EVALUATING NON-NATIVE ANNUAL BROME CONTROL WITH HERBICIDES AND FACILITATING WYOMING BIG SAGEBRUSH ESTABLISHMENT IN DEGRADED DRYLANDS

by

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DEDICATION

This thesis is dedicated to my parents for their endless love and support and for always encouraging my curiosity of the natural world.

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ABSTRACT

The degradation of drylands, through resource extraction and non-native annual brome invasion, is a major problem throughout the Intermountain West. Most restoration relies on establishing desired species from seed, but success is limited and establishing Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) is especially failure prone. This study focused on developing methods for controlling annual bromes, specifically cheatgrass (*Bromus tectorum* L.) and Japanese brome (*Bromus japonicus* Thunb.), and successfully re-introducing native sagebrush steppe species to degraded drylands.

In the greenhouse, I assessed the efficacy of glyphosate and four graminicides (clethodim, sethoxydim, fluazifop, and quizalofop) applied at two rates, to cheatgrass plants of different heights. I also assessed the same five herbicides on three accessions of cheatgrass and Japanese brome. All herbicides reduced cheatgrass biomass, with most effective control on plants less than 11 cm. Overall, glyphosate and sethoxydim treatments were least effective, and quizalofop and fluazifop treatments were most effective. Japanese brome and the disturbed accessions of both species were more susceptible to herbicides than cheatgrass and the undisturbed accessions.

My field study targeted the same annual bromes on two Montana coal mines. Four herbicide treatments (control, glyphosate, quizalofop, or glyphosate plus quizalofop) and two seeding treatments (differing in the amount of sagebrush seed) were evaluated for their effectiveness to manage annual bromes and boost seeded species establishment. Half of each herbicide plot was retreated with quizalofop the second year. All herbicide treatments reduced annual brome cover, especially in plots that received glyphosate. Wyoming big sagebrush density and cover of sown species increased in seeded plots with and without herbicides, but there was no difference between seeding treatments. Herbicide effects on seeded species were inconsistent, though generally establishment was greatest in plots receiving glyphosate. Quizalofop retreatment reduced annual brome cover, but did not impact seeded species establishment.

These results suggest that targeted herbicide applications can be used to control non-native annual bromes and increase seeded species establishment. Specifically, using glyphosate pre-seeding when plants are small and graminicides post-seeding, can decrease annual brome cover. These treatments can provide a window of opportunity for establishing species from seed, including Wyoming big sagebrush.

CHAPTER ONE

INTRODUCTION TO THESIS

The Global Distribution, Ecological Importance of, and State of Drylands

Drylands encompass arid, semi-arid, and dry-subhumid ecosystems (Bot, Nachtergaele, and Young 2000) and are a critical part of the natural world; globally they cover 5.4 billion hectares, or over 40% of the Earth's land mass (Desertification and Drought 1997). Around 2 billion people, or 40% of the world's population (Millennium Ecosystem Assessment 2005; White and Nackoney 2003), live in and rely on drylands for survival, so sustainable management of these systems is imperative (Koohafkan and Stewart 2008).

The number of people who rely on drylands is increasing, which will further increase the strain on the many goods and services these lands provide (Brauch and Spring 2009; Koohafkan and Stewart 2008). Despite low and variable annual precipitation, drylands are used for agriculture and livestock (Milchunas and Lauenroth 1993) and are therefore critical for global food security (O'Mara 2012). Drylands also provide a number of other ecosystem functions: they are hotspots of global biodiversity (Myers et al. 2000), provide critical habitat for endemic plant and animal species (Millennium Ecosystem Assessment 2005; White and Nackoney 2003), and are crucial areas for carbon storage (O'Mara 2012).

Drylands are rapidly becoming degraded, with recent estimates that up to 1.2 billion (of 5.4 billion) hectares are degraded globally, and that the extent is increasing by an additional 12 million hectares per year (Brauch and Spring 2009). This means up to 20 percent of drylands are classified as severely degraded worldwide (Millennium Ecosystem Assessment 2005) and in North America, over 70 percent of drylands are classified as moderate to severely degraded (Brauch and Spring 2009; Koohafkan and Stewart 2008). The economic costs associated with the degradation and desertification of drylands are massive, with an estimated that 42 billion dollars are lost every year (Brauch and Spring 2009; Dregne and Chou 1992).

The causes of dryland degradation are both natural and human (Koohafkan and Stewart 2008). Inherent in the definition of drylands is highly variable precipitation patterns and prolonged periods of drought (Bot, Nachtergaele, and Young 2000), which is being exacerbated by climate change (Mainguet and Da Silva 1998). These fluctuations are prompting the often poor human populations (White, Tunstall, and Henninger 2002) that rely on drylands to further alter and damage them by overgrazing livestock (Akiyama and Kawamura 2007), converting unsuitable areas to croplands (Török et al. 2012) often with poor irrigation methods (Brauch and Spring 2009). Other factors that cause degradation to drylands include deforestation and desertification (Brauch and Spring 2009), as well as resource extraction (Howard, Schuman, and Rauzi 1977).

In North America, drylands cover almost 600 million hectares or roughly 28% of the continent and are home to 60 million people (Desertification and Drought 1997). Most of the drylands exist within the United States, more specifically in the western half of the country, encompassing the Great Plains, Great Basin, Colorado Plateau, and the Mojave and Sonoran Deserts. These areas include grasslands, agricultural areas, forests, and urban landscapes (Koohafkan and Stewart 2008). One of the ecosystems included in the grasslands, and the focus of this study, is the sagebrush steppe. Total cover estimates for sagebrush steppe in the western United States vary, from as little as 43 million hectares up to 300 million hectares (Rowland, Suring, and Wisdom 2010; Welch 2005). It is, however, the largest semiarid plant community present in North America (Anderson and Inouye 2001; Miller, Svejcar, and West 1994) and extends from southern Idaho and the western edge of the Cascades and Sierra Nevada mountain ranges, to eastern Wyoming and Montana, and up to southern British Columbia (Morris, Kelsey, and Griggs 1976; Vale 1974). The sagebrush steppe is characterized by a dry climate with highly variable precipitation and long periods without fire activity (West and Goodall 1983).

Vegetation within the sagebrush steppe is a heterogeneous combination of perennial grasses, shrubs, and forbs (Seipel 2006; Young and Evans 1973), where sagebrush (*Artemisia tridentata*) is the dominant shrub. There are five subspecies of sagebrush*:* Wyoming big sagebrush (*A. tridentata* ssp. *wyomingensis*), basin big sagebrush (*A. tridentata* ssp. tridentata), mountain big sagebrush (*A. tridentata* ssp.

vaseyana), subalpine big sagebrush (*A. tridentata* ssp. *spiciformis*), and xeric big sagebrush (*A. tridentata* ssp. *xericensis*). The distribution of these subspecies varies based on ecological characteristics and habitat type (McArthur and Sanderson 1999; Kolb and Sperry 1999; McArthur and Goodrich 1986; West 1983) within the sagebrush steppe. Other dominant shrubs include prairie sagewort (*Artemisia frigida* Willd.), winterfat *(Krascheninnikovia lanata* [Pursh] A. Meeuse & Smit), and rubber rabbitbrush (*Ericameria nauseosa* [Pall. ex Pursh] G.L. Nesom & Baird).

Shrubs play a number of critical roles in determining dryland ecosystem processes (West 1983). They provide canopy structure, habitat (Knick, Hanser, and Preston 2013; Hess and Beck 2012; Rowland et al. 2006; Knick et al. 2003), forage for many species (Shipley et al. 2006; Ngugi et al. 1992), increased primary production (Aguiar and Sala 1999), resilience (Minnick and Alward 2015) and soil stabilization (Linstädter and Baumann 2013). Of these shrubs, sagebrush is often considered the species of greatest interest and concern (Prevéy et al. 2010; Noss, LaRoe, and Scott 1995; West 1983), as it is considered a keystone species in many areas (McAdoo, Boyd, and Sheley 2013).

Sagebrush steppe is only found in half of its historical range (Pyke et al. 2014; Miller et al. 2011; McArthur and Plummer 1978). The most notable human causes of decline are sagebrush removal for irrigated agricultural-lands (Welch 2005) and improved grazing (Wambolt, Walhof, and Frisina 2001; Watts and Wambolt 1996), often with the addition of sown non-native, fast growing, perennial grasses (Larson 2002;

Weaver, Gustafson, and Lichthardt 2001; West 1983). Unfortunately, the removal of sagebrush usually fails to increase forage (Welch 2005; Ratzlaff and Anderson 1995), and the disturbance caused by removal often prompts invasion by non-native species (Ratzlaff and Anderson 1995). Wildfires, non-native species invasions, conifer encroachment, conversion to agricultural or livestock grazing lands, desertification, and energy (i.e. oil, gas, and coal) development (Michaelides et al. 2012; Davies et al. 2011; Miller et al. 2011; Chambers et al. 2007; Shaw, DeBolt, and Rosentreter 2005; Miller and Rose 1999; Knapp 1996; Vale 1974) have all contributed to a significant decrease in sagebrush over the last half century. Additionally, this already diminished and fragmented range is expected to decline another 40% by midcentury due to climate change (Still and Richardson 2015) as well as other anthropogenic and natural drivers.

The loss of robust sagebrush steppe is a major concern, because of the role the system plays within the Intermountain West. Studies have found over 350 species of plants and animals associated with sagebrush (Suring, Rowland, and Wisdom 2005; Suring et al. 2005). One of these animal species, the greater sage-grouse (*Centrocercus urophasianus*), is a sage-obligate (Knick et al. 2003). Sage-grouse winter diet consists of 99% sagebrush, and they need these shrubs for their leks and successful breeding (Hess and Beck 2012; Rowland et al. 2006). The decrease and fragmentation of suitable breeding habitat and forage has led to a decline in the species and significant conservation efforts (Knick, Hanser, and Preston 2013; Hess and Beck 2012; Connelly and Braun 1997).

In addition to animal species, Wyoming big sagebrush benefits other plant species. In drylands, where water is a limiting resource, sagebrush can facilitate the growth and survival of neighboring plants by creating microsites or 'nutrient islands' (Boyd and Davies 2012). These microsites have greater soil organic carbon (Minnick and Alward 2015), temperatures (Davies, Bates, and James 2009), and water levels (Davies, Bates, and Miller 2007) which are favorable to surrounding plants. Belowground, Wyoming big sagebrush plants have both a deep tap root and shallow roots that spread laterally near the surface. This creates a hydraulic lift in which the tap root brings otherwise unavailable moisture from deep in the soil to the surface where it can be released at night, providing additional moisture to the sagebrush plant though its diffuse, shallow root system as well additional moisture to nearby plants (Davies, Bates, and Miller 2007). The woody structure above ground also provides benefits such as canopy structure and wind barriers to neighboring plants (Davies, Bates, and Nafus 2011; Davies, Bates, and James 2009).

One of the most influential system-changing phenomenon (Levine et al. 2003) to the remaining sagebrush steppe, and one of the largest drivers of shrub loss, is the invasion of non-native species, specifically the invasion of annual grasses that alter fire regimes (Brooks et al. 2004; D'Antonio and Vitousek 1992). Annual grasses have invaded vast portions of the sagebrush steppe (Davies et al. 2011; Pellant 1996) where they often form dense monocultures causing an increase in fire fuel loads (Mack 2011). These same species are then able to quickly re-colonize and thrive post-fire, thus creating a

positive fire feed-back loop (Eiswerth et al. 2009; Brooks et al. 2004) that significantly reduces sagebrush populations and hinders their potential recovery and reestablishment (Davies et al. 2011; Whisenant 1990a). This positive fire feed-back loop is responsible for extensive sagebrush loss but is not present everywhere in the sagebrush steppe (Taylor et al. 2014). Specifically, areas in the northern-eastern portion of the steppe have not exhibited positive fire-feedback responses (Larson, Lehnhoff, and Rew 2017).

Dryland Restoration Efforts and Challenges Preventing Success

Roughly 25% of once ecologically robust drylands are now degraded globally (Brauch and Spring 2009). It is increasingly acknowledged that there is a pressing need to restore degraded systems (Jones and Schmitz 2009) with an emphasis on drylands (James et al. 2013) because of the goods and services they provide, and in recent years there has been a significant rise in the number of large-scale restoration efforts (De Groot et al. 2013). Despite the interest in restoration of degraded drylands, outcomes vary widely and efforts are complicated because the criteria for success are often not clearly defined nor are restoration efforts monitored or evaluated quantitatively for effectiveness (Suding 2011).

Dryland restoration specifically is highly unpredictable and failure-prone (James et al. 2013). Because dryland systems are characterized by high annual variability, restoration efforts can be successful in some years and not in others in part due to the

fact that year-to-year variation in climate and rainfall influences seed germination, plant establishment, and long-term community assembly (Fehmi et al. 2014; MacDougall, Wilson, and Bakker 2008). Other obstacles to restoration success are competition with non-native species (Bakker et al. 2003), year of restoration efforts' initiation (Vaughn and Young 2010), and inconsistency in management decisions (Grman, Bassett, and Brudvig 2013). There are also economic barriers such as large up-front costs and additionally long-term monitoring requirements (De Groot et al. 2013) which are needed to confirm restoration goals and objectives have been met.

Dryland restoration efforts typically revolve around attempting to reintroduce desired plants from seed. Unfortunately, the grasses, forbs, and shrubs sown as part of dryland restoration efforts commonly fail to establish (Mangold et al. 2015; Davies 2010; James and Svejcar 2010). Some efforts, however, can help managers reach desired restoration outcomes. For example, controlling weedy vegetation with herbicides can increase seeded grass establishment (Mangold et al. 2015; Hirsch-Schantz et al. 2014; Kyser et al. 2013; Elseroad and Rudd 2011; Davies 2010; Whitson and Koch 1998), but not seeded shrubs (Brabec et al. 2015; Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011). In contrast, herbicides have been shown to be successful in facilitating the survival of transplanted shrubs (McAdoo, Boyd, and Sheley 2013), but on a large scale this method is not feasible.

Restoring and protecting sagebrush habitats has become a major goal throughout the western U.S., unfortunately sagebrush and other steppe species are

extremely difficult to restore. One of the reasons shrubs like sagebrush are so difficult to restore is inter-species competition. There is competition between seeded sagebrush and other seeded species (i.e. grasses and forbs), as well as between seeded sagebrush and non-seeded weedy species (Boyd and Svejcar 2011; Bailey et al. 2010; Williams et al. 2002; Eliason and Allen 1997). Competition also exists between seeded shrubs and existing grass stands and weeds (Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011; Boyd and Davies 2010; Blaisdell 1949), which can hinder shrub establishment.

Decreasing grass seed rates (Rinella, Espeland, and Moffatt 2016; Rinella et al. 2015), seeding grasses after shrubs have established (Blaisdell 1949), increasing shrub seed rates (Hild et al. 2006; Williams et al. 2002), and transplanting mature shrubs (Lambert 2005) are all strategies to facilitate sagebrush success in dryland restoration by minimizing or delaying competition with other plant species. Many of these strategies allow time for shrubs to develop tap roots so they can access resources in deeper soil layers inaccessible to grasses (Cipriotti et al. 2014; Ward, Wiegand, and Getzin 2013). Competition, however, is not the only factor to influence seeded shrub success. Factors indirectly or less related to plant competition (e.g. spring precipitation or soil temperature) can also play a role in establishment (James, Rinella, and Svejcar 2012; Mangla et al. 2011).

Troublesome Non-Native Annual Bromes

Cheatgrass (*Bromus tectorum* L.) is a winter annual grass that is native to Eurasia but spread to the United States as a grain contaminant in the mid to late 1800's (Knapp 1996; Morrow and Stahlman 1984; Mack 1981). Cheatgrass was spread through the Intermountain West along rail lines and transportation corridors, as well as by livestock (Mack 1981). Currently, cheatgrass has invaded over 22 million hectares throughout the Intermountain West (Duncan et al. 2004), and is also the dominant invader in the Great Basin (Chambers et al. 2007; Knapp 1996) where it has colonized over 20 million hectares.

Invasions of cheatgrass have been associated with loss of native biodiversity (Bansal and Sheley 2016; Humphrey and Schupp 2001; Knapp 1996), alteration of fire regimes (Epanchin-Niell, Englin, and Nalle 2009; Brooks et al. 2004; Whisenant 1990a), nitrogen (Rimer and Evans 2006; Sperry, Belnap, and Evans 2006) and water availability (Boxell and Drohan 2009; Morrow and Stahlman 1984), amount of soil organic matter (Norton et al. 2004; Ogle, Ojima, and Reiners 2004) and nutrient cycling (Norton et al. 2004; Belnap and Phillips 2001).

In general, successful invasive species often have high fecundity (i.e. high seed production) (Sutherland 2004; Baker 1965), which allows them to increase their density quickly and build up a large seed bank (Crawley, Harvey, and Purvis 1996) and outcompete native vegetation in disturbed areas. An individual cheatgrass plant can produce up to 6,000 seeds (Young and Evans 1978) which has created seedbank

densities of up to 20,000 seeds per square meter in cheatgrass dominant areas (Hempy-Mayer and Pyke 2008). Most cheatgrass seeds germinate within the first two years, but in favorable soil conditions seeds can remain viable for up to five years (Germino, Chambers, and Brown 2016). Additionally, cheatgrass exhibits a faster relative growth rate compared to many native perennial grasses (Mangla, Sheley, and James 2011). These characteristics, along with the lack of a dominant native annual grass have allowed cheatgrass to fill that unoccupied niche (Knapp 1996) and become ubiquitous throughout drylands.

Other reasons cheatgrass is successful as an invasive species is high phenotypic plasticity and life cycle traits. Sexual reproduction allows for gene flow within a population and genetic recombination (i.e. novel combinations of traits between generations). Sexual reproduction, outcrossing, and cross pollination between populations increases the likelihood of successful establishment (Ashley and Longland 2007). The high number of seeds and hybrid vigor associated with those seeds allows cheatgrass to thrive in a variety of conditions (Mack and Pyke 1983). As a winter annual, cheatgrass can germinate in the fall, winter, or spring (Knapp 1996), but most often will germinate in the fall after sufficient precipitation, it overwinters in a vegetative state (Espeland et al. 2016) which gives it a competitive advantage over other species that do not germinate or start to photosynthesize until the spring. Cheatgrass can survive cold temperatures (Bykova and Sage 2012) and relies on winter seedling and seedbank dormancy as part of its life history strategy (Roundy et al. 2007; Allen and Meyer 2002);

however its growth and spatial distribution in the sagebrush step is cold-limited (Brummer et al. 2016; Chambers and Pellant 2008). Cheatgrass germination, growth, and reproduction have all been shown to decrease with deceasing temperatures (Bradley, Curtis, and Chambers 2016; Chambers et al. 2007; Roundy et al. 2007; Thill, Schirman, and Appleby 1979). Conversely, warming temperatures have been shown to increase cheatgrass germination (Thill, Schirman, and Appleby 1979), biomass and seed production (Blumenthal et al. 2016), fecundity and survival (Compagnoni 2014), and growth rate, particularly at higher elevations (Compagnoni and Adler 2014).

Cheatgrass' life history and phenotypic plasticity can help cheatgrass succeed in plant communities within its current range, and based on future climate predictions, it may expand to some new areas. The many different climate change models have slightly different outcomes, but the overall predictions are consistent; temperatures will increase in the northern portion of the Intermountain West (Polley et al. 2013; Chambers and Pellant 2008) and while total precipitation will not change, seasonality may: winter precipitation is expected to increase and summer precipitation is expected to decrease (Polley et al. 2013). Given these predictions, and the climate changes that have already been recorded over the last 100 years, the range of cheatgrass is expected to expand to areas that are warming (Bradley, Curtis, and Chambers 2016), receiving less summer precipitation (Bradley 2009), and to higher latitudes (Bromberg et al. 2011). In some portions of the Intermountain West, including higher elevations and

areas in the northeast, changes in climate are unlikely to favor further cheatgrass invasion (Larson, Lehnhoff, and Rew 2017).

Wetter winters and drier summers are predicted to increase the areas in the Intermountain West that are susceptible to invasion (Bradford and Lauenroth 2006). Cheatgrass has responded positively to these precipitation patterns, which make soil moisture available at a time cheatgrass can utilize it (Prevéy and Seastedt 2015, 2014). Not only do these changing precipitation patterns and soil moisture conditions increase cheatgrass germination, growth, and reproduction, but they negatively impact many of the native perennial grasses that are commonly in competition with cheatgrass (Hull 1963). The current realized niche and the predicted expansion of it, combined with the invasive characteristics of cheatgrass make it a major problem throughout the sagebrush steppe.

Japanese brome (*Bromus japonicus* Thunb.) is another annual grass native to Eurasia (Beck 2016) that has become prevalent in North America. Japanese brome is sometimes classified as a synonym of field brome (*Bromus arvensis*) (Beck 2016; NRCS 2016), but this paper separates them as classified by Lesica, Lavin, and Stickney (2012). Like cheatgrass, it is a member of the Poaceae family (Whitson et al. 1991) and typically behaves as a winter annual germinating in the fall and overwintering as a seedling (Baskin and Baskin 1981). Where dominant, Japanese brome seed production can reach up to 94,000 seeds per square meter (Whisenant 1990b). It can invade both disturbed and undisturbed sites (Karl, Heitschmidt, and Haferkamp 1999; Whisenant 1990b; Reed

1952; Osborn and Allan 1949) in a number of different types of plant communities including drylands, prairie, pinyon-juniper, sagebrush steppe, and desert shrub-grasslands (Beck 2016). Japanese brome is far less studied than cheatgrass, but could potentially have similarly damaging impacts on the Intermountain West landscape.

