.583
$Fung + Phobic$	0.74	0.297	1.183
GA_3 + Fung + Phobic	0.86	0.417	1.303
Scarified (Scar)	0.00	$\mathbf{0}$	0.443
$Scar + Blank$	0.06	θ	0.503
$Scar + GA3$	0.00	$\mathbf{0}$	0.443
$Scar + Fung$	0.00	$\overline{0}$	0.443
$Scar + Phobic$	0.08	$\boldsymbol{0}$	0.523
$Scar + GA_3 + Fung$	0.00	$\mathbf{0}$	0.443
$Scar + GA3 + Phobic$	0.04	$\mathbf{0}$	0.483
$Scar + Fung + Phobic$	0.00	θ	0.443
$Scar + GA3 + Fung +$ Phobic	0.00	$\boldsymbol{0}$	0.443

Table 1-8. Statistical comparisons for *Cercocarpus ledifolius* summer survival density, collected 15 July 2021, from the field trial planted at the Bingham Canyon Mine in November 2020. Acid scarification treatments were removed for clarity due to negligible germination.

	Control	Blank	GA ₃	Fung	Phobic	$GA_3 +$ Fung	$GA_3 +$ Phobic	Fung $+$ Phobic	$GA_3 + Fung$ $+$ Phobic
Control	.0000								
Blank	.0000	.0000							
GA ₃	0.9954	0.9984	1.0000						
Fung	.0000	.0000	0.9797	.0000					
Phobic	.0000	.0000	0.9939	.0000	1.0000				
$GA_3 + Fung$	0.9998	0.9993	0.9103	0000.	0.9999	.0000			
$GA_3 + Phobic$	0.9994	0.9999	1.0000	0.9954	0.9991	0.9651	1.0000		
$Fung + Phobic$	0.9987	0.9960	0.8370	0.9999	0.9991	.0000	0.9221	1.0000	
$GA_3 + Fung + Phobic$.0000	.0000	0.9882	.0000	1.0000	0000.	0.9978	0.9997	1.0000

Table 1-9. *Cercocarpus ledifolius* seedling fall survival density, collected 15 October 2021, for seed planted at Bingham Canyon Mine in November 2020, unamended and amended overburden sites were pooled for analysis.

Treatment	Density (plants/m)	Lower conf. interval	Upper conf. interval
Control	0.48	0.09	0.87
Seed Coating (Blank)	0.86	0.47	1.25
Gibberellic Acid (GA3)	1.12	0.73	1.51
Fungicide (Fung)	0.68	0.29	1.07
Hydrophobic (Phobic)	0.74	0.35	1.13
$GA_3 + Fung$	0.56	0.17	0.95
$GA_3 + Phobic$	0.74	0.35	1.13
$Fung + Phobic$	0.72	0.33	1.12
GA_3 + Fung + Phobic	0.76	0.37	1.15
Scarified (Scar)	0.00	0.00	0.39
$Scar + Blank$	0.06	0.00	0.45
$Scar + GA3$	0.00	0.00	0.39
$Scar + Fung$	0.00	0.00	0.39
$Scar + Phobic$	0.04	0.00	0.43
$Scar + GA3 + Fung$	0.00	0.00	0.39
$Scar + GA3 + Phobic$	0.02	0.00	0.41
$Scar + Fung + Phobic$	0.00	0.00	0.39
$Scar + GA3 + Fung +$ Phobic	0.00	0.00	0.39

Table 1-10. Statistical comparisons for *Cercocarpus ledifolius* fall survival density, collected 15 October 2021, from the field trial planted at the Bingham Canyon Mine in November 2020. Acid scarification treatments were removed for clarity due to negligible germination.

	Control	Blank	GA ₃	Fung	Phobic			GA_3 + Fung GA_3 + Phobic Fung + Phobic	$GA_3 + Fung + Phobic$
Control	0000.								
Blank	0.9737	.0000							
GA ₃	0.8531	.0000	1.0000						
Fung	0.9998	0.9998	0.9889	.0000					
Phobic	0.9660	.0000	1.0000	0.9996	.0000				
$GA_3 + Fung$.0000	0.9940	0.9357	.0000	0.9914	0000.1			
GA_3 + Phobic	0.9768	.0000	1.0000	0.9998	.0000	0.9949	1.0000		
$Fung + Phobic$	0.9999	0.9996	0.9849	.0000	0.9993	1.0000	0.9997	1.0000	
GA_3 + Fung + Phobic	0.9979	0000.1	0.9978	1.0000	.0000	0.9998	1.0000	1.0000	1.0000

Stratification	Treatment	FGP $(\%)$	T_{50}
None	Control	5.3	37.6
None	$Control + Blank$	4.2	21.9
None	$1x-GA_3$	10.3	46.1
None	$2x-GA_3$	9.2	18.0
None	$4x-GA3$	12.6	30.9
None	2.5 min	21.1	74.9
None	$2.5 \text{ min} + \text{Blank}$	24.4	217.4
None	2.5 min + 1x- GA_3	11.1	19.3
None	2.5 min + $2x-GA_3$	18.2	72.1
None	$2.5 \text{ min} + 4x - GA3$	32.1	70.1
None	8 min	25.2	56.9
None	$8 \text{ min} + \text{Blank}$	12.9	40.2
None	$8 \text{ min} + 1 \text{x-GA}_3$	25.3	161.8
None	$8 \text{ min} + 2x - GA_3$	17.1	25.2
None	$8 \text{ min} + 4 \text{x-GA}_3$	15.9	23.1
None	15 min	21.1	16.8
None	$15 \text{ min} + \text{Blank}$	19.4	18.3
None	15 min + $1x-GA_3$	26.2	16.7
None	$15 \text{ min} + 2x - GA_3$	27.2	15.3
None	$15 \text{ min} + 4 \text{x-GA}$	23.4	18.2
Stratified	Control	49.5	10.3
Stratified	$Control + Blank$	34.0	11.8
Stratified	$1x-GA_3$	35.0	12.4
Stratified	$2x-GA_3$	33.0	11.8
Stratified	$4x-GA_3$	45.6	12.7
Stratified	2.5 min	56.0	10.1
Stratified	$2.5 \text{ min} + \text{Blank}$	42.5	10.9
Stratified	2.5 min + 1x- GA_3	52.5	10.9
Stratified	2.5 min + $2x-GA_3$	49.5	11.1
Stratified	2.5 min + $4x-GA_3$	53.0	10.5
Stratified	8 min	47.0	10.1
Stratified	$8 \text{ min} + \text{Blank}$	35.0	9.9
Stratified	$8 \text{ min} + 1 \text{x-GA}$	48.0	10.2
Stratified	$8 \text{ min} + 2x - GA_3$	45.5	10.6
Stratified	$8 \text{ min} + 4 \text{x-GA}_3$	28.5	9.8
Stratified	15 min	49.5	10.7
Stratified	$15 \text{ min} + \text{Blank}$	44.5	10.0
Stratified	$15 \text{ min} + 1 \text{x-GA}_3$	51.5	10.1
Stratified	$15 \text{ min} + 2x - GA_3$	49.0	9.7
Stratified	$15 \text{ min} + 4 \text{x-GA}$	58.0	9.9

