Spring 2016

Seed Germination Requirements for Four Fire-Recruiter Chaparral Shrubs

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Abstract

Wildfires are a natural part of chaparral ecosystems, as they are essential to vegetative regrowth and plant recruitment. For many chaparral species, fire stimulates germination of dormant seeds in the seed bank. This study explored fire-related cues necessary for seed to break dormancy, germinate, and emerge in four chaparral species (*Ceanothus cuneatus, C. jepsonii, Arctostaphylos manzanita, A. viscida*). Seed were exposed to various treatments mimicking wildfire conditions, including boiling water and charred wood (charate), as well as physical scarification by concentrated acid, and monitored for germination. Our data indicate that *Ceanothus* seed require heat cues to germinate, but fire-related chemical cues did not induce germination in any of the species studied. However, pre-treatment with harsh acid was necessary to break physical dormancy in the two *Arctostaphylos* species, which mimics passage through the mammalian gut. Additionally, all species required two to three months of cold stratification to promote germination. These findings may support restoration and conservation efforts in chaparral areas in which germination from seed is required.

Introduction

Chaparral systems are globally distributed and highly biodiverse ecosystems (Brady 2005). They are found in the western United States, northern and southern Africa, South America, and Australia, and many countries surrounding the Mediterranean Sea. The climate of chaparral systems is often described as Mediterranean, with mild, wet winters and hot, dry summers. The main drivers of chaparral systems are limited precipitation, limited nutrients, and wildfires (Valeron and Meixner 2009). As a result, these systems are dominated by shrub flora as the main form of vegetation, due to their resilience to these limitations (Vankat 2013).

Wildfires are natural components of chaparral systems due to their hot, dry summers. Fire plays a crucial role in these systems, driving the renewal and regeneration of these areas (Keeley 1998). However, these fire cycles are changing drastically due to human activity and the climate crisis (Keeley 2009), becoming more frequent and burning hotter. This change in fire regimes does not allow for the system to fully recover between each wildfire event, which greatly alters the biota (Keeley 2009). Therefore, although fire is an essential driver of chaparral ecosystems, fire also has the potential to be very harmful due to human-driven changes to fire regimes.

Post-fire, recruitment can occur in one of two ways: via resprouting or from the seed bank (Keeley 1987). Resprouting occurs when a new plant shoot regrows from the intact root system, replacing the shoot that was destroyed by the fire (Thomas 1989). Fire-induced seed germination from the seed bank is another important regeneration mechanism in chaparral systems (Keeley 1987). During most years, plants produce seed, which lie dormant in the topsoil as part of the seed bank until they receive the proper germination cues (Garwood 1989). Typically, wildfire cues are often needed to break the seed coat (i.e., the seeds' physical

dormancy). In nature, these cues are often related to the intense heat, referred to as heat shock, associated directly with the wildfires or from the chemicals produced from the burning flora, which scarify the seed coat. Heat shock has the ability to crack the seed coat and allow the seed to imbibe water. Chemical cues can be produced by either smoke or charred wood, referred to as charate. Although the mechanism of how these chemicals break the seed dormancy is still unknown, research suggest these chemicals induce metabolic processes that break down the seed coat (Keeley 1987).

Some species require additional cues to break dormancy that do not coincide with fire. For example, some seeds also must experience cues to initiate metabolism, or break physiological dormancy, to germinate. These cues often include non-fire associated chemical or enzymatic cues, as well as environmental cues such as precipitation and vernal cycles (Baskin 1998). Furthermore, a number of chaparral species produce seeds that are consumed by herbivores. The seeds of these species often require the strong acids found in the stomachs of vertebrates in order to break their seed coats and induce germination (Keeley 1987).

Maternal effects are another factor which can influence seed behavior and seedling fitness in post-fire recruitment from the seed bank. These maternal effects are influenced by genetic and environmental factors (Wolf and Wade 2009) and can affect the quality of the seed produced. For example, the maternal environment (e.g., soil nutrient and water availability) can influence seed size, seed quality, and seed number (Galloway 2004). Maternal plants that have sufficient nutrients and water will tend to produce larger seed, more seed, and the seed will typically germinate faster and have better rates of germination (Wulff 1987). In contrast, maternal plants growing in a stressful environment tend to produce less seed and smaller seed, but these seeds may be predisposed to withstand stressful environments (Fenner 2005).

For successful recruitment, seedlings must successfully emerge from the soil surface after germinating. Previous work in other systems, such as grasslands, has suggested that emergence is a key bottleneck to recruitment (James et al. 2011). Work in fire-driven systems indicates that fire-related cues may influence seedling emergence and fitness (Gómez-González et al. 2011), as well as escape from herbivores and plant competition following fire (Pausas 2009). Certainly, multiple and potentially related factors influence post-fire seedling recruitment and success.

