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WARM STRATIFICATION INCREASES GERMINATION OF DENDROMECON RIGIDA AND EHRENDORFERIA CHRYSANTHA

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WARM STRATIFICATION INCREASES GERMINATION OF *DENDROMECON*
RIGIDA AND *EHRENDORFERIA CHRYSANTHA*

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

by
Cesar Luis Garcia

June 2019

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ABSTRACT

We explored the seasonal factors that might play a role in triggering germination of *Dendromecon rigida* and *Ehrendorferia chrysantha*. *D. rigida* and *E. chrysantha* have been found difficult to germinate using common greenhouse techniques, Keeley and Fotheringham successfully germinated both species after storing their seeds in the field over a year and treating them with smoke. Identifying the specific seasonally changing factors that stimulated germination could have implications for understanding germination requirements for these and other hard-to-germinate chaparral species.

Dendromecon rigida and *Ehrendorferia chrysantha* are part of the Papaveraceae family and both are found in the chaparral environment in Southern California. Both species are known to increase in numbers after fire events. Both are believed to have morphophysiological dormancy based on their miniscule embryo and increase in seedling presence after fire events. Climate within the chaparral environment consists of hot, dry summers and cold, wet winters.

Storing seeds of *D. rigida* and *E. chrysantha* in the field over winter and spring months resulted in increasing germination for *D. rigida* seeds that were imbibed in smoke-water. Germination of *D. rigida* seeds occurred within six weeks and no further germination was noted beyond that. *Ehrendorferia chrysantha* seeds failed to germinate in the field.

Lab studies tested effects of stratification at different temperatures (5°C, 10°C, 18°C, 25°C and 30°C), stratification for different durations (0, 2, 4, 8, and 12 weeks), heat-shock, and fluctuating moisture and temperature conditions on a weekly time scale, on germination of both species. Of all these treatments the only combination that was effective in germinating seeds of *D. rigida* and *E. chrysantha* was warm stratification at 30°C for 8 weeks following smoke-water imbibition. Under these conditions seeds of *D. rigida* and *E. chrysantha* germinated to 10% and 9.3%, respectively.

Further studies on *D. rigida* indicated a stratification temperature optimum between 30-40°C with germination increasing with lack of light. These tested conditions corresponded to the daily peak soil temperatures measured at shallow depth in an area of chaparral inhabited by *D. rigida*. The period immediately after the first rain event after a fire may provide the chemical cues and warm stratification required to germinate buried seeds in this species.

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CHAPTER ONE

INTRODUCTION

Restoration ecologists and horticulturalists, alike, face challenges when dealing with native species that are difficult to propagate. Restoration ecologists try to restore disturbed sites using plants native to the area. Horticulturalists increasingly attempt to propagate native plants as interest in native plant gardens has increased. Both encounter difficulties when faced with a plant species with dormancy mechanisms that are poorly understood. Seed dormancy is the state at which minimal metabolism is occurring within the seed preventing it from developing into a mature plant even if given ideal conditions. Many seeds that are difficult to germinate require an intricate process to break seed dormancy. Extensive research has been done to investigate all the requirements a variety of seeds need to release them from dormancy (Baskin and Baskin, 2014, and references therein).

Seed dormancy allows plants populations to persist through unsuitable environmental conditions. Many plants produce a large quantity of seeds annually, not all of which germinate. Consequently, this produces a soil seed-bank that accumulates seeds until the requirements for various forms of dormancy are met and seeds germinate.

Despite all the research that has focused on understanding the types of seed dormancy, there are many plant species whose germination requirements

have yet to be identified. Two such plant species are *Dendromecon rigida* Benth. and *Ehrendorferia chrysantha* (Hook and Arn.) Rylander. These two species are perennial plants commonly found growing in post-fire areas in southern California chaparral. Both species have been difficult to germinate with common greenhouse techniques even when fire cues were applied (Keeley and Fotheringham, 1998b). Both species are believed to have morphophysiological dormancy, characterized by underdeveloped embryos and the need for an environmental trigger. Seedlings of both species are more numerous after fire events.

In this chapter, I review treatments that have been successful to increase the germination of chaparral plants with seed dormancy and of other similar ecosystems with common ecological traits. I will introduce the types of dormancy that have been studied, dormancy classification schemes, and studies performed on species exhibiting similar dormancy to our plant species of interest.

Seed Dormancy

A living and mature seed that is not germinating may be either in a quiescent or dormant state. Quiescent seeds (as defined by Jann and Amen, 1977) are in a suspended state of growth awaiting favorable environmental conditions such as sufficient moisture, favorable temperature or adequate oxygen levels. Dormant seeds, on the other hand, are suspended from growth even if favorable environmental conditions are present. Dormancy is a state in which seed germination is suppressed, even if seeds are exposed to favorable

conditions for growth (Escombe, 1897; Khan, 1977). Seeds with dormancy require additional environmental cues and/or physiological changes for germination to occur. Consequently, until these requirements are met, germination is inhibited. Seeds with delayed germination could potentially survive intolerable conditions and may synchronize germination after disturbance events, at appropriate times, to maximize seedling survival. Minimal respiration occurs during this state of dormancy (Crocker, 1916). Dormant seeds require a physiological or physical change to initiate germination.

The state of dormancy can be classified as either exogenous or endogenous dormancy. Exogenous dormancy is caused by chemicals or seed structures that prevents germination. Endogenous dormancy is caused by characteristics of the embryo that prevents germination. Physical and physiological properties of seeds known to induce dormancy include an impermeable seed coat and abscisic acid within the seed or pericarp (Khan, 1977). Impermeable seed coats are an advantageous trait that prevents seed desiccation and allows seeds to endure droughts of long durations. Breakage of the seed coat triggers germination by allowing water to hydrate the inner layers of the seed. Seed coats can be broken by rock abrasions during flash flood events and similar actions of force. Germination triggers for physiological properties include stratification, exposure to selective hormones, temperature fluctuations, and light fluctuations (Khan, 1977). Stratification is a pre-germination treatment done by storing seeds for a period in cold or warm temperatures in

moist conditions. This triggers a physiological change within seed embryos that initiates germination. Selective seed-germination hormones that have been used to promote germination are cytokinin and gibberellin. These promoters play a role in increasing RNA production and DNA replication in embryo tissue (Baskin and Baskin, 2014).

Several authors have created various categories and keys to classify dormancy types based on different physical and physiological seed properties. Characterizing the type of dormancy in seeds gives ecologists a framework for understanding factors that control seed dormancy. Understanding these factors benefits propagation programs that depend on the techniques to propagate seeds.

Five frequently cited dormancy classification systems are briefly summarized below and in Table 1. Crocker (1916) characterized the morphological causes of dormancy as seeds having rudimentary embryos, complete inhibition of water absorption, mechanical restriction of embryo growth, seed structures affecting gas diffusion, or a state of dormancy in the embryo itself or some organ of it, even if given germinative conditions. He noted that combinations of two or more of these causes can exist, and that seeds are capable of entering a secondary dormancy. Crocker (1916) distinguished these five functional types of dormancy with anticipation that with new knowledge, this classification would be modified. Harper (1957) categorized dormant seeds based on when dormancy was acquired: innate, enforced, and induced (see

Table 1 for definitions). These three categories became too restrictive to describe the diversity of dormancy patterns and causes as new dormancy types were studied (Baskin and Baskin, 2004). Lang (1987) categorized dormant seeds based on their physiology: endodormancy, paradormancy and ecodormancy (see Table 1 for definitions). This system was purely based on physiology which excluded the seeds with underdeveloped embryos and impermeable seed coats (Baskin and Baskin, 2004). Nikolaeva (1977) classified dormant seeds based on the seed coat permeability, embryo morphology and whole seed physiology (Table 1). Nikolaeva acknowledged Crocker's systematic investigation of dormancy as important, but it became inadequate as new information of dormancy accumulated (Nikolaeva, 1977). Baskin and Baskin (2014) created a newer hierarchical classification in which they adopted Nikolaeva's classification and updated the classification to include six hierarchical levels; division, subdivision, class, subclass, level and type;. This hierarchical classification accommodates the variation of seed dormancy found in nature and allows for new additions as factors affecting dormancy become better understood (Table 2).

Table 1. Summary of dormancy classifications from different authors.

Harper's Classification of Dormancy	
Dormancy Type	Definition
Innate	Dormancy of seeds that exists as seeds are dispersed from the mother plant
Enforced	Prevention of seed germination by an unfavorable environment (= quiescent seeds)
Induced	Seeds that acquire dormancy after dispersal from the parent plant
Lang's Classification of Dormancy	
Dormancy Type	Definition
Endodormancy	Dormancy regulated by physiological factors inside the affected structure
Paradormancy	Dormancy regulated by physiological factors outside the affected structure
Ecodormancy	Dormancy regulated by environmental factors
Nikolaeva's Classification of Dormancy	
Dormancy Type	Definition
Endogenous	pertaining to specific anatomical, morphological or physiological peculiarities of the embryo itself
Exogenous	pertaining to various physical or chemical properties of seed coats
Combination	Containing both endogenous and exogenous dormancy

Source: Crocker, 1916; Harper 1957; Lang, 1987; Lang et. al. 1985, 1987; Nikolaeva, 1977; Baskin and Baskin, 2004, 2014

Table 2. Summarized version of Baskin and Baskin's (2014) dormancy classification scheme. Classifications below "Level" are not displayed

Division 1. Imposed Dormancy – quiescent seeds	
	No subcategories
Division 2. Organic Dormancy	
Subdivision 1. Exogenous	
	Class 1. Physical dormancy
Subdivision 2. Endogenous	
	Class 2. Morphological Dormancy
	Class 3. Physiological Dormancy
	Subclass 1. Regular
	Level 1. Non-deep
	Level 2. Intermediate
	Level 3. Deep
	Subclass 2. Epicotyl
	Level 1. Non-deep
	Level 2. Deep
	Class 4. Morphophysiological Dormancy
	Subclass 1. Simple
	Level 1. Non-deep
	Level 2. Intermediate
	Level 3. Deep
	Level 4. Non-deep epicotyl
	Level 5. Deep epicotyl
	Level 6. Deep simple double
	Subclass 2. Complex
	Level 1. Non-deep
	Level 2. Intermediate
	Level 3. Deep
	Class 5. Combinational
	Level 1. Non deep
	Level 2. Intermediate
	Level 3. Deep

Source: Baskin and Baskin, 2014

Baskin and Baskin (2014) identified the following dormancy classes: physical, physiological, morphological, morphophysiological dormancy and combinational. Physically dormant seeds have impermeable seed coats that

require a type of physical abrasion or chemical dissolution of the outer layer. The removal of the seed coat makes the seed permeable to water and gases, and sensitive to light (Khan, 1977). One example of scarifying seeds is by using sulfuric acid or dipping the seeds in boiling water (Li *et al.*, 1999). Boiling water does not change the anatomy of the seeds, but makes the seeds more permeable to water. Physiological dormancy is caused by a physiological inhibiting mechanism that prevents radicle emergence (Baskin and Baskin, 2014). An underdeveloped embryo causes morphological dormancy. Morphophysiological dormancy is a combination of both physiological and morphological dormancies. For seeds that do not fall under a specific dormancy category, they are referred to as having combinational dormancy. Furthermore, the degree of dormancy varies between species and even individuals within the species (Baskin and Baskin, 2004). Therefore, the treatments used to break dormancy have potential to produce inconsistent results when used repeatedly.

Within the levels of physiological dormancy, the main difference of treatments are the amount of time needed for stratification, temperature requirements and storage time (Baskin and Baskin, 2004, 2014). Seeds with deep physiological dormancy cannot germinate with only gibberellic acid, instead they require an additional 3-4 months of cold stratification, but even then, excised embryos tended to show abnormal seedlings (Baskin and Baskin, 2004). Seeds with intermediate physiological dormancy require 2-3 months of cold stratification to germinate, excised embryos tended to show normal seedlings, and most

seeds can be germinated by gibberellic acid. Seeds with non-deep physiological dormancy require either cold or warm stratification depending on species, gibberellic acid to promote germination, and scarification may promote germination (Baskin and Baskin, 2004). Morphologically dormant seeds only require time, sufficient moisture, adequate temperatures and light/dark conditions for the embryo to grow (Baskin and Baskin, 2014). Seeds with morphophysiological dormancy require 1) a type of physical or chemical treatment to break hard seed coat, 2) a dormancy-breaking treatment to initiate embryo growth, and/or 3) time to allow for the embryo to grow (Baskin and Baskin, 2004, 2014).

The key to identifying the germination requirements of morphophysiological dormant seeds is to examine the environmental conditions in the seeds' natural habitat (Hidayati, 2012). Hidayati et. al. (2012) found that wet/dry cycles and exposure to smoke stimulated germination in two species with underdeveloped embryos from a Mediterranean climate. In such semi-arid climates, fires are common and rainfall, although seasonal, may be sporadic. Examining the ecological and environmental conditions in a species' natural habitat is generally a useful starting place for exploring the germination requirements of a species.

Although understanding and characterizing the biology of seed dormancy both for ecological and agricultural/horticulturalist reasons is important, there is much that is unknown. The various attempts of classification schemes illustrate

the complexity of seed dormancy. There is much yet to discover about the mechanisms and ecology of seed germination.

Reproduction and Persistence in Southern California Chaparral

California chaparral occurs in a region with Mediterranean-type climate, experiencing long, hot summers and cold, wet winters. Fire has historically occurred in the chaparral normally after the summer months. Many plant species have evolved a close relationship with fire requiring, to some degree, a fire to germinate and grow. This relationship with fire has prompted attempts to break dormancy in chaparral species by replicating fire-cues in controlled environments (Brown and van Staden, 1997; Keeley, 1987; Keeley and Fotheringham, 1998a, 1998b; Sweeney, 1956; Thanos and Rundel, 1995; van Staden *et al*, 2000).

Chaparral is known for having many species with long-lived soil seed banks that respond readily after fires. The types of dormancy-breaking treatments that have been investigated have included exposure to smoke, smoke-water, specific chemicals in plant derived smoke (e.g. nitrous oxide, karrikins, and butenolides), heat-shock, and leachate from charred wood (Brown and van Staden, 1997; Chiwocha *et al.*, 2009; Keeley, 1987; Keeley and Fotheringham, 1998a, 1998b; Light *et al.*, 2009; Nelson *et al.*, 2009; Sweeney, 1956; Thanos and Rundel, 1995; van Staden *et al*, 2000). Smoke-water consists of an aqueous solution containing compounds found in smoke. Heat-shock treatment on seeds is performed by immersing the seeds in high temperatures

(heating oven or near-boiling water) short enough not to kill the seeds (Keeley and Fotheringham, 1998b).

These various approaches to experimental dormancy-breaking in chaparral species are grounded in an appreciation for the ecological importance of fire in Mediterranean climate ecosystems.

Importance of Fire in Chaparral

Fire plays an important role in the diversity and succession of chaparral vegetation. Composition of mature chaparral stands varies geographically in California but *Adenostoma fasciculatum* is the most widespread species (Hanes, 1971). Other dominant genera include *Quercus*, *Arctostaphylos*, *Ceanothus*, *Heteromeles*, *Rhamnus*, *Cercocarpus*, *Prunus*, *Yucca*, and *Rhus*. Fires have historically occurred every 20-70 years in chaparral (Keeley and Fotheringham, 2001; Minnich and Chou, 1997). After a fire event in chaparral, the entire landscape is usually burned down to the ground. This produces an open plain for species with seeds stored in the soil seed bank, mainly annual wildflowers and small perennial sub-shrubs, along with resprouters, to recolonize the landscape (Hanes, 1971; Keeley *et al*, 1981). The first few years following a fire, plant diversity is high but decreases as species with fire-related requirements to germinate dwindle (Keeley and Zedler, 1978; Keeley *et al*, 1981). Most of the species that recolonize the landscape require a fire cue for germination, either by fire scarification or some type of chemical produced directly by the fire. These plant species are considered to be “fire-recruiters” (Keeley, 1991).

Many plants species exhibit mechanisms that allow them to persist after disturbances such as fires. Noble and Slatyer (1980) identified three possible mechanisms that allow plants to persist after a disturbance; 1) propagule based mechanism, 2) vegetative based mechanism and 3) combination and hierarchies of mechanisms. Plants with propagule based mechanisms produce seeds that survive past a disturbance by either germinating immediately after the disturbance or are stored in the seed bank until conditions are ideal. Plants with vegetative based mechanisms have the ability to resprout from underground organs or have thick bark that prevents damage to the plant (Noble and Slatyer, 1980; Keeley and Zedler, 1978). Most chaparral plant species cope with fire disturbance and persist after fire by either resprouting from underground root crowns or by germinating from the soil seed bank, (Keeley and Zedler, 1978; Jepson, 1916, 1939). Jepson first classified different responses of chaparral shrubs to fire. Shrub species that survived fire through underground organs, he named empyrophytes, and species that died in fires and regenerated through abundant seed germination after fire, he named daptophytes (Jepson, 1916 1925). The latter species are now more commonly called obligate seeders and typically require fire cues for germination (Keeley and Zedler, 1978). Wells (1969) further identified the sprouting behavior on *Arctostaphylos* and *Ceanothus* species a primitive trait suggesting the “obligate-seeding” trait is an evolutionary response to frequent fire events.

Chaparral species that only germinate after fire may be eliminated from a site if 1) the fire kills the plant, 2) the fire depletes the soil seed bank, and 3) a second fire occurs before the plant can set seed and reestablish in the soil seed bank. For this reason, the time between fire events influences the ability of chaparral plants to respond. Within a normal fire cycle, stands of chaparral would be able to achieve the mature stage of long-lived shrubs. Any shorter fire-return interval could potentially change the composition of species by depleting the soil seed bank.

Many studies on obligate seeder chaparral species have been done to examine the requirement for fire cues in order to germinate (Brown and van Staden, 1997; Keeley, 1987, 1991; Keeley and Fotheringham, 1998b). Some of these fire cues include seed scarification through intense heat, promotion of germination by exposure to leachate from charred wood, smoke and smoke chemicals (eg. Keeley and Keeley, 1989; Keeley and Fotheringham, 1998a; Thanos and Rundel, 1995; Van Staden *et al*, 2000). Chaparral seeds that require a fire cue to germinate have been called “refractory seeds” (Sweeney, 1956).

Despite attempts to break dormancy using fire-cues, there are many species that have failed to germinate in the lab environment. They have a complex set of germination requirements that are still not well understood. Keeley and Fotheringham (1998b) had no success germinating *Dendromecon rigida* Benth., *Ehrendorferia chrysantha* (Hook. and Arn.) Rylander, *Trichostema lanatum* Benth. and *Phacelia brachyloba* (Benth.) A. Gray using smoke-water

treatments and heat shock. However, they found that after long-term soil-storage and smoke treatment, germination increased up to over 23% for *D. rigida*, 77% for *E. chrysantha*, and 33% for *T. lanatum*. Seeds stored in soil for extensive periods may experience many conditions that can promote germination, such as time length, temperature fluctuations, and moisture fluctuations. *D. rigida* and *E. chrysantha* are believed to have morphophysiological dormancy based on their underdeveloped embryos and response to smoke (Berg, 1966, 1967; Bullock, 1989; Van Staden et. al, 2000). Long-term soil storage could simply provide sufficient time for the embryo to develop, but seasonal environmental fluctuations could be the main trigger to promote germination. In the following studies, I explored seasonal factors involved in breaking dormancy in these species. Due to limited seed availability of *T. lanatum* at the time of this study, this investigation focused on the germination requirements of *D. rigida* and *E. chrysantha*.

The Ecology of the Study Species

Dendromecon rigida (bush poppy) is a woody, evergreen, chaparral shrub from 1 to 3 meters tall found along dry slopes and washes throughout cismontane shrublands in California below 1900 meters in elevation (Baldwin et.al., 2012). Plants contain simple leaves and terminal inflorescences with single, yellow, four-petal flowers. *D. rigida* can be found flowering as early as March and as late as September. Population dynamics are greatly affected by fire-occurrences. There is an increase in individuals within post-fire areas indicating a strong relation to fire-cues. This species is generally considered an

obligate seeder, dying during fires and recruiting from seed after fire (Bullock, 1989). Wells (1962) and personal observations, however, suggest *D. rigida* is a facultative seeder, occasionally resprouting from root crowns (Figure 1).



Figure 1. *Dendromecon rigida* resprouting after fire. This *D. rigida* individual was found in the Cajon Pass (San Bernardino County) within the Blue Cut fire perimeter on April 7, 2017. This picture was taken 19 months after the fire swept through the area. Other individuals in the area were also observed to be resprouting.

Seeds contain an eliasome that facilitates for seed dispersal by ants (Berg, 1967; Bullock, 1989). The seeds require a fire-cue to germinate (Bullock, 1989; Keeley and Fotheringham, 1998b) and due to their rudimentary embryo, we believe these seeds have a morphophysiological dormancy.

