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Using Herbicide and Planting Techniques to Restore a Native Bunchgrass to Cheatgrass Invaded Systems

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Using Herbicide and Planting Techniques to Restore a Native Bunchgrass to Cheatgrass Invaded Systems

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Master of Science

This thesis explores potential seeding techiniques to limit harmful effects of preemergent herbicide on a seeded species while simultaneously reducing abudance of annual invasive grasses. The first chapter examines the use of activated carbon seed coatings and furrows to limit herbicide effect on seeds of a perrenial bunchgrass. We found that both carbon coatings and furrows mitigated some of the herbicide effects, but that only when the two techniques were combined did we observe unaffected seedling emergence, plant density, and aboveground growth. Therefore, we suggest to management that use of carbon coatings and furrows after herbicide application can likely be used to reduce invasive annual grasses while simultaneously establishing a native bunchgrass. In chapter 2, we examine the effects of a novel preemergent herbicide indaziflam, on native seeds and compare it against a common preemergent herbicide, imazapic. We found that indaziflam provides superior long-term control of annual invasive grasses than imazapic, but that it is also more detrimental to native seeds. Our results suggest that indaziflam is best suited for control purposes only, and is hard to incorporate in restoration seeding efforts due to its strong effects on native seed.

Keywords: activated carbon, furrow, imazapic, invasion, restoration, cheatgrass, indaziflam

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CHAPTER 1

Furrows and Activated Carbon Seed Coatings Allow for the Simultaneous Establishment of a Native Perennial Bunchgrass while Controlling an Invasive Annual Grass with Preemergent Herbicide

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ABSTRACT

Exotic grass introductions are transforming dryland ecosystems worldwide, including state changes to native plant communities. Efforts to restore native vegetation in invaded areas have been largely unsuccessful. Control of invasive grasses is possible with pre-emergent herbicide, but these chemicals can also inhibit restoration efforts using native seed. Seed technologies that mitigate herbicide effects on native seed could allow for the restoration of native species while controlling invasive annual grasses. In this study, we evaluated two approaches for mitigating the effects of the pre-emergent herbicide, imazapic, on a native perennial, Pseudoroegneria spicata, at sites invaded by Bromus tectorum in the sagebrush steppe ecosystem. Activated carbon was applied as a seed coating with the intent of absorbing the herbicide within the area directly around the seed, and furrows were used to side sweep soil sprayed with herbicide away from the planted seed. Our results indicate that imazapic application had a strong negative effect on the invasive annual grass, reducing B. tectorum cover 91% after one year, and 39% after two years. Imazapic also had strong negative effects on P. spicata without a carbon coating or furrow treatment, reducing seedling emergence 38%, 2yr plant density 65%, and 2yr total growth 90%. Activated carbon seed coatings did not protect P. spicata from herbicide effects on seedling emergence or 2yr survival, but reduced herbicide effects on 2yr aboveground growth. Furrow treatments mitigated herbicide effect and improved seedling emergence 286%. Furrows did not significantly mitigate herbicide effect on 2yr plant densities. Furrows limited herbicide effects on 2yr growth, producing similar biomass as non-herbicide controls. Combining carbon seed coatings and furrow treatments fully mitigated all harmful herbicide effects on emergence, plant densities, and growth. Our results suggest that activated carbon seed coatings and furrow treatments alone do not mitigate pre-emergent herbicide effects on all P. spicata life stages, but that combining both treatments results in similar establishment of a native perennial as a non-herbicide seeding and lowers B. tectorum abundance. Our technique could likely be applied with large scale restoration seedings with commercially coated seed and using cultivator sweeps to allow growth of native bunchgrasses while reducing exotic annual grasses.

INTRODUCTION

Dryland ecosystems comprise 35-40% of the Earth's terrestrial surface. Many of these areas are being transformed through human disturbances, which promote the invasion of exotic plant species (Schlesinger et al. 1990; Brooks et al. 2004; D'Antonio and Vitousek 1992). Annual grass invasion can quickly spread through a system (Bradley et al. 2017; Balch et al. 2013) and often result in reduced plant and animal diversity (Freeman et al. 2014; St Clair et al. 2016), accelerated fire regimes (Bradley et al. 2017), and changes in soil nutrient cycling (Ehlert 2017; Bishop et al. 2016; Kerns and Day 2017). The positive response of annual grasses to fire allow them to competitively displace many native perennial plant species in post-fire landscapes (Knapp 1996; Baker, Garner, and Lyon 2009). This is largely due to high seed production (Hempy-Mayer and Pyke 2008), the ability to germinate opportunistically with soil moisture availability, and fast growth rates (Young, Evans, and Eckert Jr 1969; Beckstead, Meyer, and Allen 1996; Beckstead et al. 2010).

Efforts to restore native vegetation in areas invaded by the invasive annual grass, *Bromus tectorum* L. (cheatgrass) have shown limited success (Mangold et al. 2013; Orloff, Mangold, and Menalled 2015). It has been suggested that *B. tectorum* abundance should be reduced before seeding takes place to increase the success of the restoration treatment (Davies 2011). Imazapic is a popular pre-emergent herbicide used to reduce *B. tectorum* on rangelands (Mangold et al. 2013). Imazapic kills plants by inhibiting the activity of the enzyme acetohydroxy acid synthase (AHAS or ALS), an enzyme responsible for creating branched-chain amino acids isoleucine, valine, and leucine (Umbarger 1978). At proper application rates and timing, imazapic can reduce *B. tectorum* density over 95% after one year (Elseroad and Rudd 2011). However, pre-emergent herbicide also affects the seeds of native species (Sheley, Carpinelli, and Morghan 2007), which is problematic to restoration efforts where *B. tectorum* control and direct seeding are both desirable goals.

Seed technologies could limit the impact of herbicide on native species while allowing control of invasive annual grasses (Davies, Madsen, and Hulet 2017). Carbon amendments have been used historically to absorb and neutralize organic compounds including herbicides in both agricultural and natural systems (Kadirvelu, Thamaraiselvi, and Namasivayam 2001; Li, Quinlivan, and Knappe 2002; Uchimiya et al. 2010). Activated carbon is porous and absorptive, enabling it to neutralize herbicides in conjunction with direct seeding efforts (Coffey and Warren 1969; Madsen et al. 2014b; Davies, Madsen, and Hulet 2017). Madsen et al. (2014b) showed that activated carbon seed coatings could reduce the impact of the pre-emergent herbicide imazapic on *Pseudoroegneria spicata* (Pursh) Á. Löve. (bluebunch wheatgrass) but only when the herbicide was applied at relatively low application rates. Madsen et al. (2014a) also showed that large amounts of activated carbon could be incorporated around seeds within an extruded pellet to protect the seeds from pre-emergent herbicide. Termed in the literature as “herbicide protection pods” these activated carbon extruded pellets, have been shown to improve native plant establishment with soils with high concentrations of pre-emergent herbicide (Madsen et al. 2014, Brown et al. 2019, Clenet et al. 2019). While herbicide protection pods show promise for restoring degraded rangelands, the large pellets are not compatible with traditional seeding equipment.

Another possible approach is to use soil furrows to create a safe-site from herbicide for seeded species. Creating a furrow after herbicide application side sweeps the surface soil with high herbicide concentration, leaving a safe-site with low herbicide concentrations at the bottom of the furrow (Eckert and Archives 1974). Eckert Jr et al. (1974) showed that making and planting with furrows one year after herbicide application reduced herbicide effect of atrazine on the seeded species. Though this technique successfully reduced herbicide effect of atrazine (designed for pre and post emergence control of broadleaf weeds), it has never been tested with pre-emergent herbicide used for annual grass control. Use of a furrow when planting can also improve seedling success by improving soil water relations through accessing deeper and more consistent soil moisture (Call and Roundy 1991; Witharama, Naylor, and Whytock 2007).

Combining carbon seed coating and furrow treatments in herbicide areas may enhance protection from herbicide and improve establishment over either treatment alone. Using activated carbon as a seed coating can be easily implemented in rangeland seedings, but likely will only work at low herbicide concentrations. Furrows may reduce herbicide concentration enough that individual carbon seed coatings may provide adequate protection to be seeded simultaneously as herbicide is being applied.

Herbicide mitigation strategies have to effectively protect native species at each stage of plant development from germination, to emergence, and into the growth and recruitment phases of the life cycle. Herbicide has different effects on each plant growth stage (Shinn and Thill 2004; Sebastian et al. 2017). The proposed herbicide mitigation techniques (activated carbon seed coatings and furrows) differ in their mechanisms and may differentially affect sensitivity to herbicides in different stages of development. In addition to neutralizing the effects of herbicide, carbon additives have been shown to alter soil properties by increasing cation exchange capacity, providing habitat for microorganisms, and increasing water retention (Gaskin et al. 2007). Furrows can alter soil microclimate, providing a microsite with more consistent soil moisture and lower temperatures that could increase germination success and enhance early growth (Winkel and Roundy 1991; Winkel, Roundy, and Cox 1991). Beyond the immediate benefits of seed coating and furrows to seedling germination and emergence, their potential mitigation of herbicide could provide a growth window free from annual grass competition that would likely improve growth and recruitment success (Davies, Madsen, and Hulet 2017; Sebastian et al. 2017).

This study was conducted in the semi-arid sagebrush steppe, a system heavily impacted by annual grass invasion (Bradley et al. 2017) that has experienced declines in several native plant species (Boyte, Wylie, and Major 2016). The objective of this study was to test the potential re-establishment of *P. spicata*, an, important native bunchgrass in the sagebrush steppe ecosystem, using herbicides to reduce *B. tectorum* dominance while reducing collateral effects of herbicides by coating seeds with activated carbon, or planting them in furrows. We hypothesized that: 1) herbicide applications would control the establishment of *B. tectorum* due to its effectiveness in previous studies; 2) coating seeds in activated carbon and planting in furrows would limit exposure of *P. spicata* seeds to herbicide, resulting in better seedling emergence, survival, and growth of *P. spicata*; and 3) the combination of activated carbon seed coatings and furrows would result in better establishment of *P. spicata* than either treatment alone as a result of less herbicide effect and improved microsite.

MATERIALS AND METHODS

Study Sites

The study was conducted at three sites in the sagebrush steppe system of North America. Two sites are located in the boundaries of Great Basin National Park in Nevada, and one site is located in Provo, Utah (Table 1-1S). Elevation between sites was 1448 m at the Provo site (Utah), 2013 m at Lehman Flats site (Nevada), and 2135 m at the Kious Basin site (Nevada). Soil types across sites vary from stony loam (Lehman Flats), to gravelly loamy coarse sand (Kious Basin), and gravelly loam (Provo). Our study took place over two years (October 2017 to August 2019), with plantings each fall. First-year precipitation totals ranged from 81-120% across sites, and a dry summer (68%), with no June precipitation at 2 sites (Lehman and Kious) and 64% total precipitation of the historic 30-year average (PRISM). Second-year spring precipitation was abundant (158% of average) followed by low summer precipitation (64% of average) (PRISM). Each of these sites were formerly dominated by native sagebrush communities but have been invaded by *B. tectorum* to the extent that it comprised 30-50% relative cover. One site (Provo), was fully invaded to the point of virtual monoculture with *B. tectorum*, *Aegilops cylindrica* (Love.), with no *Artemesia spp.* present. Site vegetation at the Nevada sites was dominated by *B.*

tectorum, and also contained sparse native species of *Elymus elymoides* (Raf.), *Artemesia tridentate* (Nutt.), *Pinus monophyla* (Torr. & Frem.), *Gutierrezia sarothrae* (Pursh.), and *Purshia tridentate* (Pursh.). All of the sites were on relatively flat terrain (5-10% slope) with aspects ranging northeast, southeast, and west.

