AN ABSTRACT OF THE THESIS OF

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Title: Restoration of Annual Grass-invaded Landscapes in the Sagebrush Steppe using Perennial Grass Seed Technologies and Wyoming Big Sagebrush Transplanting.

Abstract approved:

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Within the sagebrush steppe ecosystem, invasive annual grasses are of growing management concern as they outcompete native vegetation, change the fundamental nutrient cycling processes, decrease biodiversity, and increase frequency of wildfires. The most widely used and effective management tool to decrease invasive annual grass abundance, is the use of pre-emergent herbicides like imazapic. Although this herbicide is effective at annual grass control, it can have negative effects on native or desired seedlings. Thus, land managers often wait a year or more to seed native vegetation following herbicide treatment. In that time, cheatgrass and other invasive annual grasses have the opportunity to re-establish, making the herbicide treatment ineffective. By seeding and herbicide-treating annual grasses simultaneously (i.e., a single-entry approach), land managers can save a year or more in restoration costs. However, the native or desired seed needs to be protected in some sort of coating to prevent negative herbicide effects. An herbicide protection coating containing activated carbon has shown to successfully protect desired seeds in the form of

pellets, and pods. This thesis outlines a new method of coating individual native grass seed in an herbicide protection formula containing activated carbon to test its effectiveness in both greenhouse and field settings. In the greenhouse study, we used a randomized block factorial design to test the efficacy of activated carbon-based herbicide protection coatings applied to individual bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Love) seeds for protecting seedlings from injury associated with low and high rates of pre-emergent herbicide (imazapic) application. The emergence of coated seed averaged 57% ± 5% (mean ± SE) compared to bare seed which had 14% ± 10% emergence with herbicide application. Seedling height for coated seed averaged 7.56 ± 0.6 cm compared to 2.26 ± 0.4 cm in uncoated bare seed in the presence of herbicide. Coated seeds produced 87% more plant biomass than uncoated seeds. The field study, a randomized block factorial design, repeated in two years, however, had two consecutive low precipitation and high temperature years resulting in low emergence and low survival of bare and coated seeds. Further field studies with either an added irrigation treatment or in more favorable climate conditions are suggested to determine effectiveness of carbon-based seed coatings in protecting desired seedlings from herbicide damage.

This thesis also discusses a method of restoring Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* (Beetle & A. Young) S.L. Welsh) following wildfire. Sagebrush transplants have a higher establishment rate than seeding in low to mid elevation sagebrush steppe sites, however, their success is widely variable. Chapter four of this thesis outlines how 1) different ages of transplant at time of planting, 2) different seasons of planting (fall and spring planting), and 3) competition

with invasive annual grasses, affect the survival and vigor (measured by transplant volume) of sagebrush transplants over two years. This completely randomized factorial design, repeated over two years, used ten age classes of transplants at time of planting (6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 weeks of age). Age classes 10 weeks and older in the first planting year and 12 weeks and older in the second planting year had the highest survival. Spring-planted transplants had higher survival than fall-planted transplants in both years; however, fall-planted transplants had increased vigor (measured by transplant volume) in both years. Competition did not affect survival of transplants in either year but it did affect volume of transplants. Volume was 54-fold greater when not competing with annual grasses compared to transplants competing with annual grasses in the first year, and nine-fold greater in the second year. Overall, the second year of the study had much lower sagebrush survival compared to the first year. Transplants are typically grown in a greenhouse for 6 months to a year before being planted in the field. This study demonstrated that reducing greenhouse growing time to 12 weeks compared to the traditional 24+ weeks, would cut greenhouse growing costs in half making this method of restoration more cost effective and a more favorable alternative to traditional restoration methods.

Restoration of Annual Grass-invaded Landscapes in the Sagebrush Steppe using Perennial Grass Seed Technologies and Wyoming Big Sagebrush Transplanting.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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CHAPTER 1: INTRODUCTION AND BACKGROUND

Introduction

Rangeland managers today face many challenges in western North America. Two such issues are the invasion of exotic annual grasses and the expansion of woody vegetation such as *Juniperus* and *Pinus* species (Davies et al. 2011). The annual grass invasion issue can seem overwhelming and there are few effective solutions to this problem or the cadre of indirect impacts resulting from it. The purpose of this document is to 1) introduce the ecology of the sagebrush steppe ecosystem 2) describe the severity of invasion of exotic annual grasses and the threats they pose to sagebrush ecosystems, and 3) discuss restoration techniques for overcoming challenges associated with both herbaceous and woody plant restoration on sagebrush rangelands.

Great Basin Desert

The Great Basin stretches from the Sierra Nevada Mountains on the west, and the Rocky Mountains in the east, to the Columbia river drainage in the north, and the Colorado river in the south, encompassing all or portions of the states of Utah, Idaho, Nevada, California and Oregon (Grayson 2011). This desert is unique in that its water flows drain internally and do not drain towards the Atlantic or Pacific oceans (Pellant et al. 2004). The Great Basin is one of the most arid regions in the United States with an average annual precipitation of 33 centimeters, mostly occurring in the winter and spring months in the form as both rain and snowfall (U.S. Climate Data 2020). The Great Basin also displays extreme temperatures with approximate minimum temperatures of -20°C and maximums of 40°C (NOAA 2020), although this varies widely based on elevation and topographic differences (Flaschka et al. 1987). Floristically, the more arid parts of the Great Basin are dominated by salt desert shrub communities in the Chenopodiaceae family. The semi-arid or more mesic portions are dominated by *Artemisia* species (Sagebrush) and the upland areas are dominated by *Juniperus* or *Pinus* sp. with *Artemisia* species and a variety of perennial bunchgrasses throughout (Pellant et al. 2004).

Sagebrush Steppe

The relatively mesic areas within the Great Basin Desert dominated by *Artemisia* spp. are referred to as the sagebrush steppe ecosystem, which encompasses more than 45% of the Great Basin Desert (Knapp 1996). This ecosystem is dominated by both shrubs and herbaceous species which is partly due to the variation in precipitation. Low precipitation or drought years favor deep-rooted shrubs whereas higher precipitation years favor fibrous rooted herbaceous growth (West 1999). Herbaceous plants develop earlier in the year and take advantage of the spring precipitation but the shrubs are slower to develop because they draw their moisture further down in the soil profile from the previous fall and winter snowmelt (West 1999).

The sagebrush steppe historically covered over 62 million hectares of the western United States and southwestern Canada (Davies et al. 2011). The ecosystem has been steadily decreasing and now only covers 56% of its historic range with much of the remaining sagebrush steppe being fragmented (Davies et al. 2011; Schroeder et al. 2004). The decrease in prevalence of this ecosystem has had adverse effects on wildlife, resulting in over 350 plant and animal species of conservation concern inhabiting the sagebrush ecosystem (Suring et al. 2005). One species in particular, relies heavily on this ecosystem and the sagebrush within it, the greater sage-grouse (*Centrocercus urophasianus*). This bird is a sagebrush-obligate species that has been imperiled since the early 1900s (Schroeder et al. 2004). The habitat along the Snake River for example, likely supported sage-grouse and observations from the late 1800s indicated sage-grouse presence. The only specimens of sage-grouse found near the Snake River were collected in 1933 and indicated that the bird was likely extirpated from the area prior to 1900 (Schroeder et al. 2004). Further north in their historic range, sage-grouse were extirpated from Canada as early as 1918, reintroduced in 1958, and extirpated again by 1966 (Schroeder et al. 2004; Campbell et al. 1989). This species has continued to struggle for a long period of time most likely due to habitat degradation in the form of annual grass invasion, conifer encroachment, altered fire regimes, and anthropogenic disturbances (Schroeder et al. 2004; Miller and Eddleman 2000; Rowland et al. 2006).

The greater sage-grouse was considered as a candidate for listing as threatened or endangered under the U.S. Endangered Species Act and was found to be not warranted for listing (U.S. Department of the Interior 2015). Although the species was not listed, the continued conservation of this umbrella species and its habitat is a high priority for land managers in the west. Many efforts are put forth to save this bird from extinction and restoring much of the sagebrush steppe ecosystem is a large part of it. By focusing conservation efforts on annual grass invasion, conifer encroachment, and causes of altered fire regimes, the three main factors threatening greater sage-grouse, the sagebrush steppe ecosystem is being restored because these same factors challenge the integrity of this ecosystem (Miller and Eddleman 2000; Davies et al. 2011; Bradley et al. 2018).

Many other wildlife species rely on the sagebrush steppe including over 100 avian species and 70 mammal species (West 1999). Some species are sagebrush obligates and rely heavily on the sagebrush in this ecosystem. This includes the sage sparrow, sage thrasher, Brewer's sparrow, sagebrush vole, pygmy rabbit, sagebrush lizard, and pronghorn (Knick et al. 2003; West 1999). Additionally, over 1,000 insect species have been found in this ecosystem, 76 of which rely heavily on sagebrush (West 1999). Other wildlife species rely on this ecosystem such as mule deer, elk, badgers, many rodent species, as well as an abundance of raptors and songbirds.

The sagebrush steppe not only provides crucial habitat for many wildlife species but also provides other valuable resources. This land is important to the public for recreational use such as bird-watching, hiking, camping, and hunting (Plieninger et al. 2012). Additionally, ranchers use much of the sagebrush steppe for grazing through means of leasing land from the Bureau of Land Management (BLM). Approximately 70% of the sagebrush steppe is federally managed by the BLM and U.S. Forest Service (Gordon et al. 2014) and leased out to private ranchers for grazing allotments. This ecosystem also provides countless ecosystem services such as water and air purification, carbon sequestration, soil conservation, biological diversity and more (Hunt et al. 2004; Havstad et al. 2007).

An Ecological History of the Sagebrush Steppe

Westward expansion and the increase in agricultural needs including farming and livestock grazing in the mid to late 1800s, brought novel disturbance regimes to the sagebrush steppe ecosystem. At present, the sagebrush steppe is one of the most imperiled ecosystems in the United States (Noss et al. 1995; Davies et al. 2011). With the implementation of the homestead act in the late 1800s early settlers began to settle in the west. With very low precipitation and extreme temperatures, the west was difficult to cultivate. Settlers often relied on livestock production in order to keep their land. Many settlers used grazing practices common in the Great Plains region of the United States which can handle heavier stocking rates and more continuous use than the Great Basin (Knapp 1996).

Historically, the Great Basin Desert did not evolve with consistent heavy grazing pressure prior to European settlement. Native fauna such as pronghorn antelope, mule deer, bighorn sheep, lagomorphs, rodents, and insects were the primary source of herbivory on native vegetation (Knapp 1996). The impact of these animals was light and less damaging compared to Bison or other large grazers in the Great Plains region for example. Additionally, when the native fauna grazed, they exerted heavy grazing pressure only episodically, unlike that of livestock that can exert heavy grazing pressure consistently, when managed improperly.

In the mid to late 1800s as mining activity increased, the demand for beef increased and ranchers relied heavily on cattle grazing to produce that beef. In Nevada for example, the increase in precious ores led to an increase in demand for beef, and subsequently cattle operations (Knapp 1996; Young and Sparks 1985). The state also had limited fence laws which allowed cattle to roam freely on the land (Knapp 1996; Centner 2000). This “open range” technique attracted many ranchers from the surrounding states and further increased the productivity of cattle operations. In Nevada alone, by 1874, there were at least 180,000 cattle grazing the land leading to overcrowded and overstocked conditions (Knapp 1996; Hazeltine et al. 1965).

Cattle were not the only livestock to utilize the sagebrush steppe. Sheep became a large part of the ranching industry and were valued for their wool and mutton. The sheep industry grew very rapidly due to the high demand in mining towns. In the 1850’s there were only a few thousand sheep in these areas, but by 1890 there were almost 400,000 sheep grazing in Nevada alone (Knapp 1996).

Although the sheep and cattle operations were both large, the sheep had more adverse effects on the landscape compared to the cattle. Sheep not only rely on grasses but also on browse (shrubs) and will often consume a plant down to the root (Knapp 1996). Additionally, sheep are able to graze on steeper terrain in higher elevation zones compared to cattle (Knapp 1996; Hopewell et al. 2005). All of these factors, as well as the high stocking rates used by early settlers, heavily degraded native Great Basin vegetation.

The grazing practices used by early settlers were unsustainable and especially detrimental to the sagebrush steppe due to its arid and semi-arid climate resulting in slow-recovery from such practices. Continuous heavy grazing (>50% utilization of aboveground biomass) has many negative side effects including compaction in the soil leading to lower water infiltration rates, decreased organic matter in the soil, higher soil temperatures, decreased plant growth and eventually desertification (Whisenant 1999; Naeth et al. 1991; Zhao et al. 2005). One study found that the use of continuous heavy grazing resulted in 50% lower grass cover and 35% lower overall biomass compared to ungrazed pastures (Mata-González et al. 2007). Another important component of the sagebrush steppe landscape is biological crusts or assemblages of living organisms such as cyanobacteria, fungi, lichens, and algae (Condon et al. 2018) that live on the soil or rock surfaces in the high desert. They increase soil fertility, reduce erosion, and enhance establishment of vascular plants by improving water retention in the soil and altering soil temperatures (Muscha and Hild 2006; DeFalco et al. 2001). In more arid regions of the sagebrush biome, livestock can have negative effects on biological crusts, which can reduce an areas' ability to recover from disturbance events (Condon et al. 2018; Muscha and Hild 2006).

Overgrazing continued in the Great Basin through the early 1930s until the enactment of the Taylor Grazing Act in 1934. This legislation was designed to "stop injury to the public grazing lands by preventing overgrazing and soil deterioration" (Vale 1975). The Taylor Grazing Act began to improve the range conditions across the west and led to improving management techniques for the future. This legislation was an important step for reversing the legacy of overgrazing and mismanagement of grazing practices (Knapp 1996).

In 1960 the enactment of the Multiple Use Sustained-Yield Act changed the way the land was utilized. With this act, land was considered as having "multiple use" which implies not only for grazing, timber etc. but also for ecosystem services (Multiple-Use Sustained-Yield Act

1960). This act built on the Taylor Grazing Act by expanding the focus of management to the larger ecosystem. This led to more sustainable means of land use on a larger scale. Later, the National Environmental Policy Act of 1970 required federal agencies to report any environmental impact of proposed actions (U.S. Forest Service, 2020). Additionally, the Public Rangelands Improvement Act of 1978 identified current rangeland conditions and sought to improve their condition in accordance with management objectives (U.S. Forest Service, 2020). Although these laws slowed degradation of public lands in the west and began restoring some, much of the land remains in a deteriorated state. According to the BLM over 50% of rangelands remains in “unsatisfactory” (fair or poor) condition (Muir 2012). While the legacy of historic overgrazing and early settlement techniques left much of the western United States in an imperiled state, management of this ecosystem has greatly improved. Although these improvements have been beneficial to the sagebrush steppe, new challenges have become apparent.

Management challenges facing the Sagebrush Steppe

Conifer Encroachment

One major threat the sagebrush steppe faces today is conifer encroachment. Juniper (*Juniperus osteosperma* [Torr.] Little, *Juniperus occidentalis* Hook., *Juniperus scopulorum* Sarg.) and piñon (*Pinus monophylla* Torr. & Frén, *Pinus edulis* Engelm.) woodlands inhabit roughly 19 million ha in the Intermountain West (Davies et al. 2011). Prior to European American arrival, as much as 90% of these woodlands were sagebrush plant communities (Davies et al. 2011; Miller et al.

2008). Western juniper alone has increased from 0.3 million ha to 3.5 million ha since the 1870s (Davies et al. 2014*a*). This increase in juniper prevalence is associated with a number of factors including increasing atmospheric CO_2, climate change, improper livestock grazing, the interaction between improper livestock grazing and fire, and fire suppression (Davies et al. 2014*a*; Miller and Rose 1999). With the increase in improper livestock grazing, perennial bunchgrass prevalence decreased and woody plants increased. At higher elevations, this change in plant composition reduced fine fuel continuity, reduced fire frequency, and allowed juniper to encroach on previously occupied niches (Burkhardt and Tisdale 1976; Miller and Eddleman 2000). This interaction with livestock grazing and fire in combination with fire suppression led to an increase in juniper prevalence. Historically, juniper was limited to fire-safe sites (Davies et al. 2014*a*). European settlement resulted in fire suppression which led to an increase in potential juniper habitat. Additionally, juniper has directly benefitted from additional atmospheric CO_2 due to its physiology. Juniper is a C_3 plant meaning that its growth is directly correlated to the amount of carbon and oxygen in the air (Shaw et al. 1995). The more CO_2 in the atmosphere, the more C_3 plants are able to grow. As atmospheric CO_2 continues to rise, juniper growth rates increase and the potential for juniper expansion increases (Shaw et al. 1995).

The use of heavy grazing practices by early settlers depleted native bunchgrasses in the sagebrush steppe and left unoccupied niches available which juniper trees have been able to exploit. Once established (and in the absence of fire), juniper can dominate a landscape due to its deep root structure and efficient ability to uptake water (Grossiord et al. 2017). It will outcompete native grasses, shrubs and forbs and form monocultures. Juniper encroachment leads to increased runoff and erosion and decreases diversity (Davies et al. 2014*a*). This is detrimental to wildlife, especially sagebrush obligate species like the greater sage-grouse. Woody encroachment also leads to decreased available forage for livestock (Miller et al. 2005; Bates et al. 2005) and can change the fire regime (Bates et al. 2014). As sagebrush plant communities are altered to become predominantly juniper and pinyon, the higher tree canopy and high amounts of woody fuel can lead to more intense and hotter fires (Bates et al. 2014). If these juniper-dominated sites lacked native perennial grasses in the understory prior to woody encroachment, they are more susceptible to post-fire annual grass invasion (Bates et al. 2011; Bates et al. 2014). The vegetation community present prior to juniper invasion in combination with these intense fires can result in an annual grass invasion and a transition from intact sagebrush steppe plant communities to disturbed and invaded sites.

Exotic Annual Grass Invasion

Invasive annual grasses pose a major threat to the recovery of the sagebrush steppe ecosystem. There are three annual grasses that have invaded much of this ecosystem: cheatgrass (a.k.a. "downy brome"; *Bromus tectorum* L.), ventenata (*Ventenata dubia* (Leers) Coss.), and medusahead (*Taeniatherum caput-medusae* (L.) Nevski) (Brummer et al. 2018; U.S. Department of Agriculture 2020). Approximately 50-60% of the sagebrush steppe has a presence of invasive annual grasses or has been converted entirely to non-native annual grassland (West 1999; Knick et al. 2003). Among these invasive annual grasses, cheatgrass is conceivably the single most widely invasive and troublesome weed across the Intermountain West in terms of distribution and density (Mack 1981; Litt and Pearson 2013). Cheatgrass is present across the majority of the Great Basin Desert making the control of this invasive grass a top priority (Bradley et al. 2018).

Cheatgrass is native to Mediterranean Europe and central and southwestern Asia (Knapp 1996). This grass was first identified in the United States in 1861 and is thought to have invaded through contamination of imported grain or in the wool/hide of old-world sheep or cattle (Klemmedson and Smith 1964; Knapp 1996). By 1914 this grass had spread throughout the United States and by 1928 it reached its present distribution (Morrow and Stahlman 1983). The spread was heightened by the construction of the intercontinental railroad system which allowed for rapid transportation of propagules across a broad area. Grazing by cattle and feral horses further disseminated the grass (Knapp 1996).

Cheatgrass not only spreads quickly but it also establishes in and dominates a degraded sagebrush landscape fairly easily. There are several reasons for its successful establishment. First, the Great Basin Desert has a similar climate to areas where cheatgrass originally evolved (Knapp 1996). There also is a near absence of native annual grasses in the Great Basin Desert so cheatgrass quickly took over this unoccupied niche (Knapp 1996). Additionally, cheatgrass has a high phenotypic plasticity due to its quick annual reproductive cycle (Knapp 1996). This allows cheatgrass to quickly evolve to environmental changes and adapt easily to new environments. Cheatgrass seed also has an extremely high germination rate of up to 99.5% which is higher than that of most native grasses which ranges between 60% to 90% (Knapp 1996; Morrow and Stahlman 1983; James et al. 2012). Cheatgrass also can germinate, emerge, and establish in the fall or spring and it germinates earlier in the spring season than native grasses so it can easily deplete the resources on a landscape before the native plants have a chance to germinate (Knapp 1996; Harris 1967; Roundy et al. 2007).

Not only can cheatgrass establish quickly and easily but it persists for very long periods of time. The increase in fire frequency and intensity promotes the persistence of cheatgrass. The sagebrush steppe ecosystem historically had fire return intervals of 50-100 years (Baker 2006; Wright and Bailey 1982; Mensing et al. 2006). An undisturbed sagebrush steppe ecosystem consists of sagebrush shrubs, perennial bunchgrasses, forbs, biological crusts and a lot of bare ground. Historically, following a lightning strike, a fire may start but would not necessarily spread quickly due to a high relative amount bare ground, which resulted in relatively low fuel continuity (Miller et al. 2011; Hull and Hull 1974; Vale 1975; Mensing et al. 2006). With the invasion of cheatgrass, these previously bare ground spots are now often occupied by cheatgrass (Reed-Dustin et al. 2016). This means when a fire does start, it spreads rapidly due to the increase in cheatgrass cover which provides a more continuous fuel bed (Brooks et al. 2004; Miller et al. 2011).

In addition to creating fuel conditions that promote more frequent fires, cheatgrass can promote hotter and more intense fires due to its interaction with woody shrubs such as sagebrush. Once cheatgrass has spread across a landscape, it will not only invade the interspaces between established shrubs but will also appear underneath shrubs, especially following a disturbance event such as overgrazing. When fires start, the cheatgrass underneath the shrubs will cause the woody material to ignite which burns much hotter due to the high amount of calorically-dense woody fuel compared to a lower fuel grass fire. These fires are devastating to woody native shrubs and can eradiate woody plants such as sagebrush in the community, resulting in more unoccupied niches, which allows for more cheatgrass to move in and restart the cycle (Brooks et al. 2004; Larson et al. 2017; Porensky and Blumenthal 2016). These positive feedbacks accelerate the invasion of cheatgrass as well as promote the persistence of this invasive grass in the ecosystem (Brooks et al. 2004; Larson et al. 2017).

When a fire does occur, it will kill most cheatgrass seeds but the seed that survives may produce larger, more productive plants (Hassan and West 1986; Young et al. 1972). A study conducted in 1990 found that cheatgrass growing in a recently burned area grew twice as many seed stalks as plants in an unburned area, indicating cheatgrass seed will quickly take advantage of the increased nutrients following fire, which leads to a rapid recovery of cheatgrass seed (Pellant 1990). Cheatgrass is an annual plant so its reproductive success changes based on environmental changes every year (Mack and Pyke 1983; Knapp 1996). This means an increase in nutrients will help facilitate reproduction in annuals more than perennials. Cheatgrass also evolved with more frequent fire and therefore will take advantage of excess nitrogen in the soil following fires (Johnson et al. 2010) compared to native perennial sagebrush steppe vegetation that does not respond to increased nutrients in the soil as quickly and did not evolve with frequent fire.

Another aspect of cheatgrass persistence and fire frequency is a site's resilience and resistance. A sites resilience can be defined as the capacity for it to regain its fundamental structure when exposed to stress or a disturbance whereas a site's resistance is the ability for it to resist change or retain its fundamental structure despite stress or a disturbance (Chambers et al. 2014). A resilient and resistant site will be able to resist the invasion or decrease the potential for invasion of cheatgrass. Once a site has degraded either through overgrazing, climate change, woody encroachment, etc., unoccupied niches allow for cheatgrass to invade making these sites neither resilient nor resistant. Once cheatgrass has invaded the system, it will continue to contribute fine fuels and promote the spread of fire, further contributing to the persistence of this grass in the system and further impacting resilience and resistance (Brooks et al. 2004).