Ehrendorferia chrysantha (golden eardrops) is a short-lived herbaceous perennial from 0.5 to 1.60 meters tall found along dry slopes below 2300 meters in elevation (Figure 2; Baldwin *et.al.*, 2012). Plants contain pinnately compound leaves and the inflorescences are pannicles of yellow flowers. *E. chrysantha* can be found flowering as early as March and as late as September (Baldwin *et.al.*, 2012). It is an obligate seeder, abundantly found months after fire events, but populations decline the following years after fire. Seeds have small, linear, underdeveloped embryos and a requirement of fire cues for germination (Keeley and Fotheringham, 1998b; Van Staden *et al.*, 2000).

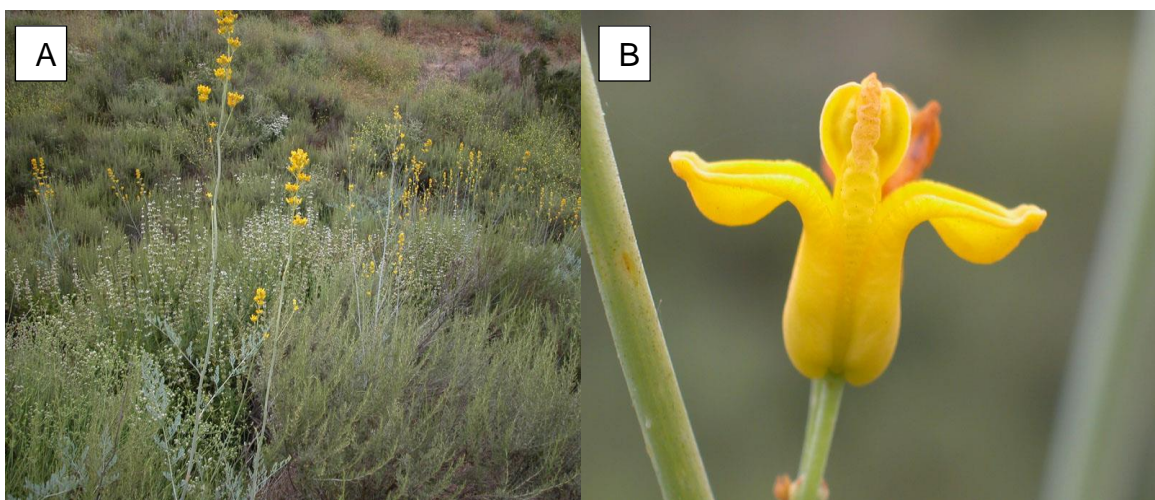


Figure 2. Representative photos of *Ehrendorferia chrysantha*, whole plant (a) and flower (b).

Thesis Overview

Chapter two presents a pilot study on seed germination responses and field conditions during winter and spring months. Chapter three demonstrates controlled lab studies on germination response to various temperature and moisture regimes. Chapter four provides the characterization of environmental conditions during fall months.

CHAPTER TWO

PILOT STUDIES: FIELD CONDITIONS AND RESPONSES

Introduction

Seeds are exposed to a wide array of environmental conditions in nature that are difficult to replicate in a lab setting, such as varying moisture conditions, fluctuating temperatures, fluctuating light levels, varying soil pH levels, and fluctuating oxygen. This can lead to unsuccessful attempts to germinate seeds in greenhouse and lab settings. Keeley and Fotheringham (1998b) had minimal success germinating *Dendromecon rigida* and *Ehrendorferia chrysantha* seeds using known dormancy-breaking pre-treatments. Only after storing the seeds in soil in the field for one year and treating the seeds with aerosol smoke did the seeds germinate, achieving approximately 23 and 77 percent germination for seeds of *D. rigida* and *E. chrysantha*, respectively. This chapter describes pilot studies conducted for the purpose of directing the design of lab studies to explore factors involved in breaking dormancy in *D. rigida* and *E. chrysantha*.

In the fall of 2014, we conducted a field experiment to determine the effectiveness of our techniques and the design parameters for performing lab studies. We tested the following questions:

- Could we replicate the results of Keeley and Fotheringham (1998b) with a shorter period of seed incubation in the field than one year, and could we use smoke water rather than aerosol smoke to stimulate seed germination?

- How early can initiation of germination in the field be detected?
- What are the temperature conditions seeds are exposed to during the winter and spring months (when moisture is available)?

In this pilot study, seeds were pre-treated with either smoke-water or de-ionized water and buried in the field in December, 2014. Seeds were retrieved from the field every 6 weeks over the following 18 weeks. Radicle emergence and embryo growth in seeds retrieved from the field were measured as indicators that dormancy had broken. Soil and air temperatures were also measured continuously to determine the conditions seeds are exposed to during winter and early spring.

Methods

Seed Collection

Seeds of both species were collected in July 2013 in Swarthout Canyon (elevation 3650 ft MSL) in San Bernardino County. Seeds from *D. rigida* and *E. chrysantha* were collected from 50 and 30 plants, respectively. The vegetation in the area is classified as Birchleaf mountain-mahogany- California buckwheat series with representative species of *D. rigida*, *Cercocarpus betuloides*, *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Prunus ilicifolia*, and *Fremontodendron californicum* (Sawyer and Keeler-Wolf, 1995). Through most of the 20th century, the collection site had experienced fire return intervals of 15-24 years. Fire had occurred more frequently in the 21st century (Table 3; CalFire FRAP Mapping).

Table 3. Fire history for seed collection site, Swarthout Canyon, San Bernardino County.

Fire Name	Year
Unnamed	1940
Cozy Dell	1964
Unnamed	1979
Louisiana	2002
Sheep	2009
Blue Cut	2016

Seed Imbibition

An initial imbibition study was done to determine if seeds of *D. rigida* and *E. chrysantha* would absorb water without being scarified and to determine the length of time required for maximal imbibition. This information was needed to ensure seeds would fully absorb the smoke-water before placing them in the field. Sets of 25 seeds were placed in petri dishes with de-ionized water over a period of 30 hours. Seeds sets were weighed every 2 hours for the first 8 hours, then again every 2 hours after 24 hours from the start of the imbibition. Seeds were patted dry with paper towels and placed on aluminum boats for weighing. After the mass was recorded the seeds sets were placed back into the petri dishes with de-ionized water (n=5 for *D. rigida*, n=3 for *E. chrysantha*). Percent imbibition was calculated by the following equation:

$$\text{Percent imbibition} = \frac{(\text{imbibed seed mass} - \text{dry mass})}{\text{dry mass}} * 100$$

Field Study Seed Treatments

Seeds of both species were imbibed on December 3, 2014 in either deionized water or 10% smoke water. Results from seed imbibition study were used to determine the amount of time required for imbibition methods. Smoke-water had been produced by bubbling smoke from burning dried *Adenostoma fasciculatum* cuttings through water for one hour. Seeds imbibed in smoke water were removed from the smoke water after 7 hours and rinsed with deionized water. This was done to prevent any over-exposure of smoke-water on the seeds. Seeds imbibed in deionized water remained in the treatment for the 7 hours. After seeds were fully imbibed, they were transferred into stainless steel mesh packets containing 30 seeds per packet. The deionized water and 10% smoke water solutions had pH levels of, approximately 5.5 and 4.8, respectively. Seed packets were stored in moist graded washed sand for two nights until the seeds were transferred into the field.

Burial of Seed Packets

The seed packets were transferred into the field on December 6, 2014 near the area of seed collection. Seed packets were buried 3 cm deep. Four seed-placement sites were chosen based on similarity of field conditions, open canopy and sandy soil. The soils series found in this site are both Soboba and Hanford series ("Soilweb," 2018). Soboba series are soils classified as sandy-skeletal, mixed, thermic typic xerofluvents formed in alluvium from predominantly granitic rock sources. Hanford series are soils classified as coarse-loamy, mixed,

superactive, nonacid, thermic typic xerorthents formed in moderately coarse textured alluvium dominantly from granite. At each site, we buried three replicates sets of each species-treatment combination (two species x two pre-treatment solutions). Each set consisted of four packets, one from each species-treatment combination, that were tethered to a central stake by buried wires to facilitate retrieval spaced a minimum of 20 cm apart. One group of packets from each location was retrieved from the field at each sampling date.

Seed Packet Retrieval and Germination Test

One seed packet per treatment per block was retrieved from the field at 6 weeks, 12 weeks, and 18 weeks after burial. Seeds were recorded for radicle emergence immediately after being removed from the seed packets. Five seeds per packet were chosen indiscriminately (20 seeds total) to be dissected and analyzed. Based on the percentage of germinated seeds, a fraction of the germinated seeds were included in the dissection proportional to the germinated fraction (see Table 4). Chosen seeds were bisected using a box cutter blade and the embryos were measured using a 40x compound microscope. The remaining 25 seeds were then placed into pots with a 50/50 mixture of graded washed sand and potting soil (Miracle Gro Potting Mix, .21N-.11P-.16K). Seeds from each treatment were combined into a single pot (100 total) to monitor subsequent germination. Pots were transferred into the campus greenhouse and the seed pots were monitored for three months after the last retrieval as shoots emerged above soil level.

Table 4. Seed dissection criteria

Number of germinated seeds	Number of germinated seeds dissected
< 6	0
7-12	1
13-18	2
19-24	3
25-30	4

Temperature Monitoring

Temperature loggers (two EL-USB-2 and one EL-USB-1, Lascar Electronics, Inc., Wiltshire, UK) were placed in the field on January 13, 2015 to track the temperature of the field conditions. Data loggers were not placed until 6 weeks after initial seed placement. One was placed 1.5 meters aboveground, in a shrub to track shaded air temperature (EL-USB-2), a second one was buried 3 cm under the soil surface in a canopy opening (EL-USB-1), and a third was buried 3 cm under the soil surface below a shrub canopy to track shaded soil temperature (EL-USB-2).

Results

Seed Coat Permeability

Seed mass increased and plateaued after 6-8 hours of being imbibed in de-ionized water (Figure 3 and Figure 4).

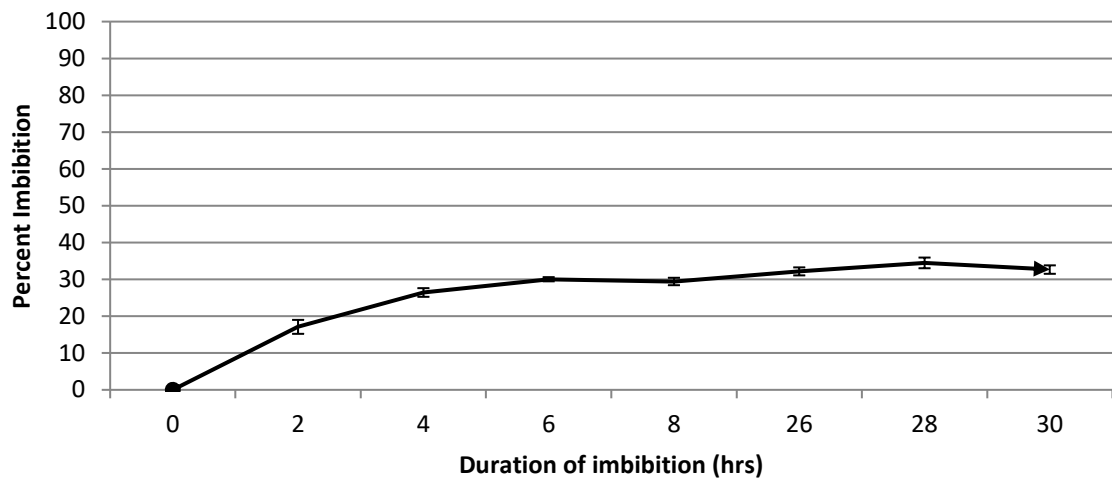


Figure 3. Percent imbibition in distilled water of *E. chrysantha* over a 30 hour period (mean \pm SEM, n=3).

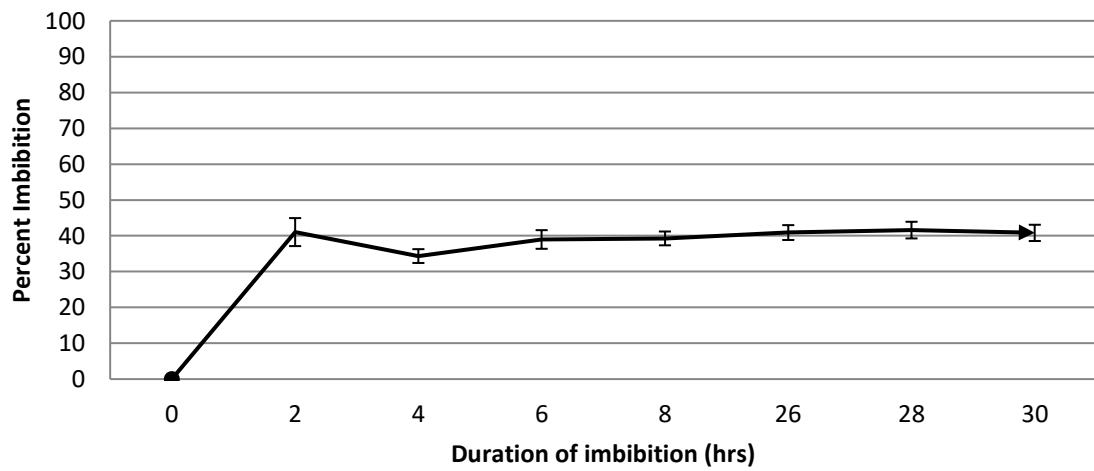


Figure 4. Percent imbibition in distilled water of *D. rigida* over a 30 hour period (mean \pm SEM, n=5).

Embryo Growth Measurements

After six weeks of field exposure, there was a clear difference between the embryo sizes in the *D. rigida* seeds that were imbibed in smoke-water and those in the seeds treated with de-ionized water (Table 5 and Figure 5).

Table 5. Two-way ANOVA results for *D. rigida* embryo length after field retrievals. (Two-way ANOVA, $p < 0.0001$).

	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio	Prob > F
Imbibition Treatment	1	0.97123130	0.9712313	14.5404	0.0002*
Weeks in the Field	3	0.67468440	0.2248948	3.3669	0.0209*
Imbibition Treatment x Weeks in the Field	3	0.73005233	0.2433508	3.6432	0.0147*

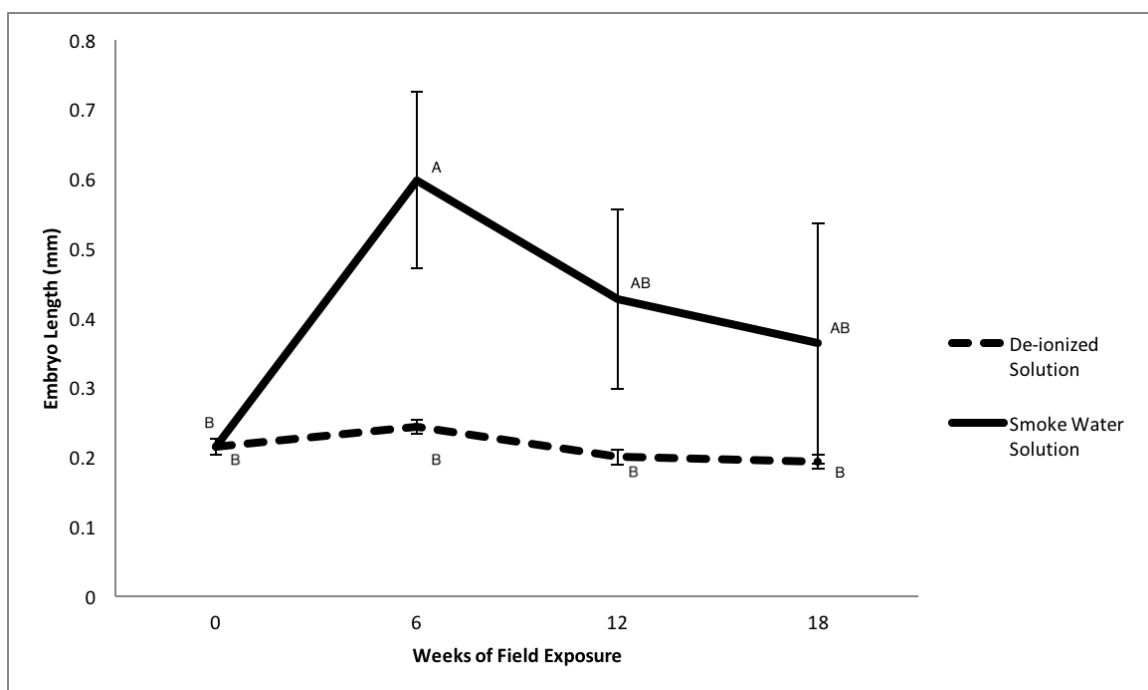


Figure 5. Average embryo length of *D. rigida* seed after field exposure (sample sizes are not equal due to degradation of seeds post-retrieval). Bars indicate standard error of the mean. Different letters indicate significant differences by Tukey-Kramer HSD.

The initial average length of *D. rigida* seed was 0.21 mm. *D. rigida* seeds treated with de-ionized water appeared to not have changed in length with field exposure and a minimal number of seeds were degraded (Figure 5 and Table 6). We defined a degraded seed as a non-viable seed in which the endosperm and embryo had become a pulp and discolored. The average embryo length of *D. rigida* increased during the first 6 weeks and decreased after that (Figure 5 and Figure 6).

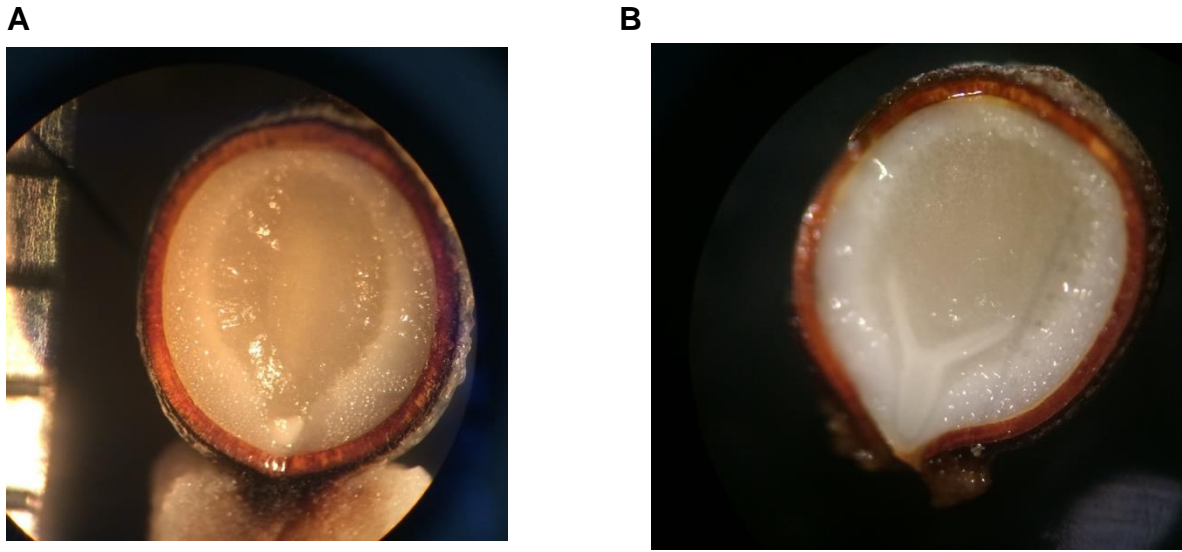


Figure 6. Longitudinal view of *D. rigida* seed pretreatment (a) and 6 weeks after field exposure (b). 40x magnification

Table 6. Number of intact *D. rigida* seeds at time of embryo length measurement. Degraded seed is a seed that the embryo and endosperm were a white mush.

Weeks of Field Exposure	Seeds imbibed in smoke-water (n=20)			Seeds imbibed in de-ionized water (n=20)		
	Embryo Present	Degraded Seed	N	Embryo Present	Degraded Seed	N
6	16	4	20	20	0	20
12	14	6	20	19	1	20
18	6	9	15	14	1	15

The time of field exposure did not affect the embryo length of *E. chrysantha* for either treatment (Figure 7, Figure 8 and Table 7). There was no difference in the degradation of *E. chrysantha* seed between the treatments (Table 8).

