2021

Heat and Chemical Cues Enhance Germination Rates in Fire-Dependent Chaparral Species

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Abstract

Climate change has caused an increase in the frequency and intensity of fire cycles in chaparral ecosystems. In environments impacted by wildfires, some plant species depend on complex fire cues to germinate. Determining how to best replicate wildfire cues is essential to using fire-recruiter species in restoration efforts. This study examines the effects of various simulated fire cues on four chaparral species: *Arctostaphylos manzanita, A. viscida, Ceanothus cuneatus,* and *C. jepsonii*. Seeds were exposed to heat, charate, liquid smoke, or combinations of treatments. Other germination cues, such as cold exposure for all species and acid exposure for *Arctostaphylos* species, were also examined. We found that *Arctostaphylos* species germinate best when exposed to acid and that *A. viscida* has greater germination rates than *A. manzanita*. The *Ceanothus* species responded best to charate and boiling water exposure. Additionally, all species require a 2-3 month cold stratification period prior to treatment. Using these conditions when planting seeds can increase the chance of successful germination.

Introduction

Fire can be a natural process in some ecosystems, such as the California chaparral (Keeley 1991). Wildfires can be damaging, but they can also have positive impacts on chaparral ecosystems. For example, fires can increase nitrogen availability near the soil surface that benefits recovering plants and bacteria (Goodridge et al. 2018). However, fire cycles have become more frequent and intense due to human suppression. In areas with high amounts of human activity, human influence has a greater effect on an ecosystem's fire cycles than the climate of the region (Syphard et al. 2017). This change in the natural fire cycles can have negative effects on plant species that have adapted to these conditions. For example, more frequent fires can make it difficult for some species to establish after a disturbance, since there is less time between fire events (Pratt et al. 2017).

In ecosystems that experience natural wildfires there are two main strategies for seed survival. Plants can be characterized as either having refractory seeds (fire-recruiters) or non-refractory seeds (fire-resisters) (Keeley 1991, Pratt et al. 2017). Refractory seeds typically establish in the year following a fire because their dormancy is broken by cues from the environment. These environmental cues include heat shock and exposure to chemicals found in charred wood (Keeley et al. 1985). However, germination cues are often very complex and fire cues alone may not be sufficient to promote germination. Other germination cues may also be necessary to imitate various environmental conditions. For example, cold stratification may be required to simulate exposure to winter temperatures, and acid scarification, to mimic the digestive tract of herbivores (Keeley 1991). Non-refractory seeds do not germinate following a fire event, but instead require an extended time with an absence of fire (Pratt et al. 2017).

Many fire-resister species do not have any seed dormancy and germinate in response to wetting (Keeley 1991). These plants often have other traits, such as vegetative resprouting, to resist frequent fires (Pratt et al. 2017). Although these strategies differ in their response to wildfires, both have been shown to be effective adaptions in areas with frequent fire cycles (Thomas and Davis 1989).

Application of refractory seed species is one method that can be used to promote new growth in damaged areas following fire. Frequently, these seeds require treatment with fire-related cues prior to planting them at the restoration site (Wilkin et al. 2013). Common treatments for refractory seeds include exposing them to heat, charate, or liquid smoke (Keeley and Fotheringham 1998, Laskowski et al. 2013, Wilkin et al. 2013). Other restoration methods that encourage germination of refractory seeds include cold stratification or treating the seeds with acid prior to planting (Keeley 1991).

The California chaparral is a Mediterranean ecosystem that evolved with fire (Wilkin et al. 2013). In the Coastal Range, plant communities adapted to a variety of soil types, including serpentine, can be found. These soils are characterized by high amounts of metals, including magnesium, nickel, chromium, cobalt, and manganese (Drenovsky et al. 2013, Gunarathne et al. 2019). These soils also have very high magnesium concentrations and very low calcium levels (Kay et al. 2016, Arthurita et al. 2017, Gunarathne et al. 2019). These conditions can create a nutrient poor environment that can limit plant growth (Arthurita et al. 2017, Gunarathne et al. 2019). Despite these harsh conditions, some plants have adapted for survival on serpentine soils (Drenovsky et al. 2013, Kay et al. 2016).