Many grass, forb, and shrub species native to the sagebrush steppe rely on arbuscular mycorrhizae fungi (AMF) in the soil for nutrient and water uptake (Wicklow-Howard 1994). These native species are often obligate mycotrophic meaning they need the AMF in the soil to survive. Cheatgrass did not evolve with AMF and is therefore only a facultative mycotrophic, meaning it can thrive with or without it (Knapp 1996). Cheatgrass has also been shown to decrease AMF diversity in the soil and reduce mycorrhizal inoculum compared to uninvaded soils (Hawkes et al. 2006). Overgrazing can also negatively affect the AMF in the soil and deplete it (Hovland et al. 2019). Overgrazed or degraded soils further promote the persistence of cheatgrass in these systems.

Once exotic annual grasses establish, they have detrimental effects on the ecosystem. Exotic annual grasses can outcompete native perennial bunchgrasses, increase fire ignition and frequency, and can degrade the soils by modifying fundamental nutrient cycling processes in ecosystems (Norton et al. 2004; Reed-Dustin et al. 2016). Large perennial bunchgrasses native to the sagebrush steppe have very large root structures that not only help to stabilize and create structure in the soil but also contribute organic matter into the soil (Burke et al. 1998). Invasive annual grass roots in comparison, do not contribute as much soil organic matter due to their small size.

Another factor that promotes structure in the soil is the presence of soil microorganisms. Exotic annual grass invasion has shown to decrease the quantity of burrowing fauna, and microbial species (Belnap and Phillips 2001; Kuske et al. 2002), both of which contribute to soil organic matter (SOM) decomposition as well as soil structure (Norton et al. 2004). Mycorrhizal fungi also contribute towards soil structure by increasing the soil's stability and water holding capacity (Hovland et al. 2019). As mentioned previously, cheatgrass is not obligate mycotrophic, resulting in reduced presence of associate mycorrhizal fungi in exotic annual grass ecotones which reduces the development of soil structure and decreases nutrients in the soil (Hovland et al. 2019).

Cheatgrass also is associated with changes in the available nutrients in the soil. One study found that in cheatgrass stands, the available Ca, Mg, and Mn were decreased compared to native grass stands (Perkins et al. 2011). Cheatgrass invasion also resulted in higher litter accumulation compared to native grass stands (Evans et al. 2001). The cheatgrass litter had significantly greater lignin:N ratios (Evans et al. 2001) meaning that it is more difficult and takes longer for microorganisms to break down the litter into available nutrients. The difference in litter accumulation and chemistry in cheatgrass stands resulted in lower rates of net N mineralization by decreasing nitrogen available for microbial decomposition (Evans et al. 2001). Additionally, overtime, the loss in nitrogen in areas invaded with exotic annual grasses was greater due to increased fire frequency and increased volatilization of nitrogen during these fire events (Evans et al. 2001).

Cheatgrass also changes the morphology and classification of the soil underlying it. The soil under cheatgrass stands was found to have a thinner A horizon (Norton et al. 2004). This is likely due to the change in microbial activity and therefore decreased soil organic matter. Not only were the A horizons thinner but they were lighter in color as well. This could also suggest a decrease in humus or organic matter. Cheatgrass has a short life cycle and only has roots in the soil for a short portion of the year. The lack of organic matter and lack of root abundance relative to perennial-dominated communities have both been shown to decrease the soils' water holding capacity (Norton et al. 2004).

Once cheatgrass has invaded a landscape, the fundamental nutrient cycling processes within the soil are altered. This makes restoration of the site more difficult and expensive (Mata-Gonzalez et al. 2008). The lack of organic matter or humus also decreases the cation exchange capacity (CEC) of the site making it less fertile (Norton et al. 2008). The water infiltration and water holding capacity are both decreased which could promote soil erosion at the invaded site.

Cheatgrass invasion also negatively affects wildlife habitat. As an aggressive competitor, cheatgrass can quickly turn sagebrush steppe habitat into a cheatgrass monoculture. This provides little to no forage for pronghorn, lagomorphs, greater sage-grouse, and even livestock. Livestock will eat cheatgrass, but once it has seeded-out doing so can cause injury due to its long and sharp awns (needle-like structure at the end of grass seeds) that can get lodged in the mouths, throats, and eyes of livestock (Knapp 1996; Morrow and Stahlman 1983). Cheatgrass also has low nutritive value after senescence, making it undesirable forage for livestock. A study conducted in the state of Washington found that the small mammal richness in cheatgrass stands was 14-fold lower than that of native grass stands (Gitzen et al. 2001). Small mammals such as rodents provide an important food source for raptors, canids, and reptiles. Exotic annual grasses have a strong negative affect on the overall biodiversity of a system (Bansal and Sheley 2016).

Managing Exotic Annual Grasses

Seeding

Invasive annual grasses pose a clear threat to the sagebrush steppe ecosystem, making the management of these grasses, and restoration of native plant communities, a top priority for land managers in the west. Seeding of native perennial bunchgrasses within the exotic annual grass ecotone is one of the best options for reclaiming disturbed and invaded areas, however, seeding generally has very low success rates (Madsen et al. 2016). Cheatgrass, as previously mentioned, is highly competitive, and has higher survival and growth rates compared to native species, making seeding efforts problematic. Additionally, due to the underreporting of negative results in literature, the actual effectiveness of seeding native perennial grasses is not known (Madsen et al. 2016). Seeding efforts may also not have high success rates due to the effect of exotic annual grasses on nutrient cycling processes prior to seeding (James et al. 2011). Seeding efforts are also extremely expensive, especially for large scale operations and since the success rates are so low, the cost of seeding is often not economical.

Established mature native perennial bunchgrasses will outcompete cheatgrass but, at the seedling demographic stage, native grasses will typically not outcompete invasive annual grasses. However, non-native grasses such as crested wheatgrass (*Agropyron cristatum*) can be seeded in invasive annual grass stands and are better competitors with invasive annual grass seedlings. These non-native grasses also provide forage for livestock, restore perennial bunchgrasses to the system, and help stabilize the soil. Some land managers use this non-native grass because it establishes quickly and often has much higher success rates when seeded (Boyd and Davies 2012). Since this species has such high seeding success rates, it often forms monocultures which reduce biodiversity, wildlife habitat and ecosystem functionality (McAdoo et al. 2017). Restoring native grasses in non-native crested wheatgrass stands first requires the removal of the non-native grass. Unfortunately, mechanical and chemical means of removal have shown to be unsuccessful at eradicating crested wheatgrass (McAdoo et al. 2017). This grass can successfully establish where native vegetation has difficulty establishing, which reduces potential exotic annual grass establishment however, crested wheatgrass may impair wildlife habitat, decrease biodiversity and decrease the ability for native vegetation to establish.

Biological Controls

Since seeding native perennial vegetation into invasive annual grass stands is often unsuccessful, the removal of invasive annual grasses prior to seeding or restoration is necessary. Unfortunately, invasive annual grasses like cheatgrass can be difficult to remove. The removal method used depends on the severity of the infestation, the site conditions, and the current land use (U.S. Forest Service 2014). One option that is not yet widely used and somewhat controversial in the ecological field is the use of biological controls or the use of living organisms to control pest species (Barbosa 1998).

Fungi such as *Ustilago bullata* and *Pyrenophora seminaperda* both are native or naturally occurring in the soils of the western United States. These fungi are often host specific and have no known negative effects on many native perennial grasses but have been shown to negatively affect cheatgrass (Meyer et al. 2001). *Pyrenophora seminaperda* primarily kills dormant seeds and has been shown to effectively decrease the cheatgrass seedbank five-fold in dry sites compared to sites without the presence of this fungus (Meyer et al. 2007). Although this fungus has shown to decrease cheatgrass stands in isolated events, it has not proven to be a useful biological control on a larger scale thus far.

Another native fungus that could be used as a biological control is *Ustilago bullata* or the Head smut pathogen. This fungus causes an infection in cheatgrass plants and results in the prevention of seed production through sporulation of the fungus in the inflorescence of cheatgrass plants (Meyer et al. 2001). This fungus is more specific to cheatgrass than native vegetation and has not caused significant disease in native vegetation (Meyer et al. 2008).

Alternative Options

Another option for removal of invasive annual grasses is changing the chemistry of the soil, which has shown some promise at reducing the prevalence of cheatgrass specifically. Cheatgrass responds well to high amounts of phosphorous in the soil compared to native vegetation (Newingham and Belnap 2006). By reducing the amount of available phosphorous in the soil, cheatgrass can effectively be controlled. Soil nutrients such as zinc (Zn^{2+}), manganese (Mn^{2+}), and iron (Fe^{2+}) oxides, can bind with available phosphorus in the soil making it locally inactive and unavailable to plants. A field study conducted in southeast Utah found that by adding a mixture of $CaCl_2$, $MgCl_2$, NaCl and zeolite to the soil, phosphorus was reduced and cheatgrass emergence and biomass were much lower (Newingham and Belnap 2006). This soil amendment was not harmful to native vegetation.

Other cheatgrass removal techniques consist of early-season burning to destroy seed in the fall prior to seeding and tillage (Meyer et al. 2001). Hand removal of the grass is also an option for small infestations although removal is required for several consecutive years before results can be seen, which is often not feasible on a large scale (U.S. Forest Service 2014). Additionally, livestock grazing in the early to mid-spring can reduce the production of cheatgrass seeds and help control the spread of this invasive grass, however if not highly controlled, heavy grazing could have the potential to decrease native vegetation vigor and promote further dissemination of the invasive grass seeds by introducing unoccupied niches that invasive annual grasses could establish in (Belsky and Gelbard 2000; Vallentine and Stevens 1992).

Pre-emergent Herbicides

Unfortunately, the use of biological controls, soil amendments, burning, and livestock grazing have not shown to be effective in large scale restoration efforts (Brooks 2002; Ditomaso et al. 2006). Some land managers have used contact herbicides such as glyphosate on annual grass invaded zones in the early spring when native vegetation has not started growing. This method can be effective but if not timed right, could negatively affect the native vegetation. One method that has had the most success in the management of invasive annual grasses is the use of pre-emergent herbicides (Sheley 2007; Davidson and Smith 2007). A pre-emergent herbicide prevents growth of recently germinating seeds through the inhibition of the production of branched chain amino acids which are vital to the plant's cell growth and production of proteins (Tu et al. 2001). If the herbicide can be applied before seeds have emerged, the reduction of the seedbank can effectively reduce and theoretically ultimately eradicate this invasive grass (Vollmer and Vollmer 2008; Clements et al. 2017).

The use of herbicide to control cheatgrass has proven to be the most effective long-term control of this invasive grass, with pre-emergent herbicides having the most success in control (Sheley 2007; Davidson and Smith 2007; Vollmer and Vollmer 2008). Pre-emergent herbicides selectively control annual and perennial grasses and some broadleaf weeds with the most success in annual weeds (Tu et al. 2001; Global Rangelands 2021). Imazapic, a pre-emergent, apoplastically-translocated herbicide has shown the most promise in exotic annual grass control and is the most widely used herbicide for exotic annual grass control by land mangers (Vollmer and Vollmer 2008; Davidson and Smith 2007). Other pre-emergent herbicides such as rimsulfron, indaziflam, and sulfometuron methyl + chlorsulfuron are some new alternatives to using imazapic for exotic annual grass control, however, some of these herbicides have not been approved for use on rangelands and are therefore not preferential for exotic annual grass control. Additionally, imazapic is so widely used because it is effective at exotic annual grass control. According to several studies, imazapic was the most effective at exotic annual grass control compared to other herbicides (Shinn and Thill 2002; Vollmer and Vollmer 2008; Davidson and Smith 2007). The herbicide selected for control of cheatgrass or other exotic annual grasses also depends on several factors such as the restoration site soil, the native plant community, and the degree of annual grass invasion.

The use of pre-emergent herbicides to manage invasive annual grasses can be effective, however these herbicides can have negative effects on native vegetation. Pre-emergent herbicides used to control exotic annual grasses can harm desired seedlings, and when used at high enough rates, these herbicides can also harm native established plants, requiring land managers to wait at least a year or more after herbicide application to seed desired species (Davies et al. 2014*b*). This method of herbicide application is referred to as a multiple-entry approach. Although in some cases effective, often a year or more of waiting to revegetate will allow annual grasses to re-invade and dominate once again, therefore making the previous herbicide application ineffective (Davies et al. 2014*b*). A potential solution to this problem is a single-entry approach wherein herbicide is applied at the same time native vegetation is seeded, which would significantly reduce restoration costs relative to a traditional multiple-entry approach (Sheley 2007). Unfortunately, the native seed will also suffer the effects of the herbicide unless it is protected in some type of seed coating. Seed coatings offer the native seed protection from herbicide while still allowing the seed to germinate and emerge. Seed coatings and other seed technologies (pellets, pods, etc.) offer a new method of restoration in exotic annual grass-dominated landscapes.

Activated Carbon

Activated carbon (AC) has been used in previous studies as a type of seed coating in the form of pellets, pods, or individual seed coatings that successfully protect desired native seeds from herbicide (Davies et al. 2017; Davies 2018; Clenet et al. 2019; Holfus et al. 2021). Activated carbon is a type of organic material such as wood, coal, or shells, for example, that has been either chemically or thermally "activated" to form a new highly adsorptive substance (Marsh and Rodríguez-Reinoso 2006). This process removes any volatile non-carbon constituents resulting in a new material with an extremely high surface area. This high surface area creates a very high adsorptive effect, allowing it to bind to many materials including herbicide (Foo and Hameed 2010). Once the activated carbon binds to the herbicide, the herbicide cannot affect the native seed within the activated carbon coating, making the herbicide locally inactive (Foo and Hameed 2010; Coffey and Warren 1969).

Due to the high adsorptive capacity of activated carbon, it has been used in other forms of restoration, for example, roots of transplants have been dipped in activated carbon to protect them from herbicide application (Kratky et al. 1970). Activated carbon is also used in agriculture to protect crops, and is often applied in strips or bands over crops seeded in rows to protect them from herbicide. Another study found that this technique protected tomato plants (*Lycopersicon esculentum* Mill.) from herbicide (Morgan and Morgans 1992). This technique has also been used to offer herbicide protection to other crops such as asparagus (*Asparagus officinalis* L.), alfalfa (*Medicago sativa* L.), beans (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), potatoes (*Solanum tuberosum* L.), spring wheat (*Triticum aestivum* L.), and sugar beets (*Beta vulgaris* L.) (Ogg 1978; Ogg 1982). Another study applied activated carbon in bands over grass seed and applied the pre-emergent herbicides, diuron, atrazine, simazine, and terbacil, at different rates (Lee 1973). Banding, although effective in agricultural settings is not preferential in rangeland restoration settings. Activated carbon banding protects all seed under the carbon layer including weed seed which would decrease the effectiveness of restoration efforts by allowing for increased establishment potential of invasive seeds (Lee, 1973; Ogg, 1978). Additionally, rangeland restoration efforts often cover a larger area compared to agricultural uses, so banding would be difficult to utilize at larger scales due to the increased amount of material needed.

An alternative to banding that has more direct benefits to native seed and is more beneficial in a rangeland setting, is the use of herbicide protection pods (HPPs). This seed technology has been used in rangeland restoration to protect grass seed from herbicide in a single-entry herbicide approach and, unlike carbon banding, does not offer direct protection to seeds of non-desired species. These HPPs combine activated carbon, bentonite clay, compost, and worm castings along with desired grass seeds in the form of a pellet. HPPs can then be broadcast seeded directly over exotic annual grass monocultures that are concurrently sprayed with herbicide. The desired grass seed within the HPPs will grow while the herbicide will kill the emerging exotic annual grasses. This newly developed seed technology has shown to be effective in both greenhouse and field studies (Davies 2018; Clenet et al. 2019). Although effective, production of HPPs requires the use of specialized equipment, and requires a lot of materials and time to make. Furthermore, activated carbon pellets result in decreased emergence and growth of some desired seeded species in the absence of herbicide application, likely because of their size, and compaction during production (Clenet et al. 2019).

A potential alternative to producing HPPs is coating individual grass seeds with the materials used to construct HPPs. A study conducted in 2014 directly coated individual grass seed with activated carbon. This study showed that the amount of coating per seed was not enough to protect the seed from high amounts of herbicide (Madsen et al. 2014). This technique showed some promise although more activated carbon or herbicide protection material would need to adhere to each grass seed to offer more protection from herbicide. In the current effort, we test a newly developed seed coating technique for attaining more activated carbon or protection material per grass seed in an attempt to protect it from imazapic pre-emergent herbicide (Holfus et al. 2021).

Sagebrush Restoration

Annual grass-invaded landscapes within the U.S. sagebrush steppe require revegetation of not only perennial bunchgrasses but also shrubs such as sagebrush (*Artemisia* L.). Greater sage-grouse and other wildlife rely heavily on sagebrush for survival. Sagebrush also provides soil stabilization, ecosystem stability, and resilience to disturbances, making the restoration of this species a high priority (McArthur 1992). Unfortunately, with annual grass invasion, there is an increase in continuous fuel beds, which leads to increased fire frequency and larger scale fires that eliminate sagebrush as well as the sagebrush seed bank (Dettweiler-Robinson et al. 2013; Wijayratne and Pyke 2009). Sagebrush is not adapted to frequent fire and its seed has short-term viability (1-2 years) making post-fire recovery problematic (Mata-Gonzalez et al. 2018; Brabec et al. 2015; Wijayratne and Pyke 2009). Once the seed bank is removed, natural re-establishment of this species is nearly impossible for sagebrush plant communities within the exotic annual grass zone due to the highly competitive nature of invasive annual grasses. Ecologists have tried to re-establish Wyoming big sagebrush through traditional seeding methods such as drill or broadcast seeding, however, these efforts are often unsuccessful with failure rates exceeding 70% (Lysne and Pellant 2004; Knutson et al. 2014), but see Davies et al. (2018). There are several reasons for such low establishment rates of sagebrush including the source of seed, soil type, vegetation present at the restoration site, and year to year variation in climate (Brabec et al. 2015; Shaw et al. 2005; McAdoo et al. 2013). Sagebrush is also genetically diverse and has many subspecies, each of which have their own specific adaptations to abiotic environmental factors, making them more suited for particular climates or sites (Brabec et al. 2015).

Additionally, sagebrush seed has very specific requirements for germination and establishment. If the seed is planted on the soil surface, the lack of seed-soil contact can result in low rates of establishment and is one reason broadcast seeding has such low success rates (Lambert 2005). The seed requires soil contact but cannot be planted too deep (>2mm deep) in the soil either (Jacobsen and Welch 1987; Lysne and Pellant 2004; Lambert 2005). If the seed is planted too deep, it will not be able to germinate due to its small size and therefore small energy storage, making it difficult for the newly emerged cotyledon to reach the soil surface as well as penetrate the hard soil surface. This is why many restoration practitioners use drill seeding that packs the seed underneath the soil surface (about 1.6mm below the surface) (Lambert 2005).

Prior to European settlement, the sagebrush steppe experienced occasional fire however, sagebrush could naturally recover more easily than present day partly because of the lack of competition with invasive annual grasses (McAdoo et al. 2013; Brabec et al. 2015). This recovery process prior to annual grass invasion was slow and could take decades however, sagebrush could eventually naturally recover. After European settlement, the presence of invasive annual grasses increased throughout the sagebrush steppe and in turn caused alterations to the fire frequency and intensity in this system which made post-wildfire recovery more challenging for sagebrush (Whisenant 1990; Dettweiler-Robinson et al. 2013). Sagebrush seed does not compete well with invasive annual grasses and therefore has difficulty naturally recovering (Shaw et al. 2005; Davies et al. 2020). Additionally, post-wildfire re-establishment of sagebrush is often unsuccessful due to its short-lived seedbanks, and dependence on seed from unburned areas (Lesica et al. 2007; Shinneman and McIlroy 2016; Ott et al. 2017). With the increase in fire frequency and intensity due to annual grass invasion, post-wildfire revegetation is an increasing priority. Since seeding success rates are so low, the use of sagebrush transplants or outplantings have shown to be a more successful alternative (Dettweiler-Robinson et al. 2013).

The use of sagebrush transplants is a rather new technique that requires growing sagebrush seedlings in a greenhouse and transplanting them in the field sometime later. Sagebrush and many other plants are most vulnerable in the seedling stage. By growing sagebrush in a greenhouse until they are mature, the potential for seedling mortality in the field is reduced. Seedlings are very vulnerable to abiotic factors such as freezing temperatures in the winter, and extreme temperature and soil moisture conditions during summer drought (Fenner 2000).

Sagebrush transplants, if successful, could provide sagebrush islands in recently burned or disturbed areas and would reintroduce a seed bank in the system. This could accelerate vegetation recovery in disturbed areas and increase biodiversity (McAdoo et al. 2013). Although this technique is rather new, numerous studies have been conducted on the success of using sagebrush transplants. The effects of site conditions, such as soil type and depth, topography, site preparation, seed source, and the herbaceous community at the restoration site have all been tested (Davidson et al. 2019; McAdoo et al. 2013; Brabec et al. 2015). Most of these studies, have used a technique of growing out the sagebrush transplants for six months to a year in a greenhouse before planting them in the field (Fleege 2010; Moore 2015; Shaw et al. 2015; Davidson et al. 2019; Brabec et al. 2015). Although the cost to grow sagebrush transplants in the greenhouse introduces additional costs compared to seeding, it has much higher success rates compared to direct seeding, which can create an economic advantage. However, if growing the transplants for a shorter period of time in the greenhouse resulted in similar success rates, this would save a great deal of time and money by limiting greenhouse growing costs. Some studies have higher success when transplants are planted in the spring whereas other studies have higher success when transplants are planted in the fall (Monsen et al. 2004; Clements and Harmon 2019). Both the timing of year and age of transplant at the time of planting affect the success of sagebrush transplant establishment. These topics have not been studied in depth and warrant further testing. This thesis examines the survivability and vigor of transplants 1) planted at different times of year, 2) at different ages at time of planting, and 3) in differing herbaceous communities to determine the effects of exotic annual grass competition on sagebrush transplants.

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CHAPTER 2: PRE-EMERGENT HERBICIDE PROTECTION SEED COATING: A PROMISING NEW RESTORATION TOOL

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Abstract

Invasive annual grasses such as cheatgrass (*Bromus tectorum* L.) outcompete native grasses, increase fire frequency, and impact the functionality and productivity of rangeland ecosystems. Pre-emergent herbicide treatments are often used to control annual grasses but may limit timely restoration options due to negative effects on concurrently-planted desired seeded species. We tested the efficacy of activated carbon-based herbicide protection coatings applied to individual bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Love) seeds for protecting seedlings from injury associated with pre-emergent herbicide (imazapic) application in a laboratory environment. Emergence of coated seed averaged 57% ± 5% compared to bare seed which had 14% ± 10% emergence with imazapic application. Seedling height for coated seed averaged 7.56 ± 0.6 cm compared to 2.26 ± 0.4 cm in uncoated bare seed in the presence of imazapic. Coated seeds produced 87% more plant biomass than uncoated seeds. Our laboratory results suggest treating individual seeds with an activated carbon-based coating dramatically reduces negative effects of pre-emergent herbicide on desired seeded species. Field studies are needed to confirm these results in an applied restoration context.