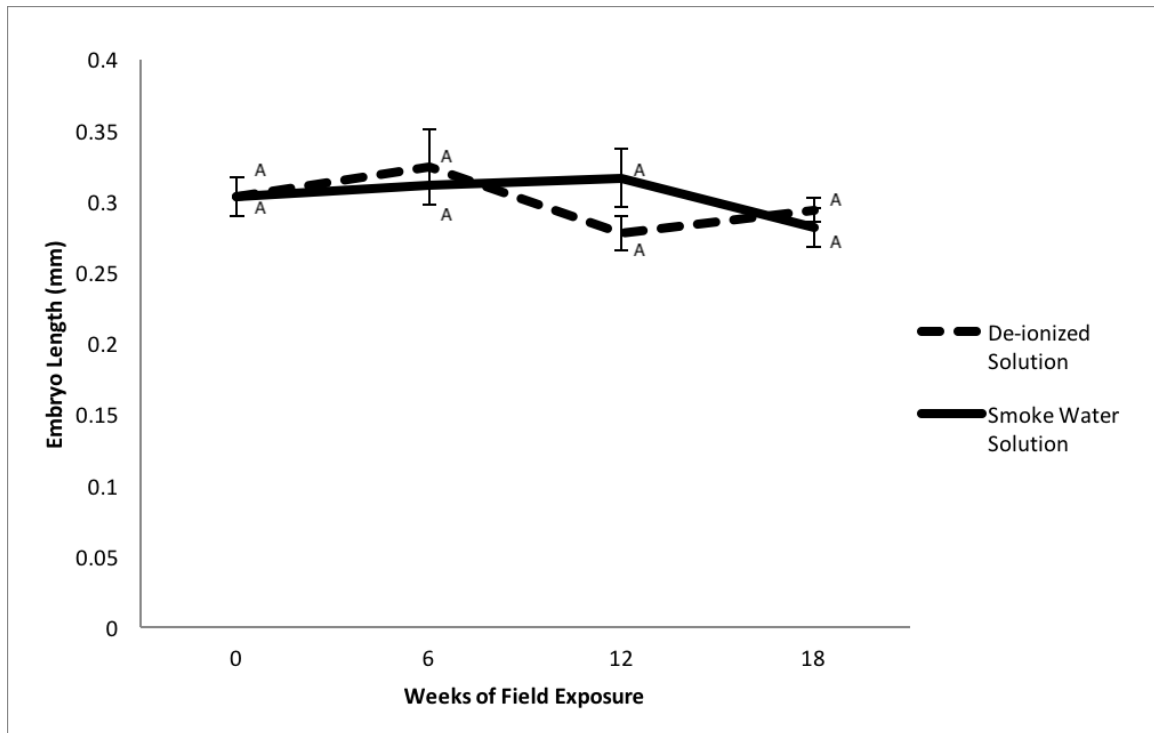


Figure 7. Average embryo length of *E. chrysantha* seed after field exposure (sample sized based on numbers from Table 7 of embryos present). Bars indicate standard error of the mean. Different letters indicate significant differences by Tukey-Kramer HSD.

Table 7. Two-way ANOVA results for *E. chrysantha* embryo length after field retrievals.

	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio	Prob. > F
Imbibition Treatment	1	0.00053312	0.0005331	0.1341	0.7148
Weeks in the Field	3	0.01501999	0.0050067	1.2593	0.2911
Imbibition Treatment x Weeks in the Field	3	0.01689341	0.0056311	1.4164	0.2409

Table 8. Number of intact *E. chrysantha* seed at time of embryo length measurement. Five seeds per location. Degraded seed is a seed that the embryo and endosperm were a white mush.

Weeks of Field Exposure	Seeds imbibed in smoke-water			Seeds imbibed in de-ionized water		
	Embryo Present	Degraded Seed	N	Embryo Present	Degraded Seed	N
6	20	0	20	18	2	20
12	19	1	20	20	0	20
18	11	4	15	11	4	15

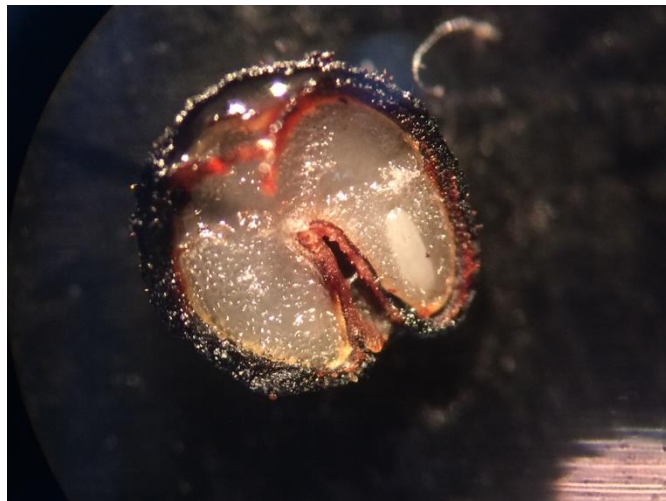


Figure 8. Longitudinal view of *E. chrysantha* seed before treatment and burial. No images of seeds after retrieval from the field are shown because no embryo growth was observed. 40x magnification

Germination of Seeds Post-Field Retrieval

There were significant differences in final germination for *D. rigida* between seeds imbibed in smoke-water and seeds imbibed in de-ionized water,

regardless of the length of time the seeds remained in the field (Table 9; Fisher's exact tests). Six weeks of field exposure produced 14% germination which was not significantly different from 12 and 18 weeks, 10% and 16% respectively. *D. rigida* seeds treated with de-ionized water did not germinate even after 18 weeks of field exposure. Neither *E. chrysantha* seeds imbibed in smoke-water nor de-ionized water germinated after any amount of field exposure.

Table 9. Final cumulative germination of *D. rigida* seeds after seed burial and subsequent planting in greenhouse (n=100 for weeks 6 and 12 and n=75 for week 18). Week 18 had a lower sample size due to vandalism of one of the replicates. Different letters indicate statistical differences among exposure times for seeds treated with smoke water (3 pairwise comparisons, Fisher's exact test, $p < 0.05$) and pairwise comparisons between treatments for each exposure time (3 comparisons, Fisher's exact test, $p < 0.05$).

Weeks of Field Exposure	Imbibition Treatment	
	Smoke-Water	De-ionized
6	14% ^a	0% ^b
12	10% ^a	0% ^b
18	16% ^a	0% ^b

Field Temperatures

Air temperature and shaded soil temperature exhibited a smaller temperature fluctuation compared to soil temperature in the open (Table 10). Generally, between the months of January and February soil temperatures in

direct sunlight dropped below 10°C during the night and usually reached between 20°C to 30°C during the day. Starting in March, the fluctuations of soil temperatures in the sun increased, exceeding 40°C during the day by late March and falling to approximately 10°C during the night (Table 10 and Appendix A).

Table 10. Soil and air temperatures in Swarthout Canyon, 2015. Values indicate means and ranges of hourly readings. Temperature loggers were buried 3 cm in the soil.

		Soil temperature – Full Sun (°C)	Soil Temperature – Under Shrub (°C)	Air temperature - Above Ground (°C)
January 13- 31st	Average (range)	9.1 (1.5-22)	9.6 (5.5-15)	11.5 (3-25)
February 1- 28th	Average	12.4 (1-31.5)	11.1 (4.5-18.5)	12.9 (1.5-29)
March 1- 31st	Average	16.6 (0.5-45)	13.7 (3.5-31)	14.8 (-0.05-30.5)
April 1- 12th	Average	17.5 (2-42.5)	14.7 (3.5-37)	11.1 (-0.05 -32.5)

Discussion and Conclusion

We achieved almost as high germination rates for *D. rigida* seeds as Keeley and Fotheringham (1998b; ~25%) did despite treating them with smoke water, rather than aerosol smoke and storing them in field conditions for six weeks, rather than a year. Seeds treated with de-ionized water did not germinate, consistent with their results of seeds only germinating with a smoke related trigger. There were no significant differences between germination

success for seeds exposed to six weeks, twelve weeks, and eighteen weeks of field conditions. By the time we retrieved embryos after six weeks of field exposure, they were as large as embryos retrieved after twelve and eighteen weeks. *D. rigida* seeds are capable of being germinated with a shorter treatment duration than anticipated from previous studies.

The apparent decrease in embryo size for *D. rigida* seeds after six weeks of field exposure could have been an artifact of embryos responding to the smoke water then degrading. If embryo growth and degradation are linked to smoke water stimulation, then larger embryos may have been selected out of the samples measured artificially decreasing embryo growth.

On the other hand, we did not achieve any germination of *E. chrysantha* seeds across all treatments tested. The reasons for these results were unclear. The seeds could have required a longer soil storage time to initiate germination and/or a different method of smoke application.

There were high temperature fluctuations observed in the field during the winter and early spring months. Seeds were exposed to temperatures as low as 0.5°C to as high as 45°C. Using historical average temperature records for the region and comparing them to NOAA's 1981-2010 temperature normals, we determined December 2014 through March 2015 were above normal in temperature months (Arguez *et al.*, 2018). Despite this being a relatively warm winter, temperatures approached freezing temperatures during multiple months. Since seeds are exposed to both near freezing temperatures and hot

temperatures during the moist months, either cold or hot temperatures could be the trigger for germination.

These pilot studies provided parameters for designing controlled lab studies that were aimed at identifying the germination requirements of *D. rigida* and *E. chrysantha*. Temperatures ranging from the single digits to the mid-thirties were realistic moist season temperatures to test as well as testing stratification durations as short as 6 weeks. These experiments are described in the following chapters.

CHAPTER THREE

GERMINATION RESPONSE TO SPECIFIC ASPECTS OF SEASONAL
TEMPERATURE AND MOISTURE VARIATION IN COMBINATION WITH FIRE
CUES

Introduction

Seeds in the field are exposed to a range of environmental conditions including temperature and moisture fluctuations, and various degrees of light exposure. A single factor or a combination of these conditions may be required to initiate germination. Each environmental condition acts upon the seed in different ways, such as causing a physical change of the seed (e.g. breaking the seed coat) or physiological change within the seed (e.g. initiating embryo growth). The order in which seeds become exposed to these environmental conditions can possibly be key for initiation of germination.

Investigators use various methods to replicate environmental conditions that seeds are exposed to in the field to initiate germination. Heat-shock and smoke exposure have been widely documented to initiate germination of chaparral plant species (Keeley, 1987). Storing seeds in cold or hot moist conditions have been shown to increase germination (cold and warm stratification; Baskin and Baskin, 2004). Furthermore, wet/dry cycling may stimulate germination (Hidayati et. al. 2012).

A fire event causes seeds to be exposed to a wide variety of environmental conditions, including high heat and exposure to smoke. Typically

fires occur in chaparral prior to the rainy season. This produces conditions for seeds present in the seedbank to be exposed to high heat and smoke before conditions may be ideal for germination. For seeds that require both fire cues and seasonally varying conditions, this raises the question whether the order of treatment application is important.

Our study was conducted under a controlled environment to investigate which pre-treatments would increase the germination of *D. rigida* and *E. chrysantha* seeds. Our study addressed the following questions:

- 1) Does cold or warm stratification increase germination of *D. rigida* and *E. chrysantha* seeds?
 - a. Does the length of stratification affect germination rates?
- 2) Does heat-shock promote germination?
- 3) Does the order of treatments affect germination rates?
- 4) Does wet/dry cycling increase germination rates?

Because smoke chemicals have been implicated as a germination cue for these two species (Keeley and Fotheringham, 1998b; Chap. 2), we included smoke-water treatments and their controls in each of the experiments designed to test the factors above.

Methods

Seed Collection

Seeds of both species were collected in July 2013 in Swarthout Canyon (elevation 3650 ft MSL) in San Bernardino County. Seeds from *D. rigida* and *E.*

chrysantha were collected from 50 and 30 plants, respectively. This area of collection is classified as Birch leaf mountain-mahogany- California buckwheat series with representative species of *D. rigida*, *Cercocarpus betuloides*, *Adenostoma fasciculatum*, *Eriogonum fasciculatum*, *Prunus ilicifolia*, and *Fremontodendron californicum* (Sawyer and Keeler-Wolf, 1995).

Effect of Cold Stratification Following Heat-Shock

Seeds of each species, *D. rigida* and *E. chrysantha*, were visually inspected for defects of the seed coat. Seeds that had insect damage, were hollow, or were significantly smaller than others were discarded. Sound seeds were sorted into plastic mesh packets with 50 seeds per packet. Packets of each species were exposed to one of four treatments: imbibition in 10% smoke-water, imbibition in deionized water, heat shock with de-ionized water, or heat shock with smoke-water (Figure 9). Heat shock consisted of boiling de-ionized water or smoke-water, removing the water from the heat and dipping the seeds for 1 minute. All seeds were then imbibed using the same protocol discussed in the previous chapter (refer to Field Seed Treatments).

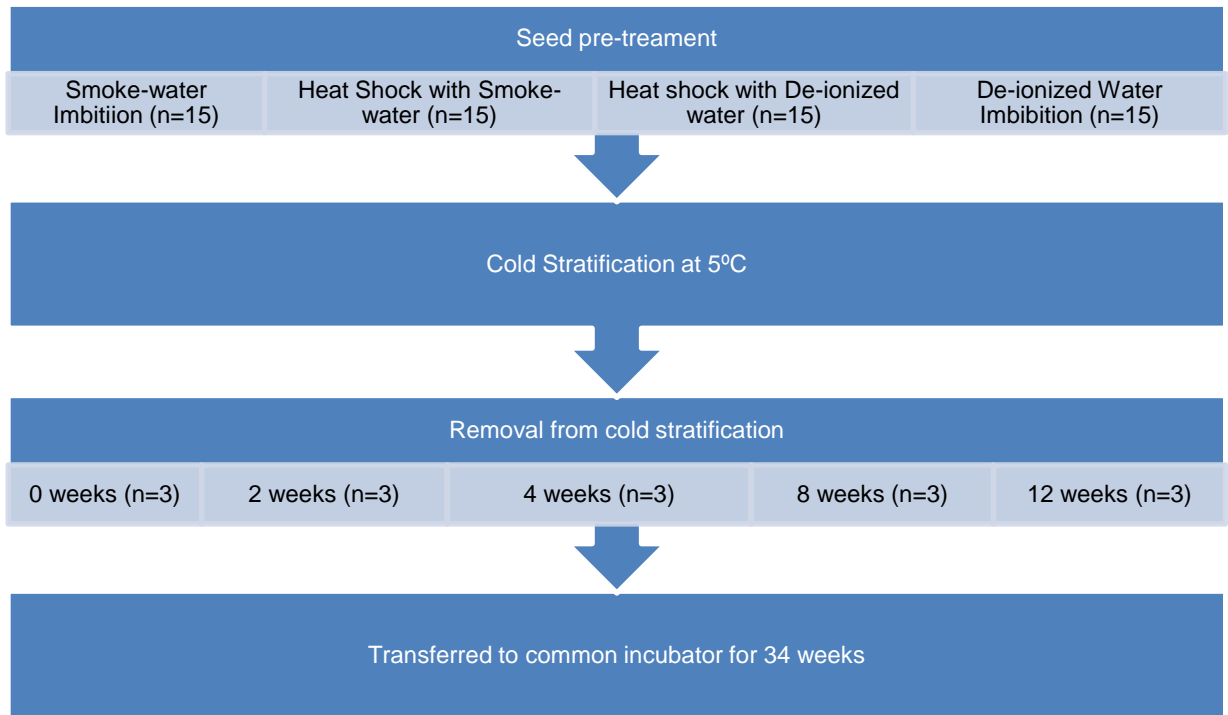


Figure 9. Schematic of heat shock experimental design. “N” indicates number of seed packets in each treatment combination at each step.

After the treatments, seeds were removed from packets and placed on 0.7% agar in petri dishes, 100mm diameter by 15mm deep, 50 seeds per dish, and wrapped with plastic wrapping to prevent evaporation. Seed plates were stratified at 5°C for; 0, 2, 4, 8 and 12 weeks in a refrigerator. Three replicate plates from each smoke and heat shock treatments were transferred into a common plant incubation chamber (Model: RF 2015, VWR, Radnor, PA) at the end of each cold-stratification period. The plant incubation chamber had daily cycles of 11 hours of light and 13 hours of darkness and 18°C/7°C, conditions that simulated average early springtime conditions in southern California

(Astronomical Applications Department, 2017; Arguez *et al.*, 2018). Germination was scored every week following transfer into the common plant incubation chamber. Monitoring ceased after thirty-four weeks of beginning of experiment.

Effects of Moist Warm Stratification and Smoke-Water Imbibition on Seed

Germination

Prior to pre-treatments, 3600 sound seeds of each species, *D. rigida* and *E. chrysantha*, were sorted into mesh packets with 50 seeds per packet. The packets were imbibed in 10% smoke-water or de-ionized water (refer to Field Seed Treatment). After the imbibition, 50 seeds of each species were placed on 0.7% agar in petri dishes and then wrapped with plastic wrapping to prevent evaporation. Nine seed plates from each imbibition treatment of each species were placed in one of four separate incubation chambers (Model:RS IF-203, Revolutionary Science, Shafer, MI) set at 10°C, 18°C, 25°C, and 30°C and sets of 3 dishes were transferred into the common plant incubation chamber after 2, 4, and 8 weeks. Germination was monitored weekly after transfer into the common incubation chamber; the experiment was terminated after thirty-four weeks (Figure 10).

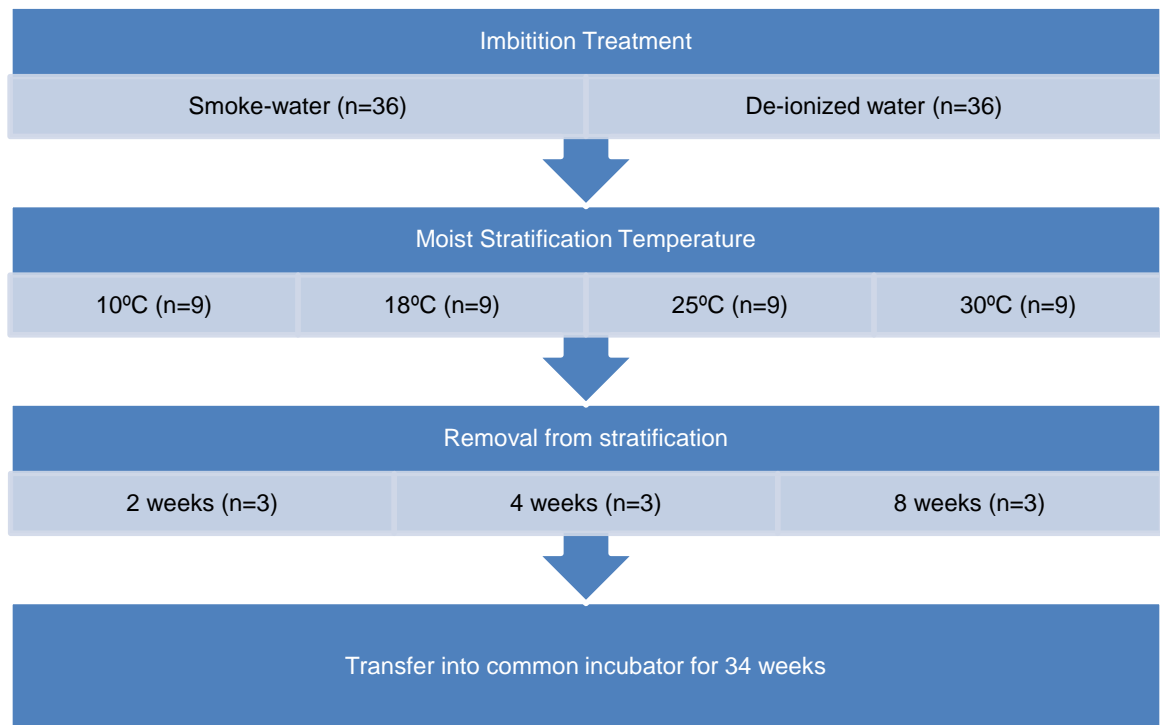


Figure 10. Schematic of moist stratification experimental design. “N” indicates number of seed packets in each treatment combination at each step.

Effects of Order of Treatment on Seed Germination

Seeds were treated with smoke-water before and after cold stratification at 5°C. One set of petri dishes with de-ionized water treated seeds were stratified in 5°C and transferred into a common incubator after 0, 2, 4, 8 and 12 weeks. A second set of petri dishes with smoke-water treated seeds were stratified in 5°C and transferred into a common incubator after 0, 2, 4, 8 and 12 weeks. A third set of petri dishes were initially treated with de-ionized water and stratified in 5°C and transferred after 0, 2, 4, 8 and 12 weeks; upon transfer into the common incubator these seeds were treated with 10 mL of 10% smoke-water. After initial

imbibition, seeds were placed on 0.7% agar in petri dishes, and the dishes were wrapped with plastic wrapping to prevent evaporation. Each dish contained 50 seeds for germination monitoring. Additionally, petri dishes from sets one and two contained additional seeds to track embryo growth after each transfer. Seed plates were placed in an incubation chamber set at 5°C and three replicates of each treatment were removed after 0, 2, 4, 8 and 12 weeks and placed into the common plant incubation chamber (Figure 11).

At each transfer time, triplicate sets for each species in each treatment were transferred from the cold stratification chamber into the common incubation chamber. At this time, seeds from sets one and two were removed for embryo measurements and 10 mL of 10% smoke-water was added to petri dishes from set three using a pipet aid. Twenty seeds per species were chosen indiscriminately from triplicate sets of each species from sets one and two at each transfer. Chosen seeds were bisected with a razor blade and embryos were measured using a compound microscope equipped with an ocular micrometer. Germination percentage was monitored bi-weekly for all treatments following transfer into the common plant incubation chamber; the experiment was terminated after thirty-four weeks.

