Fire Response and Germination Patterns of a Washington Endemic Species, *Eriogonum codium*

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A project submitted in partial fulfillment of the requirements of the Master of Environmental Horticulture

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ABSTRACT

Eriogonum codium (Polygonaceae) is a G1, S1 (Globally endangered, State endangered) species endemic to eastern Washington, with a single population existing at the Hanford Reach National Monument in Benton County. The species is a long-lived "sub-shrub" with high variability in flowering and seed set, as well as low recruitment rates. Along with limiting reproductive factors, an increased incidence of wildfire in its native shrub-steppe habitat is a threat to the species, which is not fire adapted. Following a 2017 wildfire that damaged portions of the population, seeds were collected from plants assessed as unburned, lightly burned and partially burned. The primary focus of this study was to test whether seed viability and germination were affected across categories of burn severity. A secondary goal of this project was to conduct a deeper exploration of the germination patterns of *E. codium* in order to inform propagation work. Results indicate a significant decrease in viability and germination for seeds from lightly and partially burned individuals compared to those categorized as unburned. However, there was also a large amount of variability between the unburned groups for these measures. Germination testing revealed that seed lots of *E. codium* contain a portion that are non-dormant, with the remainder exhibiting varying degrees of physiological dormancy that can be partially broken by cold-moist stratification, and some that remain deeply dormant.

INTRODUCTION

Eriogonum (Polygonaceae) is one of the largest and most diverse genera in North America, with roughly 250 species (Reveal, 2005). Influenced by the heterogeneous landscapes of western North America (all but three species exist west of the Mississippi River), species inhabit environments ranging from mountaintop to shoreline (Reveal, 2005). As a genus native to a region that is relatively young in terms of geological time, *Eriogonum* has undergone rapid evolution, with species and varieties often restricted to unique edaphic conditions and other environmental niches (Welsh, 1984). Accordingly, of the roughly 250 species of *Eriogonum*, an estimated one-third are considered uncommon or rare in their distribution (Reveal, 2005).

Eriogonum codium (Umtanum desert buckwheat) is a G1, S1 (Globally endangered, State endangered) species with a single population existing at the Hanford Reach National Monument in Benton County, Washington (Fertig, 2018). Now recognized as an important protected area for shrub-steppe habitat within eastern Washington, the Hanford Reach holds a highly biodiverse collection of plant and animal communities (Dunwiddie, Beck, & Caplow, 2000; Evans, Lih, & Dunwiddie, 2003). Originally identified as a candidate for federal listing in 1999, the US Fish and Wildlife Service determined to list *E. codium* as threatened under the 1973 Endangered Species Act in April of 2013 (U.S. Fish and Wildlife Service, 2013a) and 344 acres were designated as critical habitat in the same year (U.S. Fish and Wildlife Service, 2013b).

The species was discovered by Florence Caplow and Kathryn Beck during a 1995 biodiversity survey of the Hanford Site prior to designation of the Monument, and was taxonomically described shortly after being found (Reveal, Caplow, & Beck, 1995). *E. codium* is defined by its tomentose flowers and sparsely tomentose achenes, and is currently not considered to be closely related to any of its nearest relatives (Reveal et al., 1995). Growing as a low, mat-forming, perennial "sub-shrub", demographic studies of the population have shown it to be a slow growing and long-lived species, with some individuals over 100 years of age (Dunwiddie et al., 2000).

Distribution of *E. codium* is very limited, with the single population occupying a roughly 1 mile long and very narrow (25-150 meter) stretch on the northern crest of Umtanum Ridge at roughly 340-400 meters elevation, with individuals distributed discontinuously throughout this area (Camp & Gamon, 2011; Dunwiddie et al., 2000). The ridgeline on which the population grows is composed of exposed basaltic flow top specific to the Lolo Flow of the Wanapum Basalt Group (U.S. Fish and Wildlife Service, 2013a). High winds and weathering of the ridgeline have created gravel-sized pieces of basaltic material, and *E. codium* grows exclusively on exposed areas, with distribution of the population interrupted by absence of exposed flow (Reveal et al., 1995). When population monitoring began, five subpopulations were delineated by these breaks in exposure of the flow top (Dunwiddie et al., 2000).

Demographic studies began shortly after the species was discovered, and 26 permanent quadrats were established for long-term monitoring (Dunwiddie et al. 2000). A full census of the population was conducted in 1997, and data on flowering, growth rates, seedling recruitment, and mortality were also collected over the course of three years (Dunwiddie et al., 2000). A population viability analysis conducted using data from 1997-2006 showed that the population was experiencing a gradual decline, with a projected annual decline of slightly less than 1% (Caplow, Kaye, & Arnett, 2007). While this analysis found no chance of extinction within the next 100 years, the probability of the population declining by 50% in the same time frame was 72% (Caplow et al., 2007). However, this did not take into account stochastic events such as wildfire, which could increase this probability substantially (Caplow, Kaye, & Arnett, 2007).

Biology of *Eriogonum codium*

As with many species of *Eriogonum*, *E. codium* exhibits an extended period of flowering, often from June to September. Often one flower may be completing seed set while another next to it may just be opening (Dunwiddie et al., 2000). Flower production can be very high for individual plants, with one individual producing a count of 1,700 flowers in a single inflorescence by mid-July of one year. An estimate based on flower and inflorescence counts puts the potential production of a single plant at over 250,000 flowers per year (Dunwiddie et al., 2000). However, the number of inflorescences produced per individual and across the population can vary widely from year to year, and records show that a small number of individuals generally produce a high number of inflorescences relative to the rest of the plants (Caplow, 2003). Some individuals that were recorded as producing hundreds of flowers in one year produced almost none the following year (Dunwiddie et al., 2000).

Despite high rates of flower production, low reproduction and recruitment rates are a concern for continued existence of the species. Reveal et al. (1995) note in their original treatment that less than 5% of flowers produced mature and viable seed during that year. Other reports indicate that seed set occurs in roughly 10% of flowers (Dunwiddie et al., 2000; U.S. Fish and Wildlife Service, 2013a). However, it is unlikely that low seed production is the main limiting factor for the species, with lack of seedling recruitment being a greater concern (Dunwiddie et al., 2000).

Achenes are single-seeded fruits that are the seed unit for *Eriogonum* (Meyer & Paulsen, 2000). Dispersal from the parent plant likely occurs by gravity and may be further facilitated by wind, high levels of which often occur at Umtanum Ridge (Dunwiddie et al., 2000). While monitoring the population from 1997-1999, researchers observed many seeds being carried away by harvester ants, and thousands of seeds collected at burrow entrances. However, no seedlings were observed to be germinating at these sites (Dunwiddie et al., 2000).

Seed germination follows a similar pattern to flower production, with high variability from year to year, and the majority of seedlings often produced by a small number of adult individuals each year (Caplow, 2003). Plants within four plots out of 24 produced 55% of counted seedlings throughout a 9 year monitoring period (Caplow, 2005). Yearly seedling counts have also varied significantly. Out of 22 years of monitoring, zero seedlings have been produced during 2 years, and a range of 3-154 seedlings have been produced during the rest (Fertig, 2018).

Numbers of recruitment events are consistently low, with seedlings rarely surviving a few months after germination (Arnett, 2012). Seedling mortality rates between May and June following spring germination has ranged from 67-91%, and generally there is full mortality from one year to the next, with the exception of a few seedlings that have survived 1-2 years and then died (Caplow, 2005). A single seedling, recorded in the spring of 1999, and assumed to have germinated in the spring of 1998, has survived and been tracked to flowering in 2003 after 5 years (Beck & Caplow, 2006; Caplow, 2005). In a few cases where seedlings survived a few years, Caplow (2005) notes that they were not discovered until the spring following the July seedling monitoring period, indicating that they may have germinated later in the summer or fall.

Knowledge of *Eriogonum* Germination

An understanding of germination at the genus level has potential to provide insight into species-specific patterns. Species of *Eriogonum* generally exhibit either physiological dormancy, non-dormancy, or a combination of both, with patterns related to habitat and life history strategy (Baskin & Baskin, 2014; Meyer & Paulsen, 2000). Experiments conducted by Meyer and Paulsen (2000) on 10 species of cold-desert Utah *Eriogonum* found a correlation between elevation, and therefore winter climate, and stratification requirements. Species inhabiting lower elevations generally needed

0-8 weeks of cold-moist stratification, while those from higher elevations required 12-24 weeks of cold-moist treatment, indicating a later winter to early spring timing of seedling emergence for these species (Meyer & Paulsen, 2000).

Temperate semi-desert/desert *Eriogonum* typically exhibit physiological dormancy as their primary strategy (Baskin & Baskin, 2014). However, none are currently known to have an extended period of physiological dormancy, requiring multiple cold-moist cycles to achieve full germination. Out of 24 collections of the 10 species of Utah *Eriogonum* that underwent germination testing, only seven did not reach 100% germination after 24 weeks of cold-moist stratification, with those seven reaching a minimum of 94% gemination, indicating that a single cycle of cold-moist stratification is enough to fully relieve dormancy for these species (Meyer & Paulsen, 2000). Many *Eriogonum* also show a certain level of non-dormancy in combination with physiological dormancy, with a portion of seeds from a given seed lot requiring no cold-moist stratification for germination (Meyer, 2008). A period of dry after-ripening has also been shown to relieve dormancy for a number of species in the genus, facilitating germination without a period of stratification (Meyer, 2008; Stevens et al. 1996).

Current Knowledge of *Eriogonum codium* Germination

The first germination test for *E. codium* was conducted by the Berry Botanical Garden in 1999. Seed was stored for 2 years in cold, dry conditions (1997-1999) until testing began. Four treatments were tested, although a small sample size was used, with only 25 seeds tested across the treatments (Ed Guerrant, personal communication). Testing yielded a range of 17-86% (52% average) germination across all treatments. The most successful treatment (86%) was a cold-moist stratification period of 5°C in darkness for 8 weeks, followed by incubation in a diurnal regime of 20/10°C (day/night) and 16 hours light/8 hours dark for a further 8 weeks (Table 1). The second-highest germination (67%) occurred using cold-moist stratification at 5°C in darkness for 8 weeks followed by warm stratification in constant 20°C and 16 hours light/8 hours dark (Ed Guerrant, personal communication).

Viability testing was conducted by Ransom Seed Lab in 2002, and again in 2003 as part of a seed bank study conducted by Florence Caplow. Forty seeds (50 in 2003) were incubated at 20°C for three weeks, and any germination recorded (Caplow, 2005). Following this treatment, the remainder of seeds were cut through the cotyledons and exposed to a 400ppm solution of gibberellic acid and incubated for an additional three days at 20°C (Caplow, 2005). Seeds that remained un-germinated following this treatment were tested with tetrazolium chloride and final viability recorded (Caplow, 2005).

From the 2002 tests, 5% of seed germinated without requiring cold-moist stratification, 65% were viable but dormant, and 30% were non-viable (empty or dead) (Caplow, 2005; Table 1). In the 2003 test, 2% of seed germinated without requiring cold-moist stratification, 76% were viable but dormant, and 22% were non-viable (Caplow, 2005; Table 1).

Table 1. Summary of prior viability and germination testing for E. codium. For germination testing (Berry Botanical Garden and Rare Care), the most successful treatment from each test is listed.

