Smoking Grass:

Germination responses of six native *Poaceae* species to smoke water treatments

by

Conrad Ely

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Conrad Ely

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by

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Kevin Francis Ph. D.
Member of the Faculty

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ABSTRACT

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The South Puget Sound prairies are one of the most threatened ecosystems in the United States due to fragmentation, land use changes, non-native species invasion, conifer encroachment and climate change. From 2009 through 2015, the Sustainability in Prisons Project (SPP) has grown plugs for the revegetation of this ecosystem. During that time they have found a number of species to have particularly low germination rates, including species from the family Poaceae. Historically, these prairies were burned for thousands of years by Native Americans to promote the growth of plants used for food, fiber and medicine. The health of the ecosystem has declined in the last 150 years since European settlers implemented fire suppression practices. In 2004, two separate research teams discovered a chemical in smoke, karrikinolide (KAR_1) that promotes germination in some species. From 2004 through 2014, 1,355 species from 120 families have been tested for their responses to smoke and smoke derived products. Of those, 95 species of *Poaceae* have been tested, with 46 species having significant increases in germination. This thesis examines six native *Poaceae* species that have had poor germination rates in SPP nurseries, and their responses to plant-derived smoke water. Using the stratification protocols of SPP, followed by a five-week germination period, one of our six species (*E. glaucus*) was found to have a statistically significant ($p< 0.029$) increase in germination. Four of our six species (*E. glaucus, E. trachycaulus, D. californica, & B. carinatus*) had an average germination rate above 50% in our control group. These species appear not to be germination limited. Two of our six species (*D. oligosanthes var. scribnerianum & D. acuminatum var. fasciculatum*) had an average germination rate below 30% in our control group. These species may be germination limited.

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Introduction

Restoration of the South Puget Sound Prairies is a challenging endeavor. These grasslands are highly fragmented, existing as small islands amidst a sea of anthropogenic land use. Geographically separated by roads, farms, neighborhoods, towns and conifer forests, much of the challenge in restoring this habitat comes from a lack of natural regeneration of the plant community (Dunwiddie & Bakker, 2011). In order to augment this ecosystem, restoration organizations plant out native seeds and plugs to bolster the diversity. However, limited seed banks and low germination rates continue to pose a problem for restoration ecologists.

Ecosystem fragmentation is one of the major factors driving biodiversity loss today. This can occur by way of deforestation, construction of roads, fences and buildings, land use changes, natural disasters, and climate change. Fragmentation is clearly linked to habitat decline due to alteration of critical ecosystem processes such as plant dispersal, plant community dynamics, plant and animal reproduction, and animal movement patterns (Collinge, 2000). These processes become limited and altered as an ecosystem is reduced to patches. The greater the distance between these patches, the more unlikely cross pollination of separated plants or recolonization by lost species becomes. The smaller the area of remaining contiguous habitat left, the greater the likelihood of species extinction (MacArthur & Wilson, 1967).

Endemic species are especially sensitive to such trends. As we lose endemic species, we lose the potential for further evolutionary steps towards recovery of heterogeneous ecosystems (Wolf, 2001). Seed dispersal, as well as pollinator diversity and abundance are heavily affected by fragmentation, and clearly impact endemic flora. However, seed longevity and vegetative reproduction act as buffers allowing endemic species to persist within small patches (Wolf, 2001). Regardless, as patches decrease in area, limited resources will become monopolized by dominant species and diversity will drop (MacDougall and Turkington, 2007). As fragments become smaller, the system is more vulnerable to collapse due to trophic simplicity and single species dominance (Collinge, 2000). The loss of ecosystem complexity pressures endemic species more heavily as their range is finite and competition becomes increased.

Here in the Pacific Northwest, the South Puget Sound prairies are an ecosystem suffering from fragmentation and non-native species invasion. These prairies are home to many rare, endemic, and declining species, including four state-endangered species (spsp.org). In Washington State, this ecosystem has been reduced to 2-4% of its historical extent (Hegarty, Zabowski & Bakker, 2011). Restoration ecologists are using a variety of techniques to combat the degradation of these prairies, but the results have been mixed thus far.

One conservation organization, the Center for Natural Lands Management, bolsters the prairie ecosystem using direct seeding and revegetation using plugs. The purpose of this practice is to increase biodiversity and limit non-native species and conifer encroachment. These treatments can be an effective means of increasing prairie health, however limited seed sources and poor germination rates of both direct sown and nursery raised seeds are limiting factors to potential success.

Although the limitations described affect plants from many families, the focus of my research is on species of this distinction within the *Poaceae* family. They are important early seral species, which colonize disturbed prairie sites, providing critical food and cover for a variety of prairie fauna (Tollefson, 2006). They also play an important role in erosion control, keeping valuable topsoil bound in their complex root systems and protecting against soil transport by wind and water. These are some of the first species to be planted out in prairie revegetation projects, and thus are an important target for improved germination rates.

In order to understand what may be hindering germination of these plants, it is critical to first understand their natural history. Fire regimes played a significant role in the ecology of the region. Native Americans burned the prairies every 2-3 years for millenia in order to easily collect food, fiber and medicine as well as to combat against native conifer encroachment (Rook et al, 2011). This practice continued into the nineteenth century until western colonizers imposed burn bans. The omission of annual fire from this ecosystem is a serious change in the disturbance regime these plants experience.

While the use of prescribed fire is a proven and effective means of prairie restoration, there are a number of factors that make it unfeasible as a restoration tool during certain seasons of the year. The major factors that limit the use of prescribed fire are the number of people necessary to safely implement a controlled burn, the associated costs of equipment and training for that crew, the litigation against burning during certain seasons of the year, and its potential effectiveness in small-scale application. In response to these concerns, this thesis will delve into the components of fire that contribute to prairie regeneration and attempt to isolate one variable that may prove to be an effective surrogate to be used in the instances where prescribed fire is not a viable option.

Recent studies have identified a class of compounds released in plant-derived smoke called Butenolides. These compounds have been tested on 1,355 species of plants from 120 families for their germination responses. Within *Poaceae*, 95 species (of more than 10,000 species) have been tested, with 46 resulting in significant germination increases (Jefferson, Pennacchio & Havens, 2014). Those that have been tested come from a variety of ecosystems on six continents, with germination increases found primarily in species of fire-prone ecosystems. Treatment of seeds for this purpose have been applied both by aerosol smoke as well as plant-derived smoke water.

This thesis will explore whether the addition of plant-derived smoke water will increase germination rates for six species of *Poaceae* that have a history of poor germination in our nurseries. The seeds from each species were imbibed with plant-derived smoke water during their stratification stage. The species I have chosen are regularly grown for South Puget Sound restoration and represent some of the local diversity in the *Poaceae* family.

My hypothesis for this experiment was that the addition of plant derived smoke-water would increase the rate of germination for each species when compared to a control of deionized water. My definition for germination is the emergence of a 2mm radicle from the seed after germination is induced, by alternating light and temperature regimes. I monitored germination for 35 days (5 weeks). In my literature review, I synthesize the current science regarding all variables of my experiment including prescribed fire, seed germination, germination cues related to fire, plant-derived smoke water, my study species and future implications. The following chapters outline my materials and methods for the experiment, summarize my results and discuss their potential causal factors, and share the conclusions we can garner from this experiment.

Literature Review

Introduction

This thesis was inspired by my work as a conservation nursery coordinator with the Sustainability in Prisons Project (SPP). We are partnered with the Center for Natural Lands Management (CNLM) to raise plugs for the revegetation of the South Puget Sound (SPS) Prairies. It is through this work that we identified the species of focus for this thesis, which we have had difficulty growing in our nurseries from 2009-2015. The literature on the SPS prairies as a threatened ecosystem is vast and well documented. However, our knowledge of smoke-derived germination-inducing chemicals is relatively recent, and is still becoming understood. This literature review details the basis for the work we are doing in the SPS prairies and explores previous studies in the field of fire ecology that motivated our decision to experiment with plant-derived smoke water as our treatment.

SPS Prairie Environmental History

In order understand the energy and resources being put into prairie revegetation, it will be helpful to understand the natural history of the current ecosystem as well as historical changes in ecosystemic conditions that lead to the current state of the SPS prairies. In particular, the disturbance regime today is completely altered from what was experienced over the last five to seven thousand years (Rook et al., 2011). This change is at the heart of how the ecosystem functions now as opposed to the thousands of years prior to European arrival.

Historically, Native Americans burned these grasslands on a semi-annual basis until colonists put into effect fire suppression practices. Fire served many purposes, both culturally and ecologically. It was an effective means of control against encroaching conifer saplings, as well as against many non-native herbs and grasses (Hamman et al., 2011). Having open non-forested areas was advantageous for hunting, and the fires would drive game into the open range (Kruckeberg, 1991). After burning the prairies, Native Americans could more easily harvest plants used for food, fiber and medicine from the soil (Dunwiddie & Bakker, 2011).

In the SPS prairie ecosystem regular fire return intervals act as a pulse disturbance, where stand-replacing events reset the system back to an early successional stage (Bender, Case & Gilpin, 1984). As the system regenerates, colonizing species are the first to fill in the landscape, and tend to grow at a faster rate, as there is little competition and an abundance of nutrients, light and space. Fires promote these plants' ability to sprout, soil seed bank adaptations, and their ability to disperse seeds (Agee, 1996). The local tribes understood this process, and used it to their benefit. Not only did fires encourage the growth of important forage species, but it also made collection and harvest of these easier.

Burning the prairies specifically encouraged the growth of Camas (*Camassia quamash*), which was a culturally important plant used for food and medicine (Kruckeberg, 1991). But Camas was not the only native species of this region that evolved adaptations to fire. Species of the genera *Castilleja* are fire adapted and were used medicinally. *Balsamorhiza deltoidea* is a large flowering species that is promoted by fire and whose seeds were eaten by many tribes. *Solidago* roots were used medicinally for a number of maladies and sicknesses. Species of the *Lupinus* genera are also fire adapted and were regularly used as a food source by tribes as well as Europeans.