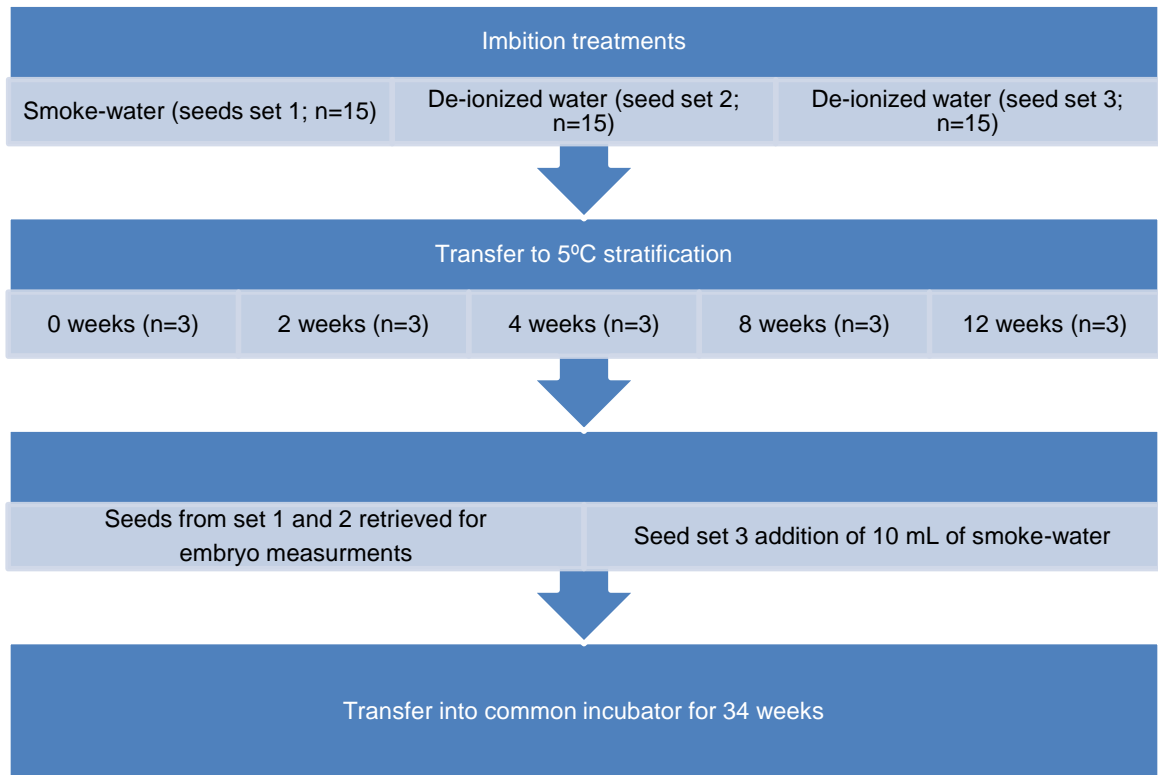


Figure 11. Schematic of experimental design for testing order of treatments.

Effects of Dry/Wet Cycling on Seed Germination

We replicated Baker *et al's* (2005) weekly regime of dry/wet cycling. Seeds of both species were imbibed in 10% smoke-water or de-ionized water (refer to Field Seed Treatment). After the imbibition treatments, seeds were placed in petri dishes with glass-fiber filter paper wrapped with plastic wrap to prevent desiccation. Four different cycles were tested. The first cycle replicated Baker *et al's* methods that consisted of 5 days of storage at 37°C with no moisture, 1 day at room temperature with the petri dishes moistened with 5 mL de-ionized water, and 1 day at room temperature with no moisture. The second

cycle eliminated moisture fluctuations, consisting of dry conditions for 5 days at 37°C and 2 days at room temperature. The third cycle eliminated temperature fluctuations, consisting of 6 days at room temperature with no moisture and 1 day at room temperature with the petri dishes moistened with 5 mL de-ionized water. The last treatment controlled for both temperature fluctuations and moisture levels consisting of 7 days at 20°C with continuous moisture in the petri dishes. Sets of three dishes from each cycle were transferred into the common plant incubation chamber after 2, 4, and 8 weeks. All dishes from the incubation chamber were moistened when needed (Figure 12). Germination percentage was monitored bi-weekly for all treatments following transfer into the common plant incubation chamber; the experiment was terminated after thirty-four weeks.

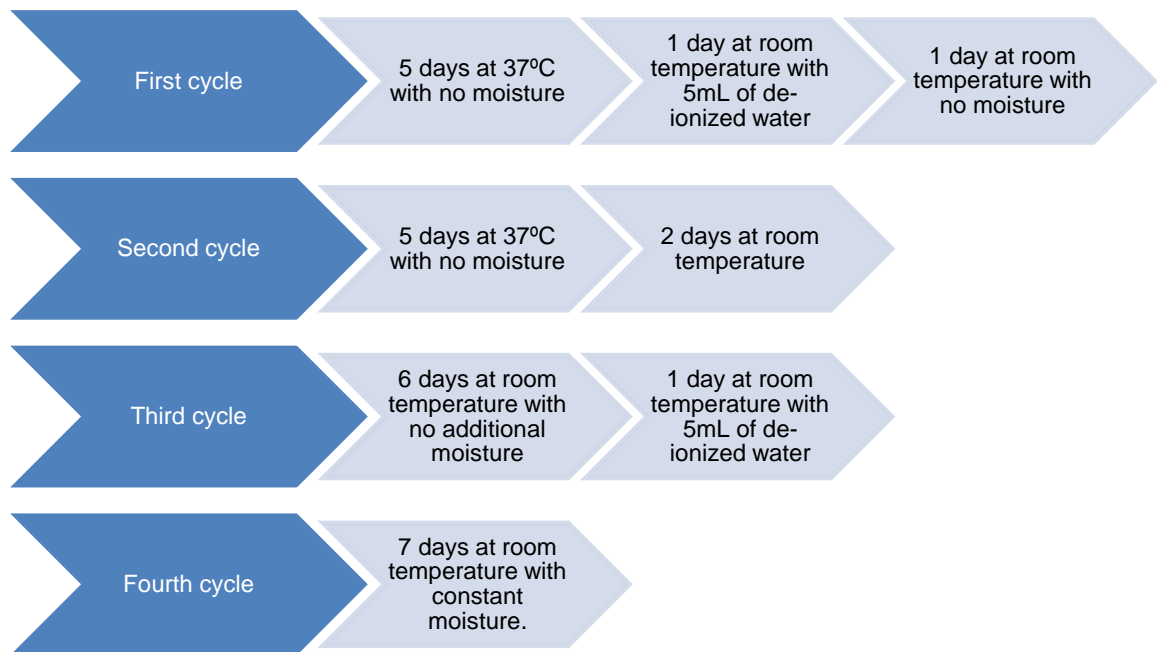


Figure 12. Schematic of dry/wet cycling experimental design.

Results

Effect of Cold Stratification following Heat-Shock and Smoke-Water Imbibition

None of the tested combinations of cold stratification, heat shock and/or smoke-water imbibition treatments were effective in triggering germination. Only two seeds from *E. chrysantha* and three seeds of *D. rigida* germinated across the entire experiment (See Appendix B).

Effects of Moist Warm Stratification and Smoke-Water Imbibition on Seed Germination

Although this experiment was designed as a 3-way factorial experiment testing effects of smoke-water, stratification temperature, and stratification duration on germination, large numbers of zeros (petri dishes with no germination) prevented the use of ANOVA. Therefore, effects of different factors were tested separately. The effect of smoke-water imbibition was tested using a Wilcoxon rank sum test (JMP Pro 13 Statistical Package), treating data from every petri dish as an independent sample without respect to stratification temperature and stratification duration. Within a smoke-water treatment, effects of stratification temperature were tested using a Kruskal Wallis test that pooled data from all stratification durations. Within a smoke-water/stratification-temperature treatment combination, effects of stratification duration were tested using a Kruskal-Wallis test followed by a Dunn's multiple comparison test.

Smoke-water exposure increased germination of both species. *D. rigida* seeds treated with smoke-water had significantly higher germination than those treated with de-ionized water (an average of 0.47 germinants per plate across all stratification temperatures and durations for smoke-water treated seeds versus zero for de-ionized water treated seeds, $Z = 2.52607$, $p < 0.0115$; Wilcoxon rank sum test). Similarly, *E. chrysantha* seeds treated with smoke-water had significantly higher germination than those treated with de-ionized water (an average of 0.66 germinants per plate across all stratification temperatures and durations for smoke-water treated seeds versus zero for de-ionized water treated seeds, $Z = 3.36379$, $p < 0.0008$; Wilcoxon rank sum test).

Effects of stratification temperature were only analyzed for seeds treated with smoke-water. This is because there was no germination of seeds treated with de-ionized water. Stratification temperature had a marginally significant effect on the germination of *D. rigida* (Figure 13; $\chi^2_{(3)} = 7.7362$, $p = 0.0518$) and a highly significant effect on the germination of *E. chrysantha* (Figure 14; $\chi^2_{(3)} = 12.4962$, $p = 0.0059$). Stratifying *D. rigida* seeds at 30°C produced higher germination percentages than stratifying at 18°C, but this difference was only marginally significant ($p = 0.0643$, Table 11). More conclusively, stratifying *E. chrysantha* seeds at 30°C produced significantly higher germination percentages than stratifying them at either 10°C or 18°C (Table 12).

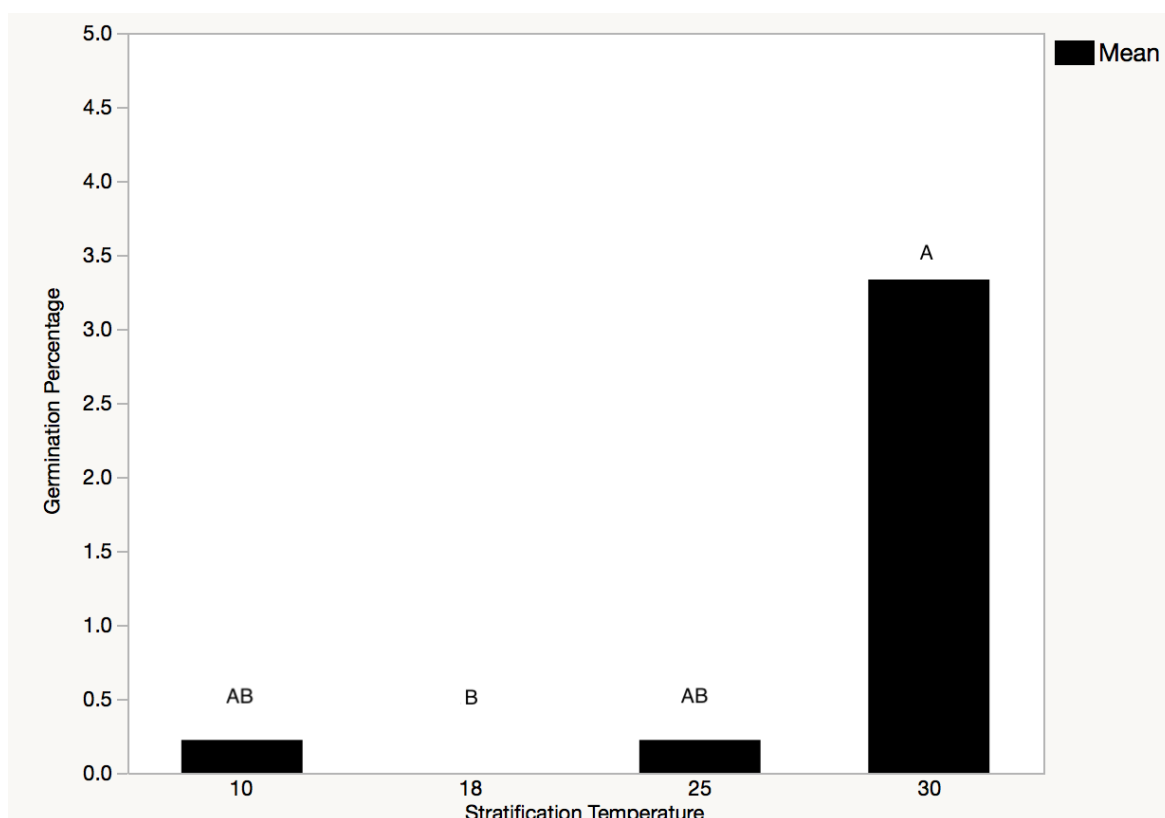


Figure 13. Final mean germination percentage of *Dendromecon rigida* seeds after being imbibed in smoke water (SW) and stratified in 10, 18, 25 or 30°C for 2, 4 or 8 weeks (n=9). Percentages were pooled within a stratification temperature treatment. Different letters indicate significant differences as identified by Dunn’s multiple comparison test at $p < 0.065$ (not quite significant).

Table 11. Results from Dunn’s multiple pairwise comparisons test for *D. rigida* seeds imbibed in smoke-water and stratified in different temperatures.

Pair Comparison	Score Mean Difference	Standard Error Difference	Z-value	p-value
30 vs 25	6.38889	3.222441	1.98262	0.2845
30 vs 18	8.22222	3.222441	2.55155	0.0643*
30 vs 10	6.38889	3.222441	1.98262	0.2845
25 vs 18	1.72222	3.222441	0.53445	1.0000
25 vs 10	0.00000	3.222441	0.00000	1.0000
18 vs 10	-1.72222	3.222441	-0.53445	1.0000

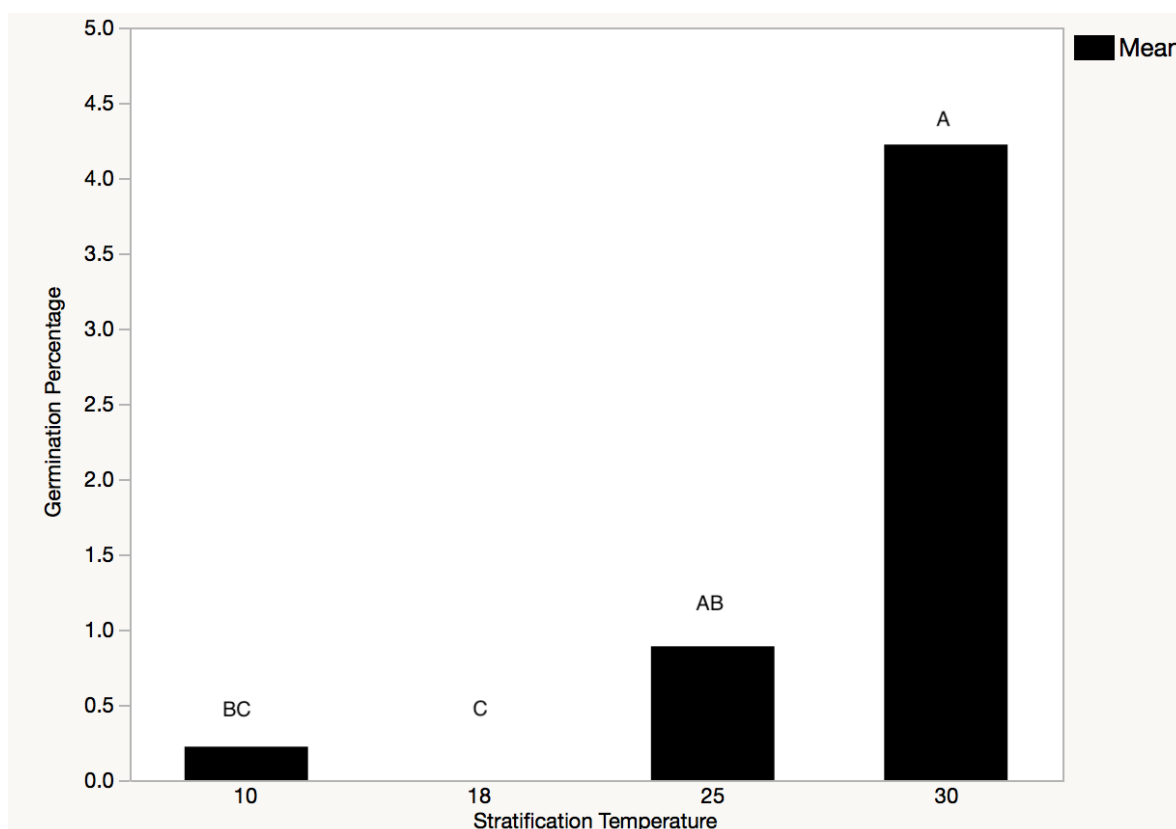


Figure 14. Final mean germination percentage of *Ehrendorferia chrysantha* seeds after being imbibed in smoke water (SW) and stratified in 10, 18, 25 or 30°C for 2, 4 or 8 weeks (n=9). Percentages were pooled within a stratification temperature treatment. Different letters indicate significant differences as identified by Dunn's multiple comparison test at $p < 0.05$.

Table 12. Results from Dunn's multiple pairwise comparisons test for *E. chrysantha* seeds imbibed in smoke-water and stratified in different temperatures.

Pair Comparison	Score Mean Difference	Standard Error Difference	Z-value	p-value
30 vs 25	7.0556	3.913347	1.80295	0.4284
30 vs 18	12.6111	3.913347	3.22259	0.0076*
30 vs 10	10.8889	3.913347	2.78250	0.0324*
25 vs 18	5.4444	3.913347	1.39125	0.9849
25 vs 10	3.7222	3.913347	0.95116	1.0000
18 vs 10	-1.6111	3.913347	-0.41170	1.0000

Effects of stratification duration were tested within each stratification temperature. Significant differences were only noted on *D. rigida* seeds stratified at 30°C (Table 13). Stratifying *D. rigida* seeds in 30°C for 8 weeks produced significantly higher germination percentages than 4 weeks (Figure 15 and Table 14). *E. chrysantha* seeds stratified in 30°C for 8 weeks appeared to have higher germination than seeds stratified for shorter periods but this was not quite significantly different ($p=0.0591$; Figure 16; Table 15; Table 16).

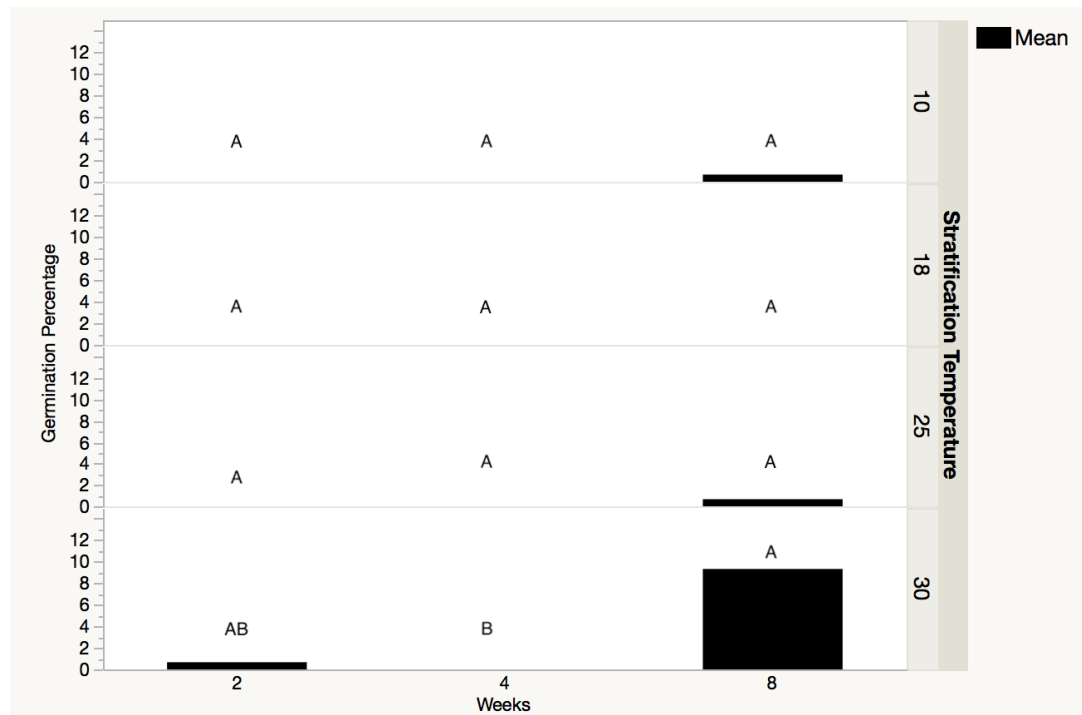


Figure 15. Mean germination percentage of *Dendromecon rigida* seeds imbibed in smoke-water after being stratified in 10, 18, 25 or 30°C for 2, 4, or 8 weeks (n=3). Different letters indicate significant differences as identified by Dunn's multiple comparison test.

Table 13. Results from Kruskal-Wallis Test for effects of stratification duration on germination of seeds imbibed in smoke-water for each stratification temperature.

Species				
<i>D. rigida</i>		ChiSquare	DF	P-value
Stratification Temperatures	10°C	2.0	2	0.3679
	18°C	0	2	1.00
	25°C	2.0	2	0.3679
	30°C	6.72	2	0.0347

Table 14. Results from Dunn Method test pair-wise comparison of Incubation duration within a stratification temperature for *D. rigida* seeds imbibed in smoke-water.