Managing Non-Native Annual Bromes

There are a wide variety of management tools used to control annual bromes, including physical, biological, and chemical approaches. Physical control methods include prescribed burns (Brooks et al. 2016; Harmoney 2007; DiTomaso et al. 2006), grazing (Vermeire, Heitschmidt, and Haferkamp 2008; Harmoney 2007), and tillage (Cox and Anderson 2004). Biological control includes fungal pathogens (Ehlert, Mangold, and Engel 2014; Beckstead et al. 2010; Meyer et al. 2007) and revegetation and restoration of native species (Mangold et al. 2015; Davies 2010; Cox and Anderson 2004; Bakker et al. 2003; Whitson and Koch 1998). Lastly, chemical control, which is the most common strategy (Radosevich, Holt, and Ghersa 2007), uses herbicides to manage cheatgrass (Ehlert, Mangold, and Engel 2014; Mangold et al. 2013; Rinella et al. 2010; Morris, Monaco, and Rigby 2009; Whitson and Koch 1998; Lym and Kirby 1991).

A class of post emergence broad-spectrum herbicides commonly used to control cheatgrass are acetolactate synthase (ALS) inhibitors (i.e. primisulfuron, sulfometuron-methyl, rimsulfuron, imazapic, and imazamox). These herbicides can be absorbed by leaves, stems, and roots (Peterson et al. 2010). One of the most widely used is imazapic;

which has been shown to control cheatgrass up to 95% relative to the control, when applied to plants in the one to two-leaf stages, though long-term efficacy can be variable (Mangold et al. 2013). However, greenhouse studies using resistant and susceptible accessions from Oregon found ALS-inhibitor resistance, specifically PSII inhibitors, in cheatgrass (Park and Mallory-Smith 2004; Mueller-Warrant, Mallory-Smith, and Hendrickson 1999) due to an increase in the rate of herbicide metabolism (Park, Fandrich, and Mallory-Smith 2004).

A second type of post emergence broad-spectrum herbicide commonly used to control cheatgrass inhibits amino-acid synthesis, specifically 5-enolpyruvylshikimate-3-phosphate (Amrhein, Schab, and Steinrücken 1980), which is absorbed only through plant leaves. Like imazapic, glyphosate has been successful at reducing cheatgrass up to 97% compared to the control (Cox and Anderson 2004; Park and Mallory-Smith 2004; Whitson and Koch 1998). Because both ALS inhibitors and glyphosate are non-selective, applications of imazapic (Morris, Monaco, and Rigby 2009; Shinn and Thill 2004) and glyphosate (Owen, Sieg, and Gehring 2011; Baker, Garner, and Lyon 2009; Morris, Monaco, and Rigby 2009) can harm non-target native species. Therefore, applications of these herbicides to control cheatgrass is a tradeoff between intended effects and damage to non-target species.

Graminicides (i.e. sethoxydim, clethodim, fluazifop, and quizalofop) are a type of post emergence herbicide that is only phytotoxic to graminiod species (Kukorelli, Reisinger, and Pinke 2013). They inhibit acetyl CoA carboxylase, specifically phospholipid

production, which is required for cell membranes (Délye, Wang, and Darmency 2002). Graminicides have been successful at controlling cheatgrass both in the greenhouse (Ball, Frost, and Bennett 2007; Park and Mallory-Smith 2004) and in the field (Brewster and Spinney 1989). Unlike ALS inhibitors and glyphosate, they are not commonly applied in the field to control cheatgrass, though a few studies used them in annual cropping systems (Marquardt and Johnson 2013; Foy and Witt 1992) and forestry (Clay, Dixon, and Willoughby 2006). Graminicides could play a unique role in cheatgrass management; while native grass species would still be impacted during applications, forbs and shrubs would not be damaged.

Goals and Objectives

In this study, I investigate the ability of glyphosate and graminicides to control the invasive annual bromes, cheatgrass and Japanese brome, in the greenhouse and in restoration scenarios in the field.

The second chapter describes my greenhouse experiment, where in a controlled setting I analyzed the effect of herbicide type, application rate, and target plant height at time of application on the efficacy of different cheatgrass and Japanese brome accessions. In my first of two greenhouse experiments, my objectives were to 1) evaluate the efficacy of glyphosate and four graminicides (sethoxydim, clethodim, fluazifop, and quizalofop), 2) compare low and high label recommended application rates of these herbicides, and 3) compare efficacy differences of each herbicide type

and rate combination across five cheatgrass heights on plant biomass 45 days post treatment. In the second greenhouse experiment, my objectives were to 1) evaluate the efficacy of glyphosate and four graminicides (sethoxydim, clethodim, fluazifop, and quizalofop), 2) compare low and high label recommended application rates of these herbicides, and 3) compare efficacy differences of each herbicide type and rate combination at one plant height between cheatgrass and Japanese brome and 4) compare the efficacy of these treatments across three accessions of each species. I used preliminary results from the greenhouse study to inform my choice of graminicide and application rate in the field study.

The third chapter describes the field study which evaluated herbicide applications and sowing of native seed mixes to facilitate the establishment of desired native species, particularly Wyoming big sagebrush. The sites were on coal mines where previous sagebrush steppe restoration efforts failed to meet revegetation goals, and as a result supported low species diversity, and were near monocultures of grasses, particularly cheatgrass and Japanese brome. My specific objectives were to evaluate four seed mixes in conjunction with three herbicide treatments (fall glyphosate, spring quizalofop, and a combination of both) applied in the first year of the experiment, and a herbicide retreatment (spring quizalofop) in the second season, on 1) the abundance of both cheatgrass and Japanese bromes, 2) the abundance of non-native annual forbs, 3) Wyoming big sagebrush establishment, and 4) the abundance of seeded species.

The final chapter brings together the knowledge gained from the greenhouse and field studies. It also considers the results from those studies and how they can inform future dryland restoration research.

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CHAPTER TWO

CONTROL EFFICACY OF CHEATGRASS AND JAPANESE BROME USING GLYPHOSATE AND FOUR GRAMINICIDES: EFFECTS OF PLANT SIZE, RATE, SPECIES, AND ACCESSION

Introduction

Controlling non-native annual grasses is a major concern in agriculture, rangelands, forestry and wildlands (Duncan et al. 2004; DiTomaso 2000). Cheatgrass (*Bromus tectorum* L.) and Japanese brome (*Bromus japonicus* Thunb.) are two non-native winter annuals that have invaded the western Unites States, with cheatgrass present in the cold deserts, western Great Plains, and western forests; and Japanese brome found mainly in the western Great Plains (Germino et al. 2016). Cheatgrass was unintentionally introduced at the turn of the 19th century (Knapp 1996; Morrow and Stahlman 1984; Mack 1981) and is now widespread throughout the United States. It has spread through severe drought in combination with overgrazing and livestock movement (Mack 1981) as well as other vectors, and now occupies over 22 million hectares throughout the Intermountain West (Duncan et al. 2004) and is the dominant invader in the Great Basin (Chambers et al. 2007; Knapp 1996). Japanese brome is less widespread but became common in northern mixed grass prairies and the western Great Plains sometime after the 1950's (Haferkamp and Heitschmidt 1999; Haferkamp et al. 1992; Whisenant 1990b). Based on the changing climate in the western United States, the invaded range of brome species is likely to expand into areas that are

warming (Bradley, Curtis, and Chambers 2016) and receiving less summer precipitation (Bradley 2009).

These annual grasses can have substantial impacts in cropped (Blackshaw 1993; Rydrych and Muzik 1968) and rangeland areas (Ogle, Reiners, and Gerow 2003; Haferkamp and Heitschmidt 1999). Cheatgrass has been found to reduce winter wheat biomass up to 59% and yield by up to 68% (Blackshaw 1993). And, Japanese brome has been shown to impact grass yield in rangelands; it's removal from a western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Love) rangeland increased standing crop yield by 220 kg ha^{-1} and tillers by 153 m^{-2} (Haferkamp and Heitschmidt 1999). Cheatgrass also impacts ecosystem processes by competing with native grasses (Vasquez, Sheley, and Svejcar 2009; Francis and Pyke 1996; Nasri and Doescher 1995), changing fire regimes (Brooks et al. 2004; Whisenant 1990a), altering available nitrogen (Rimer and Evans 2006; Sperry, Belnap, and Evans 2006), increasing soil organic carbon storage (Norton et al. 2004; Ogle, Ojima, and Reiners 2004), and nutrient cycling (Norton et al. 2004; Belnap and Phillips 2001). There are currently no studies assessing these ecological impacts of Japanese brome.

Although there have been attempts to manage cheatgrass and Japanese brome with prescribed fire, grazing, tillage, and biological control in range- and wild-lands (Brooks et al. 2016; Germino et al. 2016; Ehlert, Mangold, and Engel 2014; Vermeire, Heitschmidt, and Haferkamp 2008; Harmoney 2007; DiTomaso et al. 2006; Cox and Anderson 2004; Monsen, Stevens, and Shaw 2004; Masters and Sheley 2001; Whitson

and Koch 1998), herbicides are the most widespread management tool in these and cropping systems (Radosevich, Holt, and Ghersa 2007). Within cropping systems, there are fewer logistical constraints on herbicide application with higher and more consistent efficacy compared with range- and wild-lands. In range- and wild-lands, weed control is often inconsistent due, presumably, to variation in environmental conditions and logistical barriers such as difficulty accessing sites during the optimal herbicide application window when weather is suitable and target plants are at a specific growth stage. For these reasons, this study evaluates the effect of brome plant height at time of herbicide application, in addition to herbicide rate and type.

Glyphosate is commonly used to control non-native annual grasses in range- and wild-lands. It is a broad-spectrum post-emergence herbicide that inhibits amino-acid synthesis, specifically by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (Amrhein, Schab, and Steinrücken 1980). Glyphosate has proven effective at controlling cheatgrass both in rangeland studies (Cox and Anderson 2004; Whitson and Koch 1998) and in the greenhouse (Park and Mallory-Smith 2004). The rangeland field studies reported very high levels of cheatgrass control after one (> 97%) (Cox and Anderson 2004; Whitson and Koch 1998) and three (> 92%) consecutive applications (Whitson and Koch 1998). In the greenhouse, Park and Mallory-Smith (2004) found an average of 85% reduction of cheatgrass biomass when treated with glyphosate compared to an untreated control. Less is known about the efficacy of glyphosate on Japanese brome, though Waller and Schmidt (1983), reported glyphosate provided good

control of Japanese brome. However, because glyphosate is a broad-spectrum herbicide, it also can damage or kill desired non-weedy species of grasses, forbs, and shrubs (Owen, Sieg, and Gehring 2011; Baker, Garner, and Lyon 2009; Morris, Monaco, and Rigby 2009) and crops. In most situations non-native bromes are present along with other more desired native grasses, forb and shrub species, and using glyphosate in these situations could outweigh the control of brome species because of the damage to these non-target species (Owen, Sieg, and Gehring 2011). Glyphosate has no soil residual activity, so some selectivity is possible if glyphosate is applied when annual bromes are growing but native species are dormant.

Graminicides, are grass specific post-emergence herbicides that inhibit acetyl CoA carboxylase. Specifically, production of phospholipids, which is required for cell membranes, is halted (Délye, Wang, and Darmency 2002). Graminicides are used in annual cropping systems (Marquardt and Johnson 2013; Foy and Witt 1992) and forestry (Clay, Dixon, and Willoughby 2006), but their use is not widespread in rangeland and restoration scenarios (James et al. 2013) that require weed management. These herbicides, including sethoxydim, clethodim, fluazifop-p-butyl, and quizalofop-p-ethyl, are phytotoxic to grasses, but unlike ALS-inhibitors or glyphosate they do not affect forbs or shrubs (Kukorelli, Reisinger, and Pinke 2013). For this reason, they may be particularly useful at rangeland and restoration sites where some desired species are present, but non-native grasses are dominant.

Graminicides have been found to provide good control of cheatgrass both in field and greenhouse studies. High levels of cheatgrass cover reduction was achieved over a five year period with sethoxydim (~70%), fluazifop (95%), and quizalofop (99%) applied at label rates in a field study at Oregon State University (Brewster and Spinney 1989). Similarly high rates of biomass control were observed in a greenhouse study, for sethoxydim (85%), clethodim, fluazifop, and quizalofop (all over 98%) when applied at the recommended herbicide label rates (Ball, Frost, and Bennett 2007).

Control of cheatgrass and Japanese brome is difficult partly because of the annual life cycle of the species. High annual seed production (Young and Evans 1978) and hybrid vigor (Mack and Pyke 1983) permits survival in a variety of conditions. Additionally, seeds can germinate in the fall, winter, or spring with adequate soil water and temperature (Knapp 1996), although greatest germination occurs in the second season after planting, under certain conditions seeds remain viable for up to five years (Chambers et al. 2016). This means high levels of control are often required for a number of years to adequately reduce the seedbank. Choosing the most appropriate herbicide and rate, plant growth stage, and re-application timing is therefore imperative to ensure optimal long term control and to minimize chance of herbicide resistance development.

Cheatgrass and Japanese brome management success using herbicides, especially in the long term, is often highly unpredictable. In addition to life cycle traits, environmental variables (e.g. soil moisture, precipitation, temperature, wind) impact

herbicide efficacy (Kudsk and Kristensen 1992). Nozzle type (Ramsdale and Messersmith 2001a), spray angle (Jensen 2007), carrier volume (Ramsdale and Messersmith 2001b), adjuvants (Kudsk and Mathiassen 2007), herbicide combinations (Jordan, Frans, and McClelland 1993), application rate (Ball, Frost, and Bennett 2007; Park and Mallory-Smith 2004) plus application timing (Marquardt and Johnson 2013), plant growth stage at time of application (Brewster and Spinney 1989), and plant cover and density (Pfleeger et al. 2012; Dalton and Boutin 2010; Katovich, Becker, and Kinkaid 1996), all influence herbicide performance. Because of the large number of potentially interacting variables that can impede herbicide efficacy, controlled studies provide an effective way to evaluate specific strategies to manage these invasive brome species. There are no studies that evaluate the ability of graminicides to control Japanese brome, and because of the increasing need for control of cheatgrass in rangelands, further research on graminicides for both cheatgrass and Japanese brome control is needed.

The goal of this greenhouse study was to examine the ability of glyphosate and graminicides to control cheatgrass and Japanese brome. More specifically we aimed to evaluate, in a controlled setting, the effect of herbicide type, application rate, and target plant height at time of application, on the efficacy of different cheatgrass and Japanese brome accessions. Our first objective was to evaluate the efficacy of glyphosate and four graminicides (sethoxydim, clethodim, fluazifop, and quizalofop) on cheatgrass biomass, at high and low label recommended application rates of each herbicide, when applied across five different plant heights using one cheatgrass accession. Our second objective

was to compare the efficacy of glyphosate and the same four graminicides at high and low label recommended application rates across three accessions of both cheatgrass and Japanese brome.

Methods

Experiment 1:
Efficacy of Herbicide Type and Rate Applied to Cheatgrass of Different Heights

The efficacy of cheatgrass control was evaluated for four graminicides, sethoxydim, clethodim, fluazifop-p-butyl (hereafter fluazifop), and quizalofop-p-ethyl (hereafter quizalofop), and glyphosate, plus an untreated control. All herbicides were applied at two rates (low and high label recommended rates) to plants that had reached five pre-defined above ground heights (5, 8.5, 11, 15.5, and 17 cm). The experiment was established as a randomized complete block design with 11 herbicide treatments x 5 heights x 7 replicates = 385 experimental units. The experimental unit was one cheatgrass seedling per pot. The experiment was replicated twice (trial 1: November 2014 through May 2015 and trial 2: November 2015 through May 2016) at the Montana State University Plant Growth Center in a greenhouse with 16-hr photoperiod at 22 ± 4 C daytime temperatures and 17 ± 6 C nighttime temperatures. Cheatgrass seed was from the Montana State University Red Bluff Agricultural Research Ranch in Norris, Montana, USA (45°52’ N, 111°68’ W).

Initially two seeds were sown per pot (9 cm x 9 cm x 12 cm) into a pasteurized soil mixture containing 1:1:1 (by volume) loam soil, washed concrete sand, and

Canadian Sphagnum peat moss, and thinned to one seedling after germination. After seeding, plants were randomly allocated to each herbicide and height treatment. Thirty days after seeding, the plants assigned to the three tallest height groups were transferred to a cold chamber (4 C, 12-hr photoperiod) for six weeks and then returned to the greenhouse. Because cheatgrass is a winter annual, this cold phase provided a vernalization period. The two smallest heights (5 cm and 8.5 cm) were not vernalized because they would have surpassed their target height by the end of the vernalization period. Pots were watered equally and as needed.

Plants were sprayed when the average replicate height reached its predefined target. Herbicide treatments corresponded to the low and high ends of label recommended rates for cheatgrass control (Table 2.1). For all herbicide treatments, a nonionic surfactant (X-77 Spreader, Loveland Products, Inc.) was added at a rate of 0.25% v/v. Herbicides were applied using a moving nozzle sprayer (DeVries Manufacturing) calibrated to deliver 94 L ha^{-1} of spray solution (i.e. water plus herbicide plus surfactant) at 275.6 kPa.

Table 2.1: Herbicide treatments used in Experiment 1 and 2.

Herbicide	Low rate (kg ai ha^{-1})	High rate (kg ai ha^{-1})
Sethoxydim	0.210	0.315
Clethodim	0.076	0.136
Fluazifop	0.280	0.420
Quizalofop	0.077	0.092
Glyphosate	0.420	0.560
Control	NA	NA

Lengths of the three longest leaves (cm) and the total number of leaves were recorded for each plant (sampling unit) prior to herbicide treatment. The pre-defined plant application heights in our experiment, i.e. 5 cm, 8.5 cm, 11 cm, 15.5 cm, or 17 cm correspond to the following average number of leaves: 1, 3, 9, 21, and 35 respectively. At 45 days after herbicide application, the total number of leaves were recorded again, after which each plant was cut at the root crown, all tissue was dried at 40 C for 72 h, and weighed.

Experiment 2: Efficacy of Herbicide Type and Rate on Three Cheatgrass and Japanese Brome Accessions

The efficacy of the same four graminicides (sethoxydim, clethodim, fluazifop, and quizalofop), and glyphosate, applied at two application rates, and an untreated control, was assessed on three cheatgrass and Japanese brome accessions. Seed accessions of both species were collected from three grassland locations to determine if there were site-specific differences in response to herbicides. Cheatgrass and Japanese brome seeds were collected from disturbed restoration sites on Decker (45°06’ N, 106°84’ W) and Spring Creek (45°12’ N, 106°91’ W) coal mines, north of Decker, MT, in the Powder River Basin. The remaining cheatgrass site was the Montana State University Red Bluff Agricultural Research Ranch in Norris, MT (45°52’ N, 111°68’ W), and Japanese brome site was Burke Park in Bozeman, MT (45°67’ N, 111°03’ W). These two sites are hereafter referred to as “undisturbed”. This experiment was conducted over a seven-

month period, November 2015 through May 2016, in a MSU greenhouse with the same climate and light conditions as Experiment 1.

Seeds were sown and subsequently thinned as with Experiment 1. For this experiment, we used one height (11 cm) as our target plant height. After 30 days seedlings were placed in the cold chamber (4 C, 12-hr photoperiod) to vernalize for six weeks, then returned to the greenhouse. When the average height of the plants within a replicate reached 11 cm, the same herbicide treatments were applied as in Experiment 1. The experiment was designed as a randomized complete block design: 11 herbicide treatments x 2 species x 3 accessions x 7 replicates = 462 experimental units. The same metrics were sampled as Experiment 1.

Data Analysis

Data were analyzed with linear mixed-effects using the lmerTest and lme4 (Bates et al. 2015) packages. Least-squares means and Tukey pairwise comparisons were evaluated using the lsmeans (Lenth 2016) package. Data analysis was performed using R version 3.3.2 (R Core Team, 2016). The most parsimonious model was selected using Akaike Information Criterion (AIC) with a decrease in AIC score of two being considered a better fit. In all models the biomass response variable was log transformed to satisfy model assumptions.

For Experiment 1, a linear mixed-effects model was created where the response variable was natural log of plant biomass at time of harvest for each replicate. Initially a full model was run with fixed effects for treatment (all herbicide and rate combinations),

height at time of application (5, 8.5, 11, 15.5, or 17 cm), and trial (1 and 2) along with the interactions among treatment and height, trial and height, and treatment, trial, and height, as well as a random effect for replicate. Individual models were then created for each plant height group, to better elucidate the efficacy of herbicide treatments**.** For 5 cm, 8.5 cm, and 17 cm plant heights, fixed effects were herbicide, trial, and the interaction between herbicide and trial. The data from the 4 cm plant height treated with the clethodim low herbicide application during trial 2 in Experiment 1 were excluded due to a problem with the spray chamber during application. For the 11 cm and 15.5 cm plants, herbicide and trial were included as fixed effects. In all models, a random effect was included for replicate. The same models were run using the total number of leaves 45 days after treatment as the response variable.

Also for Experiment 1, a linear mixed-effects model was created where the response variable was natural log of plant biomass at time of harvest for each replicate with fixed effects for treatment (all herbicide and rate combinations), trial (1 and 2), and natural log number of leaves at time of application, along with the interactions between treatment and number of leaves, and trial and number of leaves, as well as a random effect for replicate.

Similar models were created for natural log of plant biomass at time of harvest and number of leaves 45 days after treatment for Experiment 2. Fixed effects were included for herbicide treatment (all herbicide and rate combinations), accession (Decker, Spring Creek, or undisturbed), and species (Japanese brome or cheatgrass), as

well as the interaction between herbicide and species. There was no difference between the Spring Creek and Decker mine accessions ($p = 0.3393$), so they were combined in the final analysis and are hereafter referred to as "disturbed". A random effect was included for replicate.