Experimental Design

At each of the three sites, we implemented an identical 2 x 2 x 2 full factorial randomized split block design replicated 5 times. The three treatments were: 1) activated carbon coated seeds, uncoated control seeds; 2) furrowed soil, or non-furrow control; and 3) herbicide treated and a no herbicide control. Each experimental block was split into two herbicide sub-block treatments: one treated with a pre-emergent herbicide imazapic, and one with no herbicide (Fig. 1-1). Within each herbicide/non-herbicide sub-block, we created furrows on half of the rows and left the others with no furrows. We then planted seed (control seed or carbon-coated seed) in all rows. Treatments were randomized and replicated within blocks with three replicate rows for each treatment. Each row was 1.2 m long, with 35 cm between each row. Furrow depth was 15 cm. Within each furrow type we planted carbon-coated or uncoated seed. We left a buffer zone of 1.05 m between sub-blocks to limit herbicide effects from neighboring treatments. Our seeding rate was 208 (pure live seed) PLS/m for the first fall planting and was reduced to 104 PLS/m during the second fall planting to reduce intraspecific competition and replicate suggested seeding rate according to the United States Department of Agriculture (USDA) plant fact sheet (Ogle, John, and Jones 2010).

We used *Pseudoroegnaria spicata* as our restoration species. It is a perennial bunchgrass native to the western US, and is often used in restoration seed mixes because of its drought tolerance and is thought to compete with annual invasive grasses (Melgoza, Nowak, and Tausch 1990). It is a slow growing perennial bunchgrass, so we report measurements after 2 years of growth knowing that small seedlings can survive and have high growth in subsequent years (Miller, Seufert, and Haferkamp 1994).

Activated Carbon Seed Coating

We coated bluebunch wheatgrass seeds with Nuchar AG® powdered activated carbon (MeadWestVaco Corporation, Richmond, VA). The formulation used for producing the coated seeds by dry weight was 67% activated carbon and 33% bulk seed. Using standard seed-coating methods, activated carbon was attached to the seeds with the partially hydrolyzed polyvinyl alcohol binder Selvol-205s (Sekisui Specialty Chemicals, Dallas TX; Table 1) that was prepared with a 12% solid content. We used a rotary seed coater to apply the treatment, and then placed the seed on a forced air drier for 10 min at 32°C (Brace Works Automation and Electric, Lloydminster, SK, CAN).

Herbicide Application

One of the two sub-blocks within each block were treated with a mixture of imazapic (Panoramic 2SL, Alligare, Opelika, AL), and glyphosate (Big and Tough, Gordon's Farm, Kansas City, MO). These two herbicides were mixed and applied at their respective recommended rates (acid equivalent) for *B. tectorum* control at 350 a.e. ha^{-1} and 840 a.e ha^{-1}. We applied herbicide with a electronic backpack sprayer to ensure even application rates of herbicide over treatments. During the herbicide application at the sites, wind never exceeded 5 kph, and maximum daily temperatures ranged 15-20 °C. We excluded granivory by rodents from the study plots in Great Basin National Park with fencing made of metal flashing placed around the plots. Rodent fencing was not used at the Provo site.

Furrows

Immediately after the herbicide was applied, furrows were formed by hand using a hoe. Seeds were planted in bottom of the furrows and were covered with 1 cm of soil. Each furrow/non-furrow row was 1.2 m long with 35 cm between rows. Excavated soil from furrows was deposited along the outside edge of the furrow.

Field Measurements

Each treatment (seed coating, furrow) was replicated three times within each sub-block (herbicide or non-herbicide). Our 2-year measurements were taken from the center of the 3 side-by-side replicate rows to limit edge effect bias in our measurements. Seedling emergence was monitored by weekly trips in the month of April, and then measured by one count in mid-late April according to peak emergence by site. Emergence data represents a compilation of two October plantings (2017 & 2018) and their respective emergence counts the following spring (2018 & 2019). Ocular *B. tectorum* cover estimates were made annually using a circular Daubenmire hoop that was laid over three replicate rows that had the same treatment. We visually estimated what percent of the area within the hoop was occupied by *B. tectorum*, using a smaller reference frame that represented 1% of the total hoop area (Bonham, Mergen, and Montoya 2004). We measured in absolute cover, estimated to the nearest 1%, considering bare ground in the space of the hoop, such that total plant cover may not occupy 100% of the area within the hoop. *B. tectorum* cover was visually estimated during the last week of May each year. Aboveground biomass was destructively sampled after two years growth from the middle row, by clipping and collecting all P. spicata aboveground biomass 5 cm above the ground. We collected 5 cm above the ground to eliminate dead plant material that may bias the sample. Plant density was determined by counting plants during 2-year biomass collection within each row and calculated as plants per square meter.

Statistical Analysis

We analyzed results using a linear mixed model in R version 3.4.2 (R Core Development Team, R Foundation for Statistical Computing, Vienna, AU). We fit models with log-transformed response variables and a gaussian error distribution. Modeling distributions were chosen by comparing residuals of the model under different error distributions and analyzing actual vs. predicted values plots. Response variables included in the model were seedling emergence, plant density, aboveground biomass 2 years after planting, and *B. tectorum* cover 1 and 2 years after planting. Our fixed effects were herbicide, furrow, carbon seed coatings and the two-way interaction of herbicide with furrows and carbon seed coatings. We split the analysis to better understand how furrows and carbon coatings function within herbicide environments, limiting noise in response from non-herbicide sub-blocks, and to better compare treatment effect. Site and block were included in the model as random effects, with block nested within site.

We were unable to build a model that tested the three-way interaction of herbicide x carbon coatings x furrow and met assumptions of normalized residuals. All statistics relating to the combination treatment use our pairwise analysis of treatments. Our model used for treatment pairwise comparison used treatment as the only fixed effect (8 levels) and block nested within site as a random variable. This analysis used a tukey adjustment for p values and the results table can be found in Table 1-2S and Table 1-3S.

RESULTS

Bromus tectorum Control

Bromus tectorum comprised 42% of plant cover in the first year and 46% in the second year in non-herbicide control plots (Fig. 1-2). Herbicide treatments (imazapic) reduced *B. tectorum* cover 91% (4% cover) at the end of the first spring (Table 1-1). Herbicide effects on *B. tectorum* weakened in the second year to 39% reduction (27% cover) (Fig. 1-2). Carbon coatings had no effect on *B. tectorum* cover in either herbicide or non-herbicide areas over the two-year study period (Table 1-1). Furrows reduced *B. tectorum* cover 30% in non-herbicide controls in year 1, but had no effect after two years (Table 1-1). Furrows did not significantly affect herbicide control of *B. tectorum* in herbicide treated plots (Fig. 1-2, Table 1-1).

Seedling Emergence 1st Year

Herbicide reduced *P. spicata* emergence by 38% in the absence of seed coating or furrow treatments (Table 1-1). Furrows mitigated the herbicide effect on seeds, producing similar emergence rates as furrow treatments without herbicide (Fig. 1-3a). Carbon seed coatings did not reduce herbicide effects on seedling emergence of *P. spicata* (Fig. 1-3b, Table 1-1). The combination of furrow and carbon coatings resulted in similar emergence to furrow treatments alone (P=0.97, Z Value = 0.04). In the absence of herbicide, furrows increased seedling emergence of *P. spicata* 1.8-fold compared to non-furrow controls, whereas carbon coatings had no effect on *P. spicata* seedling emergence (Table 1-1, Fig. 1-3). Combining carbon coating and furrow treatments in non-herbicide plots did not enhance or decrease emergence, producing similar emergence as furrow treatments alone (P=0.57, Z Score = 0.56).

Plant Density 2nd Year

Herbicide reduced *P. spicata* plant density 65% after two years when no seed coating or furrow was applied (Fig. 1-4, Table 1-1). Within herbicide treatments neither carbon seed coatings or furrow treatments significantly mitigated the effects of the herbicide P. spicata density as indicated by the insignificant interaction terms (Table 1-1). In herbicide treated plots, carbon coated seeds and furrow treatments produced 60% and 40% lower plant density than non-herbicide controls (Fig. 1-4, Table 1-1). The combination of carbon coatings and furrows produced similar plant densities in herbicide treatments as control seed (no coating, furrow, or herbicide). In absence of herbicide, both carbon coatings, furrow treatments, and their combination had no effect on plant density of *P. spicata*, producing similar plant density as control seed (no coating or furrow) after two years (Fig. 1-4).

Aboveground Biomass 2nd Year

Herbicide reduced *P. spicata* aboveground biomass 10-fold after two years when no seed coating or furrow was applied (Fig. 1-4, Table 1-1). Carbon coating and furrow treatments partially mitigated herbicide effects on *P. spicata* biomass as indicated by the significant interaction terms of carbon coatings and furrows with herbicide (Table 1-1). In herbicide treated plots, carbon-coated seeds and furrow treatments produced 11 and 13-fold more *P. spicata* biomass relative to unprotected seeds (no coating or furrow) (Fig. 1-4, Table 1-1). Despite high growth compared to the unprotected seed, total aboveground biomass after two years was similar to control seed planted in non-herbicide plots (Fig. 1-4). Combining carbon coatings and furrows in herbicide areas produced the most aboveground biomass on average of all treatments in herbicide and non-herbicide areas, but was highly variable resulting in statistically similar levels as control seed in non-herbicide areas (Fig. 1-4). In the absence of herbicide, both carbon coatings, furrow treatments, and their combination had no effect on aboveground growth of *P. spicata* relative to control seed (no coating, furrow, or herbicide) (Fig. 1-4).

DISCUSSION

Review of Hypotheses

This study tested the effects of herbicide, furrows and activated carbon coatings on control of *B. tectorum* and the emergence, growth and establishment of *P. spicata*. The data partially supported our first hypotheses that 1) herbicide application reduced establishment of *B. tectorum*. The application of the herbicide imazapic did reduce *B. tectorum* cover in both years following application, but the quick recovery of *B. tectorum* indicates that the control was only short-term. The data partially supported our second hypothesis, that 2) coating seeds in activated carbon and planting in furrows would limit exposure of *P. spicata* seeds to herbicide, resulting in better seedling emergence, survival, and growth. Both carbon coatings and furrow treatments improved growth (aboveground biomass) of *P. spicata* in herbicide treatments, but neither increased survival (plant density). We also saw an improvement in seedling emergence from furrow treatments, but neither treatment provided protection from herbicide effects to all plant life stages. This mixed response shows that both carbon coatings and furrows offer protection to some life stages, but not others. Our data support our third hypothesis, that 3) the combination of carbon coating and furrow treatments would protect *P. spicata* seeds from herbicide better than carbon coating or furrow treatments alone. Only with the combination of treatments did we see similar or improved growth and survival of *P. spicata* of all plant stages in herbicide plots compared to control seed (no carbon coating or furrow) in non-herbicide plots.