Arctostaphylos manzanita, A. viscida, Ceanothus cuneatus, and *C. jepsonii* are refractory seed chaparral species that require complex fire cues to germinate (Keeley 1991). These species represent congener pairs in which one species is found on serpentine soils (*A. viscida* and *C. jepsonii*) and the other is found off serpentine (*A. manzanita* and *C. cuneatus*). Previous studies have shown that liquid smoke can improve germination in other *Arctostaphylos* species (Laskowski et al. 2013). In *Ceanothus* species, heat shock after cold stratification has been shown to be effective in increasing germination (Keeley 1991). However, which fire cues are the most important for promoting the germination of these species is still unknown. To develop effective restoration efforts, it would be beneficial to know how to best germinate the seeds. We conducted germination trials to determine how liquid smoke, cold stratification, heat, charate, and acid scarification would affect the germination of *A. manzanita, A. viscida, C. cuneatus,* and *C. jepsonii*. We hypothesized that charate, acid scarification, and liquid smoke will promote germination in the *Arctostaphylos* species, and that heat shock will increase germination in the *Ceanothus* species.

Methods

Study Site and Study Species

Seeds were collected from the University of California Davis Donald and Sylvia McLaughlin Reserve near Lower Lake, California (38.8739°N, 122.44317°W). The Reserve is located 167.1 kilometers northwest of San Francisco and experiences a Mediterranean climate with cool, wet winters and warm, dry summers. The mean annual precipitation at the site is 620

mm. The reserve spans 2800 hectares and contains both serpentine and non-serpentine soils. Species were selected from this site because of this patchwork of soil types.

The species in this study form two congener pairs in that one species in each pair is adapted to serpentine soils and the other is found off serpentine. All four species are woody shrubs and obligate seeders that cannot resprout following a fire (Keeley 1991). One of the pairs consists of two species from the family Ericaceae. *A. manzanita* is found off serpentine and *A. viscida* is found on serpentine. Both of these species have fleshy fruits that are attractive to mammalian herbivores. The other pair consists of two species from the family Rhamnaceae. *C. cuneatus* is found off serpentine and *C. jepsonii* is found on serpentine. These species have dry, dehiscent fruits that are not eaten by herbivores.

Plant material was collected from the Reserve on July 18, 2012. Sixteen maternal plants were selected for each species to obtain seed. The *Ceanothus* species have dehiscent fruits, so the maternal plants were bagged earlier in the season to support seed collection. For *Arctostaphylos*, the fruits were collected, and the seeds were removed later in the lab. After the seeds and fruits were collected, they were stored in an oven at 21°C for approximately four months. This was done to mimic field conditions and promote after-ripening. Following storage in the oven, the *Arctostaphylos* fruits were broken open by hand, and the seeds were removed. Seeds were inspected visually to determine if they were filled. After removing the seeds from the fruits, they were stored at room temperature.

Germination Trials

2013 Experiment

Three separate experiments were conducted between 2013 and 2016. The first experiment conducted in the fall of 2013 examined the effect of acid and charate on *A. manzanita* and *A. viscida,* as well as the effect of boiling water and charate on *C. cuneatus* and *C. jepsonii*. For the *Arctostaphylos* species, treatments included charate exposure, concentrated sulfuric acid exposure, charate and sulfuric acid exposure, and control. For both species 33 plates were prepared per treatment with 20 seeds per plate. Prior to plating, seeds were prepared with their respective treatments. Charate was prepared by burning branches of *Adensostoma fasiculatum* collected from the Reserve. 0.5 g of charate was applied to moistened filter paper on a petri dish as described in Keely (1991). Seeds in the acid treatment were placed in concentrated sulfuric acid for six hours. The seeds were rinsed to remove the acid before plating. Following treatment, the seeds were plated on 10 cm filter paper moistened with deionized water. The plates were sealed with parafilm and placed in the John Carroll University cold room, where they were stored for three months at 4°C. Following the cold stratification period, the seeds were transferred to the John Carroll University greenhouse on February 25, 2014. The plates were arranged in a complete randomized design and placed under full spectrum high pressure sodium lamps on a 12-hour light cycle (average temperature: 23°C, average PPFD: 355.2 µmol $m^{-2}s^{-1}$). Supplemental lighting was used, as conditions over the duration of the experiment were cloudy. Seeds were checked daily for germination (presence of a radicle) and moistened with deionized water as needed.