Keywords: Imazapic; herbicide; restoration; seed technologies; activated carbon; cheatgrass

Introduction

The invasion of exotic annual grasses such as cheatgrass (*Bromus tectorum* L.) has impacted ecosystem function across millions of acres in US sagebrush (*Artemisia* L.) steppe and these species are now present throughout most of the Great Basin region (Davies et al. 2011). Exotic annual grasses can outcompete native perennial bunchgrasses, increase fire ignition and frequency, and degrade soils by modifying fundamental nutrient cycling processes (Norton et al. 2004; Reed-Dustin et al. 2016), making restoration efforts extremely expensive and challenging (Mata-Gonzalez et al. 2008). Given the impact of annual grass invasion on resource uses and values within the Great Basin ecosystem, and the spatial extent of the problem, more effective methods for controlling cheatgrass and restoring degraded plant communities are imperative to maintaining ecosystem services.

Research has shown that an effective method for reducing annual grass abundance is to apply pre-emergent herbicide (Sheley 2007; Davidson and Smith 2007). However, pre-emergent herbicide application can have adverse effects on seedlings of desired species (e.g., native perennial bunchgrasses) since the herbicide will injure or kill all emergent seedlings (Davies et al. 2014). Consequently, managers must wait up to a year following pre-emergent herbicide application before seeding desired species. In that time, exotic annual grasses can reestablish, reducing efficacy of the previous herbicide application (Davies et al. 2014). If a single-entry herbicide approach (i.e., simultaneously spraying herbicide and seeding desired species) were possible, this would significantly decrease costs relative to a double-entry approach and would afford seeded species the maximum window of opportunity to establish in a relatively competition-free environment (Sheley 2007).

In previous studies, the use of activated carbon (AC) in the form of pellets or herbicide protection pods (HPPs) incorporated with seeds of desired species was successful in protecting seedlings of seeded species from pre-emergent herbicide injury (Davies et al. 2017; Davies 2018; Clenet et al. 2019). Activated carbon in HPPs has a high surface area, which promotes adsorption, effectively making the herbicide locally inactive (Foo and Hameed 2010). These products allow desired seeds to be seeded simultaneous with application of herbicide (i.e., a true single-entry system). Although effective, these products can be difficult to produce without specialized equipment. Furthermore, activated carbon pellets, likely because of their size and compaction, have been shown in the absence of herbicide to decrease emergence and growth of seeded species (Clenet et al. 2019). To combat these issues, individual grass seeds have been coated in previous studies, unfortunately, with low success (Madsen et al. 2014). Getting enough activated carbon to adhere to each seed has proven to be difficult and resulted in poor protection from herbicide. In this paper, we evaluate the potential for a new method of coating individual grass seeds with an herbicide protection formula containing more activated carbon compared to previous studies, and determine the potential for seed germination and initial seedling establishment as affected by coating and by varying rates of imazapic pre-emergent herbicide. We hypothesized: 1) seed coating would not influence seedling emergence in the absence of herbicide, and 2) in the presence of herbicide, coated seed would have increased seedling emergence, shoot height, and above-ground biomass compared to bare seed, but 3) efficacy of the seed coating would decrease as herbicide amount increased.

Materials and Methods

Study Site and Materials

Soil was obtained from a Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* (Beetle & A. Young) S.L. Welsh) steppe community type, located at the Northern Great Basin Experimental Range, 16 km southwest of Riley, Oregon. Excavated soil was sifted (1.62 mm mesh) to remove remaining seeds and used to fill pots that were placed in a grow room at the Eastern Oregon Agriculture Research Center, Burns, Oregon. Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Love) seed was used in this study (Anatone; Granite Seed, Inc.; Lehi, UT) and is a major component of native plant communities in sagebrush steppe ecosystems (and restoration efforts therein) of western North America (Madsen et al. 2014; Rodhouse et al. 2014; Clenet et al. 2019). Germination potential of bluebunch wheatgrass was

92% as determined using five replicates of 25 seeds in a petri dish containing moistened blotter paper and maintained for 19 days at 22ºC and a 12 hour light/dark cycle.

Seed Coating

Approximately 200 g of bluebunch wheatgrass seeds were first coated with 192 g of powdered activated carbon (Darco GroSafe; Cabot, Billerica, MA) and 255 mL of an 8% partially hydrolyzed polyvinyl alcohol binder (Selvol-205; Sekisui Specialty Chemicals, Dallas TX) using a 14" commercial rotary seed coater (SedPell RP14-DB; BraceWorks Automation and Electric; Lloydminster, SK, Canada) and standard coating protocols. This base layer provided a surface for adhesion of herbicide protection material. Seed was subsequently coated with an herbicide protection formula consisting of: 33% activated carbon, 14% compost, 6% worm castings, and 45% bentonite clay by weight which was mixed with a 4% partially hydrolyzed polyvinyl alcohol binder (Selvol-205) using a new coating method referred to as "Vortexing". This method is comprised of layering rotary-coated seed, powdered herbicide protection material, and atomized 4% partially hydrolyzed alcohol binder on a vibrating plate and hand mixing for approximately one minute. Seed is then dried on a forced air dryer at 26°C for one hour and the process repeated until the desired application rate is achieved. For a step-by-step protocol, please see Appendix A. The development of this method was required to overcome the small particulate size of the herbicide protection formula, specifically the activated carbon. This alternative method is less abrasive than traditional rotary coating and allows additional layers of material to be added until a desired amount is coated on each seed.

Study Design

We used a randomized-block 2 x 3 factorial design with five blocks. Treatments were coated or bare (non-coated) seed, and growing pots (14 x 14 x 15 cm) were sprayed with a high herbicide application of imazapic (Plateau BASF Corporation; Research Triangle Park, NC) at 210 g ai·ha^{-1}, a low herbicide application rate at 105 g ai·ha^{-1} or not sprayed with herbicide (control). The coating method was not precise and each seed did not coat evenly. Because of this we divided coated seeds by size class and amount of activated carbon per size class was calculated. Coating material was estimated by weighing subsets of 25 seeds and averaging their weight. This weight was compared to the average weight of uncoated seed to determine coating material attained per seed and then multiplied by 33% (the amount of activated carbon in the coating material). For purposes of this study, 12 mg of activated carbon per seed was used to protect seeds from herbicide application. Seeds in the 4 mm size class had at least 12 mg (12-15 mg) of activated carbon and were used in this study. Samples were placed in a climate controlled common grow area (16 - 22°C) under Platinum LED P1200 lights (Platinum LED Lights, LLC, Kailua, HI) with a 12 hour light/dark cycle.

Each growing pot was watered to field capacity and planted with 25 bluebunch wheatgrass seeds. Seeds were placed on the soil surface and a small amount of field-collected soil was sprinkled over the top until seeds could no longer be seen. After planting, pots were sprayed with their designated herbicide application rate using a hand operated backpack sprayer (Solo, Newport News, VA), and allowed to air dry outside for one hour before being brought back inside the grow room. This was done to decrease contamination of unsprayed pots. Throughout the study, pots were kept moist and hand watered as needed. Following a four-week period, total seedling emergence was counted for each pot. Living and dead bluebunch wheatgrass seedlings were counted separately. Seedlings were considered living if they had any remaining green tissue and were considered dead if they did not contain any green tissue. Seedling blade height of live seedlings was measured and averaged by pot and above-ground biomass of live and dead seedlings was calculated per pot. Analysis of variance was used in R to compare the response of measured variables across treatments. When significant treatment or interactive effects were found, means were separated using the Tukey Honest Significant Difference method and reported with their associated standard errors. Differences were considered significant at $p \leq 0.05$.

Results

Coating and herbicide application interacted to affect seedling emergence ($p \leq 0.001$; Fig. 2.1). Percent emergence was similar across herbicide levels for coated seeds, but for bare seed herbicide application reduced emergence approximately five-fold (Fig. 2.1). Coating and herbicide application also interacted to affect seedling height ($p = 0.006$; Fig. 2.2). Seedling height decreased with herbicide application for both coated and non-coated seeds but coated seeds were approximately 2-fold taller than non-coated seeds. Plant biomass, was affected by coating ($p = 0.008$) and herbicide application rate ($p \leq 0.001$; Table 2.1). Coated seeds produced 87% more plant biomass than uncoated seeds.

Discussion

Our results indicate individual bluebunch wheatgrass seeds coated with herbicide protection material were moderately protected from imazapic herbicide application. Vigor of seedlings grown from bare seed was decreased by herbicide application as evidenced by decreased emergence, seedling height, and biomass in comparison to coated seed. In support of our first hypothesis, coating did not influence seedling performance in the absence of herbicide. Our second hypothesis was also supported since plant height and emergence were higher in coated seed than uncoated seed in the presence of herbicide. Our third hypothesis however, was not supported given that measures of seedling performance did not vary by herbicide amount among coated seeds.

Although there was no difference in emergence between herbicide and no herbicide treatments in coated seed, there was a difference in coated seed plant height. Coated seed without herbicide treatment was twice as high as coated seed in the presence of herbicide. This suggests the herbicide is having a negative effect on vigor of coated seeds. We suspect some seeds had slightly less coating (protecting material) than others even though on average they had the same amount of coating per seed. The coating method used in this study does not coat each seed evenly and therefore results in some seeds attaining more protection material than others. Since activated carbon protects seed by an adsorption effect (Foo and Hameed 2010), seeds with less coating were likely more susceptible to injury from herbicide. Thus, greater amounts of activated carbon may be needed around seeds to increase the level of herbicide protection. Additionally, once seedlings begin emergence and root elongation, they may come into contact with herbicide in the soil (Clenet et al. 2019).

For this study, the herbicide application rates were chosen based on the EPA's recommendations for rangeland use. According to the EPA, 70 g $ai \cdot ha^{-1}$ to 105 g $ai \cdot ha^{-1}$ of imazapic is enough to control cheatgrass in rangelands but 210 g ai·ha^{-1} is the maximum amount of herbicide allowed for use on rangelands (US EPA). This study used 105 g ai·ha^{-1} for the low herbicide and 210 g ai·ha^{-1} for the high herbicide rate, meaning coated seed was still offered some protection at the maximum herbicide rate allowed for rangeland use.

Results of this study add to literature showing advantages of using activated carbon in seed enhancement technologies to reduce effects of pre-emergent herbicide on seeded species. Increasing the amount of activated carbon (protection material) on individual grass seeds in this study, offered more protection from herbicide than observed by Madsen et al. (2014) with half the amount of activated carbon coating. This study is the first to coat individual seeds with the herbicide protection formula rather than activated carbon alone. This protection formula as well as the increase in activated carbon coated on individual seeds resulted in higher emergence than seeds coated individually with activated carbon only (Madsen et al. 2014). Our study begins to delineate how much activated carbon or protection material is needed to protect seeds from herbicide; which is much lower than previously thought. Similar to our grow room study, field studies that used HPPs containing activated carbon demonstrated use of activated carbon limited the effects of herbicide use on seeded species (Davies et al. 2017; Davies 2018; Clenet et al. 2020). Activated carbon seed enhancements are clearly an effective tool to reduce effects of pre-emergent herbicide on seeded native vegetation and warrant further refinement.

Management Implications

This proof of concept study shows individually coated seeds can be protected from herbicide under controlled conditions. We recommend field studies to determine success rates in natural environments. If effective in the field, activated carbon-based seed coatings could be a cost-effective alternative for restoration of annual grass-invaded landscapes of the sagebrush steppe ecosystem.

Use of herbicide protection coated products (seeds, pellets, or pods) allows for the possibility of performing seeding and weed control at the same time, which can save a year or more in restoration projects (Davies et al. 2017; Davies 2018; Clenet et al. 2019). This extra year will allow time for additional desired species growth with relatively minimal competition from exotic annual grasses, resulting in optimal restoration conditions (Madsen et al. 2014). Additionally, although some loss of seedling vigor was evident for herbicide protection coated seeds exposed to herbicide (e.g., reduced seedling height), use of individually coated seed in an applied restoration context may be an attractive management option relative to pellets or pods due to less material needed, potentially reduced negative effects from pellet compaction and size on emergence and growth, and better flow through in machinery used for seed planting (e.g. rangeland seed drills).

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Figures

Herbicide level (g)		Coating (g)	
No Herbicide	0.37 ± 0.16a	Coated seed	0.23 ± 0.16a
Low Herbicide	0.08 ± 0.07b	Uncoated seed	0.12 ± 0.06b
High Herbicide	0.08 ± 0.11b		

Table 2.1. Plant biomass (g) as affected by herbicide application level and seed coating. High herbicide = 210 g ai·ha^{-1}, low herbicide = 105 g ai·ha^{-1}. Treatments without a common letter are different ($p < 0.05$).

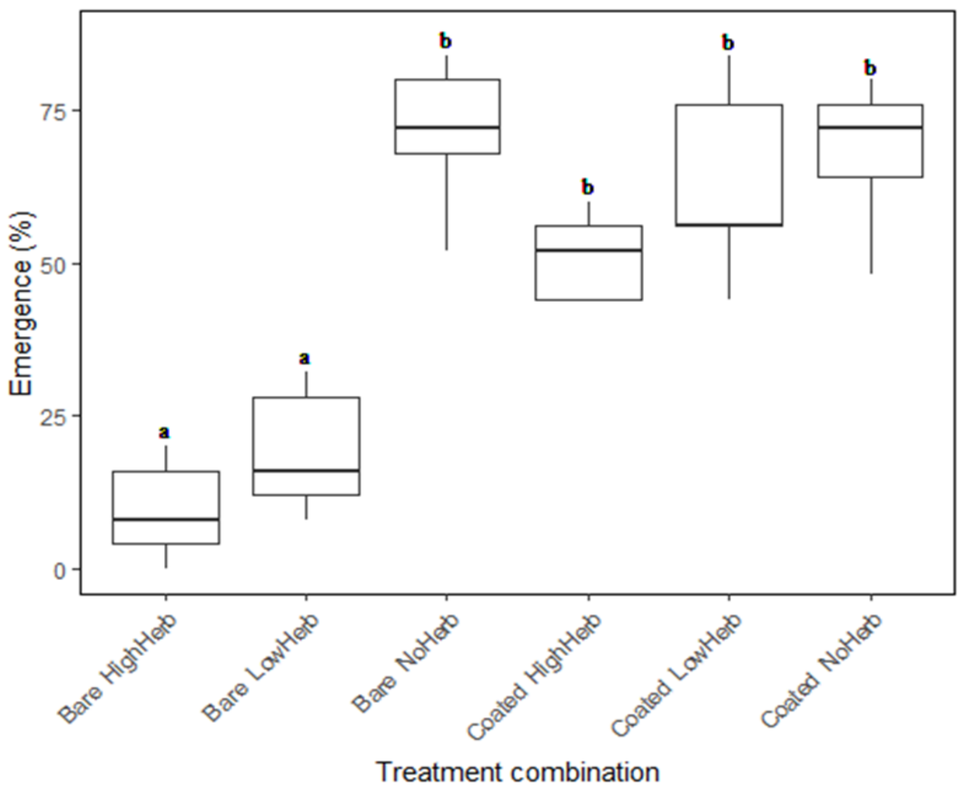

Figure 2.1. Box and whisker plots showing percent emergence of seedlings by treatment type (Bare = uncoated bare seed, Coated = herbicide protection coated seed; HighHerb = high herbicide treatment (210 g ai·ha^{-1}), LowHerb = low herbicide treatment (105 g ai·ha^{-1}), NoHerb = no herbicide application treatment). Boxes in each treatment combination represent the 25th through 75th percentiles and each whisker represents the minimum and maximum data points. The line in the middle of the box shows the median. Treatments without a common letter are different ($p \leq 0.05$).

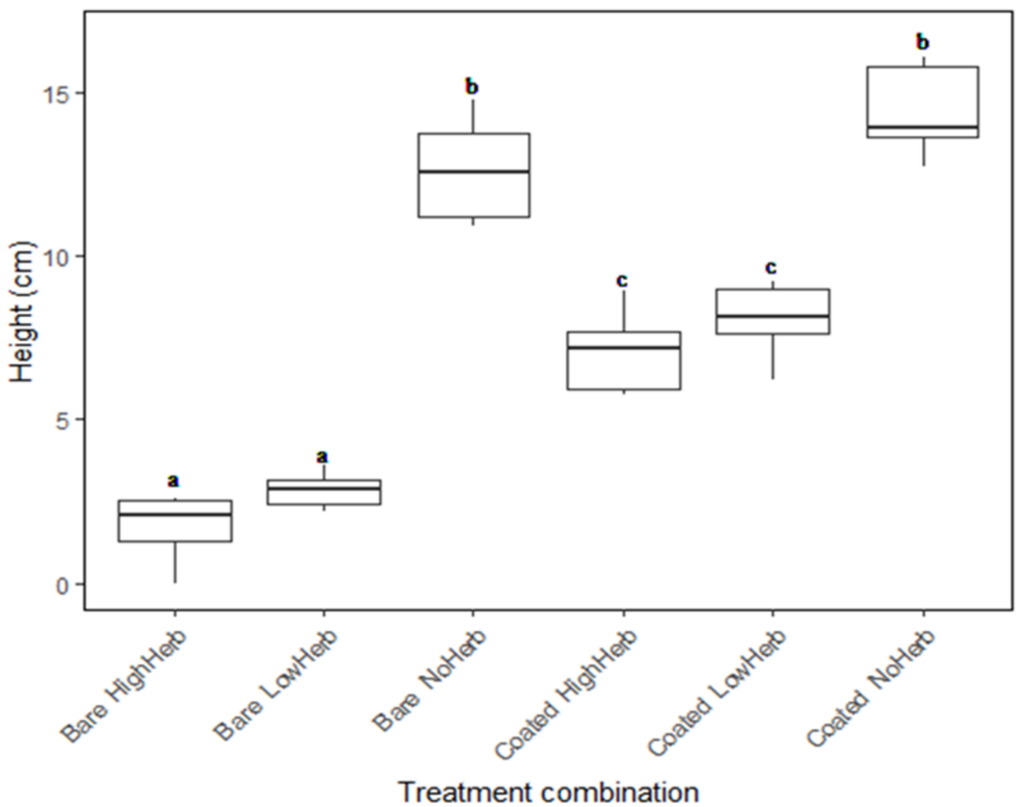

Figure 2.2. Box and whisker plots showing height of seedlings by treatment type (Bare = uncoated bare seed, Coated = herbicide protection coated seed; HighHerb = high herbicide treatment (210 g ai·ha^{-1}), LowHerb = low herbicide treatment (105 g ai·ha^{-1}), NoHerb = no herbicide application treatment). Boxes in each treatment combination represent the 25th through 75th percentiles and each whisker represents the minimum and maximum data points. The line in the middle of the box shows the median. Treatments without a common letter are different ($p \leq 0.05$).

CHAPTER 3:
PRE-EMERGENT HERBICIDE PROTECTION SEED COATING: A RESTORATION TOOL IN ANNUAL-INVADED RANGELANDS

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Abstract

Invasive annual grasses pose a major threat to the ecology and land use of the sagebrush steppe by altering fundamental nutrient cycling and disturbance processes, outcompeting native vegetation, decreasing available habitat for wildlife and reducing livestock forage. The use of pre-emergent herbicides can provide effective control of invasive annual grasses, however these herbicides negatively affect desired native seedlings. Herbicide protection coatings on native grass seed have been shown to be effective at protecting native grass seeds against low and high rates of herbicide in a greenhouse setting but have not yet been tested in the field. This study used a randomized block factorial design to test the effectiveness of three levels of herbicide protection seed coating (4, 8, and 12 mg of activated carbon coated seed) sprayed with a low and high rate of imazapic herbicide post-planting, by collecting seedling emergence and survival data in each of two years within five blocks. Due to two consecutive low precipitation years,

emergent seedling density was extremely limited and therefore, the effectiveness of seed coating protection against herbicide could not be inferred. Further studies should be conducted to test efficacy of seed coatings in the field.

Introduction

The modern sagebrush steppe ecosystem covers only 56% of its historic range (Schroeder et al. 2004). One of the major threats to the ecosystem is the invasion of exotic annual grasses (D'Antonio and Vitousek 1992; Mack 1981). Cheatgrass (a.k.a. "downy brome"; *Bromus tectorum* L) and medusahead (*Taeniatherum caput-medusae* (L.) Nevski) are the most prevalent and problematic invasive grasses in the Intermountain West (Bradley et al. 2018; D'Antonio and Vitousek 1992; Davies et al. 2011; Mack 1981; Litt and Pearson 2013).

Exotic annual grasses are highly competitive and successfully outcompete native perennial grass seedlings in the sagebrush steppe. These invasive species alter fundamental nutrient cycling processes within the soil (Knapp 1996; D'Antonio and Vitousek 1992; Norton et al. 2004), decrease available habitat for wildlife as well as forage for wildlife and livestock, and decrease biodiversity (Bansal and Sheley 2016; Davies and Svejcar 2008; Davies 2011). Annual grasses alter the fire regime in the sagebrush steppe by creating a fuel structure that increases fire frequency (Davies and Nafus 2013*a*; Reed-Dustin et al. 2016). In low to mid elevation sagebrush habitats, historical fire return intervals were every 50-100 years or more (McIver and Brunson 2014; Whisenant 1990; Rau et al. 2011; Miller et al. 2011). With the invasion of annual grasses, fire return intervals are often <10 years (Whisenant 1990; Miller et al. 2011). Most native perennial species cannot tolerate these frequent fires which further promotes the persistence of invasive annual grasses. This transition to an invasive annual grass dominated landscape causes more fires resulting in a positive feedback loop that ensures the continued presence of these invasive species (Brooks et al. 2004; Larson et al. 2017).

Restoring native perennial grasses in exotic annual grass communities has proven difficult. Due to the highly competitive nature of exotic annual grasses (Stevens et al. 2014; Shinneman and Baker 2009; Monsen 1994), the removal of these species is necessary prior to planting native vegetation. Many management methods do not provide adequate control of these invasives, however, the use of herbicide has shown the most promise (Davidson and Smith 2007; Vollmer and Vollmer 2008). Pre-emergent herbicides selectively control annual and perennial grasses and some broadleaf weeds (Tu et al. 2001; Global Rangelands 2021). Imazapic, a pre-emergent, apoplastically-translocated herbicide has shown the most promise in exotic annual grass control and is the most widely used herbicide for annual grass control by land mangers (Vollmer and Vollmer 2008; Davidson and Smith 2007).

Pre-emergent herbicides are effective at controlling exotic annual grasses; however, they can have adverse effects on native perennial vegetation. These herbicides prevent the production of branched chain amino acids which is detrimental to native seedlings and when used at high enough rates, these herbicides can kill mature plants (Davies et al. 2014; Shinn and Thill 2004). Because of this, land managers must use a multiple-entry approach which requires them to wait at least a year or more to reseed native vegetation following annual grass control, in order to prevent nontarget plant injury due to the active herbicide in the soil (Davies et al. 2014). In that year, exotic annual grasses have a chance to reinvade, making the previous herbicide application less effective. (Davies et al. 2014). A solution to this problem is a single-entry approach in which herbicide is applied at the same time as native seeding, which reduces restoration costs compared to a traditional multiple-entry approach (Sheley 2007). However, the native seed will also suffer the effects of the herbicide unless it is protected in some type of seed coating. Seed coatings and other seed technologies offer the native seed protection from herbicide while still allowing the seed to germinate and emerge (Madsen et al. 2014; Holfus et al. 2021).