Herbicide Treatments

Herbicide treatments dramatically reduced *B. tectorum* cover in year 1 (Fig. 1-2), and continued to control *B. tectorum* cover in year 2, but the extent of control decreased in the second year (Fig. 1-2). This decline is likely herbicide specific, where the herbicide used in this study (imazapic) has high soil mobility and can leach to lower depths losing its effect on *B. tectorum* (Sebastian, Nissen, and De Souza Rodrigues 2016). The low control in year two may also be explained by high propagule pressure, a factor that enables *B. tectorum* to establish and spread quickly (Chambers et al. 2016). We anticipate that reinvasion occurred more quickly in our plots due to high *B. tectorum* density around our experimental plots (Fig. 1-5). When larger areas are treated with pre-emergent herbicides in conjunction with restoration seedings, the treated areas would most likely experience less propagule pressure, likely resulting in lower re-establishment of annual invasive grasses.

Carbon Coatings

The sensitivity of *P. spicata* seedling emergence to herbicide and the lack of protection provided by carbon coatings suggests that activated carbon seed coatings may not fully neutralize herbicide effects on seeds and young seedlings, but the positive effects of carbon coating on later growth provided some protection that benefits plant establishment over time (Figs. 1-3&1-4). The low 2-year growth of uncoated seed in herbicide treatments combined with the lack of a carbon coating effect on growth in non-herbicide areas suggests that the carbon coating reduced longer-term effects of the herbicide (Fig. 1-4). Activated carbon has been shown to neutralize herbicide (Davies, Madsen, and Hulet 2017), but individual seed coatings only provided a thin protective cover that may not fully eliminate herbicide effects. This was seen in a past study where individual carbon seed coatings did not fully eliminate herbicide effects at medium and higher herbicide application rates due to insufficient quantities of carbon surrounding the seed (Madsen et al. 2014a). We applied imazapic at a medium application rate (Morris, Monaco, and Rigby 2009) and found that carbon coating's resistance to the herbicide was not enough to completely mitigate the impacts on seedling emergence (Fig. 1-3) and subsequent survival (Fig. 1-4). However, for those seedlings that did emerge, carbon coatings had positive impacts on plant biomass after two years (Fig. 1-4). Mature plants that were initially impacted by imazapic application (without carbon-coatings) causing a drop in cover, have shown the ability to recover in growth over time (Sheley, Carpinelli, and Morghan 2007; Shinn and Thill 2004). This delayed recovery highlights the need for long-term monitoring in restoration studies involving herbicide, where initial effects may not indicate long-term trends.

Furrows

Restoration success in annual grass invaded areas has historically been variable, and usually involves separating control of invasive annual grasses and seeding efforts to reduce herbicide injury to seeded species (Sbatella et al. 2011; Davies 2010). Our data suggest that planting in a furrow created after herbicide application allowed simultaneous reduction of *B. tectorum* cover while mitigating herbicide impacts on *P. spicata* emergence and 2-year aboveground biomass (Fig. 1-3). The creation of the furrow side sweeps the soil that has been treated with herbicide, leaving a safe site for native species (Eckert and Archives 1974). It also creates a more suitable microenvironment by increasing water availability and reducing temperature variability (Eckert Jr and Evans 1967; Gupta et al. 1990). Past studies have shown that improvements to seedbed conditions are key to seedling success (Tessema, de Boer, and Prins 2016; Snyman and van Wyk 2005; Kassahun, Snyman, and Smit 2009), which is consistent with the near 2-fold increase in emergence we observed in furrowed rows (Fig. 1-3).

The ability of furrow treatments to minimize herbicide effect on seeded species while partially controlling annual grass cover creates a window of reduced competition that can result in more growth of a native species (Fig. 1-4). It has been shown that in the absence of competition with exotic annual grasses, native plants are more likely to establish and successfully recruit (Eckert Jr and Evans 1967). This was consistent with our results in which herbicide reduced *B. tectorum* cover, and the release of competition from *B. tectorum* allowed the lower plant densities of *P. spicata* to experience higher growth rates and produce similar aboveground biomass per row as the higher density non-herbicide treatments (Figs. 1-4).

Combining Carbon Coatings and Furrows

Combining herbicide mitigation technologies of carbon seed coatings and furrows was the only treatment to eliminate herbicide effect at all growth stages (Fig. 1-4). Both carbon coatings and furrows partially mitigated herbicide effect, but neither was able to maintain similar plant densities as non-herbicide plots. Past studies have shown that individual carbon seed coatings did not have enough carbon to mitigate herbicide effects at high application rates (Madsen et al. 2014b), and that furrows limited herbicide effect when created one year after herbicide application(Eckert and Archives 1974). Using the side-sweeping mechanism of furrow creation, we lowered the concentration of herbicide in the soil surrounding the seed such that carbon coatings were adequate to protect seeds. The addition of furrows also resulted in more seedling emergence that may have helped with subsequent growth and survival.

Implications and Management Recommendations

Bromus tectorum is transforming landscapes across the western United States in what many refer to as the most significant plant invasion in North America (Knapp 1996; Chambers et al. 2007; D'Antonio and Vitousek 1992; Corbin and D'Antonio 2004; Boyte, Wylie, and Major 2016). Here we show how *B. tectorum* can be controlled (at least in the short term) with the pre-emergent herbicide imazapic, while simultaneously establishing a native bunchgrass. Carbon seed coatings and deep furrow treatments, may also have application for mitigating herbicide effects on other species. Carbon seed coatings can be applied on seeds of different sizes, and furrows are likely to work with most species. However, additional research is needed to evaluate these technologies on other species, soil types, and climates before they can be recommended as a restoration treatment. For example, some species are more sensitive to pre-emergent herbicide (Shinn and Thill 2004; Kyser et al. 2013), and the protection from carbon coatings and deep furrows may not be adequate to promote survival. The deep furrow treatment worked well with our model species, which has a relatively large seed. Smaller seeded species, in particular, may be limited with this treatment if the sidewalls of the furrow slough off and cover seeds to a depth that limits seedling emergence.

Imazapic reduced cheatgrass cover, but the effect was only temporary, indicating that more research is needed to achieve longer lasting control of invasives. The success seen in our study may not apply to all herbicides, as they differ in soil mobility, seed lethality, and persistence within the soil that may reduce the protective efficacy of furrows or carbon coatings. Notwithstanding, this method could prove valuable if herbicide control of annual invasive grasses can be optimized to control for longer periods.

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FIGURES

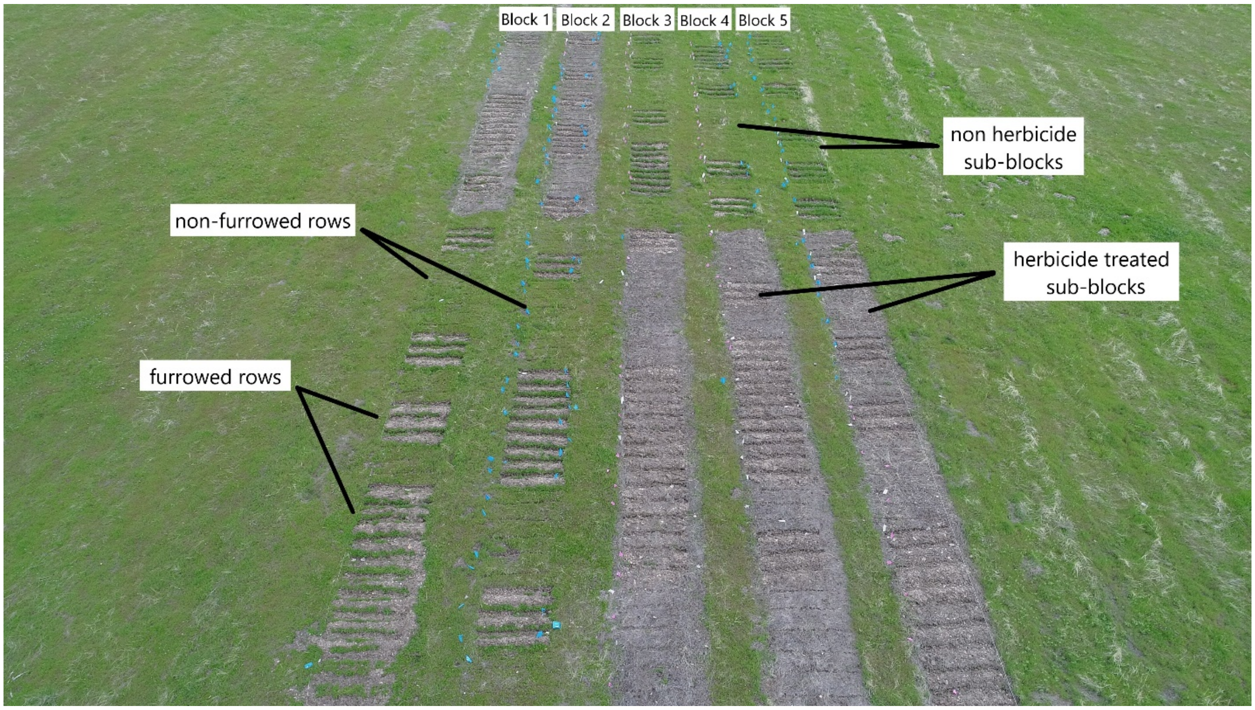

Figure 1-1. Aerial photo of Provo study site showing the layout of the experimental design.