For the *Ceanothus* species, treatments included charate exposure, boiling water exposure, charate and boiling water exposure, and control. For both species, 18 plates were prepared per treatment with 20 seeds per plate. Prior to plating, seeds were prepared with

their respective treatments. Charate was prepared as described previously. Seeds in the boiling water treatment were boiled for 10 minutes on a hot plate. Following treatment, the seeds were plated on 10 cm filter paper moistened with deionized water. The plates were sealed and placed in the cold room for three month stratification at 4°C. The plates were removed from the cold room and transferred to the greenhouse on February 22, 2014. The plates were arranged in a complete randomized design and placed under full spectrum high pressure sodium lamps on a 12-hour light cycle, with conditions similar to those for the *Arctostaphylos* experiment.

2015 Experiment

A second experiment was initially conducted in the summer of 2015, investigating the efficacy of a shorter stratification time period (2 weeks at 4°C). However, no seed germination occurred; therefore, the experiment was repeated in the fall of 2015 with longer stratification times. This experiment examined the effect of different acid concentrations and exposure times in *A. manzanita* and *A. viscida,* as well as the effect of different types of heat exposure on *C. jepsonii*.

Both of the *Arctostaphylos* species treatments involved a 2 x 2 x 2 factorial design. The factors were acid (two levels: concentrated and 90%), exposure time (two levels: three and six hours), and species (two levels: *A. manzanita* and *A. viscida*). Each species had 30 plates per treatment combination of acid concentration and exposure time, with 15 seeds per plate. The plates were divided evenly for two separate cold stratification experiments, so that 15 of the plates would be exposed to two month cold stratification and the other 15 exposed to three

month stratification. Seeds were soaked in acid based on their treatment as described previously. Following acid scarification seeds were rinsed, plated, and transferred to the cold room where they were stored at 4°C. Following two or three months, the seeds were transferred to the greenhouse on September 7, 2015 or October 5, 2015, respectively. Seeds were kept in the greenhouse and checked for germination as previously described.

The *C. jepsonii* treatments included boiling water, hot water, hot sand, and control. Only *C. jepsonii* was included in this trial, as germination rates in *C. jepsonii* were much lower in our fist experiment and thus we wanted to investigate the efficacy of other heat treatments. A total of 30 plates per treatment were prepared with 15 seeds per plate. As with the *Arctostaphylos* species, 15 of the plates were exposed to two month cold stratification and the other 15 were exposed to three month stratification. The boiling water treatment was applied as described previously. In the hot water treatment, water was boiled on a hot plate. The seeds were placed in the water then immediately removed from the hot plate. The seeds remained in the cooling water for 24 hours. In the hot sand treatment, seeds were covered by a minimum of 2 cm of sand in a tin container. The seeds were placed in a 100°C oven for 30 minutes and then removed from the oven and allowed to cool prior to plating. Following treatment, seeds were plated and stored in the cold room at 4°C as described previously. Following two or three months of stratification, the seeds were transferred to the greenhouse on September 8, 2015 or October 6, 2015, respectively. Seeds were kept in the greenhouse and checked for germination as previously described.

2016 Experiment

Given consistently low gemination rates in response to treatment in the two *Arctostaphylos* species, we conducted a third experiment in the fall of 2016 examining the effect of different concentrations of liquid smoke on *A. manzanita* and *A. viscida* germination. Seeds of both species were first soaked in concentrated sulfuric acid for six hours. Seeds were then treated with one of four liquid smoke concentrations (0%, 0.5%, 1%, 2%) for four hours. Seeds were plated on Hoaglands number two agar medium and placed under full spectrum growth lights (average PPFD: 100 µmol $m^{-2}s^{-1}$). For *A. manzanita*, 27 plates were prepared per treatment. For *A. viscida*, 24 plates were prepared per treatment. Each plate contained between 6 and 24 seeds. The seeds were checked for germination for three weeks. Seeds were considered to have germinated if the presence of a 2 mm radicle was observed.

Statistical Methods

Data were analyzed in R Version 4.0.2. Multiple logistic regression was used to determine the effect of treatments on germination. Models were created using the lme4 (1.1-23) and aod (1.3.1) packages. For each model, germination was the response variable. For the first experiment, two models were created. The *Arctostaphylos* model had the factors of species, acid, charate, and all potential interaction terms. The *Ceanothus* model had the factors of species, boiling water, charate, and all potential interaction terms. For the second experiment four models were created. The *Arctostaphylos* 2 months stratification model and the *Arctostaphylos* 3 months stratification model had the factors of species, acid concentration, exposure time, and all potential interaction terms. The *C. jepsonii* 2 month stratification model and *C. jepsonii* 3 month stratification model had the factor of heat treatment. For the third experiment one model was created that had the factors of species, liquid smoke, and the

interaction of these two treatments. The interaction effects were removed from models where there was no significant interaction, effect so the models would not be overfit.