Table 1-11. Estimates of final germination percentage (FGP) and time to 50% germination (T₅₀) from time to event model for the seed coating concentrations trial. Includes both unstratified and stratified seed.

Stratification	Treatment	FGP	T_{50}
None	Control	11.0	36.0
None	Blank	11.7	53.5
None	Acetone	12.7	58.2
None	DCM	14.6	39.2
None	15 min	27.5	18.3
None	$15 \text{ min} + \text{Blank}$	23.9	20.4
None	$15 \text{ min} + \text{Acetone}$	26.7	12.2
None	$15 \text{ min} + \text{DCM}$	31.1	18.5
None	5 sec	12.7	49.7
None	$5 \text{ sec} + \text{Blank}$	17.5	42.6
None	$5 \text{ sec} + \text{Acetone}$	24.6	38.1
None	$5 \text{ sec} + \text{DCM}$	19.6	28.6
None	10 _{sec}	17.5	37.5
None	$10 \text{ sec} + \text{Blank}$	6.8	35.7
None	$10 \text{ sec} + \text{Acetone}$	15.0	35.6
None	$10 \text{ sec} + \text{DCM}$	20.3	28.6
Stratified	Control	44.5	11.8
Stratified	Blank	27.0	12.3
Stratified	Acetone	26.6	13.6
Stratified	DCM	36.5	13.1
Stratified	15 min	32.0	15.1
Stratified	$15 \text{ min} + \text{Blank}$	28.0	13.1
Stratified	$15 \text{ min} + \text{Acetone}$	44.0	11.4
Stratified	$15 \text{ min} + \text{DCM}$	44.5	11.2
Stratified	5 sec	51.0	11.7
Stratified	$5 \text{ sec} + \text{Blank}$	34.5	13.1
Stratified	$5 \text{ sec} + \text{Acetone}$	33.0	12.9
Stratified	$5 \text{ sec} + \text{DCM}$	28.0	12.8
Stratified	10 _{sec}	40.0	12.0
Stratified	$10 \text{ sec} + \text{Blank}$	27.5	12.4
Stratified	$10 \text{ sec} + \text{Acetone}$	25.0	13.2
Stratified	$10 \text{ sec} + \text{DCM}$	41.0	12.1

Table 1-12. Estimates of final germination percentage (FGP) and time to 50% germination (T₅₀) from the time to event model for the seed coating formulations trial. Includes both unstratified and stratified seed.

Table 1-13. Statistical comparisons for FGP from time to event model for the seed coating concentrations trial with unstratified seed.

`. ` - 0.10

`. ` - 0.10 $'$ *` - 0.05

	Control	Blank	$1x-GA$	$2x-GA$	$4x-GA$	2.5 min	$2.5 \text{ min} +$ Blank	$2.5 \text{ min} +$ $1x-GA$	$2.5 \text{ min} +$ $2x-GA$	$2.5 \text{ min} +$ $4x-GA$
Control	1.0000									
Blank	0.7531	1.0000								
lx-GA	0.9310	0.7849	0000.1							
$2x-GA$	0.6813	0.8499	0.7483	1.0000						
$4x-GA$	0.9013	0.7812	0.8673	0.6547	1.0000					
2.5 min	0.8348	0.7601	0.8818	0.7424	0.8013	1.0000				
$2.5 \text{ min} + \text{Blank}$	0.9040	0.8956	0.9086	0.8935	0.9004	0.9243	1.0000			
$2.5 \text{ min} + 1 \text{x-GA}$	0.7389	0.9394	0.7698	0.9672	0.7697	0.7508	0.8942	1.0000		
$2.5 \text{ min} + 2x - GA$	0.8553	0.7851	0.8983	0.7684	0.8241	0.9909	0.9229	0.7760	1.0000	
$2.5 \text{ min} + 4 \text{x-GA}$	0.7952	0.6816	0.8690	0.6550	0.7425	0.9814	0.9215	0.6712	0.9927	1.0000
8 min	0.8268	0.6489	0.9255	0.6063	0.7440	0.9236	0.9143	0.6392	0.9388	0.9238
$8 \text{ min} + \text{Blank}$	0.9706	0.7507	0.9543	0.6907	0.8789	0.8484	0.9054	0.7359	0.8679	0.8164
$8 \text{ min} + 1 \text{x-GA}$	0.9379	0.9300	0.9422	0.9281	0.9345	0.9568	0.9797	0.9287	0.9554	0.9542
$8 \text{ min} + 2 \text{x-GA}$	0.8058	0.8993	0.8143	0.7385	0.8636	0.7749	0.8973	0.8650	0.7992	0.7029
$8 \text{ min} + 4 \text{x-GA}$	0.7684	0.9591	0.7945	0.7832	0.8033	0.7652	0.8962	0.9079	0.7900	0.6884
15 min	0.6586	0.7889	0.7367	0.9140	0.6126	0.7367	0.8928	0.9326	0.7630	0.6467
$15 \text{ min} + \text{Blank}$	0.6874	0.8651	0.7511	0.9856	0.6671	0.7437	0.8937	0.9745	0.7696	0.6571
$15 \text{ min} + 1 \text{x-GA}$	0.6587	0.7902	0.7365	0.9145	0.6140	0.7366	0.8928	0.9319	0.7628	0.6465
$15 \text{ min} + 2x - GA$	0.6336	0.7183	0.7233	0.7899	0.5683	0.7301	0.8921	0.8909	0.7567	0.6371
$15 \text{ min} + 4 \text{x-GA}$	0.6840	0.8566	0.7501	0.9865	0.6581	0.7433	0.8936	0.9729	0.7693	0.6563

Table 1-14. Statistical comparisons for T50 from time to event model for the seed coating concentrations trial with unstratified seed.