One commonly used and effective method of herbicide protection is the use of herbicide protection pellets or HPPs (Clenet et al. 2019; Madsen et al. 2014; Davies et al. 2017). These pellets contain desired seeds along with activated carbon, bentonite clay, compost, and worm castings. They can be directly broadcast seeded prior to spraying the area with pre-emergent herbicide, making it a truly single-entry approach. The activated carbon within these HPPs has very high adsorptive capacity and binds to the herbicide, making it locally inactive (Foo and Hameed 2010; Coffey and Warren 1969). Although effective, these HPPs require specialized equipment as well as a substantial amount of material to create, making them expensive to produce. Additionally, likely due to their compaction during production, they have been shown to decrease emergence and growth of some seeded native plants in the absence of herbicide (Clenet et al. 2019).

An alternative to HPPs is the coating of individual grass seeds. Unfortunately, in previous studies the use of activated carbon seed coatings has had low success (Madsen et al. 2014). Activated carbon, due to its light weight and consistency, does not easily adhere to the surface of seeds, therefore, getting enough activated carbon to coat each seed is difficult. Recently, the use of seeds with an herbicide protection coating containing more activated carbon than previous studies was shown to be effective in a greenhouse setting (Holfus et al. 2021). The objective of this study was to test the efficacy of this herbicide protection formula seed coating in a field setting and determine how much activated carbon is needed to provide herbicide protection to desired seeds. We hypothesized that 1) seed coating would not influence seedling emergence in the absence of herbicide, and 2) in the presence of herbicide, coated seed would have increased seedling emergence and seedling size compared to bare seed, but 3) efficacy of the seed coating would decrease as herbicide amount increased and coating material decreased.

Methods

Study Design

We used a randomized-block 4 x 3 factorial design with five blocks applied in each of two years. Treatments included seed coated with 4, 8, and 12 mg of activated carbon, or bare (non-coated) bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Love) seed. Plots (200 cm x 50 cm) were sprayed with a high herbicide application of imazapic (Plateau BASF Corporation; Research Triangle Park, NC) at 210 g ai·ha^{-1}, a low herbicide application rate at 105 g ai·ha,$^{-1}$ or not sprayed with herbicide (control). The amount of coating material per seed was estimated by weighing subsets of 25 seeds and averaging their weights. This weight was then compared to the average weight of uncoated or bare seed and then multiplied by 33% (the amount of activated carbon in the coating material). In November of each year, plots were broadcast seeded with 400 seeds and covered with a small amount (approximately 1 cm) of soil collected from the study site that had been sifted to remove any weed seed. Plots were then treated with the designated herbicide application in the fall of each year (November 9, 2019 and November 4, 2020).

Study Site and Materials

The study site was located at the Northern Great Basin Experimental Range, approximately 16 km southwest of Riley, Oregon. The site was a Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh) steppe community type with annual grass (cheatgrass) invasion. The site was at 1400 m elevation with 0-2% slope and a Gochea sandy loam soil (USDA NRCS 2021). It was located on a Sandy-loam 10-12 PZ Ecological Site (R023XY213OR).

Long term (1979-2021) mean annual precipitation is 294 mm; our site received 264 mm in the first year of this study (89% of the mean) and 166 mm in the second year of this study (56% of the mean; Great Basin Weather Applications 2021). During the seedling growing season (February-May) of 2020 and 2021 the site received 77%, and 58%, respectively, of the long-term mean seedling growing season precipitation (Great Basin Weather Applications 2021). Air temperature and precipitation were recorded at a US Climate Reference Network site approximately 3.5 km from the study site at the same elevation and similar topographic position

(Diamond et al. 2013). Soil moisture and temperature were recorded with a soil probe (Em50 Data Logger; Decagon Devices Inc.; Pullman, WA, USA with 5TM sensor; Campbell Scientific Corp.; Edmonton, AB, Canada) at hourly intervals and varying depths (5, 10, 15, 20, 25 cm below the surface).

Prior to seeding in November of 2019 and 2020, the 25 x 10 m rabbit-fenced enclosure was burned and rototilled in September to clear existing vegetation and seeds as well as provide a better seedbed for target seeds. Bluebunch wheatgrass seed was used in this study (Anatone; Granite Seed, Inc.; Lehi, UT), and is commonly used in regional restoration projects due to its availability and high prevalence in regional native plant communities in the sagebrush steppe (Madsen et al. 2014; Rodhouse et al. 2014; Clenet et al. 2019). Seed viability was determined using a germination test by placing 25 seeds in each of five petri dishes containing moistened blotter paper that were maintained for 19 days at 22ºC and a 12 h light/dark cycle. The resulting viability was 92% in year one and 89% in year two.

Seed Coating

The bluebunch wheatgrass seeds used in this study were first coated with powdered activated carbon (Darco GroSafe; Cabot, Billerica, MA) and a partially hydrolyzed polyvinyl alcohol binder (Selvol-205; Sekisui Specialty Chemicals, Dallas TX) using a 35.5-cm commercial rotary seed coater (SedPell RP14-DB; BraceWorks Automation and Electric; Lloydminster, SK, Canada) and standard coating protocols. This process allowed for a subsequent herbicide protection material layer to adhere to each seed. The seed was then coated with an herbicide protection formula consisting of: 45% bentonite clay, 33% activated carbon, 14% compost, and 6% worm castings by weight which was mixed with a 4% partially hydrolyzed polyvinyl alcohol binder (Selvol-205) using a newly developed coating method referred to as "Vortexing". Details of this coating method can be found in Holfus et al. (2021).

Seedling measurements

Seedling emergence and leaf area were measured in the first spring and mid-summer post-planting, respectively. Living and dead seedlings were counted separately; seedlings were considered living if they had any visible green tissue and were considered dead if they did not contain any visible green tissue. Seedling emergence was determined in the spring (April 2020 and 2021) by marking living emergent seedlings with colored tooth-picks and noting survival and death the following mid-summer at the second monitoring event in July 2020 and 2021. Seedling leaf area was also determined in the second monitoring event. For seedlings with only one tiller, the midpoint width and overall length of each leaf were measured and then multiplied together for each leaf to determine individual leaf area. All leaf areas calculated were then summed to determine overall leaf area for a seedling. For seedlings with more than one tiller, a single tiller was randomly selected for measurement. Overall leaf area for that tiller was measured as described above. The overall leaf area for the single tiller was then multiplied by the total number of tillers to estimate leaf area for the seedling (Boyd and Davies 2012).

Statistical Analysis (see "Discussion" for caveats)

All analyses were conducted in R software version 4.0.2 (R Core Team, 2020). An analysis of variance using a linear mixed effects model was used to compare the response of measured variables across treatments using the {nlme} R package (Pinheiro et al. 2021). Fixed effects were coating treatment, herbicide treatment, and their interactions, with blocking and its interactions as random effects. When significant treatment or interactive effects were found, means were separated using the {emmeans} package in R (Tukey Honest Significant Difference method) and reported with their associated standard errors. Differences were considered significant at $p \leq 0.05$.

Results

The first and second year of this study had below average precipitation and above average temperatures. The total precipitation during the seedling growing season (February, March, April, and May) in year one was 35%, 104%, 71%, and 88% of the long-term mean (Fig. 3.1). Year two of this study was 123%, 36%, 61%, and 26% of the long-term average. The temperature during the seedling growing season (February, March, April, and May) in year one was an average of 0.8 ± 0.67 °C, 0.6 ± 0.5 °C, 0.8 ± 0.8 °C, and 0.3 ± 0.9 °C above the long-term average (Fig. 3.2). The temperatures in year two of this study were an average of 0.4 ± 0.5 °C, 0.8 ± 0.4 °C, 1.1 ± 0.7 °C, and 0.9 ± 0.8 °C above the long-term average respectively (Great Basin Weather Applications 2021). Soil moisture was generally lower in year two of the study compared to year one (Fig. 3.3). In association with an abnormally low precipitation and higher than normal temperatures there was little to no emergence or growth of bluebunch seedlings (see Fig 3.4, 3.5, 3.6, and 3.7).

Total number of surviving seedlings per treatment is reported instead of means due to low seedling numbers. In the first year of the study, seedling emergence and survival under no herbicide application ranged from three seedlings emerging and three surviving for bare seed, four seedlings emerging and surviving for 4 mg coated seed, two seedlings emerging and surviving for 8 mg coated seed, and five seedlings emerging and four seedlings surviving for 12 mg coated seed (Fig 3.4). Under low herbicide application, two bare or non-coated seedlings emerged and survived, one 4 mg coated seedling emerged but did not survive, one 8 mg coated seedling emerged and did not survive, and 12 mg coated seed exhibited comparatively high emergence with six seedlings emerging in the spring, however none of these six seedlings survived. Under high herbicide application, only the 12 mg coated seed emerged in the spring with four seedlings however, all of these seedlings died. Surviving seedling leaf area averaged by treatment in the first year, was 1,378.5 ± 1,273.5 mm^2 for bare seed with no herbicide application treatment, 144.5 ± 57.5 mm^2 for bare seed under low herbicide application treatment, 2,335.5 ± 1,144.9 mm^2 for 4 mg coated seed under no herbicide application treatment, 29,073.5 ± 2,257.5 mm^2 for 8 mg coated seed under no herbicide application treatment, and 12,363.2 ± 6,207.4 mm^2 for 12 mg coated seed under no herbicide application treatment (Fig 3.5).

In the second year of the study, similar results were recorded. Under no herbicide application, one bare or non-coated seedling emerged but did not survive, five 4 mg coated seedlings emerged in the spring and three survived, one seedling emerged and survived in the 8 mg coated seed, and one 12 mg coated seedling emerged and survived (Fig 3.6). Under low herbicide application, only two seedlings in the 8 mg coated seed, and three seedlings in the 12 mg coated seed emerged, however the two 8 mg coated seedlings did not survive and the three 12 mg coated seedlings did not survive. Lastly, under high herbicide application conditions, one 8 mg coated seedling emerged and died, and three 12 mg coated seedlings emerged and died. Surviving seedling leaf area averaged by treatment in the second year of the study was 1,167 ± 634.5 mm for 4 mg coated seed under no herbicide application. Leaf area for seed coated with 8 mg of activated carbon was 975 mm under no herbicide application, and 12 mg coated seed under no herbicide application was 400 mm (Fig 3.7).

Discussion

With such low emergence, the data are mostly comprised of zeros and only a portion of the sample (i.e., emergent seedlings) survived. Having a high number of zeros in this dataset means that the data is highly skewed. Additionally, this is a very small dataset to work with since only about 0.08% of planted bluebunch wheatgrass seeds emerged. If inferences were to be made from this data, this would be misleading and the results may or may not be representative of a larger population (the degree to which they are representative would be unknown due to the high amount of unexplained variation associated with small sample sizes). The small sample size in this study decreases the statistical power of any analysis and could result in a type II error in which a finding of no significance or a failure to reject the null hypothesis is concluded when the alternate hypothesis is actually correct. The small sample size increases the likelihood of making this mistake therefore decreasing the power of the study. Small sample sizes also result in an increased amount of unexplained variation associated with a statistical model. If there is a high amount of unexplained variation, there is likely a covariate not being accounted for in the model resulting in decreased model fit and therefore unreliable results. For these reasons, planned statistical analyses (see methods) cannot be run with confidence and statistical results such as p-values or associated F-statistics cannot be provided.

Our hypotheses were not supported due to the lack of emergence and survival of bluebunch wheatgrass seedlings. However, the success rate of seeding native bunchgrasses in the sagebrush steppe is often very low (James et al. 2012). Year one and year two of this study had lower than average precipitation combined with higher than average temperatures (see results; Fig 3.1 and 3.2). In a year with favorable conditions the rate of emergence in semi-arid climates, like that of the sagebrush steppe, is extremely low (James et al. 2010). A study conducted in the sagebrush steppe found that 90% of germinated native grass seeds died prior to emergence and indicated that the time period between a germinated seed and an emergent seedling is the largest bottleneck limiting native plant establishment (James et al. 2011). The abiotic conditions during this critical growth period are crucial for seedling emergence, survival, and eventual establishment. Seedling emergence at our study location typically occurs from late February through May (Boyd and James 2013). During this critical growing period, the study site experienced below average precipitation and above average temperatures (see results), likely resulting in drier insufficient soil water content during key growing periods (Fig 3.3). These conditions could prevent seeds from imbibing with moisture and beginning the germination process or, alternatively, there was insufficient water content to sustain seedlings through the emergence process. Not only did the vast majority of seeds in this study not emerge but of the ones that emerged, most of them died. In the first year, 48% of the seedlings that emerged in the spring did not survive and 70% died in the second year.

The vortex coating method did provide protection in a controlled greenhouse setting (Holfus et al. 2021), meaning with more favorable abiotic conditions in the field, this method may provide protection against herbicide. Additionally, coated seed exhibited comparatively high emergence and survival compared to bare seed without the presence of herbicide. This indicates that seed coating alone did not negatively affect seedling emergence or survival and could even improve seedling establishment. Previous studies indicate that seed coatings can negatively affect seedling emergence (Ullah et al. 2019; Clenet et al. 2019). Our method of coating does not decrease emergence or survival without the presence of herbicide, which differs from previous studies.

Further field studies should be conducted to test the efficacy of herbicide protection coating on individual native grass seeds in the presence of imazapic herbicide. To test the efficacy of seed coatings in a field setting with potentially limiting abiotic conditions, manual irrigation may be added which would allow coated seeds to overcome limiting field conditions. Another alternative would be to use multiple locations to increase sample size and decrease unexplained variation. By adding more locations with varying abiotic conditions, it is possible to better test the efficacy of seed coatings by evaluating their efficacy in different conditions and across sites that have the potential to exhibit more favorable abiotic conditions. Conducting studies over a long period of time is not always feasible. When more locations are used, space is being substituted for time.

Rangeland seeding restoration efforts are often unsuccessful (Madsen et al. 2016; James et al. 2012). Our study had low emergence and survival which we believe was associated with lower than average precipitation and higher than average temperatures (see results) however, with climate change, restoration challenges associated with abiotic conditions may intensify (Huber and Gulledge 2011), indicating the success of rangeland seeding efforts is likely to continue to decrease. If restoration managers could seed only in years with more favorable abiotic conditions, the success rate of seeding would likely increase. Further research is needed in accurately predicting more favorable seeding years. Additionally, the use of non-native seed (such as Crested Wheatgrass; *Agropyron cristatum* (L.) Gaertn.) has much higher success than the use of native grass seed (Davies et al. 2013*b*; Hull 1974; Richards et al. 1998). If the goal of land managers is to restore ecosystem function, the use of non-natives may be preferential. With increasing climate change and decreasing native seeding success, methods that improve the success of seeding or new alternatives to seeding need further refinement.

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Figures

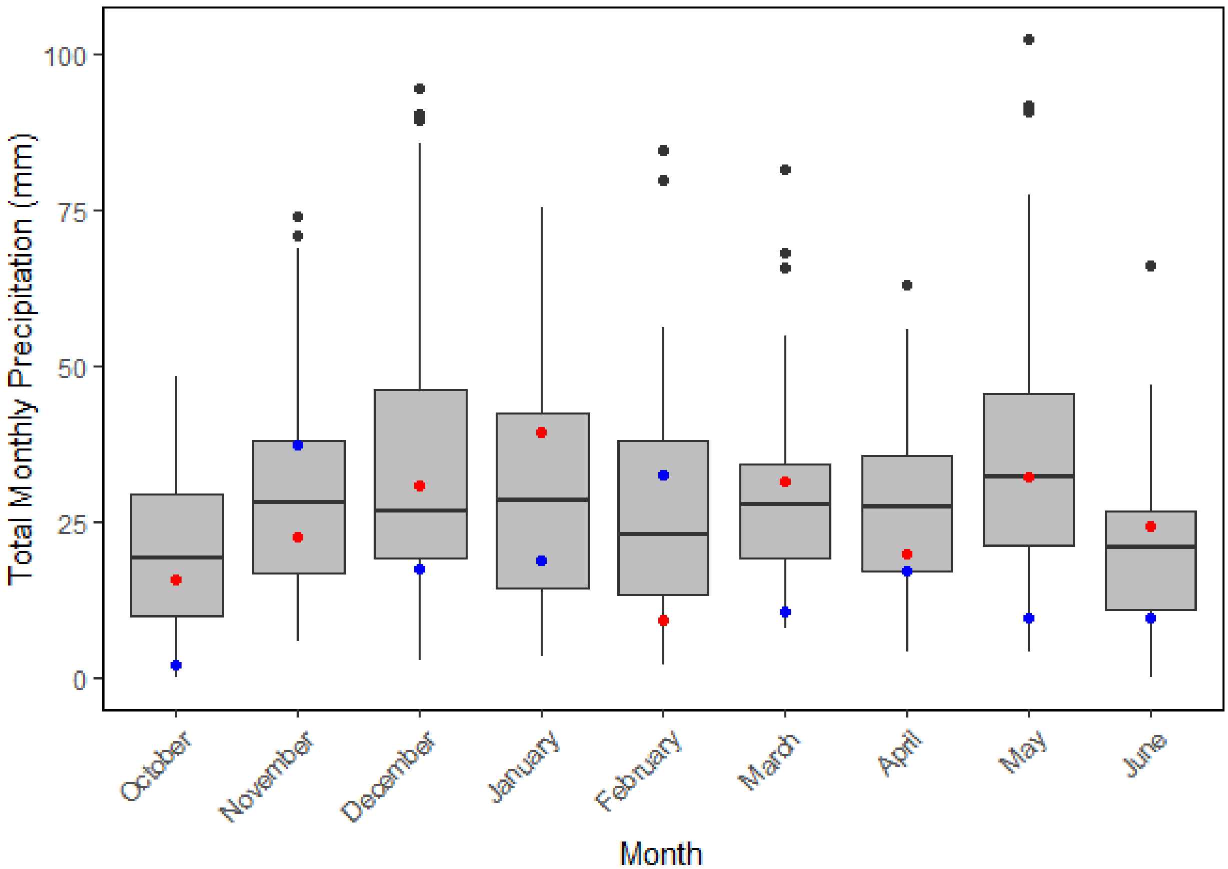

Figure 3.1. Forty-year average (1979-2020) precipitation (mm) per month in a hydrologic year (October-June) for the study site. The red dots signify the first year's total precipitation per month, the blue dots signify total precipitation per month in the second year of the study, and the black dots signify outliers of the long-term average (Great Basin Weather Applications 2021).

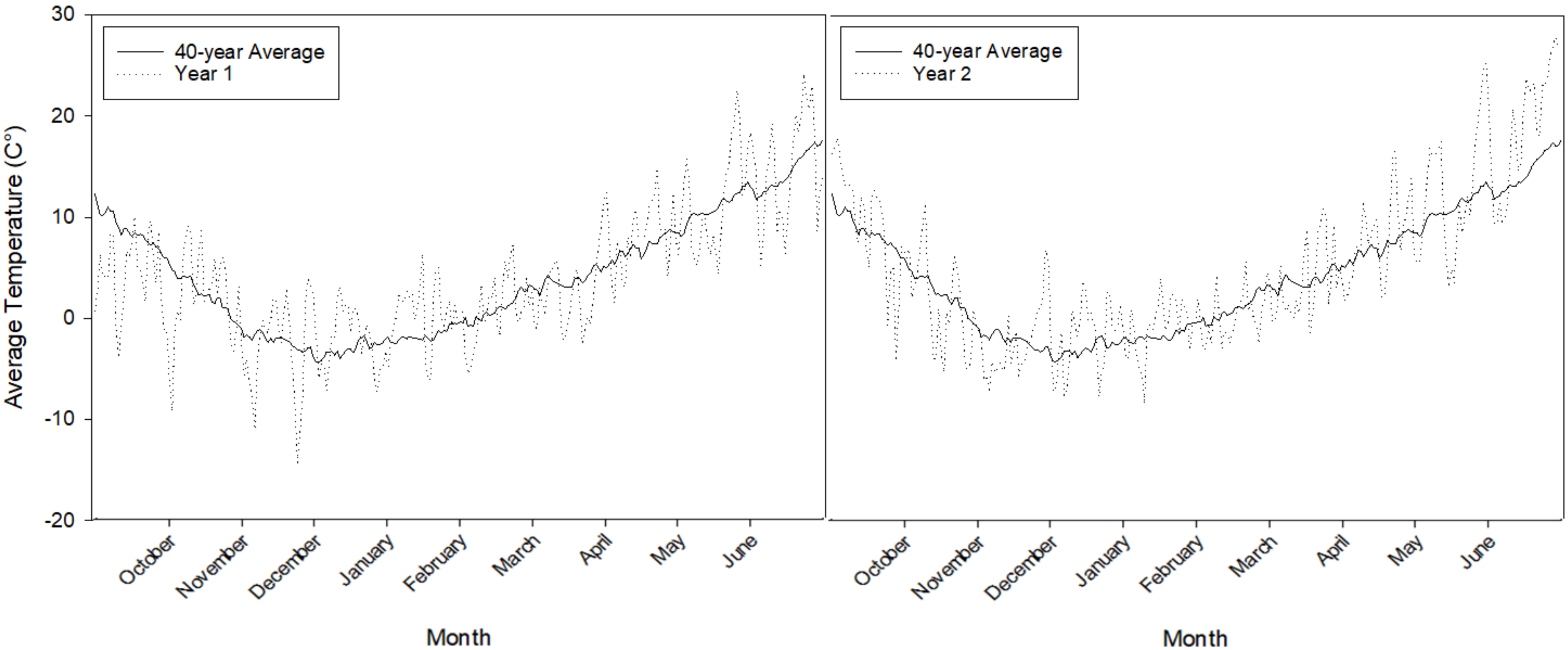

Figure 3.2. Forty-year average (1979-2020) air temperature (C°) in a hydrologic year (October-June) for the study site. The graph on the left signifies the first year's average temperature per month compared to the long-term average and the graph on the right signifies the average temperature per month in the second year of the study compared to the long-term average (Great Basin Weather Applications 2021).