Results

2013 Experiment

For the *Arctostaphylos* species there was a significant effect of acid treatment on the germination of the seeds ($p=0.035$; $CI_{0.95}$= -6.127,-0.695; Figure 1). For seeds undergoing acid scarification, the odds of germinating increased by a factor of 12.794, compared to seeds that were not exposed to acid. For *Ceanothus* species, there was a significant effect for boiling water ($p=0.003$; $CI_{0.95}$= -3.545,-0.782) and charate exposure ($p=0.024$; $CI_{0.95}$= 0.235,2.594), but no interaction effect for these treatments ($p=0.898$; Figure 2). The odds of germinating increased by a factor of 7.323 for seeds that were boiled, and by a factor of 3.800 for seeds exposed to charate, compared to the control. Emergence for *Ceanothus* was too low to perform statistical analysis (Table 1). Emergence for *Arctostaphylos* was even lower with only 3 seeds emerging out of 193 total seeds planted.

2015 Experiment

Seeds exposed to cold stratification for only two or four weeks did not germinate. Extending the stratification period to two and three months resulted in increased germination. However, for the *Arctostaphylos* experiments there were no significant differences between

species (p=0.345), acid concentration (p=0.572), or exposure time (p=0.109; Figure 3). For *Ceanothus jepsonii*, there was no significant effect of heating method (p=0.163; Figure 4).

2016 Experiment

For *Arctostaphylos* seeds exposed to liquid smoke there was a significant species effect ($p<0.001$; $CI_{0.95}$= 0.546,1.933; Figure 5). The odds of germinating increased by a factor of 3.387 for *Arctostaphylos viscida,* compared to *Arctostaphylos manzanita*. There was no significant effect of liquid smoke concentration on germination (p=0.285).

Discussion

Some treatments had a positive impact on germination rates, whereas others were ineffective at promoting germination in these trials. Both *Ceonothus* species had increased germination rates when exposed to boiling water and charate, supporting our hypothesis that heat shock and chemical cues promote germination in these species. These findings are consistent with previous studies that have found heat cues to play an important role in fire-dependent refractory seeds (Keeley and Fotheringham 1998, Wilkin et al. 2013). This makes sense given the structure of seeds in the Rhamnaceae. Seeds in this family have a thick seed coat, which prevents water and gas uptake (Stone and Juhren 1951). Intense heat can weaken parts of this impervious seed coat to break dormancy (Keeley 1991). In addition to heat, these results indicate that chemical cues also play an important role in *Ceanothus* germination. The chemicals found in charred wood can further break the seed coat and stimulate germination (Keeley et al. 1985).

Our hypothesis that acid scarification would promote germination in *Arctostaphylos* species was also supported by the germination trials. Exposure to acid mimics the process seeds would be exposed to in the digestive tract of herbivores. Fruits of plants in the Ericaceae have fleshy fruits that are eaten by mammals. The process of digestion by herbivores is important because it aids in seed dispersal (Karimi et al 2020). Acid exposure may be a prerequisite to germination to ensure proper dispersal is occurring. The acid also helps promote germination by weaking the seed coat (Keeley 1991). Concentrated and 90% sulfuric acid were equally effective in promoting germination in both *Arctostaphylos* species. *Arctostaphylos viscida* had significantly better germination rates than *A. manzanita*. One potential explanation for this result could be that *A. viscida* has larger seeds than *A. manzanita*. Contrary to our hypothesis, liquid smoke did not affect the germination rates in either species. This is inconsistent with previous research that has shown that liquid smoke does increase germination in other *Arctostaphylos* species (Laskowski et al. 2013). However, it may be possible that our study did not use high enough concentrations of liquid smoke. For *A. manzanita,* there is a nonsignificant trend of increasing germination rates at higher liquid smoke concentrations (Figure 5). Perhaps this trend continues at even higher concentrations, but further research would be needed.