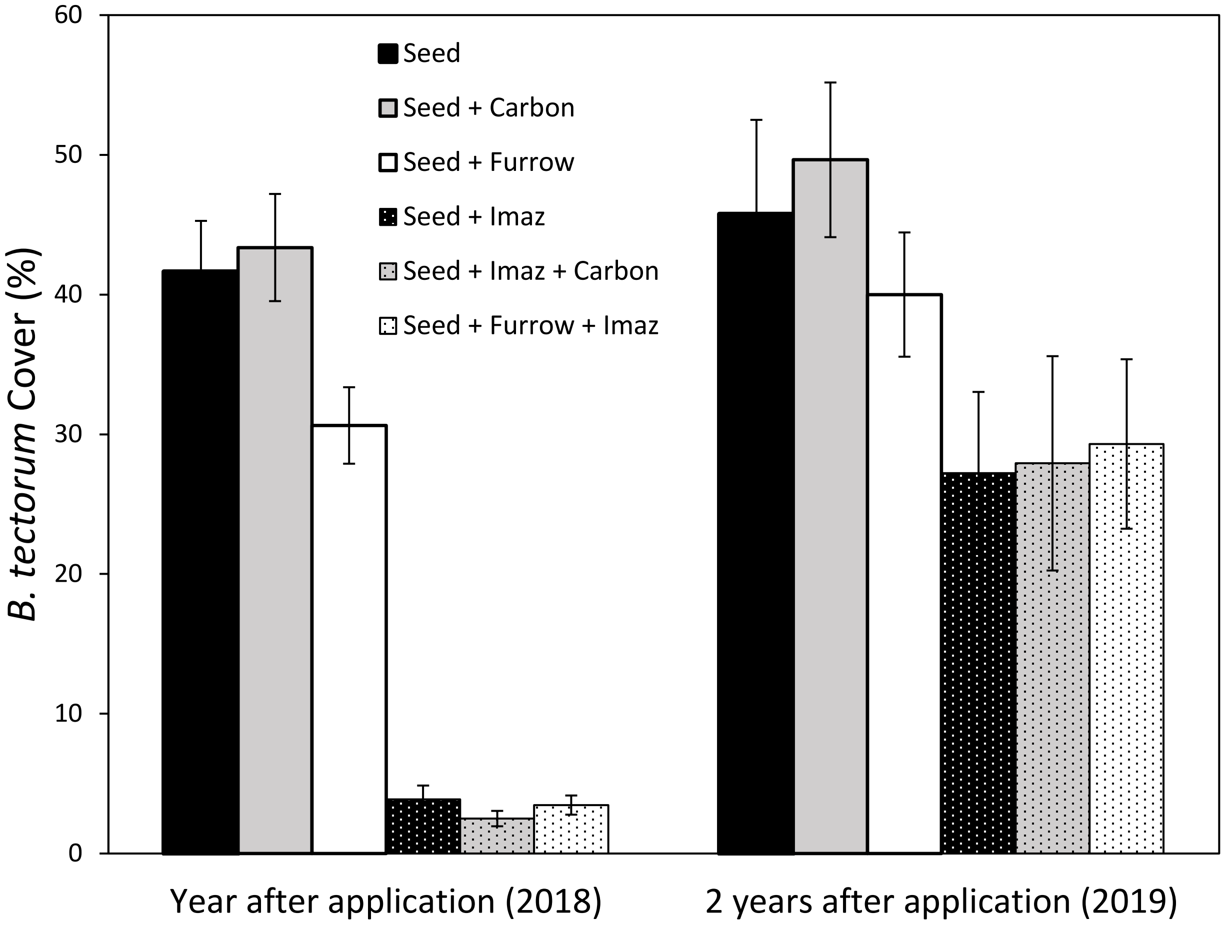

Figure 1-2. Bromus tectorum cover by treatment over the two years of the study. Data represent mean and standard error (bars) averaged across the three research sites in the Great Basin, in June 2018 and June 2019.

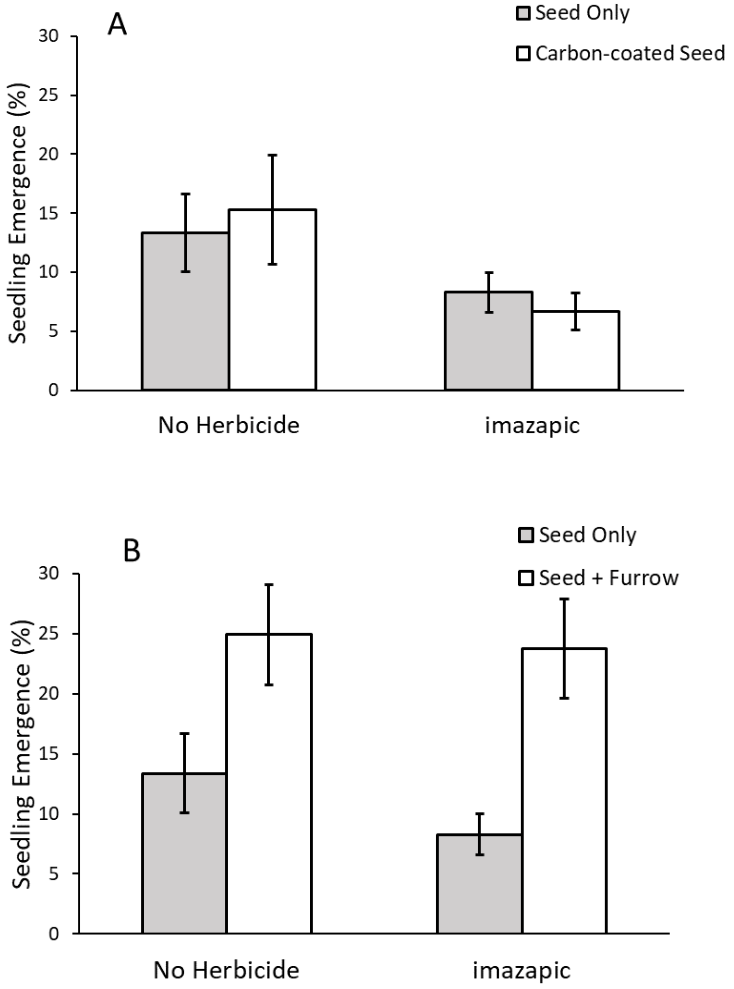

Figure 1-3. Seedling emergence % (seedlings emerged/seeds planted) of Pseudoroegnaria spicata in response to herbicide (imazapic), carbon seed coating and furrows. Data represent the average response from three research sites and two independent plantings that occurred in 2017 and 2018.

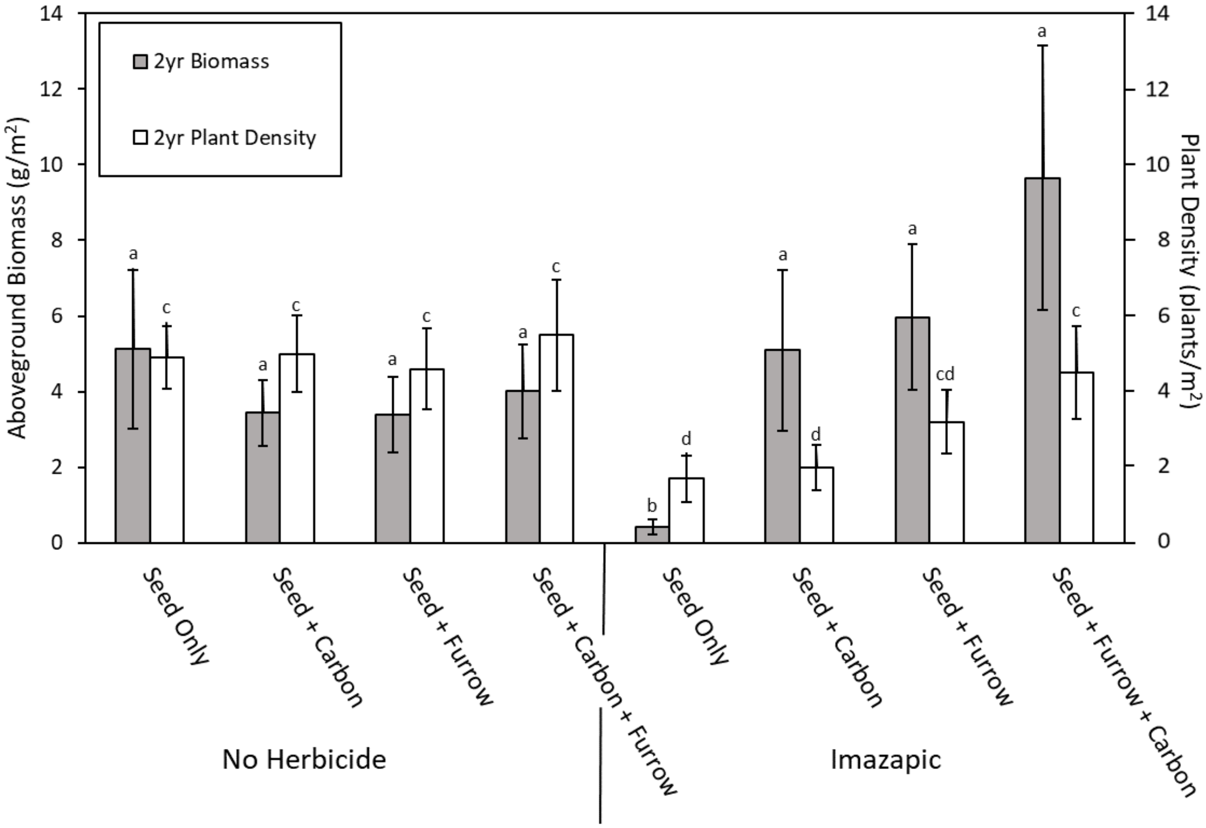

Figure 1-4. Average aboveground biomass and plant density of Pseudoroegnaria spicata in response to herbicide (imazapic), carbon seed coating and furrows. Data represents averages of 2 sites two years after planting in 2017.

Figure 1-5. Photo taken at the lower elevation site in Great Basin National Park showing growth at the end of summer, one year after herbicide application, and the high invasive propagule pressure from areas that received no herbicide treatment.

TABLES

Table 1-1. Summary of statistical results of regressions for response variables of *Pseudoroegnaria spicata* (bluebunch wheatgrass) and *Bromus tectorum* (cheatgrass): 2-year *P. spicata* aboveground biomass size (g/m2), 2-year *P. spicata* plant size (g/plant), *P. spicata* seedling emergence (% emergence), 1st year and 2nd year *B. tectorum* cover (%).