As Native Americans used fire to improve hunting, manage the ecosystem and increase supplies of food and medicine, the regular disturbances began to shape the species makeup and successional trajectory of the region. Fire characterized the region by opening up niche space, which in turn reduces single species dominance (Rook et al., 2011). Over millennia, the SPS prairies became conditioned to regular disturbance by fire. Prairie ecosystems are most limited by light, and therefore most affected by the encroachment of fast growing shrubs and trees that can quickly rise above and shade out shorter, slow growing native species (Tillman, 1997). Today some of the most prevalent examples are invasive Scot's Broom (*Cytisus scoparius*) and native Douglas Fir (*Pseudotsuga menziesii*). And as a disturbance-dependent ecosystem, the break in that disturbance pattern can pose a risk to the region as single species dominance can become prevalent (MacDougall & Turkington, 2007).

Prescribed Fire

With the knowledge that stand replacing fires were a regular event in the SPS prairies for millennia, yet are practically absent today, it follows that this ecosystem is lacking components related to fire that could influence the imbalance we witness today. Fires are recognized to maintain species diversity, eliminate non-native fire-intolerant plants, remove allelopathic substances, increase light intensity, change nutrient cycling and pH, as well as increase net primary production (Jefferson et al., 2008). Many of these environmental conditions work in succession with each other and can produce convergent properties that are more substantial than any taken individually. Due to these influences, restoration ecologists working in the SPS prairies favor prescribed fire as a restoration tool, when possible.

Managing the SPS prairies as an altered ecosystem today is a complex task that incorporates controlled fire, herbicide application, mowing, tillage, grazing and manual removal of non-native species. Controlled burns have become a major tool for prairie restoration in this region (Rook et al., 2011). However, controlled burns can only be administered under specific regulations and are also labor and equipment intensive. In order to effectively use prescribed fire, managers must understand the scale that is necessary for restoration, have a knowledgeable and collaborative burn team of at least twenty five individuals, as well as proper programmatic and political backing (Hamman et al., 2011). Prescribed fires pose the known risk of potential escape to surrounding areas, which could cause substantial economic damage (MacDougall & Turkington, 2007). In addition to the known logistical challenges, a number of questions remain regarding the consequences of using fire in this novel ecosystem.

The benefits from fire historically are clear, but whether or not those carry over to SPS prairies in their current state remains to be seen. One unknown is prairie size, and whether the use of fire can be effective at the small scale that many remnant prairies exist in today. Stand replacing disturbances such as fire normally occur on a large scale (typically >10ha). Therefore using such treatments in a habitat that is greatly fragmented, having isolated patches smaller than 10 ha, could negatively affect rare plants and animals that were formerly wide ranging, but now exist as an isolated, threatened or endangered species (MacDougall & Turkington, 2007). Another unknown is the potential germination of non-native fire adapted seed banks that could exist in the soil (Rook et al., 2011). When an area has been invaded and the species makeup has shifted, the seed bank can be as rich with invasive species as it may be with natives. While fire can be an incredibly effective tool for restoration goals, it also poses many challenges, which could be limiting depending on the scale of a restoration project.

Many of the benefits associated with fire are the product of a stand-replacing disturbance. Exposure to light and bare soil, elimination of non-native plants and elimination of single dominant species are all disturbance-based changes to grasslands. But fire is unique from other forms of natural disaster with regards to the chemical changes it can impart on a system. Its ability to remove allelopathic substances from soil, cycle nutrients, and change pH are all evidence of chemical transformations that can occur in and after a fire (Jefferson et al., 2008). But fires influence on seed germination is an area of particular interest to this thesis.

Seed Germination

Seed germination is the first stage of plant growth and is one of the few mechanisms for plant reproduction. Environmental variability is critical to this process and largely controls how and when a seed may germinate. Moisture content, light availability, soil chemistry, temperature regimes, seed viability and predation are all factors that can work independently or in succession to promote or inhibit this process.

The manner is which these variables affect seed germination is dependent upon the seed's physiology.

Serotiny is one such physiological trait that can differentiate germination mechanisms. It is defined as those seeds, often held in cones or fruit on trees, which remain attached to their mother plant after seed maturation and, when released, are immediately ready for germination (Baskin & Baskin, 2001). Serotinous seeds do not rely on a state of dormancy prior to germination, and instead rely on environmental cues that trigger their release from the parent plant. Conversely, non-serotinous seeds are released at the time of maturation regardless of the environmental conditions and instead rely on dormancy to remain viable in the soil bank until the proper conditions present themselves. Non-serotinous seeds can wait months or years for the appropriate cues prior to germination.

Dormancy within the soil bank is a trait only exhibited by non-serotinous species. But in order to understand the type of dormancy, these plants can be further split into two groups - those that possess a thick, hard, water and gas impermeable seed coat and those that can readily absorb water. The species that have an impermeable seed coat experience a state of physical dormancy. They are most likely to break this state by extreme heat shock, generally caused in the natural environment by a fire (Keeley & Fotheringham, 1998). This occurs when the hard, water-impermeable, seed coat is cracked by heat and water is allowed to penetrate and thus facilitate germination (Light, Daws & Van Staden, 2009). This cracking can also occur through freezing, extreme temperature fluctuations, or passage through animal digestive systems.

The species that do not have an impermeable seed coat, such as those from the *Poaceae* family, rely on a different mechanism to trigger their germination. They can readily absorb water and have no limitations to gas exposure. *Poaceae* species tend to experience nondeep physiological dormancy, which requires a chemical change to promote embryonic growth (Baskin & Baskin, 2001). The most common chemical changes are triggered through variation of moisture content, temperature and light exposure. However, other mechanisms exist that can alter seed chemistry, and fire is well documented to promote such chemical changes.

Fire and Germination

Documentation of seeds being treated with smoke prior to sowing date back to 1632, although the practice itself could be much older than the written record. This was observed of the Huron people of New France, who suspended germination boxes with soil and pumpkin seeds above their fires, which reportedly “increased the number of sprouters” (Jefferson, Pennacchio & Havens, 2014). Since that initial observation, similar instances of smoke-preparation of seeds have subsequently been witnessed of indigenous people in South Africa and Guatemala. Fire and smoke have been ubiquitous throughout human history, and practices of this nature have been prevalent throughout many cultures. Nonetheless, formal studies of the association between fire and increased seed germination are a relatively recent development.

One of the first published studies on the subject was in 1977, by D.T. Wicklow. The experiment tested the seeds of *Emmenanthe penduliflora* with two treatments, one of burned (3/4 charred) plant stem segments and the other of incinerated plant stem ash. The seeds touching the burnt stems germinated while those covered in ash rarely germinated. Burnt stems were then placed on ungerminated seeds that were previously used in the control, and subsequently began to germinate. The take-away from this experiment was twofold – there is an association between burnt plant material and seed germination, but plant material that is completely incinerated does not garner the same effect.

Later the association between charred plant material and seed germination was extended to smoke, which carries many of the same chemicals as the burnt plant material itself. For the sake of experimentation smoke can be easier to control for than the inconsistencies of charring plants uniformly. However, this shift does not show up in the literature until 1990 when de Lange and Boucher observed significant increases in the germination of *Audouinia capitata* after exposing the seeds to aerosol smoke. As traction began to increase on the subject, studies from the Cape Floristic region of South Africa, the Southwest Botanical Province of Western Australia, and the Californian Floristic Province became prevalent as each are biodiversity hotspots with associated fire-prone ecosystems (Jefferson, Pennacchio & Havens, 2014). Further studies have yielded increased germination of species from Mediterranean, semiarid, arid, temperate, subtropical and tropical regions.

As more articles became published, indicating a connection between smoke treatments and increased germination, researchers began to investigate the mechanism driving these results. Initial studies concluded that the stimulatory effect could be produced by a wide variety of plant materials and is not dependent on light. It is water soluble, active 24 hours after exposure, and can be produced at 175° C for 30 minutes

(Keeley & Pizzorno, 1986). It took researchers decades to investigate the thousands of compounds that can be present in smoke. One study successfully isolated and tested seventy-one different compounds, but none individually produced as significant of results as aggregate smoke treatments (Baldwin et al., 1994).

Finally in 2004, two separate research teams independently identified a water-soluble compound, produced by the combustion of cellulose that stimulated germination in lettuce seeds. One group (Flametti et al.) worked in Western Australia while the other (van Staden et al.) was in South Africa. The product, later named karrikinolide (KAR_1), belongs to the butenolide class of compounds. Butenolides are documented to drive a variety of biological activities, including “promoting and inhibiting seed germination, inhibiting shoot branching, inducing hyphal branching in arbuscular mycorrhizal fungi, toxicity and antibiosis” (Jefferson, Pennacchio, and Havens, 2014).

Since the discovery of KAR_1, studies have been done in Australia, Europe, South Africa, and the United States to test its effectiveness of increasing seed germination (Reyes & Trabaud, 2008). Although many of these trials have shown positive results, some tests of KAR_1 exclusively, have not had the success that a plant derived smoke-water solution had (Kulkarni et al., 2011). This is indicative of the number compounds that can be found in smoke, and the fact that germination cues often compound upon each other. Isolating an individual synthesized compound may not be as effective as a solution that holds many compounds and thus, potentially, multiple cues for germination. This is the basis for our choice of testing plant-derived smoke water for our experiment.