Stratification Temperature	Incubation Duration Comparison (Weeks)	Score Mean Difference	Standard Error Difference	Z-value	p-value
10	8 vs 4	1.166667	1.224745	0.9525793	1.0000
	8 vs 2	1.166667	1.224745	0.9525793	1.0000
	4 vs 2	0.000000	1.224745	0.0000000	1.0000
18	8 vs 4	0.000000	0.000000	0.000000	1.0000
	8 vs 2	0.000000	0.000000	0.000000	1.0000
	4 vs 2	0.000000	0.000000	0.000000	1.0000
25	8 vs 4	1.166667	1.224745	0.9525793	1.0000
	8 vs 2	1.166667	1.224745	0.9525793	1.0000
	4 vs 2	0.000000	1.224745	0.0000000	1.0000
30	8 vs 4	4.66667	2.041241	2.28619	0.0667
	8 vs 2	3.66667	2.041241	1.79629	0.2173
	4 vs 2	-0.66667	2.041241	-0.32660	1.0000

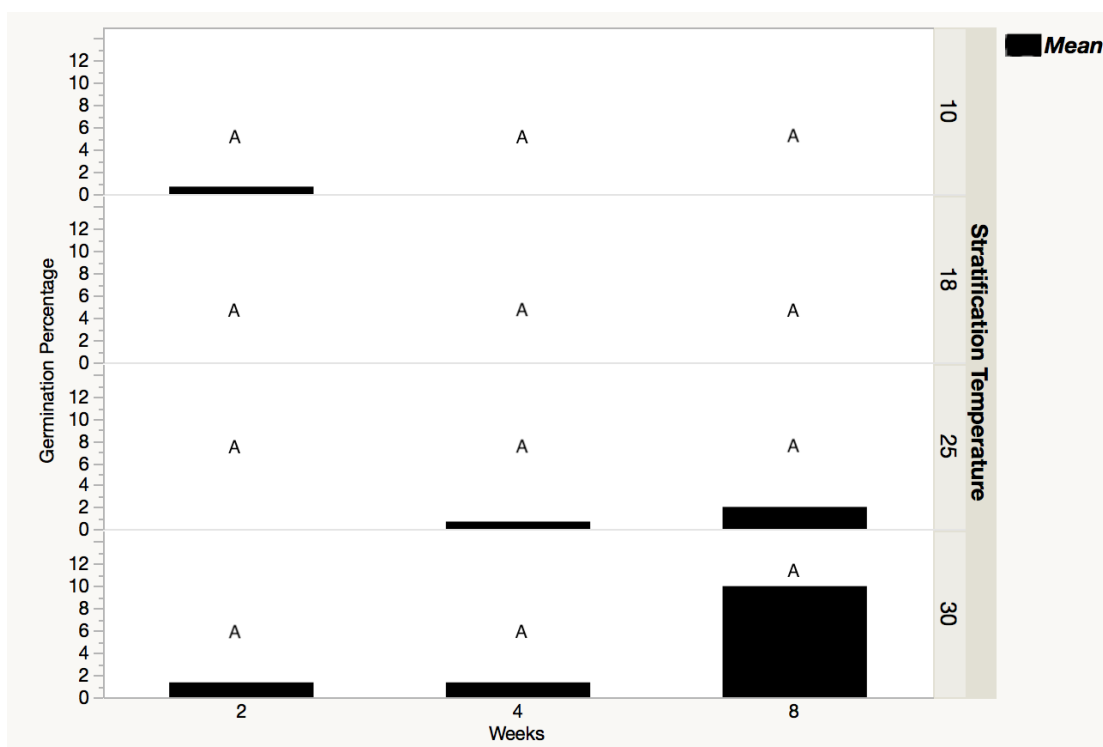


Figure 16. Mean germination percentage of *Ehrendorferia chrysantha* seeds imbibed in smoke-water after being stratified in 10, 18, 25 or 30°C for 2, 4, or 8 weeks (n=3). Different letters indicate significant differences as identified by Dunn's multiple comparison test.

Table 15. Results from Kruskal-Wallis Test for effects of stratification duration on germination of seeds imbibed in smoke-water for each stratification temperature.

Species				
<i>E. chrysantha</i>		ChiSquare	DF	P-value
Stratification Temperatures	10°C	2.0	2	0.3679
	18°C	0	2	1.00
	25°C	2.8889	2	0.2359
	30°C	5.6580	2	0.0591

Table 16. Results from Dunn Method test pair-wise comparison of Incubation duration within a stratification temperature for *E. chrysantha* seeds imbibed in smoke-water.

Stratification Temperature	Incubation Duration Comparison (Weeks)	Score Mean Difference	Standard Error Difference	Z-value	p-value
10	8 vs 4	0.00000	1.224745	0.000000	1.0000
	8 vs 2	-1.16667	1.224745	-0.952579	1.0000
	4 vs 2	-1.16667	1.224745	-0.952579	1.0000
18	8 vs 4	0.000000	0.000000	0.000000	1.0000
	8 vs 2	0.000000	0.000000	0.000000	1.0000
	4 vs 2	0.000000	0.000000	0.000000	1.0000
25	8 vs 4	2.833333	1.870829	1.514480	0.3897
	8 vs 2	1.500000	1.870829	0.801784	1.0000
	4 vs 2	1.000000	1.870829	0.534522	1.0000
30	8 vs 4	4.333333	2.188988	1.979606	0.1432
	8 vs 2	4.000000	2.188988	1.827329	0.2030
	4 vs 2	0.000000	2.188988	0.000000	1.0000

Effects of Order of Cold Stratification and Smoke-Water Treatment on Seed

Germination

No combination of cold stratification and smoke-water treatment elicited much germination of either species. Only six seeds of *D. rigida* and only one seed of *E. chrysantha* germinated across all treatments (Appendix B). Consistent with the lack of germination, seed embryo size did not change significantly over time (Figure 17 and Figure 18).

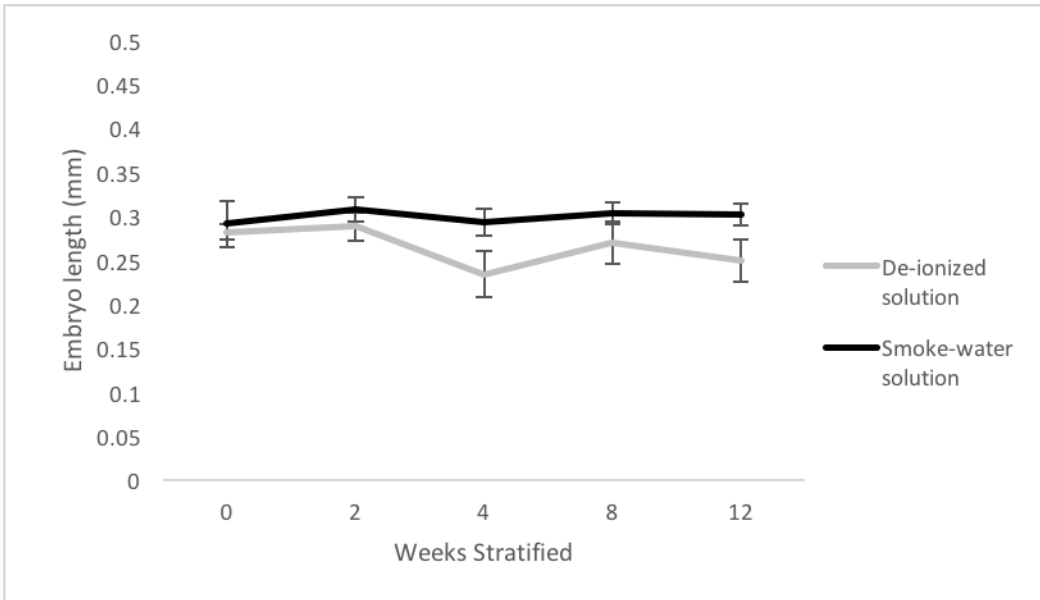


Figure 17. Embryo length of *E. chrysantha* after imbibition and 5°C cold stratification of 0, 2, 4, 8, and 12 weeks.

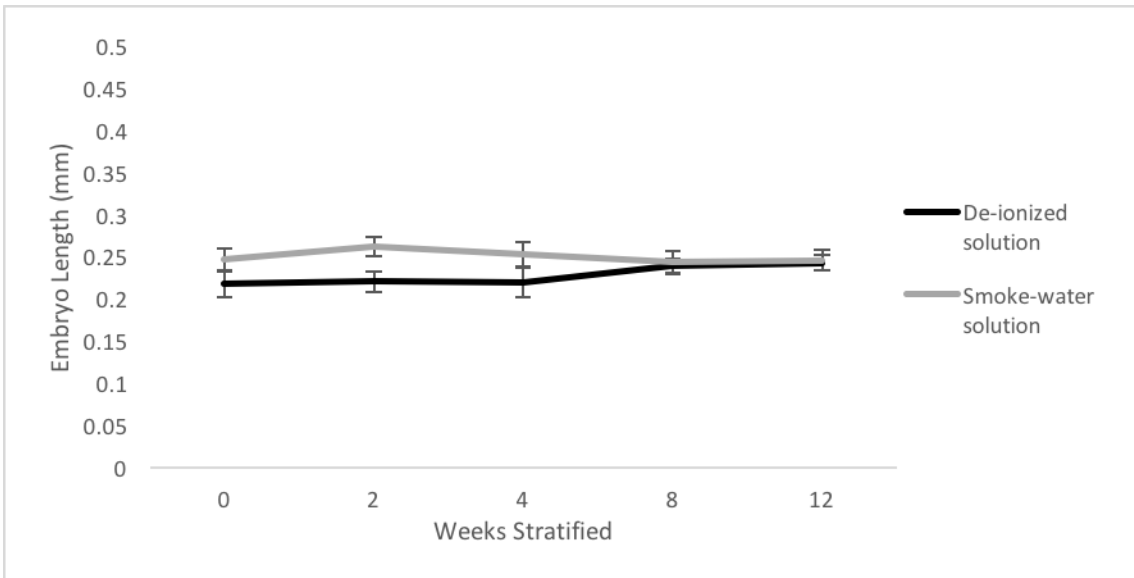


Figure 18. Embryo length of *D. rigida* after smoke-water or de-ionized water imbibition and 5°C cold stratification of 0, 2, 4, 8 and 12 weeks.

Effects of Dry/Wet Cycling on Seed Germination

Dry/wet cycling with temperature fluctuations had no effect on the germination of either species. Only five out of 3600 *D. rigida* seeds germinated, and these were distributed over five treatments. Ten out of 3600 *E. chrysantha* seeds germinated and these were distributed over seven treatments (Appendix E).

Discussion

Smoke-water imbibition combined with warm stratification gave substantial germination of *D. rigida* and *E. chrysantha* seeds. As others have found, exposure to smoke water solutions stimulated germination for both species (Keeley and Fotheringham, 1998b). Of the environmental factors that were captured in our experiments, such as exposure to cool temperatures, temperature fluctuations, moisture fluctuations, and exposure to compounds in smoke before and after cold stratification, only warm moist stratification stimulated germination. Mediterranean climate environments are considered to have cool, wet winters and hot, dry summers, but the periods in between the seasons with conditions of elevated temperatures and high moisture could be the optimal conditions to trigger germination in these species. These periods of elevated temperatures and high moisture were captured in our experimental design and were demonstrated to stimulate germination.

Based on these experimental results, a statistically significant rate of germination can be stimulated if seeds are subjected to a stratification period in elevated temperature and exposure to smoke prior to germinating. Without a period of warm temperatures, seeds are unlikely to germinate at all. Our experiment only tested stratification temperatures up to 30°C, although results from Chapter 2 indicated seeds are exposed to higher temperatures in early spring. More than one month of warm stratification was required for either species to germinate in these studies. It is possible that exposure to higher temperatures could shorten the duration of warm stratification required for germination. Further investigation is required to test the extent of fall conditions to provide the conditions required to germinate these species after a fire.

CHAPTER FOUR

INCREASING SEED GERMINATION AFTER WARM STRATIFICATION

Introduction

It appears that *D. rigida* and *E. chrysantha* seeds require elevated stratification temperatures combined with exposure to smoke to germinate (Chapter 3). In the previous study, the temperature optimum for warm stratification could not be identified because we achieved the greatest germination only with our highest temperature tested, 30°C. The fact that germination after stratification in 30°C was still low and the fact that data loggers from the field showed conditions exceeding 40°C during early spring months, raised the question of whether higher stratification temperatures would produce greater germination. The main goals of the following studies were to determine the temperature optimum for warm stratification, to determine whether the necessary stratification duration decreased with increasing stratification temperature, and to determine whether fall months provided sufficient conditions for warm stratification prior to the cold months of winter.

One additional environmental factor studied was light, because it has been shown to have positive and negative effects on seed germination (Nelson *et al.*, 2009; Keeley and Fotheringham 2000). Investigators have shown some species to have a positive germination response to light, but in the absence of light, smoke chemicals can induce germination (Nelson *et al.*, 2012 and references therein). Others have shown that germination in some species is inhibited by light

(Keeley, 1991). Nelson *et al.* (2012) suggested that smoke and light act independently on seed germination, but light can have synergistic effects or block germination when combined with smoke chemicals (Baskin and Baskin, 2014). In the event of a flood, seeds are capable of being buried eliminating sunlight, whereas in an event of a fire, the canopy of trees and shrubs are removed potentially exposing the seeds aboveground to sunlight. This variability of responses to light prompted the inclusion of light treatments within this study.

These questions were addressed by lab germination studies conducted in a full factorial experiment with two light levels, two smoke-water treatments, five stratification temperatures, and four stratification durations, and a study monitoring soil temperature and moisture in the field during the fall and winter months. Thus we were able to compare stratification requirements tested in the lab to field conditions during fall and winter months.

Methods

Seed Collection of *Dendromecon rigida* and *Ehrendorferia chrysantha*

Seeds of *Dendromecon rigida* and *Ehrendorferia chrysantha* were collected in July 2015 near Lone Pine Canyon road and Highway 138 (Refer to Chapter 2 for site description). Seeds were cleaned and dried in room temperature then stored in paper bags for 14 months.

Germination Experiments for *D. rigida* and *E. chrysantha* Seeds

The treatments consisted of imbibing the seeds in 20% smoke-water or de-ionized water, stratifying them in different temperatures for different lengths of

time and incubating them in either light or dark conditions. Prior to imbibition, undamaged seeds were placed in mesh packets, with 50 seeds in each packet. All seeds were imbibed in 20% smoke-water or de-ionized water for 24 hours and were rinsed twice in de-ionized water prior to plating on the 0.7% agar plates. Smoke-water concentration was determined by preliminary assays conducted with *Salvia mellifera* and *Emmenanthe penduliflora*; both are smoke-responsive species with more easily germinable seeds (Appendix F).

Agar plates were made by mixing de-ionized water with BP1423-500 agar to create 0.7% agar concentration (1000 mL of de-ionized water for every 7g of agar powder). The agar solution was dissolved by heating the solution up to 95°C or once the agar completely dissolved. Petri dishes (Fisher, polystyrene, 90mm x 15mm) were filled using a pipet aid with 27mL of the agar solution and were stored overnight to allow to solidify.

For the factorial experiment, seeds imbibed in smoke-water or de-ionized water were removed from their mesh packets and placed in individual petri dishes. Petri dishes were wrapped with plastic to prevent evaporation. Light was eliminated from half of the plates by wrapping them with aluminum foil. Petri dishes were incubated in 20°C, 30°C, 40°C, 50°C, or 60°C for a period of up to 8 weeks. Three replicates of each treatment were removed from the incubators and transferred into a common incubator after 0, 2, 4, and 8 weeks. The common incubator had a diurnal cycle of twelve hours of light, twelve hours of dark and daily temperature fluctuations of 20°C during the day and 10°C during

the night. These conditions simulated typical soil field conditions under a shrub during February and March (Appendix A). Germination was monitored bi-weekly for all treatments following transfer into the common plant incubation chamber; experiment was terminated after fifty-two weeks.

Identifying Optimum Warm Stratification Using Disinfected *D. rigida* Seeds

Due to fungal contamination of *D. rigida* plates in the experiment above, a smaller experiment was conducted to identify optimum warm stratification using disinfected seeds. Seeds of *D. rigida* were sterilized before being imbibed in 20% smoke-water for 24-hours. Seeds were disinfected with 1.5% calcium perchlorate solution by dipping the seeds for 15 minutes and rinsing with de-ionized water. Seeds were placed on petri dishes with 0.7% agar, thirty-five seeds per plate, and wrapped with plastic wrap to prevent evaporation. Three replicates of all treatments were incubated in 20°C, 35°C and 50°C for a period of up to 8 weeks. Three replicates of each treatment were removed at 0, 2, 4, and 8 weeks. The common incubator had a diurnal cycle of 20°C/10°C and 12-hour light conditions. Germination was monitored bi-weekly for all treatments following transfer into the common plant incubation chamber; experiment was terminated after fifty-two weeks.

Monitoring of Soil Temperature and Humidity

On October 1, 2016, two data loggers (EL-USB-2, Lascar Electronics, Inc., Wiltshire, UK) were buried near the seed collection area to monitor hourly changes in soil temperature and soil humidity. Data loggers were located 0.44

km apart following a survey of midday, unshaded, soil surface temperatures across a range of slopes and aspects. Data-logger locations were determined by taking 58 soil temperatures measurements with a fine-wire thermocouple thermometer and identifying relatively warm, flat areas; midday soil surface temperatures ranged from 32 C to 62 C on that day. One data logger was buried near the bottom of the valley, and the second was buried on top of a small ridge near the edge of the valley, where midday soil surface temperatures were recorded as 56 C and 55 C, respectively. Data loggers were buried horizontally with the top surface 2 cm below the surface of the ground, checked periodically for disturbance, and retrieved on January 28, 2017, after four months in the field.

Statistical Analysis

The analysis was carried out using the JMP Pro 13 statistical package. Due to lack of germination for a large number of treatments, the data did not meet the assumptions for a 2-way ANOVA (e.g. equality of variance). Therefore a set of non-parametric tests addressing different factors were used. Germination differences between seeds treated with smoke-water and seeds treated with de-ionized water were compared with a Wilcoxon signed rank test, pooling data for replicates and pairing data from the same treatment combinations of temperature, duration and light. Because seeds treated with de-ionized water failed to germinate, they were not included in the following analyses. Germination differences between light treatments were compared with a Wilcoxon signed rank test, pooling data from replicates and pairing data from the same treatments of

stratification temperature and duration. Germination differences among stratification temperatures were compared by chi-squared test of homogeneity, pooling data for seeds stratified for 2, 4, and 8 weeks for each temperature. Separate chi-squared tests were run for seeds stratified in the dark and seeds stratified in the light. Post hoc pair-wise comparisons to identify temperatures with significantly higher germination were carried out by methods of Cox and Key (1993). To investigate the effect of stratification duration on germination, separate Kruskal Wallis tests were performed on germination at each stratification temperature in the light and in the dark. Post hoc Dunn's Test were used to identify the highest germination produced for each light and temperature combination.

Results

Final Germination for *Ehrendorferia chrysantha*

No combination of smoke-water, stratification temperature, stratification duration or light condition was effective in stimulating germination of *E. chrysantha* seeds. Only 16 out of 3000 seeds germinated across all treatment combinations. Therefore effects of different factors were not analyzed.

Final Germination of Disinfected *D. rigida* Seeds

Seeds of *D. rigida* that were disinfected failed to germinate across all treatment combinations.

Increase of Germination for *D. rigida* with Smoke-Water, Warm-Stratification and Light Elimination

Although the maximum germination in any treatment combination was 10%, we were able to identify factors that promoted germination. As found previously, smoke-chemicals were required for germination. Only seeds of *D. rigida* imbibed in smoke-water germinated across all stratification temperatures and light treatments. There were significant differences between *D. rigida* seeds imbibed in smoke-water and seeds imbibed in de-ionized water across all treatment combinations (Wilcoxon signed rank test, $t=3.3013$, $p=0.0021$, $DF=39$).

Light appeared to inhibit germination across all treatments. *D. rigida* seeds treated with smoke-water kept in the dark throughout the incubation period had a significantly higher germination than seeds kept in the light (Wilcoxon signed rank test, $t=2.4459$, $p=0.0244$, $DF=19$; Figure 19).

Stratification at 30°C and 40°C yielded the highest pooled germination (Figure 19). There were significant differences in germination of seeds stratified in different temperatures both in the dark, $\chi^2(4, n=2250)=51.5$, $p<0.001$, and in the light, $\chi^2(4, n=2250)=16.1$, $p<0.01$. For seeds in the dark, post hoc comparisons identified stratification at 30°C and 40°C as producing significantly higher germination than the other stratification temperatures. For seeds in the light, post hoc comparisons identified stratification at 30°C as producing significantly higher germination than the other stratification temperatures.

