DISSERTATION

A NEW PARADIGM IN RANGELAND RESTORATION: USING A PRE-EMERGENT HERBICIDE TO ASSIST IN NATIVE PLANT ESTABLISHMENT AND RELEASE

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2019

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ABSTRACT

A NEW PARADIGM IN RANGELAND RESTORATION: USING A PRE-EMERGENT HERBICIDE TO ASSIST IN NATIVE PLANT ESTABLISHMENT AND RELEASE

Invasive winter annual grasses (IWAG), especially downy brome (*Bromus tectorum* L.), are a significant threat to rangeland ecosystems in the western United States. Invasions in natural ecosystems can cause severe negative impacts by reducing native plant diversity and lowering community productivity, increasing fire frequency, and displacing native vegetation that is critical wildlife and pollinator habitat. Herbicides currently used for IWAG management can provide adequate short-term control; however, results can be inconsistent and injury to desirable species can occur. Indaziflam (Esplanade®, Bayer CropScience) is a new herbicide option for long-term IWAG control in natural areas and rangeland. As a cellulose biosynthesis inhibitor, indaziflam stops root growth in newly germinated seedlings by preventing cellulose formation. Field studies were conducted to assess native plant tolerance, revegetation and broadleaf weed seedling control with indaziflam applications. At two sites, indaziflam did not impact perennial grass cover, native species richness, or the community composition of forbs and shrubs at one and two years after treatment (YAT). However, the abundance of native forbs and shrubs was reduced by treatments containing picloram and aminocyclopyrachlor. In a separate field study at two sites, a glyphosate dose response showed approximately three times more glyphosate was needed for a 50% reduction (GR_{50}) in feral rye biomass (GR_{50} = 126.0 g ae ha^{-1}) compared to downy brome biomass (GR_{50} = 40.4 g ae ha^{-1}). Indaziflam treatments still resulted in reduced downy brome and feral rye biomass compared to the non-treated check 3 YAT, while imazapic and glyphosate did not provide control beyond the first year. Establishment of cool-season grasses (C_3), warm-season grasses (C_4) and forb/shrubs through drill seeding was only successful across all three functional groups in treatments which included indaziflam. C_3 grasses had greater establishment, with an average frequency of 61 ± 1.7% (mean ± SE) at Site 1 and 46 ± 2.6% SE at Site 2 at 3 YAT. In a third field study, treatments containing indaziflam had increased Dalmatian toadflax [*Linaria dalmatica* (L.) P. Mill], diffuse knapweed (*Centaurea diffusa* Lam.), and common mullein (*Verbascum thapsus* L.) control 2 YAT compared to treatments without indaziflam. A laboratory assay was conducted to evaluate the impact of litter on imazapic, rimsulfuron, and indaziflam availability. Downy brome litter at 2,600 kg ha^{-1} intercepted 84.3 ± 1.0% SE of the applied herbicide. Simulated rainfall at 0 days (d) after application was able to recover 100% of the intercepted rimsulfuron and imazapic, while recovery decreased to 65 ± 1.7% at 1 d and 7 d. Only 54 ± 1.9% of indaziflam could be recovered at 0 d, and recovery decreased to 33 ± 1.1% when simulated rain was applied at 1d or 7 d after application. The multi-year winter annual grass control provided by indaziflam could provide a new strategy for rangeland restoration, allowing enough time for the release of the remnant native plant community or the establishment of native species through revegetation. Indaziflam could potentially be incorporated into management systems to manage the weed seed bank and extend biennial and perennial weed control.

ACKNOWLEDGEMENTS

I would first like to thank my amazing advisor, Dr. Scott Nissen, for always supporting my ideas and allowing me to develop and lead my own research. His style of pushing graduate students to be independent has given me several opportunities throughout my graduate student career I would not have had otherwise. He also plans the most awesome lab meetings and get-togethers, from impromptu meetings at The Rio and even a lab meeting on the beach in Brazil!

I would especially like to thank Dr. Derek Sebastian, as he took me under his wing when I started as a grad student and he was finishing his PhD. He started several research projects I was able to continue for my PhD and taught me how to design experiments that answered critical questions. He has been a mentor for me throughout my graduate studies. I also have to thank Jim Sebastian, the man who started it all with indaziflam and has devoted so much time to assisting me in my research. I also would like to thank my committee members: Dr. Franck Dayan, Dr. Paul Meiman, and Dr. Lou Bjostad for their support along the way.

A big thank you and hug to Dr. Paulo da Silva as he provided so much assistance to my projects during his time at CSU. Also thank you to my awesome lab group Mirella, Rachel, and Kristen, post doc Eric, and all the Weed Science graduate students and hourlies for all your help and support, especially for driving vans on our annual weed tour!

I want to thank my grandparents, Edy and Al, for always encouraging and supporting me through my studies and taking care of my kids, even into wee hours of the night as I worked. My biggest thank you is to my husband Adam for his support throughout my studies as I traveled to numerous meetings or spent evenings at the lab, and my children Lincoln and Lilliana. I would never have been able to achieve what I have without their love and support.

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INTRODUCTION

Downy brome (*Bromus tectorum* L.), an exotic winter annual grass, has emerged as one of the most invasive and problematic weeds in western rangeland and natural areas, with an estimated 14% annual spread rate (DiTomaso et al. 2010; Duncan et al. 2004). Although *B. tectorum* typically germinates in the fall after cool, wet weather, plants are opportunistic and can germinate anytime the growing conditions are favorable (Beck 2009). This variable germination cycle has allowed *B. tectorum* to thrive in arid and semi-arid western climates by rapidly growing and depleting available soil moisture and nutrients before most native species break dormancy in the spring (Knapp 1996; Mack and Pyke 1983). Invasions in natural ecosystems can cause severe negative impacts by reducing native plant diversity and lowering community productivity, increasing fire frequency, and displacing native vegetation that is critical wildlife and pollinator habitat (Abatzoglou and Kolden 2011; Beck 2009; Billings 1994; DiTomaso et al. 2010; Knapp 1996; Monaco et al. 2017; Whisenant 1990).

By the 1930's researchers had begun to recognize *B. tectorum* invasions as a serious issue in rangeland (Mack 1981; Price et al. 1948; Warg 1938). Since then, extensive research has been conducted on mechanical, cultural, biological, and chemical control of this exotic grass. Thus far, herbicides have been the most effective and widely used weed management strategy for *B. tectorum* on rangeland and natural areas (Diamond et al. 2012; Mangold et al. 2013; Monaco et al. 2017). Since its release in 1996, imazapic has been the primary herbicide used to control *B. tectorum* on rangeland because it has both PRE and POST activity and is selective at

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[1] This chapter contains published work from: Clark SL, Sebastian DJ, Nissen SJ, Sebastian JR (2019) Effect of indaziflam on native species in natural areas and rangeland. Invasive Plant Sci Manag, doi: 10.1017/inp.2019.4

relatively low use rates (Anonymous 1996; Kyser et al. 2013; Mangold et al. 2013). Several other herbicides including glyphosate, sulfometuron, and rimsulfuron have traditionally been used for *B. tectorum* control in non-crop sites (Kyser et al., 2013; Sebastian et al. 2016). Although adequate control is often achieved with these herbicide options the first year of application, control can be inconsistent or short-term and injury to desirable species can occur (Kelley et al. 2013; Kyser et al., 2013; Mangold et al., 2013; Morris et al. 2009; Thacker 2009; Whitson et al. 1997; Whitson and Koch 1998). In many invasion situations, short-term control (< 2 years) does not allow the remnant native plant community enough time to re-establish/recover and become competitive (Chambers et al. 2014; Elseroad and Rudd 2011). As *B. tectorum* infestations continue to spread, shifting native perennial grass systems to ecosystems dominated by winter annual grasses, land managers need strategies that provide long-term control of this weed.

Indaziflam is a broad-spectrum, pre-emergence herbicide first released in 2011 for use in several perennial cropping systems and later used for weed control in turfgrass, ornamentals, forestry and non-crop industrial sites (Anonymous 2011a, b; Brabham et al. 2014). In 2016 a supplemental label for indaziflam was approved for the release or restoration of desirable vegetation in natural areas, open spaces, wildlife management areas and fire rehabilitation areas, specifically targeting invasive winter annual grass control in these sites (Anonymous 2016).

Indaziflam is a cellulose biosynthesis inhibitor, representing a unique site of action with no reported cases of resistance in the field (Brabham et al. 2014; Tateno et al. 2016). Indaziflam has a longer soil residual than other commonly used herbicides for *B. tectorum* management, providing three or more years of control (Sebastian et al. 2016; Sebastian et al. 2017a). In most rangeland situations, this length of control allows enough time for release of the remnant native perennial community (Chambers et al. 2014; Sebastian et al. 2016; Sebastian et al. 2017a). Sebastian et al. (2016, 2017a) found that indaziflam will selectively control *B. tectorum* without impacting perennial grass and forb biomass, even leading to significant increases in biomass due to reductions in *B. tectorum* (Sebastian et al. 2016; Sebastian et al. 2017a). This research suggests native perennial species are tolerant to indaziflam, although studies assessing impacts to community composition and native species abundance following indaziflam applications have not been conducted.

The objective of this study was to evaluate tolerance of several native species to indaziflam applications and compare tolerance with other commonly used grass and broadleaf rangeland herbicides. We hypothesized that indaziflam would significantly reduce *B. tectorum* cover without decreasing native species abundance compared to the other herbicides evaluated.

MATERIALS AND METHODS

Site Description

The experiments were established in 2015 at two sites in Jefferson County, CO, containing *B. tectorum* with a co-occurring native grass, forb and shrub community. Site 1 (latitude 39° 45’ 37” N, longitude 105° 14’ 21” W) was located on Mt. Galbraith Open Space, and Site 2 (latitude 39° 53’ 40” N, longitude 105° 16’ 14” W) was located on El Dorado Mountain Open Space. Sites were approximately 15 km apart and both in the Western High Plains region of the Great Plains ecoregion. In June 2015, before herbicide application, we conducted an initial inventory of the plant species by recording all plant species present at each site within the boundaries of the plots. A visual estimate of *B. tectorum* canopy cover (%) was also done at both sites. Site 1 was categorized as ~30-40% *B. tectorum* cover with 33 native species and 5 co-occurring non-native species (Table 1.1). By the following year (2016), *B.*

tectorum cover at Site 1 decreased to ~5% and maintained a similar cover level throughout the course of the study. Site 2 had ~60-70% *B. tectorum* cover with 35 native species and 6 co-occurring non-native species (Table 1.1).