`. ` - 0.10 $'$ *` - 0.05

Table 1-16. Statistical comparisons for T₅₀ from time to event model for the seed coating concentrations trial following stratification for 30 d at 4° C.

`. ` - 0.10

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\therefore -0.10
$$

	Control	Blank	Acetone	DCM	15 min		$15 \text{ min} + \text{Blank}$	$15 \text{ min} + \text{Acetone}$	$15 \text{ min} + \text{DCM}$
Control	1.00000								
Blank	0.97389	00000.							
Acetone	0.95541	0.97657	1.00000						
DCM	0.82343	0.89981	0.95145	1.00000					
15 min	0.15424	0.43427	0.61379	0.44004	1.00000				
$15 \text{ min} + \text{Blank}$	0.26976	0.54741	0.70306	0.57942	0.77945		1.00000		
$15 \text{ min} + \text{Acetone}$	0.17501	0.45719	0.63240	0.46805	0.95216		0.82578	1.00000	
$15 \text{ min} + \text{DCM}$	0.15264	0.37252	0.54595	0.37344	0.81419		0.63611	0.77487	1.00000
5 sec	0.93809	0.96957	0.99862	0.94047	0.50689		0.61690	0.52945	0.43796
$5 \text{ sec} + \text{Blank}$	0.66410	0.79501	0.87632	0.87983	0.52479		0.68522	0.55737	0.44143
$5 \text{ sec} + \text{Acetone}$	0.54951	0.64599	0.73560	0.69670	0.89923		0.97696	0.92556	0.79165
$5 \text{ sec} + \text{DCM}$	0.53361	0.71411	0.81974	0.78456	0.59196		0.77161	0.62873	0.49372
10 sec	0.66824	0.79579	0.87629	0.88020	0.53143		0.69027	0.56373	0.44726
$10 \text{ sec} + \text{Blank}$	0.68010	0.80375	0.84036	0.62594	.0.07633		0.14651	.0.08833	0.08539
$10 \text{ sec} + \text{Acetone}$	0.73080	0.87045	0.93734	0.98079	0.32416		0.48567	0.35531	0.28243
$10 \text{ sec} + \text{DCM}$	0.48974	0.68748	0.80128	0.75255	0.61601		0.80245	0.65428	0.85979
	5 sec	$5 \text{ sec} + \text{Blank}$	$5 \text{ sec} + \text{Acetone}$		$5 \text{ sec} + \text{DCM}$	10 sec	$10 \text{ sec} + \text{Blank}$	$10 \text{ sec} + \text{Acetone}$	$10 \text{ sec} + \text{DCM}$
Control									
Blank									
Acetone									
DCM									
15 min									

Table 1-17. Statistical comparisons for FGP from time to event model for the seed coating formulations trial with unstratified seed.

5 sec + Blank 1.00000
5 sec + Blank 0.84418

5 sec + Blank 0.84418 1.00000
5 sec + Acetone 0.68859 0.77651

5 sec + Acetone 0.68859 0.77651 1.00000
5 sec + DCM 0.77030 0.90350 0.83800

5 sec + DCM 0.77030 0.90350 0.83800 1.00000
10 sec 0.84450 0.99917 0.77833 0.90548

10 sec 0.84450 0.99917 0.77833 0.90548 1.00000

10 sec + Blank 0.78542 0.47442 0.43362 0.35561 0.48067 1.00000
10 sec + Acetone 0.91933 0.87398 0.67926 0.75407 0.87481 0.47986

10 sec + Acetone 0.91933 0.87398 0.67926 0.75407 0.87481 0.47986 1.00000
10 sec + DCM 0.74600 0.87053 0.85868 0.96654 0.87294 0.31751 0.71273