Results

Experiment 1:
Efficacy of Herbicide Type and Rate Applied to Cheatgrass of Different Heights

For all heights, the main effect of trial was significant, with greater biomass reduction in the first than second trial for the 5 cm and 8.5 cm heights (Tables 2.2 and 2.3), and the opposite for the 11 cm, 15.5 cm, and 17 cm heights (Tables 2.4 through 2.6). Overall, in Experiment 1 for all but one of the heights in both trials, the biomass of plants treated with herbicides was lower than the control (Figure 2.1): in the second trial in the 17 cm height, many of the herbicide treatments resulted in greater biomass than the untreated control. In both trials, the larger the plant height at time of herbicide application, the lower the efficacy for every herbicide treatment (Figure 2.1). A very similar pattern was found across herbicide treatments and height groups for number of leaves 45 days after treatment (Figure A.1 and Tables A.1 through A.5 in the Appendix). All plants sprayed at 5 cm (Table 2.2) and 8.5 cm (Table 2.3) heights had significantly reduced plant biomass compared to the control. Biomass was significantly reduced in the 11 cm height for all treatments except the glyphosate low application (Table 2.4), and in the 15.5 cm height except the glyphosate low and sethoxydim low applications

(Table 2.5). For the largest plant group height (17 cm) in the first trial, all treatments except glyphosate low, glyphosate high and sethoxydim low applications significantly reduced plant biomass compared to the control, however, none of the treatments had an effect in the second trial (Table 2.6).

In general, glyphosate was not as effective at reducing biomass as the graminicides. There were no biomass differences between low and high rates of any herbicide treatments for the 5 cm height in either trial (Figure 2.1). For the 8.5 cm height, in both trials the glyphosate low treatment had significantly higher biomass than both fluazifop treatments (trial 1 low $p = 0.0496$; trial 2 low $p < 0.0001$; trial 1 high $p = 0.0028$; trial 2 high $p < 0.0001$) and quizalofop treatments (trial 1 low $p = 0.0044$; trial 2 low $p < 0.0001$; trial 2 high $p = 0.0008$; trial 2 high $p = 0.0002$). In the second trial, the glyphosate low treatment had significantly higher biomass than both sethoxydim (low $p = 0.0016$; high $p < 0.0001$) and clethodim (low $p = 0.0002$; high $p = 0.0029$) applications. For the 11 cm height, the glyphosate low treatment had significantly higher biomass than both fluazifop treatments (low $p < 0.0001$; high $p < 0.0001$), both quizalofop treatments (low $p < 0.0001$; high $p < 0.0001$), both clethodim treatments (low $p < 0.0001$; high $p < 0.0001$), the sethoxydim high treatment ($p = 0.0006$), and the glyphosate high treatment ($p < 0.0001$). The 15.5 cm height had a similar trend: glyphosate low treatment had significantly higher biomass than both fluazifop treatments (low $p < 0.0001$; high $p = 0.0102$), both quizalofop treatments (low $p < 0.0001$; high $p < 0.0001$), both clethodim treatments (low $p = 0.0044$; high $p = 0.0007$),

and the sethoxydim high treatment ($p = 0.0235$). For the 17 cm height, trial was again significant, and there was an interaction with herbicide. In the first trial, the glyphosate low treatment had significantly higher biomass than both fluazifop treatments (low $p < 0.0001$; high $p < 0.0001$), both quizalofop treatments (low $p < 0.0001$; high $p < 0.0001$), both clethodim treatments (low $p = 0.0121$; high $p = 0.0002$), and the sethoxydim high treatment ($p = 0.0003$). However, in the second trial the glyphosate low treatment had significantly lower biomass than both fluazifop treatments (low $p = 0.0008$; high $p = 0.0414$), both clethodim treatments (low $p < 0.0001$; high $p = 0.0131$), both sethoxydim treatments (low $p < 0.0001$; high $p < 0.0001$), and the glyphosate high treatment ($p < 0.0001$).

All graminicides performed well overall. There was no difference among graminicides for the 5 cm height, but at the other heights, fluazifop and quizalofop outperformed sethoxydim and clethodim, although they did not vary from one another (Figure 2.1). In the first trial for the 8.5 cm height, quizalofop ($p = 0.0062$) and fluazifop ($p = 0.004$) low treatments had significantly lower biomass than the clethodim low treatment and the quizalofop high treatment had significantly lower biomass than the clethodim low ($p = 0.0012$) and sethoxydim low ($p = 0.0309$) treatments. There were no differences among graminicides in trial 2. For the 11 cm height, in both trials quizalofop ($p = 0.0002$) and fluazifop ($p = 0.0026$) low treatments had significantly lower biomass than the sethoxydim low treatment, as did the fluazifop high ($p = 0.0048$) treatment. For the 15.5 cm height in trials 1 and 2, both fluazifop treatments (low $p < 0.0001$; high $p =$

0.0009) and both quizalofop treatments (low $p < 0.0001$; high $p < 0.0001$) had significantly lower biomass than the sethoxydim low treatment. Similarly, for the 17 cm height in trial 1, both fluazifop treatments (low $p = 0.0007$; high $p < 0.0001$) and both quizalofop treatments (low $p = 0.0177$; high $p = 0.0004$) had significantly lower biomass than the sethoxydim low treatment. In the second trial the fluazifop high ($p = 0.0365$) treatment had significantly lower biomass than the sethoxydim low treatment and both quizalofop treatments had significantly lower biomass than the sethoxydim low (low $p < 0.0001$; high $p < 0.0001$) and high (low $p < 0.0001$; high $p < 0.0001$) treatments. The only difference between fluazifop and quizalofop was in 17 cm plant group in trial 2 where both quizalofop treatments (low $p = 0.0058$; high $p = 0.0046$) outperformed the fluazifop low treatment.

When the data from Experiment 1 was analyzed using number of leaves at time of spraying (continuous variable) instead of height at time of spraying, the results yielded similar patterns (Figure 2.2). As the number of leaves at time of spraying increased, the efficacy of all herbicide treatment decreased (Table 2.7; $p = 0.0018$), and generally the herbicides worked best on plants with less than 12 leaves. There was little difference among herbicide treatments when applied at the high rate, but when applied at the low rates, fluazifop and quizalofop were more effective at reducing plant biomass (Figure 2.2; Table 2.7).

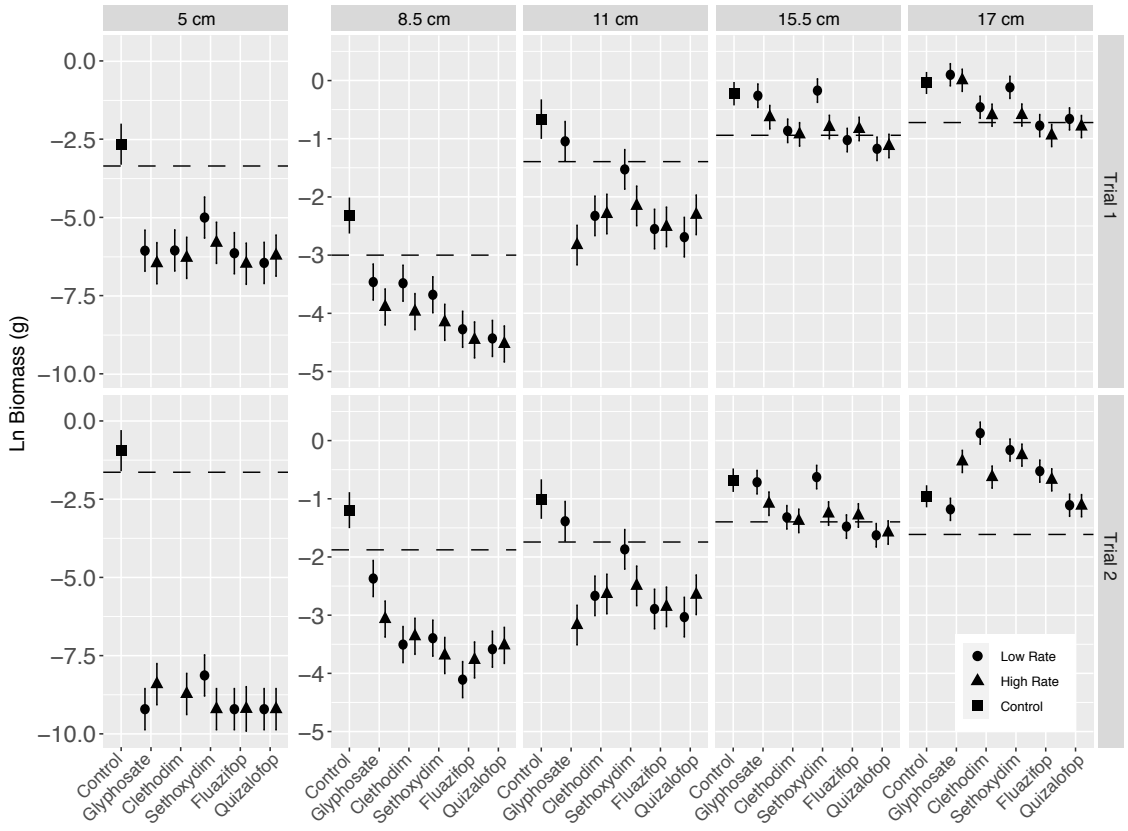

Figure 2.1: Natural log of individual plant biomass (g) using least-squares means within herbicide and rate treatments for each cheatgrass height at the time of herbicide application, for both trials of Experiment 1. Heights represent the means of the individual plants within a group. Dashed lines represent a 50% reduction in biomass compared to the control.

Table 2.2: Linear mixed-effects model results assessing log transformed individual biomass (g) for plants that were 5 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance (p <0.05). Baseline levels in the intercept are: control plants (no herbicide applications) and trial 1.

5 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**-2.649**	**0.332**	**125**	**-8.0**	**<0.0001**	Replicate	0.011
Glyphosate low	**-3.404**	**0.466**	**119**	**-7.3**	**<0.0001**	Residual	0.760
Clethodim low	**-3.397**	**0.466**	**119**	**-7.3**	**<0.0001**		
Sethoxydim low	**-2.345**	**0.466**	**119**	**-5.0**	**<0.0001**		
Fluazifop low	**-3.485**	**0.466**	**119**	**-7.5**	**<0.0001**		
Quizalofop low	**-3.795**	**0.466**	**119**	**-8.1**	**<0.0001**		
Glyphosate high	**-3.805**	**0.466**	**119**	**-8.2**	**<0.0001**		
Clethodim high	**-3.632**	**0.466**	**119**	**-7.8**	**<0.0001**		
Sethoxydim high	**-3.152**	**0.466**	**119**	**-6.8**	**<0.0001**		
Fluazifop high	**-3.821**	**0.466**	**119**	**-8.2**	**<0.0001**		
Quizalofop high	**-3.562**	**0.466**	**119**	**-7.6**	**<0.0001**		
Trial 2	**1.702**	**0.466**	**119**	**3.7**	**<0.0001**		
Glyphosate low : Trial 2	**-4.860**	**0.659**	**119**	**-7.4**	**<0.0001**		
Sethoxydim low : Trial 2	**-4.841**	**0.659**	**119**	**-7.3**	**<0.0001**		
Fluazifop low : Trial 2	**-4.779**	**0.659**	**119**	**-7.3**	**<0.0001**		
Quizalofop low : Trial 2	**-4.469**	**0.659**	**119**	**-6.8**	**<0.0001**		
Glyphosate high : Trial 2	**-3.660**	**0.659**	**119**	**-5.6**	**<0.0001**		
Clethodim high : Trial 2	**-4.146**	**0.659**	**119**	**-6.3**	**<0.0001**		
Sethoxydim high : Trial 2	**-5.112**	**0.659**	**119**	**-7.8**	**<0.0001**		
Fluazifop high : Trial 2	**-4.435**	**0.673**	**119**	**-6.6**	**<0.0001**		
Quizalofop high : Trial 2	**-4.702**	**0.659**	**119**	**-7.1**	**<0.0001**		

Table 2.3: Linear mixed-effects model results assessing log transformed individual biomass (g) for plants that were 8.5 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance (p <0.05). Baseline levels in the intercept are: control plants (no herbicide applications) and trial 1.

8.5 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**-2.321**	**0.156**	**132**	**-14.8**	**<0.0001**	Replicate	0.000
Glyphosate low	**-1.143**	**0.221**	**132**	**-5.2**	**<0.0001**	Residual	0.171
Clethodim low	**-1.163**	**0.221**	**132**	**-5.3**	**<0.0001**		
Sethoxydim low	**-1.361**	**0.221**	**132**	**-6.2**	**<0.0001**		
Fluazifop low	**-1.955**	**0.221**	**132**	**-8.8**	**<0.0001**		
Quizalofop low	**-2.112**	**0.221**	**132**	**-9.6**	**<0.0001**		
Glyphosate high	**-1.571**	**0.221**	**132**	**-7.1**	**<0.0001**		
Clethodim high	**-1.651**	**0.221**	**132**	**-7.5**	**<0.0001**		
Sethoxydim high	**-1.835**	**0.221**	**132**	**-8.3**	**<0.0001**		
Fluazifop high	**-2.138**	**0.221**	**132**	**-9.7**	**<0.0001**		
Quizalofop high	**-2.207**	**0.221**	**132**	**-10.0**	**<0.0001**		
Trial 2	**1.125**	**0.221**	**132**	**5.1**	**<0.0001**		
Glyphosate low : Trial 2	-0.032	0.313	132	-0.1	0.9190		
Sethoxydim low : Trial 2	**-1.146**	**0.313**	**132**	**-3.7**	**0.0004**		
Fluazifop low : Trial 2	**-0.839**	**0.313**	**132**	**-2.7**	**0.0083**		
Quizalofop low : Trial 2	**-0.958**	**0.313**	**132**	**-3.1**	**0.0027**		
Glyphosate high : Trial 2	-0.277	0.313	132	-0.9	0.3767		
Clethodim high : Trial 2	-0.300	0.313	132	-1.0	0.3392		
Sethoxydim high : Trial 2	-0.516	0.313	132	-1.7	0.1013		
Fluazifop high : Trial 2	**-0.663**	**0.313**	**132**	**-2.1**	**0.0359**		
Quizalofop high : Trial 2	-0.436	0.313	132	-1.4	0.1651		

Table 2.4: Linear mixed-effects model results assessing log transformed individual biomass (g) for plants that were 11 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance (p <0.05). Baseline levels in the intercept are: control plants (no herbicide applications) and trial 1.

11 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**-0.664**	**0.172**	**122**	**-3.9**	**0.0002**	Replicate	0.009
Glyphosate low	-0.382	0.227	136	-1.7	0.0949	Residual	0.361
Clethodim low	**-1.663**	**0.227**	**136**	**-7.3**	**<0.0001**		
Sethoxydim low	**-0.864**	**0.227**	**136**	**-3.8**	**0.0002**		
Fluazifop low	**-1.889**	**0.227**	**136**	**-8.3**	**<0.0001**		
Quizalofop low	**-2.028**	**0.227**	**136**	**-8.9**	**<0.0001**		
Glyphosate high	**-2.165**	**0.227**	**136**	**-9.5**	**<0.0001**		
Clethodim high	**-1.631**	**0.227**	**136**	**-7.2**	**<0.0001**		
Sethoxydim high	**-1.492**	**0.227**	**136**	**-6.6**	**<0.0001**		
Fluazifop high	**-1.853**	**0.227**	**136**	**-8.2**	**<0.0001**		
Quizalofop high	**-1.645**	**0.227**	**136**	**-7.2**	**<0.0001**		
Trial 2	**-0.341**	**0.097**	**136**	**-3.5**	**0.0006**		

Table 2.5: Linear mixed-effects model results assessing log transformed individual biomass (g) for plants that were 15.5 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance (p <0.05). Baseline levels in the intercept are: control plants (no herbicide applications) and trial 1.

15.5 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**-0.227**	**0.102**	**142**	**-2.2**	**0.0275**	Replicate	<0.0001
Glyphosate low	-0.035	0.138	142	-0.3	0.8018	Residual	0.130
Clethodim low	**-0.638**	**0.138**	**142**	**-4.6**	**<0.0001**		
Sethoxydim low	0.053	0.138	142	0.4	0.7003		
Fluazifop low	**-0.797**	**0.138**	**142**	**-5.8**	**<0.0001**		
Quizalofop low	**-0.947**	**0.138**	**142**	**-6.9**	**<0.0001**		
Glyphosate high	**-0.404**	**0.138**	**142**	**-2.9**	**0.0040**		
Clethodim high	**-0.700**	**0.138**	**142**	**-5.1**	**<0.0001**		
Sethoxydim high	**-0.573**	**0.138**	**142**	**-4.2**	**<0.0001**		
Fluazifop high	**-0.607**	**0.138**	**142**	**-4.4**	**<0.0001**		
Quizalofop high	**-0.899**	**0.138**	**142**	**-6.5**	**<0.0001**		
Trial 2	**-0.454**	**0.059**	**142**	**-7.7**	**<0.0001**		

Table 2.6: Linear mixed-effects model results assessing log transformed individual biomass (g) for plants that were 17 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance (p <0.05). Baseline levels in the intercept are: control plants (no herbicide applications) and trial 1.

17 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	-0.043	0.096	132	-0.4	0.6553	Replicate	0.0001
Glyphosate low	0.141	0.136	126	1.0	0.3015	Residual	0.0644
Clethodim low	**-0.417**	**0.136**	**126**	**-3.1**	**0.0026**		
Sethoxydim low	-0.075	0.136	126	-0.6	0.5808		
Fluazifop low	**-0.733**	**0.136**	**126**	**-5.4**	**<0.0001**		
Quizalofop low	**-0.618**	**0.136**	**126**	**-4.6**	**<0.0001**		
Glyphosate high	0.046	0.136	126	0.3	0.7342		
Clethodim high	**-0.554**	**0.136**	**126**	**-4.1**	**<0.0001**		
Sethoxydim high	**-0.551**	**0.136**	**126**	**-4.1**	**<0.0001**		
Fluazifop high	**-0.903**	**0.136**	**126**	**-6.7**	**<0.0001**		
Quizalofop high	**-0.749**	**0.136**	**126**	**-5.5**	**<0.0001**		
Trial 2	**-0.914**	**0.136**	**126**	**-6.7**	**<0.0001**		
Glyphosate low : Trial 2	-0.365	0.192	126	-1.9	0.0595		
Sethoxydim low : Trial 2	**1.501**	**0.192**	**126**	**7.8**	**<0.0001**		
Fluazifop low : Trial 2	**0.870**	**0.192**	**126**	**4.5**	**<0.0001**		
Quizalofop low : Trial 2	**1.165**	**0.192**	**126**	**6.1**	**<0.0001**		
Glyphosate high : Trial 2	**0.464**	**0.192**	**126**	**2.4**	**0.0170**		
Clethodim high : Trial 2	**0.553**	**0.192**	**126**	**2.9**	**0.0047**		
Sethoxydim high : Trial 2	**0.884**	**0.192**	**126**	**4.6**	**<0.0001**		
Fluazifop high : Trial 2	**1.258**	**0.192**	**126**	**6.6**	**<0.0001**		
Quizalofop high : Trial 2	**1.186**	**0.192**	**126**	**6.2**	**<0.0001**		

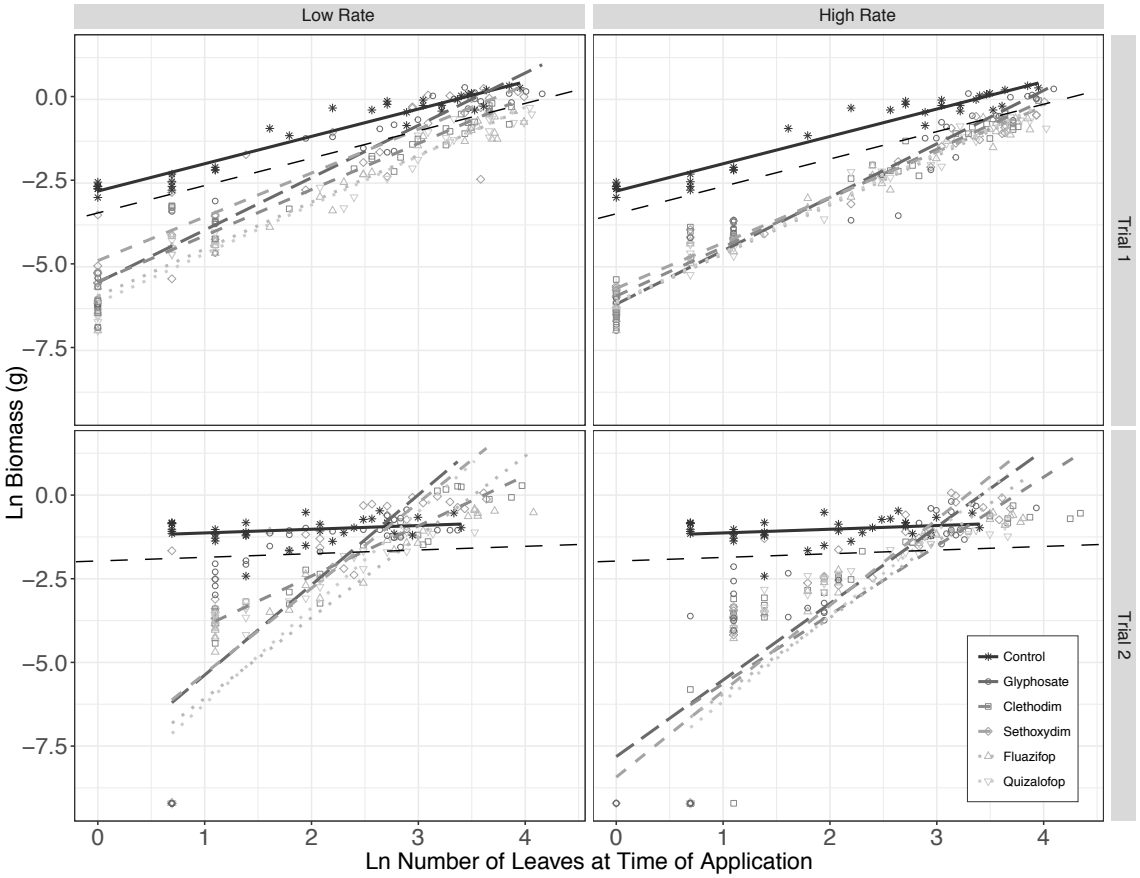

Figure 2.2: Effect of number of leaves at time of spraying on plant biomass within herbicide for both rate and trial of Experiment 1. Points represent individual plants. Black dashed lines represent a 50% reduction in biomass compared to the control.