The soil at Site 1 was Ratake rocky loam (loamy-skeletal, micaceous, frigid, shallow Typic Haplustolls), with 2.3% organic matter and 6.0 pH in the top 20 cm (U.S. Department of Agriculture, Natural Resources Conservation Service 2014). Site 1 was located on a 30° rocky slope and the average elevation was 1839 m (6035 ft). The soil at Site 2 was Flatirons stony sandy loam (clayey-skeletal, smectitic, mesic Aridic Paleustolls), with 4.9% organic matter and 6.6 pH in the top 20 cm (U.S. Department of Agriculture, Natural Resources Conservation Service 2014). Site 2 was located on a 25° rocky slope and the average elevation was 1995 m (6544 ft).

Mean annual precipitation based on the 30-yr average (1981-2010) was 468 mm at Site 1 and Site 2 based on the closest weather station (approximately 8 km from each site) (Western Regional Climate Center 2018). Both sites received an additional 252 mm of precipitation above their 30-yr average in 2015. A statewide drought occurred in 2016 and total precipitation for the sites decreased to 148 mm below the 30-yr average (Western Regional Climate Center 2018). In 2017 the sites received precipitation similar to the 30-year average. The 30-yr mean annual temperature for both sites was 10.2°C, and the average temperature for 2015 was close to the 30-year average. The average temperature for 2016 and 2017 was 1.8°C and 2.2°C warmer, respectively (Western Regional Climate Center 2018).

Experimental Design

Herbicide applications were made June 2, 2015 and timed in accordance with label recommendations for Dalmatian toadflax [*Linaria dalmatica* (L.) P. Mill.] control, one of the co-occurring non-native species (Anonymous 2018). The herbicides targeting *B. tectorum* were applied as an early PRE application timing. *Bromus tectorum* was in the ripening stage and actively setting seed. Native forb growth stage ranged from post-flowering, early-flowering to pre-flowering. Ten herbicide treatments and one non-treated control were established to 3 by 6 m plots arranged in a randomized complete block design with six replications (Table 1.2). All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat-fan nozzles (TeeJet®, Spraying Systems, P.O. Box 7900, Wheaton, IL 60187) delivering 187 L ha^{-1} at 207 kPa.

Treatment Evaluations and Data Analysis

To account for variability across the study area, native forb and shrub species were counted individually throughout the entire area of each plot from May to August (2016 and 2017) to determine species richness (total number of species) and abundance (number of individuals per species). Counts were conducted bi-weekly targeting different species each time to account for varying life cycles, and individual species were counted only once per growing season. For rhizomatous or clonal plants, each clumping patch or grouping of stems was counted as one individual. To determine *B. tectorum* and native grass canopy cover, percent cover estimates of all grass species were determined by conducting visual evaluations across each entire plot (18 m^2 plot area) in August 2016 and 2017. Species richness was defined as the total number of different species occurring by plot (18 m^2 plot area) while species abundance was defined as total number of individuals per species per plot (18 m^2 plot area). Native grass cover was collected as percent cover per species, however, due to variability across the sites species were combined into cool-season (C3) and warm-season (C4) cover categories. Cover data for *B.*

tectorum and perennial grasses were arcsine square root transformed to meet the assumptions of normality.

To test treatment effects on *B. tectorum* cover, a repeated measures linear mixed-effects model was created using the 'lme4' package in R version 3.4.3, testing for treatment effects at $\alpha = 0.05$ (R Core Team 2017). The fixed factors included in the model were treatment, year, and interactions, with year as the repeated measure; block was included as a random factor. Further analysis of the treatment and year effect was performed using the 'lsmeans' package in R (R Core Team, 2017) to obtain comparisons between all pairs of least squares means by year with a Tukey adjustment ($P < 0.05$). For grass cover data, the same analysis was performed for C3 grass cover and C4 grass cover. After rejecting the null hypothesis of equal variance for Sites 1 and 2, grass cover was analyzed separately by site.

Species richness was calculated by determining the number of native species in each plot. After failing to reject the null hypothesis of equal variance ($P = 0.3401$), sites were combined. A generalized linear mixed model was used to analyze count data with treatment and year as fixed factors and block as the random factor. Count data for species richness were assumed to follow a Poisson distribution after failing to reject the null hypothesis that sample frequencies differed significantly from the expected frequencies under a Poisson distribution ($P = 0.1113$). Significant pairwise differences between richness were determined post-hoc using a least-squares means test with a Tukey adjustment ('stats' and 'lsmeans' packages, R Core Team 2017).

To test treatment effects on overall native forb and shrub community abundance, dissimilarity matrices were generated on the collected abundance data using the Bray-Curtis method in Primer v7 (Bray and Curtis 1957; Clarke and Gorley 2015). Due to varied species amounts and types occurring at each site, sites and years were analyzed separately. Count data for each species were square-root transformed before creating a resemblance matrix for each site and year by using Bray-Curtis dissimilarity measures. Homogeneity of variance (or dispersion) at each site by year was tested using permutational analysis of multivariate dispersion and was significant for Site 2 in 2017 (Site 1, 2016 P = 0.109; Site 2, 2016 P = 0.257; Site 1, 2017 P = 0.055; Site 2, 2017 P = 0.002). There was a dispersion effect at Site 2 in 2017. PERMANOVA is largely unaffected by heterogeneity for balanced designs and is more powerful than other tests in detecting actual changes in community structure (Anderson and Walsh 2013). Therefore, the resulting resemblance matrices were used to generate Principal Coordinate Analyses (PCoA) to visualize differences among treatments. Permutational multivariate analysis of variance (PERMANOVA) was conducted to test treatment effects on native forb and shrub community composition (Anderson 2001; Anderson et al. 2008). PERMANOVA can be used as a non-parametric alternative to MANOVA and allows analysis of multiple variables (i.e. species counts) when data do not meet the assumptions of MANOVA. PERMANOVA were conducted using partial sums of squares on 999 permutations of residuals under a reduced model. Factors considered in the model were treatment as a fixed factor and block as a random factor. All multivariate analyses were conducted using PRIMER v7 and PERMANOVA+ (Primer-E, Plymouth, UK). Pairwise tests were performed by treatment levels using PERMANOVA+ (Primer-E, Plymouth, UK). Similarity percentage analysis (SIMPER) (Primer v7) for significant treatments was then used to identify specific species accounting for over 60% of the dissimilarity in community composition compared to the non-treated control. The analysis revealed whether the dissimilarity was primarily due to increases or decreases in species abundance.

RESULTS AND DISCUSSION

Bromus Tectorum Control

Bromus tectorum cover decreased significantly at Site 1 during 2016 and 2017, therefore only Site 2 was analyzed for treatment impacts to *B. tectorum* cover. Treatment was the only significant factor impacting *B. tectorum* cover ($P < 0.001$) (Table A1.1). Compared to the non-treated control, all treatments containing indaziflam had less *B. tectorum* cover 1 YAT (0% to 22% cover). Indaziflam at the highest rate (102 g ai ha^{-1}) alone or tank-mixed with aminocyclopyrachlor or picloram resulted in only 0.7±0.3% (mean±SE) *B. tectorum* cover 1 YAT. The only other treatment to reduce *B. tectorum* cover 1 YAT was imazapic applied alone (21.6±0.6%) (Figure 1.1). Indaziflam at the highest rates (73 and 102 g ai ha^{-1}) alone or tank-mixed with aminocyclopyrachlor or picloram continued to reduce *B. tectorum* cover 2 YAT (0.8±0.3%) (Figure 1.1). Although our data only represent one site, they were consistent with past studies showing multi-year *B. tectorum* control with indaziflam treatments compared to short-term (<1 year) control with imazapic treatments (Kyser et al. 2007; Mangold et al. 2013; Morris et al. 2009; Sebastian et al. 2016; Sebastian et al. 2017a).

Impacts to Native Grasses

All native grasses occurring across the two sites were perennial grasses. Site 1 had significant treatment ($P < 0.001$) and year ($P = 0.02$) effects for C3 grasses; however, the interaction of year by treatment was not significant (Table A1.1). Comparisons made to the non-treated control showed increases in C3 grass cover 1 YAT for treatments containing picloram and aminocyclopyrachlor (average of 27% C3 grass cover in non-treated control plots compared to 52 to 74% cover in the picloram and aminocyclopyrachlor treated plots). By 2 YAT, the only significant difference in C3 grass cover at Site 1 was between the non-treated control and picloram plus indaziflam treatment (Table 1.3). There was no difference in warm season grass cover 1 or 2 YAT at Site 1 (Tables A1.1 and A1.2). With little competition from *B. tectorum* at

Site 1, differences in perennial grass cover were likely due to the forb reduction from the broadleaf herbicides. At Site 2 there was no treatment effect on C3 grass cover (P = 0.6324) (Table S1). For C4 grass cover a post-hoc Tukey test revealed no significant pairwise differences between individual means separated by treatment (Tables A1.1 and A1.2).

Impacts to Species Richness

The treatment by year interaction was not significant (P = 0.4609) for species richness although there was a treatment effect (P < 0.001). The only treatment to impact species richness was picloram combined with indaziflam 1 YAT, which reduced species richness compared to the non-treated control. The picloram plus indaziflam treatment had an average richness of 7.4±0.66 compared to the non-treated control with an average species richness of 12.8±0.59. By 2 YAT no impacts to species richness were observed (Figure A1.1).

Impacts to Community Composition

Visualization of the PCoA plots suggested changes in community composition due to herbicide treatments at both sites so a PERMANOVA analysis was performed to determine any treatment differences (Figure 1.2). At Site 1 PERMANOVA analysis showed impacts to the community composition of native species from herbicide treatments 1 and 2 YAT (P < 0.001). All treatments containing picloram and aminocyclopyrachlor impacted species abundance compared to non-treated control plots both 1 and 2 YAT (Table 1.4). Further analysis with SIMPER revealed that both broadleaf herbicide treatments decreased the abundance of most native forbs and shrubs included in the analysis (Supplemental file). Hairy false goldenaster [*Heterotheca villosa* (Pursh) Shinners var. *villosa*] and western ragweed [*Ambrosia psilostachya* DC. var. *coronopifolia* (Torr. & A. Gray) Farw.] were most impacted, contributing to more than 20% of the dissimilarity between the non-treated control both 1 and 2 YAT (Supplemental file).