While researchers have isolated butenolide compounds, and can synthesize KAR_1 for more controlled applications, the specific mechanism of *how* it promotes germination is still under investigation. It is hypothesized that there are similarities between the effects of smoke and other plant growth regulators, such as gibberellins; both can stimulate germination by substituting for red light (Light, Daws & Van Staden, 2009). Subsequent research suggests KAR_1 improves temperature and water potential range, which in turn can increase germination rates (Kulkarni et al., 2011). In the decade since its discovery, many theories have been posited, yet none have been fully substantiated as to how specifically butenolides increase germination at a chemical level. Nonetheless, the significant amount of research connecting smoke-treatments and increased seed germination is undeniable, and, for the purposes of this experiment, will suffice.

Plant-Derived Smoke Water

Plant-derived smoke water is a solution used in experiments as well in horticulture to control smoke application. Other forms of smoke application include aerosol smoke, aqueous ash solutions, as well as directly smoking a plant or patch of property with a large mechanized smoking apparatus and tenting. While aerosol treatments of seeds have had negligibly better results in germination treatments, it is accepted that smoke-water is a more realistic, less cumbersome mode of smoke application for restoration purposes. (Kulkarni et al., 2011; Llyod, Dixon & Sivasithamparam, 2000). Smoke water can be produced in large batches, stored easily, and applied over a larger area of land with greater ease than the other methods of smoke application.

Plant-derived smoke water is created by "bubbling smoke through a container of water" (Light et al., 267). According to Light, Burger and Van Staden active compounds are produced between 160° and 200° C, however higher temperatures may lead to volatilization of those compounds (2005). To create smoke-water for this study, I will follow the protocols laid out by Flematti et al., except I will use *Quercus garryana* for the coals and the chaff from a native perennial in place of *Banksia-Eucalyptus* (2004). As of now there is no standardization for smoke-water concentration. Although the production methods are fairly universal, dilution rates range from 2:1 to 5000:1, depending on the study (Brown and Van Staden, 1997). For this experiment we are using a 100:1 dilution, based on the prevalence of its usage in the literature (Keeley & Fotheringham, 1998; Kulkarn et al., 2011; Lloyd et al., 2000).

Smoke Treatments of *Poaceae*

According to Jefferson, Pennacchio & Havens in their meta-analysis on the subject, 1,355 species of roughly 250,000 known plant species have been tested for some reaction to aerosol smoke, smoke-water, or plant-derived smoke products (2014). These plants range from six continents of the earth, and are native to both fire-prone regions as well as areas that do not regularly experience pressure from fires. The majority of the species that had reactions to these treatments were from Australia, South Africa, California, and the Mediterranean region. There were wide ranging effects from increased germination to decreased germination.

In the restoration of the SPS prairies, there is a moderate but critical dependence on nursery raised plugs for revegetation purposes. The species that are chosen to start in a nursery, as opposed to being directly sown in situ, are those that are either especially difficult to raise, are rare and lacking abundant seed sources, or are especially valuable as threatened species. Although transplanting plugs is not without its challenges, for some species this is simply the only viable option. Different plants require a variety of growing protocols in a nursery. Many require cold-wet stratification prior to sowing. Some require scarification of their seed coat. And others have responded favorably to smoke-water applications after sowing. Nursery managers are regularly looking for any edge that can improve the germination rates, seedling vigor, and survivorship of the plants they raise. This experiment is intended to improve the ex situ growth of a group of species that have previously challenged us with their low germination rates.

Poaceae, the grass family, is a logical place to start in the restoration of grasslands. Species from this family are the glue of the ecosystem, with their intricate root systems that bind the earth together, preventing erosion and nutrient loss. Prairie sites are first cleared of invasive and non-native species prior to transplanting native plants. *Poaceae* establish quickly in their place and can jumpstart the trajectory of a restoration project. The quick rise to maturation also allows for immediate forage and habitat cover for native fauna, which creates a feedback loop as they help to further spread seed and fertilize the area.

We found it fitting to do a survey of *Poaceae* responses to smoke water, as grasses are generally well adapted to survive under frequent fire regimes. The species we have chosen and were able to acquire seed for, represent a breadth of the native *Poaceae* of this region. Although none of the six species targeted in this experiment have previously been tested for a reaction to smoke treatments, other closely related species from the family have yielded significant germination increases (Jefferson, Pennacchio & Havens, 2014; Kulkarni, Light & Van Staden, 2011). In the meta-analysis on smoke treatments to date, nearly half of the *Poaceae* species tested (46 of 95) produced such results (Jefferson, Pennacchio & Havens, 2014). Undoubtedly, 95 species is hardly representative of a family comprised of over 12,000, but smoke treatments have not been widely documented to this point.

Study Species

Bromus carinatus is a medium-sized (45-120 cm) biennial or perennial bunchgrass. It is found in western North American states and provinces from Alaska to Baja California. It is found in cool moist woods and open meadows, from foothills to mountains. It produces numerous seeds that mature in June and July. It is a competitive species that establishes in disturbed environments quickly through high seed production. It is a successful competitor of exotic and invasive species, and its fibrous root system is great for erosion control. It provides nutritious and palatable forage for all species of ungulates, specifically elk. Bear, geese and rodents also consume its foliage, while small mammals and game birds consume the seed.

Danthonia californica is a medium-sized (30-100 cm) perennial bunchgrass. It is native from British Columbia down through California and out east to the Rocky Mountains. It is broadly adapted to woodland, shrubland, grassland, and transitional wetland habitats. California oatgrass grows sporadically from seed due to dormancy and competition. It flowers between May and early July. It is recommended for revegetation and recovery of savannahs, oak woodlands, and prairies in the Pacific coast states. It improves habitat by erosion control and invasive species competition. It also provides food, nesting and cover for songbirds. Its foliage is eaten by certain species of caterpillar and its grain is eaten by birds and mammals.

Dichanthelium acuminatum is a medium-sized (15-80 cm) perennial panic grass. It is native throughout all of the United States and Canada and can be found in woodlands, savannahs, prairies, glades and bluffs. It reproduces by seed only and establishes best in early secondary succession, however its density drops a few years after establishment. It flowers in June and then dies back, but often flowers again in September. Tapered rosette grass is valuable for ecological restoration of disturbed sites and is useful as a soil stabilizer. Moth larvae and the caterpillars of skippers consume its foliage, while the seed is eaten by game birds and songbirds.

Dichanthelium oligosanthes is a medium-sized (30-60 cm) perennial panic grass. It is native to all of the United States and Canada except Nevada and South Carolina. It can be found in woodlands, savannahs, prairies and glades. Seeds are produced in June and then again in September after the plants die off during the height of the summer. Grasshoppers, moth larvae, caterpillars of skippers and beetles eat the foliage. The seeds are eaten by game birds, songbirds, mice, rabbit and deer.

Elymus glaucus is a large (150cm) perennial bunchgrass. Blue wild rye is found from Alaska to Mexico and east through the Great Plains. It is found in woodlands, prairies and chaparral. The seeds develop from May through July. It is an effective species for stream bank erosion and helps to colonize burned and disturbed sites. It is a very fire tolerant species. Mammals, birds and waterfowl use it for habitat and forage.

Elymus trachycaulus is a medium sized (50-100 cm) perennial bunchgrass. This short-lived (3-5 years) species is found across North America from Canada to Mexico, with the exception of the southeastern United States. It is adapted to basic soils (pH 8.8) as well as moderate salinity. However, it is not as tolerant of drought as other species in the genus. The seeds are produced from late July through early August. Slender Wheatgrass grows quickly as a seedling and is used for disturbed site reclamation and erosion control. It is a pioneer species in both primary and secondary succession. Game birds and small mammals consume the seeds and use this species for cover. Larger mammals such as elk and big horn sheep readily graze this species due to its high crude protein content.

Implications

With an understanding of the fire regime history of the SPS Prairies, we know that the native species in the *Poaceae* family have experienced regular exposure to fire and smoke throughout the last several millennia. In an effort to maintain, restore and expand the prairie ecosystem in Western Washington, ecologists are augmenting the ecosystem with native seeds as well as nursery-raised plugs. However, low germination rates coupled with the rarity of many prairie plants (due to habitat loss) have challenged prairie restoration efforts. That combination of challenges makes many of these species seed-limited. Prescribed fire is an effective method for restoration, but is often resource intensive. With the recent developments in our understanding of how butenolides and other compounds from smoke can increase germination in plants from the *Poaceae* family, I believe there may be a cost effective method to increasing the germination of these seeds through the use of plant-derived smoke water. This study examines the effectiveness of smoke water applications for six species of *Poaceae.*

Methods and Materials

Plant-Derived Smoke Water

Many researchers have used plant-derived smoke water for the same purpose as this thesis. However, each study has a somewhat unique methodology for producing it, as there is no set standardization in the field as of 2016. I worked with my SPP conservation nursery crew to establish our methods, which required a few trial runs to calibrate the equipment. The initial challenge was to produce a consistently thick smoke for the duration of the process, while maintaining the temperature within the desired range. We found that it was much easier to build up to the temperature threshold incrementally, than to try and reduce the heat of the fire if it became too hot. In order to carefully control the heat, we built our coals in a separate barbeque and added them into the smoker one at a time. The following section describes how to build the smoker for optimum results.

Assembling the smoker

Figures 1.1 & 1.2 below are labeled to accompany the description that follows. The process of creating plant derived smoke water is achieved using a modified meat-smoker [A] (we used a Brinkman Trailmaster BBQ - #081269 smoker). We removed the chimney from the smoker and, in its place, attached a 3-foot long 1” stainless steel pipe [B] to the exit vent. The pipe extends from the smoker horizontally and is held upright using an H.D. jack [C] – similar to a car jack, but with a V-shaped crotch for the pipe to rest in. The other end of the pipe was attached to a ¼” rubber hose [D] that was clamped to a 5L aspirator bottle [E] full of deionized water. Atop the aspirator bottle we taped a suction diffuser [F] - a broad plastic funnel with holes drilled in the sides. The diffuser is then taped to a shop vacuum [G]. The suction diffuser allows a moderate level of airflow to be pulled through the water without the vacuum actually taking any water in.