Test	Length of Test	Seeds tested	Treatment	Germination (%)
Berry Botanical Garden (1999)	16 weeks	7	8 weeks 5°C (dark) → 20/10°C with 16 hrs dark/8 light	86% (6 seeds)
Ransom Seed Lab (2002)	3 weeks + 3 days	40	3 weeks 20°C → GA3 and 20°C for 3 days	5% germ. (70% viable)
Ransom Seed Lab (2003)	3 weeks + 3 days	50	3 weeks 20°C → GA3 and 20°C for 3 days	2% germ (78% viable)
Rare Care (2013)	72 weeks	8	8 weeks 24/14°C → 4 weeks 15/8°C → 12 weeks 5/2°C → 4 weeks 15/8°C (repeats for 72 weeks) (Treatment D)	88% (7 seeds)

The most recent germination test was conducted by Rare Care in 2013 on a 2011 seed vault accession of *E. codium* (Rare Plant Care and Conservation, 2013). A winter control (A), summer control (B), 12 week winter stratification (C), and 8 week summer stratification (D) were used. Thirty-eight seeds were divided across four treatments, with 10 seeds in treatments A, B, and C, and 8 seeds in treatment D. Following stratification periods for C and D, plates were cycled through seasons in a move-along experiment for a total of 72 weeks (Baskin & Baskin, 2014). Plates in Winter conditions were exposed to a diurnal light and temperature regime of 5/2°C and 10 hours light/14 hours dark, while Spring/Fall conditions were 15/8°C with 12 hours light/12 hours dark, and Summer conditions were 24/14°C with 14 hours light/10 hours dark (Table 4).

In the Rare Care test, germination was highest for the summer stratification treatment, followed by the winter control, resulting in germination percentages of 88% and 70%, respectively (Rare Plant Care and Conservation, 2013; Table 1). The two move-along treatments from the Rare Care test (C and D), were re-tested in this study (Figure 1).

Nursery propagation trials have also been conducted for the species, with the first in 2002 by Rain Shadow Nursery in Kittitas, Washington (Caplow, 2005). Seeds were stratified in cold-moist conditions for 60 days in later winter after collection in summer, and germination was “good,” with some seeds germinating upon being pulled from stratification (Caplow, 2005). Again, it was noted that a small number of seeds germinated without needing cold-moist stratification (Caplow, 2005). Significant propagation work has also been conducted by Jane Abel, a propagator who has worked with the species for 10 years.

Site History and Fire

Ongoing threats to *E. codium* include encroachment by invasive plants, trampling, low seed set and rates of seedling establishment, and most notably, wildfire (Fertig, 2018). Shortly following discovery of the species, a 1996 wildfire burned through Umtanum Ridge, affecting three of the five subpopulations. Post-fire monitoring indicated that adult individuals of *E. codium* are negatively affected by fire. Those that were charred exhibited no resprouting from the crown, and there was no vigorous regrowth as would be expected from a fire adapted species (Dunwiddie et al., 2000). A lack of seedlings in the year following fire was also noted. Fire-related mortality across the entire population following this event was estimated at 10-20%, although this was a conservative estimate and actual mortality was likely higher (Dunwiddie et al., 2000).

On July 2, 2017, an unknown source of ignition started the Silver Dollar Fire approximately 30 miles northwest of Richland, Washington (Newsome, 2017). A high density of brush and grasses after a cool, moist spring provided plenty of fuel which, combined with low humidity and strong winds, allowed for quick spread of the fire (Newsome, 2017). By the morning of July 3, 2017, the critical habitat area and *E. codium* population were within the fire perimeter (Newsome, 2017). Incident Management Teams worked over the course of four days to fully contain the perimeter, with one of the strategic management objectives being to minimize any firefighting impacts to sensitive species and habitat (Southeast Washington Interagency Incident Management Team, 2017).

An assessment of damage to the population of *E. codium* was conducted on July 18, 2017 by ecologists from the U.S. Fish and Wildlife Service, Washington State Natural Heritage Program - DNR, and the Department of Energy (Newsome, 2017). Inspection revealed significant damage to the population, with few areas left fully unburned (Newsome, 2017). Permanent monitoring plots were assessed and recorded as unburned, lightly burned, partially burned, or completely burned. Lightly burned plots showed no direct damage to *E. codium* individuals but exhibited burning of the surrounding vegetation (Newsome, 2017; See Appendix A). For partially burned plots, adjacent vegetation was burned and there was direct fire damage to *E. codium* individuals through scorching of the foliage and flowers (Newsome, 2017; See Appendix A). Completely burned plots showed severe, direct damage to individuals (Newsome, 2017).

It was concluded that roughly 42% of the monitoring plots were unburned, 25% were lightly burned, 25% were partially burned, and 8% were completely burned (Newsome, 2017). Of the entire population it was estimated that 63% of the population had been somehow affected by fire, while 37% remained unburned (Newsome, 2017). All of the completely burned, and most partially burned individuals, were not expected to survive following this event. Taking into account the unburned and lightly burned categories, roughly 2,000-3,000 individuals are expected to have survived (Fertig, 2018).

Research Objectives

Roughly one month following the Silver Dollar Fire, on August 9, 2017 Land Manager Heidi Newsome, along with volunteer Jane Abel, conducted seed collections from unburned, lightly burned, and partially burned areas throughout the permanent monitoring plots (Newsome, 2017). Five envelopes of seed were sent to The University of Washington Botanic Gardens (UWBG) Rare Care program on October 18, 2017, with the intention of having a portion tested, and the remainder of unburned seeds accessioned and added to the Miller Seed Vault.

The main goal of this project was to quantify differences in seed viability and germination between burned and unburned seed lots. Although by now it is understood that *E. codium* is not fire adapted, data on the effects of fire on seed could be useful for understanding post-fire seed germination and potential differences in recruitment across gradients of burn severity.

A secondary goal was to test a number of germination treatments to deepen our understanding of the germination behavior of *E. codium*. Work is ongoing to supplement the extant population of *E. codium*, and to establish potential additional populations in environmentally similar habitats near Umtanum Ridge. Newsome estimates that for the current population, 3,500 to 6,000 plants will be needed each year to replace the plants lost from the 2017 fire (Fertig, 2018). Detailed information on germination measures could be useful for maximizing the number of plants produced from limited amounts of seed.

In response to the above goals, the following research questions were addressed:

- Is *E. codium* seed viability and germination affected by fire exposure, and if so, what is the strength of this effect at different burn severities?
- What is the germination behavior of *E. codium* in response to three move-along treatments?
- Does an extended cold-moist stratification period of 16 weeks versus 12 weeks increase germination?

Effects of fire were quantified through statistical analysis following germination testing, and from there inferences regarding ecological significance could be made. Viability testing was conducted to provide measures of total viability that could be expected from the number of seeds produced within each seed lot. The germination response of *E. codium* was analyzed by visualizing the distribution of germination over time, and by comparing germination percentages across seed lots and treatments. A basic measure of seedling health was also taken and compared among seed lots.

MATERIALS AND METHODS

Over the course of 10 months, seed cleaning, viability and germination testing, and data analysis were conducted for the seed lots in this study. The Materials and Methods section below describes all processes used, beginning with an explanation of seed lots involved, seed cleaning techniques, and the methods used for viability and germination testing. An analysis section follows, and describes how the resulting data were understood and synthesized.

Seed Lots

Of the five envelopes (hereafter referred to as seed lots) sent to Rare Care for testing, three contained seed collected from individuals in three separate unburned plots (Table 2). A fourth and fifth envelope were collected from individuals in a lightly burned plot, and from a partially burned plot, respectively (Table 2). The lightly burned plot had no direct damage to *E. codium* but exhibited scorching of the surrounding vegetation, while the partially burned plot exhibited some direct fire damage to *E. codium* individuals (Figure 1). Seed lots were stored in the Miller Seed Vault at UW Botanic Gardens in 15°C and 25% relative humidity prior to the beginning of the experiment.

Table 2. Summary of seed lots included in the experiment. Summary of all seed lots along with the number of maternal plants that flower material was collected from.

Seed lot (Envelope)	E2	E3	E4	E1	E5
Status	Unburned	Unburned	Unburned	Lightly burned	Partially burned
Maternal Plants	16	12	7	12	8

Seed Cleaning

Seed cleaning began in July 2018. Raw material for each seed lot was weighed prior to the start of cleaning (Table 3). Following the cleaning process, cleaned seed for each seed lot was weighed and all seeds were counted (Table 3).

Raw material from each seed lot consisted of mostly dried flower material, along with some stem segments. USA Standard Testing Sieves were used to filter plant material. A 2-millimeter sieve separated larger debris such as stems. Flower material was gently rubbed between hands in order to remove the perianth which tightly enclosed each seed. This material was then sifted through an 850 micrometer sieve and caught in a 425 micrometer sieve. Seeds were sorted from this small material by hand using tweezers in order to maximize the number recovered. Flower material was sifted and sorted through twice for each seed lot to ensure that the number of seeds recovered from cleaning provided an accurate example of the production for each seed lot. All seeds recovered during the cleaning process were kept for each seed lot, with no attempt to remove any that appeared empty or non-viable. This was intended to provide an estimate of the germination that could occur within the population of *E. codium.*

Although not a scalable process, hand-rubbing proved to be the best method of cleaning, as seeds were small, fragile, and potentially brittle due to fire exposure. Furthermore, the end of the achene containing the radicle was slender and easily damaged, warranting care when handling. Meyer (2008) notes that while achenes of some *Eriogonum* species separate from the perianth at dispersal, achenes of other species are tightly enclosed within this structure. It is likely that *E. codium* falls within this second group of species for which the achene and perianth together is the dispersal unit. Seeds of *Eriogonum* exhibit anatropous orientation of the ovule, with the radicle pointing upward, which allows for germination if this perianth remains attached (Meyer, 2008). However, because seeds would be in moist and occasionally warm conditions throughout the 7 months of germination testing, any flower material was removed during cleaning with the hope of preempting fungal growth during the experiment.

Table 3. Weights and counts for each seed lot. Uncleaned weight, cleaned seed weight, and cleaned seed count are listed.

Seed lot	Unburned (E2)	Unburned (E3)	Unburned (E4)	Lightly burned (E1)	Partially burned (E5)
Raw material weight	15.784g	20.898g	8.597g	15.374g	7.496g
Cleaned seed weight	.487g	.203g	.295g	.434g	.133g
Cleaned seed count	1,397	552	848	1,036	364

Viability Testing

Initial Seed Lot Viability Testing

Although the The Association of Official Seed Analysts recommends using a minimum of 200 seeds per seed lot as a stand-alone test of viability (Peters, 2000), the limitations on the number of seed available made viability testing with this number of seeds impossible. Three hundred seeds from each seed lot were allocated to germination testing, and two-thirds of each unburned seed lot would be accessioned for storage in the seed vault. This left roughly 100 seeds from each seed lot for initial viability testing, with the exception of the partially burned seed lot (E5), for which 64 seeds were available.

Seeds were pulled randomly from each seed lot, wrapped in mesh, labeled with the seed lot number, and imbibed in regular, aerated, room temperature water using a bubbler for 18-24 hours. Four replicate Petri dishes (plates) were prepared for each seed lot, with 25 seeds per plate, for a total of 20 plates across the 5 seed lots. Each plate was labeled with seed lot number, numbered 1-4, and a scoring grid was attached to the bottom of each to allow for easier evaluation. A sheet of moistened filter paper (Whatman No. 2, qualitative, 9cm) was used as the substrate for each plate.