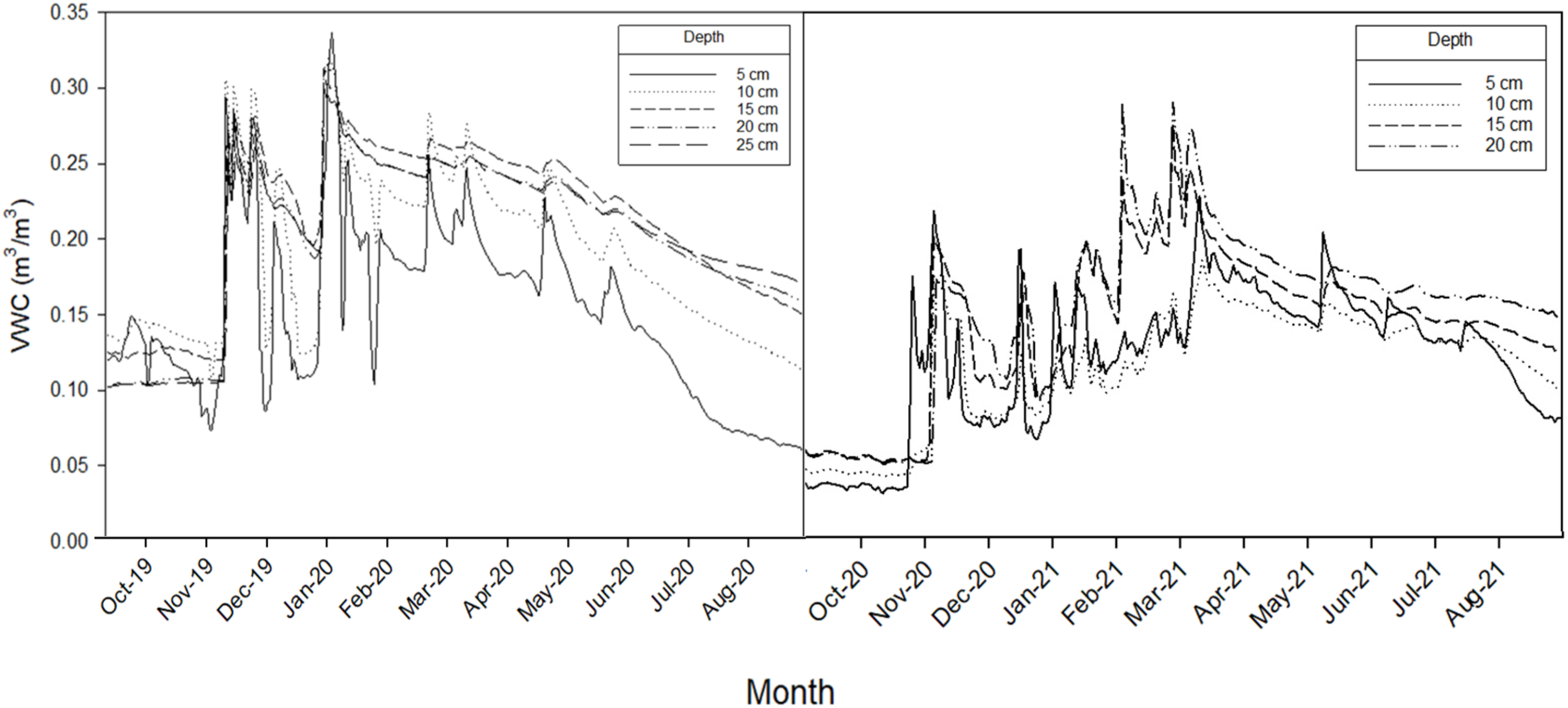

Figure 3.3. Soil moisture measurements (Volumetric Water Content m^3/m^3) for the study site by month at five different depths in the first (left) and second year of the study (right).

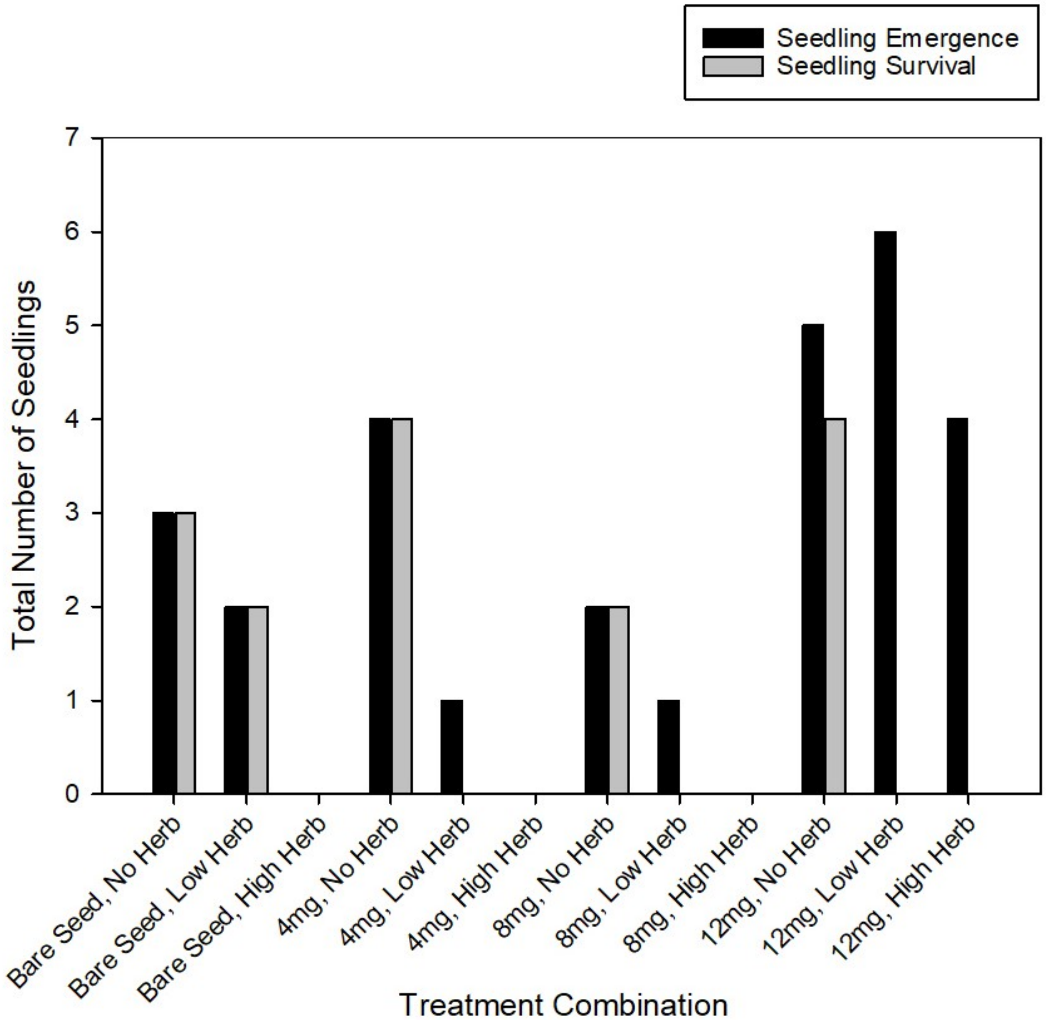

Figure 3.4. Graph of total (across blocks) number of seedlings represented by seedling emergence and survival by treatment in year one of the study. Bare = uncoated bare seed, 4mg = 4mg of activated carbon herbicide protection coated seed, 8mg = 8mg of activated carbon herbicide protection coated seed, 12mg = 12mg of activated carbon herbicide protection coated seed; HighHerb = high herbicide treatment (210 g ai·ha^{-1}), LowHerb = low herbicide treatment (105 g ai·ha^{-1}), NoHerb = no herbicide application treatment.

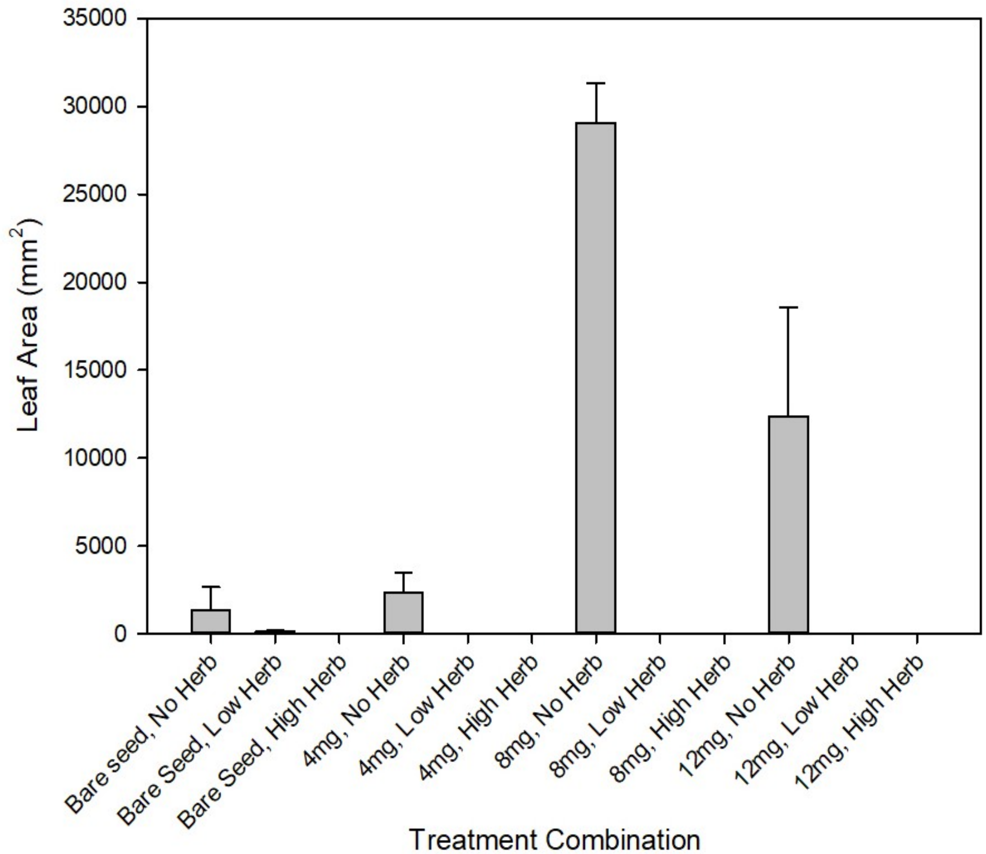

Figure 3.5. Mean surviving seedling area in year one of the study by treatment. Bare = uncoated bare seed, 4mg = 4mg of activated carbon herbicide protection coated seed, 8mg = 8mg of activated carbon herbicide protection coated seed, 12mg = 12mg of activated carbon herbicide protection coated seed; HighHerb = high herbicide treatment (210 g ai·ha^{-1}), LowHerb = low herbicide treatment (105 g ai·ha^{-1}), NoHerb = no herbicide application treatment. Error bars represent standard errors.

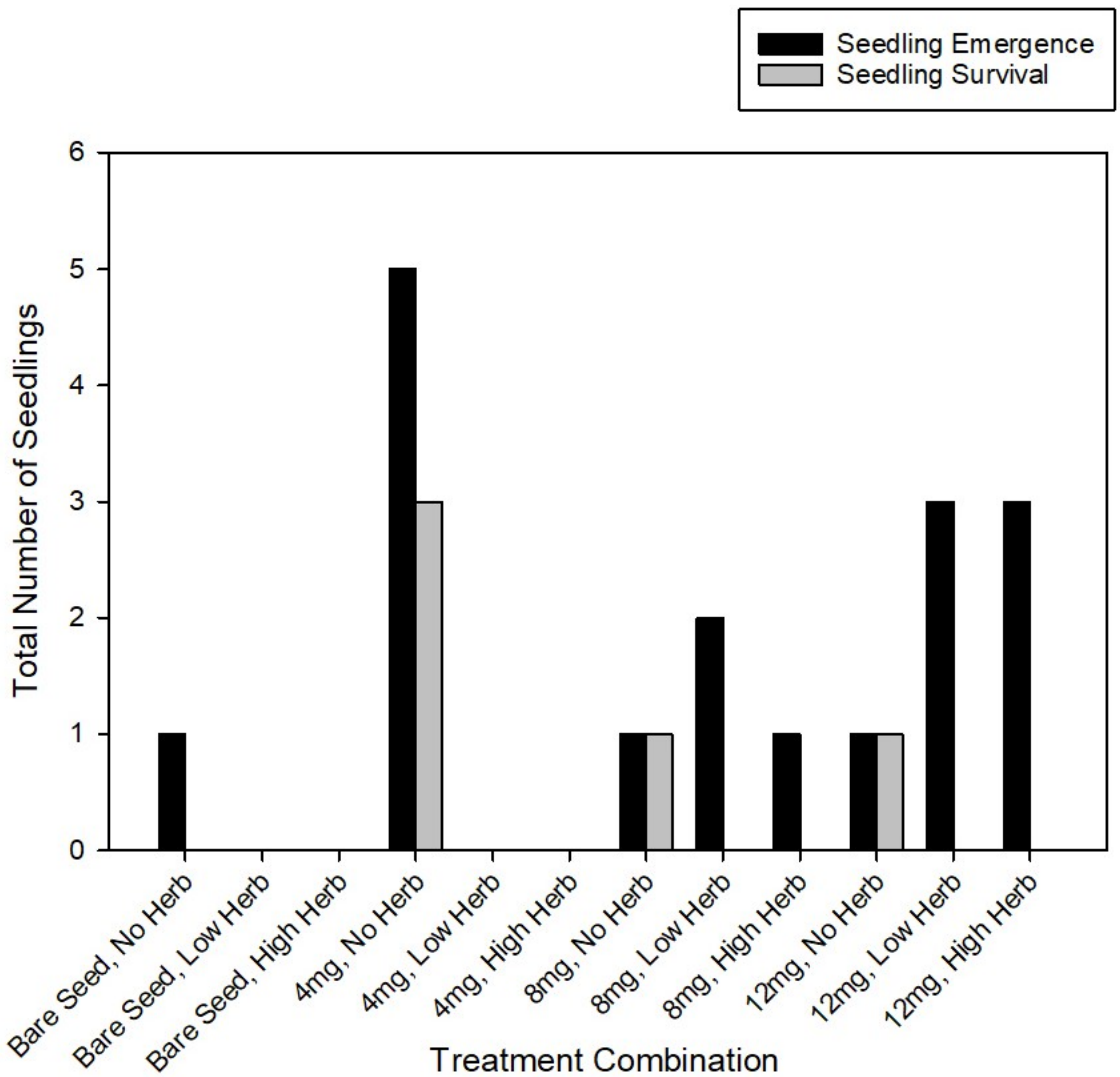

Figure 3.6. Graph of total (across blocks) number of seedlings represented by seedling emergence and survival by treatment in year two of the study. Bare = uncoated bare seed, 4mg = 4mg of activated carbon herbicide protection coated seed, 8mg = 8mg of activated carbon herbicide protection coated seed, 12mg = 12mg of activated carbon herbicide protection coated seed; HighHerb = high herbicide treatment (210 g ai·ha^{-1}), LowHerb = low herbicide treatment (105 g ai·ha^{-1}), NoHerb = no herbicide application treatment.

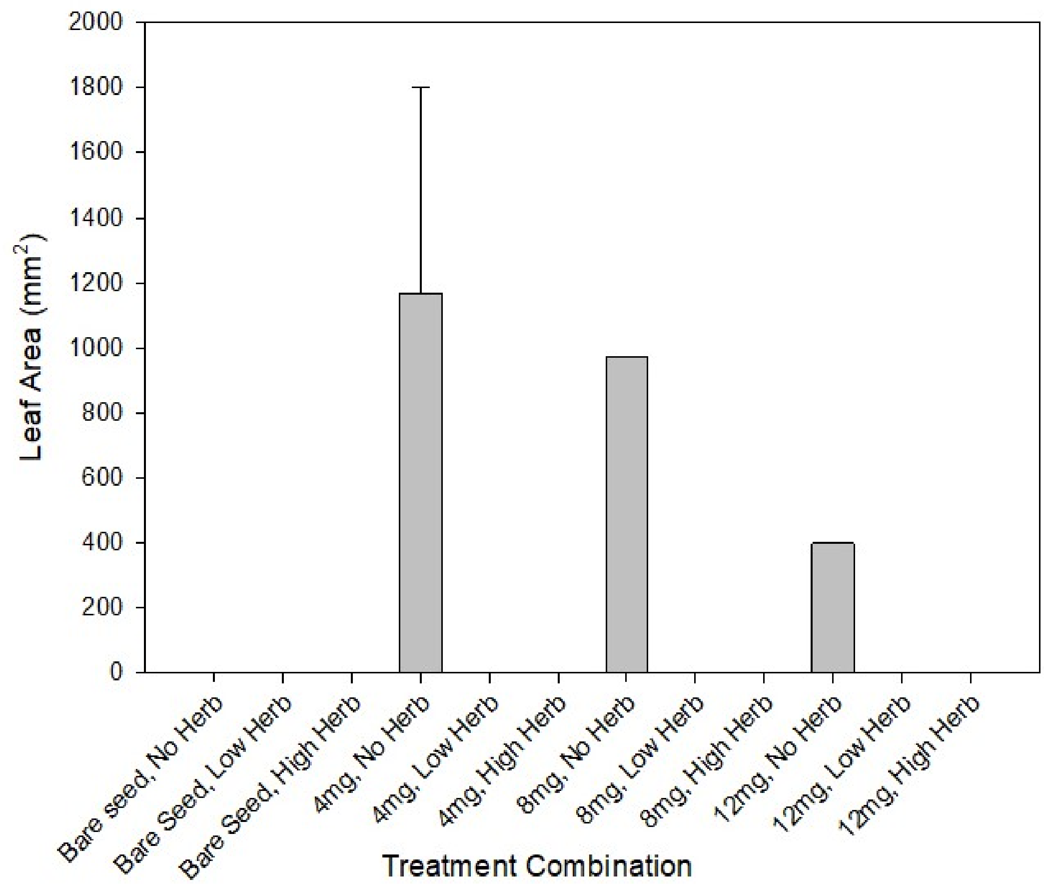

Figure 3.7. Mean surviving seedling area in year two of the study by treatment. Bare = uncoated bare seed, 4mg = 4mg of activated carbon herbicide protection coated seed, 8mg = 8mg of activated carbon herbicide protection coated seed, 12mg = 12mg of activated carbon herbicide protection coated seed; HighHerb = high herbicide treatment (210 g ai·ha^{-1}), LowHerb = low herbicide treatment (105 g ai·ha^{-1}), NoHerb = no herbicide application treatment. Error bars represent standard errors.

CHAPTER 4:
WYOMING BIG SAGEBRUSH TRANSPLANT SURVIVAL AND GROWTH AS AFFECTED BY AGE, SEASON OF PLANTING, AND COMPETITION WITH INVASIVE ANNUAL GRASSES

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Abstract

Invasive annual grasses have created a large array of management problems in the Intermountain West and not only outcompete native bunchgrasses, change the fundamental nutrient cycling process, and decrease biodiversity, but also increased frequency of wildfires. These wildfires remove native sagebrush (*Artemisia* sp.) as well as their seedbank in low to mid-elevation sagebrush habitats, making natural recovery of this species nearly impossible. Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* (Beetle & A. Young) S. L. Welsh) restoration efforts through broadcast or drill seeding have very low success rates however, transplanting sagebrush has shown higher success. This study determined how 1) different ages of transplant at time of planting, 2) different seasons of planting (fall and spring planting), and 3) competition with invasive annual grasses, affect the survival and vigor (measured by transplant volume) of sagebrush transplants. This completely randomized factorial design, repeated over two years, used ten age classes of transplants at time of planting (6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 weeks of age). Age classes 10 weeks and older in the first planting year and 12 weeks and older in the second planting year had the highest survival and spring-planted transplants had higher survival than the fall-planted transplants in both years; however the fall-planted transplants had increased volume in both years. Competition did not affect survival of transplants after the first growing season in either year but volume of transplants was 54-fold greater when not competing with annual grasses compared to transplants competing with annual grasses in the first year, and nine-fold greater in the second year. Two growing seasons post-planting, transplants competing with invasive annual grasses had decreased survival compared to transplants not competing with invasive annual grasses. This study suggests that land managers should consider reducing greenhouse growing time, and remove invasive annual grasses prior to planting sagebrush transplants to increase long-term transplant survival and vigor. Further studies should follow to determine effects of season of planting, age of planting, and transplants interaction with annual grasses.

Keywords: Sagebrush transplants; restoration; invasive annual grasses; Wyoming big sagebrush; competition

Introduction

Sagebrush (*Artemisia* sp.) provides resources and habitat for many species, including the greater sage-grouse, which rely heavily on sagebrush for their habitat needs (Pyke et al. 2015; Connelly et al. 2004; Rowland et al. 2006). Sagebrush also provides habitat for other wildlife species, and serves as a soil stabilizer in the sagebrush steppe, which helps to promote resilience to disturbances (McArthur 1992). The restoration of Wyoming big sagebrush is necessary to maintain ecosystem resilience and provide habitat for a large number of species; however, traditional seeding methods (such as broadcast and drill seeding) have very low success rates (Lysne and Pellant 2004; Knutson et al. 2014; Davies et al. 2018). Sagebrush seed production is highly variable from year to year (Nelson et al. 2013; Young et al. 1989; Young et al. 1991), sagebrush seed has short-term viability (1-2 years), very specific requirements for germination (Wijayratne and Pyke 2012; Brabec et al. 2015), and does not compete well with invasive annual grasses, making natural recovery and restoration challenging. The use of sagebrush transplants or outplantings is often the most successful method for restoration (McAdoo et al. 2013; Pyke et al. 2020). For example, Wyoming big sagebrush transplants were successful while seeding was not in highly competitive, introduced grasslands (Davies et al. 2013).

Restoration of sagebrush is made more difficult by the invasion of annual grasses across much of the sagebrush steppe. Annual grasses such as cheatgrass (*Bromus tectorum* L.), ventenata (*Ventenata dubia* (Leers) Coss.), and medusahead (*Taeniatherum caput-medusae* (L.) Nevski) not only outcompete native bunchgrass seedlings, change nutrient cycling processes, and consequently decrease biodiversity (Knapp 1996; Norton et al. 2004; Bansal and Sheley 2016), but they also have led to an increase in wildfires (D’Antonio and Vitousek 1992; Whisenant

1990; Meyer et al. 2008). Historically, lower elevation Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* [Beetle & A. Young] S. L. Welsh) plant communities had a fire return interval of approximately 50-100 years (McIver and Brunson 2014; Whisenant 1990; Rau et al. 2011; Miller et al. 2011); with the invasion of annual grasses, the fire return interval for some plant communities dominated by annual grasses has decreased to <10 years (Whisenant 1990; Miller et al. 2011).

Exotic annual grasses invade rapidly, persist for long periods of time, and alter ecosystem dynamics such as natural fire regime (Davies and Nafus 2013; Brooks et al. 2004; Bradley et al. 2018). Prior to their invasion, the sagebrush steppe had high relative amounts of bare ground or biological crusts (Miller et al. 2011; Pyke et al. 2014). Once these grasses invade, they create a more continuous fuel bed in shrub interspaces that were previously bare ground or occupied by less continuous native perennial bunchgrasses (Brooks et al. 2004; Miller et al. 2011). This change in ground cover can result in rapidly spreading wildfires following fire ignition (Link et al. 2006; Miller et al. 2011; Mensing et al. 2006). Additionally, the invasion of annual grasses underneath shrubs often results in hotter and more intense wood fuel fires which are not only detrimental to shrubs such as sagebrush but also to mature perennial bunchgrasses on the landscape (Brooks et al. 2004; Porensky and Blumenthal 2016). Wyoming big sagebrush is not adapted to frequent fire and cannot withstand above-ground combustion; which leads to sagebrush mortality (Brabec et al. 2015). Natural recovery of big sagebrush following wildfire is difficult due to its inability to resprout, its short-lived seedbank, and its inability to compete with invasive annual grasses (Dettweiler-Robinson et al. 2013; Mata-González et al. 2018).

Although restoration using Wyoming big sagebrush transplants generally has a higher success rate than seeding, survival of transplants is highly variable (Dettweiler-Robinson et al. 2013; Pyke et al. 2020). The seed source, site ecological properties, and method of planting are just a few factors that contribute to the wide range of transplant success. Furthermore, sagebrush transplants success likely varies by season of planting, but disagreement exists over whether spring or fall plantings are more successful (Monsen et al. 2004; Clements and Harmon 2019). According to some studies, fall plantings can be successful in more mild climates that allow for root development over the winter months (Wirth and Pyke 2011; Shaw et al. 2015). Under more extreme environments with harsher winter conditions, a spring planting could have more favorable results (Shaw et al. 2015). Our study site is on the wetter and colder side of the Great Basin Desert, suggesting a spring planting could have higher survival than a fall planting. Additionally, transplants are often grown out in a greenhouse or nursery for 6 months to a year before being planted in the field (Fleege 2010; Moore 2015; Shaw et al. 2015; Clements and Harmon 2019). If growing the transplants for a shorter period of time in the greenhouse resulted in similar success rates, this would save time and money by limiting greenhouse growing costs. However, there is a lack of research on how decreasing greenhouse growing time will affect sagebrush transplant survival and vigor.