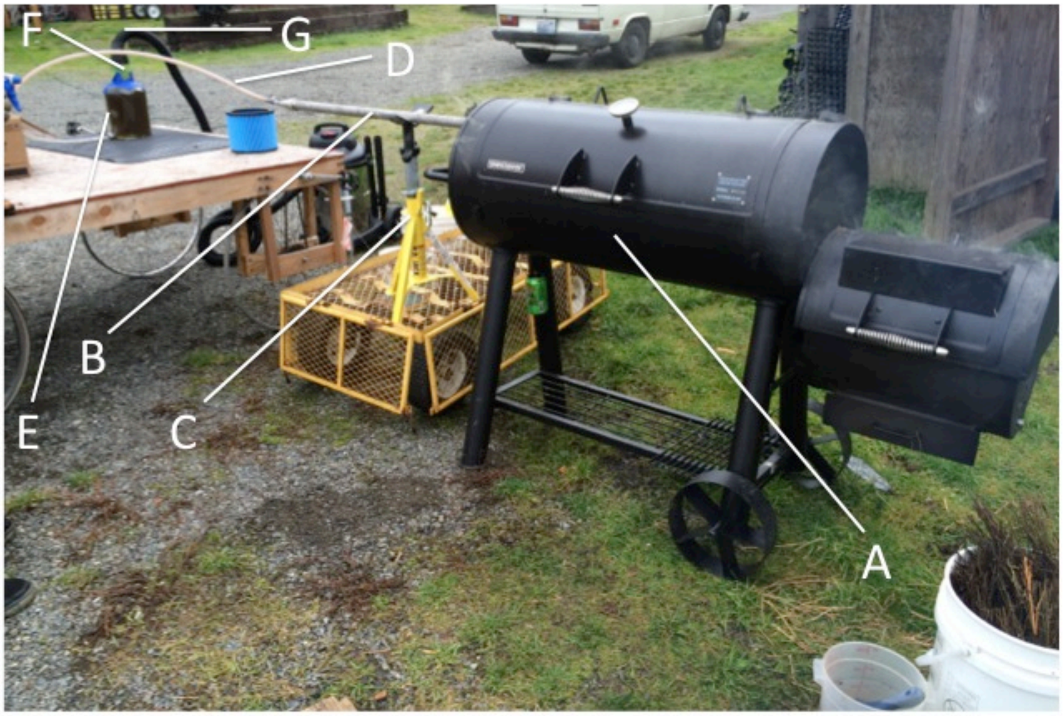


Figure 1.1 – Smoking Apparatus - Modified BBQ used to produce plant-derived smoke water. The letters represent each individual part, and correspond to the paragraph above. (See figure 1.2 for a more detailed diagram of parts D-G).

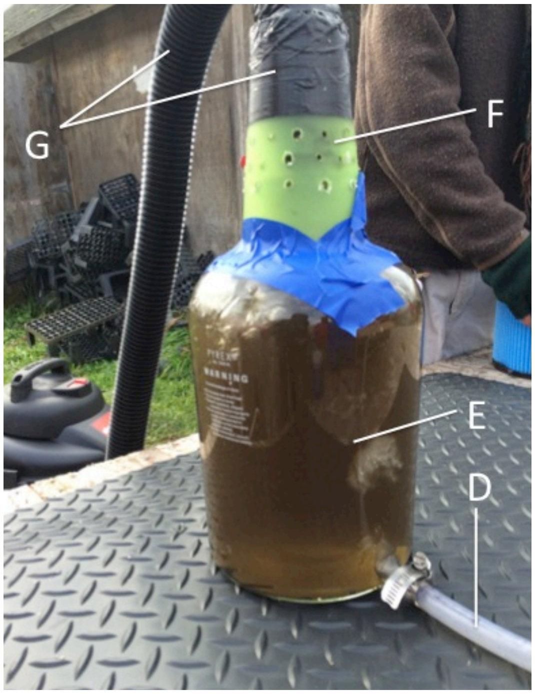


Figure 1.2 - Smoker parts D-G. Close up diagram of the 5L aspirator bottle attached to the vacuum. The letters correspond with the paragraph above.

Operation of the smoker

Once the smoking apparatus was put together we built coals in a separate grill (a Weber Smokey Joe 14” charcoal grill). We used a separate grill so we could add a single coal at a time in order to precisely control the heat in the smoker. The coals were made from chunks of *Quercus garryana* wood. We started by placing a few coals into the smoker until we got the temperature above 65° C. In order to produce smoke, we placed the chaff from native *Symphyotrichum hallii* and *Seriocarpus rigidus* onto the coals. Chaff is the leftover husks, stems, leaves and straw from plants that have been harvested and cleaned to separate out the seeds. If the temperature became too hot (above 90° C) we would add chaff, of the same two species, that had been soaked in water. We used native oak and chaff over store bought charcoal and wood chips to ensure that all compounds in the smoke were those that would be found in a prairie ecosystem. Once we had produced a thick smoke, we turned on the vacuum to pull the smoke through the deionized water. In order to maintain the high end of the heat threshold, we would add in coals one at a time throughout the process. To maintain a steady thick smoke, and to keep the temperature from elevating too quickly, we would add dry chaff followed by wet chaff. This process continued for one hour, after which the water had become saturated with smoke. The final product was transferred to 500 mL plastic bottles, labeled and stored in a lab freezer at 0° C.

Study Species

All of the seeds used in this experiment were collected at maturity from a number of sites around South Puget Sound (Table 1). The seeds of *B. carinatus* and *E. trachycaulus* were wild collected from Joint Base Lewis-McChord, site R76, in the summer of 2015. The seeds of *D. californica* were harvested from Webster's seed farm (Maytown, WA) in the summer of 2014. The seeds of *D. acuminatum* and *D. oligosanthes* were harvested from Shotwell's Landing seed farm (Littlerock, WA) in the summer of 2015. And the seeds of *E. glaucus* were harvested from the Violet Prairie seed farm (Tenino, WA) in the summer of 2015. After collection and harvest, all seeds were cleaned at Shotwell's Landing, and stored in paper envelopes at 5° C in a seed storage refrigerator until they entered stratification for this experiment.

Species	Lot	Year	Location	Site	Type
BRCA	2524	2015	JBLM	R76	Wild
DACA	2500	2014	Maytown	Webster's	Farm
DIAC	2612	2015	Littlerock	Shotwell's	Farm
DIOL	2908	2015	Littlerock	Shotwell's	Farm
ELGL	2781	2015	Tenino	Violet Prairie	Farm
ELTR	2579	2015	JBLM	R76	Wild

Table 1. Seed collection origin. The table lists the species codon, lot number, collection year, collection location, site name, and site type.

Experimental Design

The methodology for ecologically meaningful germination studies is outlined in chapter 2 of "Seeds" by Baskin & Baskin (1998), and is the protocol used in this experiment. They recommend using intact natural dispersal units, however our seeds lost their palea and lemma in the seed cleaning process. This is the condition of the seeds that are used by restoration ecologists, and as such I wanted to maintain continuity with what is being used in the field for this experiment. Baskin & Baskin defer to Dr. Lela V. Barton on the case of replication, recommending 3 replicates of 50 seeds per treatment. We will be using 10 replicates of 50 seeds per treatment in order to strengthen our results. Our experiment had separate phases, first a stratification period, followed by a germination period (Table 2).

Species Name	**Common Name**	**Stratfication**	**Germination**
Bromus carinatus	California Brome	10	35
Danthonia californica	California Oatgrass	90	35
Dichanthelium acuminatum var. fasciculatum	Western Panicgrass	15	35
Dichanthelium oligosanthes var. scribnerianum	Scribner's Panicgrass	15	35
Elymus glaucus	Blue Wildrye	10	35
Elymus trachycaulus	Slender Wheatgrass	10	35

Table 2 – Experiment Time Table - Stratification period and germination period duration for each species, in days (SPP Conservation Nursery Manual, 2014).

Seed stratification

Seeds for each of our species require cold stratification to achieve optimum germination. Cold stratification is the emulation of winter conditions, where the seeds are placed on a wet substrate and stored in a cold environment equal to the average temperature of their native winter ecosystem. Every species responds differently to stratification length. For the purpose of this experiment, we are using the stratification protocols used by SPP in their nurseries (Table 2). This will allow for a comparison of our germination results with their nursery data from 2009-2015. We used a germination chamber for both the stratification period and the germination period. This was an ideal tool for the experiment as the lights and temperature can be manually changed as needed and then maintained by timers.

The seeds for each of my six species were split into sets of 50 seeds. Each seed was examined on top of a light table and under magnification to determine that they were fully formed. Once split into sets, each set was placed into a sterilized petri dish on top of two pieces of filter paper (double-rings 90mm). One replicate was a set of 50 seeds. Each species had ten control replicates and ten experimental replicates. The total number of seeds for each species was 1000. The control replicates were imbibed with 3mL of deionized water. The experimental replicates were imbibed with 3mL of a 1:100 solution of plant-derived smoke water and deionized water. All 120 replicates were randomized and then placed into a darkened germination chamber (SG30 Controlled Environment Chamber) set at 2° C. After 24 hours all replicates were rinsed with 2mL of deionized water and returned to the germination chamber.