Both *Arctostaphylos* and *Ceonothus* species had very poor germination when exposed to cold stratification periods of only two or four weeks. Germination rates were much higher when seeds were exposed to cold for two or three months after receiving their respective treatments, suggesting that a long stratification period is beneficial to germination. Cold stratification mimics the temperature difference that the seeds experience during the winter months and ensures that the seeds germinate after the cold temperatures have passed (Keeley 1991).

Although preliminary emergence experiments were a part of this study, there was not sufficient data for analysis. One potential reason for such low emergence rates could be that the radicles tended to be very brittle, making the transplanting process problematic. Another explanation could be that the humid greenhouse environment created challenging conditions for these species, which are adapted to drier habitats (Pratt et al. 2007). Future research would involve determining which factors best promote emergence and survival following germination. Although not compared directly, seeds grown in agar tended to have higher germination rates. This was also likely due to the moisture sensitivity of these species. Additionally, the switch to agar better protects the radicles and might lead to increased emergence. Another potential next step would involve field studies using these treatments to assess whether or not these laboratory procedures are effective and practical in restoration efforts.

Using *Arctostaphylos viscida*, *A. manzanita*, *Ceanothus cuneatus,* and *C. jepsonii* as restoration plants following disturbance can be more effective with knowledge on how to best germinate their seeds. The high intensity of fires can damage seeds in the seed bank and replanting these species can help them better recover from these disturbances. Using these practices can regrow populations quicker than planting untreated seeds. The enhanced recovery of dominate shrub species such as these can help accelerate the restoration of California chaparral ecosystems affected by climate change.

Acknowledgments

Funding was provided by the Weaver Fund (V. Fung) and the Barrett Undergraduate Endowment Research Award (K. McClain). I would like to thank Kati McClain, Vivian Fung, Cathy

Wheeler, and the students of the fall 2016 BL 157 course for their laboratory contributions to the experiments analyzed in this project. I would also like to thank Dr. Rebecca Drenovsky and Dr. Rebecca Fang for their guidance throughout the project.

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Table 1. Emergence (%) for *Ceanothus* species in 2013. Emergence (%) was calculated as the number of seeds emerged divided by the number of seeds planted and multiplied by 100.

Species	Treatment	% Emergence
C. cuneatus		
	Control	0.8
	Boiling water	15.8
	Charate	0.0
	Boiling water & charate	1.9
C. jepsonii		
	Control	2.2
	Boiling water	3.6
	Charate	1.1
	Boiling water & charate	1.1