This study examines the effects of age of transplant when planted, season of planting, and competition from invasive annual grasses on Wyoming big sagebrush transplant survival and vigor (as measured by shrub volume). We hypothesized that 1) survival of sagebrush transplants would increase with transplant age at time of planting, and would be highest in the absence of competition from invasive annual grasses, 2) sagebrush transplants planted in the spring would have higher survival than transplants planted in the fall, and 3) sagebrush transplant vigor (measured as transplant volume), would increase with transplant age at time of planting, and would be highest in the absence of competition from invasive annual grasses.

Methods

Site Description

The study site was located at the Northern Great Basin Experimental Range (NGBER; 119˚71'W, 43˚49'N) at approximately 1400 m elevation in a formerly Wyoming big sagebrush steppe community type with annual grass invasion. The site had 0-2% slope and a Gochea sandy loam soil (USDA NRCS 2021). It was located on a Sandy-loam 10-12 PZ Ecological Site (R023XY213OR).

Climate data was obtained from a US Climate Reference Network site, approximately 3.5 km from the study site at the same elevation and similar flat topographic position (Diamond et al. 2013). Soil probes (Em50 Data Logger; Decagon Devices Inc.; Pullman, WA, USA with 5TM sensor; Campbell Scientific Corp.; Edmonton, AB, Canada) were used at the site to record hourly soil moisture and temperature at varying depths (5, 10, 15, 20, 25 cm below the surface) and in two locations.

Study Design

This study was a completely randomized 10 x 2 x 2 factorial design repeated in two planting years. We used Wyoming big sagebrush seed, of the Columbia [River] Basin biotype, from BFI Native Seeds (Moses Lake, WA, USA). Ten age classes of transplants were used (6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 weeks of age). Seeds were sown every 14 days starting on May 1, 2019 for the first-year fall cohort, October 2, 2019 for the first-year spring cohort, May 18, 2020 for the second-year fall cohort, and October 12, 2020 for the second-year spring cohort. Transplants were grown in an indoor climate-controlled grow room (16-22 °C) under high-intensity lights with a 12 h light/dark cycle. Transplants were grown in cone-tainers (Ray Leach 'Super Cell Classic' cone-tainers, Stuewe & Sons, Inc., Tangent, OR) with a 3.8 cm diameter at the top and 21 cm depth, filled with a 50/50 mixture of soil collected from the study site that had been sifted to remove weed seed, and a potting soil mix (MiracleGro Seed Starting Potting Mix 0.03(N) - 0.03(P) - 0.03(K); Scotts Company LLC; Marysville, OH, USA) and watered as needed.

We burned 40 m x 25 m rabbit-fenced enclosures (September 26, 2019 and September 15, 2020) and remaining vegetation was hand cleared. Each enclosure was split into two planting locations: one location had competing vegetation pre-emptively removed through the use of a pre-emergent herbicide (here and forward referred to as "zero-competition" transplants or location; Oct. 15, 2019 and September 16, 2020; imazapic, 105 g active ingredient ha^{-1}, Plateau BASF Corp., Research Triangle, NC, USA), the other planting location did not receive a pre-emergent herbicide treatment, allowing for emergence of invasive annual grasses the following growing season (here and forward referred to as "competition" transplants or location).

Transplants were planted in one of two seasons: fall (October 18, 2019 and November 2, 2020) and spring (March 20, 2020 and March 29, 2021).

At the time of planting, transplants were randomly assigned to $1m^2$ plots within each planting location. Each transplant was planted using a planting bar that created an approximately 21-cm deep hole. The hole was deep enough to prevent “J-rooting” of the planted transplant. The transplant was placed in the hole and, using a garden trowel, soil was pressed firmly against the roots of the transplant to prevent air pockets. Each transplant was marked with a numbered metal tag.

Vegetative Measurements

Herbaceous vegetation cover was measured at each location in each planting year (June 2020 and June 2021) using four evenly spaced 25 m transects and a line-point-intercept method (Herrick et al. 2009). Vegetative cover, litter and bare ground were estimated. Soil compaction was also measured at both planting locations each year using a penetrometer (Geotester pocket dial penetrometer; Geotest Instrument Corp.; Burr Ridge, IL, USA) by taking compaction measurements at 50 randomly located points throughout each planting location. Prior to planting, transplant root and shoot height were recorded to the nearest mm for a randomly selected sub-set of 25 plants in each age class; these plants were discarded after measurement. Transplant survival and surviving shrub volume were measured in the summer (August 2020 and 2021) following planting. Shrubs were considered alive if any green tissue remained and if, when bent, the transplant did not break. Volume was used as an index of shrub vigor and is commonly used in other studies (McAdoo et al. 2013; Miglia et al. 2007). Volume was measured by measuring the maximum height (ground surface to tallest point excluding reproductive stems), the major diameter (maximum diameter of shrub), and minor diameter (diameter of shrub perpendicular to major diameter). Volume was calculated using the formula, $V = 2/3\ \pi\ H\ [(\text{Major diameter}/2) \times (\text{Minor diameter}/2)]$, where H is the plant height (Thorne et al. 2002).

Statistical Analysis

All analyses were conducted in R software version 4.0.2 (R Core Team, 2020). Data were analyzed within each planting year for treatment effects on transplant survival using a binomial generalized linear model in the {nlme} R package to test the effects of age, season of planting, competition, and their interactions (Pinheiro et al. 2021). Fixed effects were age, season of planting, competition, and their interactions, and there were no random effects. When quasi- or complete separation was suspected, the {brglm} R package Firth bias correction function (a penalized likelihood method) was used to reduce bias (Heinze and Schemper 2002). Analysis of variance was used to model treatment effects on transplant volume with the fixed effects of age, season of planting, competition, and their interaction and no random effects. A repeated measures binomial generalized linear model was used to model the change in transplant survival over time for the first-year transplants after returning to the site for two consecutive years, and a repeated measures analysis of variance was used to model the change in transplant volume over time for the first-year transplants after returning to the site for two consecutive years. These analyses were done using the {nlme} R package and tested the fixed effects of age, season of planting, competition, year, and their interactions, with year as the repeated measure, and no random effects. A two-sided t-test was used to determine differences between transplant root length and shoot height differences between planting year one and planting year two. Natural log transformations were used for volume data to meet assumptions of statistical models and to increase model fit. When significant treatment or interactive effects were found, means were separated using the {emmeans} R package (Tukey Honest Significant Difference method; (Lenth 2020)). All treatment means are reported with their associated standard errors. Differences were considered significant at $p \leq 0.05$.

Results

The first and second planting year had below average precipitation and above average temperatures with year two having drier and hotter conditions than planting year one. The total precipitation in the fall months before and after the fall plantings (September, October, November, and December) in planting year one was 225%, 78%, 73%, and 87% of the long-term mean (Fig 4.1). Planting year two was 5%, 11%, 121%, and 49% of the long-term mean. The total precipitation during the critical spring growing season for transplants (March, April, May, and June) in planting year one was 104%, 71%, 88%, and 116% of the long-term mean. Planting year two spring growing season was 36%, 61%, 26%, and 49% of the long-term average. The temperature during the fall months before and after the fall plantings (September, October, November, and December) in planting year one was an average of 1.8 ± 1.1°C below, 5.1 ± 0.8°C below, 0.2 ± 1°C above, and 0.06 ± 0.8°C above the long-term average (Fig 4.2). The temperatures in planting year two were an average of 1.1 ± 0.8°C above, 0.6 ± 1°C above, 1 ± 0.9°C below, and 0.4 ± 0.7°C below the long-term average. The temperature during the critical spring growing season (March, April, May, and June) in planting year one was an average of 0.6 ± 0.5 °C, 0.8 ± 0.8 °C, 0.3 ± 0.9 °C, and 0.5 ± 0.9°C above the long-term average. The temperatures in planting year two were an average of 0.8 ± 0.4 °C, 1.1 ± 0.7 °C, 0.9 ± 0.8 °C, and 5 ± 1.1°C above the long-term average (Great Basin Weather Applications 2021). Soil moisture was generally higher in the zero-competition location for a longer duration into the summer months compared to the competition location (Fig. 4.3).

In planting year one, percentage herbaceous cover of the zero-competition planting location was 90 ± 2.6% bare ground, 6 ± 2.6% cheatgrass, and 4 ±1.3% annual forbs. The competition planting location had 15 ± 2.3% bare ground, 78 ± 5.6% cheatgrass, and 7 ± 3.5% perennial grass cover. In planting year two, percentage herbaceous cover of the zero-competition planting location was 92 ± 2.3% bare ground, 2.6 ± 0.5% native perennial forbs, and 5.3 ± 4% perennial grass cover. The competition planting location had 28.8 ± 2.3% bare ground, 53.3 ± 7.5% cheatgrass, 4.4 ± 0.5% perennial grass cover, 4.4 ± 0.5% native perennial forbs, and 8.8 ± 2.3% annual forbs. Soil compaction was 2.61 ± 0.22 kg/cm^2 in planting year one competition planting location, and 4.69 ± 0.42 kg/cm^2 in the zero-competition planting location. Soil compaction in planting year two competition location was 8.46 ± 0.75 kg/cm^2 and 4.88 ± 0.42 kg/cm^2 in the no competition planting location.

Root length and shoot height of the transplants did not present any obvious pattern of differences between age classes or season of planting in the first or second planting year. Mean root length did not differ between planting year one and planting year two ($p = 0.25$) and was 180 ± 1.8 mm across all age classes and planting seasons, in both planting years. Shoot height however, did differ between years, with mean height of 105 ± 3.7 mm in planting year one, and 55 ± 5 mm in planting year two ($p < 0.001$).

Planting Year One

In planting year one, survival of transplants was affected by season of planting, age class and their interaction ($p = 0.004$; Fig 4.4). Generally, age classes 6 weeks and 8 weeks had the lowest survival compared to the other eight age classes. Age class 6 had 7 ± 5 % survival in the fall and 42 ± 10 % in the spring and age class 8 had 58 ± 10 % survival in the fall and 46 ± 10 % in the spring planting. The other eight age classes ranged from 62% to 78% survival averaged across both the fall and spring, with the exception of age classes 16 and 18 in the fall planting with 40% and 33% survival respectively. In general, the spring planted transplants had 1.46-fold higher survival than the fall planted transplants (Fig. 4.4). Competition transplants in planting year one averaged 60 ± 0.04 % survival and zero-competition transplants averaged 67 ± 0.03 % survival (data not shown).

Volume of surviving transplants in planting year one was highly variable in accordance with treatment. Transplant volume was affected by season of planting, competition, as well as a weak interactive effect between age class and season of planting ($p = 0.08$; Fig 4.5A-C and 4.6).

Competition generally affected transplant volume in both planting seasons in planting year one with 54-fold higher volume in zero-competition transplants compared to competition transplants (Fig 4.5B and 4.6). Season of planting affected transplant volume in planting year one with transplants planted in the fall having 3.6 times greater volume than transplants planted in the spring (Fig 4.5A).

After returning to transplants from planting year one in the second growing season following planting, survival was affected by the interaction of age at planting and season of planting ($p < 0.001$; Fig 4.7A), the interaction of age at planting and competition with invasive annual grasses ($p = 0.02$; Fig. 4.7B), and the interaction between season of planting and competition ($p < 0.001$; Fig 4.7C). There was no interaction between any variable and time. Two years following planting, mortality for transplants from planting year one was low with only 5% of all transplants dying, 80% of which were competition transplants (data not shown). Shrub volume was affected by the interaction between time and season of planting ($p < 0.001$; Fig. 4.8A), time and competition ($p < 0.001$; Fig. 4.8B), season of planting and age at planting ($p = 0.002$; data not shown), and a three-way interaction between age, season, and competition ($p = 0.02$; Fig. 4.8C). The volume of shrubs increased 11-fold from the first to second year following planting (Fig. 4.8A-B). Zero-competition transplants were at least 11-fold larger than competition transplants after two years (Fig. 4.8B). Transplants planted in the fall had 1.6 times greater volume than transplants planted in the spring (Fig 4.8 A and C).

Planting Year Two

In planting year two, survival of transplants was low with only 12.67 ± 0.01 % of all transplants surviving. Transplant survival was affected by age class ($p < 0.001$; Fig 4.9A), season of planting ($p < 0.001$), and an interaction between season of planting and competition ($p = 0.003$; Fig 4.9B). Similar to planting year one, age classes 6 and 8 weeks had the lowest survival with age class 6 having 0 % survival in both planting seasons, and age class 8 having 1.7 % survival however, in planting year two, age class 10 also had comparatively low survival with only 5 ± 2.8 % survival. The other seven age classes ranged from 10% to 28% survival across planting seasons. Zero-competition transplants exhibited higher survival for the spring planting however, competition transplants exhibited no difference in survival between planting seasons (Fig 4.9B).

Volume of surviving transplants in planting year two was affected by age at time of planting, season of planting, and their interaction ($p = 0.01$; Fig 4.10A). Surviving shrub volume was also affected by competition with invasive annual grasses ($p = 0.004$; Fig 4.10B). Generally, volume was greater in the fall-planted transplants than the spring-planted transplants (Fig 4.10A). Additionally, competition greatly affected shrub volume with zero-competition transplants being nine-fold larger than competition transplants (Fig 4.10B).

Discussion

This manuscript serves as the first indication that sagebrush transplants may not need to be grown out in the greenhouse for an extended period of time (6 months to a year) before being transplanted in the field. Previous research indicated that the use of sagebrush transplants has highly variable success, with the average survival rate ranging from 15-80% (Dettweiler-Robertson et al. 2013). Planting year two of this study falls below this range (12.67% survival) in association with abiotic conditions (see discussion below) however, survival rates in planting year one are comparable with the literature and even exceed previously reported survival rates in some age classes and planting seasons (Fig.4.4). Age classes ranging from 12 weeks of age to 24 weeks of age had comparable within-planting-year survival and transplant vigor (volume) in both years of the study, suggesting that restoration practitioners could reduce growing time in a nursery or greenhouse before planting, relative to the traditional grow-out time of six months (Fleege 2010; Moore 2015; Shaw et al. 2015).

Reduced grow-out time could save land managers a substantial amount of time and money on sagebrush restoration projects, however quantifying the exact amount of money saved can be difficult due to the many differences in growing procedures, availability and cost of seed, and more. In both years of the present study, age class 12 weeks had comparable survival and volume to 24 weeks (6 months). Dettweiler-robinson et al. (2013) found that on average, the cost to successfully plant a Wyoming big sagebrush transplant from seed to a planted surviving seedling was approximately $2.66/plant or $627 per hectare (~236 transplants/ha). If transplants were grown for 12 weeks in a greenhouse (compared to the traditional 6 months), the labor cost to successfully plant transplants would be cut in half. By reducing greenhouse growing costs, land managers reduce their restoration labor expenses, making the use of sagebrush transplants a more favorable restoration option.

Season of planting also made a difference in survival and volume of transplants in the first year following planting. Spring-planted transplants had higher survival in both years, which contradicts some previous work (e.g., Clements and Harmon 2019) however, even slight differences in climatic variables between study sites could elicit different results (Barnett and McGilvray 1993). Our study site, located at the NGBER, is on the cooler and wetter spectrum of the Great Basin Desert which creates more harsh winter conditions for newly fall planted sagebrush transplants. Previous studies have shown that fall plantings may be more favorable in more mild climates and spring plantings would be more favorable in colder climates (Wirth and Pyke 2011; Shaw et al. 2015). Our study site had average or below average temperatures immediately following the fall plantings (see results) which could be why our transplants had higher survival in the spring planting compared to the fall planting. Although survival was higher for spring-planted transplants in the present study, volume was greater for fall-planted transplants, suggesting that a fall planting could result in increased transplant vigor. Alternatively, the fall-planted transplants had been in the ground longer and were growing for a longer period of time compared to spring-planted transplants, potentially leading to increased volume at the time of measurement.

Competition did not affect survival of transplants after the first growing season in either year, however it greatly affected volume of surviving transplants in the first growing season following planting, suggesting that competition with invasive annual grasses could reduce the long-term survivability and vigor of transplants. Previous studies have shown that by decreasing competing vegetation, sagebrush transplant survival and growth increased (McAdoo et al. 2013;

Brabec et al. 2015; Schuman et al. 1998; Austin et al. 1994). For example, a study conducted in 2020 found that by decreasing herbaceous cover through grazing, sagebrush transplants exhibited increased growth (Davies et al. 2020*a*), and another study found that by reducing competition, sagebrush transplant survival increased (Davies et al. 2020*b*). Two growing seasons post-planting, survival was affected by competition, indicating that competition with invasive annual grasses does decrease long-term survival which is consistent with previous research (Davies et al. 2020*a*). Transplants with reduced vigor are less likely to survive the winter thermal extremes and summer drought within the sagebrush steppe (Fenner 2000). This decrease in transplant vigor could allow invasive annual grasses to outcompete sagebrush transplants and make the restoration efforts less effective. If transplants are planted in less favorable abiotic conditions than the present study, this effect could be heightened and result in a more drastic decrease in long-term survival. Increased transplant vigor (measured by volume) in transplants not competing with annual grasses could result in more resilient and longer lasting transplants (McAdoo et al. 2013; Cook and Child 1971). Larger sagebrush shrubs will also reach maturity sooner and start producing seed earlier than smaller shrubs (Young et al. 1989; Innes 2019), indicating that competition with invasive annual grasses has the potential to influence the contribution to the sagebrush seedbank. One study found that sagebrush transplants with increased herbaceous competition had fewer reproductive stems than transplants not competing with herbaceous vegetation (Davies et al. 2020*a*). The long-term decrease in survival caused by competition with invasive annual grasses suggests that it may be beneficial if competing vegetation is removed prior to sagebrush transplant revegetation.

Year to year variation in climate variables, especially precipitation, can greatly affect survival and vigor of transplants (Barnett and McGilvray 1993). Climatic factors influencing the outcome of restoration practices are outside the control of the managers (Monsen et al. 2004). Transplants from planting year two of this study had five-fold lower survival and ten-fold lower volume than transplants from planting year one and there are many possible reasons for this difference. The annual precipitation in the second year of the study was lower than the 40-year average, especially during the critical spring growing season (March-June; Fig 4.1). The site received 42% of the long-term average precipitation during March-June in the second year, compared to 93% of the long-term average precipitation in the first year. Temperatures were also greater in the spring growing season than the long-term average with the site experiencing 2 ± 0.7 °C higher average daily temperatures than the long-term average in the second year (Fig 4.2). The abnormally low precipitation, and above-average temperatures likely led to an increase in evaporative losses in the soil, which decreased the soil moisture content (Fig 4.3). These factors likely contributed to decreased survival of transplants planted in the second year.

In addition to abiotic differences at the site, the second year of this study used a separate enclosure, with the same ecological site and soil type, located less than 1 km from the first year's study. The second-year enclosure soil likely had increased compaction, which could have led to a decrease in soil water availability (Whisenant 1999). Although the soil compaction measurements did not differ significantly between enclosures (see results), compaction is still thought to have had an effect on transplant survival and growth. Our index to soil compaction was measured using a soil penetrometer, which measures soil compaction in the top cm of soil.

During the planting process in the second-year enclosure, compaction was noted by the increased difficulty to dig planting holes using the planting bar. Planting transplants in the first-year enclosure was achieved easily with the planting bar, meaning the second-year enclosure soil likely had compaction further down in the soil profile. This perceived compaction in the second-year enclosure was likely, in part, due to the difference in soil moisture between sites. The soil moisture was much higher during the planting process in the first year (approximately 0.146 Volumetric Water Content (VWC) m^3/m^3 for the fall planting and 0.229 VWC m^3/m^3 for the spring planting; Fig 4.10) than the second year (approximately 0.032 VWC m^3/m^3 for the fall planting and 0.174 VWC m^3/m^3 for the spring planting; Fig 4.10) which likely increased difficulty to dig holes in the second year. Soil compaction could have limited transplant root growth and led to increased mortality (Kozlowski 1999).

The transplants grown for the second year of the study also had shorter shoot height compared to the first year. Although the transplant care protocol in the greenhouse remained the same in both years, even the slightest differences in watering or light exposure could have resulted in different growing rates. Even though these transplants had reduced shoot heights, they still met the minimum required shoot height (5cm) for transplanting according to previous work (Dettweiler-robinson et al. 2013; Brabec et al. 2015). Additionally, these transplants, due to their reduced shoot height (i.e., compared to planting year one) but comparable root length, exhibited a high root to shoot ratio. Many plants in arid and semi-arid regions have evolved a high root to shoot ratio to increase resource capture and decrease mortality in a precipitation limited environment (Mata-González et al. 2017; Fernandez and Caldwell 1975). For this reason, the reduced shoot height does not seem to be a contributing factor to decreased survival in the second year.

Although the root length was measured for the transplants, this data was not very informative. Root length did not differ between years or between age classes. This is likely because sagebrush seedlings send out a long taproot shortly after emergence (Welch 2005; Caldwell and Fernandez 1975). This taproot reaches the bottom of the cone-tainers quickly (in less than two weeks based on our lab observations) resulting in similar lengths of roots across all age classes (Caldwell and Fernandez 1975). Root biomass would have been a better measurement to display differences in root thickness and density, however root biomass was not recorded in the present study. Although root length did not differ between age classes, the root biomass generally appeared greater in older age classes. The youngest two age classes (6 and 8 weeks) had comparatively decreased root biomass compared to age classes 10-24 weeks, which was likely a contributing factor in decreased survival among the two smallest age classes of sagebrush transplants.

Management Implications

With increasing frequency and size of wildfires in low to mid-elevation sagebrush communities, there is an increasing need to restore sagebrush. Given the low success rates in seeding, transplants offer managers an alternative with higher success. Although transplants are more costly compared to seeding, transplants have a higher success rate, making this technique a favorable restoration option, particularly for localized high priority areas (e.g., sage-grouse winter habitat). Our data suggest a less costly alternative to traditional sagebrush transplant grow-out protocols, which would reduce the cost of using sagebrush transplants in restoration projects; specifically, growing sagebrush transplants for only 10 or 12 weeks compared to the traditional 24+ weeks.

This study also indicates that a spring planting may offer higher survival compared to a fall planting, however this conclusion is inconsistent with previous research indicating that site specific conditions will likely determine whether a fall or spring planting will result in higher survival and vigor of sagebrush transplants. For this reason, we cannot definitely suggest what planting season will exhibit higher survival. This study also indicates that competition with invasive annual grasses greatly reduces a sagebrush transplant's vigor and decreases long-term survival. All of these factors add to the ecological knowledge around sagebrush restoration and contribute to maximizing the success of sagebrush transplanting. Further studies should follow to confirm the results of this study, especially in different climate conditions to help land managers form a better understanding of sagebrush restoration options.