Table 2.7: Linear mixed-effects model results assessing log transformed individual biomass (g) for all plants for Experiment 1 using number of leaves at application as the explanatory variable. Satterthwaite approximations were used. Predictors in bold indicate significance ($p < 0.05$). Baseline levels in the intercept are: control plants (no herbicide applications) and trial 1.

Experiment 1	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**-1.579**	**0.284**	**738**	**-5.6**	**<0.0001**	Replicate	0.000
Glyphosate low	**-4.086**	**0.391**	**738**	**-10.4**	**<0.0001**	Residual	1.429
Clethodim low	**-3.345**	**0.412**	**738**	**-8.1**	**<0.0001**		
Sethoxydim low	**-3.650**	**0.389**	**738**	**-9.4**	**<0.0001**		
Fluazifop low	**-4.571**	**0.386**	**738**	**-11.9**	**<0.0001**		
Quizalofop low	**-4.770**	**0.388**	**738**	**-12.3**	**<0.0001**		
Glyphosate high	**-4.434**	**0.385**	**738**	**-11.5**	**<0.0001**		
Clethodim high	**-4.272**	**0.389**	**738**	**-11.0**	**<0.0001**		
Sethoxydim high	**-4.364**	**0.386**	**738**	**-11.3**	**<0.0001**		
Fluazifop high	**-4.592**	**0.389**	**738**	**-11.8**	**<0.0001**		
Quizalofop high	**-4.734**	**0.392**	**738**	**-12.1**	**<0.0001**		
Trial 2	**-1.965**	**0.174**	**738**	**-11.3**	**<0.0001**		
Number of Leaves	**0.391**	**0.125**	**738**	**3.1**	**0.0018**		
Glyphosate low : # Leaves	**1.264**	**0.171**	**738**	**7.4**	**<0.0001**		
Sethoxydim low : # Leaves	**0.825**	**0.172**	**738**	**4.8**	**<0.0001**		
Fluazifop low : # Leaves	**1.063**	**0.169**	**738**	**6.3**	**<0.0001**		
Quizalofop low : # Leaves	**1.087**	**0.166**	**738**	**6.5**	**<0.0001**		
Glyphosate high : # Leaves	**1.221**	**0.170**	**738**	**7.2**	**<0.0001**		
Clethodim high : # Leaves	**1.163**	**0.165**	**738**	**7.0**	**<0.0001**		
Sethoxydim high : # Leaves	**0.987**	**0.166**	**738**	**6.0**	**<0.0001**		
Fluazifop high : # Leaves	**1.108**	**0.166**	**738**	**6.7**	**<0.0001**		
Quizalofop high : # Leaves	**1.077**	**0.166**	**738**	**6.5**	**<0.0001**		
Glyphosate low : # Leaves	**1.173**	**0.170**	**738**	**6.9**	**<0.0001**		
Trial 2 : # Leaves	**0.808**	**0.074**	**738**	**10.9**	**<0.0001**		

Experiment 2: Efficacy of
Herbicide Type and Rate on Three Cheatgrass and Japanese Brome Accessions

Like the first experiment, the herbicides were effective at reducing plant biomass compared to the control (Figure 2.3; Table 2.8). A similar pattern was found across herbicide treatments, accession, and species for number of leaves 45 days after treatment (Figure A.2 and Table A.6 in the Appendix). The herbicide treatments had a greater negative impact on Japanese brome biomass compared to cheatgrass (Table 2.8; $p < 0.0001$). The herbicide treatments also had a greater negative impact on the disturbed accessions compared to the undisturbed (Table 2.8; $p < 0.0001$). Like Experiment 1, the glyphosate low treatment was the least effective at controlling cheatgrass, and the same pattern was observed for Japanese brome. For the cheatgrass accessions the glyphosate low treatment had significantly higher biomass than both fluazifop treatments (low $p < 0.0001$; high $p < 0.0001$), both quizalofop treatments (low $p < 0.0001$; high $p < 0.0025$), both clethodim treatments (low $p < 0.0001$; high $p < 0.0001$), and the glyphosate high treatment ($p < 0.0001$). For the Japanese brome accessions, the glyphosate low treatment had significantly higher biomass than all of the graminicide treatments (fluazifop low $p < 0.0001$; fluazifop high $p < 0.0001$; quizalofop low $p < 0.0001$; quizalofop high $p < 0.0001$; clethodim low $p < 0.0001$; clethodim high $p < 0.0001$; sethoxydim low $p < 0.0001$; sethoxydim high $p < 0.0001$) and the glyphosate high treatment ($p < 0.0001$).

For Japanese brome, there were no efficacy differences among the graminicide treatments, nor between the disturbed and undisturbed accessions. However, for

cheatgrass accessions, the fluazifop and quizalofop were the most effective, similar to Experiment 1. Both fluazifop treatments (low $p < 0.0001$; high $p < 0.0001$) and the quizalofop low treatment ($p = 0.0001$) had lower biomass than the sethoxydim low treatment, and the fluazifop low treatment had lower biomass than the sethoxydim high treatment ($p = 0.0168$). Within sethoxydim and clethodim, both the clethodim low ($p < 0.0001$) and high ($p = 0.0008$) treatments had lower biomass compared to the sethoxydim low treatment.

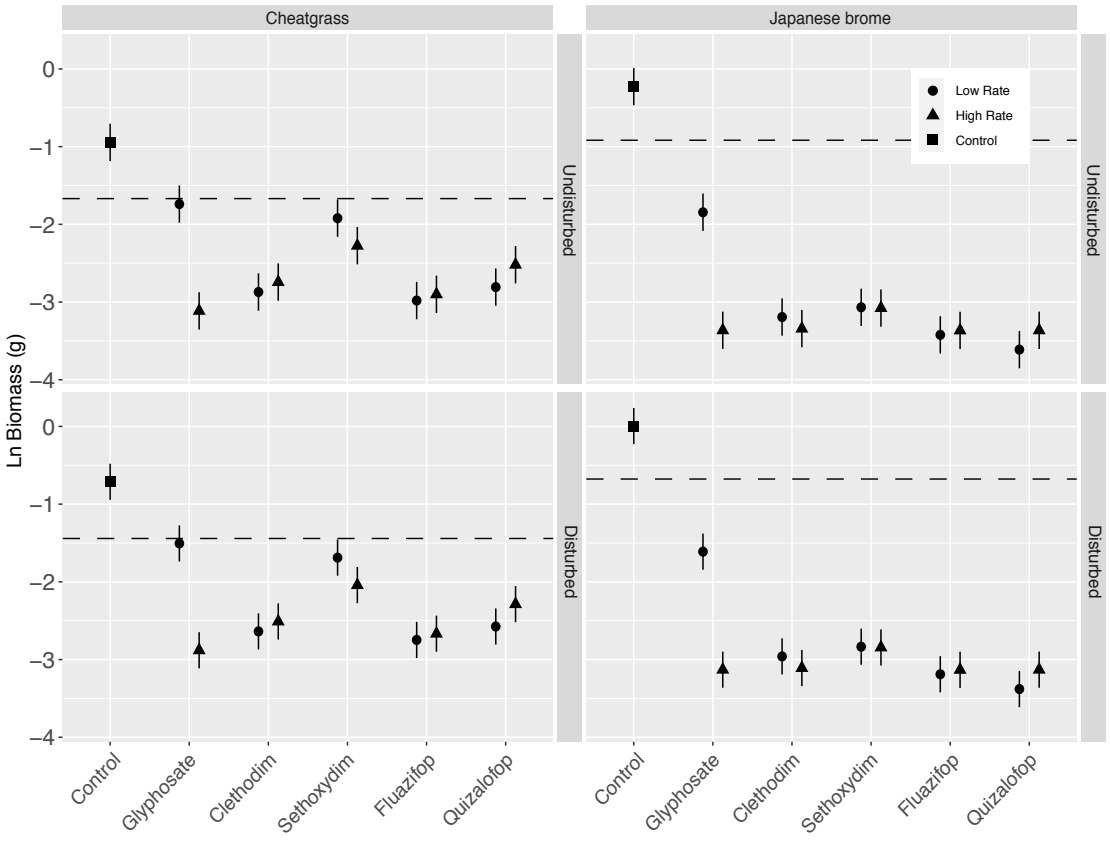

Figure 2.3: Natural log of individual plant biomass (g) using least-squares means within herbicide and rate treatments for undisturbed and disturbed cheatgrass and Japanese brome accessions treated at 11 cm mean plant height for Experiment 2. Dashed lines represent a 50% reduction in biomass compared to the control.

Table 2.8: Linear mixed-effects model results from assessing log transformed individual plant biomass (g) for Experiment 2. Satterthwaite approximations were used. Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications), undisturbed accession, and cheatgrass. BRJA is Japanese brome.

Experiment 2	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**-0.946**	**0.122**	**439**	**-7.7**	**<0.0001**	Replicate	<0.0001
Glyphosate low	**-0.793**	**0.166**	**439**	**-4.8**	**<0.0001**	Residual	0.288
Clethodim low	**-1.925**	**0.166**	**439**	**-11.6**	**<0.0001**		
Sethoxydim low	**-0.975**	**0.166**	**439**	**-5.9**	**<0.0001**		
Fluazifop low	**-2.035**	**0.166**	**439**	**-12.3**	**<0.0001**		
Quizalofop low	**-1.862**	**0.166**	**439**	**-11.2**	**<0.0001**		
Glyphosate high	**-2.168**	**0.166**	**439**	**-13.1**	**<0.0001**		
Clethodim high	**-1.797**	**0.166**	**439**	**-10.9**	**<0.0001**		
Sethoxydim high	**-1.329**	**0.166**	**439**	**-8.0**	**<0.0001**		
Fluazifop high	**-1.954**	**0.166**	**439**	**-11.8**	**<0.0001**		
Quizalofop high	**-1.574**	**0.166**	**439**	**-9.5**	**<0.0001**		
Disturbed accession	**0.233**	**0.053**	**439**	**4.4**	**<0.0001**		
Japanese brome	**0.718**	**0.166**	**439**	**4.3**	**<0.0001**		
Glyphosate low : BRJA	**-0.823**	**0.234**	**439**	**-3.5**	**<0.0001**		
Clethodim low : BRJA	**-1.039**	**0.234**	**439**	**-4.4**	**<0.0001**		
Sethoxydim low : BRJA	**-1.864**	**0.234**	**439**	**-8.0**	**<0.0001**		
Fluazifop low : BRJA	**-1.159**	**0.234**	**439**	**-5.0**	**<0.0001**		
Quizalofop low : BRJA	**-1.523**	**0.234**	**439**	**-6.5**	**<0.0001**		
Glyphosate high : BRJA	**-0.968**	**0.234**	**439**	**-4.1**	**<0.0001**		
Clethodim high : BRJA	**-1.317**	**0.234**	**439**	**-5.6**	**<0.0001**		
Sethoxydim high : BRJA	**-1.520**	**0.234**	**439**	**-6.5**	**<0.0001**		
Fluazifop high : BRJA	**-1.183**	**0.234**	**439**	**-5.1**	**<0.0001**		
Quizalofop high : BRJA	**-1.561**	**0.234**	**439**	**-6.7**	**<0.0001**		

Discussion

Glyphosate, sethoxydim, clethodim, fluazifop, and quizalofop all reduced cheatgrass and Japanese brome biomass, especially when applied shortly after germination – with a tendency for fluazifop and quizalofop to work best. In Experiment 1, at the 5 cm, 8.5 cm and 11 cm heights, or with plants with less than 12 leaves, all herbicides reduced biomass by more than 50 percent of the control, except the glyphosate low treatment at 11 cm. In Experiment 2, where treatments were applied at 11 cm, we found a similar pattern to those found in Experiment 1 across herbicides. Quizalofop and fluazifop were again the most effective, and the glyphosate low treatment the least effective at reducing biomass 45 days after treatment.

The low and high treatments for all graminicide treatments reduced plant biomass by at least 50% compared to the control in the groups sprayed when individuals were 11 cm or smaller. However, for glyphosate, application rate did matter. The high rate was more effective than the low rate for glyphosate applied at 11 cm in Experiment 1 and 2. Thus, for glyphosate, the high application rate was necessary to ensure significant control; this will likely be especially important in a field setting where target plant heights could vary. Park and Mallory-Smith (2004) applied glyphosate at a rate of 0.420 kg ai ha^{-1} to cheatgrass plants in the three to four leaf stage and found an average of 85% control. In our study, this same treatment (8.5 cm height group treated with our low glyphosate treatment) only provided an average of 68% control of cheatgrass. In a Wyoming field experiment, Whitson and Koch (1998) applied glyphosate to cheatgrass

plants at the two to eight leaf stage at 0.42, 0.55, 0.69, and 0.83 kg ai ha^{-1} and achieved >99% decrease in live canopy cover in all treatments. This is far greater control than we achieved with our glyphosate treatments in our 11 cm plant group. It has been shown that higher rates of imazapic can increase the effectiveness of cheatgrass control (Morris, Monaco, and Rigby 2009), but broad-spectrum herbicides like glyphosate and imazapic can also damage desired species (Kyser et al. 2013). Because graminicides are grass-specific, using a higher rate to control bromes, should not increase the damage to non-target shrub and broad leaf species (Kukorelli, Reisinger, and Pinke 2013), and therefore using graminicides may provide a good alternative to broad-spectrum herbicides in restoration scenarios.

Unlike rate, application timing influenced brome control for all herbicides tested. Treatments were less effective as the plant size at time of application got larger, with little or no reduction in biomass when herbicide was applied at the 17 cm height. All herbicides when applied to plants 11 cm or smaller, except the glyphosate low treatment, reduced biomass by more than 50% of the control. Often studies report timing of herbicide application in relation to germination (i.e. pre-emergence or post-emergence) or relative to each other (i.e. early or late), but herbicide label recommendations are made based on growth stage (number of leaves) and plant height. In studies that have reported growth stage or plant height at the time of application, the results vary. Owen, Sieg, and Gehring (2011) found no difference in cheatgrass control when imazapic was applied to plants with two to four leaves

compared to plants with five to 10 leaves. In contrast, Mangold et al. (2013) found that cheatgrass control increased when imazapic was applied to plants at the one to two-leaf stage compared to the three to four-leaf stage. We found that herbicide efficacy is greatest when plants are treated when they are small, and that brome control varied on a much finer scale (i.e. 2.5 cm intervals) than is often recommended (i.e. before 15 cm or in the 3 to 8 leaf stage). While logistical constraints of large-scale herbicide applications (e.g. spring precipitation and plant growth patterns, wind, tractor and ATV access, etc.) often hamper timely application, both plant height and number of leaves are simple to assess in the field. Our study demonstrates that targeting smaller plants provides more reliable results. Specifically, spraying when plants are 11 cm or smaller, with less than 12 leaves resulted in greater than 50% reduction of biomass in all but one (i.e. the glyphosate low) treatment.

In Experiment 2, we found that all herbicides were more effective at reducing biomass for Japanese brome compared to cheatgrass. The Japanese brome untreated control plant biomass was greater than the cheatgrass control plants, and in contrast, the Japanese brome plants treated with herbicides all had lower biomass than their cheatgrass counterparts that received the same application. Studies have found that glyphosate is effective at reducing cheatgrass biomass (Cox and Anderson 2004; Park and Mallory-Smith 2004; Whitson and Koch 1998), and in the only study to test effectiveness on Japanese brome, Waller and Schmidt (1983) stated that it provided excellent control of Japanese brome in a Nebraska tall grass prairie, though no data

were reported. This also agrees with our findings, but for both Japanese brome and cheatgrass the low glyphosate rate performed significantly worse than the high rate, where the biomass was 457% and 395% greater, respectively. There is limited information addressing graminicides ability to control cheatgrass (Ball, Frost, and Bennett 2007; Brewster and Spinney 1989), but in the few studies that do, graminicides provided good control. Additionally, our study agrees with Ball, Frost, and Bennett (2007) who found that quizalofop and fluazifop are generally the most effective and sethoxydim the least effective. There are no studies that test the efficacy of these graminicides on Japanese brome.

Additionally, in Experiment 2 we found there was no difference between the disturbed accessions (i.e. Spring Creek mine and Decker mine), and together they were less susceptible than the undisturbed accessions (i.e. Red Bluff or Burke Park) across all herbicides for both response variables. There is evidence to suggest that plant characteristics such as cold tolerance (Bykova and Sage 2012) and germination success (Hardegree et al. 2013) can vary across cheatgrass accession. Additionally, some cheatgrass accessions have developed resistance to both ALS inhibitors (Park and Mallory-Smith 2004; Mueller-Warrant, Mallory-Smith, and Hendrickson 1999) and graminicides (Ball, Frost, and Bennett 2007; Park and Mallory-Smith 2004). In our study, we found differences in herbicide control among accessions. The disturbed sites (Spring Creek and Decker mine) are located within 25 km of one another, so are likely to be genetically similar and this could be why there was no difference between them. The

disturbed sites did differ from the undisturbed sites (Red Bluff for cheatgrass and Burke Park for Japanese brome) which were geographically distant, and therefore are likely genetically different.

This was a greenhouse study and results would likely vary under field conditions (Fletcher, Johnson, and McFarlane 1990). Additionally, long-term impacts of herbicide applications in the field can differ from a single application in a greenhouse. Field studies that measured the impacts of a single glyphosate application over two to three years found variable results from no brome control (Cox and Anderson 2004) to moderate control (McAdoo, Boyd, and Sheley 2013) to significant control (Whitson and Koch 1998), possibly due to plants emanating from the seedbank. All of these studies, however, significantly decreased cheatgrass cover in the first year (McAdoo, Boyd, and Sheley 2013; Cox and Anderson 2004; Whitson and Koch 1998). Similarly, Brewster and Spinney (1989) found that graminicides provided high brome control in year one. There are no long-term studies that examine graminicide efficacy, though we would expect similarly variable results. This highlights the necessity of following up our greenhouse study with multi-year field observations, particularly with graminicides.

Herbicide control of annual bromes is important as they have invaded large areas (Chambers et al. 2007; Duncan et al. 2004; Knapp 1996; Haferkamp et al. 1992; Whisenant 1990a), their ranges are expanding (Bradley, Curtis, and Chambers 2016; Bradley 2009), and are negatively impacting many different systems (Ogle, Reiners, and Gerow 2003; Haferkamp and Heitschmidt 1999; Blackshaw 1993; Rydrych and Muzik

1968). Infestations of cheatgrass and Japanese brome are not limited to single individuals, but are often dense stands where both species regularly co-occur. We demonstrated that there are efficacy differences, although small, across species and accessions. While we can only provide insight into six accessions, more experiments on the local scale and adaptive management based on the results of those experiments (Rew, Lehnhoff, and Maxwell 2007; Tu, Hurd, and Randall 2001), have the potential to help increase control. Our results show that graminicides, specifically fluazifop and quizalofop, can be used to control annual bromes, and regardless of herbicide and rate, biomass reduction was significantly greater in cheatgrass when plants were sprayed early post emergence. More specifically, to ensure greatest control it is best to spray herbicides when plants are small with less than 12 leaves and no taller than 11 cm.

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CHAPTER THREE

USING HERBICIDES AND SEEDING TO RESTORE WYOMING BIG SAGEBRUSH TO ANNUAL BROME-INVADED LANDSCAPES OF THE NORTHERN GREAT PLAINS

Introduction

Drylands, defined as arid, semi-arid, and dry-subhumid ecosystems, account for over 40% of Earth's landmass. In addition to storing carbon (O'Mara 2012), these ecosystems are hotspots of global biodiversity (Myers et al. 2000) and house many endemic plant and animal species (Millennium Ecosystem Assessment 2005; White and Nackoney 2003). By providing food and habitat for livestock and game animals (Milchunas and Lauenroth 1993), drylands help support over two billon people worldwide (Millennium Ecosystem Assessment 2005), so these systems are critical to food security.

Despite their acknowledged value, drylands around the world are becoming degraded. Estimates are that 600 to 1,200 million hectares are classified as degraded (Brauch and Spring 2009), and such areas are increasing by an additional 12 million hectares per year (Brauch and Spring 2009). A number of factors have compromised native dryland plant communities including climate change (Mainguet and Da Silva 1998), energy development (e.g. mining, natural gas extraction) (Howard, Schuman, and Rauzi 1977), overgrazing (Akiyama and Kawamura 2007), and conversion of unsuitable areas to croplands (Török et al. 2012). Degradation resulting from these factors is

estimated to cause 42 billion dollars in global income and productivity losses each year (Brauch and Spring 2009; Dregne and Chou 1992).

The degradation of drylands has spawned extensive restoration efforts around the globe, including the United States where over 100 million dollars has been invested annually to restore these systems (Merritt and Dixon 2011; Sheley et al. 2011). Dryland restoration efforts typically revolve around attempting to reintroduce desired plants from seed. Unfortunately, the grasses, forbs, and shrubs sown as part of dryland restoration efforts commonly fail to establish (Mangold et al. 2015; Davies 2010; James and Svejcar 2010).