No treatments resulted in increased species abundance at Site 1 (Table 1.4) (Figure 1.2). At Site 2, the PERMANOVA also showed treatment effects to community composition of native species in both years ($P < 0.001$). For 1 YAT, treatments containing picloram reduced the abundance of most species, while treatments of imazapic alone and indaziflam at 44 g ai ha^{-1} increased species abundance compared to the non-treated control (Table 1.4). In year 2, treatments containing picloram still reduced species abundance, however, no treatments increased species abundance compared to the non-treated control (Table 1.4) (Figure 1.2). Picloram had the greatest impacts to *H. villosa* and trailing fleabane (*Erigeron flagellaris* A. Gray), accounting for over 30% and 40% of the dissimilarity to the non-treated control in 1 and 2 YAT, respectively (Supplemental file). In the imazapic alone treatment, the greatest increases to abundance were to *A. psilostachya*, western poison ivy [*Toxicodendron rydbergii* (Small ex Rydb.) Greene], and *H. villosa*, accounting for almost 40% of the dissimilarity to the non-treated control (Supplemental file). Indaziflam (44 g ai ha^{-1}) had the greatest increases to western ragweed, horned spurge (*Euphorbia brachycera* Engelm.), Nuttall's violet (*Viola nuttallii* Pursh), and sidebells penstemon (*Penstemon secundiflorus* Benth.), accounting for almost 45% of the dissimilarity (Supplemental file). Additional species contributing to the dissimilarity to the non-treated control are available in the supplemental file. Reducing *B. tectorum* abundance can lead to increases in perennial grass and forb abundance as the competition for resources is removed (Monaco et al. 2017; Sebastian et al. 2016; Sebastian et al. 2017a; Thill et al. 1984; Whitson and Koch 1998), therefore, the increases in species abundance in Site 2 in the indaziflam and imazapic treatments are likely due to the reduction in *B. tectorum* cover.

The impacts to the native plant community differed between the two sites, although some responses to treatments were the same. At Site 1 no treatments resulted in increased native forb or shrub abundance compared to the non-treated control, while broadleaf herbicides increased C3 grass cover. At the same site, both broadleaf herbicides (picloram and aminocyclopyrachlor) reduced native forb and shrub abundance, while the annual grass herbicides (indaziflam and imazapic) had no impact to the overall community composition. The shift to a more C3 grass dominated community in the plots treated with broadleaf herbicides is likely due to the reduction in forb and shrub abundance (Arnold and Santelmann 1966; Greet et al. 2016). At Site 2, which was characterized by 60-70% *B. tectorum* cover, only picloram decreased species abundance, while increases in species abundance 1 YAT were observed among a few treatments that reduced *B. tectorum* cover. Decreases in species richness were also observed from one picloram treatment at this site as well. These findings support extensive research showing decreases in native forb abundance from picloram applications and more recent work showing transient forb decreases from aminocyclopyrachlor applications (Arnold and Santelmann 1966; Carter and Lym 2018; Greet et al. 2016; Ortega and Pearson 2011; Thilmony and Lym 2017; Wagner and Nelson 2014).

Much of the research examining herbicide impacts on native species abundance is compounded by noxious weed competition at the site (Arnold and Santelmann 1966; Beran et al. 1999; Carter and Lym 2018; Davies and Sheley 2011; Elseroad and Rdd 2011). This can make separating herbicide impacts from invasion impacts difficult. In sites dominated by invasive weeds, especially long-term invasions, the diversity of the native plant community has already been compromised, while in non-invaded, intact plant communities, there is a higher potential for loss as native species have not been impacted by non-native invaders (Davies and Sheley 2011; Duncan et al. 2004). In a study conducted by Ortega and Pearson (2011), the authors presented an impact gradient for picloram which coincided with spotted knapweed (*Centaurea* *stoebe* L.) invasion levels. Their study found that native forb cover declined >20% in treated plots versus control plots at non-invaded sites, while impacts to forb cover were minimal in sites with moderate to high *C. stoebe* invasion levels. The authors concluded that differences in picloram effects on native species was due to the strength of release effects from the invasive species, as the increased diversity in sites void of *C. stoebe* had more loss potential than sites already suffering the effects of invasion. This offers a possible explanation for why decreased native species abundances were observed from aminocyclopyrachlor treatments in the site with a more intact native plant community versus the site dominated by *B. tectorum*.

To date, the two published field studies showing indaziflam treatments resulting in long-term *B. tectorum* control reported no observable negative impacts to native species (Sebastian et al. 2016; Sebastian et al. 2017a). *Bromus tectorum* control with indaziflam at 58 g ai ha^{-1} lasted 3 yr with no injury to crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] and western wheatgrass [*Pascopyrum smithii (*Rydb.) A. Love] or impacts to forb species richness (Sebastian et al. 2016). Another study by Sebastian et al. (2017a) reported 2 yr of *B. tectorum* control from indaziflam (44, 73, 102 g ai ha^{-1}), with increased perennial grass and forb biomass and no impact to forb species richness. Our data corroborates previous findings of native species tolerance to indaziflam applications, while also showing that the community composition and abundance of native species is not impacted. The literature on impacts to perennial species abundance with imazapic applications is more diverse and past findings have been variable, showing no impact to species abundance or impacts to specific perennial species, especially in areas with low annual precipitation or during periods of drought (Beran et al. 1999; Kyser et al. 2007; Monaco et al. 2005; Morris et al. 2009; Shinn and Thill 2002). Our study found no evidence of decreases in species abundance with imazapic applications. Our findings from Site 2 are consistent with previous research showing multi-year *B. tectorum* control with indaziflam applications (Sebastian et al. 2016; Sebastian et al. 2017a) and variability in control with imazapic applications (Davies and Sheley 2011; Davison and Smith 2007; Elseroad and Rudd 2011).

One important aspect land managers must take into account when considering the results from this study and developing large scale weed management plans is interannual variability in plant community composition. Although *B. tectorum* was initially a target species for control in this study, the cover at one site decreased to a negligible level (<5% cover) the year after treatments were applied. *Bromus tectorum* invasions levels can decrease during periods of drought and return with increased fall and winter moisture (Mack and Pyke 1983). Climatic variation can also impact weed control and injury to native species from herbicide treatments (Evans et al. 1969; Sebastian et al. 2017c).

Indaziflam is an effective tool for multi-year *B. tectorum* control (Sebastian et al. 20176; Sebastian et al. 2017a) and our results suggest that this herbicide can be used in non-crop sites without impact to native perennial species. Land managers should consider impacts to the plant community when using broadleaf herbicides in these sites, as there is a potential to decrease forb and shrub abundance and shift to a more grass dominated ecosystem. Integrating indaziflam into current management programs could provide the length of *B. tectorum* control needed to deplete the invasive annual grass seed bank and release the remnant plant community (Chambers et al. 2014; Elseroad and Rudd 2011; Sebastian et al. 2017b). Re-establishing the dominant native perennial plant community further increases the resistance and resilience of that ecosystem to future *B. tectorum* invasions, and decreases fine fuels from invasive annual grass that are associated with wildfires (Chambers et al. 2014). Future studies should evaluate the length of *B.*

tectorum control and native species tolerance across varying climates and soil types, specifically in more arid regions such as the Great Basin.

MANAGEMENT IMPLICATIONS

Rangeland weeds cause severe ecological impacts, including depleting soil moisture and nutrients, reducing plant diversity and community productivity, altering fire frequency, and reducing recreational land values. Several herbicides approved for use in natural areas and rangeland can negatively affect native species, while the duration of weed control can be highly variable. Long-term weed control is critical in allowing sufficient time for native species recovery, therefore, herbicide options are needed that provide multi-year control without impacting the native plant community. Indaziflam, a newer herbicide option for pre-emergent invasive winter annual grass management, can provide control for 3 or more yr, although there has been limited research on its effects to native species. A field study was conducted to evaluate changes in the native plant community composition from two annual grass herbicides, imazapic and indaziflam, as well as changes from two broadleaf herbicides, picloram and aminocyclopyrachlor, in diverse native perennial grass and forb communities. The study evaluated species richness and species abundance in the plant community for 2 yr. Picloram decreased native species abundance throughout the duration of the study across both sites, while aminocyclopyrachlor decreased species abundance at one site. Imazapic and indaziflam did not decrease species abundance or richness at either site over 2 yr. The results presented here suggest that indaziflam is an option for land managers to control winter annual grasses without negatively impacting existing native perennial species. In sites with a remnant native plant community, the multi-year winter annual grass control provided by indaziflam may allow enough time to achieve native species recovery.

Table 1.1: List of species occurring at Site 1 and Site 2 with their nativity status.

Scientific Name	Common Name[a]	Site 1	Site 2	Nativity
Allium textile	Prairie onion	X	X	Native
Alyssum simplex	Annual alyssum	X	X	Non-native
Ambrosia psilostachya var. coronopifolia	Western ragweed	X	X	Native
Andropogon gerardii	Big bluestem	X	X	Native
Aristida purpurea	Purple threeawn	X	X	Native
Artemisia campestris	Field sagewort	X	X	Native
Artemisia frigida	Fringed sagebrush	X		Native
Artemisia ludoviciana	White sagebrush	X	X	Native
Astragalus shortianus	Short's milkvetch	X	X	Native
Bouteloua gracilis	Blue grama	X	X	Native
Bromus tectorum	Downy brome	X	X	Non-native
Castilleja integra	Wholeleaf Indian paintbrush		X	Native
Cerastium arvense L.	Field chickweed		X	Native
Cirsium undulatum	Wavyleaf thistle	X		Native
Cryptantha virgata	Miner's candle	X		Native
Dalea purpurea	Purple prairie clover	X		Native
Delphinium carolinianum	Carolina larkspur	X		Native
Descurainia pinnata	Western tansymustard		X	Native
Erigeron flagellaris	Trailing fleabane	X	X	Native
Eriogonum alatum	Winged buckwheat	X		Native
Eriogonum umbellatum	Sulfur-flower buckwheat	X	X	Native
Erodium cicutarium	Redstem filaree	X		Non-native
Euphorbia brachycera	Horned spurge		X	Native
Gaillardia aristata	Blanketflower	X		Native
Helianthus pumilus	Little sunflower	X	X	Native
Hesperostipa comata	Needle and thread	X	X	Native
Heterotheca villosa	Hairy false goldenaster	X	X	Native
Iris missouriensis	Rocky Mountain Iris	X	X	Native
Koeleria macrantha	Praire junegrass		X	Native
Lappula occidentalis	Flatspine stickseed	X		Native
Lesquerella ludoviciana	Foothill bladderpod	X		Native
Leucocrinum montanum	Common starlily	X	X	Native
Liatris punctata	Dotted blazing star	X	X	Native
Linaria dalmatica	Dalmatian toadflax	X	X	Non-native
Lomatium orientale	Northern Idaho biscuitroot	X	X	Native
Noccaea fendleri	Fendler's pennycress		X	Native
Oenothera suffrutescens	Scarlet beeblossom		X	Native

Opuntia polyacantha	Plains pricklypear	X	X	Native
Oxytropis sericea	White locoweed	X		Native
Pascopyrum smithii	Western wheatgrass		X	Native
Penstemon secundiflorus	Sidebells penstemon	X	X	Native
Penstemon virens	Front Range beardtongue		X	Native
Poa compressa	Canada bluegrass		X	Non-native
Pseudocymopterus montanus	Alpine false springparsley		X	Native
Psoralidium tenuiflorum	Slimflower scurfpea	X	X	Native
Ratibida columnifera	Upright prairie coneflower		X	Native
Rosa woodsii	Woods' rose		X	Native
Schizachyrium scoparium	Little bluestem	X		Native
Senecio spartioides	Broom-like ragwort	X	X	Native
Toxicodendron rydbergii	Western poison ivy		X	Native
Tragopogon dubias	Yellow salsify	X	X	Non-native
Verbascum thapsus	Common mullein		X	Non-native
Viola nuttallii	Nuttall's violet	X	X	Native

[a] Common names based on United States Department of Agriculture PLANTS database https://plants.sc.egov.usda.gov/.