All seeds were cut under a dissecting microscope according to the second method described in the Tetrazolium Testing Handbook guidelines for the Polygonaceae family (Peters, 2000). A cut was made across the distal end of the cotyledons using a scalpel. After the distal end was removed, seeds were placed with the cut end facing down on the filter paper. A 1% tetrazolium chloride solution was mixed and a few drops applied to each plate, enough to fully saturate the filter paper. Each plate was wrapped with plastic wrap and tied with a rubber band to prevent evaporation of the tetrazolium solution during incubation. Plates were placed in an incubation chamber at 32-34°C and left for 14-16 hours. Start and end time, along with start and end temperature, were recorded for each test.

Following incubation, plates were pulled, any excess tetrazolium solution blotted from the filter paper, and seeds carefully flushed with deionized water to maintain moisture. Plates were scored under a dissecting microscope by assessing the color of each seed within a block of the scoring grid and recording Y or N on the corresponding tetrazolium testing data sheet (See Appendix B).

During the scoring process, each embryo was removed from the surrounding seed tissue with tweezers in order to fully evaluate both the radicle and cotyledons. Embryos that stained completely red were counted as viable, while seeds exhibiting partially or fully white, yellow, or green embryos were scored as non-viable (See Appendix C). If seeds had a red stained radicle but unstained cotyledons, or vice versa, they were marked as non-viable in accordance with tetrazolium testing guidelines for the genus. Endosperm health was also assessed and considered throughout the evaluation process. For example, seeds were assigned to the non-viable category if they exhibited ambiguous staining and had an underdeveloped or non-existent endosperm.

Within each seed lot test, a few seeds were almost entirely red with the exception of a small amount of white at the very tip of the radicle. For others, staining was ambiguous, often with a red radicle and light pink cotyledons, or vice versa. For this small group, it seemed appropriate to mark a third category of 50/50 when recording test results, allowing for a small range of viability. During initial germination testing, a total of 11 out of the 464 seeds tested across all seed lots were assigned to the 50/50 category. During analysis, it was decided that these seeds would be assigned to the non-viable category. Therefore this measure provides a conservative estimate of viability.

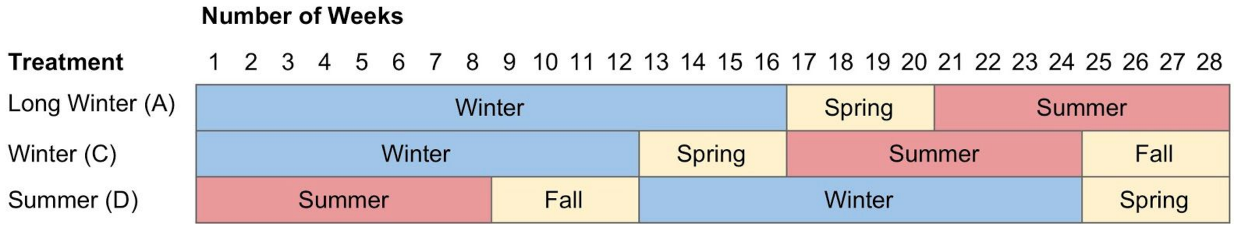
Post-Germination Viability Testing

Ungerminated seeds left at the end of each germination test were also tested for viability using tetrazolium chloride. At the end of week 28, all plates were pulled and tested using the method described above. Results were recorded using separate tetrazolium testing data sheets (See Appendix B). Seeds were kept on the original replicate plates used for germination testing so that results could be recorded and analyzed across seed lots but also for treatments within each seed lot. A 50/50 category was again designated during testing, with a total of 8 seed across all plates allocated to this category. These seeds were again assigned to the non-viable category during analysis.

Germination Tests

Germination testing was initiated on October 12 and 13, 2018. Three move-along treatments were tested across all five seed lots (Baskin & Baskin, 2014). Each treatment had 4 replicate Petri dishes (plates) of 25 seeds each per seed lot. 300 seeds from each seed lot were pulled, pretreated with a 10% bleach solution for 10 minutes, rinsed with deionized water and randomly allocated to plates assigned to the three treatments. Plates were lined with 2 sheets of filter paper (Whatman No. 2, qualitative, 9cm), moistened with deionized water, covered, and placed in growth chambers in Merrill Hall at the Center for Urban Horticulture. Growth chambers simulated Winter, Spring/Fall, and Summer conditions (Table 4).

The Winter (C) and Summer (D) treatments were a repeat of previous Rare Care treatments which were tested in 2013 and yielded two of the highest germination percentages. A third test Long Winter (A) was conducted to see if a longer winter stratification period followed by spring would increase germination. All treatments ran for a total of 28 weeks, cycling through Winter, Spring, Summer, and Fall conditions (Figure 1).



Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Long Winter (A)	Winter																Spring				Summer							
Winter (C)	Winter												Spring				Summer								Fall			
Summer (D)	Summer								Fall				Winter												Spring			

Figure 1. Duration and cycling of seasons for each germination treatment.

Table 4. Conditions of growth chambers for germination testing. Day and night temperatures and length of light/dark conditions are listed with their associated seasons.

Growth Chamber	Diurnal temperature (°C)	Day/Night Length
Winter	5/2°C	10/14 hours
Spring/Fall	15/8°C	12/12 hours
Summer	24/14°C	14/10 hours

Plate Evaluation

Plates were checked twice a week, on Tuesdays and Fridays, and any new germination or removal activity recorded. Data were recorded on spreadsheets adapted from Rare Care germination testing sheets (See Appendix E). Four columns were used for each seed lot: germination, cumulative germination, removed, cumulative removed. This allowed for detailed recording of the point in time that each seed on a plate germinated or was removed, along with tracking cumulative germination and removal throughout the duration of each test. A 0 was used when no germination or removal activity happened for that week, while recording 1 indicated that one seed germinated or was removed. For all seeds, germination was defined as the point at which both radicle and cotyledons were visible.

Along with this data, reasoning for removal was also recorded in the removed column. If a seed was removed due to becoming soft/mushy and molding, 1-ng ("not-germinated") was recorded. A seed that germinated and then died on the plate was recorded as 1-gx. For some seeds, a radicle began to emerge and then stopped before continuing onto full germination. If these partial germinants died before continuing on to full germination, 1-rgx was recorded.

Fungal growth was an issue on some of the plates. The Summer treatment which began with 8 weeks of summer, exhibited recurring growth on a number of plates. The other two treatments did not experience this issue during their winter stratification period and only occasionally showed fungal growth when moved into spring and summer. Seeds that exhibited fungal growth were tested with a tweezers for firmness during bi-weekly observations. Those that were soft and exhibited recurring fungal growth were removed and recorded as -ng, assuming that they were non-viable.

While seed removal data were not used for analysis, recording the timing and reasoning for removal provided context for how the three treatments may have influenced seed viability throughout the duration of testing. Overall, a higher number of -ng seeds were removed from the Summer treatment relative to Winter and Long

Winter. For Summer, 104 seeds were removed during the 28 weeks of testing, and for Winter and Long Winter 55 and 24 seeds were removed, respectively (See Appendix F).

Seedling Propagation and Health Evaluation

While some seedlings died in the days following germination, the remainder of seedlings were removed from plates, typically within a week of germination, and transplanted. Upon removal from a plate, surviving seedlings were qualitatively evaluated for healthy or abnormal characteristics and all data recorded in a spreadsheet (See Appendix G). The cotyledons and radicle of each were visually examined and defined as either normal or abnormal. Radicles that were stunted or did not elongate normally during germination were labelled as abnormal. As almost no cotyledons appeared abnormal during examination, data collection and analysis focused primarily on radicle health.

During this evaluation, each seedling was assigned an identification code based on the seed lot number, treatment used, and number of seed potted up (ie. Seed lot 2, plate A1, third seed removed and potted up would be 2A1-3). Seedling mortality was also recorded for all plants that were transplanted throughout the duration of the experiment. Recording individuals in this manner allowed for detailed tracking of each plant through the duration of the experiment to provide information on how seedling health and mortality might differ among seed lots.

Seedlings were potted into rose pots or 164ml cones (Ray Leach cone-tainers) with a mixture of 7 parts Sunshine #4 and 3 parts horticultural sand. Each individual was labelled with the species name, date potted, and its individual identification code. After potting up, seedlings were returned to their growth chamber for continued cycling through seasons, and ultimately to the Douglas Research Conservatory Greenhouse at the Center for Urban Horticulture, which stays at 68-72°F and an average of 60% relative humidity. These seedlings were grown with the intention of transporting them back to Hanford Reach for outplanting or use as seed increase plants.

Analysis

Fire Response

Results of the germination testing were analyzed and visualized in R (version 3.5.1) using RStudio (RStudio Team, 2015). A two way analysis of variance (ANOVA) was used to understand the effect of fire on seed germination across the three germination treatments (Long Winter, Winter, and Summer) and the five seed lots, with seed lots and treatments as independent variables, and cumulative germination at the end of the experiment as the response variable. A 95% confidence interval was used for all testing. Significant terms from the ANOVA were followed by pairwise comparisons tests (compact letter display) to identify differences in mean cumulative germination among seed lots. Due to the small sample size, multiple comparison corrections were not made.

A measure of seedling health was intended to provide data on how fire might affect seedling health and potential for survival following germination. Data on radicle health were visualized using the mean number of germinants with normal and abnormal radicles per seed lot.

Viability Testing

Viability testing provides a measure of the potential germination for a given seed lot before germination testing. It also provides a measure of any continued seed dormancy following germination testing, and therefore, an idea of how successful a germination treatment has been in breaking dormancy. For the purposes of analysis, two measures of viability were defined. **Initial viability** was used to understand the viability of seed lots independently of any treatments used. **Total viable seed** provided a measure of total viability for each seed lot following germination testing. These measures were defined as follows:

Initial viability (VI%):
VI% = number of viable seeds per plate / number of seeds TZ tested per plate

Total viable seed (VT%):
VT% = (number of germinated seeds per plate + number of viable seeds per plate from post-germination TZ test) / total number of seeds per plate.

Germination Response

Analysis was conducted on the germination responses of the three move-along treatments (Winter, Long Winter, and Summer) in order to visualize long-term patterns and to provide a measure of how successful each treatment was in facilitating germination. As with fire response, pairwise comparisons were used to understand differences in mean cumulative germination among the three treatments.

Germination distribution over time was explored in order to understand the response of *E. codium* germination to seasonal changes. For the three move-along treatments, cumulative germination over time for each seed lot within treatments, along with mean cumulative germination across all seed lots, were visualized to understand how the length and timing of stratification regimes influenced germination.

Two measures of germination percentage were used in this study to provide a comprehensive view of *E. codium* germination. A third measure provided an understanding of any continued dormancy following testing. Because measures of germination percentage were intended to provide a view of the germination that might be happening within the population or could be expected during propagation, only the three Unburned seed lots (E2, E3, and E4) were included in these analyses.

Germination percentage included all seeds tested (non-viable and viable), and was intended to provide a view of the amount of germination that might be occurring within areas of the population. Germination percentage was visualized with Total viable seed (VT%) for each treatment.

Because the viability of seed lots was low relative to the number of seeds tested, **Viability-adjusted germination** was used to provide a better understanding of germination percentages that can be expected from viable seeds (Tieu, Dixon, Meney, Sivasithamparam, & Barrett, 2001). This was intended to provide a more useful measure of germination for propagation efforts, and also to facilitate comparisons with prior tests of *E. codium*.

Finally, **Dormant seed** provided an understanding of any continued dormancy following germination testing. It was understood as the percentage of seed that remained viable and ungerminated out of the number of total viable seed. All measures are defined as follows:

Germination percentage (G%):
G% = mean total number of seeds germinated per plate / total number of seeds tested per plate.