In the California Coast Range, a combination of factors, including drought, climate change, and anthropogenic pressures, are altering fire regimes in chaparral ecosystems (Pausas and Keeley 2009). This region is host to unique and diverse serpentine plant communities, which exist in an edaphic mosaic with non-serpentine chaparral communities. Found in these areas are related species that differ in their adaptations to serpentine soils. My study consisted of four species in two congener pairs which occur on and off serpentine (first species in each pair is found off serpentine; second species in each pair is found on serpentine): (1) *Ceanothus cuneatus* and *Ceanothus jepsonii* and (2) *Arctostaphylos manzanita* and *Arctostaphylos viscida*. Although extensive germination work has been conducted for many prominent California chaparral species, particularly within the genus *Ceanothus* (Keeley 1987), there are still many gaps in our knowledge of how seeds in these unique systems germinate. Systematizing post-fire germination cues for a broader range of species is essential for further studies of these chaparral regions. For example, in order to perform manipulative greenhouse experiments on this flora, there must be an effective and efficient method for germinating these species from seed. The objective of my thesis was to add to the body of knowledge on post-fire seed recruitment by determining the cues needed for seeds of key chaparral shrub species to break dormancy, germinate, and establish. I hypothesize that these cues will differ based on species: the *Ceanothus* seed will require cues

associated with heat shock, while the *Arctostaphylos* seed will require chemical cues from charate as well as acid scarification (to mimic mammal digestive systems) in order to germinate.

Methods

Study Site and Species Selection. The seed for this study was collected from the University of California David and Sylvia McLaughlin Reserve, located near Lower Lake, California (38.8739ºN, 122.44317ºW). The 2,800 hectare reserve is located 167.1 kilometers northwest of San Francisco, California in the California Coast Range. The reserve consists of a mosaic of serpentine and non-serpentine soils, with congeners and confamilials growing on these differing soil types. Serpentine soils produce harsh soil conditions for plants due to their characteristic low calcium to magnesium ratio, lack of nitrogen, phosphorus, and potassium availability, and high concentrations of heavy metals, such as chromium and nickel (Harrison and Rajakaruna 2011). Plant species growing within this mosaic have experienced similar climatic conditions but differing soils, and therefore, they provide an excellent model system for studying the evolution of plant adaptations to unique soil types (Anacker 2014).

Four species, representing two congener pairs, were chosen for study. These congener pairs differ in their adaptation to serpentine soils (O'Dell et al. 2006, Drenovsky et al. 2013). The first congener pair consisted of two low nutrient adapted, evergreen shrubs from the family Ericaceae: (1) *Arctostaphylos manzanita,* the non-serpentine congener and (2) *A. viscida,* the serpentine-adapted congener. Both species produce berries that are reddish-brown when ripe, which are often consumed by herbivores. Previous work on other *Arctostaphylos* species suggest that chemical cues related to fire and the acidic digestive tract of mammals are the conditions needed to break dormancy. The second congener pair consisted of two low nutrient adapted,

evergreen shrubs from the family Rhamnaceae: (1) *Ceanothus cuneatus*, the non-serpentine congener and (2) *C. jepsonii,* the serpentine-adapted congener. *Ceanothus* fruits are dehiscent capsules. Previous work on other *Ceanothus* species suggest that heat shock cues related to wildfire conditions are needed to break physical dormancy (Thomas and Parnell 1974).

Seed Collection and Storage. Seed was hand-collected on 18 July 2012 from the University of California McLaughlin Reserve from 16 maternal plants per species. Following collection, seeds were placed in a drying oven for approximately four months at 21ºC, mimicking field conditions, to promote after-ripening. The seeds were then cleaned by breaking open the fruits by hand and visually inspecting the seeds to ensure they were filled, indicating presence of an embryo. After cleaning, the seeds were stored at ambient temperature.

Ceanothus *experiments.* Three successive experiments were conducted to determine optimal conditions for germination. The first experiment, initiated on 22 November 2013, explored the relative roles of heat cues and maternal effects. In this experiment, *Ceanothus* seeds received one of four treatments: (1) control (no pre-treatment), (2) charate exposure, (3) boiling water exposure, and (4) boiling water and charate exposure. The control seeds received no pre-treatment. For all seeds that received the charate treatment, the filter paper was coated with approximately 0.5 g of charate following the methods of Keeley (1991). Charate is the charred wood remaining after a fire. For this treatment, branches of *Adenostoma fasciculatum* were collected at the University of California David and Sylvia McLaughlin Reserve, burned, and milled to a fine powder prior to use. For the boiling water treatment, a 100-mL beaker of deionized water was brought to boil on a hot plate. Seeds were placed in the boiling water for 10 minutes before being strained and plated (Keeley 1991). These treatments were applied to seed

from six maternal plants per species to observe the effects of each treatment as well as differences between maternal plants. An additional treatment of maternal plants could not be included in the study, due to insufficient filled seed from some of the maternal plants. All seed were placed on qualitative filter paper-lined plastic petri dishes (10 mm), moistened with deionized water. After the seeds were plated, they were sealed and placed in a cold room at John Carroll University at 4ºC for three months, which mimicked cold stratification. After the stratification period, the seeds were transferred to the greenhouse on 22 February 2014, where they were laid out in a completely randomized design (2 species x 6 maternal plants per species x 3 replicates per treatment per maternal plant per species x 4 treatments x 20 seeds per plate) under full spectrum high pressure sodium lamps, which were on a 12 hour light/dark cycle (average temperature: 23ºC average and average PPFD: 355.2. μmol m^{-2} s^{-1}). Supplemental lighting was used in this experiment due to cloudy conditions during the duration of the experiment. Seed were monitored for germination daily; germination was determined by presence of the radicle. Deionized water was added to the plates as needed. After germination, seeds were transplanted to SC7 Ray Leach Cone-Tainers, which were 3.8 cm in diameter by 14 cm deep (Stuewe and Sons Inc., Tangent, OR) filled with a 1:1 ratio of sand and fritted clay and covered by a layer of Glad Press and Seal wrap to maintain soil moisture. The seeds were buried less than 1 mm from the soil surface. Seeds were then watered and monitored daily for 30 days until emergence.