Table 1.2: Herbicides and rates applied in evaluating *Bromus tectorum* control and native species tolerance.

Common name	Rates applied[a] g ai ha^{-1}	Manufacturer
Picloram	561	Dow AgroSciences, Indianapolis, IN
Aminocyclopyrachlor	57	Bayer CropScience, Research Triangle Park, NC
Imazapic	105	BASF Specialty Products, Research Triangle Park, NC
Indaziflam	44	Bayer CropScience, Research Triangle Park, NC
Indaziflam	73	Bayer CropScience, Research Triangle Park, NC
Indaziflam	102	Bayer CropScience, Research Triangle Park, NC
Aminocyclopyrachlor	57 + 102	Bayer CropScience, Research Triangle Park, NC
+ indaziflam		Bayer CropScience, Research Triangle Park, NC
Aminocyclopyrachlor	57 + 105	Bayer CropScience, Research Triangle Park, NC
+ imazapic		BASF Specialty Products, Research Triangle Park, NC
Picloram	561 + 102	Dow AgroSciences, Indianapolis, IN
+ indaziflam		Bayer CropScience, Research Triangle Park, NC
Picloram	561 + 105	Dow AgroSciences, Indianapolis, IN
+ imazapic		BASF Specialty Products, Research Triangle Park, NC

[a] All treatments included 0.25% v/v non-ionic surfactant.

Table 1.3: Mean percentage cover of perennial cool season (C_3) grasses at both sites 1 and 2 years after treatment (YAT). Means followed by the same letter do not differ significantly within year at $P < 0.05$.

	Perennial C_3 grass cover			
	Site 1		Site 2	
	1 YAT	2 YAT	1 YAT	2 YAT
	--------------------%--------------------			
Non-treated control	27 a	43a	37 a	34 a
Picloram	55 bcd	65 ab	41 a	34 a
Aminocyclopyrachlor	68 cd	65 ab	32 a	49 a
Imazapic	39 ab	45 ab	44 a	34 a
Indaziflam 44	46 abc	50 ab	32 a	62 a
Indaziflam 73	42 abc	53 ab	28 a	51 a
Indaziflam 102	37 ab	54 ab	38 a	65 a
Aminocyclopyrachlor + indaziflam	52 a-d	56 ab	42 a	52 a
Aminocyclopyrachlor + imazapic	56 bcd	63 ab	39 a	53 a
Picloram + indaziflam	68 cd	70 b	45 a	50 a
Picloram + imazapic	74 d	69 ab	33 a	42 a

Table 1.4: Pairwise comparisons of herbicide treatments versus non-treated control for native forb abundance from PERMANOVA analysis.

Treatment versus non-treated control	Site 1		Site 2	
	2016	2017	2016	2017
	P-value[a]		P-value[a]	
Picloram	0.017*	0.019*	0.01*	0.015*
Aminocyclopyrachlor	0.004*	0.006*	0.358	0.146
Imazapic	0.367	0.35	0.018⁺	0.776
Indaziflam 44	0.581	0.557	0.045⁺	0.177
Indaziflam 73	0.552	0.407	0.199	0.676
Indaziflam 102	0.453	0.162	0.324	0.2
Aminocyclopyrachlor + indaziflam	0.015*	0.032*	0.456	0.071
Aminocyclopyrachlor + imazapic	0.008*	0.012*	0.085	0.24
Picloram + indaziflam	0.026*	0.014*	<0.001*	0.037*
Picloram + imazapic	0.012*	0.028*	0.033*	0.028*

[a] P-values marked with asterisks are considered significant abundance reductions at the <0.05 level. P-values marked with plus signs are considered significant abundance increases at the <0.05 level.

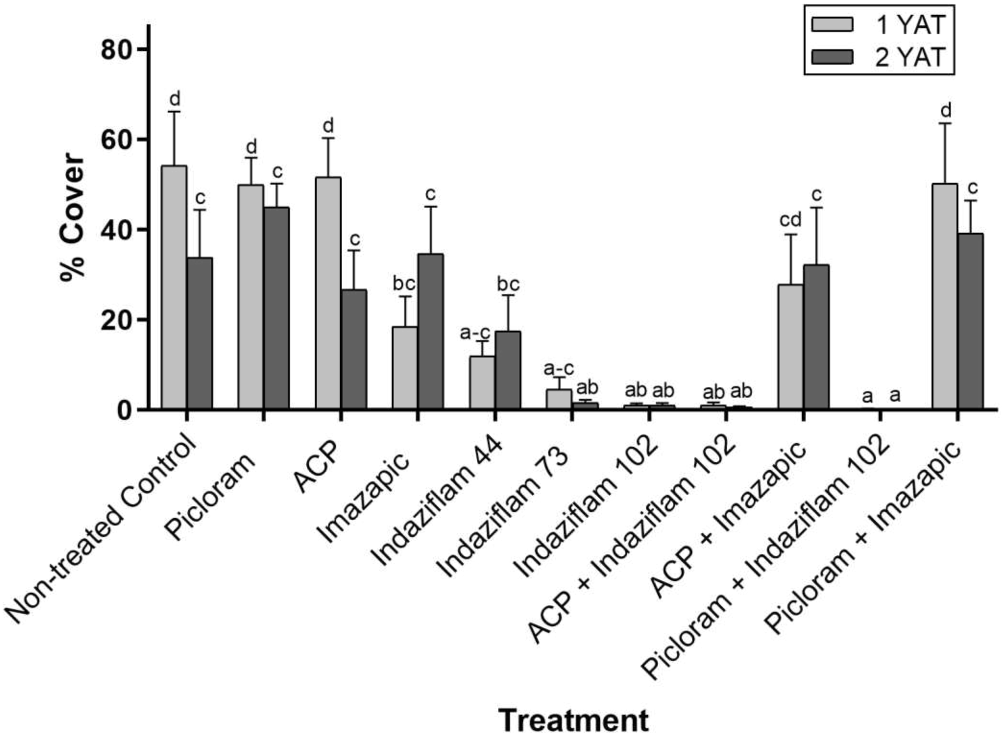

Figure 1.1: Percentage *Bromus tectorum* cover at Site 2, 1 year after treatment (YAT) (2016) and 2 years after treatments (YAT) (2017). Letters indicate significant differences among herbicide treatments across years, using least-squares means ($P < 0.05$). Herbicide treatment rates are as follows: picloram (561 g ai ha^{-1}), aminocyclopyrachlor (ACP, 57 g ai ha^{-1}), imazapic (105 g ai ha^{-1}), indaziflam (44, 73 and 102 g ai ha^{-1}), and non-treated control.

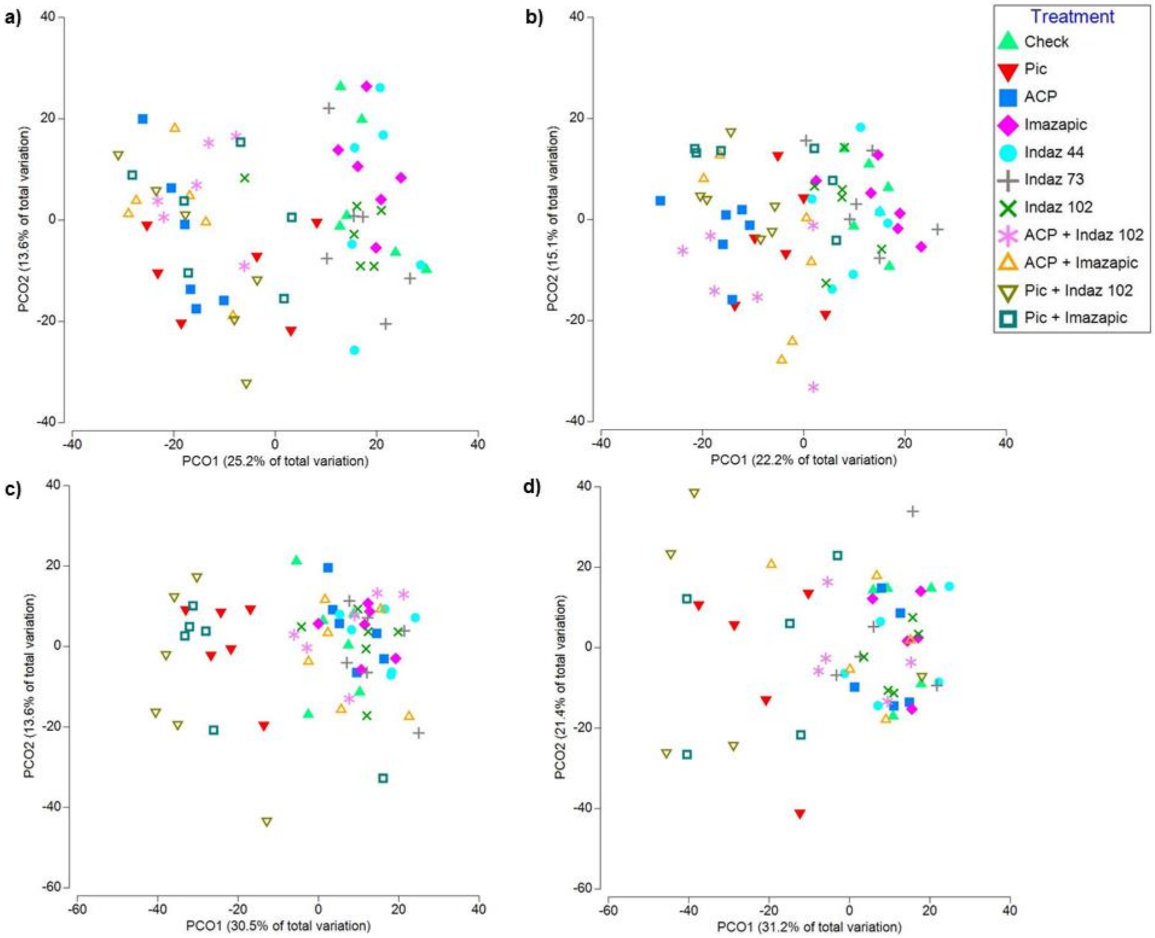

Figure 1.2: Principal coordinate analysis (PCoA) of native forb and shrub species abundance separated by treatment. Treatments farther away from the non-treated (check- represented by the green triangle) had more dissimilarities in community composition. The analysis was based on the Bray–Curtis dissimilarity matrix constructed using the square-root-transformed species counts from Site 1 in 2016 (a) and 2017 (b); Site 2 in 2016 (c) and 2017 (d). The percent of variation explained is given in brackets on the x- and y-axes. ACP, aminocyclopyrachlor; Indaz, indaziflam; Pic, picloram.