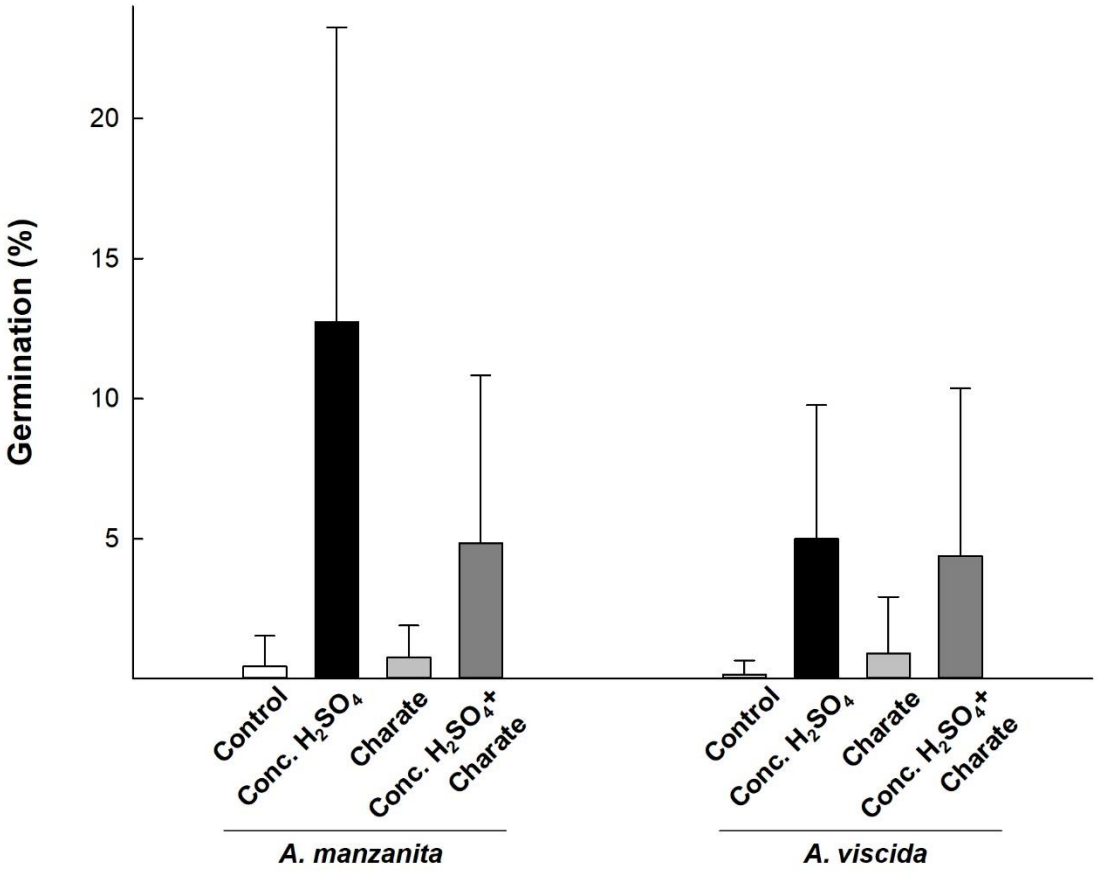

Figure 1. Germination (%) for *A. manzanita* and *A. viscida* in 2013. Data are means + SD (n=11).

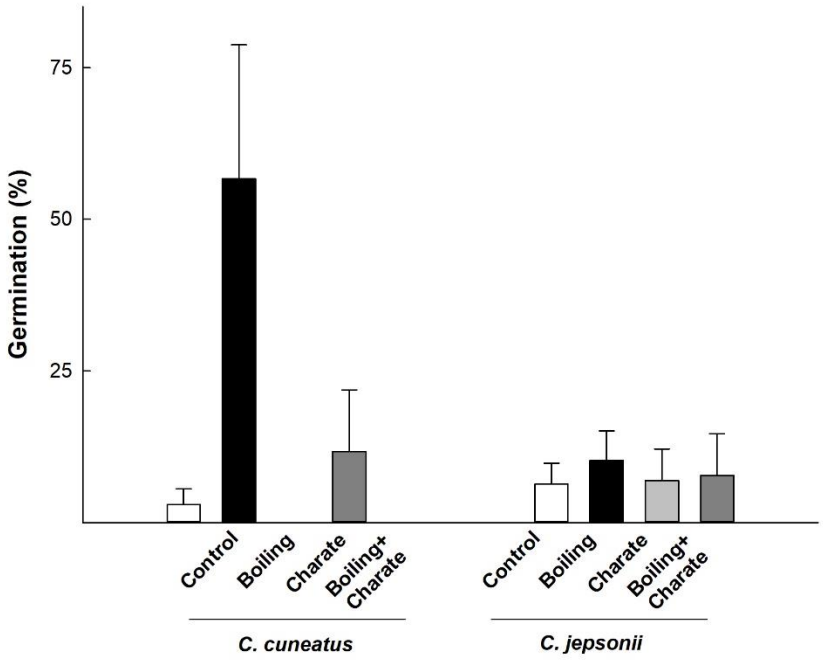

Figure 2. Germination (%) for *C. cuneatus* and *C. jepsonii* in 2013. Data are means + SD (n=6).