 $15 \text{ min} + \text{Blank}$ 15 min + Acetone 15 min + DCM

10 sec + DCM 0.74600 0.87053 0.85868 0.96654 0.87294 0.31751 0.71273 1.00000

	Control	Blank	Acetone	DCM	15 min			$15 \text{ min} + \text{Blank}$	$15 \text{ min} + \text{Acetone}$	$15 \text{ min} + \text{DCM}$
Control	1.0000									
Blank	0.7899	1.0000								
Acetone	0.8849	0.9771	1.0000							
DCM	0.9481	0.8566	0.9050	1.0000						
15 min	0.1963	0.5855	0.7944	0.6557	1.0000					
$15 \text{ min} + \text{Blank}$	0.2950	0.6092	0.8049	0.6902	0.8066			1.0000		
15 min + Acetone	0.0824	0.5221	0.7638	0.5641	0.3076			0.3328	1.0000	
$15 \text{ min} + \text{DCM}$	0.3136	0.5922	0.7956	0.6661	0.9906			0.8885	0.6109	1.0000
5 sec	0.8829	0.9731	0.9618	0.9185	0.7336			0.7510	0.6842	0.7365
$5 \text{ sec} + \text{Blank}$	0.8182	0.8754	0.9198	0.9478	0.3510			0.4053	0.2433	0.3915
$5 \text{ sec} + \text{Acetone}$	0.9693	0.8516	0.9005	0.9874	0.7034			0.7347	0.6179	0.7113
$5 \text{ sec} + \text{DCM}$	0.7734	0.7148	0.8482	0.8381	0.6457			0.7221	0.4641	0.6827
10 sec	0.9630	0.8228	0.8945	0.9770	0.5333			0.5849	0.4116	0.5593
$10 \text{ sec} + \text{Blank}$	0.9933	0.8071	0.8857	0.9523	0.6149			0.6621	0.4964	0.6338
$10 \text{ sec} + \text{Acetone}$	0.9821	0.7862	0.8827	0.9414	0.2744			0.3679	0.1391	0.3706
$10 \text{ sec} + \text{DCM}$	0.7516	0.7114	0.8478	0.8346	0.6016			0.6891	0.4058	0.6512
	5 sec	$5 \text{ sec} + \text{Blank}$	$5 \text{ sec} + \text{Acetone}$		$5 \text{ sec} + \text{DCM}$	10 _{sec}		$10 \text{ sec} + \text{Blank}$	$10 \text{ sec} + \text{Acetone}$	$10 \text{ sec} + \text{DCM}$
Control										
Blank										
Acetone										
DCM										
15 min										
$15 \text{ min} + \text{Blank}$										
$15 \text{ min} + \text{Acetone}$										
$15 \text{ min} + \text{DCM}$										
5 sec	1.0000									
$5 \text{ sec} + \text{Blank}$	0.9411	1.0000								
$5 \text{ sec} + \text{Acetone}$	0.9122	0.9368	1.0000							
$5 \text{ sec} + \text{DCM}$	0.8240	0.6794	0.8668		1.0000					
10 _{sec}	0.9003	0.8986	0.9932		0.8131		1.0000			
$10 \text{ sec} + \text{Blank}$	0.8866	0.8714	0.9696		0.8624	0.9679		1.0000		
$10 \text{ sec} + \text{Acetone}$	0.8795	0.8128	0.9630		0.7960	0.9536		0.9970	1.0000	

Table 1-18. Statistical comparisons for T₅₀ from time to event model for the seed coating formulations trial with unstratified seed.

10 sec + DCM 0.8229 0.6634 0.8644 1.0000 0.8054 0.8576 0.7782 1.00000

 $'$ *` - 0.05

Table 1-20. Statistical comparisons for T₅₀ from time to event model for the seed coating formulations trial following stratification for 30 d at 4ºC.

APPENDIX

Use of Seed Coating Technologies to Improve *Cercocarpus ledifolius* (Curl-Leaf Mountain Mahogany) Seed Germination and Emergence to Reclaim Mine Lands

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INTRODUCTION

Native plants are an important tool in restoring degraded drylands because they are adapted to the climate of the area, perform important ecosystem functions, and reduce degradation caused by invasive species (Davies, 2011). One desirable native species in the mountain browse zones in the Western United States is *Cercocarpus ledifolius* (curl-leaf mountain mahogany). *Cercocarpus ledifolius* is an evergreen species that ranges in height from 2-9 m. It is often found on warm, dry, rocky slopes in the mountain brush zone of the Intermountain West in North America, and frequently found colonizing habitats where few other desirable woody species grow (Brotherson, 1990; Davis & Brotherson, 1991; Ibanez & Schuup, 2002). *Cercocarpus ledifolius* is an important browse species for large game such as mule deer, elk, and bighorn sheep, and is an important species for smaller wildlife (Paschke et al., 2003; Scheldt & Tisdale, 1970; Wood et al., 1995). In addition to its value for wildlife, *C. ledifolius* is an actinorhizal plant species that influences the nitrogen cycling and status of the surrounding plant communities (Freund et al., 2018; Lepper & Fleschner, 1977).

While there are many beneficial characteristics of *C. ledifolius*, most attempts at establishing *C. ledifolius* by seeding fail (Schultz, Tausch, & Tueller, 1996; Young et al., 1978), which results in colonization by undesirable and invasive species in the area. With establishment

failure of seeded species up to 90%, reduction of seed dormancy is necessary for revegetation efforts (James et al., 2013; Kildisheva et al., 2020; Merritt & Dixon, 2011). Failure of this species to establish has generally been attributed to seed dormancy inhibiting germination (Liacos & Nord, 1961; Young et al., 1978), but in addition to the challenges presented by seed dormancy, this species also has slow natural regeneration rates and low seedling survival rates (Ex et al., 2011; Ibanez & Schuup, 2002; Scheldt & Tisdale, 1970). Good seed crops of *C. ledifolius* may only occur a few times per decade and, of those seedlings that emerge, many are lost to summer drought or winter browsing (Ibanez & Schuup, 2002; Shaw et al., 2004) *Cercocarpus ledifolius* seed dormancy issues, in combination with low seedling survival and slow natural regeneration, cause challenges when utilizing this species in revegetation projects (Ex et al., 2011).

To use *C. ledifolius* in restoration efforts, we need to understand its seed dormancy and germination traits. *Cercocarpus ledifolius* seeds exhibit traits of physiological dormancy, which can include several mechanisms by which germination is inhibited (Stidham et al., 1980; Young et al., 1978). Low growth potential of the embryo, restriction of radicle emergence by the structures covering the embryo, and chemical inhibitors, are all aspects of physiological dormancy that may be limiting *C. ledifolius* seed from germinating in a given season and contribute to its low success rates in seeding efforts (Baskin & Baskin, 2014).

To help overcome *C. ledifolius* seed dormancies, several chemical treatments have been attempted (Kitchen, 2008). Dormancy has been targeted with scarification methods such as soaking seed in sulfuric acid (Heit 1970; Liacos & Nord, 1961; Young et al., 1978). Liacos and Nord (1961) soaked seed in concentrated (\approx 98%) sulfuric acid for durations ranging from 5- to 20-min followed by a 4-hr soak in thiourea, resulting in a 62% increase in germination. Young et

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al. (1978) soaked seed in concentrated (\approx 98%) sulfuric acid for durations ranging from 1- to 30min, they found that those treatments reduced germination up to 15%. Acid scarification for 5- to 10-min, has also been used with a related species, *Cercocarpus montanus* (alder-leaf mountain mahogany), where it was successful in improving germination in 4 of 7 seed sources (Rosner et al., 2003). However, it was noted in this study that *C. montanus* does not exhibit typical traits of physical dormancy for which acid scarification is generally used to treat, but instead contains germination inhibitors in the seed coat. The variable success of these previously tested acid scarification seed treatments is often attributed to ecotypic variation of the species and macroclimatic differences in the seed source (Rosner et al., 2003; Young et al., 1978), but may also be influenced by the research method variation. These method variations may be the amount of time seed was rinsed in water following treatment, whether seed was also rinsed in a neutralizing bicarbonate solution, or other variations that may not have been specified.