Shrubs have proven exceedingly difficult to restore. The difficulty of establishing shrubs is troubling because of the important roles they play in drylands (Prevéy et al. 2010; West 1983). Shrubs provide essential habitat for threatened and endangered species (Knick, Hanser, and Preston 2013; Hess and Beck 2012; Rowland et al. 2006; Knick et al. 2003), forage for certain wildlife and livestock species (Shipley et al. 2006; Ngugi et al. 1992), and soil stabilization (Linstädter and Baumann 2013). The presence of shrubs can increase primary production (Aguiar and Sala 1999), increase resilience to disturbance (Minnick and Alward 2015) and help reduce exotic plant invasions (Prevéy et al. 2010). Additionally, shrubs can sometimes benefit neighboring plants by creating microsites or 'nutrient islands' (Boyd and Davies 2012) with high soil organic carbon (Minnick and Alward 2015), and soil temperatures (Davies, Bates, and James 2009) and water levels (Davies, Bates, and Miller 2007).

In the western U.S., sagebrush *(Artemisia tridentata*) is a shrub of particular conservation concern. This species currently occupies only about half of its historic approximately five million hectare range (Miller et al. 2011; Pyke et al. 2014), and sagebrush habitat is expected to decline another 40% by midcentury due to climate change (Still and Richardson 2015). Restoring and protecting sagebrush habitats has become a major goal throughout the western U.S., in large part because greater sage grouse (*Centrocercus urophasianus*) and a number of other wildlife species are reliant on sagebrush habitat for survival (Knick et al. 2003). Despite the acknowledged necessity of sagebrush, it remains one of the most difficult species to restore.

Plant competition is a key barrier preventing establishment of sagebrush and other shrubs (Schuman, Booth, and Cockrell 1998; Cook and Lewis 1963; Blaisdell 1949) in natural and restoration settings. In dryland restoration when seed mixes comprising of grasses, forbs, and shrubs are sown into bare soil, shrub establishment is often diminished because of competition with seeded grasses and forbs as well as weedy species . Additionally, in areas where past disturbances have depleted shrubs, competition from existing grass stands and weeds can prevent reintroduction of shrubs from seed (Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011; Blaisdell 1949). A variety of techniques have been used to improve shrub establishment. These include decreasing grass seed rates (Rinella et al. 2015; Rinella, Espeland, and Moffatt 2016), seeding grasses after shrubs have established (Blaisdell 1949), increasing shrub seed rates (Williams et al. 2002; Hild et al. 2006), and transplanting mature shrubs (Davies,

Boyd, and Nafus 2013; Lambert 2005). These strategies can reduce or delay grass competition, overcome safe site limitations, and/or allow shrubs to develop tap roots so they can access plant growth resources in deeper soil layers inaccessible to grasses (Cipriotti et al. 2014; Ward, Wiegand, and Getzin 2013).

A number of studies have shown controlling weedy vegetation with herbicides can increase seeded grass establishment (Mangold et al. 2015; Hirsch-Schantz et al. 2014; Kyser et al. 2013; Elseroad and Rudd 2011; Davies 2010; Whitson and Koch 1998), suggesting herbicides could be used to increase seeded shrubs as well. Additionally, McAdoo, Boyd, and Sheley (2013) found that using herbicides to control weeds benefitted transplanted shrubs, suggesting it may also benefit seeded shrubs. However, results have been disappointing in the few studies that have evaluated herbicides as a means for improving seeded shrub establishment. Davies, Boyd, and Nafus (2013) broadcast seeded Wyoming big sagebrush *(Artemisia tridentata* spp. *wyomingensis* [Beetle & A. Young] S. L. Welsh) at six sites after brushing herbicide onto 0, 25, 50, or 75% of crested wheatgrass (*Agropyron cristatum* [L] Gaertm) plants. Wyoming big sagebrush density was ~0.2 plants m^{-2} where 75% of crested wheatgrass plants were treated with herbicide, whereas no Wyoming big sagebrush was observed in the other treatments. Similarly, after combining herbicide, tillage and seeding treatments in crested wheatgrass stands, Fansler and Mangold (2011) observed very little establishment of Wyoming big sagebrush or other shrubs. Likewise, after using mowing and herbicides to control crested wheatgrass and other weeds, Brabec et al. (2015)

were unable to establish Wyoming big sagebrush and other native species from seed. It is not clear why herbicides failed to allow adequate shrub establishment in these studies. Although, herbicides provided limited long-term control of existing vegetation in all three studies (Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011; Brabec et al. 2015); competition from existing plants may have prevented establishment and survival. Another possibility is that factors indirectly or less related to plant competition (e.g. spring precipitation, seed viability, or soil temperature) prevented establishment (James, Rinella, and Svejcar 2012; Mangla et al. 2011).

The overall goal of this study was to evaluate previously untested herbicide strategies for boosting seeded Wyoming big sagebrush establishment. In addition to seeding Wyoming big sagebrush, we evaluated establishment of seed mixes that varied in the number of grass, forb and shrub species. Field sites were on drylands previously disturbed by coal mining and where restoration efforts failed to meet revegetation goals, supported low species diversity, and were near monocultures of grasses. Some areas were dominated by the perennial native grass western wheatgrass (*Pascopyrum smithii* [Rydb.] Á. Löve). These areas allowed us to explore ways to reintroduce shrubs into perennial grass-dominated lands, a major priority in ours and other systems (Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011). Other areas were dominated by the non-native winter annual grasses cheatgrass (*Bromus tectorum* L.) and to a lesser extent Japanese brome (*Bromus japonicus* Thunb.). Cheatgrass has invaded over 22 million hectares throughout the Intermountain West (Duncan et al.

2004), and it's range is predicted to continue to expand into the cooler climate areas (Bradley, Curtis, and Chambers 2016). Restoring shrubs and other desired plants to cheatgrass-dominated landscapes is widely recognized as a difficult and important challenge (Knutson et al. 2014; Eiswerth et al. 2009).

Although there have been attempts to manage cheatgrass with prescribed fire, grazing, tillage, and biological control agents (Brooks et al. 2016; Germino et al. 2016; Ehlert, Mangold, and Engel 2014; Vermeire, Heitschmidt, and Haferkamp 2008; Monsen, Stevens, and Shaw 2004; Cox and Anderson 2004), herbicides are the most common management tool (Radosevich, Holt, and Ghersa 2007). Herbicides commonly used to control cheatgrass and other unwanted grasses in drylands are acetolactate synthase (ALS) inhibitors (sulfometuron-methyl, rimsulfuron and imazapic), and an enolpyruvylshikimate-3-phosphate synthase inhibitor (glyphosate). A major obstacle to using these herbicides to aid shrub establishment is that, in addition to damaging or killing grasses, they are phytotoxic to shrubs (Owen, Sieg, and Gehring 2011; Morris, Monaco, and Rigby 2009; Baker, Garner, and Lyon 2009). Glyphosate is quickly metabolized in soil, while the ALS herbicides can be absorbed by roots of emerging forbs and shrubs for prolonged periods following application. Therefore, none of these herbicides can be safely used after shrubs emerge, and only glyphosate can be safely used before shrubs emerge.

Another class of post-emergence herbicides are the acetyl CoA carboxylase (ACCase) inhibitors including sethoxydim, clethodim, fluazifop, and quizalofop. These

herbicides are commonly referred to as graminicides because they are phytotoxic to grasses but have no activity against forbs and shrubs (Kukorelli, Reisinger, and Pinke 2013). In a greenhouse study, Ball, Frost, and Bennett (2007) found sethoxydim, clethodim, fluazifop, and quizalofop applications reduced cheatgrass biomass between 85 and 99% at label-recommended rates. They are widely used in annual cropping systems (Marquardt and Johnson 2013) and forestry (Clay, Dixon, and Willoughby 2006), but not drylands. Their selectively for grasses suggests they could play a unique role in dryland restoration because they have the potential to control unwanted grass species, while not harming native shrubs and forbs. To date, graminicides have not been tested in the field for use against annual or perennial grasses in dryland restoration.

In this study, we tested the ability of herbicides to increase establishment of Wyoming big sagebrush and other desirable seeded species at sites dominated by cheatgrass and Japanese brome (hereafter referred to as annual bromes). Additionally, we performed the same test at sites with near monocultures of the perennial native western wheatgrass. Our objectives were to evaluate how four seed mixes factorially combined with three herbicide treatments (the graminicide quizalofop, glyphosate, or both) influenced nonnative annual bromes and forbs, as well as Wyoming big sagebrush and other seeded species.

Methods

Site Description

Study sites were in the northern Great Plains on Decker (45°06’ N, 106°84’ W) and Spring Creek (45°12’ N, 106°91’ W) coal mines in the Powder River Basin north of Decker, MT. Both mines are within the sagebrush steppe biome (West 1983; Johnson 1979). Natural vegetation in areas near the mines is characterized by combinations of warm and cool season perennial grasses including blue grama *(Bouteloua gracilis* [Willd. ex Kunth] Lag. ex Griffiths), prairie Junegrass (*Koeleria macrantha* [Ledeb.] Schult.), alkali sacaton (*Sporobolus airoides* [Torr.] Torr.), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.), sand dropseed (*Sporobolus cryptandrus* [Torr.] A. Gray), sandberg bluegrass (*Poa secunda* J. Presl), and western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Love), along with forbs including western yarrow *(Achillea millefolium* L. var. *occidentalis* DC), prairie coneflower *(Ratibida columnifera* [Nutt.] Wooton & Standl.), white prairie clover (*Dalea candida* Michx. ex Willd.), scarlet globemallow (*Sphaeralcea coccinea* [Nutt.] Rydb.), and blacksamson echinacea (*Echinacea angustifolia* DC.). The dominant shrubs include Wyoming big sagebrush *(Artemisia tridentata* spp. *wyomingensis* [Beetle & A. Young] S. L. Welsh), prairie sagewort (*Artemisia frigida* Willd.),winterfat *(Krascheninnikovia lanata* [Pursh] A. Meeuse & Smit), and rubber rabbitbrush (*Ericameria nauseosa* [Pall. ex Pursh] G.L. Nesom & Baird). The topography consists of rolling plains fragmented by small drainages and rock outcroppings. At Spring Creek and Decker, elevation, annual mean temperature, wind

speed, and precipitation are 1061 m, 9.0 °C and 11.3 km hr^{-1}, and 355 mm and 1,075 m, 8.0 °C, 12.1 km hr^{-1}, and 339 mm respectively.

Experimental Design

We began an experiment involving both mines in fall 2014 (2014 Experiment), and we repeated that experiment beginning fall 2015 (2015 Experiment). Each experiment included six sites at Decker and six sites at Spring Creek. These sites were seeded between 2009 and 2011 as part of the reclamation process but few seeded plants established, and sites became dominated by non-target species. At Spring Creek, half of the 2014 and 2015 experiments were dominated by western wheatgrass and remaining sites were dominated by annual bromes. At Decker, all 12 sites were dominated by annual bromes.

Ten plots (12 x 12 m) were established at each site (n = 60 plots per mine and experiment). Eight plots had litter removed to increase light availability and improve seed-soil contact. These eight plots received a factorial combination of four herbicide treatments (control, glyphosate, quizalofop, or both) and two seeding treatments differing in Wyoming big sagebrush seed rate (Table 3.1). A ninth plot received no treatments and a tenth plot received litter removal but no other treatments.

Glyphosate (Roundup PRO®, Monsanto at 0.877 L per ha^{-1}) was applied 10/7/2014 (2014 Experiment) and 10/13/2015-10/14/2015 (2015 Experiment) when annual brome seedlings were actively growing and most native vegetation had become dormant. Even though western wheatgrass was beginning to enter dormancy, we

expected glyphosate would provide some control of this species. Quizalofop (Assure II®, DuPont at 0.950 L per ha^{-1}) was applied 4/24/2015-4/28/2015 (2014 Experiment) and 4/4/2016 (2015 Experiment) when annual bromes were growing and native vegetation remained largely dormant. Herbicides were mixed with water and applied with an ATV-mounted sprayer with even flat spray tips (TeeJet 8002E) calibrated to deliver spray solution at 128 liters ha^{-1}. A nonionic surfactant (Brewer 90-10, Brewer International) was added at a rate of 0.25% v/v for all herbicide applications.

Litter was removed shortly after herbicide applications; i.e. 4/28/2015-5/18/2015 (2014 Experiment) and 4/7/2016-4/14/2016 (2015 Experiment). Litter was removed using a chain harrow pulled by a tractor, except in three sites of the 2014 experiment at Decker where accidental burning on 4/28/2015 removed litter.

Seeding occurred 5/18/2015-5/19/2015 (2014 Experiment) and 4/8/2016-4/14/2016 (2015 Experiment). The species and seeding rates differed by mine, but within a mine the two seed mixes that were tested differed only in Wyoming big sagebrush seed rate, with the rates being 3.4 and 5.6 kg pure live seed (PLS) ha^{-1} (Table 3.1). The Wyoming big sagebrush rates used maintained the required functional group (i.e. grasses, forbs, and shrubs) ratios in dryland restoration. Seeds were broadcasted with cracked corn and rice hulls as carries using a Flex II Seeder™, (Traux Company, Inc.) at Decker and a Sunflower Seeder™ (AGCO Corporation) at Spring Creek. The total seed rate excluding Wyoming big sagebrush was 7.61 kg PLS ha^{-1} at Decker and 6.73 kg PLS ha^{-1} at Spring Creek.

Approximately 1 year after seeding of the 2014 experiment (i.e. 4/4/2016), the six plots at each site that had previously received herbicide were divided, and one half of each plot was retreated with quizalofop using the same methods and rate used the year before.

Table 3.1: Pure live seed rates (kg ha^{-1}) used in the 2014 and 2015 experiments.

Functional group	Species	Spring Creek	Decker
Cool-season grasses	Sandberg bluegrass	0.56	0
	Prairie junegrass	1.40	0
Warm-season grasses	Alkali sacaton	0.73	1.68
	Blue grama	1.12	1.12
	Sideoats grama	0	2.24
	Sand dropseed	0.56	1.68
Forbs and subshrubs	Prairie coneflower	1.12	0
	Scarlet globemallow	0.17	0.22
	Western yarrow	0.34	0.28
	Blacksamson echinacea	0.45	0
	White prairie clover	0	0.39
	Prairie sagewort	0.28	0
Shrubs	Wyoming big sagebrush	Mix 1: 3.36 Mix 2: 5.60	Mix 1: 3.36 Mix 2: 5.60

Vegetation and Environmental Sampling

Within both halves of each plot, four randomly chosen locations were permanently marked. Prior to applying any treatments [10/6/2014-10/7/2014 (2014 Experiment) and 10/5/2015-10/12/2015 (2015 Experiment)], annual brome cover was recorded at all marked locations, in 0.10-m^2 circular frames. After treatment, Wyoming big sagebrush density and cover of all other species, litter, rock, and bare ground were sampled at the same marked locations using 0.25-m^2 circular frames. Sampling took

place 7/5/2015-7/22/2015 (2014 Experiment) and 7/12/2016-7/29/2016 (2014 and 2015 Experiments).

Data Analysis

Western wheatgrass-dominated sites were excluded from analysis because herbicide treatments proved ineffective against this species, and seeded species establishment was extremely low. We detected no differences between the two Wyoming big sagebrush seed rates (Table 3.1) and no differences between the control and litter removal only treatments, so terms controlling differences between these treatments were excluded from all models. Additionally, it was not necessary to include seeding treatments as predictors in models for annual bromes and other weedy species, because seeded species remained too small to impact weed cover over the course of the study. Many total seeded species cover values were zero (67%), so these data were analyzed with a Tobit model we fit using a FORTRAN program (Intel Corporation 2013) that implements methods of Chib (1992). All other response variables were analyzed with linear mixed-effects models fit and evaluated using R version 3.3.2 (R Core Team, 2016) and the lme4 (Bates et al. 2015), lmerTest (Kuznetsova, Brockhoff, and Christensen 2014), and lsmeans (Lenth 2016) packages. The Akaike Information Criterion (Burnham and Anderson 2004) was used for model selection.

Annual brome cover the summer after herbicide applications was evaluated with a linear mixed-effects model. The response variable was (natural log) annual brome cover averaged by measurement frames within plots. The model had fixed effects for

herbicide treatment (control, glyphosate, quizalofop, or both), (natural log) standardized (mean = 0 and standard deviation = 1) pre-treatment brome cover, experiment (2014 or 2015), mine (Spring Creek or Decker), and the interaction between experiment and mine. Random effects were included for site and the interaction between site and herbicide treatment. In the 2014 experiment, annual brome cover the second summer after herbicide applications was evaluated with a similar model. Fixed effects were included for herbicide treatment, retreatment (control or quizalofop), the previously described pretreatment brome cover variable, and mine. Random effects were included for site, the site by herbicide interaction, and plot. Plot was included to account for two observations on individual plots (i.e. retreated, not retreated half of plot). Controlling annual bromes caused major increases in non-native annual forbs, particularly kochia (*Kochia scoparia* [L.]) and Russian thistle (*Salsola tragus* [L.]). We quantified these changes using models with the same terms as models for annual bromes.

Wyoming big sagebrush density averaged over measurement frames was analyzed with a Poisson count model. For the summer following seeding, fixed effects were included for herbicide and seeding treatments (seeded or not) as well as mine, experiment, and their interaction. For the second summer after seeding, fixed effects were the same, except terms involving experiment were omitted and a term for retreatment (control or quizalofop) was included. Both models had random effects for

site, site by herbicide, and site by seeding interactions. For the second summer after seeding, an additional random effect was included for plot.

The model for total seeded species cover (except Wyoming big sagebrush) the summer after seeding included fixed effects for herbicide treatments, seeding treatments, mine, and experiment, as well as mine by herbicide and mine by experiment interactions. Random effects were included for site, site by herbicide, and site by seeding treatment interactions. A similar model was used for the 2014 experiment second summer after seeding, except that all terms involving experiment were omitted, retreatment (control or quizalofop) was added as a fixed effect, and plot was added as a random effect.

Results

Annual Brome Cover

Seeded species remained small and did not impact cover of annual bromes or broadleaf weeds over the course of this study. Within an herbicide treatment, annual brome cover did not vary appreciably between mines ($p = 0.535$) or between 2014 and 2015 experiments ($p = 0.579$) (Figure 3.1; Table 3.2). The summer after initial herbicide treatments were applied, annual brome cover was 22% (13%, 36%) (parentheses show 95% confidence interval) in the control, which was significantly greater ($p = 0.0001$ to 0.034) than in the glyphosate [11% (5%, 25%)], quizalofop [16% (7%, 35%)] and glyphosate plus quizalofop [9% (4%, 20%)] treatments. Initial herbicide treatments

ceased affecting annual brome cover the second summer after application (Figure 3.1; Table 3.2), but retreatment with quizalofop just prior to that summer reduced annual brome cover from 40% (29%, 55%) in all plots not retreated to 28% (17%, 46%) in retreated plots at both mines (Figure 3.2; Table 3.2; $p < 0.001$).

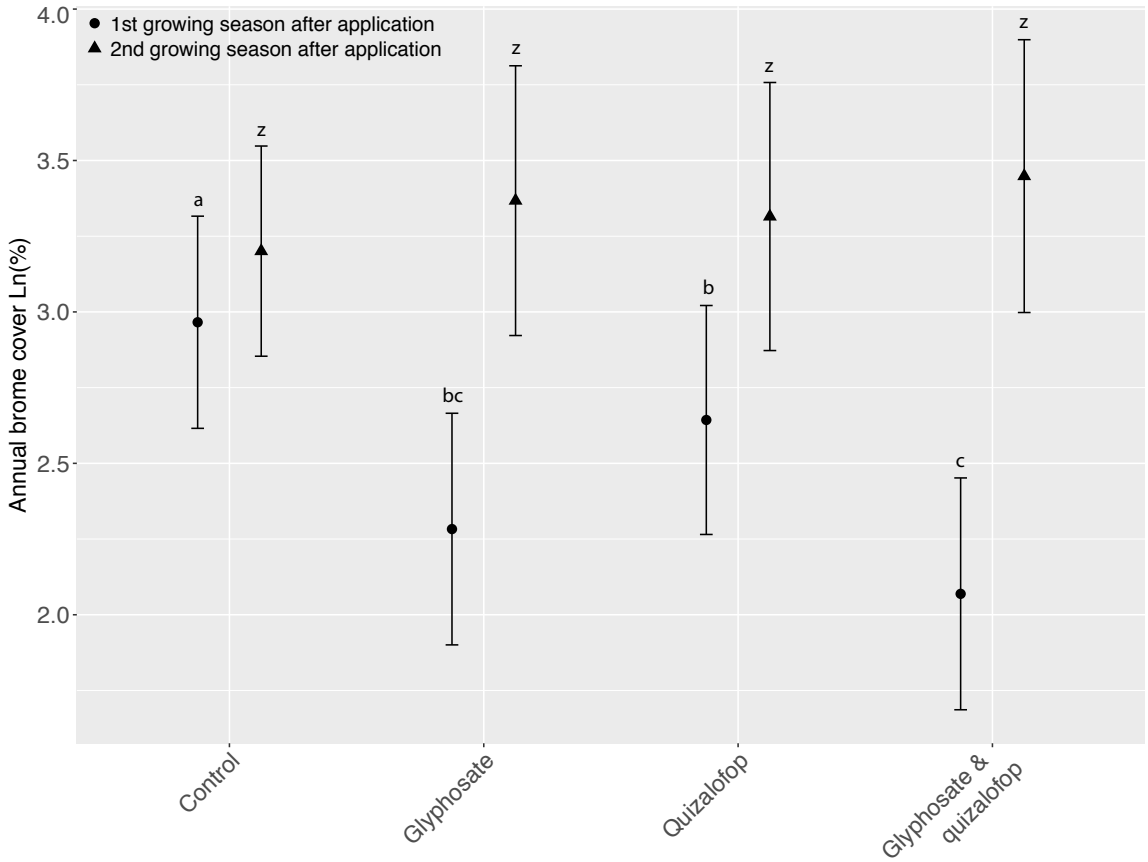

Figure 3.1: Annual brome cover means (dots and triangles) and 95% CIs (bars) in the first summer after single herbicide applications in two experiments (one initiated 2014, the other initiated 2015) at Decker and Spring Creek mines. Results did not differ by experiment or mine. Within a summer, means with different letters are significantly different ($p < 0.05$).