Cheatgrass (*B. tectorum*)

	Treatment	Estimate	Std. Error	df	t value	Pr(>|t|)
1st yr Cheatgrass Cover	Seed Only	35.867	5.943	2.7	6.04	0.012
	Carbon Coating	0.171	3.499	70.9	0.05	0.961
	Furrow	-6	3.489	70.2	-1.72	0.090
	Herbicide	-31.467	3.489	70.2	-9.02	2.38E-13
	Furrow + Herbicide	-32	3.489	70.2	-9.17	1.25E-13
	Carbon Coating + Herbicide	-32.733	3.489	70.2	-9.38	5.15E-14

	Treatment	Estimate	Std. Error	df	t value	Pr(>|t|)
2nd yr Cheatgrass Cover	Seed Only	43.721	7.525	7.0	5.81	0.001
	Carbon Coating	4.279	7.818	81.0	0.55	0.586
	Furrow	-4.321	7.818	81.0	-0.55	0.582
	Herbicide	-15.655	7.818	81.0	-2.00	0.049
	Furrow + Herbicide	-13.655	7.818	81.0	-1.75	0.084
	Carbon Coating + Herbicide	-11.188	7.818	81.0	-1.43	0.156

Bluebunch Wheatgrass (*P. spicata*)

	Treatment	Estimate	Std. Error	df	t value	Pr(>|t|)
Seedling Emergence	(Intercept)	14.086	3.727	3.7	8.34	0.003
	Herbicide	-5.419	2.594	87.0	-2.03	0.046
	Carbon Coating	1.599	2.594	87.0	0.02	0.841
	Herbicide x Carbon Coating	-3.199	3.706	87.0	-0.68	0.498
	(Intercept)	13.767	3.478	3.4	8.53	0.002
	Herbicide	-5.1	3.168	87.0	-1.72	0.089
	Furrow	11.633	3.168	87.0	3.54	0.001
	Herbicide x Furrow	3.967	4.481	87.0	0.97	0.334

Treatment	Estimate	Std. Error	df	t value	Pr(>|t|)

2 yr Aboveground Biomass	(Intercept)	5.123	1.559	31.9	7.78	7.34E-09
	Herbicide	-4.697	1.823	27.0	-3.01	0.006
	Carbon Coating	-1.679	1.823	27.0	-0.44	0.663
	Herbicide x Carbon Coating	6.345	2.578	27.0	2.13	0.042
	(Intercept)	5.123	1.507	36.0	8.37	5.78E-10
	Herbicide	-1.723	2.131	36.0	-0.50	0.624
	Furrow	-4.697	2.131	36.0	-2.88	0.007
	Herbicide x Furrow	7.271	0.4004	36.0	2.71	0.010

	Treatment	Estimate	Std. Error	df	t value	Pr(>|t|)
2 yr Average Plant Size	(Intercept)	0.8781	0.3039	33.8	11.68	2.05E-13
	Herbicide	-0.7416	0.3853	27.0	-2.31	0.029
	Carbon Coating	-0.2998	0.3853	27.0	-0.72	0.481
	Herbicide x Carbon Coating	1.5057	0.5448	27.0	2.69	0.012
	(Intercept)	0.8781	0.2504	36.0	13.85	5.4E-16
	Herbicide	-74.16	0.3541	36.0	-2.53	1.6E-02
	Furrow	-0.2697	0.3541	36.0	-0.71	4.8E-01
	Herbicide x Furrow	1.6738	0.5008	36.0	3.61	9.2E-04

	Treatment	Estimate	Std. Error	df	t value	Pr(>|t|)
2 yr Plant Density	(Intercept)	1.8419	0.1654	35.4	11.13	4.10E-13
	Herbicide	-0.6476	0.2247	27.0	-2.88	0.008
	Carbon Coating	-0.0241	0.2247	27.0	-0.011	0.915
	Herbicide x Carbon Coating	0.1012	0.3178	27.0	0.32	0.753
	(Intercept)	1.842	0.169	36.0	10.87	6.40E-13
	Herbicide	-0.648	0.24	36.0	-2.7	0.012
	Furrow	-0.119	0.24	36.0	-0.5	0.623
	Herbicide x Furrow	0.469	0.339	36.0	1.38	0.185

SUPPLEMENTAL MATERIAL

Table 1-1S. Description of study sites.

Site	Slope	Elevation (m)	Aspect	MAT (°C)	MAP (mm)	2018 Precip	2019 Precip
Kious	9%	2041	SE	9.33	307.2	236 (76%)	356 (115%)
Lehman	6%	2069	East	9.06	344.5	254 (64%)	394 (114%)
Provo	8%	1448	West	11.4	485.4	286 (59%)	659 (135%)

Table 1-2S. Summary of statistical results from pairwise comparison of 2-year plant density of *Pseudoroegnaria spicata* (bluebunch wheatgrass) with treatments of herbicide (imazapic), activated carbon seed coatings, and furrows. Results represent data from 2 sites in the sagebrush steppe system.

Comparison	Estimate	SE	df	Z ratio	P value
Seed - Seed + Carbon	-0.0202	0.2	-	-0.101	1
Seed - Seed + Carbon + Furrow	-0.1155	0.196	-	-0.591	0.999
Seed - Seed + Furrow	0.0632	0.204	-	0.309	1
Seed - Seed + Furrow + Imaz	0.4261	0.226	-	1.883	0.5629
Seed - Seed + Furrow + Imaz + Carbon	0.0852	0.206	-	0.414	0.9999
Seed - Seed + Imaz	1.0586	0.28	-	3.777	0.004
Seed - Seed + Imaz + Carbon	0.8961	0.264	-	3.392	0.016
Seed + Carbon - Seed + Carbon + Furrow	-0.0953	0.195	-	-0.49	0.9997
Seed + Carbon - Seed + Furrow	0.0834	0.203	-	0.41	0.9999
Seed + Carbon - Seed + Furrow + Imaz	0.4463	0.225	-	1.98	0.4955
Seed + Carbon - Seed + Furrow + Imaz + Carbon	0.1054	0.205	-	0.515	0.9996
Seed + Carbon - Seed + Imaz	1.0788	0.28	-	3.859	0.0029
Seed + Carbon - Seed + Imaz + Carbon	0.9163	0.264	-	3.478	0.0119
Seed + Carbon + Furrow - Seed + Furrow	0.1787	0.199	-	0.898	0.9863
Seed + Carbon + Furrow - Seed + Furrow + Imaz	0.5416	0.221	-	2.446	0.219
Seed + Carbon + Furrow - Seed + Furrow + Imaz + Carbon	0.2007	0.2	-	1.003	0.9742
Seed + Carbon + Furrow - Seed + Imaz	1.1741	0.276	-	4.249	0.0006
Seed + Carbon + Furrow - Seed + Imaz + Carbon	1.0116	0.26	-	3.891	0.0025
Seed + Furrow - Seed + Furrow + Imaz	0.3629	0.229	-	1.583	0.7606
Seed + Furrow - Seed + Furrow + Imaz + Carbon	0.022	0.209	-	0.105	1
Seed + Furrow - Seed + Imaz	0.9954	0.283	-	3.522	0.0102
Seed + Furrow - Seed + Imaz + Carbon	0.8329	0.267	-	3.123	0.038
Seed + Furrow + Imaz - Seed + Furrow + Imaz + Carbon	-0.3409	0.23	-	-1.481	0.8181
Seed + Furrow + Imaz - Seed + Imaz	0.6325	0.299	-	2.117	0.404
Seed + Furrow + Imaz - Seed + Imaz + Carbon	0.47	0.284	-	1.656	0.7158
Seed + Furrow + Imaz + Carbon - Seed + Imaz	0.9734	0.283	-	3.434	0.0138
Seed + Furrow + Imaz + Carbon - Seed + Imaz + Carbon	0.8109	0.268	-	3.03	0.0501
Seed + Imaz - Seed + Imaz + Carbon	-0.1625	0.329	-	-0.495	0.9997

Table 1-3S. Summary of statistical results from pairwise comparison of 2-year aboveground biomass of *Pseudoroegnaria spicata* (bluebunch wheatgrass) with treatments of herbicide (imazapic), activated carbon seed coatings, and furrows. Results represent data from 2 sites in the sagebrush steppe system.

Comparison	**Estimate**	**SE**	**df**	**T ratio**	**P value**
Seed - Seed + Carbon	0.1313	0.433	63	0.303	1
Seed - Seed + Carbon + Furrow	0.1839	0.433	63	0.425	0.9999
Seed - Seed + Furrow	0.1597	0.433	63	0.369	1
Seed - Seed + Furrow + Imaz	-0.163	0.433	63	-0.376	0.9999
Seed - Seed + Furrow + Imaz + Carbon	-0.2851	0.433	63	-0.658	0.9978
Seed - Seed + Imaz	1.1038	0.433	63	2.549	0.1948
Seed - Seed + Imaz + Carbon	0.2095	0.433	63	0.484	0.9997
Seed + Carbon - Seed + Carbon + Furrow	0.0527	0.433	63	0.122	1
Seed + Carbon - Seed + Furrow	0.0285	0.433	63	0.066	1
Seed + Carbon - Seed + Furrow + Imaz	-0.2943	0.433	63	-0.679	0.9973
Seed + Carbon - Seed + Furrow + Imaz + Carbon	-0.4164	0.433	63	-0.961	0.9782
Seed + Carbon - Seed + Imaz	0.9726	0.433	63	2.245	0.3398
Seed + Carbon - Seed + Imaz + Carbon	0.0782	0.433	63	0.181	1
Seed + Carbon + Furrow - Seed + Furrow	-0.0242	0.433	63	-0.056	1
Seed + Carbon + Furrow - Seed + Furrow + Imaz	-0.347	0.433	63	-0.801	0.9925
Seed + Carbon + Furrow - Seed + Furrow + Imaz + Carbon	-0.4691	0.433	63	-1.083	0.9581
Seed + Carbon + Furrow - Seed + Imaz	0.9199	0.433	63	2.124	0.4115
Seed + Carbon + Furrow - Seed + Imaz + Carbon	0.0255	0.433	63	0.059	1
Seed + Furrow - Seed + Furrow + Imaz	-0.3228	0.433	63	-0.745	0.9951
Seed + Furrow - Seed + Furrow + Imaz + Carbon	-0.4449	0.433	63	-1.027	0.9685
Seed + Furrow - Seed + Imaz	0.9441	0.433	63	2.18	0.3778
Seed + Furrow - Seed + Imaz + Carbon	0.0497	0.433	63	0.115	1
Seed + Furrow + Imaz - Seed + Furrow + Imaz + Carbon	-0.1221	0.433	63	-0.282	1
Seed + Furrow + Imaz - Seed + Imaz	1.2669	0.433	63	2.925	0.0846
Seed + Furrow + Imaz - Seed + Imaz + Carbon	0.3725	0.433	63	0.86	0.9885
Seed + Furrow + Imaz + Carbon - Seed + Imaz	1.389	0.433	63	3.207	0.0414
Seed + Furrow + Imaz + Carbon - Seed + Imaz + Carbon	0.4946	0.433	63	1.142	0.9447
Seed + Imaz - Seed + Imaz + Carbon	-0.8944	0.433	63	-2.065	0.4484

CHAPTER 2

Herbicide Effects on the Establishment of a Native Bunchgrass in Cheatgrass Invaded Areas: Indaziflam vs. Imazapic

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Master of Science

ABSTRACT

Annual grass invasion is transforming the western US and driving a need for restoration techniques that can both reduce the abundance of exotic annual grasses and allow revegetation of native species. Pre-emergent herbicides can provide control of annual grasses, but when applied concurrently with direct seeding efforts, the herbicide can also impact seeded species. Indaziflam is a relatively new pre-emergent herbicide that may provide extended control of exotic annual grasses, but little is known about its effects when applied at the time of seeding. In this study, we compared indaziflam to imazapic, a popular herbicide used in restoration efforts, to understand how indaziflam affects plant establishment of a native species, bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Á. Löve). We created furrows on half of our treatments to limit herbicide concentrations and potentially create a safe-site for seeding. During the two-year study, indaziflam provided consistent control of the annual weed, cheatgrass (*Bromus tectorum* L.), whereas imazapic control decreased sharply with time. Indaziflam and imazapic decreased bluebunch wheatgrass seedling emergence by 96 and 46%, and two-year plant density by 91 and 65%, respectively. Both herbicides reduced aboveground biomass by over 85% two years after seeding/herbicide application. Furrow treatments mitigated the effects of imazapic on bluebunch wheatgrass, but did not limit the impacts by indaziflam. Future research is now merited for

evaluating the combined use of imazapic and furrows at larger scales to determine if this treatment can be used in restoration efforts. Indaziflam does not appear to work currently with seeding efforts and alternate application strategies should be found for this herbicide, such as applying after seeded species are established to provide long-term control of invasive weeds.