Seed germination

After the stratification period ended, the germination chamber was reset to alternate its temperature from 10° C with no grow lights for 12 hours, followed by 20° C with grow lights (40 μmol photons m^{-2} s^{-1} PPFD) for 12 hours. This alternation of light and temperature emulates spring conditions in order to trigger germination responses in the seeds. During this germination period, each replicate was removed and opened to survey for germination. Germination in this experiment is defined by the emergence of a 2mm radicle – the embryonic root (Figure 2). Once identified, the number of germinated seeds per sample was recorded for that day, and those seeds were then removed to eliminate duplicate counting in the future. This process was repeated every other day during a 35-day germination period.

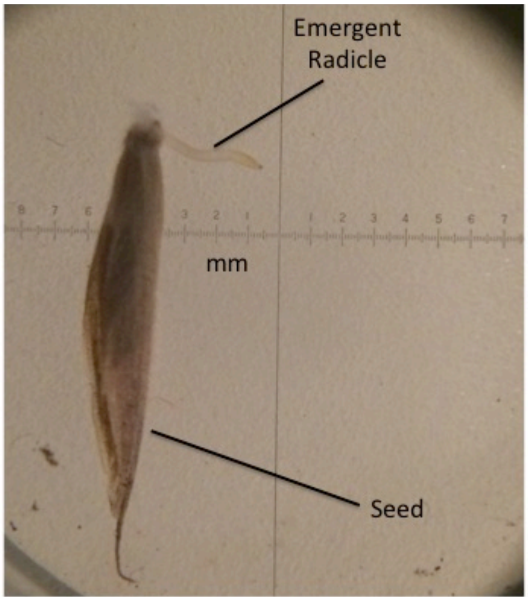


Figure 2 – Emergent Radicle - Germination is defined by the emergence of a 2mm radicle, or embryonic root.

Data Analysis

We analyzed the data from this experiment using JMP software. We ran a one-way ANOVA to determine whether the difference of means between each treatment was statistically significant for each species. The mean for each replicate was determined as a percentage of x/50 germinated seeds. We also calculated the time to 50% germination (t50) for each treatment of every species. The mean t50 between treatments determines a difference in seedling vigor as a measurement of growth speed. For all tests, statistical significance was determined based on $p < 0.05$.

Results

Germination Rates

Elymus glaucus

Plant-derived smoke water increased germination rate significantly (p < 0.029) for one of the six study species, *Elymus glaucus* (Figure 3.1). This data was especially telling when you look at table 3.1 and see 6/10 experimental replicates had germination rates equal to or above 90%. None of the control replicates reached 90%. Also only one of the experimental replicates was below 50%.

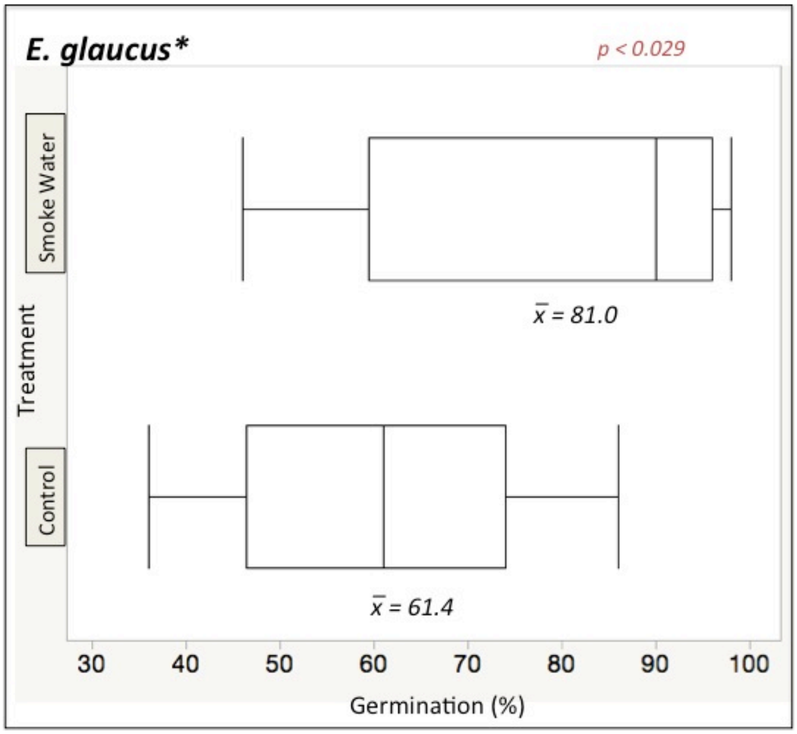
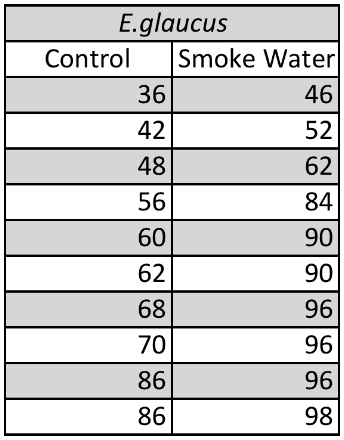


Figure 3.1 Germination responses by *E. glaucus* to plant-derived smoke water treatments. Each box and whisker plot represents the distribution of the data, and x-bar indicates the mean. Significance was determined based on p < 0.05.



E.glaucus	
Control	Smoke Water
36	46
42	52
48	62
56	84
60	90
62	90
68	96
70	96
86	96
86	98

Table 3.1 Germination percentages for each replicate of *E. glaucus*

Elymus trachycaulus

Plant-derived smoke water did not significantly increase germination rates for *E. trachycaulus* ($p < 0.199$). The spread of the data was very low for this species, especially in the experimental group. Smoke water appears to have had a very uniform effect on the experimental treatment group, with 9/10 replicates within the 64-70% germination range. The control group had a little more variability, and a higher ceiling with half of the treatments achieving a greater germination percentage than the highest experimental replicate. But both groups produced germination rates over 50% across the board.

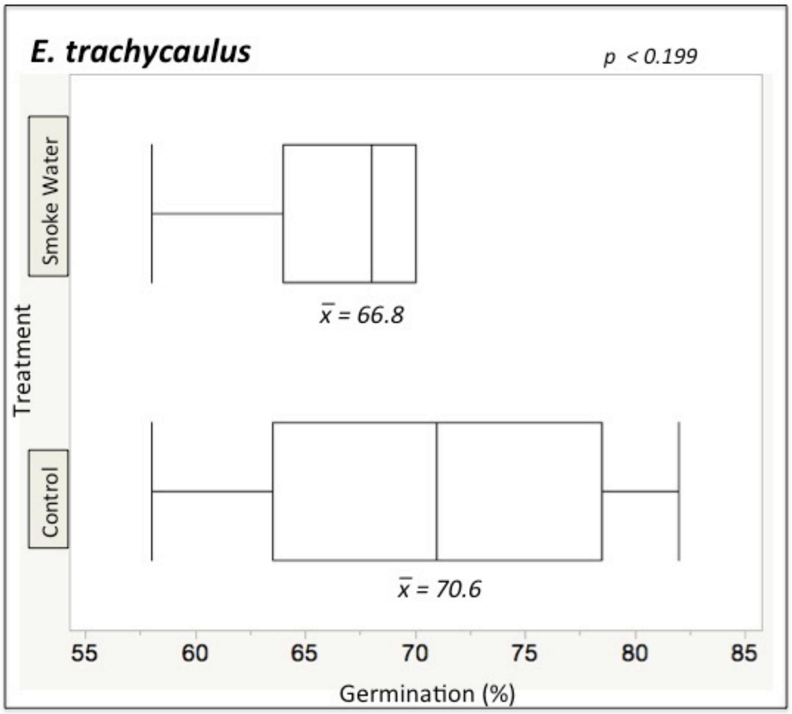


Figure 3.2 Germination responses by *E. trachycaulus* to plant-derived smoke water treatments. Each box and whisker plot represents the distribution of the data, and x-bar indicates the mean. Significance was determined based on $p < 0.05$.

E. trachycaulus	
Control	Smoke Water
58	58
62	64
64	64
66	66
70	66
72	70
74	70
78	70
80	70
82	70

Table 3.2 Germination percentages for each replicate of *E. trachycaulus*

Dichanthelium oligosanthes var. scribnerianum

Plant-derived smoke water had no significant increase in germination for *D. oligosanthes var. scribnerianum*, ($p < 0.245$). The difference in means here is higher than for any of the other non-significant species. However, the range of the data is also one of the highest in this experiment. The smoke water group has an especially large range, including an outlier of 0% germination. Overall the means here seem representative of the spread of the data. The high variability of the smoke water group interests me for further research.

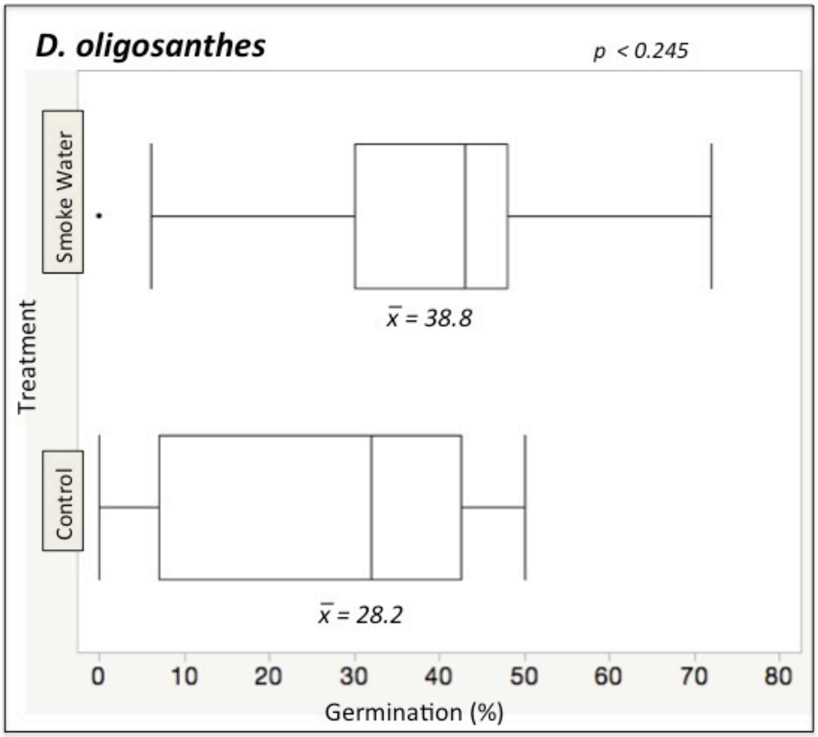


Figure 3.3 Germination responses by *D. oligosanthes* to plant-derived smoke water treatments. Each box and whisker plot represents the distribution of the data, and x-bar indicates the mean. Significance was determined based on $p < 0.05$.