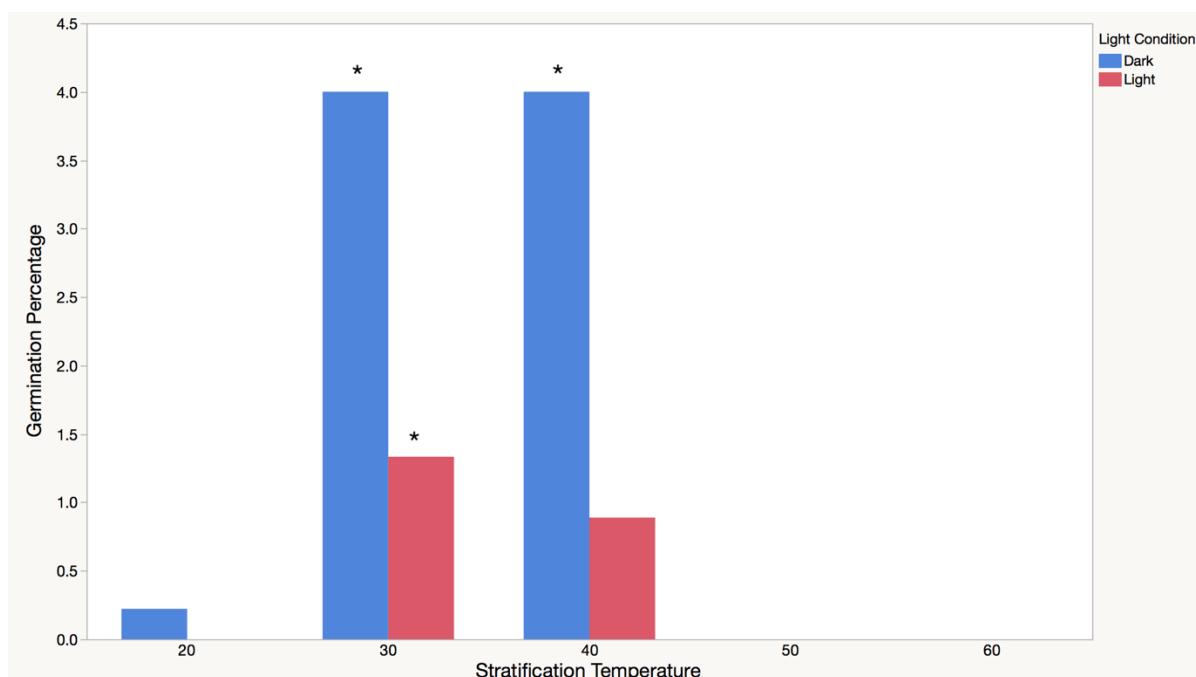


Figure 19. Germination percentages for *D. rigida* seeds stratified in different temperatures with pooled data within stratification temperatures of stratification durations 2, 4, and 8 weeks (n=9). Asterisks indicate significant difference from other treatments within a light condition.

Stratification duration significantly affected germination of *D. rigida* seeds stratified in the dark at 30°C. This effect was marginally significant for seeds stratified at 40°C in the dark (Figure 20; Table 17). Stratification for eight weeks produced significantly more germination than stratification for only two weeks when seeds were stratified at 30°C (Table 18). Stratification at 40°C produced the highest germination when seeds were stratified for only two weeks and the lowest when seeds were stratified for eight weeks, but that difference was only marginally significant (Dunn's test, $p=0.064$; Table 18).

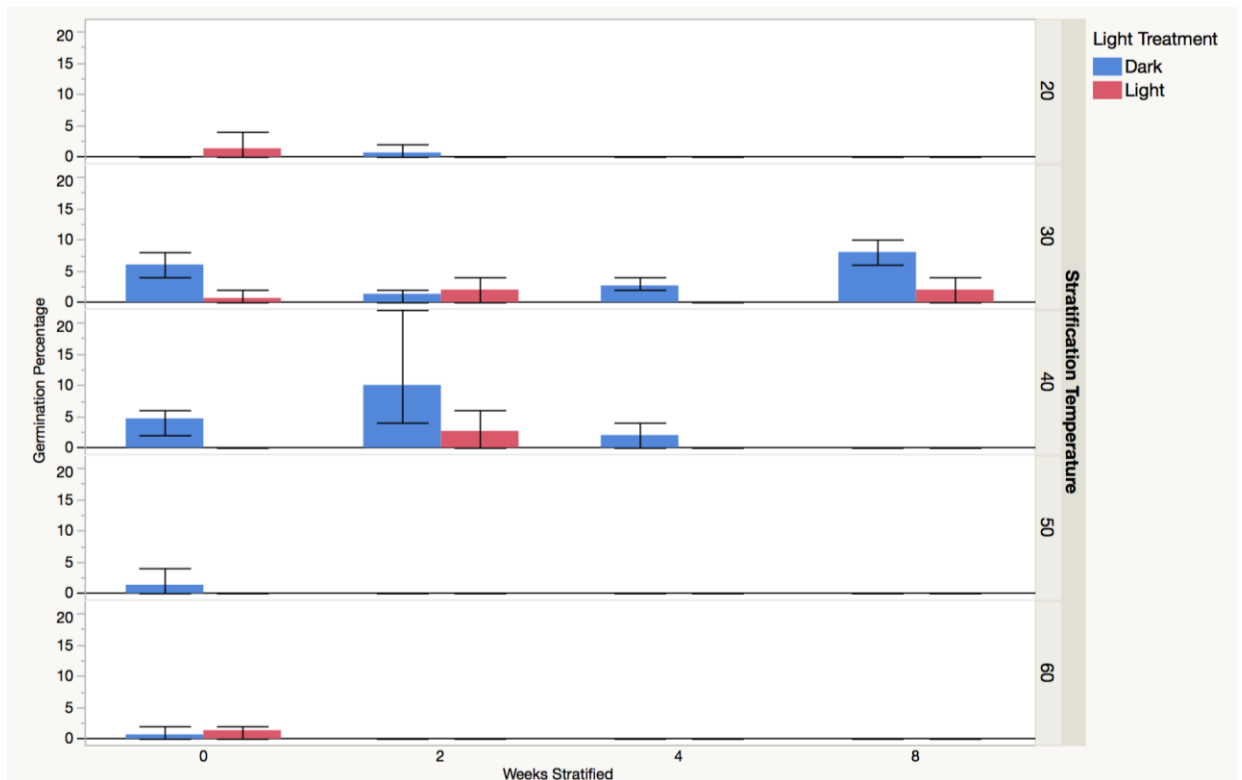


Figure 20. Germination percentages for *D. rigida* seeds stratified in different temperatures and stratification duration combinations. Bars indicate range of germination for replicates within a treatment combination.

Table 17. Results from Kruskal-Wallis Test for effects of stratification duration on germination of *D. rigida* seeds imbibed in smoke-water for each stratification temperature.

Stratification Temperature	Light Condition	chi-square	DF	p-value
30°C	Dark	9.0391	3	0.0288
40°C	Dark	7.5370	3	0.0566

Table 18. Results of Dunn's post-hoc pairwise comparisons for effects of stratification duration on germination of seeds imbibed in smoke-water for each stratification temperature. P-values are adjusted by the family-wide error rate (FWER) method of Holm to reduce Type-I errors. Durations that did not produce significantly different germination rates at $p \leq 0.05$ are joined by an underscore.

At 30°C:	8 wk	0 wk	4 wk	2 wk
	<hr/>			
At 40°C:	2 wk	0 wk	4 wk	8 wk
	<hr/>			

Elevated Temperature Conditions in the Field During Fall and Winter Months

Data loggers placed in the field over fall and winter months of 2016 showed that soil temperatures exceeded 30°C during the day for several weeks after the first fall rains occurred. Minor rainfall occurred on September 28, before data-logger installation, and on October 17; the first major rainfall of the season occurred on October 24 (Figure 21 and Figure 22). Both data loggers were found on the soil surface on October 15 and reburied. Although erratic readings of humidity and unusually low nighttime temperatures during the second week of October suggest that data for that week may have been affected by disturbance, general patterns from both data loggers indicated that daily maximum temperatures in shallow soil regularly exceeded 40°C during the three weeks following the first minor rainfall (September 28th) and daily maximum temperatures exceeded 30°C on most days during the three weeks following the first major rainfall (October 24th).



Figure 21. Temperature and humidity data of Data Logger #1 from Swarthout Canyon between October 1, 2016 to January 30, 2017. (Data loggers were removed from soil on January 28.).

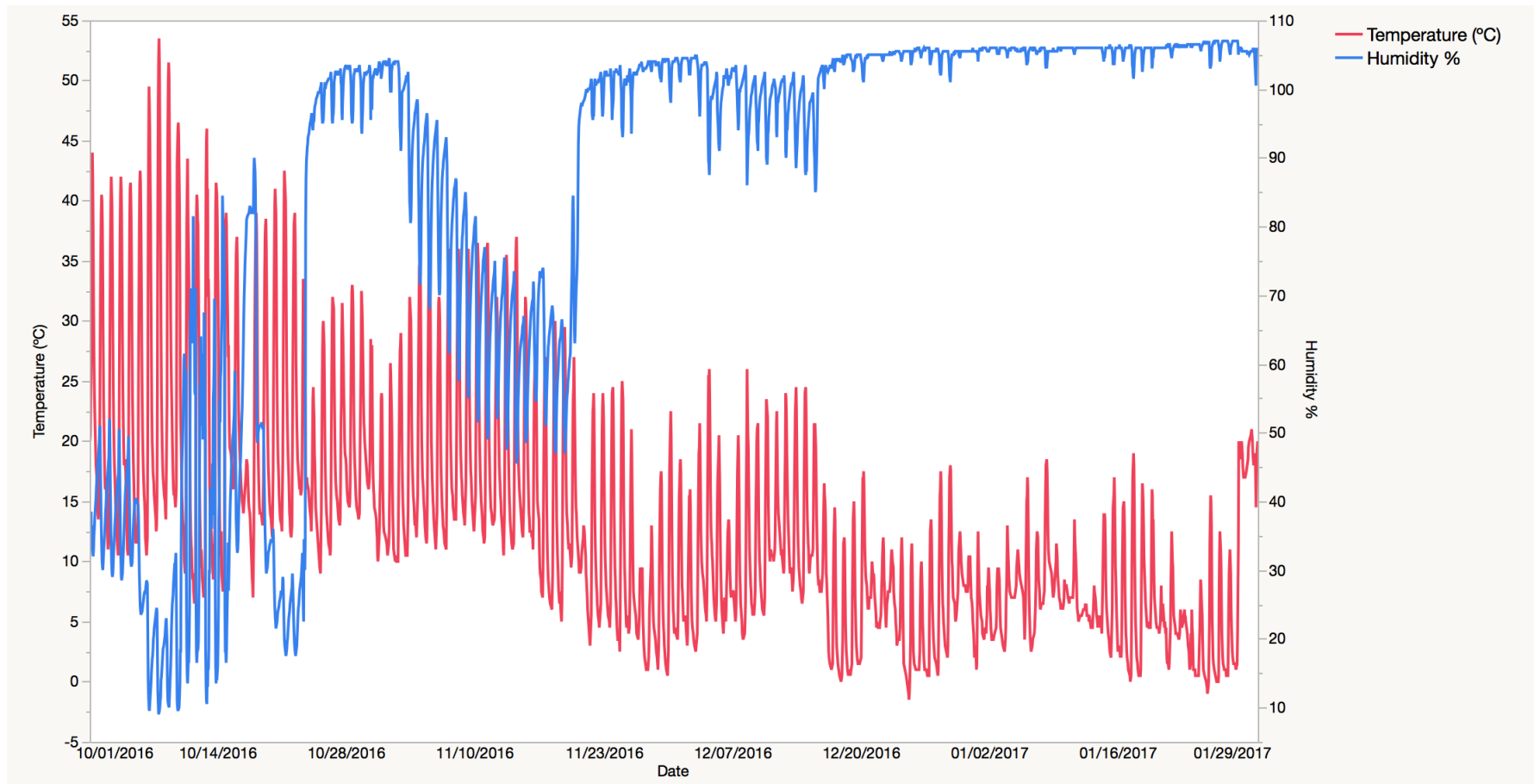


Figure 22. Temperature and humidity data of Data Logger #2 from Swarthout Canyon between October 1, 2016 to January 30, 2017. (Data loggers were removed from soil on January 28.).

Discussion

No combination of warm stratification, smoke-water imbibition, or dark/light conditions was effective in germinating *E. chrysantha* seeds. We could only speculate on reasons as to why there was no germination; seeds may have undergone a secondary dormancy after seed collection or seeds may have required a different treatment. These results contradicted what we found in Chapter 3 suggesting further testing needs to be done to identify the environmental factors required to germinate *E. chrysantha*.

Stratification temperatures that corresponded to peak daily soil temperatures in the field during the fall increased germination of *D. rigida* seeds. In the lab experiments, there was an apparent stratification temperature optimum between 30°C and 40°C, with germination being hindered at temperatures above 50°C. The field conditions could be separated into two periods. Between the first minor rain event on September 28 and the first major rain event on October 24th, the soil was only moistened briefly and mid-day temperatures exceeded 40°C. After the first major rain event, soil moisture levels remained elevated and mid-day temperatures normally exceeded 30°C for three weeks.

There was weak support for the hypothesis that the effective stratification duration decreased with higher temperatures. At 30°C, stratification for eight weeks yielded significantly more germination than stratification for two weeks. In contrast, stratifying seeds at 40°C for two weeks had the highest germination but the significance of the difference between the durations was weak (maximum

p=0.06). Mid-day soil temperatures did hit or exceed 40°C in the field, however for 3 weeks following the first minor rains in 2016. If the initial rains in the field hydrated the seeds enough for them to be susceptible to stratification, the period following early rains might be sufficient for stimulating germination.

Unexpectedly, some *D. rigida* seeds did not require a stratification period to germinate and began germinating four months after exposure to smoke-water. This phenomenon and its significance in the field require further investigation.

CHAPTER FIVE

CONCLUSION

Overall, two factors that proved to be the most effective in germinating *D. rigida* and *E. chrysantha* seeds were warm stratification in combination with smoke-water imbibition. Other factors that were tested but were not as effective on germinating were cold stratification, stratification above 50°C, heat-shock, and moisture cycling.

For *D. rigida* the optimum stratification temperature was 30-40°C, corresponding to daily peak field temperatures in open field sites during the fall months. For *E. chrysantha*, initial studies indicated that stratification at high temperature, 30°C, stimulated germination, but follow-up studies to determine the optimum stratification temperature failed due to lack of germination. The reasons for the failure of this experiment are unknown. Keeley and Fotheringham (1998b) suggested low pH levels in smoke-water solutions (i.e. oxidizing agents) could play a role in germination by increasing the permeability of seed coats.

We did not achieve high germination percentages for either species under any condition. Maximal germination was only 10-14% suggesting there is another factor that was not included in our studies. One obvious factor that was not studied was daily temperature swings. Field conditions naturally fluctuate, and seed germination is often higher under fluctuating temperatures than constant temperatures (Baskin and Baskin, 2014). Keely (1986) demonstrated that strong

diurnal fluctuations significantly increased germination of *Salvia mellifera*, another smoke-stimulated species. While stratification under constant conditions between 30-40°C proved to stimulate *D. rigida* seeds, these temperatures were only reached during mid-day in the field. The way these temperature fluctuations affect stratification is unknown and merits further research.

Fires typically occur before rain events during the fall months, providing crucial conditions for the seeds to germinate (e.g. smoke chemicals, moisture, and warm temperatures). Baker (2005) and Hidiyati (2012) showed that replicating seasonal conditions may break dormancy. Immediately after seed dispersal of *D. rigida*, seeds are exposed to fall month conditions of warm temperatures and periodic rain events. Seeds can exploit the short period immediately after a rain event, when temperatures are still above 30 to 40°C, to successfully germinate.

Fire history could have played a role in the fecundity of the plant population leading to a lower germination rate overall, not necessarily the failure of our germination treatments. A short fire interval could have selected for re-sprouters decreasing the trait of seeders.

Furthermore, *D. rigida* seeds kept in the dark increased germination, demonstrating a requirement in the field for seeds to be buried. *D. rigida* is found in a fire prone environment susceptible to floods increasing the chance of seeds being buried after dispersal. In this case, it appears that smoke-chemicals and

lack of light interact in a synergistic manner increasing germination only when seeds were kept in the dark.

APPENDIX A
DATA LOGGERS FOR ENVIRONMENTAL CONDITIONS AT SWARTHOUT
CANYON FROM JANURARY THROUGH APRIL 2015

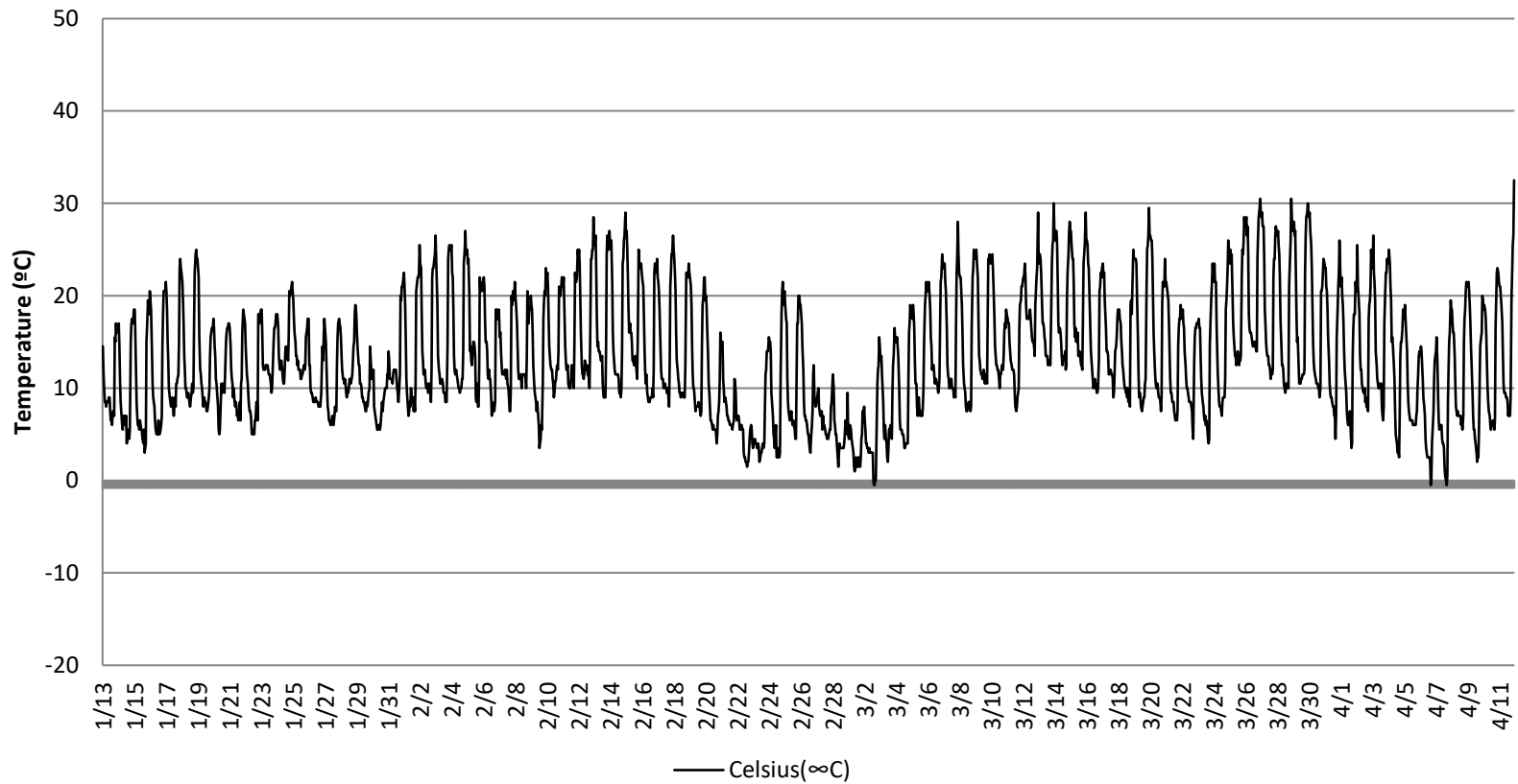


Figure 1. Data logger displaying the change in daily air temperature over the period of field storage in Swarthout Canyon, 2015.