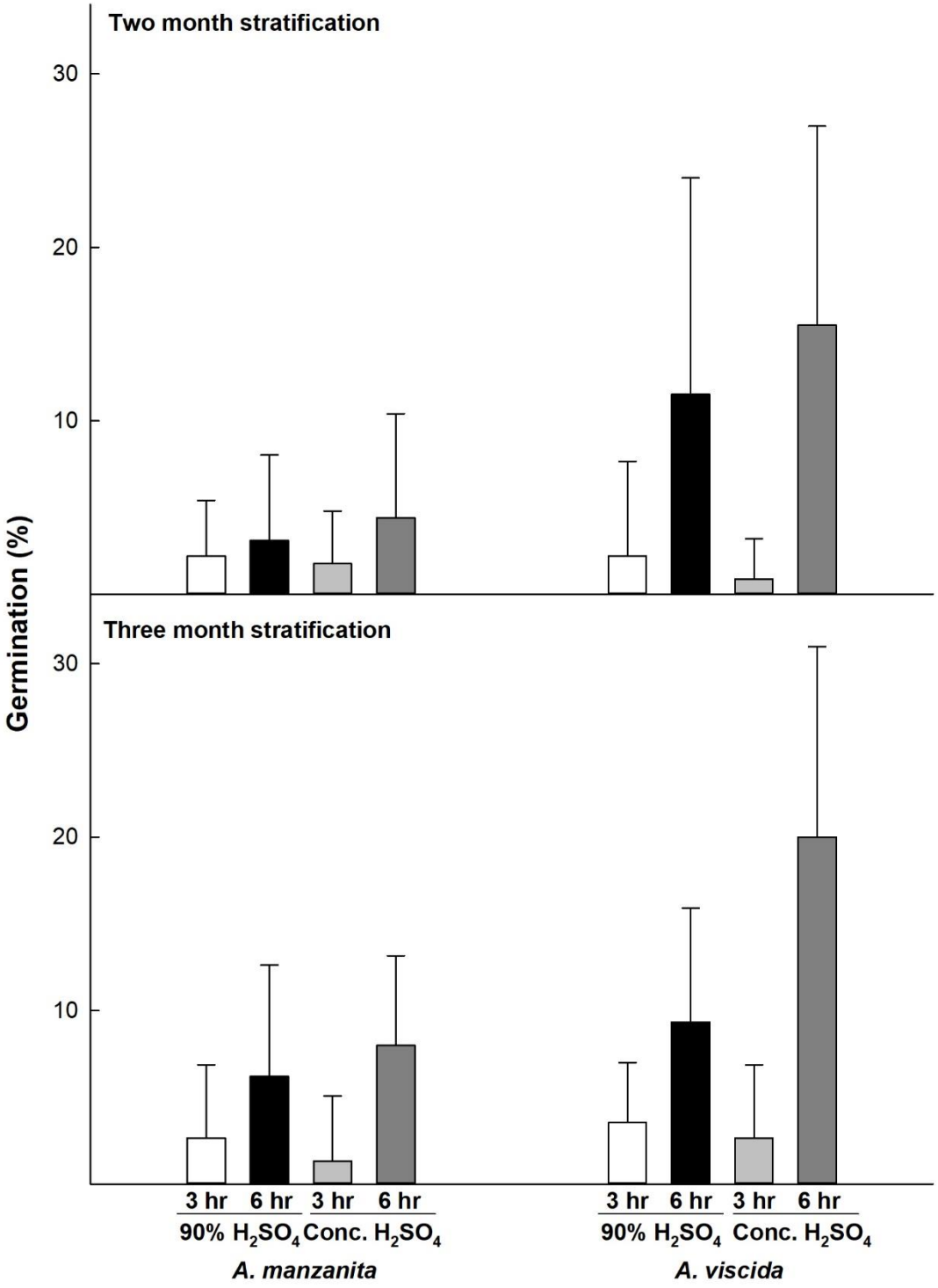

Figure 3. Germination (%) for *A. manzanita* and *A. viscida* in 2015. Data are means + SD (n=15).