Due to equivocal treatment effects for *C. jepsonii* in the previous round of experiments, additional treatments were tested for this species during Summer 2015. The stratification period was also shortened. *C. jepsonii* seed received one of four different treatments: (1) control (no pre-treatment), (2) boiling water exposure, (3) hot water exposure, and (4) hot sand exposure.

The control and boiling water treatments were the same as described previously. For the hot water treatment, a 100-mL beaker of deionized water was brought to a boil on a hot plate. The seeds were placed in the water, and the beaker was immediately removed from the hot plate and allowed to cool. The seeds remained in the water for 24 hours. The seeds were then strained and immediately plated. For the hot sand treatment, seeds were mixed into sand in a tin, ensuring they were completely covered by at least 2 cm of sand. The tin was placed into an oven at 100ºC for 30 minutes. After seeds were removed from the oven, they were strained from the sand and immediately plated. After treatment, all seeds were plated on 10 mm plastic petri dishes (20 seeds per plate), which contained qualitative filter paper that was wetted with deionized water. After the seeds were plated, they were sealed and placed in the cold room at 4ºC for either two or four weeks for cold stratification. Following stratification, plates were placed in the John Carroll greenhouse under ambient conditions and monitored for germination for one month, as previously described. Four week stratification plates were placed in the greenhouse on 6 July 2015, and two week stratification plates were placed in the greenhouse on 3 July 2015. In total, there were 4 treatments x 5 replicates per treatment x 2 stratification periods. Within a stratification period, plates were completely randomized. After germination, seeds were transplanted to Ray Leach Cone-Tainers (as previously described) and monitored and treated as previously.

Due to very low germination in the previous round of *C. jepsonii* experiments, the experiment was replicated again in Fall 2015. All treatments were applied as previously, except the stratification period was lengthened. Half of the replicates were exposed to a two month stratification period, and half to a three month stratification period. The replicates exposed to a two month cold stratification were moved to the greenhouse on 8 September 2015, while the

replicates exposed to a three month cold stratification were moved to the greenhouse on 6 October 2015. In total, there were four treatments x 2 stratification periods x 15 seeds per plate x 15 replicates per treatment. Following stratification, plates were placed in the John Carroll greenhouse under ambient conditions and monitored for germination for one month, as previously described. Within a stratification period, plates were completely randomized. After germination, seeds were transplanted to Ray-Leach Cone-Tainers as before, filled with a 1:1 ratio of sand and fritted clay. The seeds were buried less than 1 mm from the surface of the sand. Seeds were then watered daily and monitored daily for emergence.

Arctostaphylos *experiments.* Similar to the first round of *Ceanothus* experiments, our first experiment with *Arctostaphylos* seed tested the effects of chemical cues and maternal effects. *Arctostaphylos* seeds received one of four treatments: (1) control (no pretreatment), (2) charate exposure, (3) concentrated sulfuric acid exposure, and (4) concentrated sulfuric acid and charate exposure. The control seeds received no treatment. For all seeds that received the charate treatment, the filter paper was coated evenly with approximately 0.5 g of charate as described previously. For the concentrated sulfuric acid treatment, the seeds were immersed in concentrated sulfuric acid (18 M) for 6 hours. After this time period, they were strained and rinsed before being plated. These treatments were applied to seed from eleven maternal plants per species to investigate both the effects of the treatments as well as any differences between maternal plants. After treatment, all treated seeds were placed on 10 mm plastic petri dishes with qualitative filter paper, which was wetted with deionized water. After the seeds were plated, they were sealed and placed in the cold room at 4^{o}C for three months cold stratification. After that stratification period, the seeds were transferred to the John Carroll University greenhouse on

25 February 2014. In total, there were 2 species x 11 maternal plants per species x 4 treatments x 3 replicates per treatment per maternal plant x 20 seeds per plate. In the greenhouse, the plates were laid out in a completely randomized design under full spectrum high pressure sodium lamps, which were on a 12 hour light/dark cycle (average temperature: 23^{o}C and average PPFD: 355.2. μmol m^{-2} s^{-1}). Supplemental lighting was used in this experiment due to cloudy conditions during the duration of the experiment. Seeds were monitored for germination daily, and germination was determined by presence of the radicle. Distilled water was added to the plates as needed. After germination, seeds were transplanted to Ray-Leach containers (as described previously) filled with a 1:1 ratio sand and fritted clay. The seeds were buried less than 1 mm from the surface of the sand. Seeds were then watered daily and monitored daily for emergence.

Due to low survivorship of germinated seedlings, an additional experiment was conducted to test whether lower concentrations of sulfuric acid would improve germination and emergence. The stratification period was also shortened. In this experiment, *Arctostaphylos* seed received one of nine different treatments, which were a factorial combination of different time intervals and different concentrations of sulfuric acid. The varying exposure periods were 1 hour, 3 hours, and 6 hours. The various acid concentrations were 70% sulfuric acid, 90% sulfuric acid, and concentrated sulfuric acid. The seeds were also thoroughly rinsed three times with water, and the pH was checked to be neutral before the seeds were plated. After treatment, all seeds were plated on 10 mm plastic petri dishes which contained qualitative filter paper, wetted with deionized water. After the seeds were plated, they were sealed and placed in the cold room at 4^{o}C either two or four weeks cold stratification. After that stratification period, the seeds were transferred to the John Carroll University greenhouse. The plates receiving 2 weeks

of cold stratification were moved to the greenhouse on 10 June 2015; the plates receiving four weeks of cold stratification were moved to the greenhouse on 23 June 2015. In total, there were 9 treatments x 5 replicates per treatment x 2 species x 2 stratification periods x 20 seeds per plate. In the greenhouse, the plates were laid out in a completely randomized design under ambient conditions. Germination was monitored for one month, as previously described. After germination, seeds were transplanted to Ray Leach Cone-Tainers (as described previously) filled with a soil mixture of 60% organic potting soil, 30% organic seed starter mix, and 10% Turface Athletics MVP mix. The seeds were buried less than 1 mm from the surface of the soil, watered daily, and monitored daily for emergence.