D. oligosanthes	
Control	Smoke Water
0	0
4	6
8	38
28	40
28	42
36	44
42	46
42	46
44	54
50	72

Table 3.3 Germination percentages for each replicate of *D. oligosanthes*

Danthonia californica

Plant-derived smoke water did not significantly increase germination for *D. californica*, ($p < 0.335$). The average germination for these two groups is very close, and overall the spread of the data is very similar for both groups. The smoke water treatment yielded one replicate with a particularly high germination percentage appears to be the only minor difference. Both treatments produced germination rates above 60% across the board.

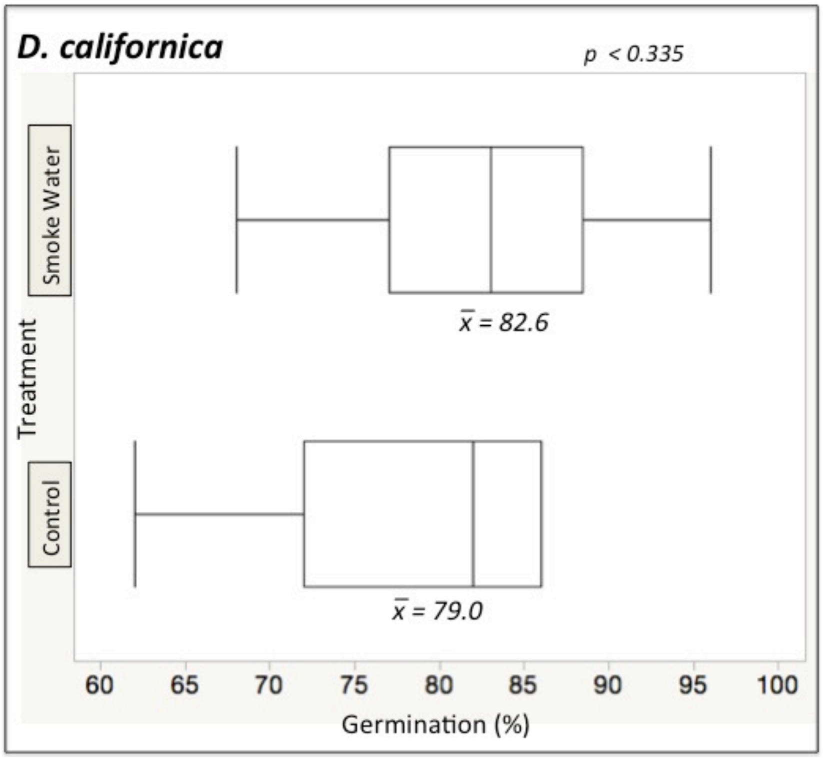
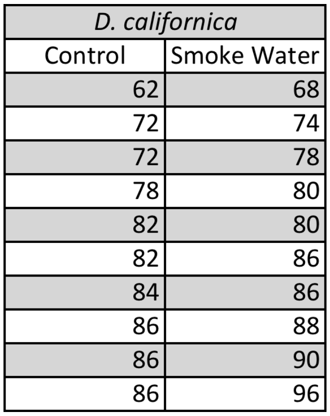


Figure 3.4 Germination responses by *D. californica* to plant-derived smoke water treatments. Each box and whisker plot represents the distribution of the data, and x-bar indicates the mean. Significance was determined based on $p < 0.05$.



D. californica	
Control	Smoke Water
62	68
72	74
72	78
78	80
82	80
82	86
84	86
86	88
86	90
86	96

Table 3.4 Germination percentages for each replicate of *D. californica*

Dichanthelium acuminatum var. fasciculatum

Plant-derived smoke water did not significantly affect the germination of *D. acuminatum var. fasciculatum*, ($p < 0.336$). This species had consistently low germination overall.

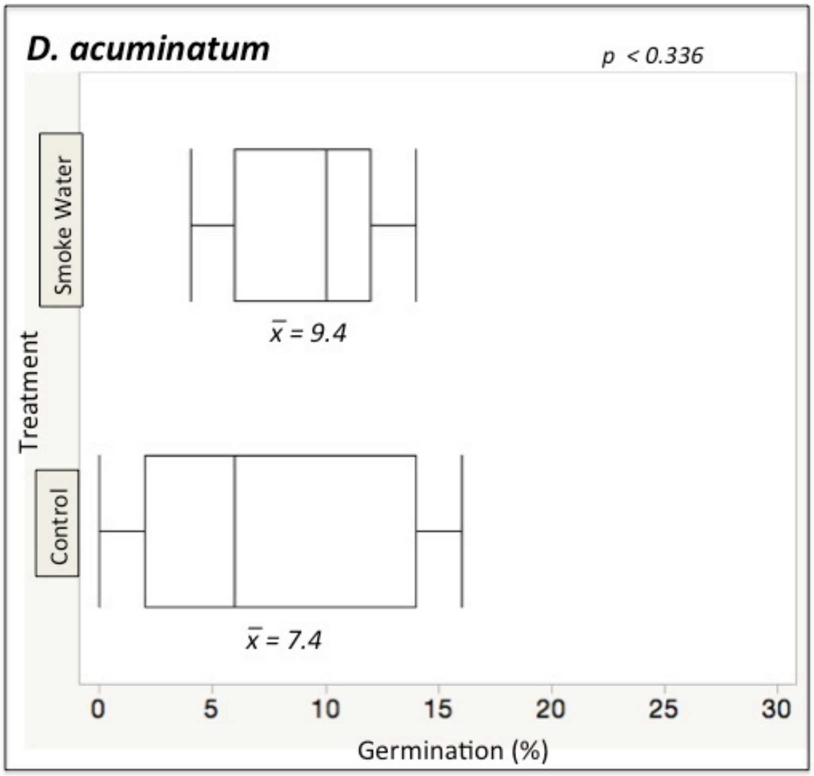


Figure 3.5 Germination responses by *D. acuminatum* to plant-derived smoke water treatments. Each box and whisker plot represents the distribution of the data, and x-bar indicates the mean. Significance was determined based on $p < 0.05$.

D. acuminatum	
Control	Smoke Water
0	4
2	6
2	6
6	10
6	10
6	10
8	10
14	12
14	12
16	14

Table 3.5 Germination percentages for each replicate of *D. acuminatum*

Bromus carinatus

Plant-derived smoke water did not significantly affect the germination of *B. carinatus*, ($p < 0.396$). The range of data some high for both treatments, but the majority 7/10 of the replicates yielded similar rates (< 16% difference) within each treatment. This is the only species where the control treatments yielded higher individual germination rates across the board. 8/10 control group treatments had a germination rate above 50%.

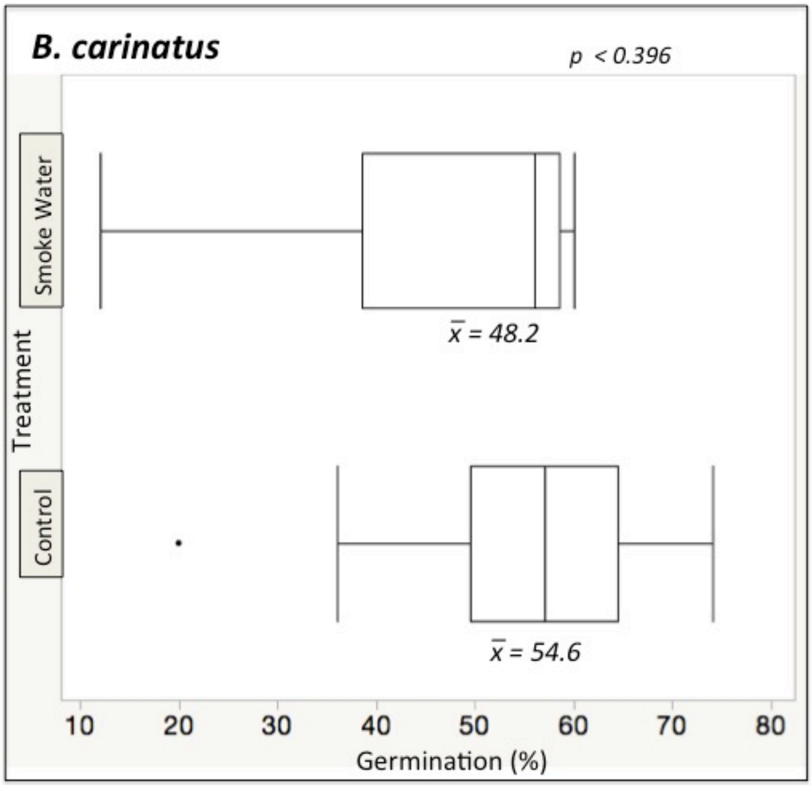


Figure 3.6 Germination responses by *B. carinatus* to plant-derived smoke water treatments. Each box and whisker plot represents the distribution of the data, and x-bar indicates the mean. Significance was determined based on p < 0.05.