Viability-adjusted germination (VG%):
VG% = total number of seeds germinated per plate / Total viable seed (VT%) per plate.

Dormant seed (D%):
D% = number of viable seeds per plate from post-germination TZ tests / number of total viable seed (VT%) per plate.

RESULTS

Response to Fire

There was a highly significant main effect for seed lot, indicating that mean cumulative germination of seed lots were significantly different from one another (Table 5). Accordingly, the null hypothesis that there is no significant difference in mean cumulative germination between unburned and burned seed lots, can be rejected. The main effect of treatment was also significant (Table 5). However, the interaction effect between seed lot and treatment was non-significant (Table 5).

A pairwise comparisons test indicated that mean cumulative germination for both the partially burned (E5) and lightly burned (E1) seed lots was significantly lower than cumulative germination for any of the unburned seed lots (E2, E3, and E4) (Figure 2). However, the mean cumulative germination of the partially burned (E5) and lightly burned (E1) seed lots were not significantly different from one another (Figure 2).

The pairwise comparisons test also indicated significant differences among the three unburned lots (E2, E3, and E4). The means of E3 and E4 were significantly different from one another, but E2 was not significantly different from either E3 or E4 (Figure 2).

Table 5. ANOVA summary table for cumulative germination. * significant at *p* < .05, * significant at *p* < .001**

	Sum of Squares	*df*	Mean Sq.	*F*	*p*
Seed Lot	1040.3	4	260.07	14.638	0.0009 ***
Treatment	210.5	2	105.27	5.925	0.02 *
Seed Lot x Treatment	35.53	8	4.44	1.048	0.4
Residuals	190.75	45	4.24		

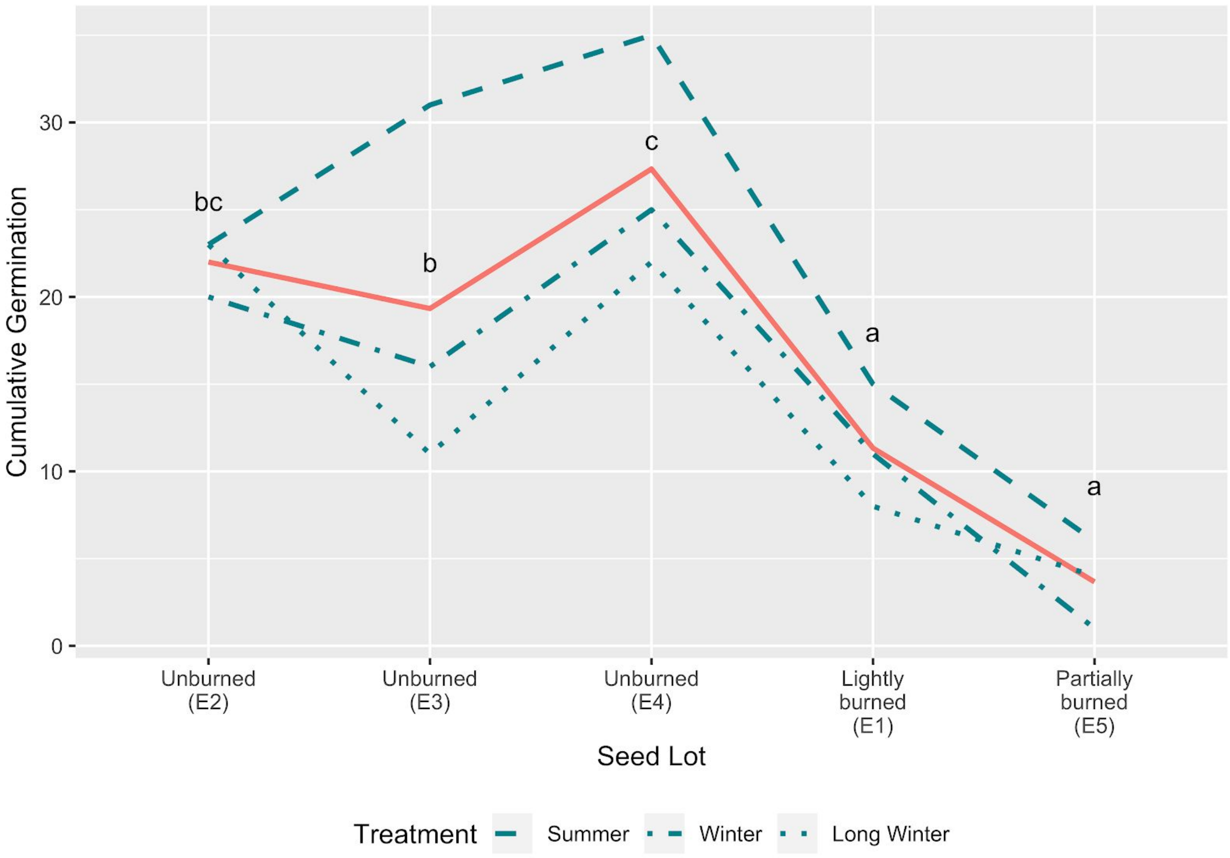


Figure 2. Cumulative germination counts for seed lots across treatments. The coral line indicates mean cumulative germination for seed lots across treatments. Letters above seed lots denote the results of a pairwise comparison test following ANOVA. Seed lots that do not share a letter have a mean difference that is statistically significant, i.e. "a" is most dissimilar to "c" and most similar to "b".

Results of Viability Testing

Initial viability (VI%) testing indicated differences among all seed lots. The burned seed lots showed large differences when compared to the unburned lots. Viability of the partially burned seed lot (E5) was much lower than for any other seed lot, though the viability of the lightly burned seed lot (E1) was between that of unburned seed lot E3 and the two other unburned seed lots (E2 and E4) (Table 6). There was high variation between the unburned seed lots; initial viability exhibited a three-fold range (Table 6).

A comparison of initial (VI%) and post-germination (VT%) testing results revealed differences between the two measures. Initial testing results were generally higher than post-germination results, with the exception of unburned E3 (Table 6). However, overall patterns of viability among seed lots were largely consistent between initial and post-germination testing. Comparisons of the three unburned seed lots between initial and post-germination results indicated less variation in viability. Unburned seed lots E2 and E3 showed comparable percentages at the end of testing, while the viability of E3 was half that of E2 following initial testing (Table 6).

Table 6. Results of initial seed lot viability (VI%) testing with post-germination viability testing (Total viable seed: VT%).

Seed lot	Initial viability (VI%)	Total viable seed (VT%)
Unburned (E2)	41%	28.3%
Unburned (E3)	20%	25.6%
Unburned (E4)	61%	41.6%
Lightly burned (E1)	25%	15.3%
Partially burned (E5)	5%	4%

Results of Germination Testing

Cumulative Germination

Cumulative germination in the Summer treatment was significantly higher than either of the winter-start treatments (Table 7). The hypothesis that a longer cold-stratification period would increase germination can be rejected, as the difference in mean cumulative germination between the Winter and Long Winter treatments was not significant (Table 7).

Table 7. Summary of pairwise comparisons results for germination treatments.

Treatment	*Mean (%)*	*Standard Deviation*	group
Long Winter	13.6	7.6	a
Winter	14.6	8.2	a
Summer	22	10.5	b

Germination Distribution Over Time

Both the Winter and Long Winter treatments exhibited a germination response during their winter (cold-moist stratification) period, reaching an average of 6 and 7 seeds germinated, respectively (Figure 3). Conversely, there was almost no germination activity during the cold-moist stratification period for the Summer treatment. Similar to both Winter and Long Winter, the Summer treatment also exhibits a germination response during the first 8-week summer (warm-moist stratification period), with an average of 10 seeds germinating across all seed lots (Figure 3).

Although the spring and fall periods of the Summer treatment experience the same diurnal light and temperature regime, they exhibited different germination responses. Germination activity slowed for most seed lots following movement from summer to fall conditions (Figure 3). Upon movement from winter to spring conditions in week 24, there was a strong germination response, with an average of 8 seeds germinating (Figure 3).

There was almost no difference in germination response following the movement of Winter and Long Winter from cold-moist stratification into spring conditions. When comparing mean cumulative germination between the two treatments following this seasonal change, both reach an average of 12-13 germinated seeds (Figure 3).

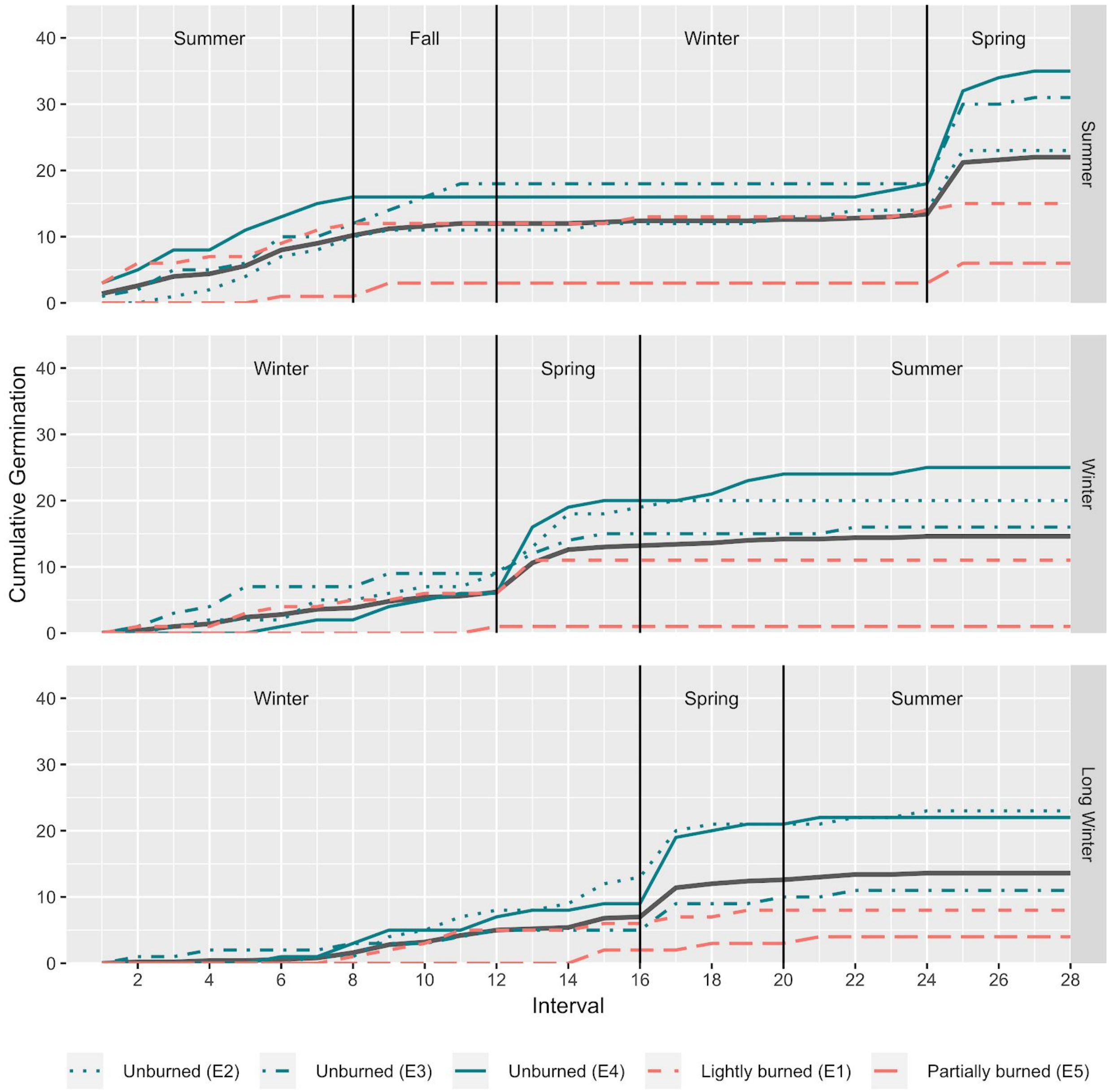


Figure 3. Distribution of cumulative germination over time. Cumulative germination counts for the full duration of testing are shown for each treatment by seed lot, along with mean cumulative germination across seed lots for each treatment. Coral lines indicate burned seed lots, blue lines indicate those that are unburned, and the solid black line indicates mean germination for all seed lots. Vertical lines indicate points at which plates were moved to the next season.