Due to very low germination in the previous round of *Arctostaphylos* experiments, the experiment was performed again in Fall 2015. The seeds were treated in a full factorial design of 90% sulfuric acid solution and concentrated sulfuric acid for 3 or 6 hours; the 70% sulfuric acid solution and 1 hour exposure times were both eliminated as treatments as these treatments were ineffective in the last round of experimentation. Treatments were applied as previously, except half of the replicates were exposed to a two month stratification period and half to a three month stratification period. The plates experiencing two months of cold stratification were moved to the greenhouse on 7 Sept 2015, and the plates experiencing three months of cold stratification were moved to the greenhouse on 5 October 2015. In total, there were 4 treatments x 15 replicates per treatment x 2 species x 2 stratification periods x 15 seeds per plate. Following stratification, plates were placed in the John Carroll greenhouse under ambient conditions and monitored for germination for one month, as previously described. Within a stratification period, plates were completely randomized. After germination, seeds were transplanted to RayLeach Cone-Tainers (as described previously) and filled with a 1:1 ratio sand and fritted clay. The seeds were buried

less than 1 mm from the surface of the sand. Seeds were then watered daily and monitored daily for emergence.

Statistical analysis. Similar statistical models were built for the first *Ceanothus* and *Arctostaphylos* experiments. Effects in the nested ANOVA model included species, treatment, species*treatment, maternal plant(species), and treatment*maternal plant(species). The second set of experiments conducted with both congener pairs (those including shortened stratification periods) had extremely low germination rates, preventing statistical analysis beyond descriptive statistics. For the third set of experiments for *C. jepsonii*, ANOVA model effects included treatment; replicates from the two stratification periods were compared using separate ANOVA models, as the replicates were randomized within stratification period. Similarly, for the third set of *Arctostaphylos* experiments, separate ANOVA models were constructed for replicates from the two month and three month stratification periods. Within a stratification period, species, exposure time and acid concentration were the main effects in the model; we also explored all potential interaction terms. The Shapiro Wilks test was used to test for normality, and the Levene's test was used to determine whether treatments had unequal variance. When necessary, data were log-transformed to meet the normality assumption and weighted models were run to meet the assumption of equal variance among treatments.

Results

Ceanothus *experiments* –

Fall 2014. The two *Ceanothus* species responded differently to seed treatments (significant species*treatment interaction: $F_{3,30}$=7.36, P=0.0008; Fig. 1). *C. cuneatus* seed exposed to boiling water had higher germination rates than other treatments, whereas germination rates were

similar and low across all *C. jepsonii* treatment groups. Although maternal lines within species responded differentially to treatments ($F_{30,143}$=2.2, P=0.002), this response was much weaker than the significant species*treatment interaction. Emergence was low for both species (Table 1), but *C. cuneatus* seed treated with boiling water had the most successful emergence (≈16%). The boiling water treatment also yielded the highest emergence rates in *C. jepsonii*, but emergence for this species was overall much lower.

Summer 2015. Germination rates from seed stratified for only two or four weeks were very low (data not shown); too few seed germinated for statistical comparison. The two week and four week long cold stratification times were too short to break dormancy in the seeds.

Fall 2015. Although not statistically comparable, germination rates were greater in the three month cold stratification period than the two month cold stratification period (Fig. 2). Germination rates differed by treatment for both stratification period lengths ($F_{3,36}$=14.4, P<0.0001 for the two-month stratification period; $F_{3,36}$=14.9, P<0.0001 for the three-month stratification period). Based on post-hoc Tukey's tests, germination rates from the control, boiling water treatment and hot water treatment were not significantly different from each other but were significantly higher than the hot sand treatment for those seed stratified for two months (Fig. 2A). For seed stratified three months, the boiling water and hot water treatments produced significantly higher germination rates than the control or hot sand treatments (Fig. 2B).

Arctostaphylos *experiments* –

Fall 2014. Both species responded similarly to treatments (species effect, P>0.05), but seed treatments significantly affected germination rates ($F_{3,20}$=4.9, P=0.007; Fig. 3). Both *A. manzanita* and *A. viscida* seeds exposed to concentrated sulfuric acid had higher germination

rates than other treatments. Although maternal lines within species responded differentially to treatments ($F_{20,112}$=2.2, P=0.003), this response was much weaker than the significant species*treatment interaction. Emergence was low for both species; only three *A. manzanita* seeds emerged (all from the control treatment), and there was no emergence for *A. viscida* seed.

Summer 2015. Germination rates from seed stratified for only two or four weeks were very low. The 70% sulfuric acid treatment and the one hour acid exposure time had little effect on germination (data not shown).