B. carinatus	
Control	Smoke Water
20	12
36	22
54	44
56	56
56	56
58	56
62	58
64	58
66	60
74	60

Table 3.6 Germination percentages for each replicate of *B. carinatus.*

Germination Rates Compared with SPP and the Literature

The basis for this experiment was the challenge SPP has had growing these species as plugs from 2009 – 2015. I compiled their data below as compared with my own control and experimental average germination rates (Table 4). I also included germination data from the Native Plant Network (NPN), from the closest reports I could find to western Washington. Laboratory based germination tests of these species were not available in the literature. Both the SPP and NPN data are based off of the total number of plants as compared to the number of seeds planted in plug trays. This data is only an approximation and is used here a proxy for comparison sake.

Four of the five applicable control group averages from this experiment are higher than the SPP averages. However, only two of the five applicable control group averages are higher than the NPN literature. *E. glaucus*, the only species with a statistically significant increase in germination from my experiment, also has a higher rate than either SPP or NPN data.

Species	(Ely, 2016) C	(Ely, 2016) E	SPP ('09-'15)	NPN	Location
B. carinatus	54.6	48.2	n/a	91	Bridger, MT (2002)
D. californica	79	82.6	10	60	San Francisco, CA (2001)
D. acuminatum	7.4	9.4	15	30	Corvalis, OR (2007)
D. oligosanthes	28.2	38.8	5	n/a	n/a
E. glaucus	61.4	81	20	40-50	Glacier, MT (2008)
E. trachycaulus	70.6	66.8	20	92	Bridger, MT (2002)

Table 4 – Germination rates as compared with the literature - Germination rates (%) of the control (C) and experimental (E) groups from this study compared with data from the Sustainability in Prisons project (SPP) from 2009-2015, and literature from the Native Plant Network (NPN) and the location and year of those studies.

Vigor

According to Soltani et al., the t50 metric is a valuable way to understand the effects of treatments on seedling vigor (2015). The t50 statistic, time to 50% germination, was calculated for all applicable species. No significant difference in vigor was observed between the control and experimental treatments for any species (Table 5). Each treatment reached 50% germination within 2 days of the other (out of 35 days) for all species.

Species	Control	St. Dev.	Smoke Water	St. Dev
B. carinatus	6.5	0.926	5.429	2.225
D. californica	12.2	0.632	12.6	0.966
D. acuminatum	n/a	n/a	n/a	n/a
D. oligosanthes	22	n/a	23	1.414
E. glaucus	4.571	0.976	4.889	1.054
E. trachycaulus	5.8	1.751	4.2	0.632

Table 5 – Time to 50% Germination (t50) - This table shows mean number of days to 50% for each treatment of each species that reached at least 50% germination. One standard deviation is given below each mean; outliers disproportionately skewed some of the data. The experiment length was 35 days (5 weeks).

Discussion

Plant-Derived Smoke Water

The data from this study indicates that there may be a relationship between plant-derived smoke water and increased germination rates for *E. glaucus*. The one-way ANOVA showed a statistically significant difference of means between the 10 control samples and the 10 experimental samples. This could bode well for future plantings of the species in nursery settings. For plug production, smoke water could be applied either at the time of seed stratification or immediately after sowing. The other five species did not yield significant differences in germination rates between the control and experimental groups. This could be due to a number of factors, which will be discussed in the subsequent sections.

Overall Germination Compared to the Literature

Although only one out of six species showed significant differences between the control and the experimental groups, it is worth noting that four out of five species had greater germination rates in the control group than previously attained in nursery plantings by SPP from 2009 to 2015 (Table 4). Furthermore, the control groups also improved upon the reported germination rates of two out of the five species there is germination literature on in the Native Plant Network (NPN), one of the most comprehensive online databases regarding native plant propagation. This is valuable in and of itself, as the goal of this study is to improve germination rates for the purpose of restoration, regardless of whether the improvement comes by way of smoke water applications.

E. glaucus

The overall germination rates for both treatments were greater than those observed by SPP and researchers in Glacier National Park. SPP raised this species on and off from 2009 through 2015, and their average germination rate for *E. glaucus* over that period was 20% (Carl Elliott, personal communication). According to the NPN, a nursery in West Glacier, MT recorded 40-50% germination for the species (Luna et al., 2008). Our own research yielded a 61.4% average germination rate for the control group, while the smoke water treatments increased that to 81%. It would follow that the conditions provided in this experiment (temperature and light settings, stratification length, and the addition of plant-derived smoke water) all contributed to increased germination when compared with the literature as well as the control group.

D. californica

The overall germination rates for both treatments were greater than those observed by SPP and a nursery in San Francisco, CA. SPP raised *D. californica* on and off from 2009 through 2015, and their average germination rate for the species over that time was 10% (Carl Elliott, personal communication). According to the NPN, a nursery in San Francisco, CA averaged 60% germination when raising this species (Young, 2001). The results of this experiment yielded a 79% germination rate for the control group, and an 82.6% rate for the experimental group. The difference between the control and the experimental groups are negligible, but I would recommend following the stratification length and temperature and light splits chosen for our control treatment to attain this high rate of germination in future plantings.

E. trachycaulus

This particular species behaved different than the majority of the others. The germination rate for the experimental group, at 66.8%, was lower than that of the control, 70.6%. Once again, the difference between the two is negligible at < 5%, but it appears clear that smoke-water is not a driving factor for germination of this species. Nonetheless, the control group germination rate is still an improvement over the average rate recorded between 2009-2015 by SPP, which was 20% (Carl Elliott, personal communication). The protocols used in this experiment should be considered for future sowing of *E. trachycaulus* in Western Washington.

It is worth noting that a nursery in Bridger, MT was able to achieve a 92% germination rate when growing this species (Winslow, 2002). But it can be difficult to compare the growing conditions in Eastern Montana with those of Western Washington, which we modeled the temperature fluctuation used in our germination chamber after. I will discuss the variable of differing temperature regimes below, but that may be a factor worth considering when comparing the robust germination found in Montana with the moderate success of our control group.

D. oligosanthes

This species has very little literature published regarding its propagation. SPP has attempted to grow it from 2009-2015 and only yielded a 5% average germination rate (Carl Elliott, personal communication). This experiment did not bring much clarity to the challenge of growing *D. oligosanthes*. The control group averaged 28.2% germination, while the experimental group did slightly better at 38.8%. The one-way ANOVA did not find the difference between the two to be statistically significant. However the JMP software determined one value to be an outlier in the experimental group. After omitting the outlier, the average germination rate improved to 43.1%, while the p-value from the one-way ANOVA changed from $p < 0.245$ to $p < 0.085$. This is still not significant but intrigues me regarding future research.

D. oligosanthes is somewhat of a mystery when it comes to growing protocols. SPP fared poorly in raising it, the NPN had no entries regarding growing it, and I would not say 38.8-43.1% germination rate for our experimental group is particularly strong when projecting out for prairie restoration. It is hard to evaluate the data from this particular species as the average rate appears accurate overall, but there are also extreme high and low individual replicate values in the experimental group (Table 3.3).

Due to the ambiguity of the results, I would like to see this species tested further for smoke and fire related treatments. One consideration is the complexity of germination cues that result from fire. Many of these cues compound upon each other to produce ideal germination conditions. Maybe this is a scenario where smoke water coupled with another fire-related cue could result in a significant increase in germination. Looking at the individual replicate values, its obvious that the experimental group improved germination rates over the control, albeit negligibly in most cases. I would still suggest nursery managers to consider further experimentation with smoke-water when growing this species as all other documentation reports extremely low germination rates comparably. Another consideration would be a longer stratification period. For this experiment we used a 15-day stratification length, but future researchers may want to experiment with 30, 45, 60 and 90-day stratification intervals as well.

B. carinatus

This was the other species from this experiment whose germination rate decreased from the control (54.6%) to the experimental group (48.2%). Once again, the difference between the two groups is not significant. A high germination percentage of 54.6% is acceptable for nursery growing purposes, but not ideal. SPP has no previous records of the germination rates for this species, so the only comparison available is the nursery from Bridger, Montana. According to the NPN, they achieved a 91% germination rate, which is certainly greater than anything in this experiment (Winslow, 2002). I will maintain the same caveat I mentioned earlier regarding the difference in climate between eastern Montana and western Washington.

Upon further research, I found a U.S. Forest Service document describing the geographic differences in growing *B. carinatus*. According to their literature, this species has been reported with germination rates of 89% when grown in the southwestern U.S., 85% in Montana and 48% in Oregon. This was followed up with data from a greenhouse experiment that yielded 85% germination when the temperature fluctuated between 20°

and 30° C. They then lowered the average temperature to 14° C and the germination rate dropped down to 46% (Tollefson, 2006). This aligns with both the results from our experiment, and the data from the NPN. Unfortunately, with limited time for this experiment and only one germination chamber, we were only able to set one temperature regime for all six species. But I would suggest that in future trials the 20° to 30° C range be used for *B. carinatus*. Also, for nursery managers in western Washington attempting to grow this species, early summer sowing may produce the best results.

D. acuminatum

This species had the lowest overall germination rate of the six, with a control group average of 7.4%, and experimental group average of 9.4%. SPP did not fare much better in their attempt to grow *D. acuminatum,* as they only reached 15% germination (Carl Elliott, personal communication). The NPN has a listing from Corvallis, OR that yielded a 30% germination rate for the species (Bartow, 2007). None of these numbers are ideal for restoration purposes. It seems there is some factor yet to be explained that holds the potential for increased germination of this species. I would highly recommend more research to explore the alternatives, but this experiment does not provide much of a starting point of where to look regarding this species.