Table 3.2: Model results assessing (natural log) annual brome cover. Bolded predictors are significant ($p < 0.05$). Terms were included for differences between a control and three herbicide treatments (i.e. glyphosate, quizalofop, glyphosate & quizalofop), between experiments initiated 2014 and 2015 (i.e. 2015 Experiment) and between Decker and Spring Creek (i.e. Spring Creek).

First summer annual brome cover	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**3.090**	**0.275**	**20.1**	**11.3**	**<0.001**	Site	0.316
Glyphosate	**-0.683**	**0.153**	**65.8**	**-4.5**	**<0.001**	Site by herbicide	0.109
Quizalofop	**-0.323**	**0.149**	**65.4**	**-2.2**	**0.034**	Residual	0.288
Glyphosate & quizalofop	**-0.897**	**0.151**	**64.4**	**-5.9**	**<0.001**		
2015 Experiment	0.200	0.352	13.7	0.6	0.579		
Spring Creek	-0.274	0.432	13.8	-0.6	0.535		
Pre-treatment brome cover	0.148	0.135	153.4	1.1	0.274		
2015 Experiment by Spring Creek	-0.626	0.639	16.4	-1.0	0.342		
Second summer annual brome cover	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**3.682**	**0.174**	**12.5**	**21.1**	**<0.001**	Plot	0.005
Glyphosate	0.167	0.240	39.6	0.7	0.491	Site	0.066
Quizalofop	0.114	0.233	35.7	0.5	0.627	Site by herbicide	0.289
Glyphosate & quizalofop	0.248	0.244	42.4	1.0	0.316	Residual	0.162
Quizalofop retreatment	**-0.340**	**0.080**	**21.7**	**-4.2**	**<0.001**		
Spring Creek	-0.318	0.258	6.9	-1.2	0.258		
Pre-treatment brome cover	**0.188**	**0.079**	**111.1**	**2.4**	**0.020**		

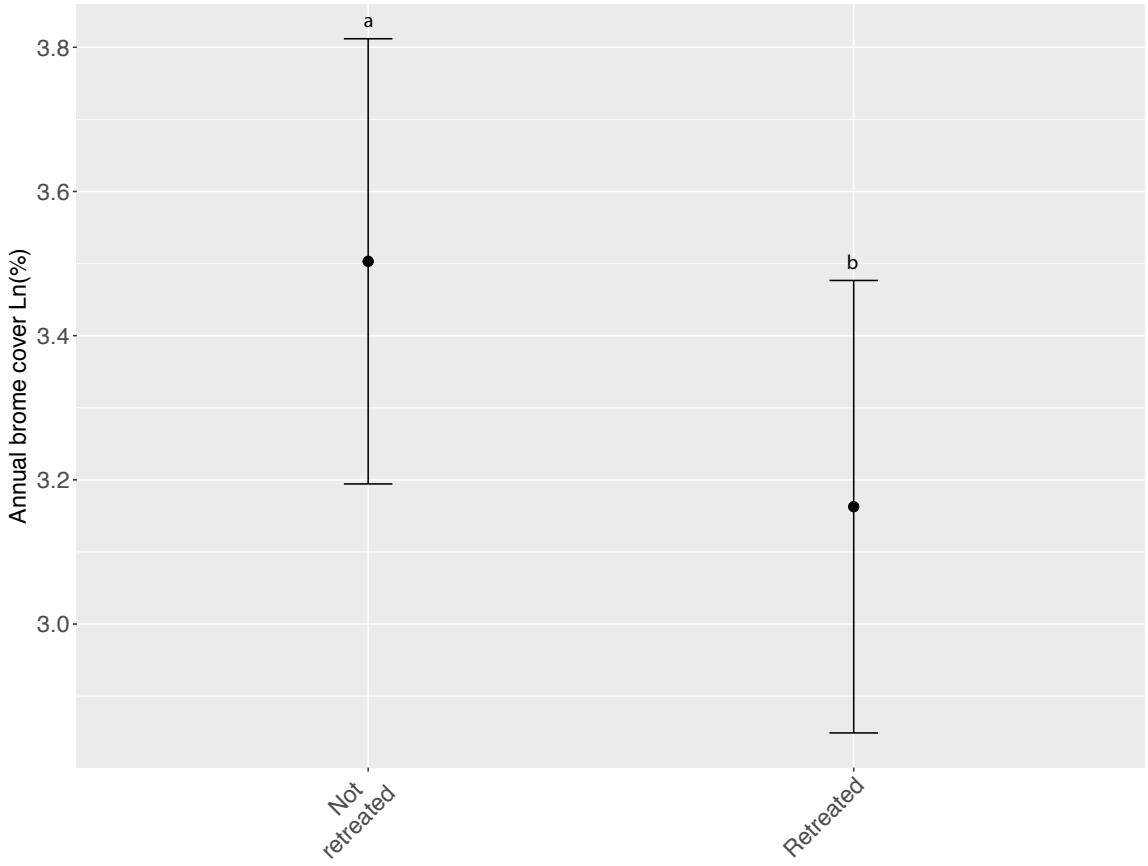

Figure 3.2: Annual brome cover means (dots) and 95% CIs (bars) for the second summer after herbicide application of the 2014 experiment at Decker and Spring Creek mines (results did not differ by mine). The “not retreated” bar describes plot halves that were treated with herbicides once, and the “retreated” bar describes plot halves that were retreated with quizalofop approximately a year after the initial herbicide treatments. Means with different letters are significantly different ($p < 0.05$).

Non-Native Forb Cover

Controlling annual bromes increased non-native forbs (Figure 3.3; Table 3.3). At Decker, in the summer following initial herbicide treatments, glyphosate ($p < 0.001$) and glyphosate plus quizalofop ($p < 0.001$) increased nonnative forb cover from 5% (4%, 6%) to 14% (11%, 19%) and 15% (12%, 21%), respectively. Corresponding increases for Spring Creek were from 8% (6%, 11%) to 24% (17%, 34%) ($p < 0.001$) and 26% (19%, 37%) ($p < 0.001$). In the next summer at Decker, in plots that received glyphosate ($p = 0.006$) and glyphosate plus quizalofop ($p = 0.029$) weedy forbs increased, in this case from 7% (4%, 10%) to 14% (9%, 22%) and 13% (8%, 20%), respectively. Corresponding increases for Spring Creek were from 7% (4%, 14%) to 15% (8%, 30%) ($p = 0.06$) and 14% (7%, 27%) ($p = .029$). Applying quizalofop alone (i.e. no glyphosate) shortly after seeding increased weedy forbs in the first but not second summer after application (Figure 3.3), and reapplying quizalofop roughly a year later did not increase weedy forbs (Table 3.3).

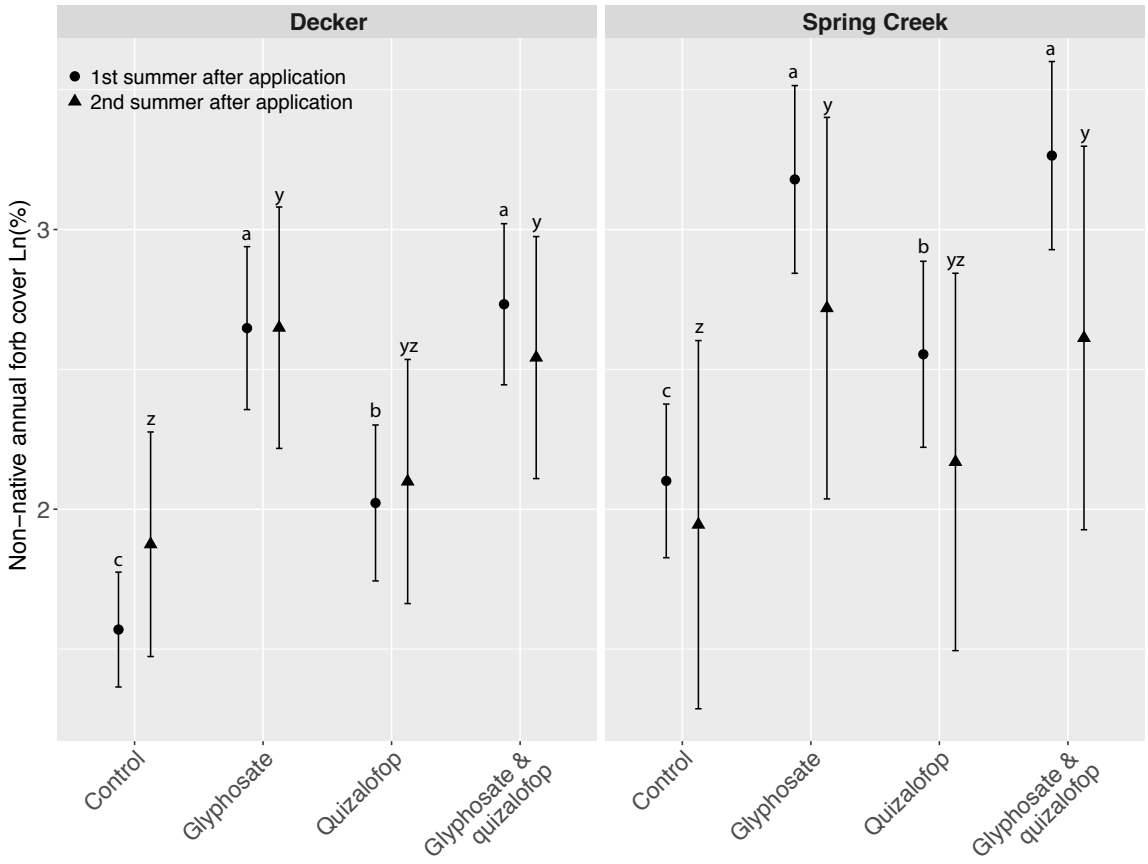

Figure 3.3: Annual non-native forb cover means (dots and triangles) and 95% CIs (bars) the first and second summer after single herbicide applications in two experiments (one initiated 2014, the other initiated 2015) at Decker and Spring Creek mines. Results did not differ by experiment. Within a summer, means with different letters are significantly different ($p < 0.05$).

Table 3.3: Model results assessing (natural log) non-native forb cover. Bolded predictors are significant ($p < 0.05$). Terms were included for differences between a control and three herbicide treatments (i.e. glyphosate, quizalofop, glyphosate & quizalofop), between experiments initiated 2014 and 2015 (i.e. 2015 Experiment) and between Decker and Spring Creek (i.e. Spring Creek).

First summer annual weed cover	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**1.413**	**0.142**	**112.6**	**10.0**	**<0.001**	Site	0.000
Glyphosate	**1.078**	**0.169**	**84.4**	**6.4**	**<0.001**	Site by herbicide	0. 237
Quizalofop	**0.453**	**0.164**	**82.8**	**2.8**	**0.007**	Residual	0.177
Glyphosate & quizalofop	**1.163**	**0.168**	**82.4**	**6.9**	**<0.001**		
2015 Experiment	0.260	0.146	84.2	1.8	0.079		
Spring Creek	**0.425**	**0.207**	**75.8**	**2.1**	**0.043**		
Pre-treatment brome cover	0.048	0.077	149.7	0.6	0.535		
2015 Experiment by Spring Creek	0.212	0.295	108.6	0.7	0.474		
Second summer annual weed cover	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**1.779**	**0.173**	**9.3**	**10.3**	**<0.001**	Plot	0.000
Glyphosate	**0.774**	**0.191**	**35.7**	**4.1**	**<0.001**	Site	0.108
Quizalofop	0.224	0.183	30.5	1.2	0.228	Site by herbicide	0.136
Glyphosate & quizalofop	**0.667**	**0.195**	**38.5**	**3.4**	**0.001**	Residual	0.139
Quizalofop retreatment	-0.003	0.076	72.1	0.0	0.966		
Spring Creek	0.070	0.315	6.5	0.2	0.831		
Pre-treatment brome cover	-0.116	0.076	107.5	-1.5	0.130		

Sagebrush Density

Wyoming big sagebrush densities were greater at Decker than Spring Creek (Figure 3.4; Table 3.4). In the summer after seeding, seeding without herbicides increased Wyoming big sagebrush density from 0.08 (0.03, 0.27) to 1.08 (0.54, 2.19) plants m^{-2} at Decker ($p < 0.001$) and from 0.01 (0.00, 0.05) to 0.17 (0.06, 0.44) plants m^{-2} at Spring Creek ($p < 0.001$) (Figure 3.5; Table 3.5). Combining quizalofop with seeding did not increase Wyoming big sagebrush densities above seeding alone. Conversely, combining glyphosate with seeding increased densities at Decker from 1.08 (0.54, 2.19) to 3.48 (1.87, 6.48) and from 0.76 (0.27, 2.11) to 3.05 (1.42, 6.56) plants m^{-2} in the first ($p = 0.0148$) and second ($p = 0.0451$) summer after seeding, respectively. Corresponding increases for Spring Creek were from 0.17 (0.06, 0.44) to 0.53 (0.22, 1.31) and from 0.11 (0.03, 0.43) to 0.43 (0.13, 1.40) plants m^{-2} in the first ($p = 0.0148$) and second ($p = 0.0451$) summer after seeding, respectively. Glyphosate boosted Wyoming big sagebrush densities similarly whether or not it was combined with quizalofop. Reapplying quizalofop about a year after the original application did not significantly increase Wyoming big sagebrush densities ($p = 0.912$; Table 3.4). Wyoming big sagebrush seedlings were between one to seven centimeters tall the first season after seeding, and between one and 15 centimeters tall the second season after seeding.

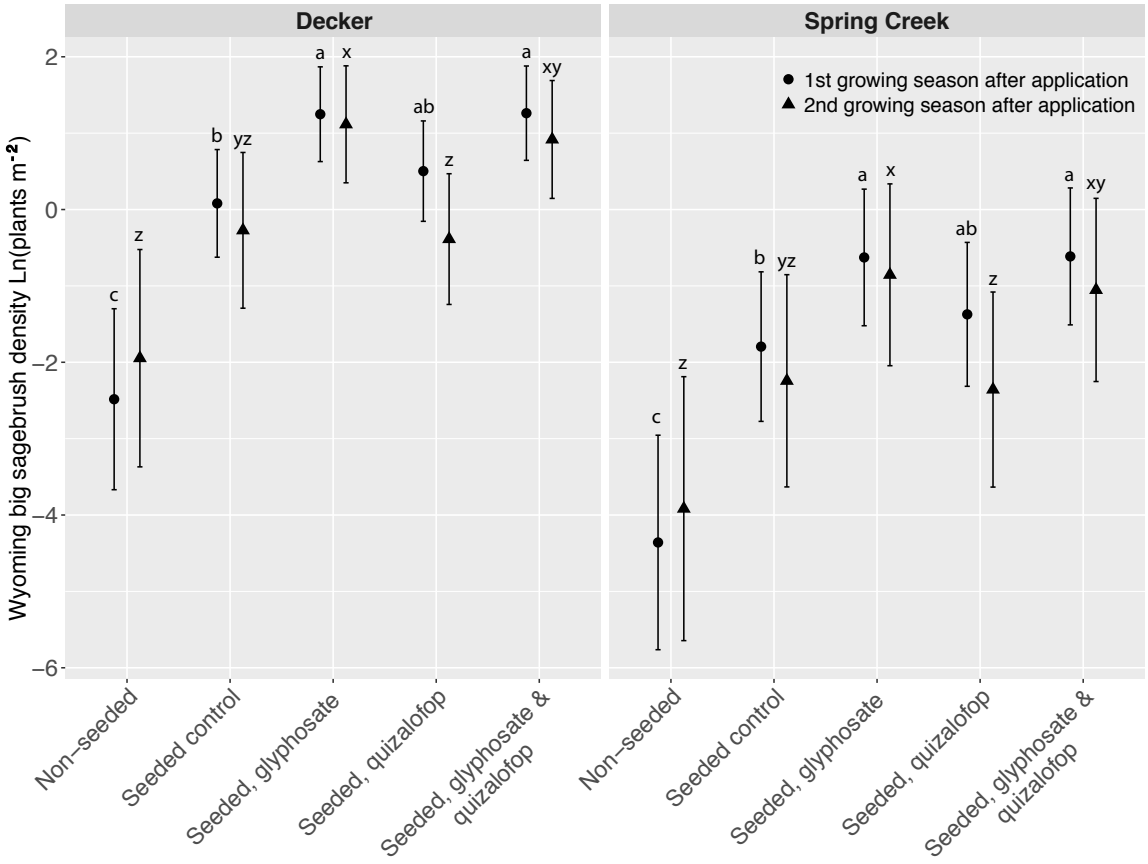

Figure 3.4: Wyoming big sagebrush density (plants m^{-2}) means (dots and triangles) and 95% CIs (bars) the first and second summer after single herbicide applications in two experiments (one initiated 2014, the other initiated 2015) at Decker and Spring Creek mines. Results did not differ by experiment. Within mine and summer, means with different letters are significantly different ($p < 0.05$).

Table 3.4: Model results assessing Wyoming big sagebrush density (plants m^{-2}). Bolded predictors are significant ($p < 0.05$). Terms were included for differences between a control and herbicide treatments (i.e. glyphosate, quizalofop, glyphosate & quizalofop), between experiments initiated 2014 and 2015 (i.e. 2015 Experiment) and between Decker and Spring Creek (i.e. Spring Creek).

First summer Wyoming big sagebrush density	Fixed effects				Random effects	
Variable	Estimate	SE	z	p	Variable	Variance
Intercept	**-2.457**	**0.653**	**-3.8**	**<0.001**	Site	0.615
Glyphosate	**1.168**	**0.330**	**3.5**	**<0.001**	Site by herbicide	0.497
Quizalofop	0.422	0.342	1.2	0.216	Site by seeding	0.100
Glyphosate & quizalofop	**1.181**	**0.330**	**3.6**	**<0.001**		
Seeded	**2.564**	**0.602**	**4.3**	**<0.001**		
2015 Experiment	-0.054	0.529	-0.1	0.918		
Spring Creek	**-2.313**	**0.707**	**-3.3**	**0.001**		
2015 Experiment by Spring Creek	0.875	0.993	0.9	0.379		
Second summer Wyoming big sagebrush density	Fixed effects				Random effects	
Variable	Estimate	SE	z	p	Variable	Variance
Intercept	**-1.933**	**0.715**	**-2.7**	**0.007**	Site	0.628
Glyphosate	**1.388**	**0.434**	**3.2**	**0.001**	Plot	0.362
Quizalofop	-0.115	0.473	-0.2	0.808	Site by herbicide	0.000
Glyphosate & quizalofop	**1.190**	**0.437**	**2.7**	**0.006**	Site by seeding	0.059
Seeding	**1.674**	**0.728**	**2.3**	**0.022**		
Quizalofop retreatment	-0.027	0.246	-0.1	0.912		
Spring Creek	**-1.970**	**0.685**	**-2.9**	**0.004**		

Seeded Species Cover

At Decker, seeding without herbicides increased seeded species cover from 0.1% (0.0%, 0.3%) to 0.3% (0.1%, 0.7%) and from 0.0% (0.0%, 0.2%) to 0.2% (0.0%, 0.7%) in the first and second summer after seeding, respectively ($p = 0.009$) (Figure 3.5). Corresponding increases for Spring Creek are from 0.1% (0.0%, 0.2%) to 0.2% (0.1%, 0.4%) and from 0.1% (0.0%, 0.4%) to 0.3% (0.1%, 1.9%), respectively ($p = 0.009$). At Decker, combining herbicides with seeding further increased seeded species cover (Figure 3.5). For example, combining glyphosate with seeding increased seeded species cover from 0.29% (0.12%, 0.69%) to 1.05% (0.44%, 2.54%) in the first summer after seeding ($p < 0.001$). Conversely, combining herbicides with seeding never further increased seeded species cover at Spring Creek. Reapplying quizalofop about a year after the original application did not significantly increase seeded species cover.

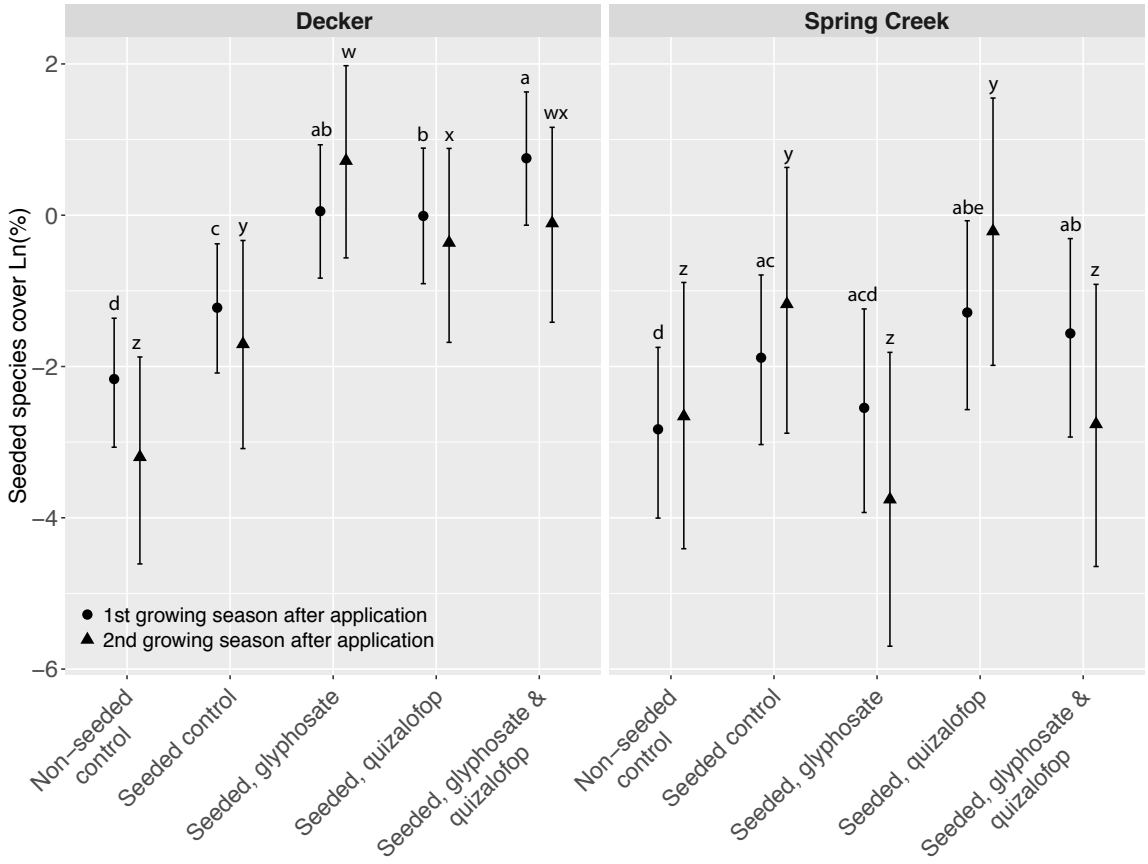

Figure 3.5: Seeded species cover means (dots and triangles) and 95% CIs (bars) the first and second summer after single herbicide applications in two experiments (one initiated 2014, the other initiated 2015) at Decker and Spring Creek mines. Results did not differ by experiment. Within mine and summer, means with different letters are significantly different ($p < 0.05$).