Fall 2015. Similar to the *Ceanothus* experiments in Fall 2015, germination rates tended to be higher for seed stratified for three, rather than two months (Fig. 4). For seed stratified for two or three months, *A. viscida* responded more strongly to longer seed pre-treatments than *A. manzanita* (species*time: $F_{1,112}$=18.8, P<0.0001 and $F_{1,112}$=4.8, P=0.03, respectively). For the three month stratification period, there was also a significant interaction between the sulfuric acid treatment and the time interval that seeds were exposed to the acid (acid*time, $F_{1,112}$=12.1, P<0.0001).

Discussion

As predicted, the *Ceanothus* species had the greatest germination rates in response to heat shock treatments, specifically boiling or hot water. Surprisingly, few *C. jepsonii* seed germinated in response to the hot sand treatment, even though this treatment should have mimicked the dry heat found in naturally occurring wildfires (Baskin and Baskin 1998). Chemical cues from charate did not influence germination in *Ceanothus*, which was consistent with the literature on other species of *Ceanothus*, suggesting members of this genus respond more strongly to heat

than chemical cues from wildfires (Keeley 1998). Heat shock causes the seed coat to split, allowing imbibition (Bonner 2008), which presumably facilitates seed germination in *Ceanothus*.

Both *Arctostaphylos* species had the highest germination rates when subjected to the concentrated sulfuric acid treatment, but did not respond to chemical cues related to fire. *Arctostaphylos* fruits are consumed by mammalian herbivores, exposing them to acidic conditions in the digestive tract (Baskin and Baskin 1998). However, emergence following acid exposure was very low, and only three seeds emerged, all of which were from the control treatment. It is likely that acid exposure damaged the embryos following germination but prior to emergence. Subsequent trials indicated lower acid concentrations and shorter exposure times were too weak to break the physical dormancy of the seed coat in both *Arctostaphylos* species. Instead, high acid concentrations and long exposure times did result in germination, but this also required better rinsing techniques, followed by pH measurement of the seeds prior to plating. Surprisingly, there are not many suggested methods for rinsing the seed after the acid treatment in the literature; however, scrubbing seeds three separate times with gloved hands in large bowls of water, followed by pH measurement to ensure water neutrality yielded the highest emergence rates.

Charate exposure did not stimulate germination in the *Arctostaphylos* seed, which was inconsistent with the literature (see Keeley 1987). In other species of *Arctostaphylos*, chemical cues from charate promoted germination (Keeley 1987, Baskin and Baskin 1998, Emery 1988). It is possible that the intensity of the concentrated acid in the present study overwhelmed any influence the charate may have had over inducing seed germination in these species. The acid treatment was very influential on germination, but it left the environment of the seed plates highly acidic. This environment may have disrupted the potential chemical interactions between

the charate and the seed coats. In a series of follow-up experiments, the charate treatment was removed, and instead focused on determining the optimal acid concentrations and exposure times. However, future studies should reassess the influence of the charate cue, as germination rates in *Arctostaphylos* species were still low in subsequent, acid-only trials.

Many studies of chaparral shrub species indicate that cold stratification periods of two to four weeks are sufficient to induce germination (Garwood 1989, Bonner 2008, Keeley 1987). Chaparral systems have mild winters, so a lengthy cold stratification period was not expected. However, through the series of experiments in the present study, germination rates were highest in seed cold stratified for three months. The prolonged cold stratification time may be due to the fact that the species examined in this study are typically found at high elevation in their natural ecosystem. Chaparral species that occur at higher elevations (such as those used in the present study) depend much more on cold stratifications to induce germination than chaparral species that naturally occur at lower elevations, due to colder winter temperatures at higher elevations (Bonner 2008). In such plants where a cold stratification period is necessary, germination rates of most chaparral species increase up to a period of two to four months cold stratification time (Keeley 1987, Garwood 1989, Bonner 2008).

In general, germination rates were low across the four species, but in particular for the two species of *Arctostaphylos*. Members of genus *Arctostaphylos* are notoriously difficult to grow from seed, due to their strong and thick seed coats (Keeley 1987, Baskin and Baskin 1998, Emery 1988). Also, because the seeds often germinate after passing through an herbivore in nature, the natural microenvironment that germination usually occurs in is difficult to mimic in a controlled setting. In fact, these seed are known to be particularly hard to germinate through any

methods other than extremely artificial methods, such as exposure to concentrated acid or physical scarification with sand paper (Keeley 1987).

Generating sufficient emergence was very challenging for all species, but particularly so with the *Arctostaphylos* species. During transplantation, the roots appeared to be very brittle, which may have contributed to the poor emergence values. Another challenge to this experiment was achieving sufficient soil moisture without leading to fungal infection. In the future, it may be necessary to pre-treat the seeds with bleach prior to their respective treatments to reduce fungal growth and directly sowing the seeds into soil after they receive their respective treatments, in order to minimize fungal and transplantation challenges. Also, charate should be reconsidered in the effects of chemical cues on the *Arctostaphylos* species. The mechanisms of how chemical cues from wildfire induce seed germination are still largely unknown. It is possible that these chemical cues induce metabolism or neutralize germination inhibitors in the seeds (Emery 1988). If these physiological responses occur in response to charate cues, the harshness from the acid treatment (without thorough rinsing) could disrupt or mask these processes, minimizing the charate's influence on seed germination.

Beyond generating basic knowledge, understanding germination cues in these species is important for conservation efforts in chaparral ecosystems. Global climate change is altering wildfire cycles in these areas, making them more frequent. These changes could, in turn, alter plant community composition, favoring invasive plant species over slow-growing native shrubs (Keeley 2009). In order to restore these areas with their native vegetation, further research on propagation from seed is needed.