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Figures

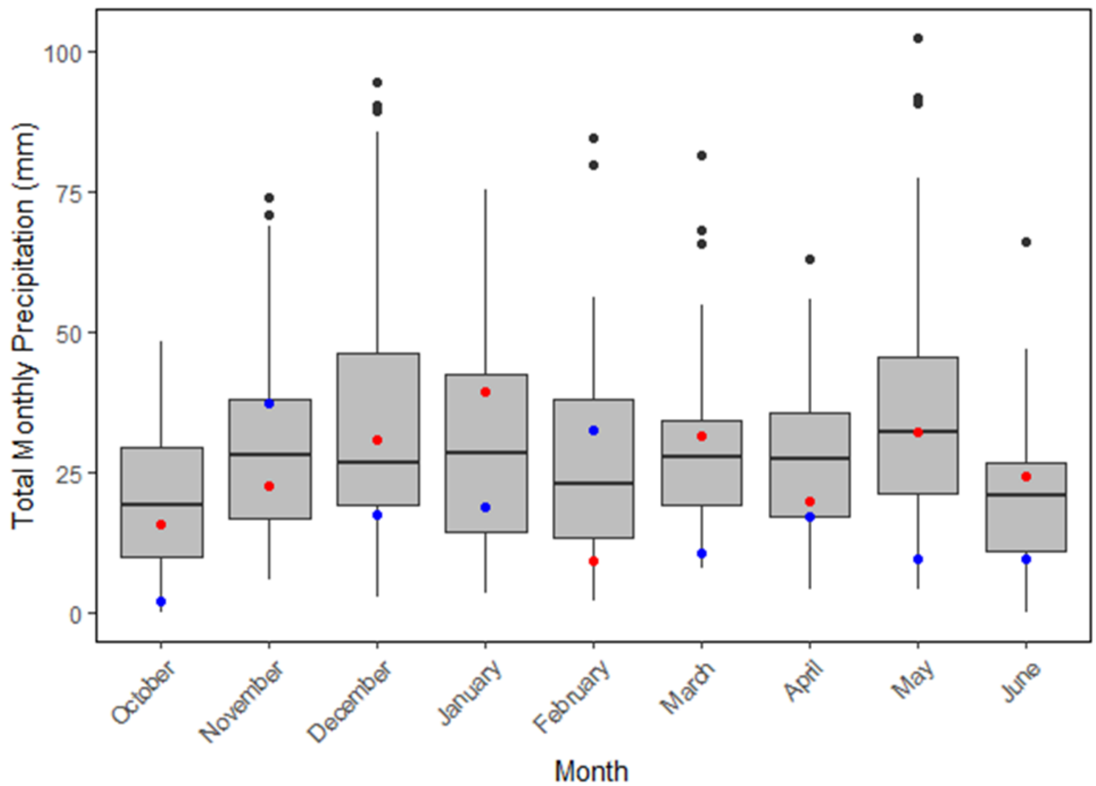

Figure 4.1. Forty-year average (1979-2020) precipitation (mm) per month in a hydrologic year (October-June) for the study site. The red dots signify the first year's total precipitation per month, the blue dots signify total precipitation per month in the second year of the study, and the black dots signify outliers of the long-term average (Great Basin Weather Applications 2021).

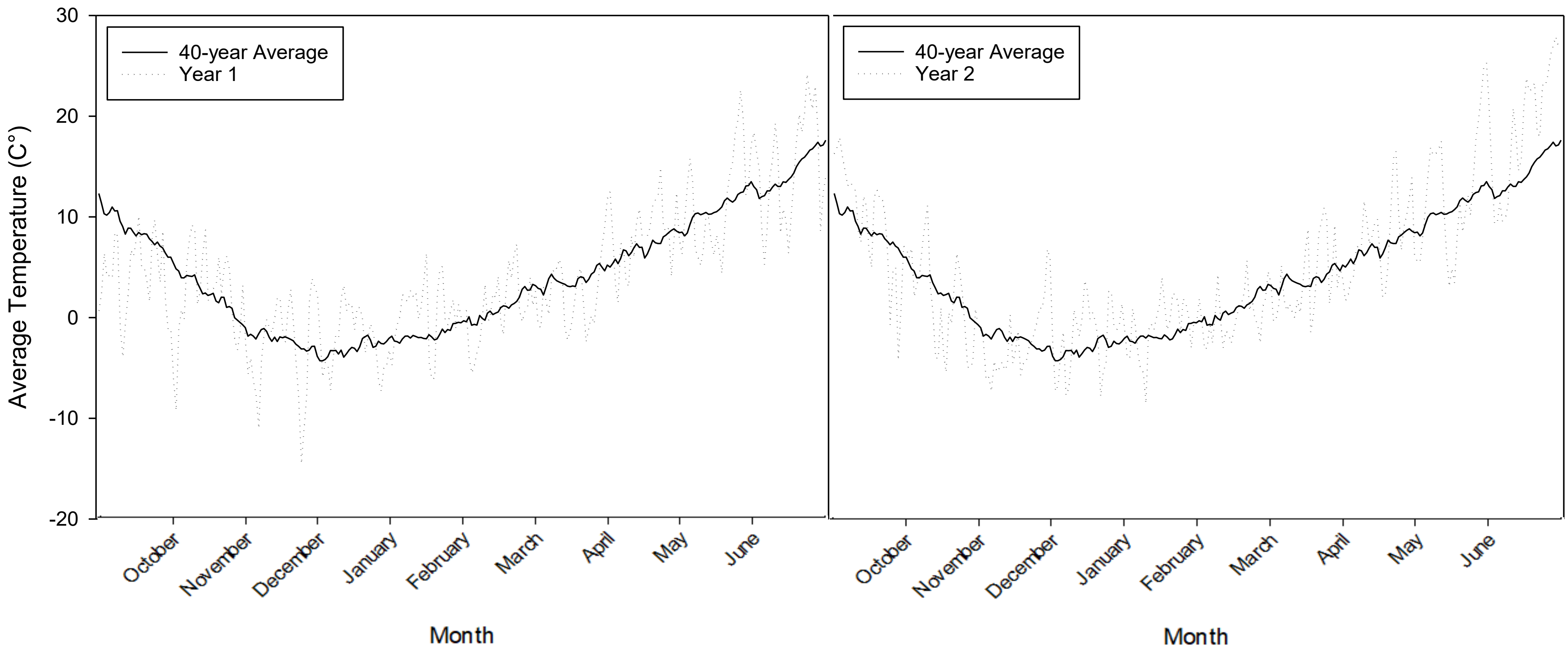

Figure 4.2. Forty-year average (1979-2020) air temperature (C°) in a hydrologic year (October-June). The graph on the left signifies the first year's average temperature per month compared to the long-term average and the graph on the right signifies the average temperature per month in the second year of the study compared to the long-term average (Great Basin Weather Applications 2021).

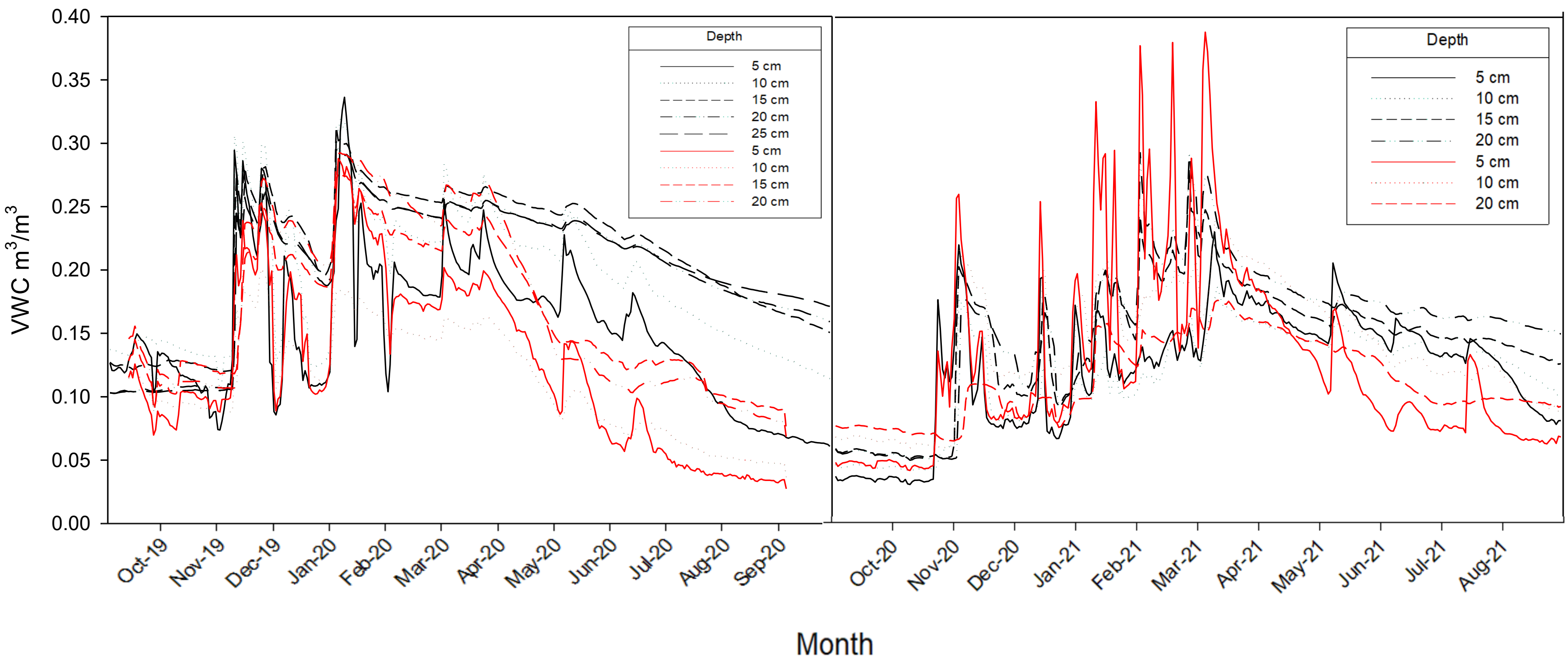

Figure 4.3. Soil moisture measurements (Volumetric Water Content m^3/m^3) by month at five different depths in the first year of the study (left) and the second year of the study (right) in the no competition landscape (black lines) compared to the competition landscape (red lines).

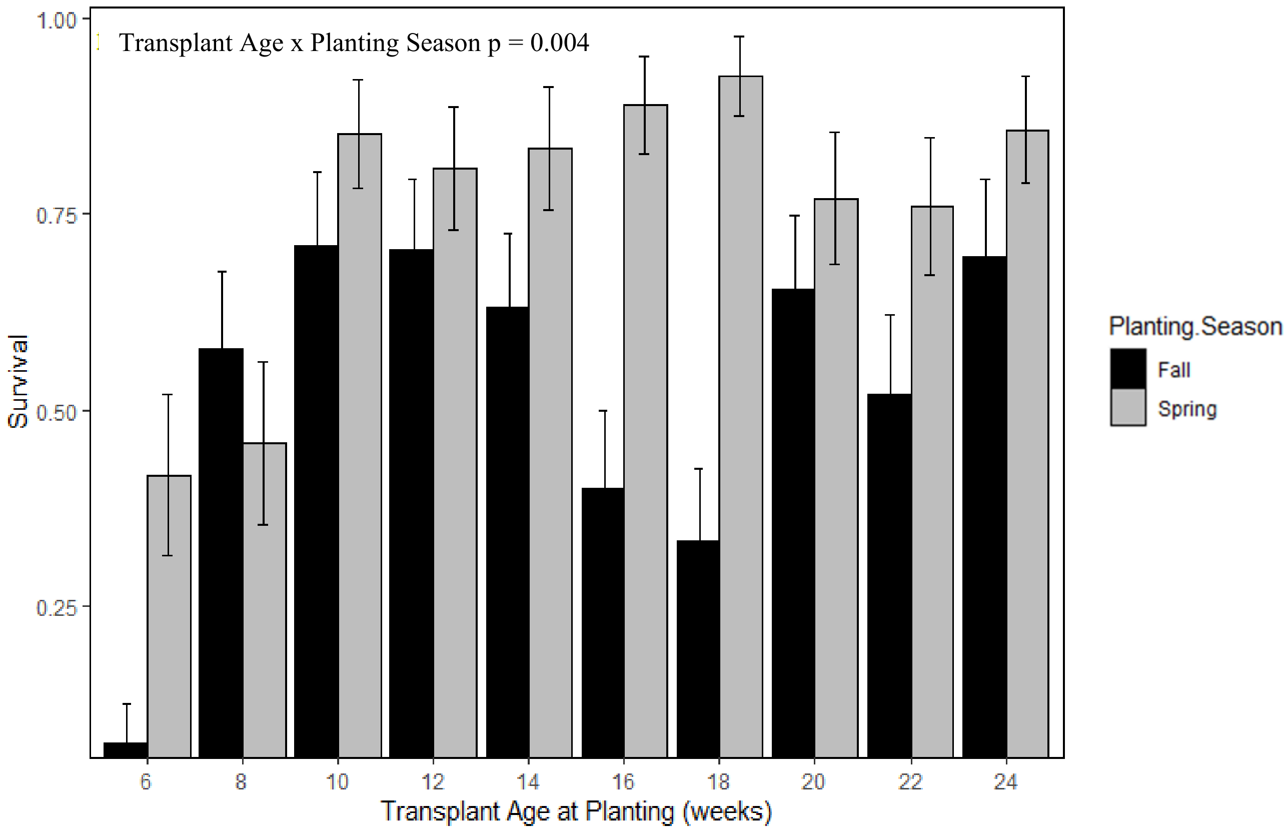

Figure 4.4. Mean survival of sagebrush transplants from planting year one as a function of the interaction of age and planting season in the first year of the study.

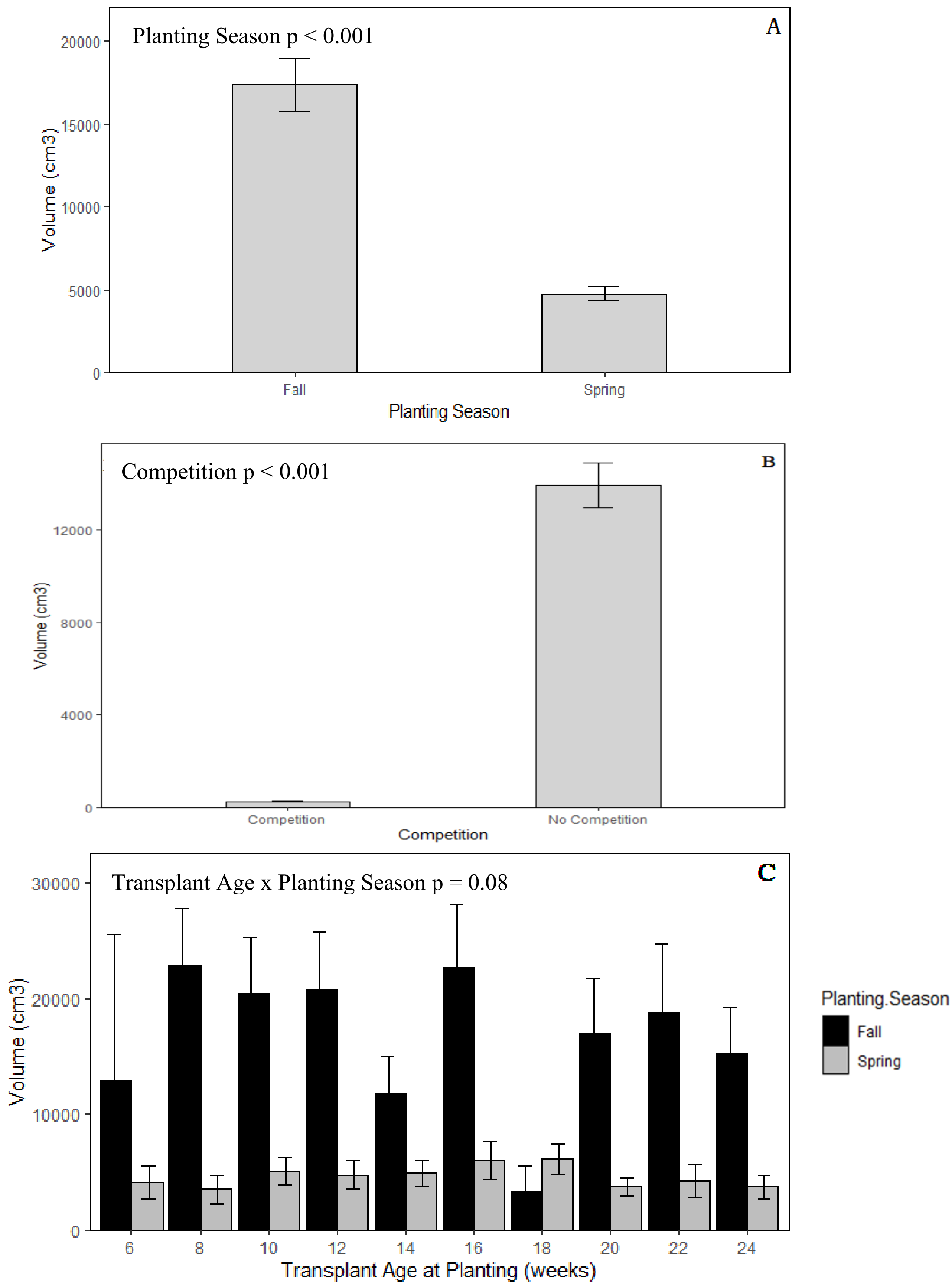

Figure 4.5. Mean volume (cm^3) of planting year one transplants **a**) by planting season, **b**) competition transplants and zero-competition transplants, and **c**) as a function of transplant age and its interaction with planting season in the first year of the study.

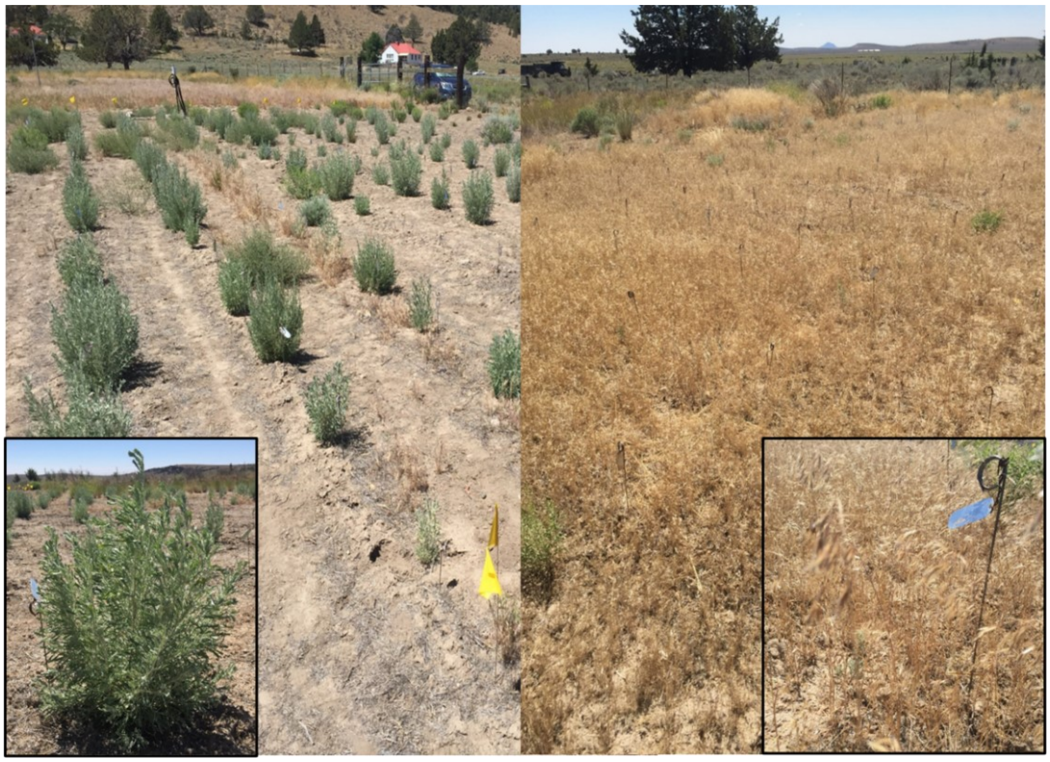

Figure 4.6. Side-by side-comparison of the zero-competition landscape (left) and competition landscape (right) taken in summer 2020.

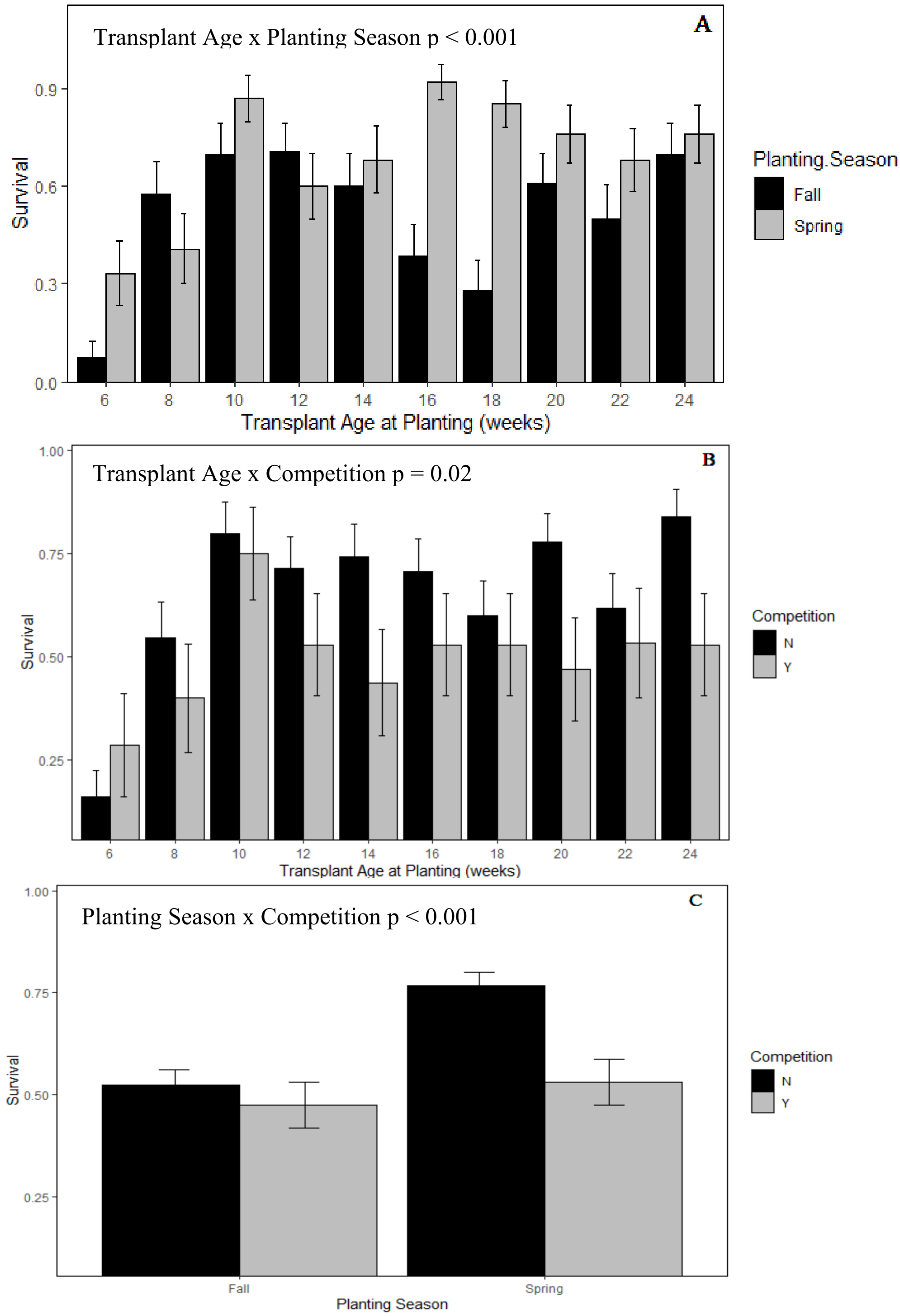

Figure 4.7. Mean survival of transplants from planting year one, two years following planting as a function of **a)** the interaction between planting season and age **b)** the interaction between age and competition and **c)** the interaction between planting season and competition.