To enhance the ability with which GA3 is able to break dormancy of *C. ledifolius*, with easier entry through the seed coat, mechanical scarification was explored. Similar to acid scarification, this mechanical scarification method is typically used with water impermeable seed; the scarification treatment alters the seed coat making it permeable to water allowing imbibition to occur (Baskin & Baskin, 2014). Several native forb species such as *Astragalus filipes* (basalt milkvetch), *Dalea ornata* (western prairie clover), and *Lupinus arbustus* (longspur lupine), have been found to respond well to mechanical scarification, reaching >90% germination (Kildisheva, et al., 2018). Since mechanical scarification can be used to enhance germination of water impermeable seed, it is possible that it could enhance the permeability to GA₃ as well.

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The purpose of our research is to identify tools to overcome *C. ledifolius* seed dormancy so it can be used to revegetate degraded regions. To accomplish this, we will address *C. ledifolius* physiological dormancies through mechanical and acid scarification, along with the addition of GA3 applied through a seed coating. Our objective is to determine durations of mechanical and acid scarification that will increase *C. ledifolius* germination in a laboratory setting. We hypothesize that the combination of treatments targeting the restrictive seed coat of *C. ledifolius* will increase the germination of *C. ledifolius* in a laboratory setting.

MATERIALS & METHODS

Cercocarpus ledifolius seed for all trials in this study was purchased from Granite Seed and Erosion Control (Lehi, UT, USA), and was collected from Utah County in 2019. Seed for the trials combining scarification methods and GA3 seed coatings was purchased from Great Basin Seed (Ephraim, UT, USA) and was also collected in 2019 from the state of Utah (Great Basin seed lot). In all trials, seeds were counted as germinated when the radicle had emerged at least 2 mm. Seeds that had germinated were removed from the Petri dish at the time of counting. Using germination data, we estimated the time to reach 50% germination (T₅₀), and final germination percentage (FGP) using a non-linear, three-parameter log-logistic time-to-event model (Ritz, Piper, & Streibig, 2013). Time-to-event models were fitted using the 'drm' function of the 'drc' package in the R programming environment (R Core Team, 2022; Ritz et al., 2015). We compared treatment effects by performing pairwise comparisons between treatments using the 'compParm' function in the 'drc' package, adjusting P-values with the Bonferroni method (α = 0.10) (Ritz et al., 2015).

Imbibition trial

Imbibition testing was used to determine if the seed coat was preventing water uptake of *C. ledifolius*. In the experiment, we compared imbibition rates of seed that was left untreated to seed that had the seed coat nicked with a razor blade on the distal end of the seed (Campbell-Martínez et al., 2019). This experiment was replicated 5 times with each treatment/replicate containing 25 seeds. Seeds were placed in Petri dishes lined with one sheet of blotter paper saturated with 5 ml of distilled water. Petri dishes were randomly distributed on a laboratory bench, covered to exclude light, and kept at room temperature (\approx 24 $^{\circ}$ C). Percentage of mass increase was calculated by subtracting the mass of the seed at 24 hr from the mass at 0 hr and multiplying by 100. The percent mass between the two treatment groups were compared using a t-test $(a = 0.10)$ in the R programming environment (R Core Team, 2022).

Acid Scarification trial

Seeds were soaked in concentrated (\approx 98.0%) sulfuric acid for seven different periods of time: 0-, 2.5-, 5-, 10-, 15-, 20-, and 25-minutes, in batches of 50 seeds at a time. Following soaking in sulfuric acid, seeds were washed under running water for approximately 3 minutes, and then air dried. Once dry, 50 seeds of each treatment group were placed in Petri dishes containing two layers of blotter paper moistened with 7 ml of distilled water, replicated 8 times. These Petri dishes were then incubated at 4ºC for 30 d prior to being exposed to a constant temperature of 10ºC with a 12 hr light cycle for germination. Germination was recorded each week up to one month, with distilled water being added to Petri dishes as needed to maintain moisture during incubation and after the temperature was increased.

Mechanical Scarification trial

Seeds were scarified in a Forsberg electric seed scarifier (Forsberg's Inc., Thief River Falls, Minnesota, USA) with 400 grit sandpaper. Durations of 0-, 1-, 2-, 5-, 10-, 30- and 60-sec of 50-g batches (\approx 4,000 seeds) in the Forsberg scarifier were tested. Following treatment, 50 seeds of each treatment group were placed in 9 mm Petri dishes containing 2 sheets of blotter paper that was moistened with 7 ml of distilled water, replicated 8 times. These Petri dishes were incubated for 30 d at 4ºC before being exposed to a constant temperature of 15ºC with a 12-hr light cycle for germination. Germination was recorded weekly up to one month, with distilled water being added to petri dishes as needed to maintain moisture during incubation and after the temperature was increased.

RESULTS

Imbibition trial

Percentage of mass increase of the control and scarified seed were 65 and 66% respectively, which were not significantly different from each other ($P = 0.897$).

Acid Scarification trial

For trial 1 of acid scarification, there were no significant differences ($P > 0.10$) between any treatment duration and the 0-minute treatment (Figure 2-1). For trial 2, the FGP of the 25 minute scarification treatment was 18.7% higher than the 0-minute treatment ($P = 0.0797$). T₅₀ estimates for sulfuric acid treatments were not significantly different ($P > 0.10$) from the 0minute treatment.