Acknowledgements

Funding for this study was provided in part by the Barrett Undergraduate Endowment Research Award and the John Carroll University Biology Department. Special thanks go to Dr. Rebecca E. Drenovsky, Dr. Ralph A. Saporito, and Dr. Angela C. Jones and the Honors Program for their guidance and assistance throughout my project. Additional thanks goes to Vivian Fung and Clare Muller for lab assistance.

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Table 1. Emergence for *Ceanothus* species from Fall 2014. Emergence (%) was calculated as number of seeds planted divided by number of seeds emerged multiplied by 100.

Species	Treatment	% Emergence
C. cuneatus		
	Control	0.8
	Boiling water	15.8
	Charate	0
	Boiling water + charate	1.9
C. jepsonii		
	Control	2.2
	Boiling water	3.6
	Charate	1.1
	Boiling water + charate	1.1

Figure Legends

Fig 1. Percent germination for both *Ceanothus* species from Fall 2014. Each individual bar represents one of the six maternal plants from which seed were collected. Data are means+SD (n=3 per maternal plant).

Fig 2. Percent germination for *C. jepsonii* from Fall 2015. Fig 2A represents data from two month cold stratification treatment, while Fig 2B represents data from three month cold stratification. Letters represent significant differences among treatments based on post-hoc Tukey's tests. Data are means+SD (n=10).

Fig. 3. Percent germination for both *Arctostaphylos* species from Fall 2014. Each individual bar represents one of the eleven maternal plants from which seed were collected. Data are means+SD (n=3 per maternal plant).

Fig 4. Percent germination for both *Arctostaphylos* species from Fall 2015. Fig 2A represents data from two month cold stratification treatment, while Fig 2B represents data from three month cold stratification. Data are means+SD (n=5).

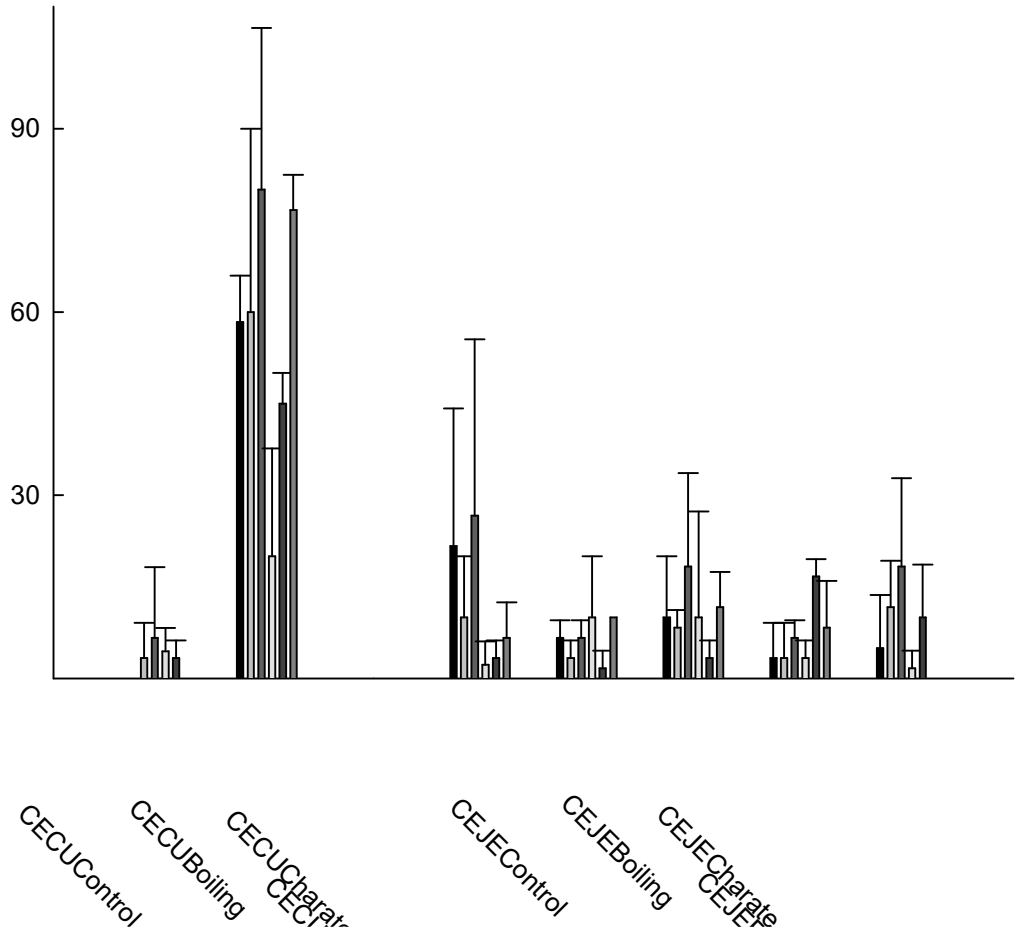
90
60
30
CECUControl
CECUBoiling
CECUCharat
CECI
CEJEControl
CEJEBoiling
CEJECharate
CEJE