Germination Percentage (Unburned Seed Lots)

When considering the three unburned seed lots, the Summer treatment provided the highest final germination percentage, reaching 29.6% (Table 8). Germination percentages for Winter and Long Winter were comparable to one another, at 20.3% and 18.6%, respectively (Table 8).

Dormancy was maintained for a portion of seeds from all unburned seed lots within each treatment (Figure 4). The Summer treatment exhibited the lowest percentage of continued dormancy following germination testing, at 22.6%, while Winter and Long Winter had higher continued dormancy at 28.2% and 35.1%, respectively (Table 8).

Table 8. Final germination, adjusted germination, and dormancy percentages for all unburned seed lots (E2, E3, and E4). Both Viability-adjusted germination and dormancy percentages are calculated from Total viable seed (VT%).

	Long Winter	Winter	Summer
Germination (G%)	18.6%	20.3%	29.6%
Total viable seed (VT%)	26%	30.3%	38.3%
Viability-adjusted germination (VG%)	64.9%	71.8%	77.4%
Dormant (D%)	35.1%	28.2%	22.6%

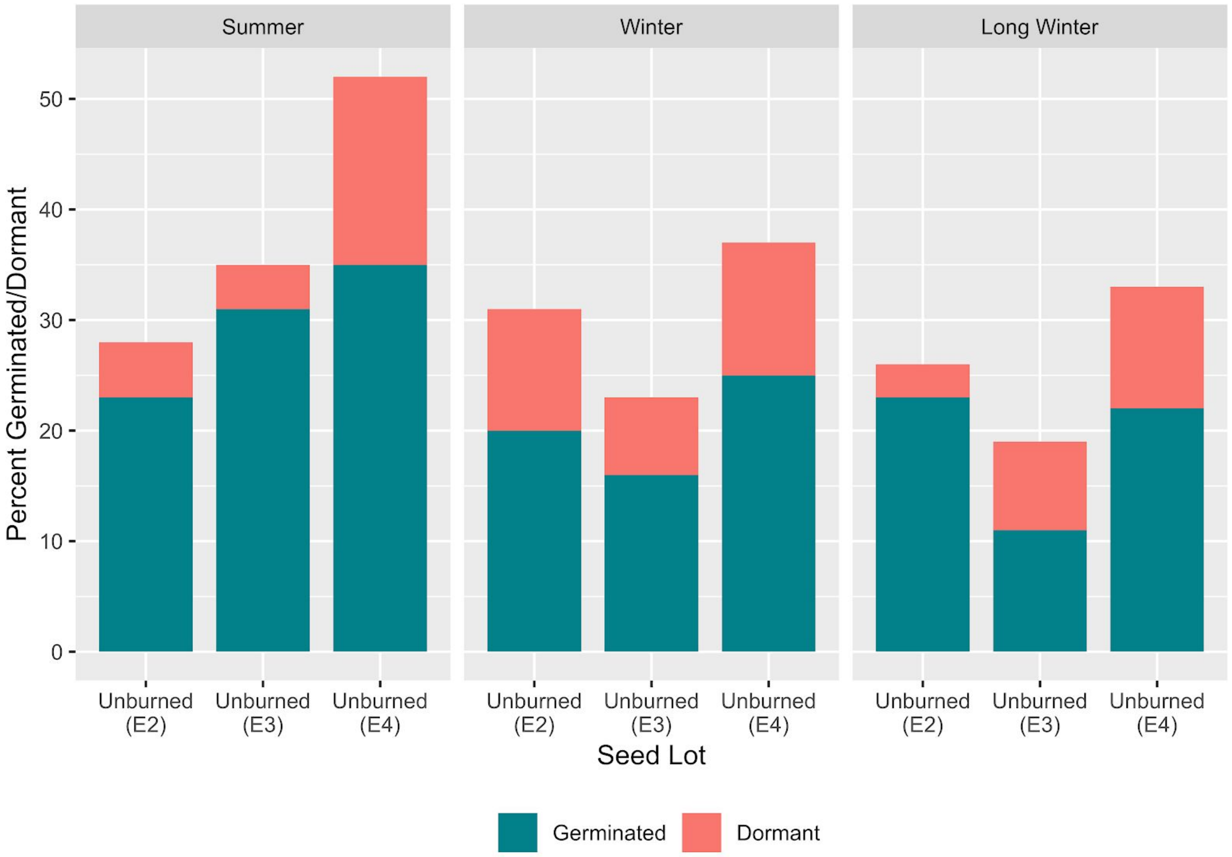


Figure 4. Germination percentage and dormant seeds for the three unburned seed lots. Final germination percentages for each treatment are compared with percent dormant seeds from post-germination TZ testing for seed lots E2, E3, and E4.

Seedling Health Evaluation

An evaluation of seedling health across all seed lots found that there were higher numbers of abnormal seeds for both lightly burned (E1) and partially burned (E5) seed lots than for the three unburned seed lots (Figure 5). The lightly burned and partially burned lots showed very little difference from one another in terms of abnormality

(Figure 5). However, it should be noted that due to differences in germination between seed lots, sample sizes for this analysis were not equal.

In terms of seedling mortality following transplanting, out of 166 seeds that germinated and were transplanted, 95 survived and 71 died; roughly a 57% survival rate.

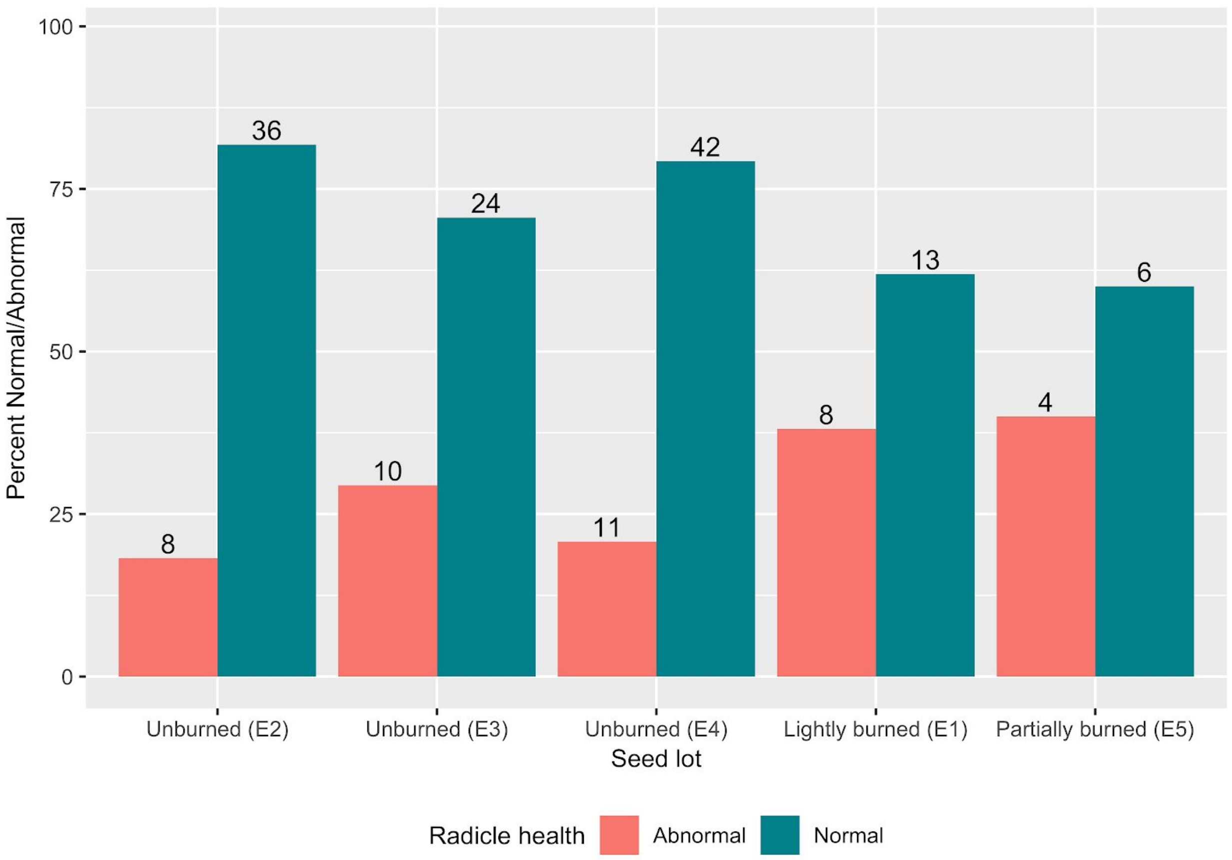


Figure 5. Seedling health by seed lot. Percentage of healthy and abnormal radicles per seed lot are shown. Numbers above bars indicate the number of seeds in each category per seed lot.

DISCUSSION

Fire Response

The response of *E. codium* seeds to testing indicated a significant decrease in both viability and germination related to fire exposure. This result was expected, considering the known negative response of adult individuals to fire and the records of lower seedling recruitment following past fire events, as well as the documented responses of seeds of other *Eriogonum* to fire (Carrington, 2010; Keeley, 1987). The magnitude of this effect is still surprising, however, with viability and cumulative germination across treatments from the partially burned seed lot (E5) at only 4% and an average of 4 seeds, respectively (Figure 2; Table 6).

Seeds of other *Eriogonum* species existing in fire-prone habitats exhibit similar responses. A study conducted by Keeley (1987) found that when seeds of *E. fasciculatum,* found in coastal sage scrub habitats, were exposed to 120°C for 5 minutes there was a significant decrease in germination. Similarly, seeds of *E. floridanum* in Florida sand pine scrub systems were completely killed following exposure to 100°C for 5 minutes (Carrington, 2010). Other studies have indicated a lack of fire adaptivity for mature *Eriogonum*, implying that seeds could be similarly affected. Multiple species of *Eriogonum* within sagebrush systems have been found to be moderately to severely damaged following wildfire, including species which also occur at Hanford Reach such as *E. douglasii* and *E. heracleoides* (Agee, 1994; Evans et al., 2003; Sugihara et al., 2006). Furthermore, although some species, such as *E. floridanum*, exhibit fire adaptations such as resprouting and prolific flowering following fire, seeds still showed a significant decrease in germination when exposed to high temperatures (Carrington, 2010).

Patterns of fire behavior in shrub-steppe systems are heterogeneous, typically with patches of low to moderate severity burning (Agee, 1994). This spatial dynamic provides potential areas of refuge in which plants may be unaffected or experience only low intensity burning within a population. Similarly, seeds can survive in patches that experience lower fire intensity (Carrington, 2010). Although mean cumulative germination was not significantly different between the lightly burned (E1) and partially burned (E5) seed lots (Figure 3), germination of the lightly burned seed lot was not as low as the partially burned lot (Figure 3). Following the Silver Dollar fire, 25% of the population was estimated to be lightly burned (Newsome, 2017). If viability and germination within this portion of the population are consistent with the lightly burned seed lot in this study, only the remaining 25% that was partially burned may have experienced very high losses of viability. However, losses of seed viability may be of minimal concern compared to even minor losses of adult plants due to fire, considering that recruitment rates are generally extremely low.