INTRODUCTION

Arid and semi-arid ecosystems comprise over one-third of earth's terrestrial surface (Schlesinger et al. 1990), with many facing the threat of exotic annual grass invasion (Brooks et al. 2004; D'Antonio and Vitousek 1992). Annual grass invasions often lead to decreased plant and wildlife diversity by means of competition for soil moisture, accelerated fire cycles, and altered soil nutrient cycling (Knapp 1996; Ehlert 2017; Peters and Bunting 1994; Bishop et al. 2016; Kerns and Day 2017). The sagebrush steppe is a representative arid/semi-arid ecosystem vulnerable to invasion due to historic of overgrazing (D'Antonio and Vitousek 1992), altered fire regimes (Knapp 1996), and fluctuations in precipitation patterns (Chambers et al. 2007; Bradley and Mustard 2005; Davis, Grime, and Thompson 2000). Plant invasion by annual grasses has transformed native plant communities in the sagebrush steppe in what many refer to as the most significant plant invasion in North America (Knapp 1996; Chambers et al. 2007; Corbin and D'Antonio 2004; Boyte, Wylie, and Major 2016). One prominent plant invader, cheatgrass (*Bromus tectorum* L.), is estimated to now cover more than 21 million hectares in the western United States, with an estimated 14% annual rate of spread (Duncan and Clark 2005; Bradley et al. 2017). Innovative restoration techniques are needed to restore native vegetation to landscapes now dominated by invasive annual grasses.

Controlling invasive species proves vital to restoring native plant species, as invasives often outcompete native species after disturbance (Sheley, Larson, and Johnson 1993), and quickly monopolize the seedbank leaving reduced opportunity for native species to re-establish (Humphrey and Schupp 2001). Use of pre-emergent herbicide is a common, and effective way to reduce invasive plant abundance (Mangold et al. 2013), but when used after seeding efforts can negatively impact the establishment of native shrubs (Owen, Sieg, and Gehring 2011), and perennial grasses (Shinn and Thill 2004a). However, when the effects of the herbicide are limited to the invasive species, herbicide can improve the establishment of native plants by reducing competition for resources (Eckert and Archives 1974; Sheley, Carpinelli, and Morghan 2007).

Seedbed preparation such as furrows could potentially mitigate the harmful effects of herbicides on native species. If herbicide effect is lowered for non-target species, it would allow restoration seedings in systems that also need control of invasives. Usually this task approached with invasive control and seeding efforts occurring separately, diminishing the opportunity to establish native plants in an environment with low invasive competition (Madsen et al. 2014). Furrows are a common practice in agriculture that improves water availability, but can also have the potential to limit exposure of non-target species to herbicide (Eckert et al. 1974). Creating a furrow after herbicide application side-sweeps surface soil that has been sprayed with herbicide, creating a potential safe site for desirable seeded species with low herbicide concentrations. The furrow may also remove weed seed within the area the seeds are planted. Subsequently, this treatment may provide protection to the seeded species without reducing the herbicide control of invasive weeds.

The development of pre-emergent herbicides could aid restoration efforts in invaded systems. Imazapic is currently the most commonly recommended pre-emergent herbicides for invasive annual grass control (Mangold et al. 2013). Imazapic kills plants by inhibiting the activity of the enzyme acetohydroxy acid synthase (AHAS or ALS), an enzyme that is responsible for the biosynthesis of the branched-chain amino acids isoleucine, valine, and leucine (Umbarger 1978). Inhibiting ALS effectively starves the plant of these essential amino acids and is thought to be the herbicide's mechanism causing plant death (Tranel and Wright 2002). While imazapic provides strong control for one year, there is some evidence that it has limited soil residual activity, which results in inferior control of invasive annual after one year (Sebastian, Fleming, et al. 2017). Short-term control of invasives poses a problem for re-invasion (Morris, Monaco, and Rigby 2009), which increases competition on young plants. A new pre-emergent herbicide called indaziflam is now being tested for the control of annual rangeland weeds (Sebastian et al. 2017). Indaziflam is an alkylazine herbicide that controls annual invasive grasses by inhibiting biosynthesis of cellulose in susceptible species (Brabham et al. 2014). This herbicide has been shown to control cheatgrass up to three years after application (Sebastian et al. 2016). Indaziflam's extended control is largely due to low soil mobility (Alonso et al. 2011; Jhala and Singh 2012), and a longer soil half-life (>150 d) than many other pre-emergent herbicides including imazapic.

Successful seeding efforts are comprised of strong emergence, survival, and growth. Pre-emergent herbicides can have different effects on each plant growth stage (Shinn and Thill 2004b; Sebastian, Fleming, et al. 2017). Imazapic and indaziflam differ in their soil mobility, persistence, and mechanism, which may affect plant growth stages differently. It remains unknown if these differences make these herbicides more or less problematic for non-target native species in restoration efforts.

Many studies have compared herbicide control of invasive annual grasses (Elseroad and Rudd 2011, Mangold et al. 2013, Sebastian et al. 2017b), but few have examined potential ways to combine invasive species control with strategies that mitigate control efforts on non-target species to restore native plant communities. Here we study two herbicides with different mechanisms and soil mobility looking for potential opportunities to limit herbicide effect to annual grasses and reduce negative effects on species seeded for restoration. Our first objective of this study was to understand how indaziflam and imazapic differentially affect a commonly seeded restoration species in the sagebrush steppe, bluebunch wheatgrass (*Pseudoroegnaria spicata* (Pursh) Á. Löve). The herbicides effect on bluebunch wheatgrass was assessed by measuring changes to the following demographic stages, emergence, plant density, and above ground biomass two years after planting. Our second objective was to compare how these herbicides control cheatgrass. The third objective of this study was to determine if we could simultaneously reduce cheatgrass densities with herbicide while protecting our seeded species, bluebunch wheatgrass. We hypothesize that: 1) indaziflam will provide superior control of cheatgrass over a two-year period based on results from other studies. 2) Indaziflam will be more lethal to bluebunch wheatgrass than imazapic due to its novel mechanism. 3) The side sweep action of furrow creation will reduce herbicide effects of indaziflam on bluebunch wheatgrass more than imazapic due to low soil mobility.

MATERIALS AND METHODS

Study Sites

This study was conducted at three sites in the sagebrush steppe system during the years 2017-2019. Sites 1 & 2 are located in the boundaries of Great Basin National Park, Nevada, and site 3 is located in Provo, Utah. Sites vary in slope, elevation, and soil type (Table 1-1S). Elevation between sites was1448m at the Provo site (Utah), 2013 m at Lehman flats site (Nevada), and 2135m at the Kious basin site (Nevada). Soils types across sites vary from stony loam (Lehman flats), to gravelly loamy coarse sand (Kious basin), and gravelly loam (Provo). All sites were invaded to the extent that cheatgrass comprised 40-80% relative cover. Site vegetation at the Nevada sites was dominated by cheatgrass, but also contained several native species: *Elymus elymoides* (Nutt.), *Artemesia, tridentate spp.*, *Pinus monophyla* (Torr. & Frem.), *Gutierrezia sarothrae* (Pursh), and *Purshia tridentate* (Pursh). Precipitation at the sites in 2018 consisted of an average spring (93-136%) and a dry summer (62-102%). In 2019 the precipitation consisted of a very wet spring (160-178%) and dry summer (46-51%) (DayMET).

Experimental Design

Research plots were installed between 30 October - 5 November 2017. We tested the establishment and growth of bluebunch wheatgrass in response to herbicide treatment using a 3 x 2 full factorial design. We had three herbicide treatments: imazapic, indaziflam, and no herbicide, accompanied by two planting methods: planting within a furrow, and planting without a furrow after herbicide application. We created five replicate blocks, split into three sub-blocks, one treated with imazapic, one treated with indaziflam, and one receiving no herbicide (Figure 2-1). Location of herbicides within each block was randomized. Immediately following herbicide applications, furrows were created in half of the rows within each sub-block, and seeds were planted in both furrowed and non-furrowed rows.

Herbicide Application

Herbicide treatments were applied as follows: no herbicide treatment (control), a mixture of imazapic and glyphosate at respective rates of 148 and 355 ml per acre (350 a.e. ha^{-1} and 840 a.e ha^{-1}), and a mixture of indaziflam and glyphosate at the rates of 148 and 355 ml per acre (350 a.e. ha^{-1} and 840 a.e ha^{-1}). Herbicide was applied using a calibrated electric backpack sprayer with a wand (model number: 63985, Chapin, Batavia, NY). Herbicide application occurred on days with little to no wind, abundant sun, and daily maximum temperatures exceeding 15-20°C.

Furrows

Furrows were created immediately following herbicide application to maximize the side-sweeping action of herbicide treated soil that occurs with furrow creation. Furrows were 15 cm deep from soil surface, and 35 cm wide. The depth and width were chosen based on capabilities of furrow creation on large-scale seedings with drill seeders using cultivator sweeps. All rows (furrowed and non-furrowed) were spaced 35 cm apart and 1.2 m long. Soil was excavated with a garden hoe, placing the soil removed from the furrow in mounds between rows in efforts to replicate furrows created by cultivator sweeps in restoration settings. Bluebunch wheatgrass seeds were buried 1 cm below the surface soil surface (control) or covered in 1 cm soil in furrow bottoms the last week of October 2017.

Plant Measurements

Seedling emergence of bluebunch wheatgrass was characterized at the end of April 2018 by individually counting all live seedlings in each row. Aboveground biomass of bluebunch wheatgrass was sampled in late August 2019 two years after the initial planting. Biomass samples were collected by clipping all aboveground biomass at ground level. Cheatgrass cover was measured visually during the last week of May 2018 and May 2019. Cover estimates were made visually to the nearest 1% using a circular Daubenmire hoop (Bonham, Mergen, and Montoya 2004). The hoop used was 1 m in diameter and placed over 3 side-by-side replicates of one treatment. Percent of total ground area occupied by cheatgrass within the hoop was estimated visually using a smaller reference frame that represented 1% of the total hoop area.

Statistical Methods

We used a mixed model linear regression for analysis of bluebunch wheatgrass and cheatgrass responses in our study. All analyses were done in R version 3.4.2 (R Core Development Team, R Foundation for Statistical Computing, Vienna, Austria). Response variables for bluebunch wheatgrass were emergence (counts), plant size (g per plant), and total aboveground biomass (g per row). Fixed variables were herbicide type, and deep furrows. Random variables were site and block, with block nested within site. Response variables were log transformed to produce near normal error distributions. Pairwise comparisons of treatments were done using a Tukey-Kramer adjustment within our ANOVA analysis. Results were deemed significant if p or z values were below 0.1.