Mechanical Scarification trial

The FGP for 0-, 1-, 2-, 5-, and 10-second treatments were significantly higher $(P < 0.10)$ than the 30- and 60-second treatments, and the 30-second treatment had a significantly higher (P < 0.10) FGP than 60-seconds (Figure 2-2). No mechanical scarification (0-seconds in electric seed scarifier) had 30.9 and 45.0% higher germination than 30- and 60-second treatments ($P \le$ 0.001 and $P < 0.0001$, respectively) (Figure 2-2). Mechanical scarification decreased germination, and the trend was maintained in T_{50} germination as well with 0-, 1-, 2-, and 5second treatments reaching 50% germination 4.01, 3.94, 4.35, and 3.43 d before the 30 second treatment ($P < 0.10$). The 0-, 1-, and 2-second treatments also reached 50% germination 6.72 (P) $= 0.0926$, 6.65 (P = 0.0960), and 7.06 (P = 0.0775) d, respectively before the 60-second treatment.

Seed Coating Concentrations trial

Unstratified. There were no significant differences $(P > 0.10)$ for FGP or T₅₀ between control seed and treatments regardless of scarification or GA3 coating (Tables 2-1, 2-2, 2-3, 2-4).

Stratified. The stratified seed had some significant differences ($P < 0.10$) between control seed and treatments for FGP (Table 2-5). The 4x GA₃ coating, and 15-minute scarification with 2x GA₃ had 21.9 (P = 0.0891), and 21.5% (P = 0.0955), higher germination than control seed, respectively (Table 2-1, Figure 2-3). The 4x GA3 coating, and 15-minute with 2x GA3 also had 23.4 ($P = 0.0660$), and 23.0% ($P = 0.0711$) higher germination than the blank coating (Table 2-1, Figure 2-3). Treatments were not significantly different ($P > 0.10$) when compared to the control for T_{50} regardless of scarification or GA_3 coating (Table 2-6).

Seed Coating Formulations trial

Unstratified. The FGP pairwise comparisons had some significant differences $(P < 0.10)$ between the control and treatments (Table 2-7). Control seed had 21.0 (P = 0.0767), 20.9 (P = 0.0783), 24.2 ($P = 0.0357$), and 22.0% ($P = 0.0603$) higher germination than 15-minute acid scarification, 15-minute with blank, 15-minute with acetone, and 15-minute with DCM coatings, respectively (Table 2-2, Figure 2-4). Pairwise comparisons for T₅₀ germination indicated treatments were not statistically different $(P > 0.10)$ from the control seed (Table 2-8).

Stratified. The stratified seed had no significant differences ($P > 0.10$) for FGP or T₅₀ between control and any combination of scarification and GA_3 coating (Table 2-1, 2-2, 2-9, 2-10).

DISCUSSION

Dryland ecosystems are important to the support of billions of people, and when these ecosystems are disturbed through anthropogenic activities it is important to initiate the reclamation of disturbed areas before invasive species cause further degradation. *Cercocarpus ledifolius* is a native plant with substantial beneficial characteristics supporting wildlife and the surrounding soils and plants. Unfortunately, *C. ledifolius* does not establish well from seed, which is likely due to seed dormancy inhibiting germination (Liacos & Nord, 1961; Young et al., 1978). Our research efforts focused on breaking seed dormancy and mechanisms to increase seed germination, thereby increasing the revegetation of disturbed lands.

Imbibition of water is vital for a seed to germinate, and past research on *C. ledifolius* seemed to suggest that its dormancy could be a result of the seed coat limiting water uptake because of the commonly recommended treatments. Sulfuric acid scarification was a frequent recommendation for treating *C. ledifolius* seed and is commonly used to treat physically dormant seed (Heit, 1970; Liacos & Nord, 1961). However, in our trials we found that seed coat permeability is not limiting water uptake as physically damaged (scarified) seed and nonphysically damaged (control) seed imbibed water at the same rate in our imbibition trial. This is corroborated by the results of the mechanical scarification trial where none of the mechanical scarification treatments alone had a positive effect on germination and increasing the duration of scarification time in the electric seed scarifier decreased germination percentages (Figure 2-2).

Acid scarification on the other hand, did not cause any significant decreases in germination, and in the second acid scarification trial, the 25-minute soak in sulfuric acid was significantly better than the control treatment with an 18.7% increase in germination ($P =$ 0.0797) (Figures 2-1). Our results are similar to the conclusions and recommendations from both Liacos and Nord (1961), and Heit (1970) who found acid scarification from 10- to 20-minutes increased germination. However, the same 25-minute scarification treatment results varied in significance and were not different from control seed in the first acid scarification trial, even though treatments were performed on seed from the same lot. These results align with conclusions made by Young et al. (1978) that the success of acid scarification treatment is highly variable as it can change the permeability of seed to gasses and change the sensitivity of the seed to light and temperature.

Cercocarpus ledifolius seed dormancy may be caused by a combination of physiological dormancy mechanisms, and by treating seed with physical treatments and plant hormones we may be able to consistently break dormancy. Therefore, in the two combination trials (seed coating concentration and seed coating formulation trials), we looked at treatments combining various scarification methods and GA3 seed coatings. Seed was tested in stratified and

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unstratified conditions. The concentrations trial tested combining acid scarification with GA3 concentrations as a seed coating. For this trial, treatments were not significant for unstratified seed. Yet, for stratified seed, the 4x GA3 coating and 15-minute scarification with a 2x GA3 coating had significantly higher germination than the control (Figure 1-6).

In the formulations trial, where both acid and mechanical scarification were combined with GA₃ coating formulations, it was found that for unstratified seed, the control treatment had significantly higher germination than any of the 15-minute acid scarification treatments even when combined with a GA₃ coating (Figure 2-4). With stratified seed, however, there were no differences for any combination of scarification and GA3 coating.

Seed germination in general was higher when seed was stratified, with germination percentages in the 40 -50% range, compared with unstratified in the 10 - 20% range (Table 2-1, 2-2). The higher germination in seed that was stratified was consistent across both combination trials and agrees with conclusions from Stidham et al. (1980) stating that *C. ledifolius* would not be likely to produce suitable stands if planted in the spring as opposed to fall planting where it would receive cold stratification over the winter months.