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Chapter 2: Evaluating Winter Annual Grass Control and Native Species Establishment Following Applications of Indaziflam on Rangeland

INTRODUCTION

Invasive winter annual grasses (IWAGs), especially downy brome (*Bromus tectorum* L.), are a significant threat to rangeland ecosystems in the western US (Monaco et al. 2017; Knapp 1996; Mack 1981). Winter annual grasses are able to dominate native perennial systems in arid and semi-arid western climates due to their opportunistic life cycle, prolific seed production, and ability to deplete early season soil moisture (Mack and Pyke 1983). Unchecked infestations can severely impact ecosystem services by decreasing habitat, native plant diversity, and forage production, while increasing fire frequency due to the buildup of fine fuels (Monaco et al. 2017; Abatzoglou and Kolden 2011; Weltz et al. 2011; Ditomaso et al. 2010).

In the past few decades, increased expansion of newer and less widespread annual grass invaders have become a greater concern for land managers (Davies and Johnson 2011; Duncan et al. 2004). Feral rye (*Secale cereale* L.), an IWAG that derived from the cultivated cereal rye, is an aggressive weed in cereal crops (Burger et al. 2006). More recently, feral rye has started to spread into and become very problematic on non-crop sites (Roerig and Ransom 2017; Ellstrand et al. 2010). A study by Roerig and Ransom (2017) showed landscape-scale expansion rates of feral rye as high as 50% in one year on natural area sites in Utah.

Remnant native plant communities still persist in much of the rangeland and natural areas invaded by IWAGs (Belnap et al. 2012; Hobbs et al. 2006; DiTomaso 2000). Sites with a robust native plant component are easier to restore than highly degraded sites lacking native species because timely weed control allows for native species recovery and resistance from future invasion (Sebastian et al. 2017a; Chambers et al. 2014; DiTomaso 2000). Several studies have shown that native plant communities respond positively to IWAG control, including increases in perennial grass and forb biomass as well as species richness (Sebastian et al. 2017a; Sebastian et al. 2016b; Ditomaso et al. 2010). In invaded sites where the native plant community is highly degraded or nonexistent, more intensive management is required (Fowers 2015; Evans and Young 1977). For example, the native perennial seedbank has been severely diminished in large areas of the shrub-steppe communities of the Great Basin, which have been plagued by dense infestations of IWAGs and frequent fire cycles (Chambers et al. 2007; Humphrey and Schupp 2001). In areas such as the Great Basin, weed management along with re-vegetation is required in order to re-establish native species and prevent reinvasion (Davies and Boyd 2018; Mangold and Parkinson 2015; Wilson et al. 2010). Re-vegetation is not only very costly and labor intensive, but often fails in western climates where moisture events are scarce and unpredictable (Mangold and Parkinson 2015; Young 2000; Ethridge et al. 1997). Herbicides used in the restoration process can also negatively impact desirable seeded species, preventing successful establishment (McManamen et al. 2018; Lym et al. 2017; Sbatella et al. 2011; Morris et al. 2009; Shinn and Thill 2004).

Herbicides currently used for IWAG control and site restoration provide adequate short-term weed control; however, most herbicide options do not provide the long-term control needed for native species establishment (Kelley et al. 2013; Kyser et al. 2013; Mangold et al. 2013; Shinn and Thill 2004; Whitson and Koch 1998). Furthermore, very few herbicides for use on rangeland or natural areas provide feral rye control. Glyphosate can be used during native species dormancy to provide POST control of overwintering winter annual grass seedlings or for broad-spectrum weed control before re-vegetation on sites without desirable species (Morris et al. 2016; Kyser et al. 2013; Kyser et al. 2012). Although this can be effective for short-term control, glyphosate has no soil residual and does not provide protection against re-establishment of invasive grasses (Sebastian et al. 2017b). Winter annuals are very opportunistic, with the ability to germinate whenever the growing conditions are favorable (Thill et al. 1984); therefore, providing soil residual control is critical to depleting the annual grass seedbank and achieving long-term restoration (Sebastian et al. 2017b).

Indaziflam, a new herbicide option for weed management on natural areas and rangeland, provides long-term downy brome and feral rye control (Sebastian et al. 2017a; Sebastian et al. 2016b). As a cellulose biosynthesis inhibitor, indaziflam stops root growth in newly germinated seedlings by preventing cellulose formation (Tateno et al. 2016; Brabham et al. 2014). Indaziflam is a long-residual, PRE herbicide that is effective in controlling both grass and broadleaf seedlings, although it is more active on grasses (Sebastian et al. 2017a; Sebastian et al. 2016a; Brabham et al. 2014). Low application rates (44 to 102 g·ai·ha-1) and perennial native species tolerance makes this an ideal herbicide for rangeland restoration (Sebastian et al. 2017a; Sebastian et al. 2016a; Sebastian et al. 2016b). Additionally, indaziflam can be combined with glyphosate when desirable perennials are dormant to achieve POST control of winter annual grass seedlings plus PRE control to prevent reinvasion from the soil seed bank. Several studies have been conducted evaluating IWAG control and native species tolerance with indaziflam applications (Sebastian et al. 2017a; Sebastian et al. 2016a; Sebastian et al. 2016b), but currently there is no published research using indaziflam in combination with seeding of native species. Therefore, the objective of this study was to evaluate long-term downy brome and feral rye control with indaziflam applications and subsequent establishment of drill-seeded grasses, forbs and shrubs.

MATERIALS AND METHODS

Site Description

The experiment was established in 2014 at two sites in Larimer County, CO; Site 1 was infested by a downy brome monoculture (100% canopy cover), and Site 2 was infested by a feral rye monoculture (100% canopy cover). In 2015 Site 3 was established in Boulder County, CO; this site consisted of a dense (~ 80% canopy cover) downy brome and Japanese brome infestation with a remnant native species understory. Site 1 (latitude 40° 42' 48" N, longitude 104° 57' 4" W) was located on Colorado Parks and Wildlife's Wellington State Wildlife Area; Site 2 (latitude 40° 43' 33" N, longitude 104° 56' 59" W) was also located on Colorado Parks and Wildlife's Wellington State Wildlife Area; and Site 3 (latitude 40° 15' 1" N, longitude 105° 12' 58" W) was located on Boulder County Parks and Open Space's Ron Stewart Preserve at Rabbit Mountain. Sites 1 and 2 were approximately 1.5 km apart and 56 km from Site 3.

The soil at Site 1 was Satanta loam (Fine-loamy, mixed, superactive, mesic Aridic Argiustolls), with 3.3% organic matter and 7.6 pH in the top 20 cm. The site was level with an average elevation of 1607 m (5271 ft). The soil at Site 2 was Nunn clay loam (fine, smectitic, mesic Aridic Argiustolls), with 1.6% organic matter and 7.5 pH in the top 20 cm. This site was also level with an average elevation of 1609 m (5278 ft). The soil at Site 3 was Baller stony sandy loam (loamy-skeletal, mixed, superactive, mesic Lithic Haplustolls), with 4.9% organic matter and 6.6 pH in the top 20 cm. The site had an approximate 9° slop with an average elevation of 1737 m (5699 ft) (U.S. Department of Agriculture, Natural Resources Conservation Service 2014).

Mean annual precipitation based on the 30-yr average (1981-2010) was 361 mm at Sites 1 and 2, and 379 mm at site 3 based on the closest weather station (approximately 14 km from

Sites 1 and 2 and 16 km form Site 3) (Western Regional Climate Center 2018). In 2014, the year Sites 1 and 2 were established, both sites received a slight increase of 49 mm precipitation above their 30-yr average. The following year (2015) when site 3 was established was a wetter year along the Colorado Front Range, with sites 1 and 2 receiving an additional 212 mm of rainfall and site 3 receiving an additional 199 mm of rainfall above the 30-yr average (Western Regional Climate Center 2018). A statewide drought occurred in 2016 with total precipitation for sites 1 and 2 decreasing to 78 mm below the 30-yr average and site 3 decreasing 144 mm below the 30-yr average (Western Regional Climate Center 2018). In 2017, the sites received precipitation close to the 30-year average. During 2014-2017, the average temperature for sites 1 and 2 was just slightly above (~0.4°C higher) the 30-year average of 8.7°C and average temperatures at site 3 were near the 30-yr average of 9.1°C (Western Regional Climate Center 2018).

Experimental Design

Herbicide applications were made on 22 March 2014 at Sites 1 and 2 and 7 April 2015 at Site 3. All winter annual grasses were actively growing when the herbicide applications were made. Downy brome was 8 to 10 cm tall with 3 to 5 tillers at Site 1 and feral rye was 13 to 18 cm tall with 1 to 4 tillers at Site 2. Both downy brome and Japanese brome were actively growing and were 4 to 8 cm tall with 1 to 5 tillers when herbicide applications were made at Site 3. There were twelve herbicide treatments and one non-treated. Herbicide treatments were applied to 3 by 9 m plots arranged in a randomized complete block design with four replications (Table 2.1). All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat-fan nozzles at 187 L ha^{-1} at 207 kPa.