RESULTS

Cheatgrass Control

Imazapic and Indaziflam reduced cheatgrass cover 88% and 70% ($P<0.001$) compared to non-herbicide control plots after one year (Figure 2-2). Despite, imazapic providing stronger control than indaziflam in the first year, indaziflam provided superior control by year 2 (spring 2019) (Figure 2-2). Indaziflam maintained 70% cheatgrass control throughout the two-year period (P<0.001), whereas imazapic control of cheatgrass decreased from 88% control in the first year to only 20% in the second year (P=0.03) (Figure 2-2).

Bluebunch Wheatgrass Emergence

Imazapic and indaziflam application decreased bluebunch wheatgrass seedling emergence 46% and 96% compared to herbicide control plots (P<0.001) (Figure 2-3a, Table 2-1). In non-herbicide plots, furrow treatments increased bluebunch wheatgrass emergence 32% (P<0.001) compared to the non-furrow treatments. Furrows reduced herbicide effects of imazapic on emergence of bluebunch wheatgrass such that they were similar to seedling emergence observed in furrow treatments within non-herbicide plots (P=0.99) (Figure 2-3), as indicated by the significant interaction between imazapic and furrow treatments (Table 2-1). In contrast, furrows did not protect bluebunch wheatgrass seeds from indaziflam, resulting in similar low seedling emergence as indaziflam treatments without a furrow (Figure 2-3). Beyond protection, indaziflam negated the positive effect of the furrow seen in non-herbicide plots as indicated by the negative indaziflam by furrow interaction term (Table 2-1).

Bluebunch Wheatgrass Plant Density

Imazapic and Indaziflam reduced plant density of bluebunch wheatgrass 65% and 91% compared to non-herbicide controls (P<0.001) (Figure 2-4a, Table 1). Furrow treatments did not affect plant density in non-herbicide treatments (Table 2-1). Furrow treatments mitigated the herbicide effect of imazapic on bluebunch wheatgrass plant density, producing similar densities as non-herbicide treatments (Figure 2-4b). Furrows did not mitigate herbicide effects of indaziflam on bluebunch wheatgrass plant density, resulting in similar low seedling emergence as indaziflam treatments without a furrow (Figure 2-4).

Bluebunch Wheatgrass Growth

Imazapic and indaziflam herbicide treatments reduced aboveground biomass of bluebunch wheatgrass by over 98% after two growing seasons when planted without a furrow ($P<0.001$) (Figure 2-5a, Table 2-1). In the absence of herbicide, furrows did not significantly affect aboveground biomass of bluebunch wheatgrass compared to non-furrowed rows (Table 2-1). In herbicide applications however, furrows mitigated the negative effects of imazapic treatments on aboveground growth, as indicated by the imazapic x furrow interaction term. Furrow treatments within imazapic treated plots produced 14-fold more aboveground biomass than non-furrow treatments in imazapic treated plots ($P<0.001$) (Figure 2-5, Table 2-1). Furrows in indaziflam treatments did not protect plants, as indicated by the insignificant indaziflam x furrow term, resulting in little to no aboveground biomass (Figure 2-5, Table 2-1).

DISCUSSION

Review of Hypotheses

Herbicide treatments had large effects on the growth of bluebunch wheatgrass, which differed depending on herbicide type (Figure 2-3). In respect to our hypotheses, our results support our first hypothesis showing that indaziflam provides better control of cheatgrass after two years, despite stronger control by imazapic in the first year after application. Our results show partially support our second hypothesis, 2) imazapic was less detrimental to bluebunch wheatgrass plant density than imazapic, but both imazapic and indaziflam applications resulted in similar bluebunch wheatgrass aboveground biomass. Our data does not support our third hypothesis, 3) creating a furrow to limit herbicide effect on our planted seed was a more effective with imazapic, showing no benefit when used with indaziflam.

Herbicide Effects on Cheatgrass

Reinvasion of areas treated with imazapic occurred quickly, with cheatgrass recovering two years after the initial application (Figure 2-2). Imazapic is a strong control agent immediately following application, but due to higher soil mobility and a shorter soil half-life it may not completely control cheatgrass 1-2 years after application (Sebastian, Nissen, and De Souza Rodrigues 2016). We anticipate that reinvasion happened more quickly in our study system than it would in a large-scale imazapic application in post-fire conditions. Our herbicide treatments were applied to the rows where seed was planted, allowing large stands of cheatgrass to grow at the edge of the herbicide treated rows. This resulted in high propagule pressure, a major factor in invasion rates (Chambers et al. 2016). In a large-scale application, high invasive propagule pressure occurs mostly near edges, whereas our small plots experienced pressure across the entire herbicide treated area.

Indaziflam provided better long-term control of cheatgrass than imazapic (Figure 2-2). As briefly described above, indaziflam has moderate to low mobility (Alonso et al. 2011) and readily persists in soil (Jhala and Singh 2012). Comparatively, indaziflam has a longer half-life in soil than imazapic, along with significant residual activity that likely extends the duration of weed control (de Barreda et al. 2013). With low soil mobility and high residual activity, indaziflam is well equipped to provide several years of control of cheatgrass, a species that has high seed production (Hempy-Mayer and Pyke 2008).

Herbicide Effects on Bluebunch Wheatgrass

Both herbicides reduced aboveground biomass of bluebunch wheatgrass similarly after two years of growth (Figure 2-5), but imazapic was less detrimental to plant density and seedling emergence than indaziflam (Figures 2-3&2-4). The reason imazapic was equally detrimental to aboveground growth compared to indaziflam, while being less detrimental to plant density and seedling emergence than indaziflam may be due to their different mechanisms. Indaziflam reduces growth by inhibiting cellulose synthesis (Brabham et al. 2014), whereas imazapic kills by inhibiting synthesis of branched-chain amino acids (Tranel and Wright 2002). Many seeds treated with imazapic emerged, and survived, but didn't grow into large plants. We hypothesize that many of the seeds affected by imazapic were able to cope with inhibited amino acid synthesis, and survive for two years, but that the legacy effects of the herbicide reduced aboveground growth.

Furrow Effects

Furrow treatments improved emergence dramatically (Figure 2-3) in non-herbicide treatments, but the growth effect did not persist into the second year (Figures 2-4&2-5). High emergence may have led to increased competition and resulted in reduced plant growth. A meta-analysis showed that intraspecific competition in grasses is four to five-fold stronger than interspecific competition (Adler et al. 2018). Also, furrows can slough in over time, potentially burying small seedlings.

Furrow treatments eliminated herbicide effect on all stages of bluebunch wheatgrass growth in imazapic treatments but provided no protection in indaziflam treatments (Figures 2-3&2-5). We hypothesize this difference is either due to 1) bluebunch wheatgrass physiology is more sensitive to indaziflam than imazapic, or 2) the difference in soil mobility between the two herbicides is making the furrow treatment less effective in herbicide removal for indaziflam. The first hypothesis is supported by our emergence data outside of furrows, where indaziflam application resulted in less than 1% seedling emergence whereas imazapic application produced 8% emergence (Figure 2-3). In a study comparing the effects of indaziflam to imazapic, indaziflam caused higher seed mortality of invasives than imazapic at the same rate (Sebastian, Fleming, et al. 2017). One explanation of their different lethality toward invasives is the different mechanisms each herbicide uses to kill plants. Herbicides inhibiting amino-acid synthesis, such as imazapic, are slow to show visible injury to plants (Devlin and Cunningham 1970). Indaziflam inhibits cellulose biosynthesis, a major structural component of plants that requires over 18-24 catalytic proteins, and can act very quickly (Brabham et al. 2014). The complexity of cellulose biosynthesis makes it vulnerable to attack by indaziflam, and may have more immediate negative effects than imazapic.

The second hypothesis of different soil mobility is conceptually possible, where the two herbicides vary largely in their soil mobility that may affect seeds as the furrows sluff in over time or as precipitation causes leaching of herbicide concentrations. Indaziflam is much less mobile than imazapic, largely due to lower water solubility (2.8 mg L^{-1}) and higher adsorption into organic matter than is seen with imazapic (Sebastian, Fleming, et al. 2017; Alonso et al. 2011). Imazapic doesn’t move much laterally but does leach vertically (de Souza et al. 2000), so the lateral movement of imazapic away from the furrow may isolate furrow bottoms from the leaching pathway of imazapic. Whereas indaziflam, being less mobile, may persist in the upper soil longer (Hunter Perry et al. 2011), leaching less than imazapic, and affect the seeds as the furrows sluff over time.

Implications and Recommendations

Here we show that herbicide may have a place in restoration efforts. If herbicide injury can be limited to target species, invasive annual competition on seeded species is reduced to produce larger plants at early growth stages. In general, restoration efforts including pre-emergent herbicides are challenging; the characteristics of indaziflam that lead to longer control of cheatgrass than imazapic, also make it difficult to reduce injury to restoration species. In contrast, imazapic injury to seeded species can be limited, but control of cheatgrass is short, resulting in eventual reinvasion.

Our results suggest that indaziflam applications strongly limit restoration of a native species, and that it is likely best suited for control of invasive annual grasses alone. Studies of indaziflam applications to sites with mature native vegetation have been shown to increase native species growth and provide 3+ years of annual grass control by reducing competition from weeds (Sebastian, Fleming, et al. 2017). Imazapic can be used in restoration seeding efforts as long as measures are taken to limit seed exposure to the herbicide. To achieve long-term control of annual invasive grasses on restoration seeding sites, imazapic application alone will not suffice. One potential option is to apply imazapic prior to seeding, plant in a furrow made after herbicide application, and then applying indaziflam two years later. Another option would be to wait (2-5 years) until indaziflam activity level has decreased, and then seed native species. Both approaches could potentially allow restoration seeding success, and long-term control of invasive annual grasses.

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FIGURES

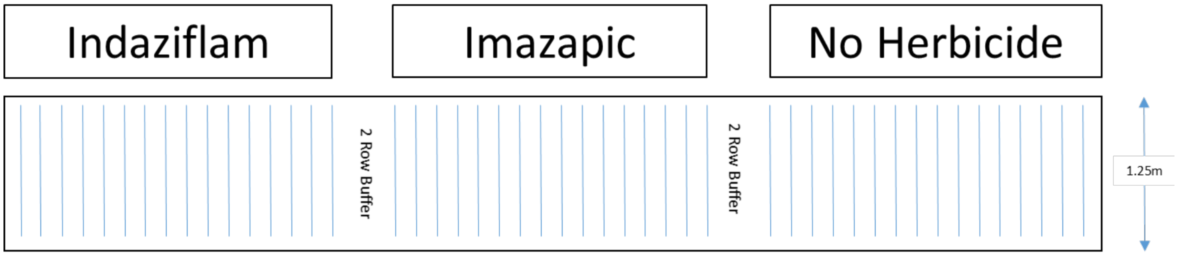

Figure 2-1. Diagram illustrating experimental block, split into three sub-blocks with each sub-block receiving herbicide treatment by indaziflam, imazapic, or no herbicide.