It has been suggested that because *E. codium* is a long-lived plant, even a single year of good germination and seedling recruitment every few decades could be enough to balance out mortality rates (Dunwiddie et al., 2000; Walter Fertig, personal communication). However, it is possible that a year with optimal climate conditions for seedling survival could coincide with a year of conditions advantageous for fire. A cool, moist spring, which would seem to favor seedling growth, was also noted as the reason for increased continuity of grasses which provided higher fuel loads for the 2017 Silver Dollar fire (Newsome, 2017).

The evaluation of seedling health also showed patterns that could be related to burn severity. Both the partially burned and lightly burned seed lots exhibited a higher incidence of radicle abnormality compared all three unburned seed lots. This measure indicated similar levels of abnormality regardless of whether seeds belonged to the lightly or partially burned lot. While radicle damage could be due directly to fire exposure, it is more likely due to increased risk of damage to seeds following fire. For this study specifically, radicles may have been injured during the seed cleaning process, as the end of the seed containing the radicle is fragile even in unburned seeds.

Viability

Beyond differences in response to fire among seed lots, viability testing revealed further patterns. Although the relative relationships of viability among the five seed lots generally remained consistent, there were large differences between initial and post-germination testing results. This discrepancy could be partially attributed to the duration of testing. Because seeds were on moist filter paper, and sometimes in warm conditions throughout the 28 weeks of testing, there was likely some mortality due to pathogens during that time period.

Temperature and moisture content are the two most important factors in determining the longevity of a seed lot, and seeds generally lose viability more quickly at higher temperatures and moisture contents (Bewley, Bradford, Hilhorst, & Nonogaki, 2013). During germination testing, twice the number of “-ng” seeds were removed from the Summer treatment than from either of the winter-start treatments, and the majority of these removals occurred during the first 12 weeks of testing, coinciding with periods of simulated summer and fall temperatures. Similarly, in the 2013 Rare Care test, the summer control treatment exhibited higher losses in viability by the end of testing compared to the winter control and winter-start move-along treatments (Rare Plant Care and Conservation, 2013). While the Summer treatment may have provided the highest germination percentage, it was likely also the treatment that incurred the highest losses in terms of viability.

Testing also revealed considerable differences in viability among the three unburned seed lots (E2, E3, and E4) of *E. codium*, namely the three-fold range in initial viability percentages (Table 6). While these differences are notable, they cannot necessarily be ascribed to natural variation within the population. Collection of seed lots from different numbers of maternal individuals may have been a factor influencing this variation. While Unburned E4 had the highest initial viability percentage, it was also collected from nearly half the number of maternal individuals as Unburned E3 (Table 2). Furthermore, it is possible that individuals categorized as unburned may have experienced impacts from fire, such as exposure to high temperatures or effects from smoke, despite a visible lack of scorching.

Overall, viability was low relative to the number of seeds recovered for the three unburned seed lots. While the primary reasoning for this is likely variable impacts due to fire exposure, the tetrazolium testing process may have impacted these results. While tetrazolium testing provides a useful method for understanding the viability of a seed lot, some limitations exist. Because the method was developed for agricultural seeds, which are bred to lack deep dormany, there may be a lack of staining due to low respiration rates for native seeds that can exhibit deeper dormancy states (Baskin & Baskin, 2014; Peters, 2000). It is possible that some of the seed lots in this study contained a portion which may have been viable, but deeply dormant, and therefore evaluated incorrectly as non-viable.

Germination Response

Non-dormancy

The seed lots in this study contained both seeds that were physiologically dormant and responded to cold-moist stratification, and seeds that were non-dormant and began germinating at the start of testing. Across all seed lots within the Summer treatment, an average of 10 seeds germinated (10.2%) in warm-moist conditions during the first 8 weeks of testing, indicating a lack of dormancy for this group of seeds (Figure 4). The germination response during the first 12-16 weeks of both the Winter and Long

Winter treatments was likely also a result of non-dormant seeds being capable of germination under low temperatures. The lack of germination activity during the cold-moist period of the Summer treatment indicates that the impulse of non-dormant seeds to germinate was satisfied prior to this chilling period.

It is likely that after-ripening plays a role in the germination ecology of *E. codium*, and facilitates non-dormancy, as seen in a portion of each seed lot in this study. The process of after-ripening is promoted by warm, dry conditions, and can occur naturally in a population or during storage (Baskin & Baskin, 2014). During the months when seeds are maturing and dispersing (roughly July through September) temperatures are warm (25-19°C) and humidity is low (32.7-41.9%) (Table 9). Considering the conditions, this process may occur naturally within the population.

Table 9. Climate data from Hanford Meteorological Station by averaged by month from 1946 - 2018. The station elevation is 222 meters, and the Umtanum Ridge elevation is roughly 300 meters (United States Department of Energy, 2018).

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Precip. (in.)	.94	.63	.52	.47	.55	.54	.19	.23	.30	.57	.85	1.01
Temp. (°C)	-.38	3.2	7.5	11.6	16.6	20.8	25	24.1	19	11.7	4.5	0
Humidity (%)	78.6	70.5	56.5	47.3	42.9	39.2	32.7	35.2	41.9	56.7	73.8	80.5

After-ripening also occurs in other species of *Eriogonum*, which have been shown to require roughly 30-45 days following dispersal in order to achieve higher levels of germination (Stevens et al., 1996). However, there have been no known studies conducted on the germination response of freshly collected versus stored seeds of *Eriogonum*. The shortest time between collection and testing for *E. codium* specifically was the Ransom Seed Lab test from 2002, begun at 3 months post-collection, and in which 5% of seeds were non-dormant at the start of the test (Caplow, 2005). However, it is not out of the question for *Eriogonum* to experience fall germination that would be encouraged by both a period of after-ripening and increased moisture availability later in the growing season. Meyer and Paulsen (2000) note that multiple species of *Eriogonum* exhibit patterns which likely place their germination, or a portion thereof, during the fall after summer seed production.

Length of Cold Stratification

One of the questions explored in this study was if an extended cold-moist stratification period would increase germination. However, a comparison of germination response between the Winter and Long Winter treatments does not support this, as both reached the same number of germination events following their respective stratification periods and did not exhibit a significantly different cumulative germination at the end of testing (Table 7).

The germination response following 12 weeks of cold-moist stratification in the Winter and Summer treatments indicates a relatively short cold-moist stratification requirement for the species when considering the climatic conditions at Hanford Reach. The area where the population is located averages temperatures roughly at or below conditions simulated by the winter growth chamber for 4 months out of the year (Table 9). However, this remains consistent with findings by Meyer and Paulsen (2000) which indicate that 0-8 weeks of cold-moist stratification are needed for lower elevation *Eriogonum*, with species generally having comparable or shorter cold-moist stratification requirements compared to their habitat.

Meyer and Paulsen (2000) note that multiple species in their study showed germination patterns that likely placed the timing of germination during late winter or early spring in wild populations. *Eriogonum niveum*, a species which also exists at Hanford Reach, has been observed to germinate under snow, indicating that seeds of this species and potentially other *Eriogonum* may be adapted to germinate under very low temperatures (Delaney, 1986; Evans et al., 2003). In this study, seeds from the Summer treatment were observed to have radicle emergence at roughly 6-8 weeks into their cold-moist stratification period, and to not continue on to full germination (cotyledon appearance and radicle elongation) until moving into spring temperatures. This activity indicates that an even shorter period of cold-moist stratification, less than the 12 weeks tested in this study, would likely be acceptable for a comparable germination response. The high germination of the 8 week cold-moist stratification treatment used in the Berry Botanical Garden test appears to verify this (Ed Guerrant, personal communication).

Continued Dormancy

Continued dormancy in some seeds of *E. codium* was another notable response in this study. Although the majority of seed exhibited either non-dormancy or non-deep physiological dormancy and germinated following cold-stratification, between 22.6% and

35.1% of seeds from the three treatments remained viable and dormant following 12-16 weeks of cold-moist stratification (Table 8). This response suggests that the seed lots of *E. codium* in this study contained a portion of seed with an extended physiological dormancy that would be relieved by subsequent periods of cold-moist stratification. While many annual and perennial species of cold-desert and semi-desert habitats have mechanisms that prevent a portion of seed from germinating until the following year, this response has not yet been found in any species of *Eriogonum* (Baskin & Baskin, 2014; Meyer & Paulsen, 2000).

Although the time limitations on this study did not allow for the testing of a second period of cold-moist stratification, results of prior testing for *E. codium* indicate both continued dormancy following an initial cold-moist stratification period, as well as germination following a second cold-moist treatment. It is well-documented that seeds with non-deep physiological dormancy can cycle through non-dormancy and dormancy states multiple times before finally germinating (Baskin & Baskin, 2014). In the 2013 Rare Care test, there was another germination response (2 out of 8 seeds) following a second cold-stratification cycle (Rare Plant Care and Conservation, 2013). Jane Abel also confirmed that in 2018, containers sown with *E. codium* seed were left out for a second winter period and there was a second, although less dramatic, germination response the following spring (Jane Abel, personal communication).

However, it is unlikely that this continued dormancy reflects a pattern that would naturally occur for seeds in the population. Extended periods of dry storage, as was the case with this study, have the potential to induce deeper dormancy in seeds, or otherwise alter germination responses, and could result in a similar reaction of maintained dormancy (Baskin & Baskin, 2014). Two years of storage preceded the 2013 Rare Care test, which found continued dormancy for a small portion of seeds even after 72 weeks and two cycles of cold-moist stratification (Rare Plant Care and Conservation, 2013). A similar response was found in the Berry Botanical Garden test conducted on seeds stored for a similar length of time (Ed Guerrant, personal communication).

However, this behavior was not the case in seeds stored for only a few months. Of two Ransom Seed Lab tests on a single accession, one was conducted shortly following seed collection in 2002, and another after one year of cold dry storage in 2003. In the 2002 test, no seeds maintained dormancy following warm-moist stratification and treatment using gibberellic acid, while in the 2003 test, 15.5% of seeds remained dormant following the same treatment (Caplow, 2005). These results point to deeper dormancy as a function of storage length rather than the behavior of seeds in the population.

Continued dormancy could also suggest the formation of a soil seed bank within the *E. codium* population which could extend germination beyond the fall or spring following seed set. Although seed dormancy is not a necessary precursor to the formation of a soil seed bank, another common factor limiting germination in this case would be light, which was provided during this study (Baskin & Baskin, 2014). However, it has been suggested that species of *Eriogonum* do not form persistent seed banks, as many have been shown to germinate without requiring stratification, and are almost fully relieved of dormancy following a single cold-moist stratification period (Meyer and Paulsen, 2000).

Although soil seed bank formation may be possible for *E. codium* specifically, a seed bank study conducted by Florence Caplow in 2003 indicates otherwise. Results of this study showed a steep loss in viability for *E. codium* seeds, from 70-78% viable to 5-7% viable following 1 year of burial at roughly 2 cm (Caplow, 2005). Based on these results, it is likely that any seed bank formed by *E. codium* would be transient (less than 1 year) to short-term persistent (Baskin & Baskin, 2014; Caplow, 2005). This would be consistent with findings indicating a lack of persistent seed banks associated with long-lived woody species (Csontos & Tamas, 2003; Meyer & Paulsen, 2000). Furthermore, burial at 2 cm has been shown to physically inhibit the germination of some *Eriogonum* species, likely due to small seed size (Carrington, 2010). Therefore, within the population of *E. codium*, it is likely that while a small portion of seed may survive and germinate following a second winter, as seen by other studies, any significant long-term maintenance of viability is unlikely.