In December 2014, Sites 1 and 2 were dill seeded using a no-till rangeland grass drill (Flex II, Truax Company, Inc, Minneapolis, MN) with a variety of native species, consisting of cool and warm season grasses, forbs and shrubs at National Resources Conservation Service (NRCS) recommended seeding rates (Table 2.2). Grasses, forbs, and shrubs were seeded in two individual rows per species perpendicular to the herbicide treatments. A native prairie forb mix which included 19 forb species was used, although only nine species established and were included in the analyses. On 22 February 2016 at Sites 1 and 2, herbicide applications were reapplied to half of each plot creating a split-plot design to evaluate differences in long-term control and plant establishment with one versus two herbicide applications. All treatments that initially contained indaziflam received a reapplication of indaziflam (102 g ai ha^{-1}) plus glyphosate (420 g ae ha-1) while the other treatments received a reapplication of the original treatment. At the second herbicide application timing, downy brome was actively growing and was 3 to 8 cm tall at the 1 to 2 tiller stage, while feral rye was 5 to 10 cm at the 1 to 3 tiller stage. All treatments were applied with the same equipment used for the first application.

Treatment Evaluations and Data Analysis

Downy brome and feral rye biomass were harvested from 2014 to 2017 at Sites 1 and 2 using randomly placed 1 m^2 quadrats in each plot; quadrats were not placed in the same location in subsequent years. In 2016 and 2017, after the second herbicide application was made to half of each plot, two biomass collections were made, one subsample from the side that received one herbicide application and one subsample from the side that received a second herbicide application. Drilled species establishment at Sites 1 and 2 was determined by taking frequency counts using a meter stick separated into ten, 10 cm segments. Frequency was taken for each drilled species individually. An occurrence of a species in a 10 cm segment counted as one, therefore counts ranged from 0 to 10. Counts were then directly converted into percent frequency by multiplying by 10. Three frequency counts were taken for each plot to determine an average percent frequency over the entire 3 m wide plot and two drilled rows. In 2016 and 2017, frequency counts were conducted the same way although two frequency measurements were collected in each sub-plot (one or two herbicide applications) to adhere with the split-plot design.

At Site 3 from 2015 to 2018, brome, perennial grass, and forb biomass were harvested using the same quadrat collection method used in Sites 1 and 2. A second herbicide application was not made at this site so only one biomass sample per plot was collected during those years. The two brome species were not separated so total biomass of both species together was recorded. Species richness was determined at Site 3 by counting the number of species occurring in each plot.

Nonlinear regression using the ‘drc’ package in R version 3.4.3 was used to determine glyphosate rates required to reduce plant dry biomass by 50% (GR_{50}) for downy brome and feral rye (R Core Team 2017). The herbicide concentrations resulting in 50% reduction in plant biomass (GR_{50}) were determined for feral rye and downy brome using four-parameter log-logistic regression. The equation used to regress herbicide concentration with percent reduction in plant dry biomass was:

$$Y = c + \frac{(d - c)}{1 + 10^{(LogGR_{50} - X)b}}$$

where c is the lower response limit, d is the upper response limit, b is the slope of the curve, and GR_{50} is the herbicide dose resulting in 50% reduction in response (biomass). For curve fitting and GR_{50} estimation, the lower limit of the model was constrained to 0. An F test of the curves for both species was conducted to determine if the difference between the GR_{50} values was statistically significant at the 5% level of probability.

To test the effect of herbicide treatment on IWAG biomass, a linear mixed-effects model was created using the ‘lme4’ package in R version 3.4.3, testing for treatment effects at $\alpha = 0.05$

(R Core Team 2017). Site and year were not included in the model and were analyzed separately due to a large variability in biomass across years from environmental factors as well as to increase data normality. For Sites 1 and 2 (the first two years) and Site 3 (all years), the fixed factor included in the model was treatment while block was included as a random factor. For Sites 1 and 2, the split-plot design was then considered in years 3 and 4 in the linear mixed-effects model with the fixed factors being treatment, number of herbicide applications, and their interaction, while block was included as a random factor. At all three sites, several treatments had zero brome and feral rye biomass which created problems with data normality; therefore, all treatments with a mean of zero were excluded from the model. Confidence intervals were then estimated for all non-zero treatment means. Further analyses of treatment effect were performed using the ‘emmeans’ package in R (R Core Team, 2017) to obtain comparisons between all pairs of least squares means for each year with a Tukey adjustment ($P < 0.05$). Treatments with a mean of zero were then grouped with treatments where the confidence interval included zero in order to have a full set of treatment comparisons. Any treatment with a confidence interval not containing zero was considered significantly different from the treatments where the mean was zero.

Drilled species were grouped into cool season grasses (C_3), warm season grasses (C_4), and forbs/shrubs for analysis. Site and year were again analyzed separately. Drilled species frequencies were square root ($n + 0.5$) transformed when needed to meet assumptions of normality. Frequency data were subjected to the same linear mixed-effects model and post-hoc analysis used to evaluate winter annual grass biomass data. Again, treatments with an average frequency of 0 were dropped from the model and included with treatments whose confidence interval included zero for post-hoc analysis.

At Site 3, to analyze treatment effects on grass and forb biomass a linear mixed-effects model was created with treatment, year and their interaction as fixed factors. Block was included as the random factor. We failed to reject the hypothesis that count data of species richness were from a Poisson distribution ($P = 0.9523$), therefore, species richness was analyzed using a generalized linear mixed model with a Poisson distribution using the same factors as the grass and forb data. Any significant treatment, year or interaction effects were determined post-hoc using pairwise comparison of least-squares means test with Fisher's Protected LSD ($P < 0.05$) ('lme4' and 'emmeans' packages, R Core Team 2017).

RESULTS AND DISCUSSION

Glyphosate Dose Response

Downy brome (Site 1) was controlled at a much lower glyphosate rate than feral rye (Site 2) (Figure 2.1). The GR_{50} value was approximately three times greater for feral rye (GR_{50} = 126.0 g ae ha^{-1}) compared to downy brome (GR_{50} = 40.4 g ae ha^{-1}). A comparison between the two GR_{50} values was highly significant ($P < 0.001$); however, a GR_{50} value could not be calculated for Site 3 (downy/Japanese brome with native species understory) because the lowest glyphosate rate reduced the biomass more than 50% (data not shown).

Invasive Winter Annual Grass Response in Highly Degraded Sites

Year to year IWAG biomass was inconsistent due to variable precipitation; therefore, year and site were analyzed separately. Treatment was highly significant ($p < 0.001$) for Sites 1 and 2. During the initial season after application (2014), only the treatments that included glyphosate reduced both downy brome and feral rye biomass, as the herbicide applications were made POST while both annual grasses were actively growing (Figure 2.2). By 1 YAT (2015), only treatments containing indaziflam provided reductions in downy brome and feral rye biomass, even when glyphosate was not included in the initial application. All other treatments were no longer providing biomass reductions (Figure 2.2).

In the season directly following the second herbicide application and two years after initial application (2016), the treatment by application number interaction was highly significant at Sites 1 and 2 ($P < 0.001$). Both one and two applications of indaziflam performed similarly in providing downy brome and feral rye biomass reductions, except for indaziflam (73 g ai ha^{-1}) plus glyphosate (140 g ae ha^{-1}) in both sites and indaziflam at the lowest rate (44 g ai ha^{-1}) in the feral rye site (Site 2), in which a second application was needed (Figure 2.3). For treatments that did not contain indaziflam, one herbicide application was not sufficient to control IWAGs two years later (Figure 2.3). At both Sites 1 and 2, the second imazapic application with glyphosate did provide better IWAG biomass reduction compared to one application, while a second application of glyphosate alone was not sufficient (Figure 2.3).

One year after herbicide reapplication and three years after initial application (2017), the treatment by application number interaction was still highly significant at both sites ($P < 0.001$). In the downy brome site (Site 1), there were no differences between one and two applications for treatments containing indaziflam (Figure 2.3). Both one and two applications continued to reduce downy brome biomass compared to the non-treated check. There was no longer a difference between one and two imazapic applications and all treatments without indaziflam had downy brome biomass comparable to the non-treated (Figure 2.3). In the feral rye site (Site 2), the lowest indaziflam rates applied without glyphosate (44 and 73 g ai ha^{-1}) and the indaziflam (73 g ai ha^{-1}) plus glyphosate (280 g ae ha^{-1}) treatments had differences in biomass with one compared to two herbicide applications, although all treatments including indaziflam (one or two applications) were still providing reductions in feral rye biomass compared to the non-treated

(Figure 2.3). Treatments that did not include indaziflam were no longer providing any reductions in feral rye biomass with one or two applications (Figure 2.3).

Native Species Establishment in Highly Degraded Sites

Species frequency was assessed by functional group: C_3 grasses, C_4 grasses and forbs/shrubs. In the year following drill seeding (2015), treatment was highly significant for all functional groups in both sites ($P < 0.001$). In the downy brome site (Site 1), there was significant C_3 grass establishment in all indaziflam treatments, except for indaziflam at 102 g ai ha^{-1}, and the imazapic alone treatment compared to the non-treated, although the indaziflam treatments had an average C_3 grass frequency of 36 ± 4.8% (mean±SE) compared to imazapic with an average frequency of 11 ± 4.1% (Table 2.3). Only treatments which included indaziflam had significant C_4 grass and forb/shrub establishment (Table 2.3). Overall establishment was lower at the feral rye site (Site 2), although drilled species in all functional groups only established in treatments that included indaziflam (Table 2.4).

After the second herbicide application was made in half of each plot, the treatment by application number interaction was analyzed for significance. Application number and the treatment by application number interaction were not significant for all functional groups of drilled species at both sites over the two years. Treatment was highly significant ($P < 0.001$) for all functional groups at both sites during both years, so establishment for each group was averaged over application number and compared across treatments.

In the downy brome site (Site 1) 2 YAT (2016), all three functional groups had significant establishment in every indaziflam treatment compared to the non-treated (Table 2.3). Both imazapic treatments also had significant C_3 grass establishment (Table 2.3). Three years after initial herbicide treatments, all treatments which included indaziflam continued to have a higher frequency of drilled species compared to the non-treated (Table 2.3). Imazapic treatments also had significant establishment of C_3 grasses 3 YAT, although C_3 frequency averaged 24 ± 5.1% for imazapic treatments and 61 ± 1.7% for indaziflam treatments (Table 2.3).