According to data published by the US Forest Service, *D. acuminatum* increased in frequency in burned sites as opposed to unburned sites. However, the frequency was greater in sites burned annually, and moderate in sites burned periodically. Other data from the same report observed that *D. acuminatum* was one of the established species most negatively affected by a 15-year fire in post-burn plant surveys (Walsh, 1995). This information would suggest that minor to moderate intensity fires may improve overall growth for this species, but it may also be vulnerable to higher intensity irregular fires. Smoke water alone did not seem to affect this species growth, but maybe moderate heat shock would improve its germination rates.

Controlled versus Variable Growth Environment

The motivation for this experiment was based from the low germination rates from the SPP conservation nurseries. The laboratory based germination test was done in order to isolate germination as clearly as possible. But the majority of the application of this data will end up being used back in the variable environment of a greenhouse or the prairies. While this experiment yielded increased germination rates over those previously observed by SPP for *D. californica, D. oligosanthes*, *E. glaucus*, and *E. trachycaulus*, it is important to acknowledge that this controlled environment eliminates a number of factors at work in an outdoor nursery. For this experiment, the seeds received thorough moisture throughout the germination phase, precise light and temperature changes, and were not encumbered by pests, pathogens, or weeds. All of these factors can contribute to lower germination rates in a nursery and should be considered when comparing the data from this experiment with germination data from a nursery. This experiment will not tell us specifically whether the lower germination rates from the SPP nurseries are due to external factors, but will only allow us to understand the effect of smoke water on germination and how germination-limited each species may be.

Nonetheless, it is beneficial to recognize the potential of each species in a controlled setting and then begin to isolate the other factors that could be holding the germination rates back. This could include predation by pests, competition by other plant species that find their way into the same tube, seasonal conditions based on time of planting or climate change, and simply length, frequency and duration of watering regimes chosen during the germination stage. Knowing the ceiling for a species germination rate with all other factors even is a foundation to build upon, but it is unrealistic to assume the same results could immediately be achieved in a plug tray as opposed to a petri dish. Future research will be beneficial to further our understanding of external variables.

Seed Limitations

Another factor worth addressing is the seeds themselves. Seed source is incredibly important in restoration. It is used for the purposes of tracking genetic lineages and knowing which seed lots produce the hardiest plants. It is also critical for creating heterogeneous ecosystems and not propagating genetic bottlenecks. For this experiment we only used one seed lot per species, so the comparison of control seeds to experimental seeds is accurate for evaluating the difference in treatments. However, the seed lots used in this experiment were not the seed lots used in the SPP records, nor those used in any of the NPN reports. Intra-species variation for plant varieties can differ significantly. For example, this season SPP is growing 8 different lots of *Castilleja hispida* – some of the seed lots have produced 0% germination and others are as high as 75%, with the rest somewhere in the middle. The results produced in this experiment may vary greatly from another identical experiment using seed sourced from another location. Seed viability can decrease with age as well, and is another consideration to take into account when comparing this study with former or future studies in the same vein. All of these seeds were collected or harvested by the Center for Natural Lands Management (Table 1).

Germination Cues from Fire

Fire can influence seed germination through a variety of chemicals, temperature fluctuations, water and nutrient availability, and exposure to light. Many of those cues work in succession with one another to provide ideal growing conditions for fire-adapted seeds. In this experiment, we controlled for each of those variables except plant-derived smoke water. The results of this study did not yield significant germination differences for five of the six species when exclusively adding smoke-water. However, there were minor increases for some species, specifically *D. oligosanthes* and *E. trachycaulus,* and such changes may not be coincidental.

Heat has been tested in conjunction with smoke treatments in much of the literature on the subject. But there is no clear consensus about the role heat plays when partnered with smoke. In one study of three native *Lupinus* species from SPS prairies, *L. Lepidus* seeds had significantly increased germination rates when immersed in 80° C water. Variable heating followed by cooling regimes to emulate spring conditions also demonstrated germination increases for the other two *Lupinus* species (Elliott, Fischer & LeRoy, 2011). For some species, temperature regime changes alone are enough to stimulate germination.

Another study examined six South African grassland species and their germination and growth when exposed to smoke water and butenolide concentrates at different temperatures. The germination results showed few trends between species, except for all species the overall germination rates increased in all treatment groups as the temperature increased; and for five of the six species, germination peaked between 25 and 30° C. They also measured the shoot and root growth of each treatment group at 15°, 20°, 25°, 30°, 35°, and 30°/15° C for each species. In the smoke water treatment group, five of the six species had a steady increase of growth as the temperature increased until peaking at 30° C and then dropping off. The same trend occurred in the control group, but with lower overall measurements (Ghebrehiwot et al., 2009). This demonstrates that heat can impact germination on its own, but can increase that effect when paired with smoke for species from fire-prone grasslands.

A separate study of 34 species from the California chaparral compared a control group, heat shock treatments of 105° and 115° C, a 5% charred wood aqueous leachate, and aerosol smoke treatments of 5 and 8 minutes. Seeds of each species were exposed to all treatments in order to measure germination rate. The smoke treatments in this experiment only raised the temperature in the treatment chamber 1° to 2° C above the ambient room temperature, so the purpose of this was to isolate heat treatments from smoke treatments. 22 of their 34 species had a statistically significant ($p < 0.001$) increase in germination from smoke exposure. They found heat shock to have no stimulatory effect for these species. Charred wood treatments also induced germination, but not as significantly as the smoke treatments. In some cases, the 8 minute smoke exposure produced a lethal effect (Keeley and Fotheringham, 1998B). The results of this experiment are somewhat counter to those of the South African study.

Each of these studies provides a different example of how heat-shock, temperature regime changes, and smoke-treatments can all work individually or compounded with one another to affect germination and seedling vigor. They each produced varying results that do not necessarily align with one another. But the purpose in all of these is to attempt to represent each of the variables present in a wild fire. Heat, burnt plant material and smoke all have been observed in some instance to increase seed germination. Each of these variables should be researched further for their affects on the germination of SPS prairie species.

Temporal Conditions

Time of year is controlled for in this experiment by use of a germination chamber, but the temperature fluctuation of 10° C to 20° C could also play role in the overall low germination rates for a few of the species, specifically *D. acuminatum* and *D. oligosanthes*. The temperature range chosen for this experiment was meant to emulate spring conditions. Both *Dichanthelium* species set their seeds in spring but often have a second flush in late summer (August-September). So perhaps a ceiling of 30° C could prove to be more beneficial for these species. If this were to have an impact it could alter the time of year nursery managers choose to sow their seeds for restoration.

Conclusion

The SPS prairie ecosystem is unique in its dependence upon humans for management. They were first formed from the glacial outwash approximately 10,000 years ago (Hegarty, Zabowski & Bakker, 2011). As the conditions turned more mesic a few millennia later, conifers began to encroach into the lowland savannahs. The Native Americans present at the time began to burn the grasslands on a three to five year interval in order to stop the conifer encroachment, allowing the people to hunt and forage in the open prairies. But regular burning stopped in the mid nineteenth century due to suppression by European Americans. The combination of development and climate change over the next 150 years further fragmented the prairies and altered their species makeup. The prairies we are left with today occupy less than 5% of their previous extent, are in poor health, dominated by non-native species, and lack diversity. The human influence on this system cannot be overlooked. The SPS prairies flourished due to human intervention, and are now seriously threatened due to human intervention. The future of this ecosystem is in our hands today.

Regular fire-return intervals play a critical and complex role in the SPS ecosystem. And the regeneration of the prairie sites on Joint Base Lewis-McChord (JBLM), where burn regulations are much more flexible, is a testament to the positive impact fire can have on the diversity of the system. Unfortunately, JBLM does not house all the prairie sites left in the northwest. And the sites outside of the military base are limited by seasonal conditions and state law, both of which restrict prescribed burns to a few months each year. Although a site may only require burning once every five years to have a positive impact, this still may not be feasible based on the resources, training, and manpower necessary to execute a safe and effective burn.

The purpose of this study was to explore a supplemental avenue to controlled burns. Restoration organizations are already working year round to produce seed, raise plugs, and regrow the SPS prairies as quickly and efficiently as possible. But many of the species are conditioned to certain disturbances that cannot be emulated in nurseries. Plant-derived smoke water provides one channel to possibly unlocking the germination potential in these fickle species.

Our hypothesis that smoke water would increase germination for six native species from the *Poaceae* family was not realized. *E. glaucus* was the lone species that appears to have a positive relationship with smoke water treatments, and I would recommend to any manager raising this species, either in a nursery or direct seeding into the prairie, that they incorporate smoke into that process in some capacity. As for the other species, there is more research left to do. We yielded healthy germination rates in our control group for *D. californica* and *E. trachycaulus*, at 79% and 70.6% respectively. Those two species can theoretically get sufficient germination following the control protocols of this paper. *B. carinatus* appears to be a species that prefers a warmer climate than we have in western Washington for most of the year, so summer sowing or heated greenhouse propagation may be the best avenue to increase its germination rate. *D. oligosanthes* may well respond to multiple cues from fire and should be explored further with smoke-water as well as heat shock tests. *D. acuminatum* is reported to occupy post burn sites, but may be vulnerable to high intensity fires. Experimentation with different stratification lengths coupled with moderate heat shock and smoke-water treatments may yield better results. But it is clear by looking at these six species alone, that ecologists cannot paint with broad strokes when propagating prairie species for restoration.

The positive takeaway from this research is that four of these six species are not germination limited, when aiming for a germination rate above 50%. Growing these species in plugs is a separate challenge, and should be further studied. For the other two species, it is very helpful to recognize that germination is a limiting factor, even in a controlled environment. They may not be economically viable to continue producing until the key to their germination limits are understood. This study can be used to more effectively produce *B. carinatus, D. californica, E. glaucus,* and *E. trachycaulus*, and can be a springboard for future research focused on *D. acuminatum* and *D. oligosanthes*.

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