Comparisons with Prior Testing

When comparing the results of this study to those of prior germination testing, the measure of viability-adjusted germination provides the most accurate comparison. For the Summer treatment in this study, Viability-adjusted germination was 77.4% (Table 8), while prior testing yielded germination percentages of 86 and 88% (Table 1). While these percentages are close to the one found in this study, differences in treatment, number of seeds, and length of testing may explain this variability.

When considering the Berry Botanical Garden test, seeds in the most successful treatment were incubated in dark conditions for 8 weeks, followed by a diurnal light/dark and fluctuating temperature regime (Ed Guerrant, personal communication). Further study would be needed to determine if dark conditions influenced seed germination for *E. codium*, but this remains a possibility.

Length of testing explains the difference compared to the 2013 Rare Care test, which involved two cold-moist stratification periods, stimulating a second germination response during the second spring (Rare Plant Care and Conservation, 2013). Finally, the number of seeds used in this study (300 total for the three unburned seed lots) is much greater than the number of seeds used in any prior tests. While the larger sample size implies more accuracy for the final percentages seen in this study, the unknown effects of fire on the three seed lots categorized as unburned may have lowered the final germination percentage.

Beyond final germination percentages, a comparison of dormancy patterns between prior tests and this study remained largely consistent. Compared to the Ransom Seed Lab tests in which 2-5% of seed germinated with warm-moist stratification within 21 days (Caplow, 2005), for the three unburned seed lots within the Summer treatment of this study, an average of 5% germinated within the same time frame. The Berry Botanical Garden and Rare Care tests also showed a percentage of non-dormant seeds that germinated without cold-moist treatment (Ed Guerrant, personal communication; Rare Plant Care and Conservation, 2013).

Implications for Conservation

Due in part to high adult mortality following fire and low seedling recruitment rates, it is likely that *ex situ* propagation and reintroduction will play an important role in the conservation of *E. codium* (Dunwiddie et al., 2000). Propagation efforts for this species are ongoing, and outplantings of nursery-grown plants have been conducted within the extant population and at similar suitable habitats (Fertig, 2018). Newsome (2017) estimates that a large number of plants will be needed to sustain the population following the 2017 fire, and methods will need to be scaled to meet this need. Results of this study can provide information to help set production targets and maximize the number of plants produced from seed.

Although measures of viability for unburned seed lots in this study were originally intended to provide an idea of percentages that could be expected of *E. codium,* the high variability among the three unburned lots indicates that other variables may have influenced these percentages. For an accurate picture of the typical viability of *E. codium*, testing would need to be conducted on seeds collected in a year without fire. However, the measure of viability-adjusted germination in this study can provide a helpful guide for expected germination percentages from viable seed during propagation. Viability-adjusted germination ranged from 64.9% to 77.4%, depending on the treatment, with the highest percentage achieved by the Summer treatment (Table 8). While this provides a helpful starting point, there is also the consideration of storage time which might influence these values. Because the continued dormancy observed in this study is likely related in part to long-term storage, seeds may be more germinable within a shorter period following harvest, thereby increasing these percentages.

Based on the results of this study, the Summer treatment, with 8 weeks of warm-moist stratification followed by a 4 week cool-moist (Fall) and 12 week cold-moist (Winter) period, can be recommended as the current best propagation treatment. Summer achieved the highest overall germination percentages, facilitating germination for the highest number of both non-dormant and physiologically dormant seeds.

While propagation efforts for this species have already considered cold-moist stratification, results of this study clarify the necessary length of stratification for the portion of seeds that are physiologically dormant. Although the winter climate at the site of the population would suggest a longer cold-moist period, testing of 12 and 16 week cold-moist periods in this study indicates that a shorter period of cold-moist stratification stimulates a germination response, with a longer period of stratification achieving comparable germination. Furthermore, radicle emergence for a portion of seeds at 6-8 weeks within these treatments suggests that an even shorter cold-moist stratification treatment would achieve a germination response.

The tracking of seedling mortality following transplanting also revealed information that could be useful for propagation. Seedlings that were transplanted were typically either able to establish in their containers, averaging a 57% survival rate, or died within the first few days following transplanting. After this point, seedlings that became established experienced very little mortality. It should be noted that all seedlings were transplanted regardless of radicle health, and therefore some of this mortality during transplanting may be due to plants that exhibited radicle damage and could not recover. The process of transplanting fragile germinants from Petri dishes to media was also a likely cause of some mortality. It is likely that this survival rate represents a lower one than would be expected from nursery production in the case where seeds would be directly sown into media and would undergo less handling during transplanting.

Study Limitations

A portion of this study was meant to provide information to aid propagation work for the species. However, it should be noted that germination testing under lab conditions may provide varying results from nursery propagation work. Often, seeds may react differently when they are tested on Petri dishes with filter paper versus in media or soil (Baskin & Baskin, 2014). Because growth chambers provide a very simplified version of diurnal temperature and light regimes that would be experienced in a natural habitat, germination activity can be expected to vary somewhat from wild populations, or from what might be experienced in a nursery setting. Therefore, the results presented may appear “cleaner” than what may actually be happening within a given population. However, studies such as this provide an opportunity to discover small patterns that may not be easily detectable otherwise.

Another factor that may have influenced the viability results seen in this study was the extended period of storage for all seed lots before testing began. Although best practice for most orthodox seeds, long periods of dry storage can result in a loss of viability or shifted germination responses (Baskin & Baskin, 2014). While viability has been successfully maintained in other Polygonaceae at both room temperature and low humidity, limited information exists on the response of this species to extended dry storage (Heather, Perez, & Wilson, 2010). However, results of the two Ransom Seed Lab tests conducted 1 year apart (November 2002 and October 2003) on the same seed collection indicate that dry stored seed had at 71% and 78% viability, respectively, implying good maintenance of viability for *E. codium* during long-term storage (Caplow, 2005).

Seed collection practices and timing may also have added variability to viability and germination results. A potential limiting factor was the lack of seed separation along maternal lines during collection. This made it impossible to tell if variation in viability and germination across seed lots was due in part to variability between individuals versus areas of the population. In germination studies of *E. niveum*, Delaney (1986) found that the main source of variation in germination percentages was within populations at the individual level. Furthermore, timing of seed collection could also play a role in variability of results. Fluctuations in pollinator availability and temperatures during the extended flowering period of E. codium allow for the possibility of similar shifts in both timing and amount of viable seed set.

Finally, measurements of burn severity on individual plants were assessed using qualitative methods, mostly through visual observation. Along with this, multiple individuals conducting plant assessments could have resulted in variation being introduced into assignment of plants to categories. Patchiness of fire may have also led to variation in burn severity between plants assigned to the same burn category. It is unlikely that every individual within a category received the same level of heat or scorching, or even that different flowers on a single individual were exposed to the same conditions. These factors may have introduced a level of variation which impacted the viability results seen between burned and unburned seed lots, as well as among the unburned seed lots.

CONCLUSION

While it is known that adult individuals of *Eriogonum codium* are not fire-adapted or fire tolerant, this study shows that fire exposure also reduces the viability and germination of its seeds. Both the partially and lightly burned seed lots in this study exhibited significantly lower viability and germination than all unburned seed lots. However, unburned seed lots also showed significant variability, indicating that the 2017 fire, or other climatic factors, may have affected seed viability and germination even for plants that appeared unburned.

The seed lots used in this study contained a percentage of seeds that were non-dormant, likely induced by a period of after-ripening. The remainder and majority of seeds exhibit non-deep physiological dormancy which was broken by a period of cold-moist stratification. Although 12 weeks was the minimum amount of time tested in this study, it is likely that an even shorter period of stratification would be acceptable based on the response of seeds in some treatments at 6-8 weeks of cold-moist stratification.

Of the three treatments tested in this study, the Summer treatment, with 8 weeks of warm-moist stratification followed by 4 weeks of cool-moist and 12 weeks of cold-moist stratification, was the most effective in maximizing germination for this species. A longer cold-moist stratification used in the Long Winter treatment did not increase the germination response.

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APPENDICES

Appendix A: Example Photos from Post-fire Plant Assessment

An individual categorized as partially burned. Photo credit: Heidi Newsome

An individual categorized as lightly burned. Photo credit: Keith Abel

Appendix B: Tetrazolium Testing Evaluation Sheet

Species	
Seed lot #	

Imbibe date/time in:	
Imbibe date/time out:	

Timeframe	Time	Date	Temperature
Start time for test			
End time for test			

Results	
Average Y	
Average 50/50	
Average N	

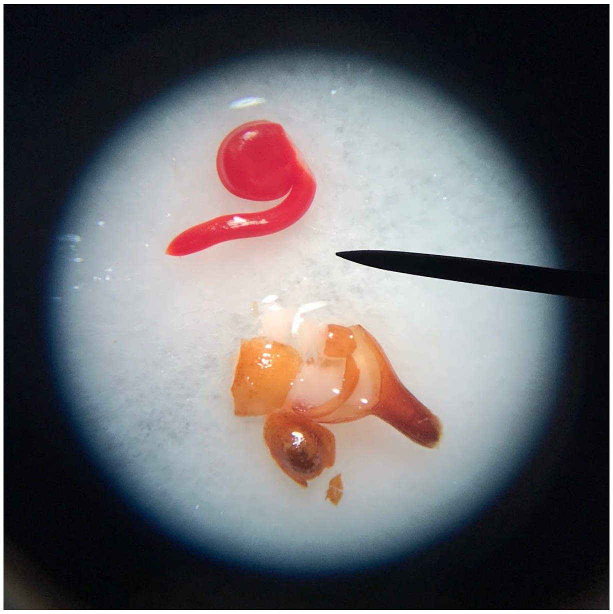
Dish #:	Seed lot #:	Date:

	1	2	3	4	5
A					
B					
C					
D					
E					

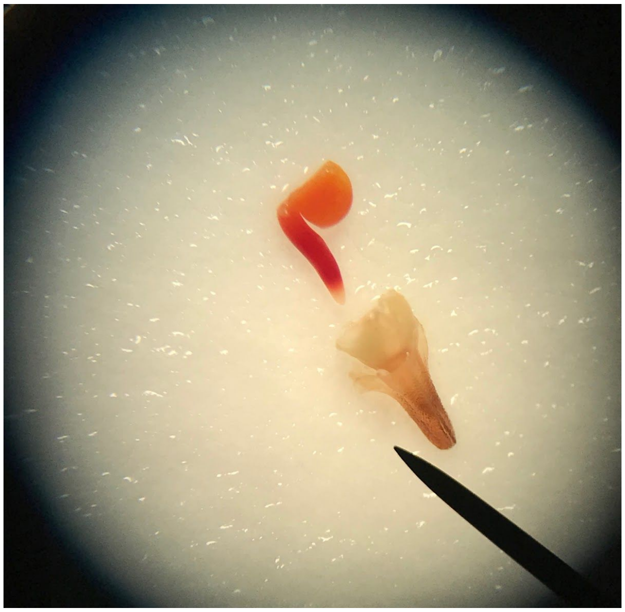
Totals

Y	
50/50	
N	

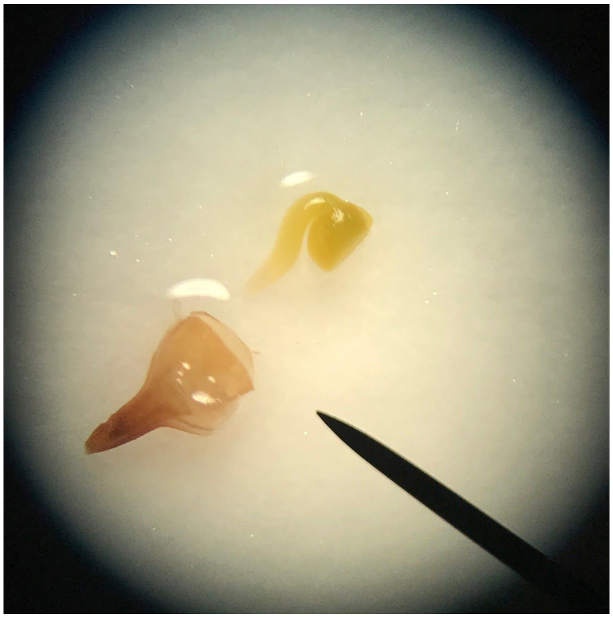
Appendix C: Sample Photographs of Tetrazolium Testing