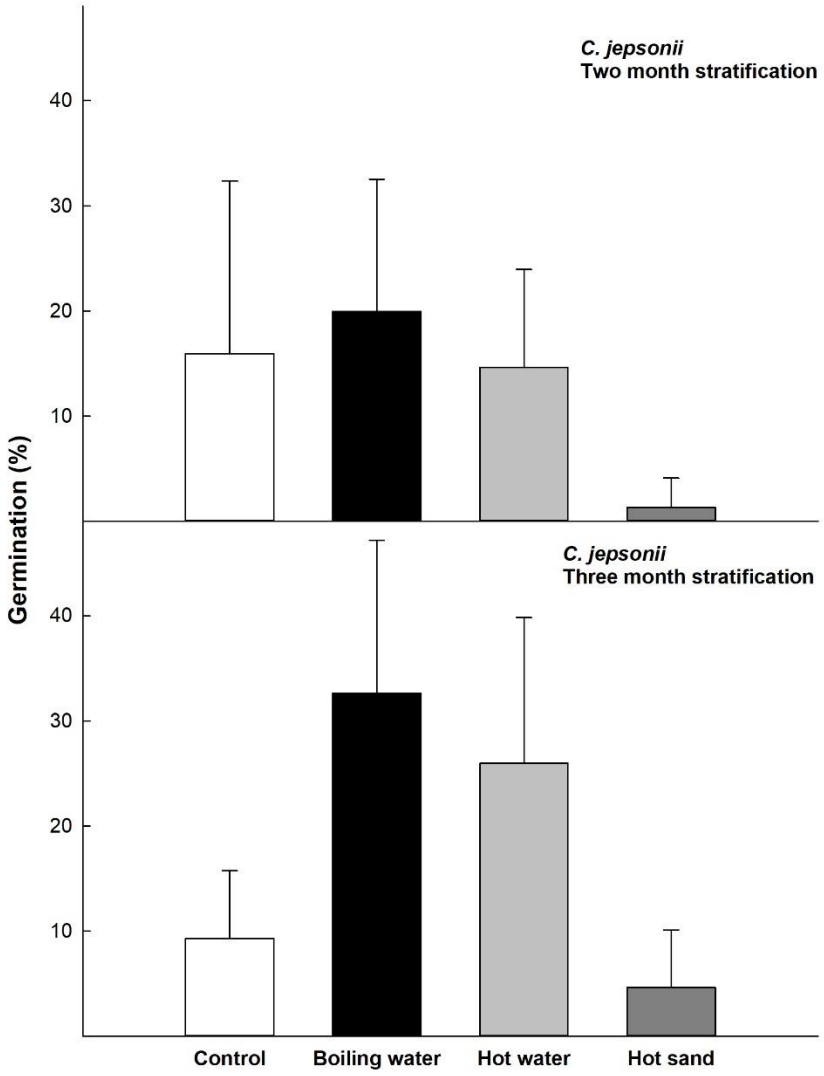

Figure 4. Germination (%) for *C. jepsonii* in 2015. Data are means + SD (n=15).

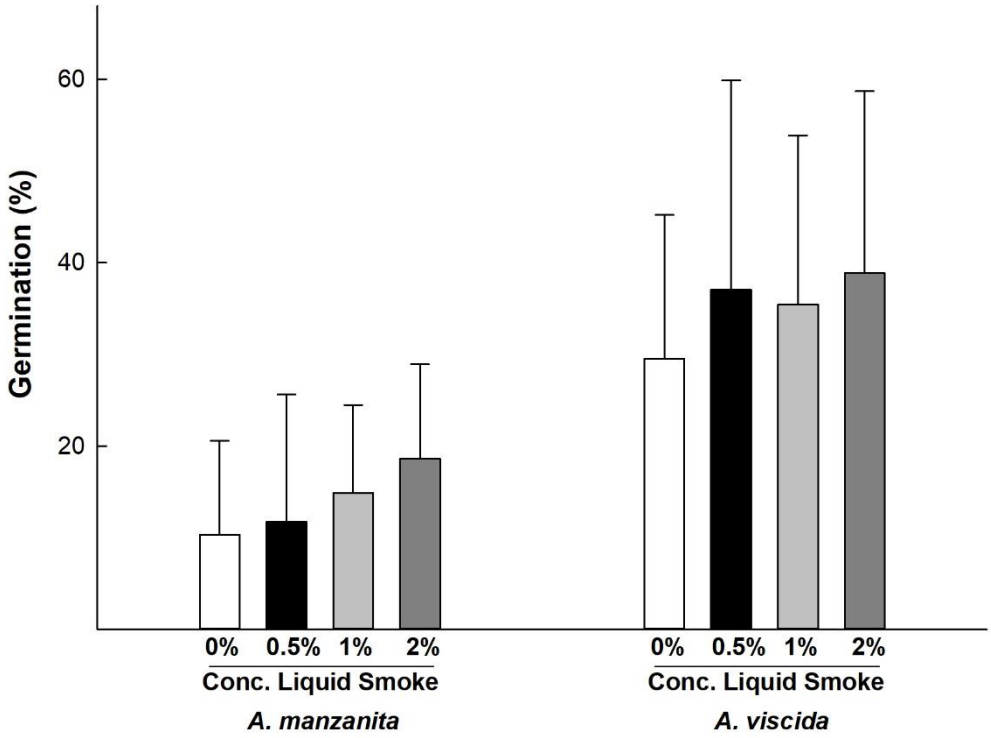

Figure 5. Germination (%) for *A. manzanita* and *A. viscida* in 2016. Data are means + SD (n=27 for *A. manzanita*, n=24 for *A.viscida*).