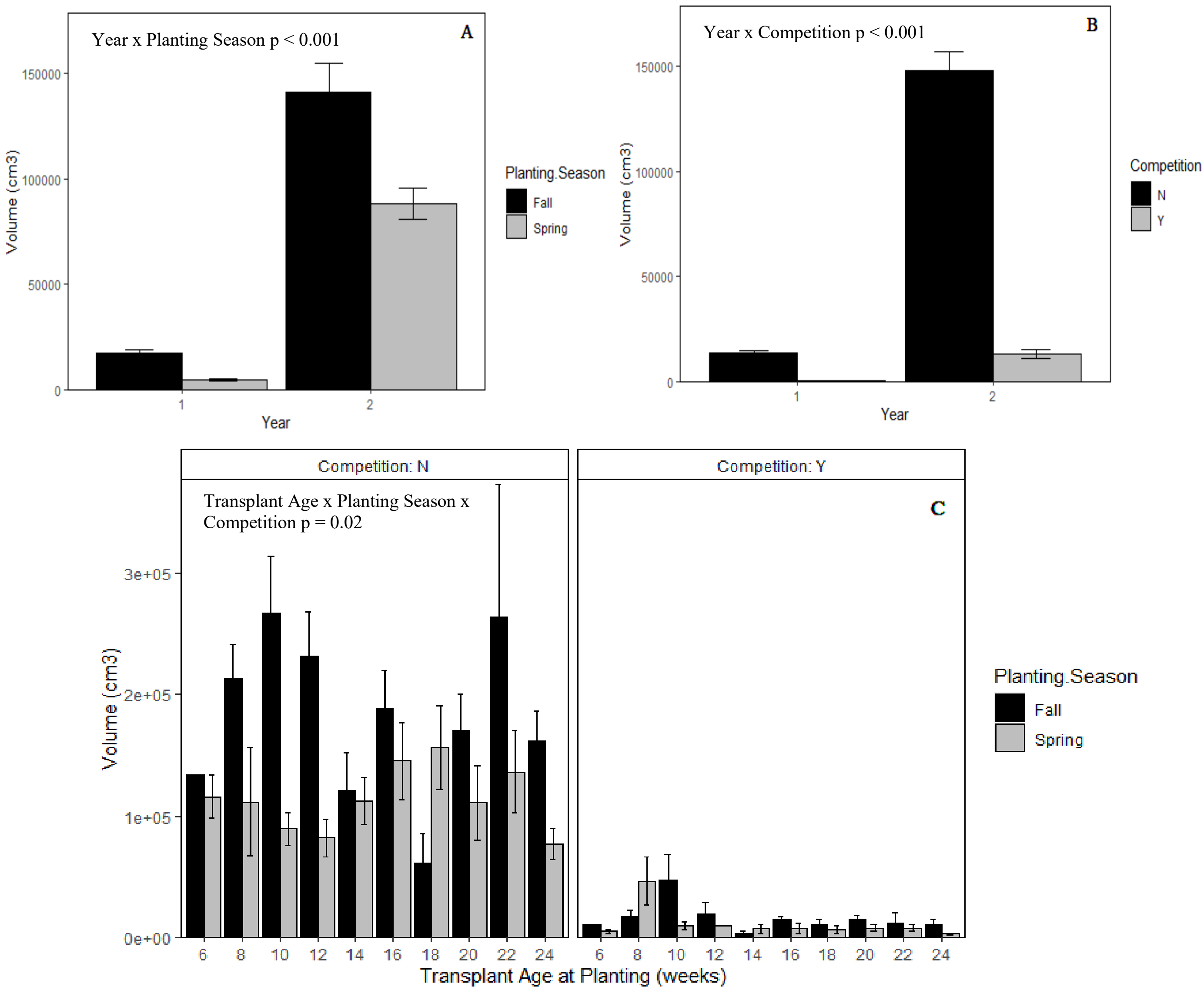

Figure 4.8. Mean volume (cm^3) of surviving transplants from planting year one after one and two years as a function of **a)** the interaction between planting season and year **b)** the interaction between competition with invasive annual grasses and year and **c)** the three-way interaction between planting season, age, and competition after two years.

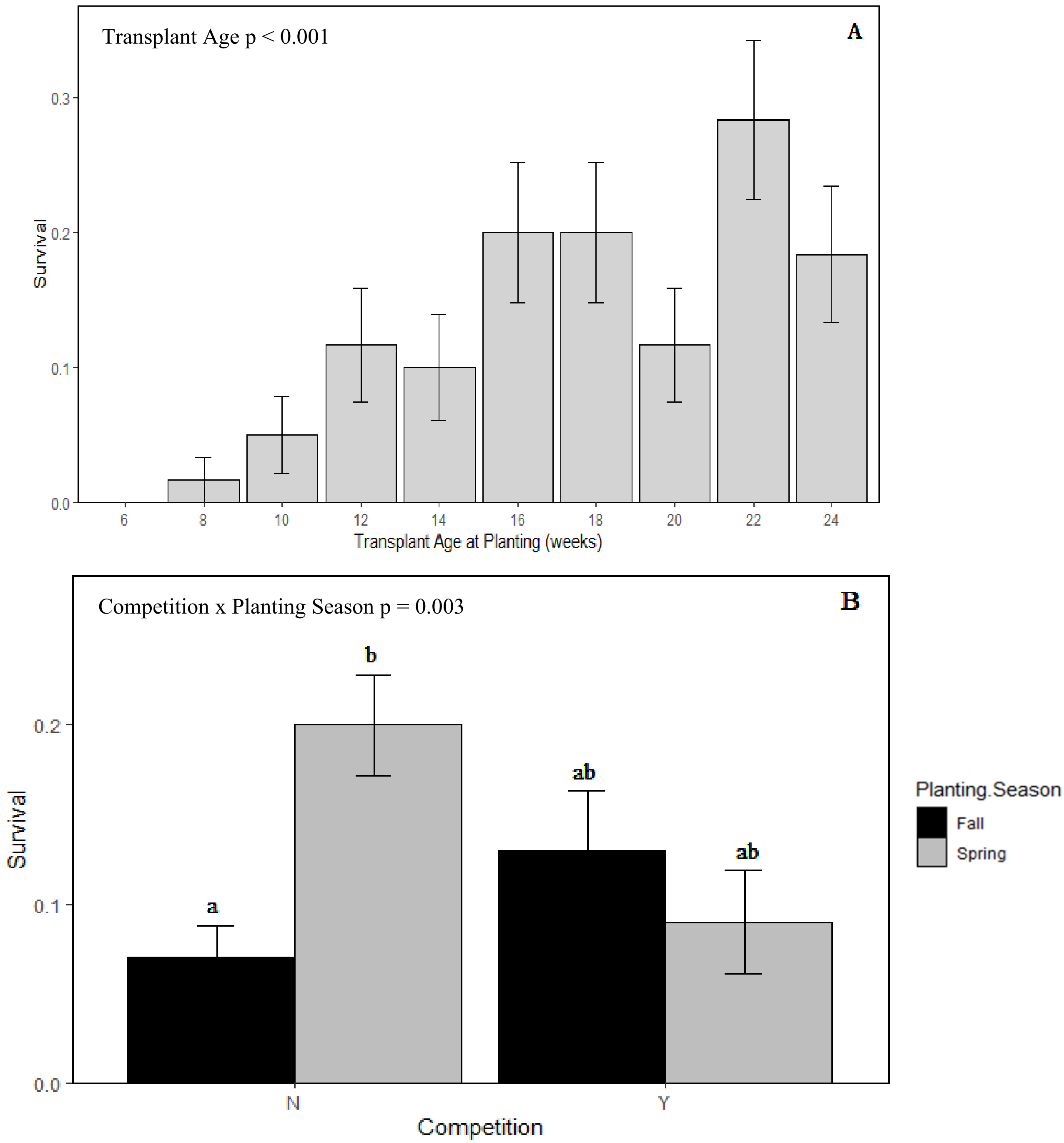

Figure 4.9. Mean survival of transplants from planting year two as a function of **a)** age at time of planting and **b)** the interaction between planting season and competition. Significant differences for panel C are denoted by differing lower case letters.

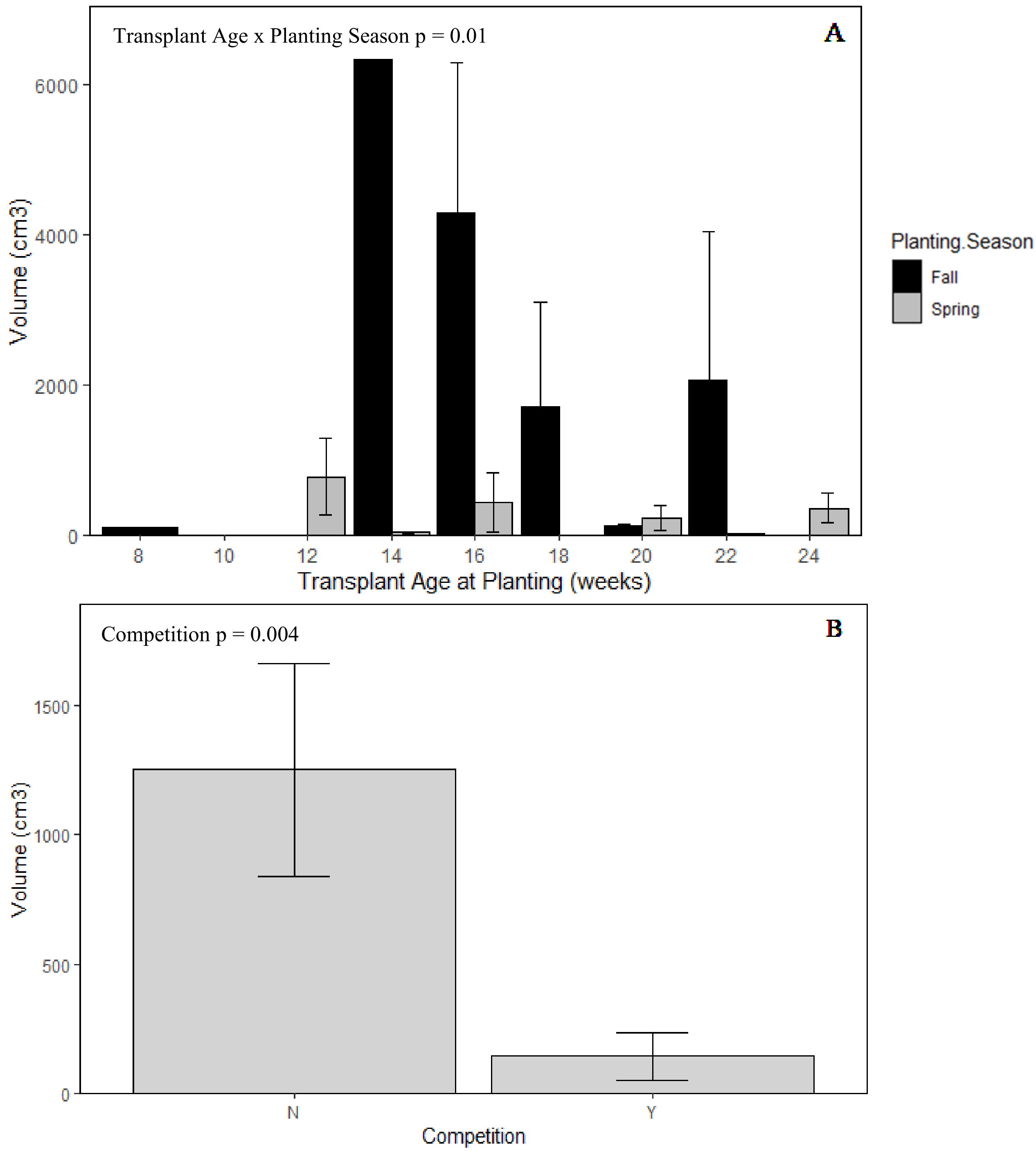

Figure 4.10. Mean volume (cm^3) of transplants from planting year two as a function of **a)** the interaction between age and season and **b)** competition with invasive annual grasses.

CHAPTER 5: OVERARCHING CONCLUSIONS AND FUTURE RESEARCH

Activated carbon has been shown to be a useful tool in minimizing harmful effects of herbicide and protecting desired seeds in the form of herbicide protection pods (HPPs) and individual seed coatings (Davies et al. 2017; Clenet et al. 2019; Holfus et al. 2021). Although HPP's are effective, they can be expensive to produce and do not easily fit into drill seeders (Holfus et al. 2021). Alternatively, individual seeds coated with an herbicide protection material containing activated carbon fit easily into a drill seeder and do not require as much material to produce. Individually coating seed has been shown to effectively protect desired seed against herbicide in a controlled greenhouse setting (Holfus et al. 2021). Under these controlled conditions, plant height and above-ground biomass were higher in coated seed in the presence of herbicide compared to bare seed. Moreover, emergence was higher in coated seed compared to bare seed in the presence of low and high herbicide application rates (Holfus et al. 2021). These results suggest that in a year with favorable climatic conditions, individually coated seed could provide protection against herbicide in a field setting, even under high herbicide application rates. Nonetheless, this method of restoration is novel and although successful in a greenhouse setting, has not yet been shown to be successful in the field. Due to the under-reporting of negative results in scientific journals (Sheley, 2007; Drayton and Primack 2012), and the recent development of this technology, the success of activated carbon seed coatings in a field setting is currently unknown.

Rangeland seeding efforts are often unsuccessful with a success rate as low as 10% when using native seed (Madsen et al. 2016). With climate change causing an increase in extreme temperatures and a decrease in precipitation in drier climates (Huber and Gulledge 2011; Trenberth 2011), there is an increasing need to find successful methods of perennial grass restoration, especially in unfavorable environmental conditions. Invasive annual grasses now cover approximately 22 million hectares of the Great Basin Desert and continue to spread (Bradley et al. 2018; Duncan et al. 2004). The need to restore perennial grasses within the invaded sagebrush steppe is vital to wildlife habitat, livestock forage availability, and the natural ecosystem function of these areas (Brooks et al. 2004; Norton et al. 2004). Invasive annual grasses spread rapidly and outcompete native vegetation however, they cannot easily outcompete adult perennial bunchgrasses making them the best defense against annual grass invasion. Conversely, at the seedling stage, perennial bunchgrasses do not compete well with invasive annual grasses and will likely be outcompeted by these invasive species. With pre-emergent herbicides showing the most promise in invasive annual grass management, herbicide protection seed coatings could be the future of perennial grass restoration however, further studies should follow to determine their success under variable abiotic conditions.

Not only have perennial grasses been negatively affected by invasive grasses but native sagebrush (*Artemisia* sp.) has also greatly declined in association with the invasive grasses' interaction with fire (Welch 2005; Dettweiler-Robinson et al. 2013). In lower elevations, Wyoming big sagebrush is not adapted to frequent fire and therefore is not able to resprout following fire (Pyke et al. 2020). Without resprouting, the recovery of sagebrush is dependent upon the seedbank, however, the sagebrush seedbank is unreliable. Many of the seeds do not survive following wildfire and those that do, have very specific requirements for germination, do not compete well with invasive annual grasses, and have an extremely short-term viability making them unlikely to germinate and establish (Lambert 2005; Pyke et al. 2020). All of these factors combined make natural recovery of this species nearly impossible.

Sagebrush provides habitat and forage for many wildlife species as well as ecosystem structure and resilience, making the restoration of this species a high priority (Knick et al. 2003; McArthur 1992). Seeding sagebrush is often unsuccessful due to its specific requirements for germination and establishment, the variability in climatic factors, and its short-lived seed (McAdoo et al. 2013; Pyke et al. 2020). Seeding of sagebrush also requires soil disturbance for effective seed-soil contact which can be too disruptive and can kill existing vegetation in partially intact plant communities making it a less favorable restoration option (Dettweiler-Robertson et al. 2013). Alternatively, the use of sagebrush transplants ensures seedling emergence in a controlled greenhouse setting, and allows the vulnerable seedling to overcome the bottleneck of increased mortality in the face of extreme temperatures in the summer drought and winter freeze within the sagebrush steppe (Fenner 2000; Roundy et al. 2016; Dettweiler-Robertson et al. 2013). The use of sagebrush transplants has exhibited higher success than seeding in lower elevation sites (Lysne and Pellant 2004; Grant-Hoffman and Plank 2021), making it a more favorable restoration option in arid and semi-arid climates, however, this rather newly developed and expensive technology has highly variable success rates in the field that range from 15-80% (Dettweiler-Robertson et al. 2013; McAdoo et al. 2013).

The wide range of success of transplants is dependent upon many factors including abiotic factors such as year to year weather variation, the site's soil type, topography, elevation, the seed source, and the method of planting (Brabec et al. 2015; Shaw et al. 2005; Davidson et al. 2019). Current research tells us that the time of year of planting affects transplant survival, with spring plantings showing higher survival rates in some instances (Shaw et al. 2015; McAdoo et al. 2013: see chapter 4) and fall plantings showing higher success in other instances (Clements and Harmon 2019; Dettweiler-Robinson et al. 2013). The research presented here resulted in higher rates of survival in the spring plantings, however transplants planted in the fall have increased volume compared to spring-planted transplants, and reach their desired height (>30cm) earlier than spring-planted transplants, which could benefit wildlife such as the greater sage-grouse sooner. Nesting sage-grouse require sagebrush to reach a minimum height of 30cm and at least 15% cover but only 5-10% cover for foraging habitat (Connelly et al. 2000; Pyke et al. 2020). In addition to season of planting, competition with invasive annual grasses was shown to negatively affect sagebrush transplant vigor, evident by decreased transplant volume, but did not affect survival after one growing season. With invasive annual grasses spreading over much of the sagebrush steppe and across the west, sagebrush restoration could benefit from the removal of these invasive grasses prior to planting.

The results from the present study are the first to show that sagebrush transplants can be successfully established at a younger age than previously thought. The standard grow time of six months or greater in a greenhouse or nursery no longer provides the highest survival rates compared to younger age classes. The present research indicates that transplants at least 12 weeks of age had comparable mean survival rates to transplants grown for six months (24 weeks; see chapter 4). This discovery could impact the feasibility of sagebrush restoration via transplanting by reducing restoration costs. Although the use of transplants has shown more success than seeding, its main downfall is the increased cost (McAdoo et al. 2013). If this cost were reduced by decreasing greenhouse growing time, this method could provide the highest success of sagebrush establishment at a reduced cost, making it potentially the most favorable sagebrush restoration technique.

It is clear that there is a vital need to re-establish sagebrush within the sagebrush steppe ecosystem, however the most successful method depends on a large array of factors. There are some restoration techniques such as the use of "wildlings," or mature sagebrush collected from the field and re-planted elsewhere, that could have more success and decreased cost compared to the use of transplants. This method has shown some success in more arid environments (Bailey 2021; McAdoo et al. 2013), but these results lack repetition making their success unreliable. Other restoration techniques such as the use of mycorrhizal enhancements in the form of root dipping, may increase transplant survival in arid and semi-arid regions but this method further increases restoration costs (Stahl et al. 1998; Dettweiler-Robertson et al. 2013).

There is a clear need for further research on narrowing down the most economical and feasible methods of sagebrush restoration. The restoration method selected depends upon the herbaceous community at the site, climatic variables, soil type, and topography of the restoration site as well as the economic constraint of land managers. The highly variable results from the literature on sagebrush restoration methods warrants further research. With the increase in climate change causing less favorable abiotic conditions and in turn, increasingly favorable environmental conditions for exotic annual grasses, there is an ever increasing need to build upon the current research so effective restoration techniques can be employed.

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APPENDICES

APPENDIX A: Vortex Seed Coating Protocol.

Vortex Seed Coating Protocol

This document outlines the vortex seed coating method employed by Holfus et. al. (2021).

Equipment & Materials

- Rotary seed coater (SedPell RP14-DB; BraceWorks Automation and Electric; Lloydminster, SK, Canada)
- Forced air dryer
- Vortex Genie-2 shaker (Scientific Industries, Inc.; Bohemia, NY)
- Spray bottle
- Round aluminum pan (30cm D x 2.5cm H)
- Small plastic putty knife (3-5cm W)
- #14 sieve
- 200g bluebunch wheatgrass (*Pseudoroegneria spicata*) seed
- Selvol-205 (Sekisui Specialty Chemicals, Dallas TX) 4% & 8% solid content solutions
- Activated carbon (Darco GroSafe; Cabot, Billerica, MA)
- Herbicide protection formula mixture (w/w):
    - 45% calcium bentonite clay (Pelbon Ca Bentonite Clay; American Colloid Company; Hoffman Estates, IL)
    - 33% activated carbon (Darco GroSafe; Cabot, Billerica, MA)
    - 14% sieved compost (Biofine; Deschutes Recycling; Bend, OR)
    - 6% sieved worm castings (Worm Gold Plus; California Vermiculture, LLC; Cardiff, CA)

Data Requirements

Prior to coating, assess seeds per bulk gram of bare seed and, once prepared, rotary coated seed. In addition, calculate the dry weight of 4% selvol applied within the surface area of the aluminum pan when atomized by a single trigger spray. The amount will vary depending on the atomizer selected, temperature of solution, distance, and angle relative to the pan. The values aforementioned are required to calculate material adhesion via the coating process, including the activated carbon attained per seed.

Method

Bare seed is prepared for vortex coating utilizing a rotary seed coater (SedPell RP14-DB) to apply a base layer of 8% selvol and activated carbon following standard rotary procedures. This initial layer provides a surface for the herbicide protection formula to readily adhere. The composition of the formula (i.e., pellet mixture) prevents its use in a rotary coater, therefore the mixture of activated carbon, compost, worm gold, and bentonite clay are applied by means of the vortex method.

The vortex method consists of layering herbicide protection formula, 33g of rotary coated seed and more herbicide protection formula in an aluminum pan, then spraying with 4% selvol. The pan is then placed on a vibrating surface (e.g., Vortex Genie-2) and gently stirred by hand with a putty knife. The vibration prevents agglomeration of the seed and stirring provides even coating. Once a layer of coating has been achieved, contents of the pan are sieved with a #14 mesh to remove excess formula and the seed placed on a forced air dryer at 26C for one hour. After drying, seed is weighed and the amount of herbicide protection formula gained, including activated carbon, is calculated. This layering process continues until the activated carbon target has been achieved (e.g., 12mg).

Calculations

Assessing the amount of activated carbon adhered per seed is based on data collected prior to and during the vortex process. This should be performed per layer so the target isn't missed, but can be also be assessed at the end. Coating weight gain per seed is calculated by subtracting the mean start-weight (i.e., previous layer's weight) for a sub-sample (e.g., 25 seeds) from the average end-weight of an equivalent sub-sample, then dividing by the number of seeds in the sub-sample. Selvol weight per seed is then subtracted and the final value multiple by 33% to determine activated carbon per seed attained. Selvol weight per seed is calculated by dividing the total dry selvol applied by the batch seed number (selvol applied is equal to the number of sprays applied multiplied by a single spray's dry weight).

Literature

Holfus, C. M., Rios, R. C., Boyd, C. S., Mata-González, R. 2021. Pre-emergent herbicide protection seed coating: a promising new restoration tool. Rangeland Ecology and Management. 76, 95-99.