Discussion

Seeding without herbicides increased establishment of Wyoming big sagebrush and other seeded species, and with some notable exceptions, combining herbicides with seeding tended to further increase seeded species establishment. In terms of efficiency for annual brome control and Wyoming big sagebrush establishment, our most effective herbicide treatment was glyphosate applied alone in fall (i.e. not followed by quizalofop the following spring). This was because quizalofop alone (i.e. not preceded by glyphosate) did not reduce annual brome cover enough to boost Wyoming big sagebrush densities and because combining quizalofop with glyphosate gave responses similar to glyphosate alone. Using glyphosate to control annual brome seedlings in fall reduced annual brome cover the following summer, thereby allowing seeded Wyoming big sagebrush to better establish. A common Wyoming big sagebrush density goal in our study system is at least 1.0 plants m^{-2} six growing seasons after seeding (Schuman, Vicklund, and Belden 2005; Hild et al. 2006). Two summers after applying glyphosate and seeding, mean Wyoming big sagebrush densities were 3.05(1.42, 6.56) and 0.43(0.13, 1.40) plants m^{-2} at Decker and Spring Creek, respectively, so our best treatment was on target to meet this goal at the average site at one or perhaps both study mines.

While glyphosate aided seeded species cover at Decker, it had the downside of reducing these plant groups at Spring Creek. Prairie coneflower *(Ratibida columnifera* [Nutt.] Wooton & Standl.) was present both in our seed mix and as

established plants from prior restoration efforts at Spring Creek, and we believe glyphosate reduced seeded species cover as a consequence of damaging preexisting coneflower plants from initial seeding efforts. Quizalofop alone had neutral to positive effects on seeded forbs, subshrubs and grasses.

These key results agree with other studies showing seeded plants sometimes establish, and herbicides sometimes improve their establishment in degraded drylands dominated by annual bromes (Mangold et al. 2015; Hirsch-Schantz et al. 2014; Kyser et al. 2013; Elseroad and Rudd 2011; Whitson and Koch 1998) and other invasive plants (Mangold et al. 2015; Kyser et al. 2013; Davies 2010). In contrast, Wilson et al. (2004) found that when unwanted annual forb cover was reduced using herbicides, native seeded species establishment did not increase. Although past studies have attempted to use herbicides to increase seeded Wyoming big sagebrush survival (Brabec et al. 2015; Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011), ours is the first study to show benefits of using herbicides for this purpose. It is not entirely evident why we had greater success at establishing Wyoming big sagebrush from seed, but it could be attributed to good seed-to-soil contact at the time of seeding or favorable precipitation patterns post seeding.

Our results were consistent across sites and mines, suggesting our treatments point to somewhat dependable methods for establishing seeded plants in annual brome-dominated areas of the northern Great Plains. However, a wealth of past research indicates there are no guarantees repeating our treatments in other years or at

other sites will give similar results. Seeded species establishment is highly dependent on weather conditions and other factors (Bansal and Sheley 2016; James, Rinella, and Svejcar 2012; Mangla et al. 2011; Morris, Monaco, and Rigby 2009; Bakker et al. 2003), and seeded species often nearly or completely fail to establish in degraded drylands (Brabec et al. 2015; Fansler and Mangold 2011). Moreover, while it is known from past research that glyphosate often adequately controls annual bromes and other invasive grassland species (Cox and Anderson 2004; Bakker et al. 2003; Whitson and Koch 1998), it is also known that this and other herbicides sometimes fail to control invaders (Mangold et al. 2013; Ambrose and Wilson 2003). Whereas our results do suggest modestly reliable seeding methods for establishing native plants on degraded drylands of the northern Great Plains, our methods, like any other seeding methods used in this system, are by no means immune to failure. It is important that land managers understand that any given dryland seeding effort may fail, in which case additional herbicide, seeding, or other treatments may be needed to meet restoration goals.

While using herbicides to control annual bromes increased establishment of Wyoming big sagebrush and other seeded species, it also increased cover of the nonnative annual forbs kochia and Russian thistle. A couple considerations lead us to believe this flush of weedy forbs will not persist beyond a few growing seasons. First, annual forb cover differences between our herbicide treatments and untreated controls shrank considerably between the first and second summer after application. Also, past studies have observed these forbs quickly becoming dominant in response to tillage and

other seeding-related disturbances but then getting displaced by seeded species and/or annual bromes over the course of a few growing seasons (Prodgers 2013).

Glyphosate and quizalofop treatments did not reduce western wheatgrass at the six of our 24 sites that were dominated by this native perennial grass instead of annual bromes. Native and nonnative perennial grasses remain a barrier to establishing diverse native dryland plant communities because they can persist long-term (Wilson and Pärtel 2003), are difficult to control (Davies, Boyd, and Nafus 2013; Fansler and Mangold 2011; Francis and Pyke 1996), and are highly competitive (Asay et al. 2001; Hull Jr and Klomp 1967). It is possible that using different herbicide type, rate, or application timing could provide greater control of western wheatgrass.

Wyoming big sagebrush seedling densities did not vary between our 3.4 and 5.6 kg ha^{-1} Wyoming big sagebrush seed rates. Likewise, Rinella et al. (2015) observed no relationship between shrub seed rate and shrub cover across hundreds of seeded fields in our study system. On the other hand, Williams et al. (2002) found Wyoming big sagebrush seedling densities increased as the seeding rate increased from 1 to 2 to 4 kg ha^{-1}. The lack of a seed rate effect in our study may be explained by seed safe sites being saturated at the lower 3.4 kg ha^{-1} rate (Fowler 1988). Conversely, safe sites may not have been saturated at the lower seed rates explored by Williams et al. (2002).

Applying quizalofop after our initial herbicide treatments (i.e. one year after seeding) helped maintain reductions in annual brome cover, but the reductions were not sufficient to increase seeded species establishment. We nevertheless believe follow

up grass-specific herbicide treatments could still prove beneficial, especially if they could be designed to more completely control annual bromes. In particular, it may still prove possible to repeatedly apply grass-specific herbicides over a number of growing seasons until Wyoming big sagebrush and other native species grow large enough to survive competitive interactions with annual bromes.

Alternatively, it may be that no additional herbicide treatments are needed. Wyoming big sagebrush seeds can remain viable and develop into seedlings up to four years after seeding, and this could allow Wyoming big sagebrush densities in our plots to remain stable or even increase somewhat over time (Schuman, Booth, and Cockrell 1998). In other northern Great Plains studies that did not employ herbicides, seeded Wyoming big sagebrush densities were measured periodically for 12 growing seasons after seeding (Schuman, Mortenson, and Vicklund 2012; Hild et al. 2006). In these studies, densities declined somewhat over time, but not dramatically, and surviving plants grew fairly rapidly. This provides some assurance our study's Wyoming big sagebrush seedlings may survive, continue growing and ultimately reproduce.

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CHAPTER FOUR

CONCLUSION TO THESIS

The historical and continuing decline and fragmentation of intact sagebrush steppe (Brauch and Spring 2009; Koohafkan and Stewart 2008) is a major problem in the United States Intermountain West. Human activity, including mining, conversion of steppe to irrigated crop-lands, and removal of sagebrush for range-land forage, have all contributed to a 40% reduction of the historical range of sagebrush (Welch 2005). These disturbances have created an opportunity for a number of non-native species to colonize vast areas of the sagebrush steppe (Duncan et al. 2004; Knapp 1996; Mack 1981), chief among them are annual bromes including cheatgrass (*Bromus tectorum* L.) and Japanese brome (*Bromus japonicus* Thunb.). The range of these invaders is predicted to continue to expand in some parts of the sagebrush steppe given the climate change predictions for the region (Bradley, Curtis, and Chambers 2016; Bradley 2009; Bromberg et al. 2011).

Restoration of the sagebrush steppe has become a priority in the Intermountain West. These restoration efforts include attempts to control unwanted non-native species and re-introduce desired native plants (Mangold et al. 2015; Hirsch-Schantz et al. 2014; Kyser et al. 2013; Davies, Bates, and Nafus 2011; Elseroad and Rudd 2011; Fansler and Mangold 2011; Boyd and Davies 2010; Whitson and Koch 1998). A common technique for managing non-native species, including annual bromes, is chemical

control with herbicides (Mangold et al. 2015; Hirsch-Schantz et al. 2014; Kyser et al. 2013; Elseroad and Rudd 2011; Whitson and Koch 1998). Widespread use of broad-spectrum herbicides (i.e. glyphosate and imazapic) have provided mixed results, but are generally successful when used to control annual bromes (Mangold et al. 2013; Cox and Anderson 2004; Park and Mallory-Smith 2004; Whitson and Koch 1998). There is limited information on how effective other available herbicides (i.e. graminicides) are at controlling annual bromes in restoration scenarios. However, there are promising results from a few greenhouse studies, showing significant control of annual bromes when treated with graminicides (Ball, Frost, and Bennett 2007; Park and Mallory-Smith 2004; Brewster and Spinney 1989). This in combination with the fact that graminicides are grass specific and therefore should not harm forbs or shrubs (Kukorelli, Reisinger, and Pinke 2013), make them a good alternative or addition to glyphosate or imazapic herbicide application in the field, where desired non-grass species are present and desired grasses are scarce.

Similar to managing annual bromes with herbicides, efforts to re-introduce native species have produced varied results (Mangold et al. 2015; Davies, Boyd, and Nafus 2013; Whitson and Koch 1998). Unfortunately, efforts to re-establish Wyoming big sagebrush from seed are often unsuccessful (Fansler and Mangold 2011). Because shrubs, particularly sagebrush, play such key roles within the sagebrush steppe ecosystem it is imperative that strategies for successful establishment are developed. One barrier to restoration success within the sagebrush steppe is competition with non-

native grasses (Bakker et al. 2003). Therefore, decreasing competition between seeded native species and annual bromes, specifically cheatgrass and Japanese brome, could aid sagebrush steppe restoration.

The goal of this study was to investigate methods to reduce annual bromes and increase seeded species establishment, specifically Wyoming big sagebrush. The first experiment in my greenhouse study compared the efficacy of glyphosate, sethoxydim, clethodim, fluazifop, and quizalofop at two rates (low and high label recommendations) on cheatgrass plants at five heights (5, 8.5, 11, 15.5, and 17 cm). The second experiment compared the efficacy of the same five herbicides at two rates applied at a single plant height (11 cm) on three accessions (Spring Creek and Decker for both species, and Red Bluff (cheatgrass) and Burke Park (Japanese brome)) of each brome species.

The findings of the greenhouse study informed the herbicide treatments I used in my field study, in which I used herbicides and seeding to control annual bromes and aimed to increase seeded species establishment. Of the four graminicides tested, quizalofop was consistently one of the best at reducing annual brome biomass. Across two mines (Spring Creek and Decker) and two, two year experiments, I used a factorial design to apply four herbicide treatments (control, glyphosate, quizalofop, or both) and two seeding treatments, plus two controls at six sites on each mine. Half of each plot that initially received an herbicide treatment was retreated with quizalofop a year after the first application.

Choosing the Most Appropriate Herbicides for Annual Brome Control

Past studies have found glyphosate to be effective at controlling cheatgrass (Cox and Anderson 2004; Park and Mallory-Smith 2004; Whitson and Koch 1998) and Japanese brome (Waller and Schmidt 1983), which is consistent with the results from the first experiment in my greenhouse study. I found that glyphosate significantly reduced cheatgrass biomass compared to the control when applied to plants at 5 and 8.5 cm, but once plants were 11 cm, the high glyphosate rate reduced biomass and low rate did not. In the two largest plant groups (15.5 and 17 cm) neither glyphosate treatments reduced cheatgrass biomass. This is consistent with another greenhouse study by Park and Mallory-Smith (2004) where glyphosate applied at a rate of 0.420 kg ai ha^{-1} to plants 7.5 cm (four leaf stage) or smaller provided greater than 50% reduction in cheatgrass biomass.

As plant height at time of application increased to 11 cm and greater, the low glyphosate treatment did not reduce cheatgrass biomass in my study. In contrast, Whitson and Koch (1998) in a field study found that glyphosate applied at 0.420 kg ai ha^{-1} to plants with up to eight leaves (equivalent to my 11 cm group) controlled >99% of cheatgrass. In the same study, they applied increasing rates (0.55, 0.69, and 0.83 kg ai ha^{-1}) and all achieved >99% control of cheatgrass. I also found that increasing the glyphosate application rate to 0.56 kg ai ha^{-1} significantly reduced cheatgrass biomass compared to the control when plants were 11 cm at time of application. Given the high control Whitson and Koch (1998) achieved with increasing rates, it is possible that rates

higher than the ones used in my experiment would be able to control cheatgrass when individuals have grown to 11 cm or larger. However, using higher rates such as 0.69, and 0.83 kg ai ha^{-1} surpasses allowable label recommendations for brome control and broad-spectrum herbicides like glyphosate can damage desired species (Kukorelli, Reisinger, and Pinke 2013). Therefore, I recommend spraying target plants when they are as small as possible to ensure sufficient control.

In most restoration scenarios, including the field component of this study, desired species are often found in conjunction with cheatgrass. These non-target shrub and broad leaf species would be harmed by glyphosate applications, so graminicides, which are grass specific post-emergence herbicides (Kukorelli, Reisinger, and Pinke 2013; Délye, Wang, and Darmency 2002) could be a good option. Currently graminicides are not commonly used in range-land and restoration (James et al. 2013), but have been adopted for weed management in annual cropping systems (Marquardt and Johnson 2013; Foy and Witt 1992) and forestry (Clay, Dixon, and Willoughby 2006).

Studies have shown that graminicides are also effective at controlling cheatgrass (Ball, Frost, and Bennett 2007; Brewster and Spinney 1989). Similar to the trends I found across heights for my glyphosate treatments, as plant height at time of application increased, the efficacy of all graminicide (sethoxydim, clethodim, fluazifop, and quizalofop) treatments decreased. At 5 and 8.5 cm, all graminicides reduced cheatgrass biomass compared to the control, which is consistent with the results reported by Ball, Frost, and Bennett (2007) where sethoxydim (85%), clethodim, fluazifop, and quizalofop

(all over 98%) reduced cheatgrass biomass when plants were sprayed at the two to three leaf stage. In my study all graminicides were still effective at reducing biomass compared to the control when plants were 11, 15.5, and 17 cm, except the sethoxydim low treatment, and overall at these heights quizalofop provided the greatest redction in biomass.

In the second experiment of my greenhouse study, I compared cheatgrass and Japanese brome across three accessions and found the same herbicide efficacy trends. When applied to cheatgrass, the glyphosate low treatment provided the smallest reduction in biomass; among the graminicides, sethoxydim performed the worst, which again agrees with the findings of Ball, Frost, and Bennett (2007). When applied to Japanese brome all herbicides reduced plant biomass equally, except for the glyphosate low treatment which provided the smallest reduction: I found no studies that evaluated graminicide efficacy on Japanese brome. Across all treatments, Japanese brome was more susceptible to herbicides. Additionally, I found there was no difference between the disturbed accessions (i.e. Spring Creek mine and Decker mine) which is likely due to geographic proximity to one another. The undisturbed accessions (i.e. Red Bluff or Burke Park for cheatgrass and Japanese brome respectively) across all herbicides were less susceptible to control. The disturbed sites were within 25 km of each other and the undisturbed sites were over 500 km away which supports the idea that these accessions were genetically different from one another. Similar to the herbicide efficacy differences I found among accessions, other studies have shown accession differences in cold

tolerance (Bykova and Sage 2012) germination success (Hardegree et al. 2013), and herbicide resistance (Ball, Frost, and Bennett 2007; Park and Mallory-Smith 2004; Mueller-Warrant, Mallory-Smith, and Hendrickson 1999).

The results from both greenhouse experiments were used to inform my field study. Though greenhouse results can vary when applied in field conditions (Fletcher, Johnson, and McFarlane 1990), they provide important insights for maximizing treatment efficacy and efficiency. I found that 1) herbicide efficacy decreased as the size of the plant at time of spraying increased, specifically spraying when plants are 11 cm or smaller resulted in greater than 50% reduction of biomass in all but one treatment; 2) when applying glyphosate the high rate is required for sufficient control; 3) within graminicides, quizalofop and fluazifop, consistently provided the greatest reduction in biomass; 4) Japanese brome was more susceptible to all herbicide treatments compared to cheatgrass; and 5) the undisturbed accessions were more susceptible to all herbicide treatments than the disturbed accessions.

Herbicide Efficacy and Seeding Success Under Field Conditions

In my field study, I found that fall glyphosate applications, especially in conjunction with spring quizalofop applications, successfully reduced annual brome cover. Seeding significantly increased sagebrush density and seeded species cover, but was not affected by herbicide application. There were differences in seeded species cover across mines, which suggest site specific attributes also impacted establishment

success. Lastly, in addition to their intended effects, herbicide application and seeding, all caused ground disturbances which increased undesirable annual non-native forb cover. Despite this unintended secondary treatment effect, using a combination of herbicides and seeding was successful at managing annual brome and facilitating the establishment of desired native species from seed.

A single treatment of glyphosate reduced annual bromes in the first but not the second summer after application. Some studies found that a single application of glyphosate failed to control annual bromes (Cox and Anderson 2004) while others reported greater control (McAdoo, Boyd, and Sheley 2013; Nyamai, Prather, and Wallace 2011). Single herbicide applications generally only provide short-term control, so, long-term control of weedy species is a barrier to successful dryland restoration (Fansler and Mangold 2011). Repeated applications are often necessary to maintain annual brome control, but using a broad-spectrum herbicide (e.g. glyphosate, imazapic) can harm non-target species (Owen, Sieg, and Gehring 2011; Morris, Monaco, and Rigby 2009). McAdoo, Boyd, and Sheley (2013) reported that a single application of glyphosate killed 98.4% (SE = 0.67%) of cheatgrass, but sagebrush plants were covered with plastic during application, which is not feasible in large scale restoration scenarios.

Graminicides are grass-specific, they can be applied directly after seeding and repeatedly post emergence without harming forbs and shrubs to help with long-term control of annual grasses. This, as well as the promising results from my greenhouse study, make graminicides a good option for restoration when multiple herbicide

applications are needed. Unfortunately, quizalofop applied alone was not as effective as glyphosate at reducing annual bromes in the first summer, but when quizalofop was applied with glyphosate the reduction of annual brome cover was greatest. Brome cover was, however, reduced in the half of each plot retreated with quizalofop prior to the second summer. While there is evidence that graminicides alone can provide control of brome species in the greenhouse, my study and Ball, Frost and Bennett (2007), and in planted monocultures in the field (Brewster and Spinney 1989), ours is the first study to use graminicides in dryland restoration. My results suggest that quizalofop applications post seeding could be used until seeded forbs and shrubs have reached sufficient numbers and sizes to compete against the annual bromes.

A secondary effect of the disturbances caused by herbicide treatments, litter removal, and seeding, was an increase in bare ground. Increased bare ground can lead to greater colonization of both non-target and target species (Fargione, Brown, and Tilman 2003; Kennedy et al. 2002; Wilson and Gerry 1995; Gross and Werner 1982). Annual non-native forb cover, specifically kochia (*Kochia scoparia* [L.]) and Russian thistle (*Salsola tragus* [L.]) increased in a pattern opposite to the pattern of annual brome reduction. Fortunately we expect that species will only persist for a few growing seasons (Prodgers 2013).

Glyphosate treatments increased seeded species at Decker compared to the un-seeded and seeded controls in the first and second summer after treatment. However, at Spring Creek seeded species cover decreased in plots receiving glyphosate. This

decrease is likely due to harmful effects of glyphosate on remnant desired species that were sown as part of the initial restoration efforts in 2009 and 2001. My field locations did not meet plant diversity goals which necessitated re-seeding; however, it is apparent that some forbs and shrubs had established. Particularly at Spring Creek, prairie coneflower (*Ratibida columnifera* [Nutt.] Wooton & Standl.) was in the original seed mix and was present in the control plots indicating that there was some successful establishment during the initial seeding. Glyphosate, because it is a non-selective herbicide, could have harmed the remnant coneflower causing a decrease in seeded species cover.