Our study of seed treatments and seed coatings reiterates the difficulty of pinpointing one treatment that will consistently overcome *C. ledifolius* dormancy and improve germination. Results of all treatments tested were variable between different trials with the same seed lot. It will be important for future research on *C. ledifolius* seed to determine more precisely the mechanism of physiological dormancy. From our findings, one potential mechanism is for it to be caused by a chemical inhibitor found in the seeds, as suggested by Liacos and Nord (1961) and suggested by Moore (1963) with *C. montanus*. Finding if an inhibitor exists in *C. ledifolius* seeds would allow for use of more accurately targeted treatments to leach or nullify effects of the inhibitor in some way. Another possible dormancy mechanism could be structures surrounding the embryo restricting the radicle emergence (Baskin & Baskin, 2014; Heit, 1970). If structures such as the seed coat were restricting radicle emergence, this could explain why seed benefitted from acid scarification, even though it is not water impermeable as seen in the imbibition trial, which is what acid scarification is generally used to treat. Overall, for revegetation efforts, we recommend testing several time durations ranging from 2.5 to 15 minutes of sulfuric acid scarification on a sample of each seed lot since this treatment had the most success and testing a range of sulfuric acid treatments will identify the best treatment for a particular seed lot.

CONCLUSION

Cercocarpus ledifolius seed has complicated dormancy mechanisms limiting revegetation efforts, and our study results further demonstrate the complexity of seed dormancy. Especially for native shrub species, dormancy can pose significant barriers to germination and potential use in land restoration efforts. The inconsistent results from seed treatments of acid scarification and the GA3 seed coatings indicate a need to understand the mechanism more precisely by which *C. ledifolius* seed is physiologically dormant. Future research should explore the potential of chemical inhibitors and restrictive structures limiting radicle emergence as mechanisms of dormancy. This information may allow for more targeted treatment selection. Another consideration is the genetic variation of the species. Any treatment which may be successful with one seed source may not be successful with another. This means that for revegetation efforts, it will be vital to test several potential seed treatments on samples of each seed lot before treating seed on a large scale.

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FIGURES

Figure 2-1. Time to event model for germination in acid scarification trial 1 (left) and trial 2 (right). Error bars represent standard error.

Figure 2-2. Time to event model for germination in the mechanical scarification trial. Error bars represent standard error.

Figure 2-3. Time to event model for germination of the great basin seed lot in the seed coating concentrations trial following stratification for 30 d at 4ºC. Error bars represent standard error. For clarity, only the treatments that were significantly different $(P < 0.10)$ than the control and control with blank coating are shown.

Figure 2-4. Time to event model of the great basin seed lot for the seed coating formulations trial. Error bars represent standard error. For clarity, only the treatments which were significantly different ($P < 0.10$) from the control are shown.

TABLES

Table 2-1. Estimates of final germination percentage (FGP) and time to 50% germination (T50) from time to event model for the seed coating concentrations trial. Includes both unstratified and stratified seed.

Stratification	Treatment	FGP $(\%)$	T_{50}	
None	Control	13.4	15.6	
None	$Control + Blank$	9.8	35.0	
None	$1x-GA_3$	21.7	46.6	
None	$2x-GA_3$	17.0	29.0	
None	$4x-GA_3$	22.9	25.7	
None	2.5 min	26.7	20.1	
None	$2.5 \text{ min} + \text{Blank}$	10.6	20.6	
None	2.5 min + 1x- GA_3	30.2	47.7	
None	2.5 min + $2x-GA_3$	23.2	50.2	
None	2.5 min + $4x-GA_3$	24.5	17.0	
None	8 min	11.9	27.9	
None	$8 \text{ min} + \text{Blank}$	8.9	24.5	
None	$8 \text{ min} + 1 \text{x-GA}_3$	17.6	18.3	
None	$8 \text{ min} + 2x - GA_3$	18.2	13.7	
None	$8 \text{ min} + 4 \text{x-GA}_3$	18.3	40.5	
None	15 min	19.7	11.7	
None	$15 \text{ min} + \text{Blank}$	13.3	23.4	
None	$15 \text{ min} + 1 \text{x-GA}_3$	23.1	18.8	
None	15 min + $2x-GA_3$	25.7	11.6	
None	15 min + $4x-GA_3$	28.2	15.2	
Stratified	Control	21.5	10.3	
Stratified	$Control + Blank$	20.0	10.1	
Stratified	$1x-GA_3$	35.0	10.3	
Stratified	$2x-GA_3$	33.0	10.2	
Stratified	$4x-GA_3$	43.4	10.2	
Stratified	2.5 min	32.0	10.0	
Stratified	$2.5 \text{ min} + \text{Blank}$	26.0	10.5	
Stratified	2.5 min + 1x - GA_3	40.5	10.0	
Stratified	2.5 min + $2x-GA_3$	36.5	9.6	
Stratified	2.5 min + $4x-GA_3$	35.0	10.0	
Stratified	8 min	22.0	9.8	
Stratified	$8 \text{ min} + \text{Blank}$	22.0	9.7	
Stratified	$8 \text{ min} + 1 \text{x-GA}$	40.5	9.3	
Stratified	$8 \text{ min} + 2 \text{x-GA}_3$	23.5	11.3	
Stratified	$8 \text{ min} + 4 \text{x-GA}_3$	39.0	9.2	
Stratified	15 min	27.0	9.6	
Stratified	$15 \text{ min} + \text{Blank}$	24.5	9.9	
Stratified	15 min + $1x-GA_3$	32.1	9.7	
Stratified	15 min + $2x-GA_3$	43.0	8.9	
Stratified	$15 \text{ min} + 4 \text{x-GA}_3$	33.0	8.5	