Russian thistle (*Salsola tragus* L.) invaded the feral rye site (Site 2) 2 YAT in plots where feral rye was controlled, and negatively impacted the continued establishment of the warm season grasses. Over the course of the study, warm season grasses that originally established decreased in frequency. By 3 YAT, less than half of the indaziflam treatments still had a greater frequency of C_4 grasses (Table 2.4). Although there was some successful C_4 grass establishment, the average frequency in indaziflam treatments was only 9% in Site 2 compared to 47% in Site 1 (Tables 2.3 and 2.4). Two and three YAT, all treatments that included indaziflam had better C_3 grass and forb/shrub establishment compared to the non-treated, with the exception of indaziflam at the lowest rate (44 g ai ha^{-1}) (Table 2.4). Overall, C_3 grasses had greater establishment and were less impacted by the Russian thistle invasion in Site 2, as frequency increased from an average of 16 ± 1.4% 1 YAT to 46 ± 2.6% 3 YAT. During the course of the study, there was no establishment of any drilled species in Site 2 among treatments that did not contain indaziflam (Table 2.4).

Invasive Winter Annual Grass Response in Site with Remnant Native Plant Community

At Site 3, an analysis of brome biomass in the non-treated plots showed a difference in biomass across years ($P = 0.0016$), with 2016 having significantly more brome than other years. Due to this variability in brome biomass, each year was analyzed separately to meet normality assumptions. All four years of the study had a highly significant treatment effect ($P < 0.001$). During the growing season following initial herbicide applications, only treatments containing glyphosate reduced brome biomass (Figure 2.4). One YAT, all treatments containing indaziflam and the imazapic with glyphosate treatment had less brome biomass compared to the non-treated. Two and three YAT, only the indaziflam treatments, with the exception of the lowest rate of indaziflam (44 g ai h^{-1}), were providing reductions in brome biomass (Figure 2.4).

Perennial Grass, Forb and Species Richness Response in Site with Remnant Native Plant Community

Year and the interaction of treatment by year were not significant for perennial grass biomass, while treatment was highly significant ($P < 0.001$). Therefore, perennial grass biomass was combined across all years and analyzed by treatment. With the exception of the indaziflam treatments with the highest glyphosate rates (420 and 560 g ae ha^{-1}), treatments containing indaziflam had increases in perennial grass compared to the non-treated. Treatments without indaziflam did not increase perennial grass biomass (Figure 2.5).

The treatment by year interaction was not significant for forb biomass ($P = 0.1017$), although treatment ($P = 0.0345$) and year ($P < 0.001$) were significant, therefore data were analyzed across treatments by year. Throughout the four years of the study, differences in forb biomass were highly variable by year. In the initial year of application (2015) very few differences were seen in forb response, with increased biomass only in treatments of the highest indaziflam rate (102 g ai ha^{-1}) and glyphosate (420 g ae ha^{-1}) alone (Figure A2.1). One YAT, treatments increased forb biomass, with the exception of indaziflam at 44 g ai ha^{-1}, indaziflam plus glyphosate at 70 g ae ha^{-1}, and imazapic alone. Although there were still significant downy brome reductions among indaziflam treatments 2 and 3 YAT, forb biomass differences were no longer observed (Figure A2.1).

Lastly, any impacts to species richness from herbicide treatments were analyzed. Since the treatment by year interaction was not significant ($P = 0.9523$), treatment effects on species richness were analyzed across treatments by year. Increases in species richness were variable throughout the study, most likely influenced by interannual variation in moisture. Increases in species richness were observed in approximately half of the indaziflam treatments the season following treatment, 1 YAT, and 2 YAT, while treatments without indaziflam did not have greater species richness throughout the course of the study (Figure A2.2). In 2017 (2 YAT) there were no differences in species richness observed among treatments, although this was following severe drought conditions in 2016 (Figure A2.2).

Glyphosate can be a critical component in IWAG management systems because it provides non-selective POST control and can be applied alone or in combination with products that provide long-term soil residual control during native species dormancy (Morris et al. 2016). Glyphosate label recommendations of rates to control downy brome and feral rye vary; 315-433 g ae ha^{-1} Roundup Weathermax (Anonymous 2017), 315-420 g ae ha^{-1} Glyphosate 5.4 (Anonymous 2015), and 210-420 g ae ha^{-1} Accord XRTII (Anonymous 2014). The results of our dose response study suggest that higher glyphosate rates are needed to control feral rye compared to downy brome. Feral rye biomass was only reduced to near zero at 560 g ae ha^{-1} of glyphosate, which is more than the highest recommended rate for downy brome control (Figure 2.1). For land managers, this information is critical as the recommended labeled rates for glyphosate may not be enough to control feral rye in highly invaded sites. Therefore, these data suggest that glyphosate rates may need to be altered to fit the target species and invasion level at each site.

Previous research has shown that IWAGs quickly reinvade areas after herbicide application, even when adequate first-year control is attained (Davies and Boyd 2018; Davies 2010; Morris et al. 2009). Sebastian et al. (2017b) looked at the downy brome seedbank longevity and found that at least four years with no additional seed rain were needed to prevent reinvasion from the soil seedbank. That research indicated that many times the reinvasion is occurring from the seedbank at the site and not from new seed moving in from adjacent areas. Our results suggest indaziflam could provide the length of IWAG control needed to deplete the soil seedbank with just one herbicide application. At the termination of this study three YAT, indaziflam (73 g ai ha^{-1} or higher) was still providing significant IWAG control at all three sites with just one application. Interestingly, similar results in long-term control were achieved at sites void of native species (Sites 1 and 2) and a site with a remnant native plant community (Site 3), although revegetation was required in the sites without a remnant community. In some sites, especially with annual grasses that are more difficult to control, such as feral rye, higher rates or two applications may be needed to achieve the goal of depleting the annual grass seedbank.

Results from the current study suggest that indaziflam could play an important role in the restoration process at sites with a remnant native plant community and in highly degraded sites where revegetation will be required. Research has shown that in sites with a remnant native plant community, native species can reestablish from the existing community through persistent IWAG control (Monaco et al. 2017; Sebastian et al. 2017a; Sebastian et al. 2016b). The native plant community responded to indaziflam treatments in our study that provided multi-year reductions in brome biomass, with increases in perennial grass biomass and species richness still evident even 3 YAT. In a comprehensive review by Monaco et al. (2017), an evaluation of perennial grass impacts after IWAG management indicated that increases in perennial grass are often only a short-term response (<2 y) (Monaco et al. 2017). Our results suggest that by providing multi-year IWAG control, increases in perennial grass biomass and native species richness can be achieved on a longer-term or more permanent basis.

Our study was the first to evaluate revegetation using drill-seeding after indaziflam applications. The successful establishment of grasses, forbs and shrubs in the indaziflam treatments demonstrates that drill-seeding after indaziflam applications is a viable option. Successful establishment of native plants depended on treatments that provided more than one year of IWAG control. Seeded species continued to persist throughout the three years of the study, although some functional groups performed better than others. Three years after seeding, there was a higher frequency of C_3 grasses in almost all treatments that continued to provide IWAG control, while C_4 grass establishment did not persist in most treatments at the feral rye site. The forb and shrub populations did persist through the three years of evaluations at both Site 1 and 2, although the overall frequency of these species was not as high as C_3 grass frequency. These results support past research indicating that species selection plays an important role in long-term persistence of seeded plants and in providing competition against reinvasion (Rinella et al. 2012).

Even when initial establishment of desirable species is achieved, revegetation efforts often fail, as weeds reinvade the site and inhibit seeded species from persisting (Rinella et al. 2012). Multi-year control efforts are needed to deplete the IWAG seedbank and allow time for native species recovery or species establishment through revegetation methods (Sebastian et al. 2017b; Chambers et al. 2014; Morris et al. 2009). In highly degraded sites without a remnant native plant community, establishing desirable species in a way that is sustainable requires long-term weed control in order to be successful. These sustainable plant communities could then potentially be resistant to reinvasion or new invasions (Rinella et al. 2012; Davies 2010; Wilson et al. 2010; Morris et al. 2009). Our results demonstrate that indaziflam is a viable tool to achieve the goal of seedbank depletion and restoration of non-crop sites where revegetation is required.

Table 2.1: Herbicides and rates applied in evaluating downy brome (*Bromus tectorum*), feral rye (*Secale cereale*), and Japanese brome (*Bromus japonicus*) control.

Common name	Rates applied[a] g ha^{-1}
Indaziflam[b]	44 ai
Indaziflam[b]	73 ai
Indaziflam[b]	102 ai
Indaziflam[b] + glyphosate	73 ai + 70 ae
Indaziflam[b] + glyphosate	73 ai + 140 ae
Indaziflam[b] + glyphosate	73 ai + 210 ae
Indaziflam[b] + glyphosate	73 ai + 280 ae
Indaziflam[b] + glyphosate	73 ai + 420 ae
Indaziflam[b] + glyphosate	73 ai + 560 ae
Imazapic[c]	122 ai
Imazapic[c] + glyphosate	122 ai + 420 ae
Glyphosate[d]	420 ae

[a] All treatments included 0.25% v/v non-ionic surfactant.

[b] Treatments received a sequential application of indaziflam (102 g ai ha^{-1}) + glyphosate (420 g ae ha^{-1}) on half of each experimental plot two years after original application (February 2016) at Sites 1 and 2.

[c] Treatments received a sequential application of imazapic (122 g ai/ae ha^{-1}) + glyphosate (420 g ha^{-1}) on half of each experimental plot two years after original application (February 2016) at Sites 1 and 2.

[d] Treatment received a sequential application of glyphosate (420 g ae ha^{-1}) on half of each experimental plot two years after original application (February 2016) at Sites 1 and 2.

Table 2.2: List of perennial grass, forb and shrub grass species drill-seeded at two locations following pre-planting herbicide application in March 2014[a].

Scientific name	Common name[b]
Cool season grasses (C_3)	
Elymus elymoides	Squirreltail
Elymus trachycaulus	Slender wheatgrass
Leymus cinereus	Basin wildrye
Nassella viridula	Green needlegrass
Pascopyrum smithii	Western wheatgrass
Warm season grasses (C_4)	
Bouteloua curtipendula	Sideoats grama
Bouteloua gracilis	Blue grama
Schizachyrium scoparium	Little bluestem
Perennial forbs[c]	
Coreopsis tinctoria	Golden tickseed
Dalea purpurea	Purple prairie clover
Gaillardia aristata	Blanketflower
Gaillardia pulchella	Indian blanket
Helianthus annuus	Common sunflower
Helianthus maximiliani	Maximilian sunflower
Machaeranthera tanacetifolia	Tanseyleaf tansyaster
Ratibida columnifera	Upright prairie coneflower
Rudbeckia hirta	Blackeyed Susan
Shrubs	
Artemisia frigida	Prairie sagewort
Krascheninnikovia lanata	Winterfat

[a] Species were seeded in December 2014 at both locations.