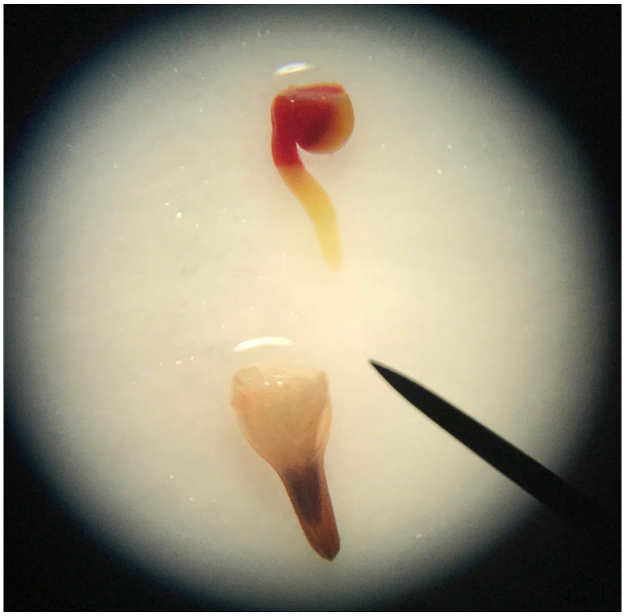
Example of seed assigned to viable category. Red staining on both radicle and cotyledons, along with healthy endosperm.



Example of seed assigned to the 50/50 category. The radicle is mostly red with a white tip, indicating that it may not elongate normally during germination



Example of seed assigned to non-viable category. Lack of red staining indicates dead tissue.



Example of a seed assigned to the non-viable category. White staining on radicle and portion of cotyledons indicates dead tissue.

Appendix D: Results of Initial and Post-germination Testing Tetrazolium Testing

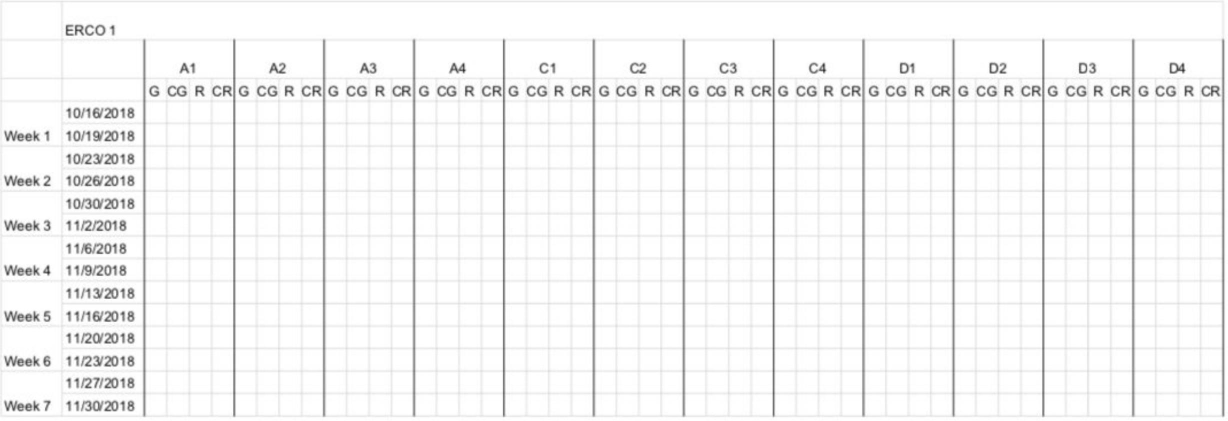
	Unburned (E4)	Unburned (E2)	Unburned (E3)	Lightly burned (E1)	Partially burned (E5)
Seeds tested	100	100	100	100	64
Total viable	61	41	20	25	3
Total non-viable	38	57	77	72	59
Total 50/50	1	2	3	3	2
Initial viable (VI%)	61%	41%	20%	25%	5%

Results of initial seed lot viability test.

	Unburned (E4)	Unburned (E2)	Unburned (E3)	Lightly burned (E1)	Partially burned (E5)
Seeds tested	300	300	300	300	300
Total germinated	82	66	58	34	11
Total dormant	43	18	19	12	1
Total non-viable	174	212	221	250	288
Total 50/50	1	3	2	4	0
Total viable (VT%)	41.6%	28.3%	25.6%	15.3%	4%

Results of viability test for each treatment after germination testing. Total dormant indicates number of seeds that remained viable at the end of germination testing.

Appendix E: Germination and Propagation Data Collection Sheets



	ERCO 1																																																
		A1				A2				A3				A4				C1				C2				C3				C4				D1				D2				D3				D4			
		G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR	G	CG	R	CR
	10/16/2018																																																
Week 1	10/19/2018																																																
	10/23/2018																																																
Week 2	10/26/2018																																																
	10/30/2018																																																
Week 3	11/2/2018																																																
	11/6/2018																																																
Week 4	11/9/2018																																																
	11/13/2018																																																
Week 5	11/16/2018																																																
	11/20/2018																																																
Week 6	11/23/2018																																																
	11/27/2018																																																
Week 7	11/30/2018																																																

Spreadsheet used for data collection during germination testing.

Date potted	Envelope (1-5)	Treatment/Replicate (A1,A2, etc)	Seedling ID	Media	Location	Date to move	Notes

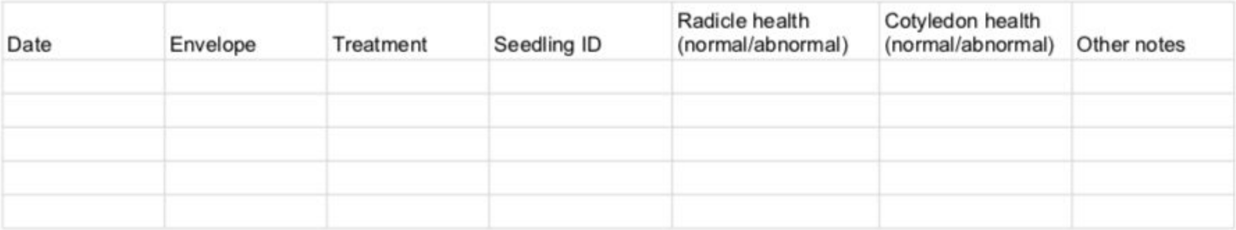
Spreadsheet used for data collection during seedling propagation.

Appendix F: Number of Seeds Removed During Germination Testing

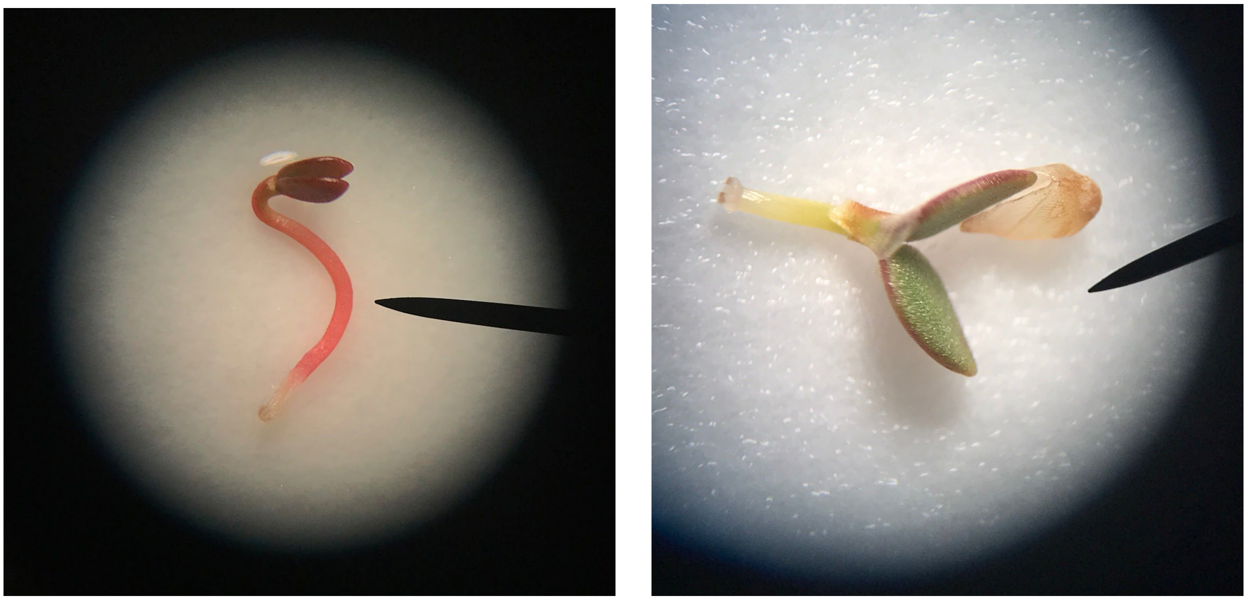
	(Unburned) E2	(Unburned) E3	(Unburned) E4	(Lightly burned) E1	(Partially burned) E5	Treatment total
Long Winter	17 ng 7 gx 2 rgx	1 ng 1 gx	5 ng 3 gx	5 ng 3 gx	1 ng 0gx	29 ng 14 gx 2 rgx
Winter	2 ng 5 gx 1 rgx	13 ng 7 gx 2 rgx	3 ng 4 gx	16 ng 4 gx 1 rgx	13 ng 1gx	55 ng 21 gx 4 rgx
Summer	27 ng 2 gx 1 rgx	22 ng 5 gx 1 rgx	7 ng 5 gx 2 rgx	22 ng 3 gx 1 rgx	24 ng 0gx	104 ng 15 gx 5 rgx
Seed lot total	46 ng 14 gx 4 rgx	36 ng 13 gx 3 rgx	15 ng 12 gx 2 rgx	43 ng 10 gx 2 rgx	38 ng 1 gx	

Summary of -ng (non-germinated), -gx (germinated and died on plate), and -rgx (radicle emergence and death) removed for each seed lot and treatment during testing.

Appendix G: Seedling Health Evaluation Sheet and Comparison Photos



Date	Envelope	Treatment	Seedling ID	Radicle health (normal/abnormal)	Cotyledon health (normal/abnormal)	Other notes



Examples of seedlings assigned to the abnormal category. Radicles show damage and did not elongate normally during germination.