In contrast, at Decker seeded species cover increased in both summers compared to the un-seeded control in the plots treated with quizalofop alone. Quizalofop increased seeded species cover at Decker in both summers compared to the un-seeded and seeded controls. In areas where inter-seeding is needed due to poor diversity outcomes of the initial restoration efforts, spraying quizalofop, will not harm remnant shrubs and forbs (Kukorelli, Reisinger, and Pinke 2013) and could provide additional control of unwanted species, potentially facilitating greater establishment of inter-seeded species. When no remnant species are present, like at Decker, glyphosate can also be used to control annual bromes and boost seeded species establishment.

At both mines, Wyoming big sagebrush densities increased when seed mixes were applied with and without herbicides. Williams et al. (2002) compared 1, 2, and 4 kg ha^{-1} PLS of Wyoming big sagebrush seeding rates and found a positive correlation. In

contrast, I found there was no benefit to increasing the rate of Wyoming big sagebrush from 3.4 and 5.6 kg ha^{-1} PLS as densities did not differ between the two seeding treatments after two years. Sagebrush seed is expensive and germination rates are usually low, so there is no benefit to using a seeding rate greater than 3.4 kg ha^{-1} PLS. Rinella et al. (2015) found that an increase in non-shrub seed rates decreased shrub cover, suggesting that using lower grass seeding rates could also improve sagebrush success. Additionally, using glyphosate post-germination is not recommended because it will be too detrimental to the newly established sagebrush (Owen, Sieg, and Gehring 2011). It is possible that continued application of quizalofop will help maintain the greater density of sagebrush long-term, but in the short-term my study found no benefit from retreating with quizalofop to sagebrush densities.

Sagebrush is often the seeded species of highest ecological and restoration interest, but lowest establishment success so developing restoration methods to facilitate germination and long-term survival of this species is imperative. For the most successful treatment (i.e. seeded plots receiving glyphosate and quizalofop at Decker), I achieved a mean Wyoming big sagebrush density of 3.53 (1.87, 6.48) plants / m^{-2}. This along with minimal sagebrush density decreases from the first to second summer after seeding for all seeded treatments at both mines, is promising for long-term sagebrush success. Presumably, even though sagebrush densities decrease year to year, individuals that do survive are growing larger so sagebrush cover will increase as will the likelihood of those larger individuals surviving. Despite these promising sagebrush density trends,

short-term results are unreliable at predicting long-term success (Rinella et al. 2012), so more years of monitoring are needed.

Given that, 1) herbicides are one of the primary tools for weed management in dryland restoration; 2) there is limited establishment of desired species from seed (Mangold et al. 2015; Hirsch-Schantz et al. 2014); 3) single applications of glyphosate provide unreliable long-term brome control so repeated applications may be necessary; and, 4) both glyphosate and other often used herbicides (e.g. imazapic) are non-selective so they could damage desired established seeded species, the need to explore novel herbicides, such as graminicides, is evident. My two-year field study showed potential for using glyphosate in the fall pre-seeding in combination with repeated spring applications of the graminicide, quizalofop, to reduce annual brome cover and increase seeded species, including sagebrush, establishment.

Future Research

My greenhouse and field studies demonstrated that graminicides can be used to control cheatgrass and Japanese brome. Additionally, I have shown that herbicides in conjunction with seeding can reduce annual brome cover and increase seeded species establishment. However, continued reapplication of quizalofop to my already established plots, as well as more years of monitoring are needed to understand the long-term impact of my treatments to re-vegetation goals.

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APPENDIX A

CHAPTER TWO SUPPLEMENTAL FIGURES

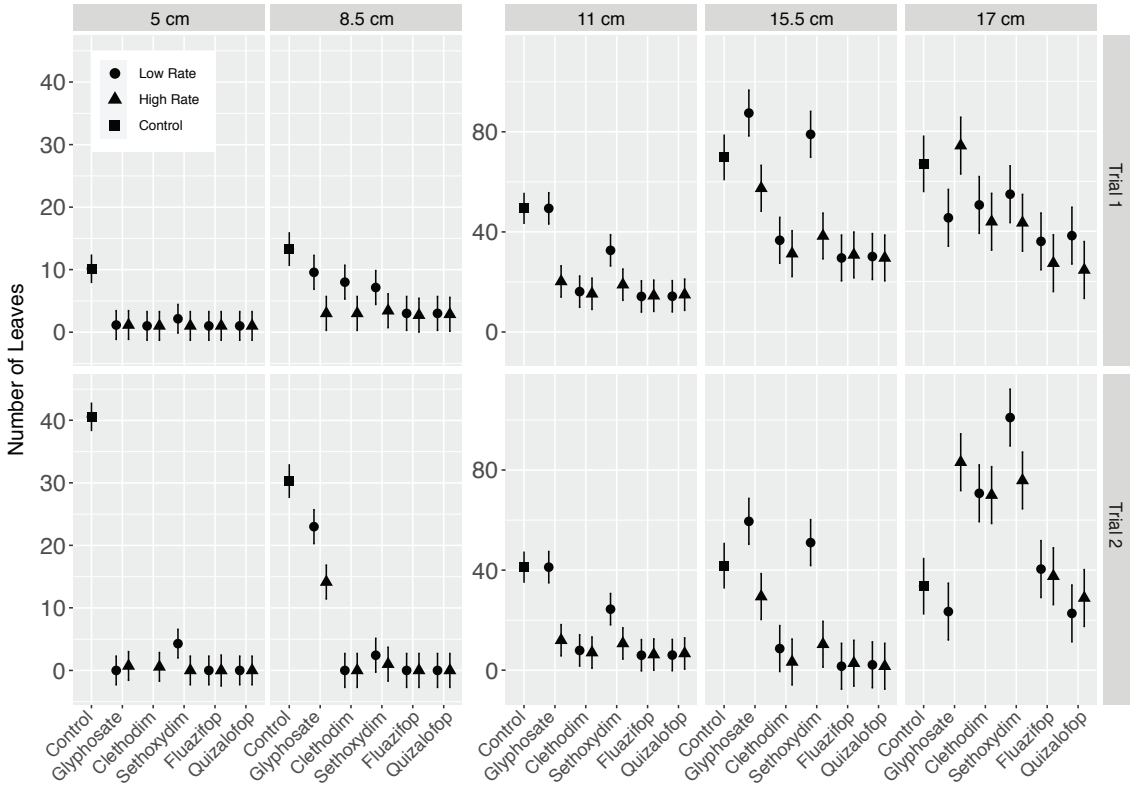

Figure A.1: Number of leaves 45 days after treatment using least-squares means within herbicide and rate treatments for each cheatgrass height at the time of herbicide application, for both trials of Experiment 1. Number of leaves represent the means of the individual plants within a group.

Table A.1: Linear mixed-effects model results assessing number of leaves 45 days after treatment for plants that were 5 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications) and trial 1.

5 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**10.143**	**1.158**	**123**	**8.8**	**<0.0001**	Replicate	0.244
Glyphosate low	**-9.000**	**1.616**	**119**	**-5.6**	**<0.0001**	Residual	9.145
Clethodim low	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Sethoxydim low	**-8.000**	**1.616**	**119**	**-4.9**	**<0.0001**		
Fluazifop low	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Quizalofop low	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Glyphosate high	**-9.000**	**1.616**	**119**	**-5.6**	**<0.0001**		
Clethodim high	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Sethoxydim high	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Fluazifop high	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Quizalofop high	**-9.143**	**1.616**	**119**	**-5.7**	**<0.0001**		
Trial 2	**30.429**	**1.616**	**119**	**18.8**	**<0.0001**		
Glyphosate low : Trial 2	**-31.571**	**2.286**	**119**	**-13.8**	**<0.0001**		
Sethoxydim low : Trial 2	**-28.286**	**2.286**	**119**	**-12.4**	**<0.0001**		
Fluazifop low : Trial 2	**-31.429**	**2.286**	**119**	**-13.7**	**<0.0001**		
Quizalofop low : Trial 2	**-31.429**	**2.286**	**119**	**-13.7**	**<0.0001**		
Glyphosate high : Trial 2	**-30.857**	**2.286**	**119**	**-13.5**	**<0.0001**		
Clethodim high : Trial 2	**-30.857**	**2.286**	**119**	**-13.5**	**<0.0001**		
Sethoxydim high : Trial 2	**-31.429**	**2.286**	**119**	**-13.7**	**<0.0001**		
Fluazifop high : Trial 2	**-31.439**	**2.334**	**119**	**-13.5**	**<0.0001**		
Quizalofop high : Trial 2	**-31.429**	**2.286**	**119**	**-13.7**	**<0.0001**		

Table A.2: Linear mixed-effects model results assessing number of leaves 45 days after treatment for plants that were 8.5 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications) and trial 1.

8.5 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**13.286**	**1.370**	**132**	**9.7**	**<0.0001**	Replicate	0.039
Glyphosate low	-3.714	1.934	126	-1.9	0.0571	Residual	13.097
Clethodim low	**-5.286**	**1.934**	**126**	**-2.7**	**0.0072**		
Sethoxydim low	**-6.143**	**1.934**	**126**	**-3.2**	**0.0019**		
Fluazifop low	**-10.286**	**1.934**	**126**	**-5.3**	**<0.0001**		
Quizalofop low	**-10.286**	**1.934**	**126**	**-5.3**	**<0.0001**		
Glyphosate high	**-10.286**	**1.934**	**126**	**-5.3**	**<0.0001**		
Clethodim high	**-10.286**	**1.934**	**126**	**-5.3**	**<0.0001**		
Sethoxydim high	**-9.857**	**1.934**	**126**	**-5.1**	**<0.0001**		
Fluazifop high	**-10.571**	**1.934**	**126**	**-5.5**	**<0.0001**		
Quizalofop high	**-10.429**	**1.934**	**126**	**-5.4**	**<0.0001**		
Trial 2	**17.000**	**1.934**	**126**	**8.8**	**<0.0001**		
Glyphosate low : Trial 2	-3.571	2.736	126	-1.3	0.1941		
Sethoxydim low : Trial 2	**-25.000**	**2.736**	**126**	**-9.1**	**<0.0001**		
Fluazifop low : Trial 2	**-21.714**	**2.736**	**126**	**-7.9**	**<0.0001**		
Quizalofop low : Trial 2	**-20.000**	**2.736**	**126**	**-7.3**	**<0.0001**		
Glyphosate high : Trial 2	**-20.000**	**2.736**	**126**	**-7.3**	**<0.0001**		
Clethodim high : Trial 2	**-5.857**	**2.736**	**126**	**-2.1**	**0.0342**		
Sethoxydim high : Trial 2	**-20.000**	**2.736**	**126**	**-7.3**	**<0.0001**		
Fluazifop high : Trial 2	**-19.429**	**2.736**	**126**	**-7.1**	**<0.0001**		
Quizalofop high : Trial 2	**-19.714**	**2.736**	**126**	**-7.2**	**<0.0001**		

Table A.3: Linear mixed-effects model results assessing number of leaves 45 days after treatment for plants that were 11 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications) and trial 1.

11 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**49.390**	**3.160**	**120**	**15.6**	**<0.0001**	Replicate	3.359
Glyphosate low	0.000	4.174	136	0.0	1.0000	Residual	121.98
Clethodim low	**-33.290**	**4.174**	**136**	**-8.0**	**<0.0001**		
Sethoxydim low	**-16.790**	**4.174**	**136**	**-4.0**	**0.0001**		
Fluazifop low	**-35.210**	**4.174**	**136**	**-8.4**	**<0.0001**		
Quizalofop low	**-35.140**	**4.174**	**136**	**-8.4**	**<0.0001**		
Glyphosate high	**-29.210**	**4.174**	**136**	**-7.0**	**<0.0001**		
Clethodim high	**-34.140**	**4.174**	**136**	**-8.2**	**<0.0001**		
Sethoxydim high	**-30.500**	**4.174**	**136**	**-7.3**	**<0.0001**		
Fluazifop high	**-34.930**	**4.174**	**136**	**-8.4**	**<0.0001**		
Quizalofop high	**-34.500**	**4.174**	**136**	**-8.3**	**<0.0001**		
Trial 2	**-8.208**	**1.780**	**136**	**-4.6**	**<0.0001**		

Table A.4: Linear mixed-effects model results assessing number of leaves 45 days after treatment for plants that were 15.5 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications) and trial 1.

15.5 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**69.779**	**4.640**	**142**	**15.0**	**<0.0001**	Replicate	0.000
Glyphosate low	17.714	6.283	142	2.8	0.0055	Residual	276.30
Clethodim low	**-33.143**	**6.283**	**142**	**-5.3**	**<0.0001**		
Sethoxydim low	9.214	6.283	142	1.5	0.1447		
Fluazifop low	**-40.214**	**6.283**	**142**	**-6.4**	**<0.0001**		
Quizalofop low	**-39.643**	**6.283**	**142**	**-6.3**	**<0.0001**		
Glyphosate high	-12.357	6.283	142	-2.0	0.0511		
Clethodim high	**-38.500**	**6.283**	**142**	**-6.1**	**<0.0001**		
Sethoxydim high	**-31.429**	**6.283**	**142**	**-5.0**	**<0.0001**		
Fluazifop high	**-39.000**	**6.283**	**142**	**-6.2**	**<0.0001**		
Quizalofop high	**-40.214**	**6.283**	**142**	**-6.4**	**<0.0001**		
Trial 2	**-27.987**	**2.679**	**142**	**-10.4**	**<0.0001**		

Table A.5: Linear mixed-effects model results assessing number of leaves 45 days after treatment for plants that were 17 cm at time of application for Experiment 1. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications) and trial 1.

17 cm at time of application	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**67.000**	**5.740**	**126**	**11.7**	**<0.0001**	Replicate	10.77
Glyphosate low	**-21.571**	**7.925**	**126**	**-2.7**	**0.0074**	Residual	219.83
Clethodim low	**-16.429**	**7.925**	**126**	**-2.1**	**0.0402**		
Sethoxydim low	-12.143	7.925	126	-1.5	0.1280		
Fluazifop low	**-31.000**	**7.925**	**126**	**-3.9**	**0.0001**		
Quizalofop low	**-28.714**	**7.925**	**126**	**-3.6**	**0.0004**		
Glyphosate high	7.286	7.925	126	0.9	0.3597		
Clethodim high	**-23.143**	**7.925**	**126**	**-2.9**	**0.0041**		
Sethoxydim high	**-23.571**	**7.925**	**126**	**-3.0**	**0.0035**		
Fluazifop high	**-39.714**	**7.925**	**126**	**-5.0**	**<0.0001**		
Quizalofop high	**-42.429**	**7.925**	**126**	**-5.4**	**<0.0001**		
Trial 2	**-33.429**	**7.925**	**126**	**-4.2**	**<0.0001**		
Glyphosate low : Trial 2	11.429	11.208	126	1.0	0.3098		
Sethoxydim low : Trial 2	**53.571**	**11.208**	**126**	**4.8**	**<0.0001**		
Fluazifop low : Trial 2	**79.571**	**11.208**	**126**	**7.1**	**<0.0001**		
Quizalofop low : Trial 2	**37.857**	**11.208**	**126**	**3.4**	**0.0010**		
Glyphosate high : Trial 2	17.857	11.208	126	1.6	0.1136		
Clethodim high : Trial 2	**42.286**	**11.208**	**126**	**3.8**	**0.0002**		
Sethoxydim high : Trial 2	**59.571**	**11.208**	**126**	**5.3**	**<0.0001**		
Fluazifop high : Trial 2	**65.857**	**11.208**	**126**	**5.9**	**<0.0001**		
Quizalofop high : Trial 2	**43.714**	**11.208**	**126**	**3.9**	**0.0002**		

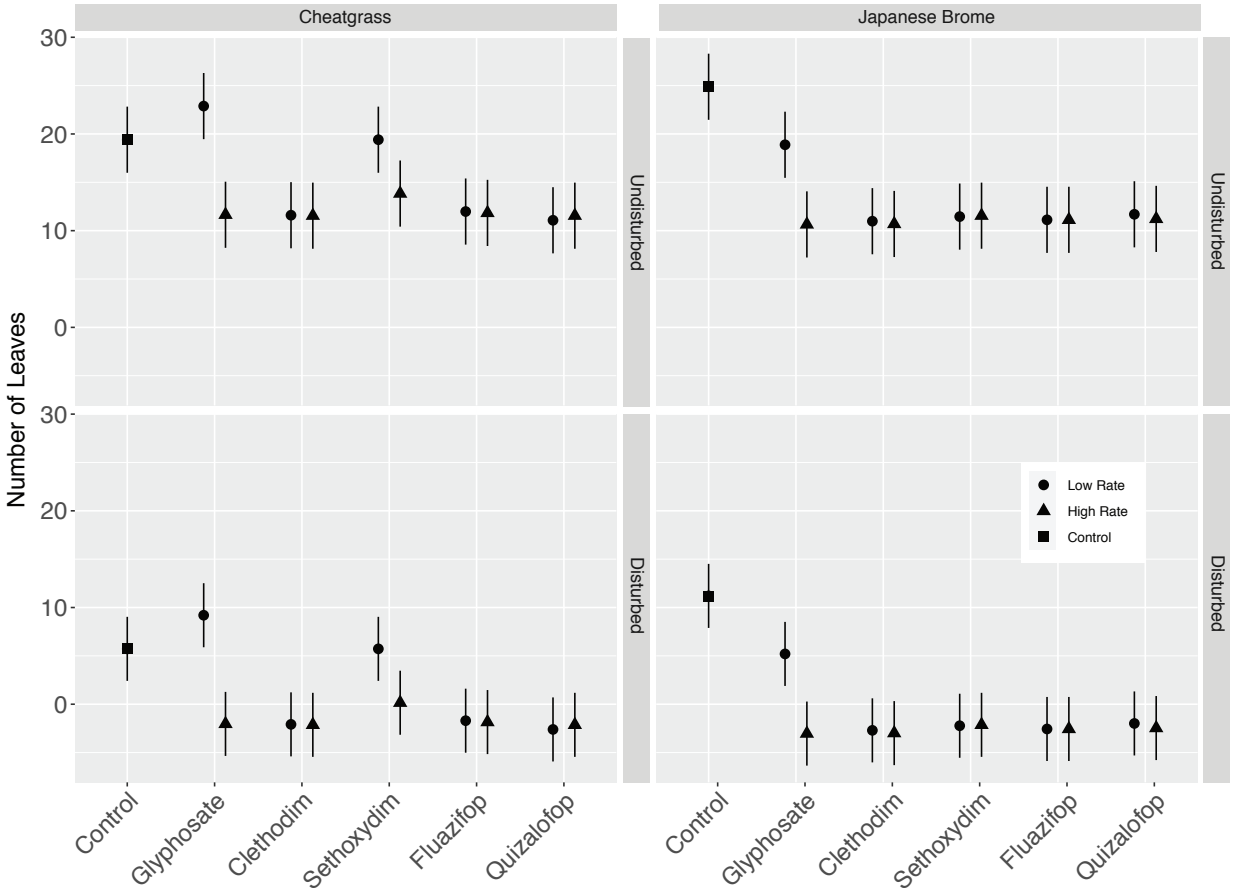

Figure A.2: Number of leaves 45 days after treatment using least-squares means within herbicide and rate treatments for undisturbed and disturbed cheatgrass and Japanese brome accessions treated at 11 cm for Experiment 2. Number of leaves represent the means of the individual plants within a group.

Table A.6: Linear mixed-effects model results from assessing number of leaves 45 days after treatment for Experiment 2. Satterthwaite approximations were used to calculate p values and degrees of freedom (df). Predictors in bold indicate significance ($p < 0.05$). Baseline levels, contained in the intercept are: control plants (no herbicide applications), undisturbed accession, and cheatgrass. BRJA is Japanese brome.

Experiment 2	Fixed effects					Random effects	
Variable	Estimate	SE	df	t	p	Variable	Variance
Intercept	**5.73**	**1.74**	**438**	**3.3**	**0.0011**	Replicate	0.00
Glyphosate low	3.48	2.36	438	1.5	0.1413	Residual	58.43
Clethodim low	**-7.81**	**2.36**	**438**	**-3.3**	**0.0010**		
Sethoxydim low	0.00	2.36	438	0.0	1.0000		
Fluazifop low	**-7.43**	**2.36**	**438**	**-3.1**	**0.0018**		
Quizalofop low	**-8.33**	**2.36**	**438**	**-3.5**	**0.0005**		
Glyphosate high	**-7.76**	**2.36**	**438**	**-3.3**	**0.0011**		
Clethodim high	**-7.86**	**2.36**	**438**	**-3.3**	**0.0009**		
Sethoxydim high	**-5.57**	**2.36**	**438**	**-2.4**	**0.0186**		
Fluazifop high	**-7.57**	**2.36**	**438**	**-3.2**	**0.0014**		
Quizalofop high	**-7.86**	**2.36**	**438**	**-3.3**	**0.0009**		
Disturbed accession	**13.68**	**0.87**	**438**	**15.7**	**<0.0001**		
Japanese brome	0.00	0.87	438	0.0	1.0000		
Glyphosate low : BRJA	**5.48**	**2.36**	**438**	**2.3**	**0.0207**		
Clethodim low : BRJA	**-9.48**	**3.34**	**438**	**-2.8**	**0.0047**		
Sethoxydim low : BRJA	-6.10	3.34	438	-1.8	0.0684		
Fluazifop low : BRJA	**-13.43**	**3.34**	**438**	**-4.0**	**0.0001**		
Quizalofop low : BRJA	-6.33	3.34	438	-1.9	0.0583		
Glyphosate high : BRJA	-4.86	3.34	438	-1.5	0.1461		
Clethodim high : BRJA	-6.48	3.34	438	-1.9	0.0529		
Sethoxydim high : BRJA	-6.33	3.34	438	-1.9	0.0583		
Fluazifop high : BRJA	**-7.76**	**3.34**	**438**	**-2.3**	**0.0204**		
Quizalofop high : BRJA	-6.19	3.34	438	-1.9	0.0642		