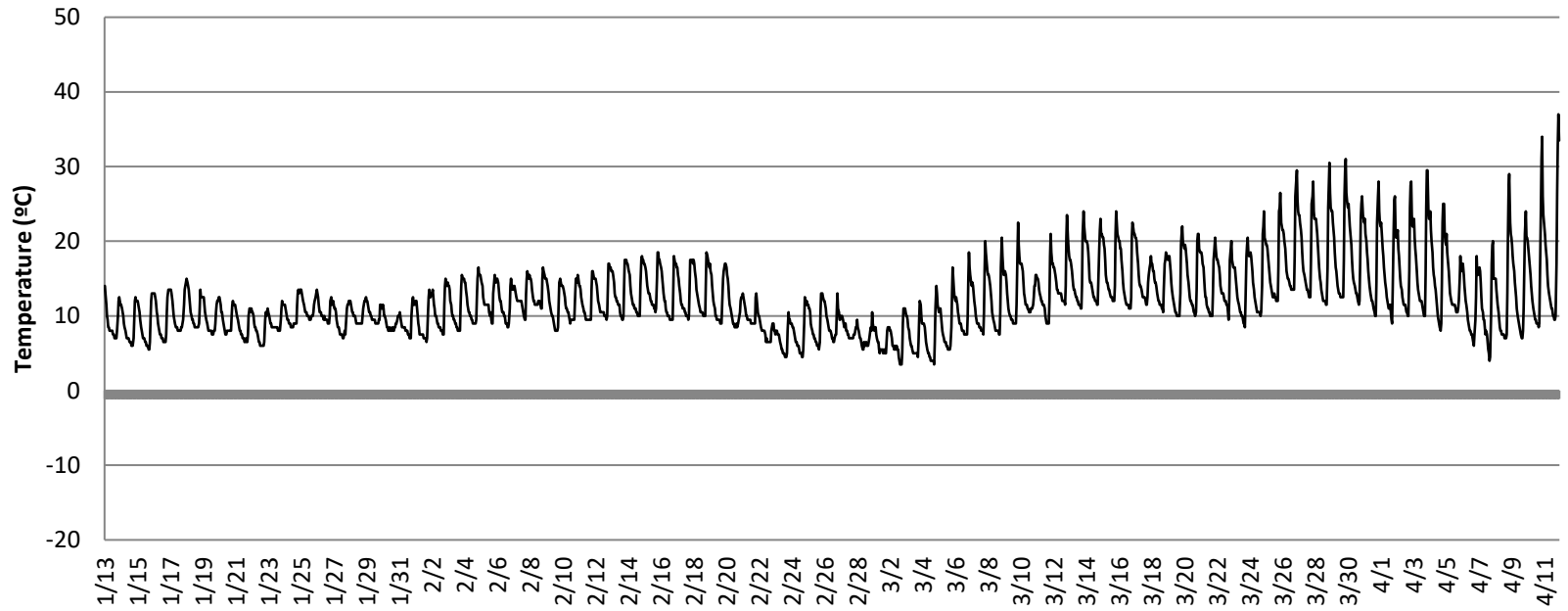


Figure 2. Data logger displaying the change in daily soil temperature buried under shrub cover over the period of field storage in Swarthout Canyon, 2015.

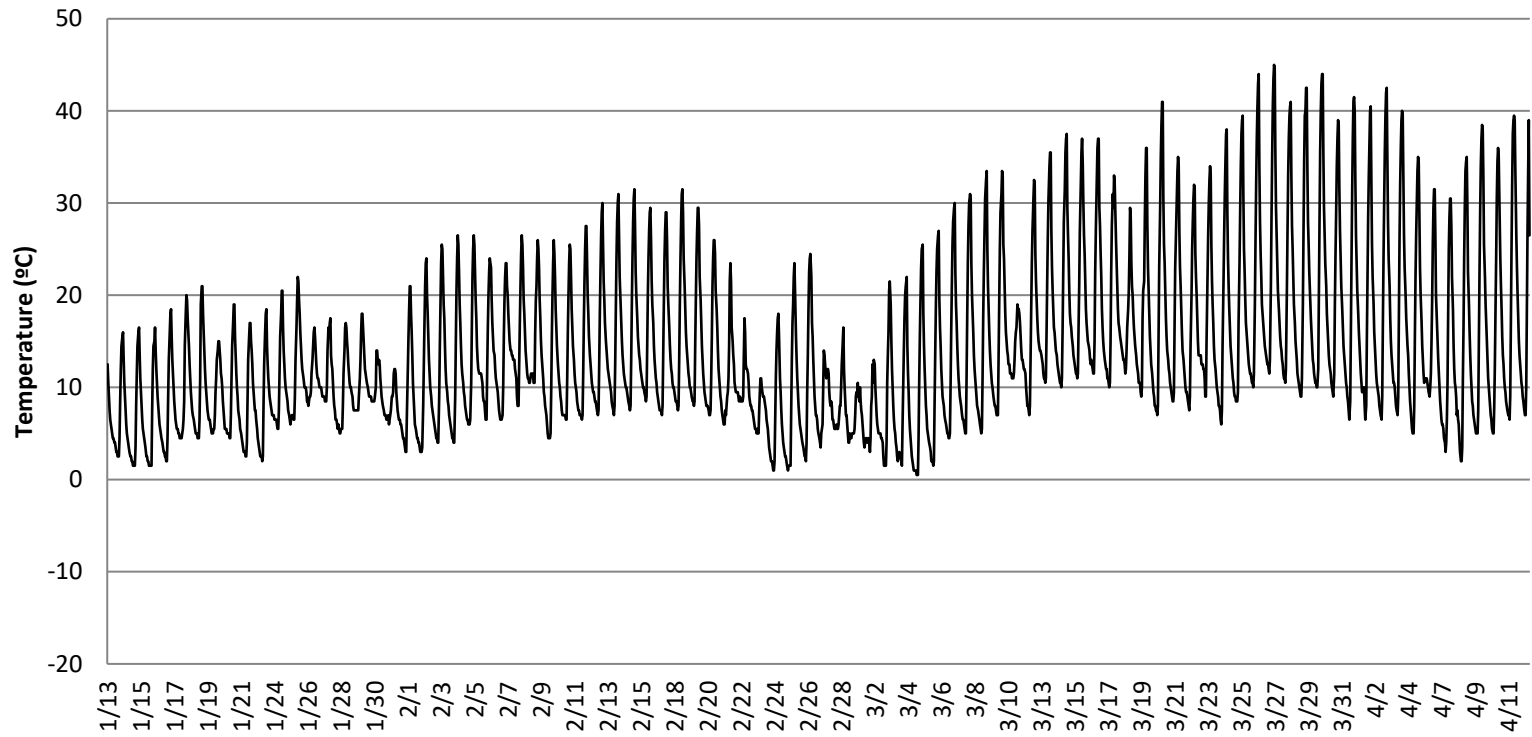


Figure 3. Data logger displaying the change in daily soil temperature buried in open canopy over the period of field storage in Swarthout Canyon, 2015

APPENDIX B

GERMINATION FOR EFFECT OF COLD STRATIFICATION FOLLOWING
HEAT SHOCK AND SMOKE-WATER IMBIBITION

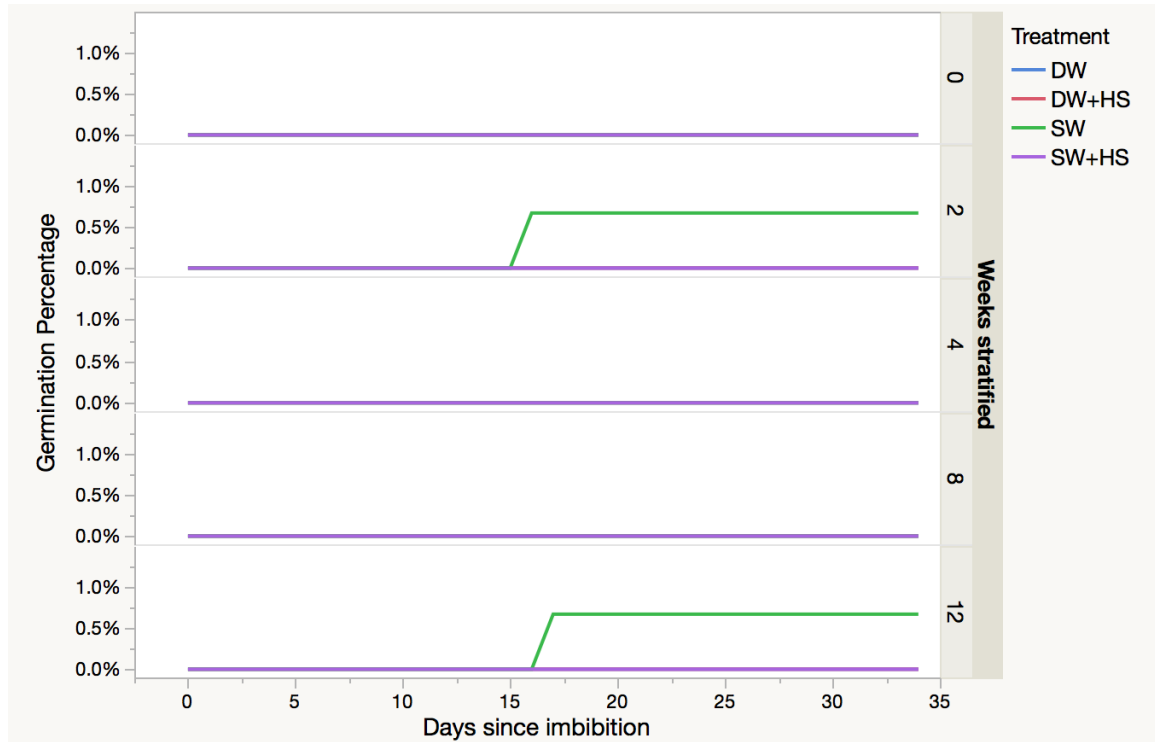


Figure 1. Cumulative germination of *E. chrysantha* seed after being cold stratified at 5°C (n=150). Seeds had four different treatments 1) 24-hour smoke water imbibition (SW), 2) 1 minute heat shock with smoke water plus 24-hour imbibition (SW HS), 3) 24-hour distilled water imbibition (DW) and 4) 1 minute heat shock with distilled water plus 24 hour distilled water imbibition (DW HS).

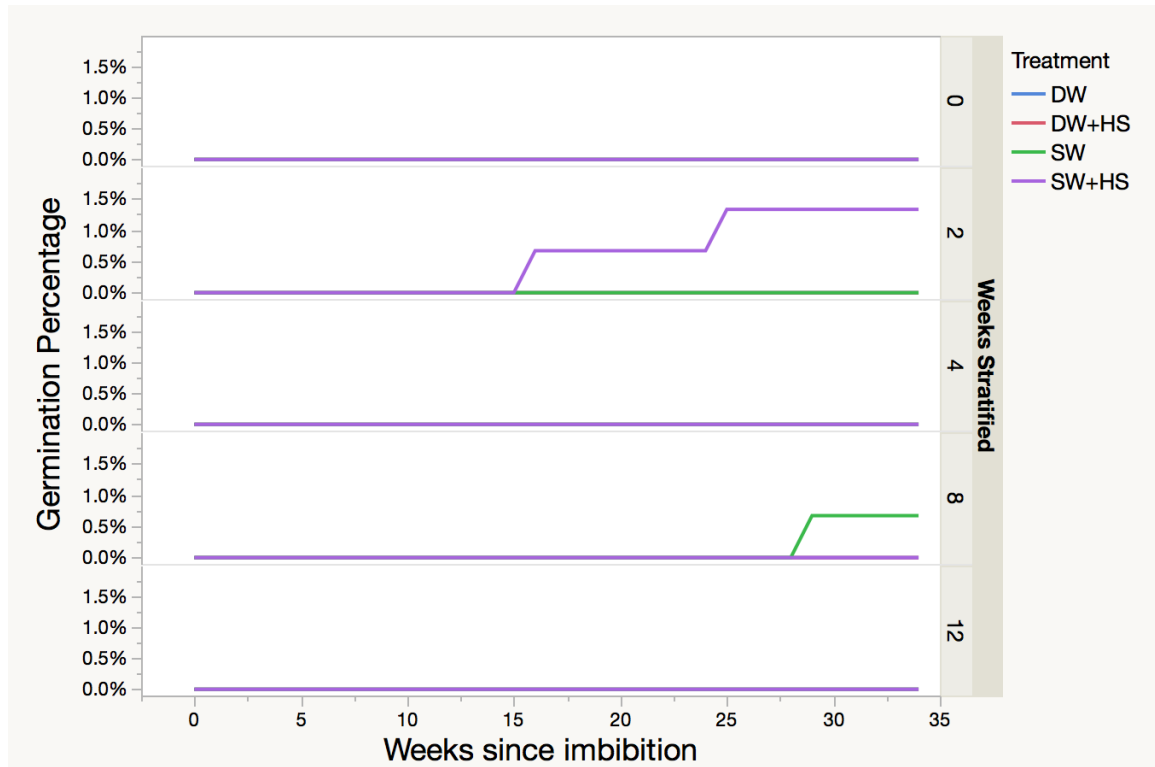


Figure 2. Cumulative germination of *D. rigida* seed after being cold stratified at 5°C (n=150). Seeds had four different treatments 1) 24-hour smoke water imbibition (SW), 2) 1 minute heat shock with smoke water plus 24-hour imbibition (SW HS), 3) 24-hour distilled water imbibition (DW) and 4) 1 minute heat shock with distilled water plus 24 hour distilled water imbibition (DW HS).

APPENDIX C

EFFECTS OF MOIST WARM STRATIFICATION ON SEED GERMINATION

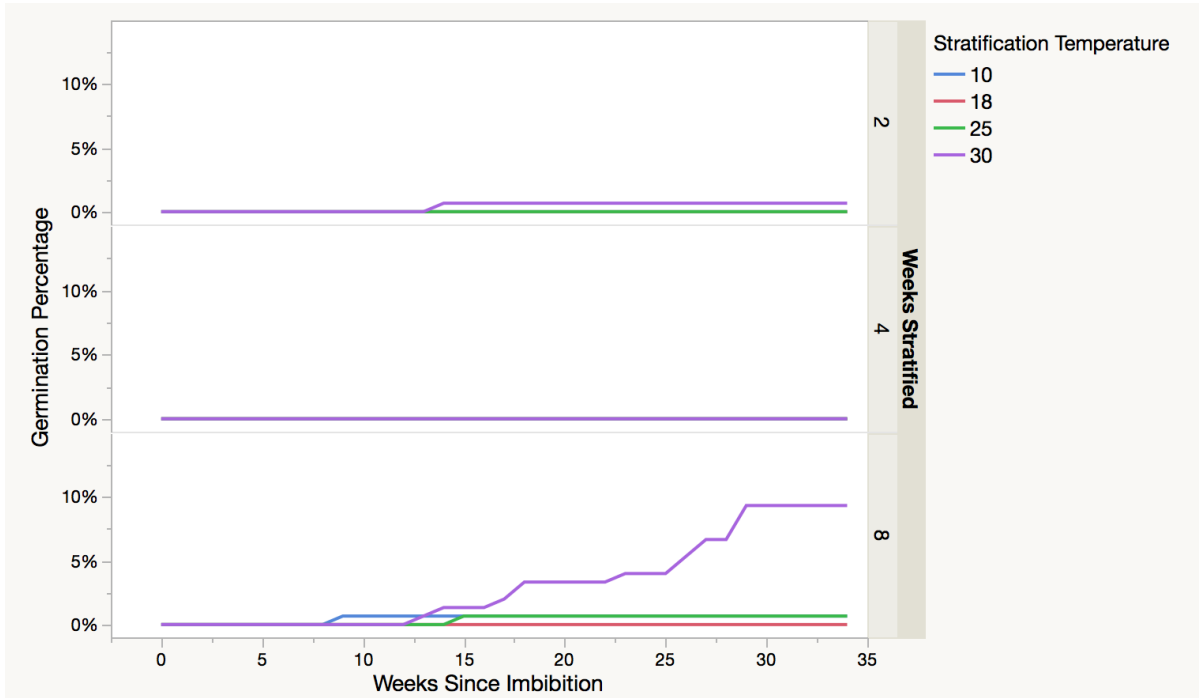


Figure 1. Cumulative germination of *D. rigida* after exposure to four different temperature regimes (10°C, 18°C, 25°C and 30°C) and different lengths of temperature exposure a) 2 weeks b) 4 weeks and c) 8 weeks (n=150).

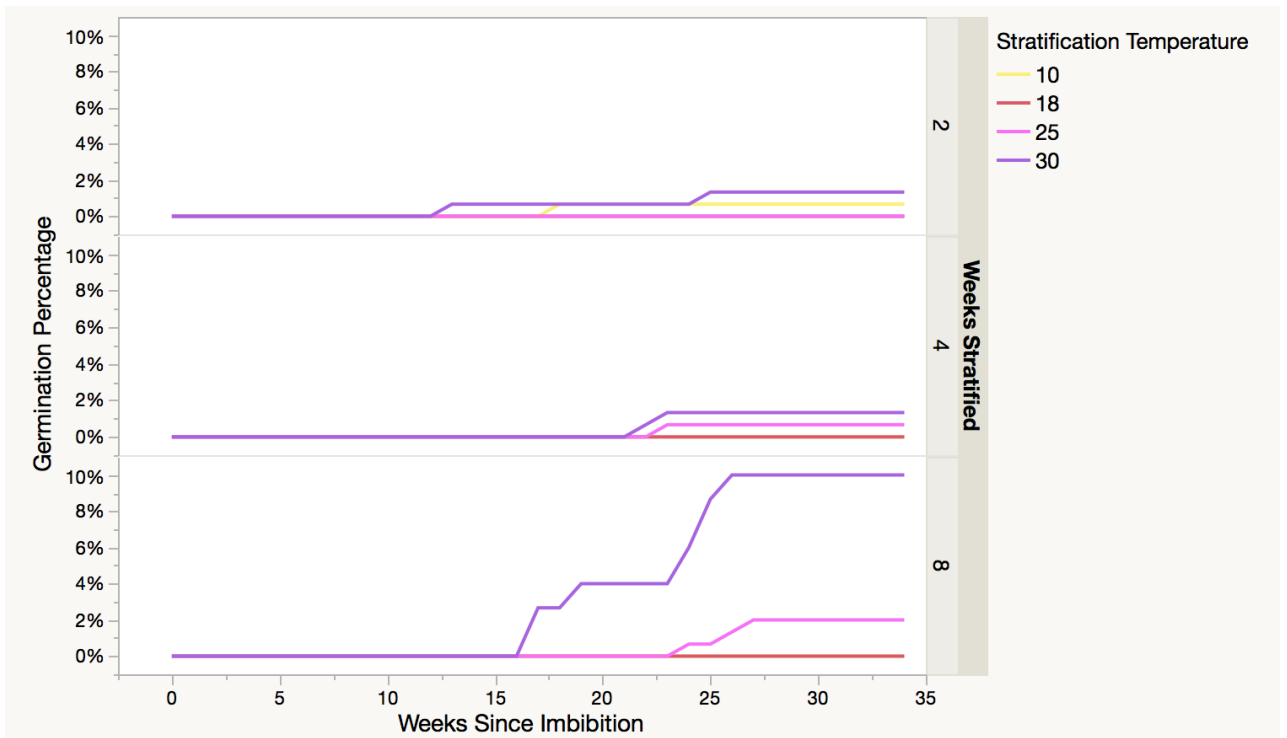


Figure 2. Cumulative germination of *E. chrysantha* after exposure to four different temperature regimes (10°C, 18°C, 25°C and 30°C) and different lengths of temperature exposure a) 2 weeks and b) 4 weeks c) 8 weeks (n=150).

APPENDIX D
EFFECTS OF ORDER OF COLD STRATIFICATION AND SMOKE-WATER
TREATMENT ON SEED GERMINATION

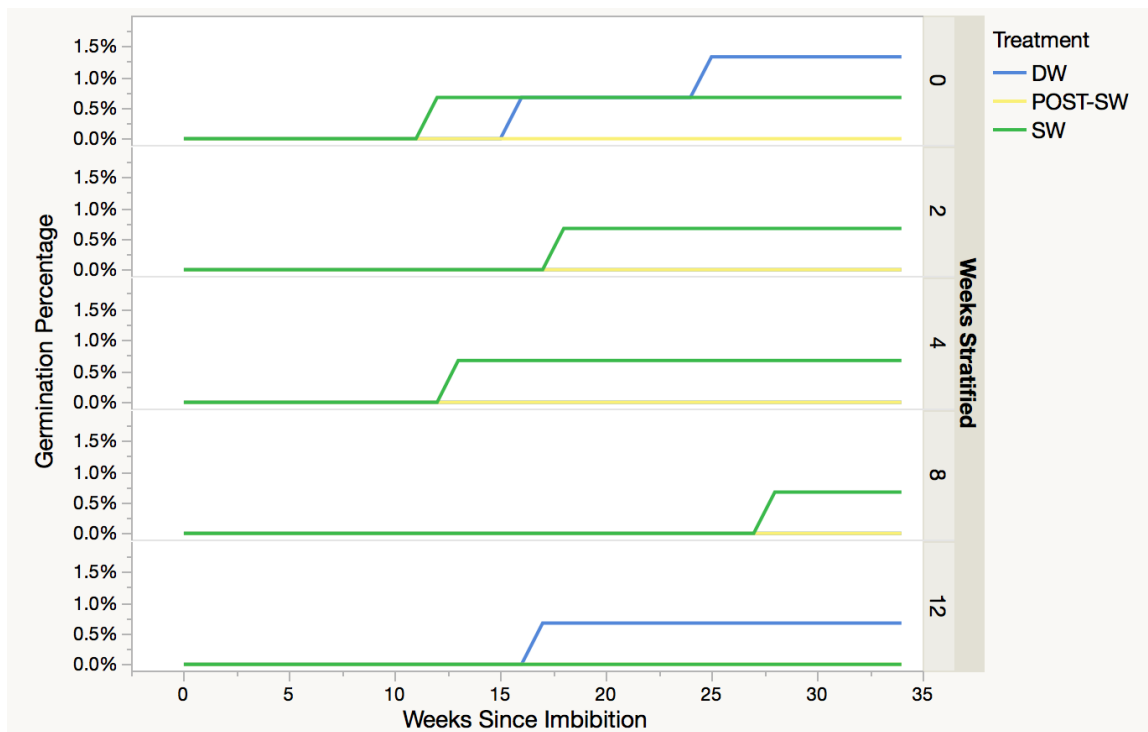


Figure 1. Cumulative germination of *E. chrysantha* seed exposed to 5°C cold stratification combined either with smoke-water imbibition (SW), de-ionized water imbibition (DW), or smoke-water exposure after removal from cold stratification (POST-SW).