Stratification	Treatment	FGP	T_{50}
None	Control	35.1	13.4
None	Blank	21.1	15.4
None	Acetone	19.7	18.4
None	DCM	27.6	20.2
None	15 min	14.1	9.9
None	$15 \text{ min} + \text{Blank}$	14.2	12.1
None	$15 \text{ min} + \text{Acetone}$	10.9	11.3
None	$15 \text{ min} + \text{DCM}$	13.1	9.3
None	5 sec	31.6	14.9
None	$5 \text{ sec} + \text{Blank}$	17.5	17.8
None	$5 \text{ sec} + \text{Acetone}$	24.7	17.1
None	$5 \text{ sec} + \text{DCM}$	24.5	18.2
None	10 _{sec}	42.8	13.0
None	$10 \text{ sec} + \text{Blank}$	24.9	16.6
None	$10 \text{ sec} + \text{Acetone}$	22.1	19.9
None	$10 \text{ sec} + \text{DCM}$	28.1	15.3
Stratified	Control	29.0	9.6
Stratified	Blank	20.5	10.1
Stratified	Acetone	26.5	11.5
Stratified	DCM	26.7	12.0
Stratified	15 min	15.0	11.3
Stratified	$15 \text{ min} + \text{Blank}$	19.6	11.3
Stratified	$15 \text{ min} + \text{Acetone}$	17.5	10.0
Stratified	$15 \text{ min} + \text{DCM}$	17.5	8.8
Stratified	5 sec	30.0	10.6
Stratified	$5 \text{ sec} + \text{Blank}$	24.0	10.5
Stratified	$5 \text{ sec} + \text{Acetone}$	21.6	10.7
Stratified	$5 \text{ sec} + \text{DCM}$	28.0	9.8
Stratified	10 _{sec}	29.8	11.4
Stratified	$10 \text{ sec} + \text{Blank}$	26.5	9.6
Stratified	$10 \text{ sec} + \text{Acetone}$	37.2	10.8
Stratified	$10 \text{ sec} + \text{DCM}$	39.6	9.7

Table 2-2. Estimates of final germination percentage (FGP) and time to 50% germination (T₅₀) from the time to event model for the seed coating formulations trial. Includes both unstratified and stratified seed.

	Control	Blank	$1x-GA$	$2x-GA$	$4x-GA$	2.5 min	$2.5 \text{ min} +$ Blank	$2.5 \text{ min} +$ $1x-GA$	$2.5 \text{ min} +$ $2x-GA$	$2.5 \text{ min} +$ 4x-GA
Control	.0000									
Blank	0.7904	.0000								
lx-GA	0.4737	0.8909	0000.1							
$2x-GA$	0.5523	0.9358	0.7092	1.0000						
$4x-GA$	0.5092	0.8987	0.6363	0.8921	1.0000					
2.5 min	0.6943	0.8378	0.5387	0.6893	0.7071	1.0000				
$2.5 \text{ min} + \text{Blank}$	0.7696	0.8453	0.5626	0.7427	0.7943	0.9762	1.0000			
$2.5 \text{ min} + 1 \text{x-GA}$	0.6361	0.8984	0.9890	0.7904	0.7477	0.6836	0.6940	1.0000		
$2.5 \text{ min} + 2x - GA$	0.6382	0.8827	0.9653	0.7796	0.7406	0.6819	0.6911	0.9793	1.0000	
$2.5 \text{ min} + 4x - GA$	0.8879	0.8048	0.4899	0.5808	0.5351	0.7495	0.8226	0.6499	0.6510	1.0000
8 min	0.4793	0.9239	0.6793	0.9688	0.9080	0.6455	0.7287	0.7749	0.7655	0.5032
$8 \text{ min} + \text{Blank}$	0.5965	0.8867	0.6221	0.8603	0.9519	0.7884	0.8502	0.7362	0.7299	0.6324
$8 \text{ min} + 1 \text{x-GA}$	0.8467	0.8203	0.5200	0.6544	0.6673	0.8985	0.9041	0.6671	0.6666	0.9184
$8 \text{ min} + 2x - GA$	0.8446	0.7691	0.4410	0.4758	0.3806	0.4866	0.6580	0.6142	0.6181	0.6599
$8 \text{ min} + 4 \text{x-GA}$	0.4215	0.9447	0.9065	0.7510	0.6467	0.5071	0.5492	0.9222	0.9017	0.4393
15 min	0.6759	0.7478	0.4124	0.4155	0.2949	0.3364	0.5607	0.5930	0.5986	0.4485
$15 \text{ min} + \text{Blank}$	0.6986	0.8770	0.6167	0.8426	0.9211	0.8664	0.9035	0.7282	0.7224	0.7395
$15 \text{ min} + 1 \text{x-GA}$	0.7867	0.8242	0.5203	0.6503	0.6507	0.9093	0.9161	0.6701	0.6695	0.8606
$15 \text{ min} + 2x - GA$	0.6644	0.7472	0.4113	0.4119	0.2872	0.3193	0.5550	0.5923	0.5980	0.4233
$15 \text{ min} + 4 \text{x-GA}$	0.9645	0.7848	0.4622	0.5209	0.4454	0.5982	0.7296	0.6299	0.6326	0.8097

Table 2-4. Statistical comparisons for T50 from time to event model for the seed coating concentrations trial with unstratified great basin seed lot.

Table 2-5. Statistical comparisons for FGP from time to event model for the seed coating concentrations trial with the great basin seed lot following stratification for 30 d at 4ºC.

Table 2-6. Statistical comparisons for T50 from time to event model for the seed coating concentrations trial with the great basin seed lot following stratification for 30 d at 4ºC.

Table 2-7. Statistical comparisons for FGP from time to event model for the seed coating formulations trial with unstratified great basin seed lot.

`. ` - 0.10

 $'$ *` - 0.05

**` - 0.01

Table 2-9. Statistical comparisons for FGP from time to event model for the seed coating formulations trial with the great basin seed lot following stratification for 30 d at 4ºC.

`. ` - 0.10

 $'$ *` - 0.05

Table 2-10. Statistical comparisons for FGP from time to event model for the seed coating formulations trial with the great basin seed lot following stratification for 30 d at 4ºC.