Figure 1

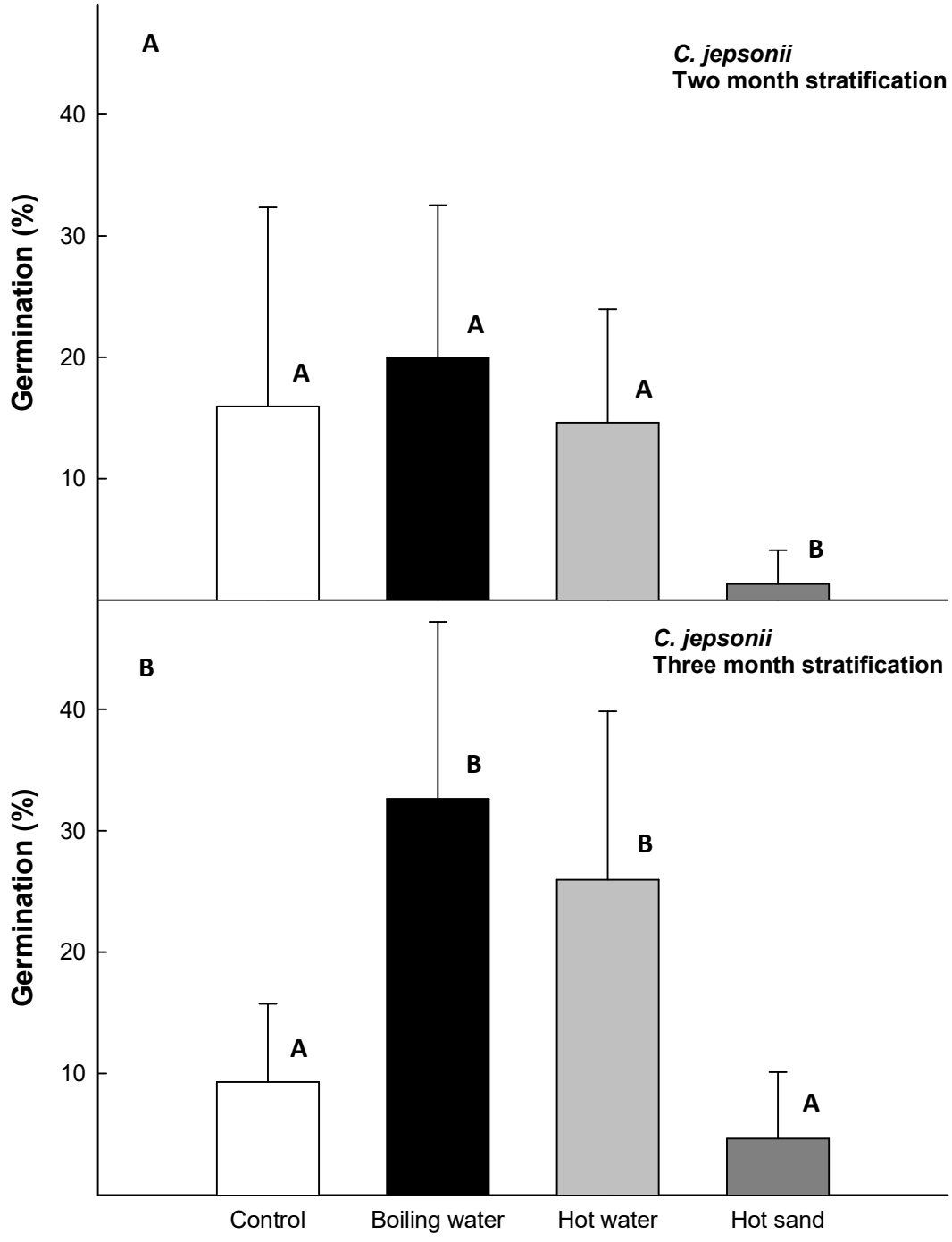
A
C. jepsonii
Two month stratification
Germination (%)
40
30
20
10
A
A
A
B
B
C. jepsonii
Three month stratification
Germination (%)
40
30
20
10
A
B
B
A
Control
Boiling water
Hot water
Hot sand