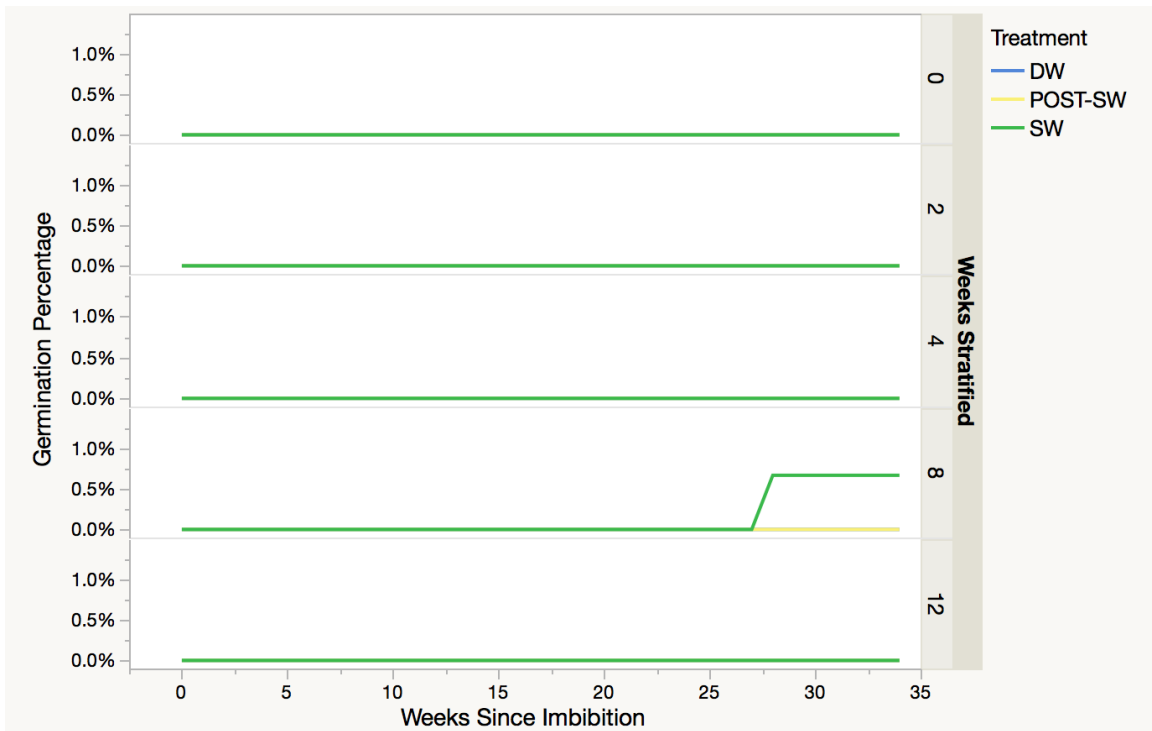


Figure 2. Cumulative germination of *D. rigida* seed exposed to 5°C cold stratification combined either with smoke-water imbibition (SW), de-ionized water imbibition (DW), or smoke-water exposure after removal from cold stratification (POST-SW).

APPENDIX E
EFFECTS OF DRY/WET CYCLING ON SEED GERMINATION

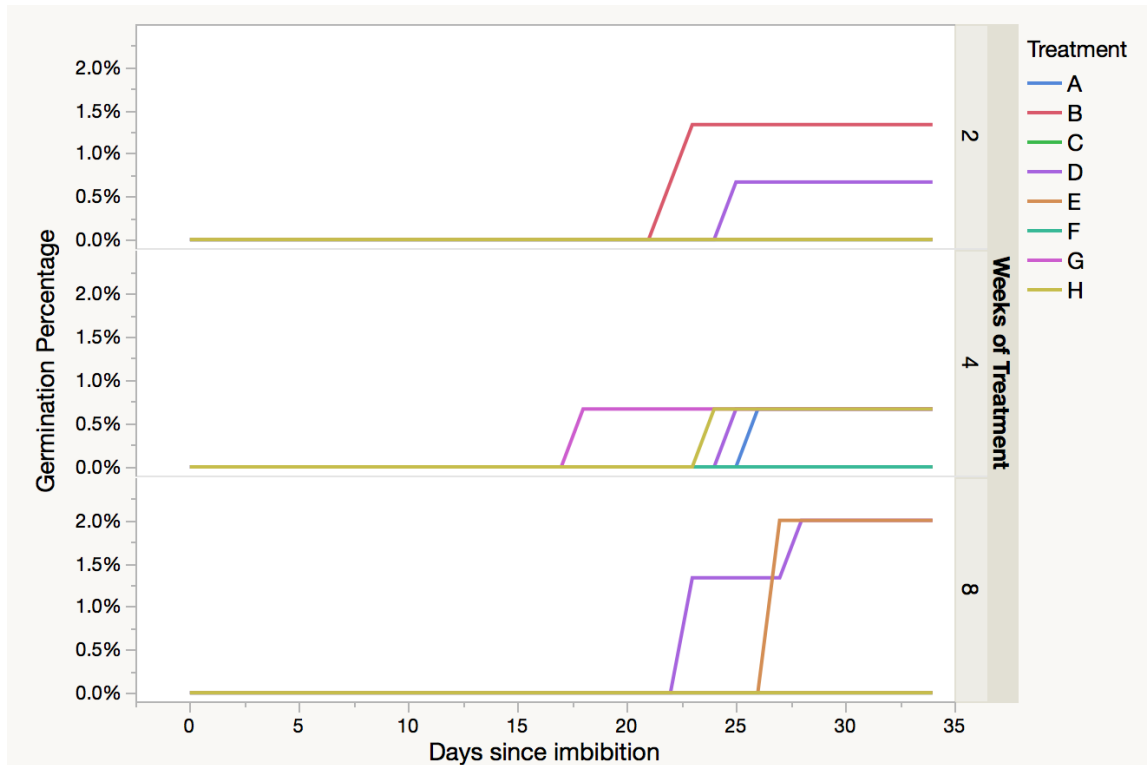


Figure 1. Cumulative germination percentage of *E. chrysantha* seed exposed to wet/dry cycling combined with smoke-water (SW) or de-ionized water (DW) treatments and different temperature regimes (RT = room temperature; n=150). (A - 5 days of storage at 37°C with no moisture, 1 day at room temperature with the petri dishes moistened with 5 mL de-ionized water, and 1 day at room temperature with no moisture. B - 5 days at 37°C and 2 days at room temperature. C - 6 days at room temperature with no moisture and 1 day at room temperature with the petri dishes moistened with 5 mL de-ionized water. D - 7 days at 20°C with continuous moisture in the petri dishes. Seeds in treatments A-D were imbibed in smoke-water prior to the cycles. E – H have the same treatments but seeds were imbibed in de-ionized water prior to the cycles.)

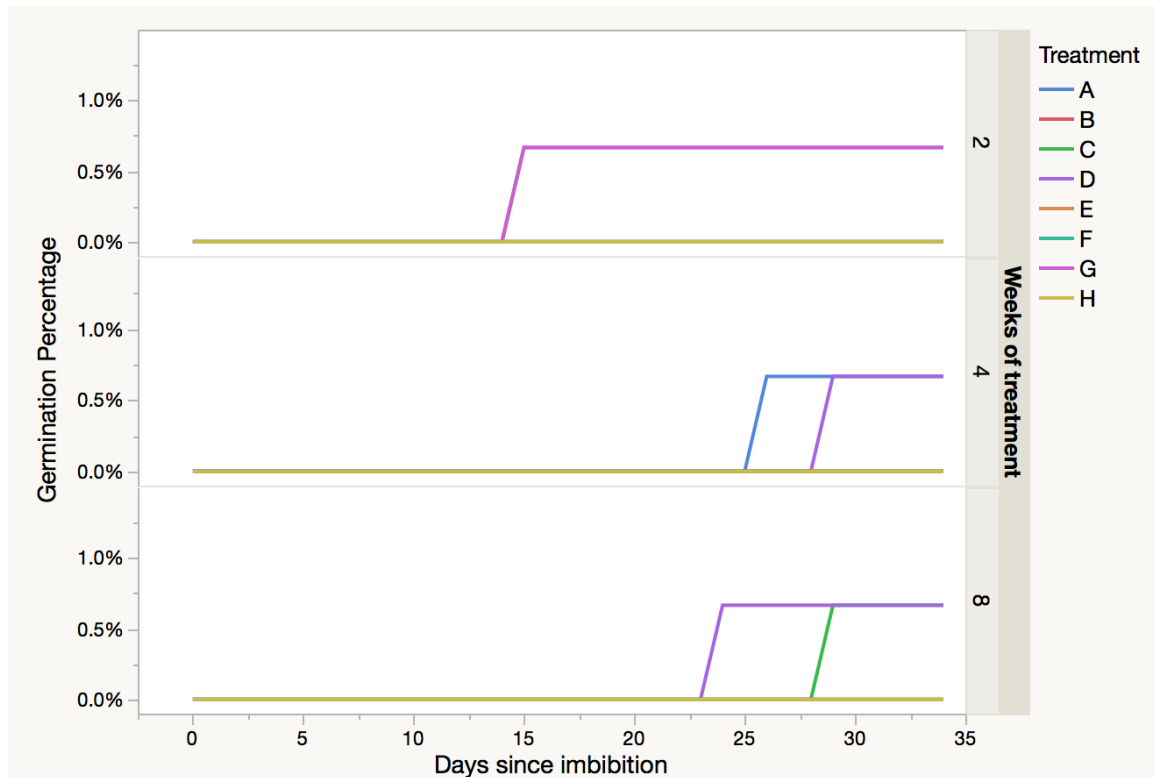


Figure 2. Cumulative germination percentage of *D. rigida* seed exposed to wet/dry cycling combined with smoke-water (SW) or de-ionized water (DW) treatments and different temperature regimes (RT = room temperature; n=150). (A - 5 days of storage at 37°C with no moisture, 1 day at room temperature with the petri dishes moistened with 5 mL de-ionized water, and 1 day at room temperature with no moisture. B - 5 days at 37°C and 2 days at room temperature. C - 6 days at room temperature with no moisture and 1 day at room temperature with the petri dishes moistened with 5 mL de-ionized water. D - 7 days at 20°C with continuous moisture in the petri dishes. Seeds in treatments A-D were imbibed in smoke-water prior to the cycles. E – H have the same treatments but seeds were imbibed in de-ionized water prior to the cycles.)

APPENDIX F
BIOASSAY FOR SMOKE WATER CONCENTRATION AND PLATING METHOD
DETERMINATION

A bioassay was required to determine the potency of a new batch of smoke-water prepared in lab. *Salvia mellifera* and *Emmenanthe penduliflora* were used because of their rapid germinability to smoke-water.

Methods

Seeds of *Salvia mellifera* (black sage) were collected from coastal sage scrub near California State University, San Bernardino and *Emmenanthe penduliflora* (whispering bells) seeds were purchased from Seeds of Success. For all experiments, 35 seeds were randomly chosen per plate by selecting visibly undamaged seeds. All experimental incubation was conducted in a diurnal common incubator (Model: RF 2015, VWR, Radnor, PA) with 20°C/10°C and 12-hour light conditions.

The first experiment consisted of imbibing the seeds of both species in 0%, 0.1%, 1%, 5%, 10%, 20% or 30% smoke-water for 6 hours and transferring the seeds into petri dishes (Fisher, polystyrene, 90 x 15 mm) with 0.7% agar made with corresponding concentration of smoke-water the seeds were imbibed in. Seeds were rinsed with de-ionized water after imbibition prior to plating the seeds. The dishes were wrapped with plastic wrap to prevent evaporation and aluminum foil to control for light inhibition.

The second experiment consisted of imbibing the seeds of both species in 0%, 0.1%, 1%, 5%, 10%, 20% or 30% smoke-water for 24 hours and transferring the seeds into petri dishes with 0.7% agar. Seeds were rinsed with de-ionized water after imbibition prior to plating the seeds. The dishes were wrapped with

plastic wrap to prevent evaporation and aluminum foil to control for light inhibition.

The third experiment consisted of plating seeds of both species on petri dishes with glass fiber filter paper. Seeds were hydrated with 4mL of 0%, 0.1%, 1%, 5%, 10%, 20% or 30% smoke-water. The dishes were wrapped with plastic wrap to prevent evaporation and aluminum foil to control for light inhibition.

Plates were checked for germination after 3 weeks of incubation in room with ambient light. Seed germination was determined by noting radicle emergence. Germination percentages were compared between trials to determine which smoke-water concentration should be used and which method should be used for germinating *Dendromecon rigida* and *Ehrendorferia chrysantha*.

Determination of Smoke-water concentration

The bioassay of *Salvia mellifera* and *Emmenanthe penduliflora* resulted in 20% smoke-water in plain agar being the optimal concentration for imbibition (Figure 1 and Figure 2).

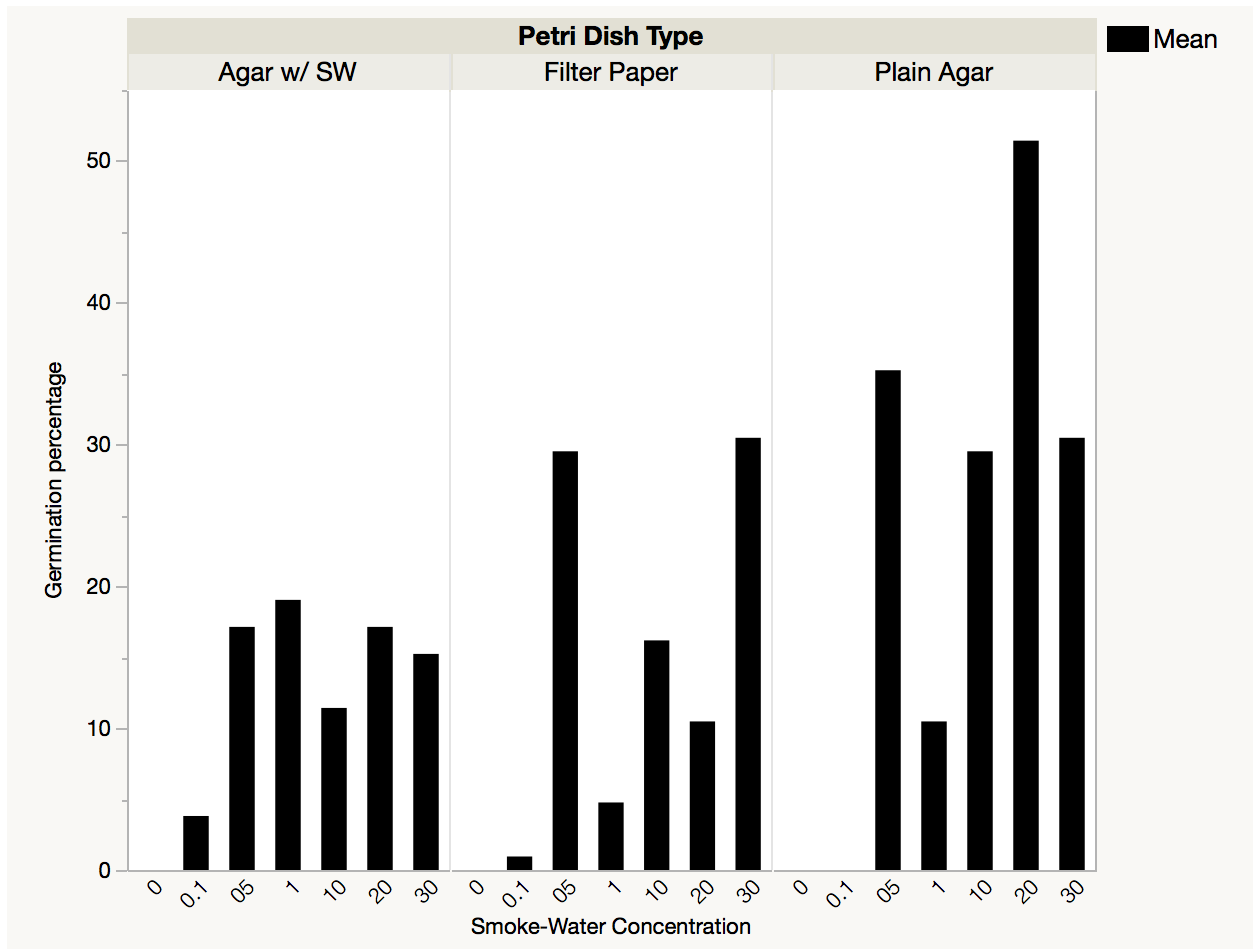


Figure 1. Bioassay of *Salvia mellifera* across different smoke-water treatments, n=35. 1) 0.7 agar plates with smoke-water, 2) plates with filter paper moistened with smoke-water, 3) plain agar with seeds imbibed in smoke-water prior to plating.

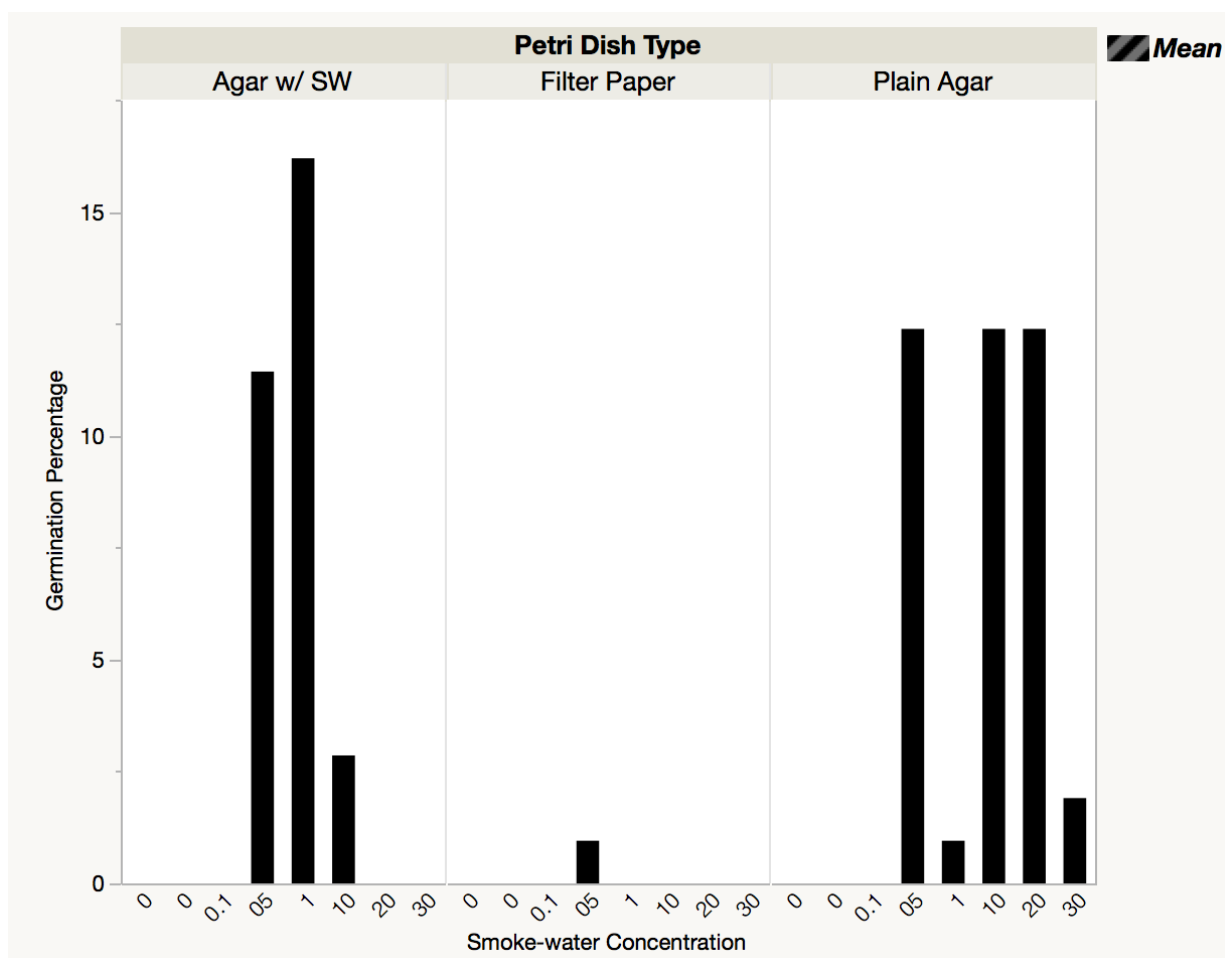


Figure 2. Bioassay of *Emmenanthe penduliflora* across different smoke-water treatments, n=35. 1) 0.7 agar plates with smoke-water, 2) plates with filter paper moistened with smoke-water, 3) plain agar with seeds imbibed in smoke-water prior to plating.

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