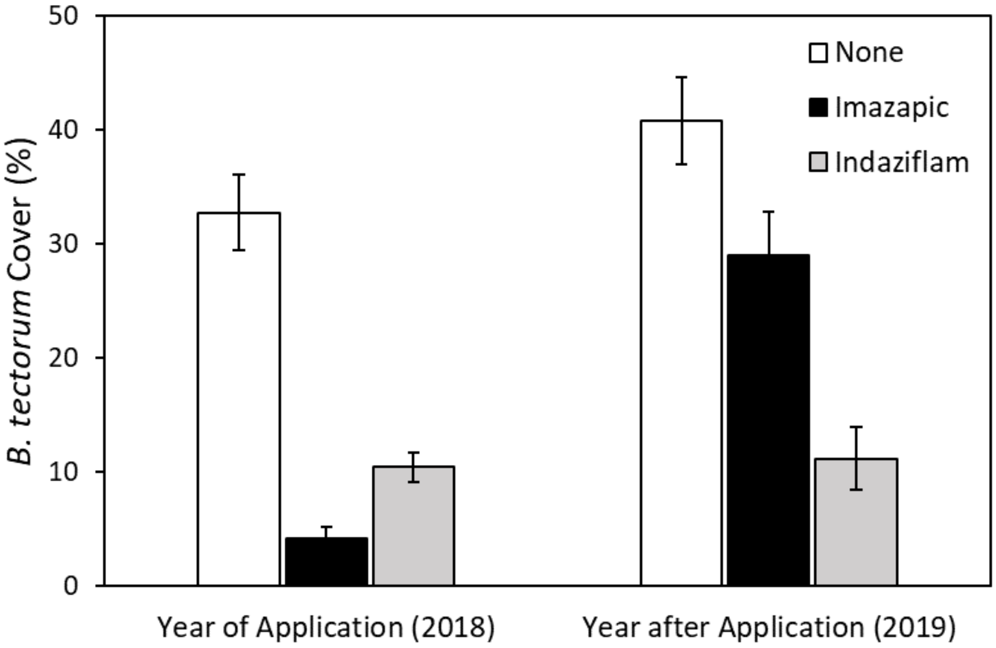

Figure 2-2. Absolute cover percentages of *Bromus tectorum* (cheatgrass) when using two pre-emergent herbicides (imazapic and indaziflam) and no herbicide at three sites in the sagebrush steppe.

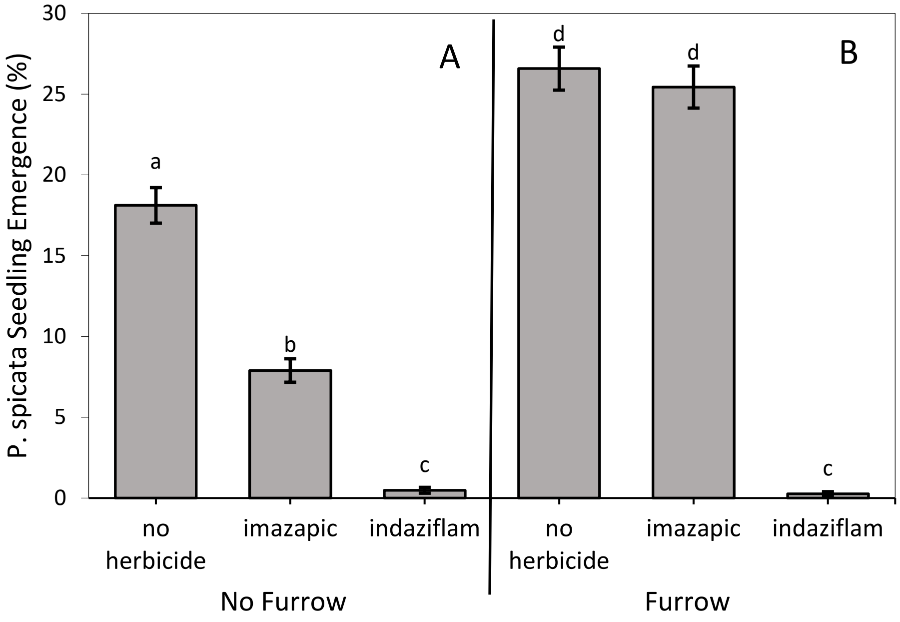

Figure 2-3. Seedling emergence (%) of *Pseudoroegnaria spicata* (bluebunch wheatgrass) when planted in different herbicide treated areas (imazapic, indaziflam, and no herbicide) without furrows (A) and with furrows (B) at 3 sites in the sagebrush steppe.

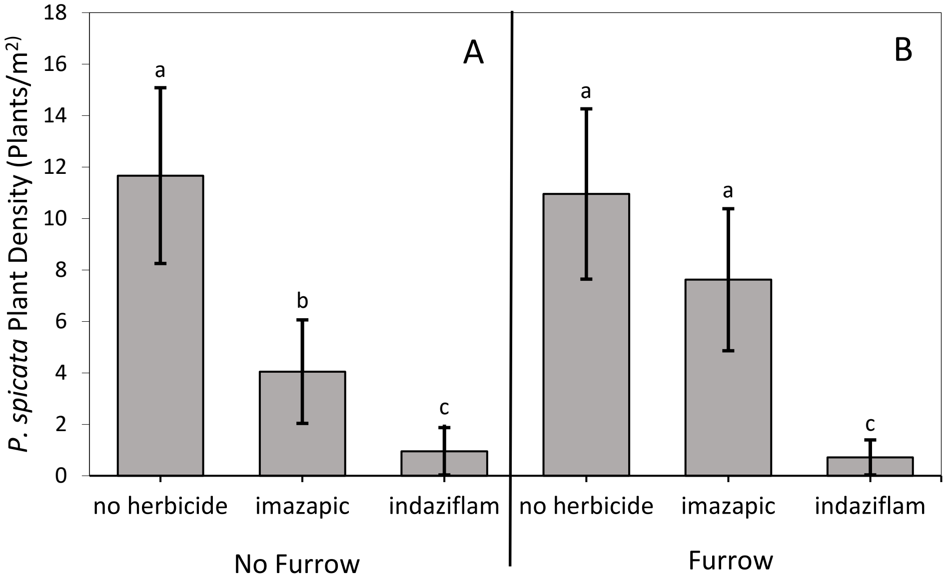

Figure 2-4. Average plant density (plants/m^2) of *Pseudoroegnaria spicata* (bluebunch wheatgrass) after 2 years growth when planted in different herbicide treated areas (imazapic, indaziflam, and no herbicide) without furrows (A) and with furrows (B) at 2 sites in the sagebrush steppe.

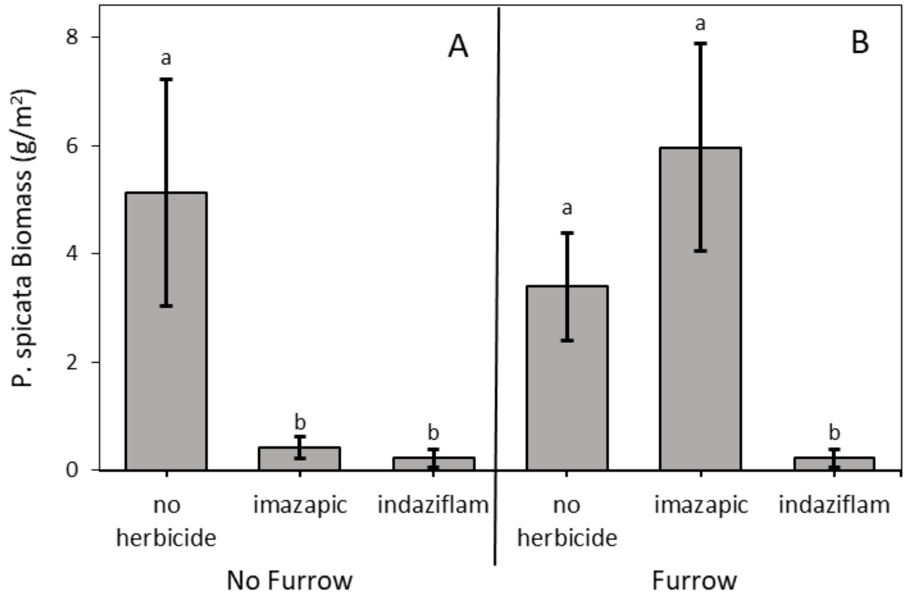

Figure 2-5. Aboveground biomass (g/m2) of *Pseudoroegnaria spicata* (bluebunch wheatgrass) after 2 years when planted in different herbicide treated areas (imazapic, indaziflam, and no herbicide) without furrows (A) and with furrows (B) at 2 sites in the sagebrush steppe.

TABLES

Table 2-1. Summary of statistical regressions for all response variables. Mean and standard error values represent the mean of individual treatments for each response variable. Data is comprised of two years of data at 3 sites in the sagebrush steppe system. Degrees of freedom are excluded from our seedling emergence and plant density data due to the nature of regression with a poisson error distribution.

Cheatgrass (*B. tectorum*)

		Estimate	Std Error	DF	T Value	Pr(>|t|)
1st Year Cheatgrass Cover (%)	No Herbicide	5.47	0.58	3	9.46	0.006
	Imazapic	-3.97	0.30	85	-13.10	2.00E-16
	Indaziflam	-2.41	0.30	85	-7.98	6.36E-12
2nd Year Cheatgrass Cover (%)	No Herbicide	6.13	0.41	7	15.06	2.92E-06
	Imazapic	-1.01	0.46	85	-2.18	0.032
	Indaziflam	-3.43	0.46	85	-7.39	1.00E-10

Bluebunch Wheatgrass (*P. spicata*)

		Estimate	Std Error	DF	Z Value	Pr(>|z|)
Seedling Emergence (#)	Intercept (Seed Only)	3.09	0.16	-	19.16	2.00E-16
	Imazapic	-0.68	0.09	-	-7.47	8.00E-14
	Indaziflam	-1.66	0.13	-	-12.53	2.00E-16
	Furrow	0.42	0.07	-	6.14	8.10E-10
	Imazapic x Furrow	0.75	0.11	-	6.89	5.50E-12
	Indaziflam x Furrow	-0.38	0.18	-	-2.06	0.04

		Estimate	Std Error	DF	Z Value	Pr(>|z|)
Plant Density (plants/m^2)	Intercept (Seed Only)	1.43	0.16	-	8.94	2.00E-16
	Imazapic	-0.91	0.29	-	-3.15	0.001
	Indaziflam	-1.34	0.34	-	-3.96	7.57E-05
	Furrow	-0.01	0.22	-	0.01	0.999

	Imazapic x Furrow	0.63	0.37	-	1.71	0.088
	Indaziflam x Furrow	-0.45	0.53	-	-0.85	0.394

		Estimate	Std Error	DF	T Value	Pr(>|t|)
Aboveground Biomass (g)	Intercept (Seed Only)	1.15	0.25	54	4.66	2.13E-05
	Imazapic	-0.87	0.35	54	-2.49	0.016
	Indaziflam	-0.77	0.35	54	-2.21	0.031
	Furrow	-0.06	0.35	54	-0.16	0.875
	Imazapic x Furrow	1.32	0.49	54	2.67	0.01
	Indaziflam x Furrow	-0.05	0.49	54	-0.11	0.915