Figure 2

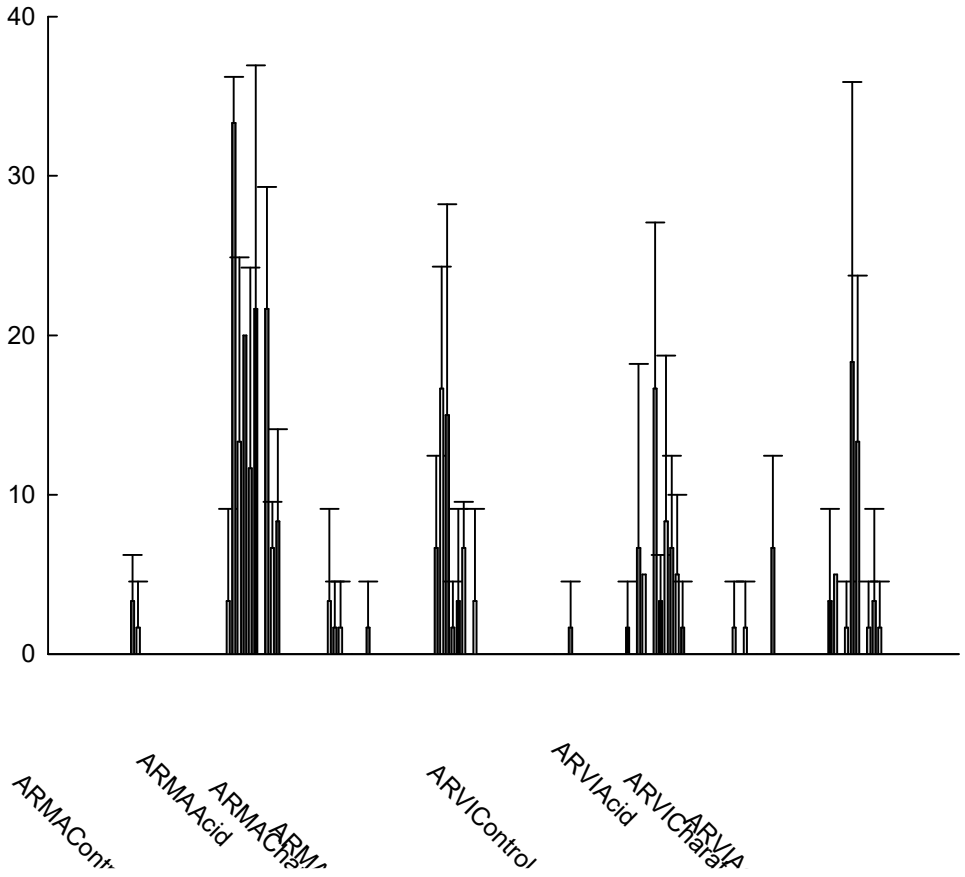

Figure 3

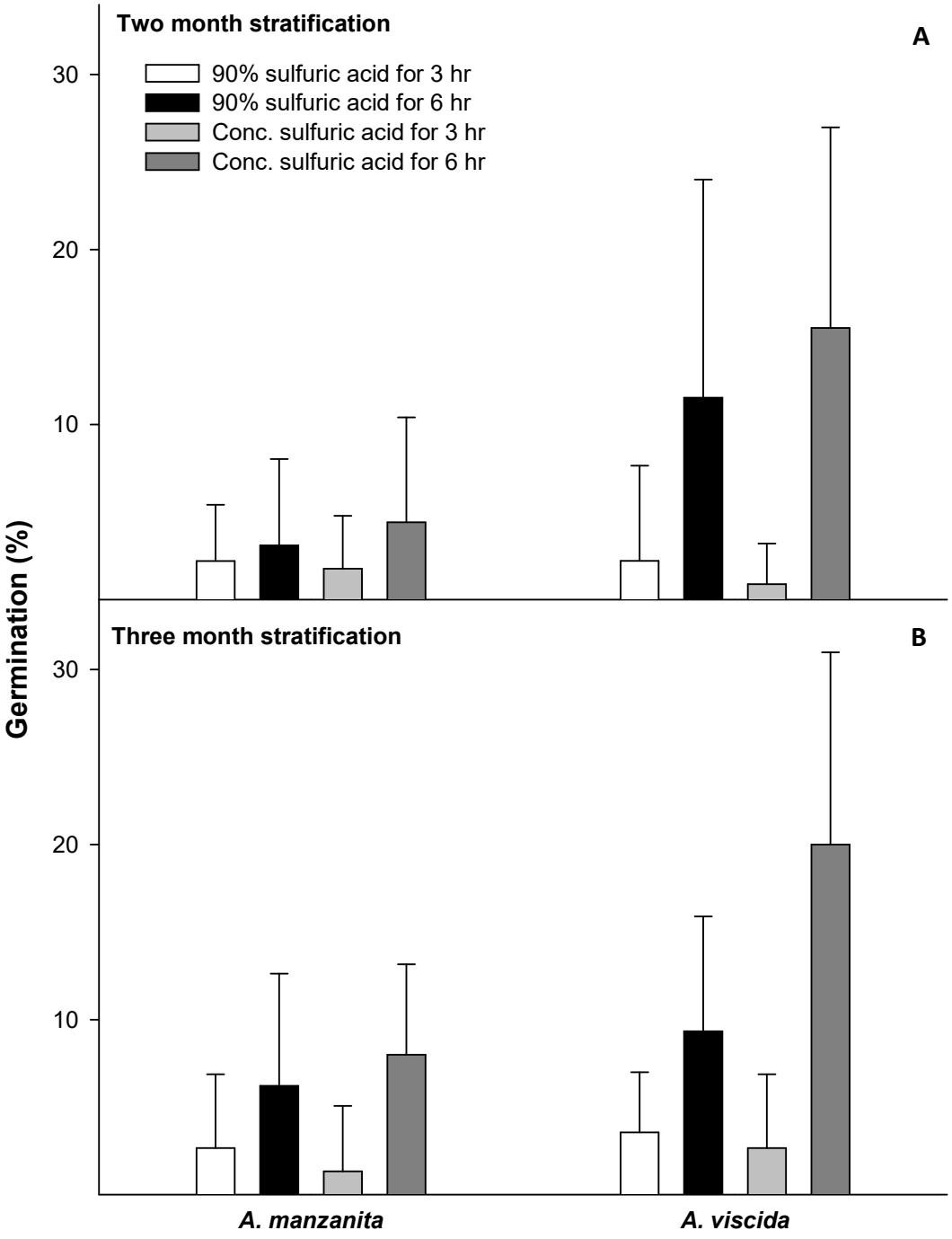
Two month stratification
A
90% sulfuric acid for 3 hr
90% sulfuric acid for 6 hr
Conc. sulfuric acid for 3 hr
Conc. sulfuric acid for 6 hr
30
20
10
Germination (%)
Three month stratification
B
30
20
10
A. manzanita
A. viscida

Figure 4