[b] Common names based on US Department of Agriculture PLANTS Database: https://plants.usda.gov/.

[c] A native prairie forb mix was used which included 19 forb species. Only the species that established and were part of the frequency counts for data analysis are included in the table.

Table 2.3: Percent frequency of cool season grasses (C_3), warm season grasses (C_4), and forbs/shrubs 1, 2 and 3 years after initial herbicide treatment (YAT) at Site 1. Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).

	1 YAT			2 YAT			3 YAT		
Treatment	C_3 Grass[a]	C_4 Grass[a]	Forb/Shrub[a]	C_3 Grass[a]	C_4 Grass[a]	Forb/Shrub[a]	C_3 Grass[a]	C_4 Grass[a]	Forb/Shrub[a]
	------------------------	------------------------	------------------------	------------------------	%	------------------------	------------------------	------------------------	------------------------
Non-treated	0 a	0 a	0 a	0 a	0 a	0 a	4 ab	0 a	3 ab
Indaz 44	41 def	38 cd	9 bc	53 ef	34 de	31 bc	62 c	40 cde	14 abc
Indaz 73	23 bcd	16 bc	9 bc	44 de	21 cd	28 b	59 c	32 cd	27 cd
Indaz 102	9 abc	13 bc	8 bc	30 bcd	12 bc	34 bcd	37 cd	19 bc	31 cd
Indaz + Glyph 70	38 def	44 d	17 bc	56 ef	36 def	38 bcd	68 e	42 cde	38 cd
Indaz + Glyph 140	31 cde	39 cd	18 c	41 cde	34 def	31 bcd	51 de	44 de	39 cd
Indaz + Glyph 210	36 def	35 cd	15 bc	54 ef	40 def	51 cd	65 e	53 de	50 d
Indaz + Glyph 280	40 def	51 d	16 bc	66 f	57 f	38 bcd	69 e	67 e	49 d
Indaz + Glyph 420	59 f	48 d	15 bc	70 f	53 ef	53 d	69 e	65 e	26 bcd
Indaz + Glyph 560	49 ef	46 d	20 c	60 ef	37 def	31 bcd	66 e	62 de	34 cd
Imaz	11 bc	6 ab	0 a	15 b	4 ab	0 a	26 c	5 ab	0 a
Imaz + Glyph 420	5 ab	3 ab	5 abc	21 bc	6 ab	1 a	23 bc	6 ab	3 ab
Glyph 420	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a

[a]Means followed by the same letter within the column do not differ significantly at the $P < 0.05$ level.

Table 2.4: Percent frequency of cool season grasses (C_3), warm season grasses (C_4), and forbs/shrubs 1, 2 and 3 years after initial herbicide treatment (YAT) at Site 2. Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).

	1 YAT			2 YAT			3 YAT		
Treatment	C_3 Grass[a]	C_4 Grass[a]	Forb/Shrub[a]	C_3 Grass[a]	C_4 Grass[a]	Forb/Shrub[a]	C_3 Grass[a]	C_4 Grass[a]	Forb/Shrub[a]
	------%								------
Non-treated	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Indaz 44	14 b	14 b	5 ab	15 b	7 b	9 ab	18 b	8 ab	10 ab
Indaz 73	17 b	16 b	13 b	31 bc	9 b	25 b	48 c	16 b	18 b
Indaz 102	13 b	9 ab	7 ab	21 b	5 ab	28 b	37 bc	7 ab	18 b
Indaz + Glyph 70	15 b	13 b	13 b	23 b	5 ab	26 b	43 c	5 ab	23 b
Indaz + Glyph 140	15 b	13 b	9 b	27 bc	6 b	18 b	39 bc	5 ab	18 b
Indaz + Glyph 210	18 b	9 ab	8 ab	28 bc	3 ab	17 b	41 bc	5 ab	14 b
Indaz + Glyph 280	14 b	14 b	7 ab	31 bc	6 b	12 b	55 cd	10 b	16 b
Indaz + Glyph 420	14 b	14 b	16 b	29 bc	5 ab	30 b	56 cd	8 ab	27 b
Indaz + Glyph 560	25 b	15 b	8 ab	45 c	9 b	21 b	72 d	16 b	18 b
Imaz	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Imaz + Glyph 420	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Glyph 420	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a

[a]Means followed by the same letter within a column do not differ significantly at the $P < 0.05$ level.

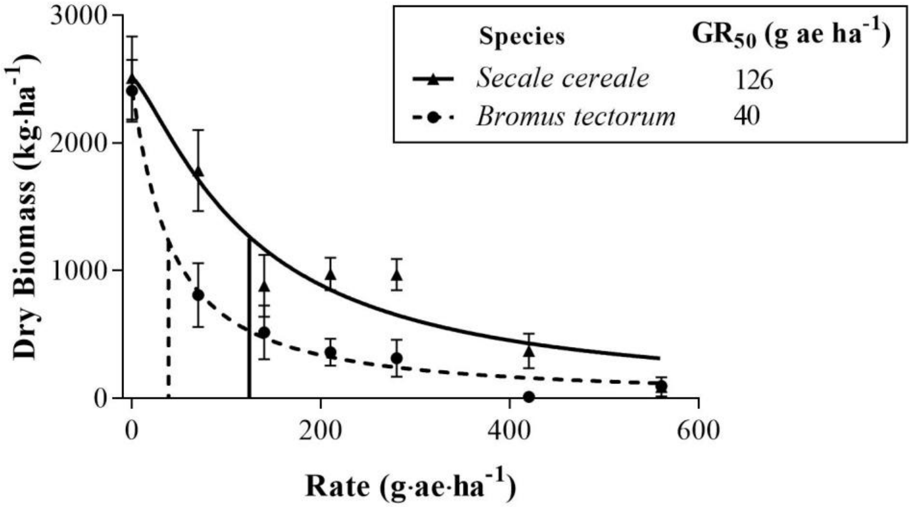


Figure 2.1: Response of feral rye and downy brome to glyphosate. Dose response curves were fit using three parameter log-logistic regression. Mean values of four replications are plotted. Vertical lines represent the herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) for each species.

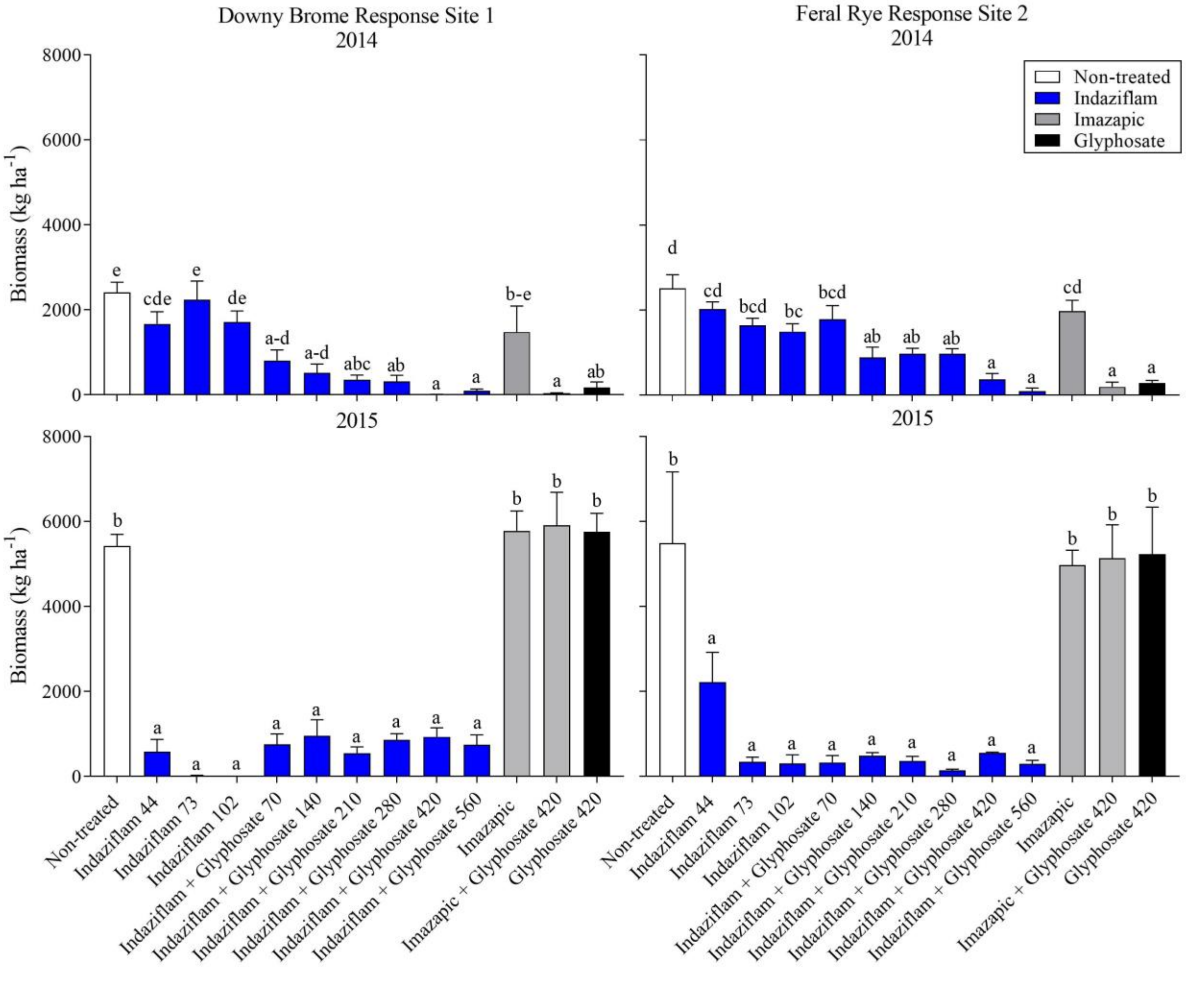


Figure 2.2: Invasive winter annual grass biomass response to herbicide treatments at Site 1 (downy brome) and Site 2 (feral rye), year of treatment (2014) and 1 YAT (2015). Application timing was after downy brome and feral rye emergence (POST) in March 2014. Letters indicate differences among herbicide treatments separated by year and by site, using least-squares means ($P < 0.05$). Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).

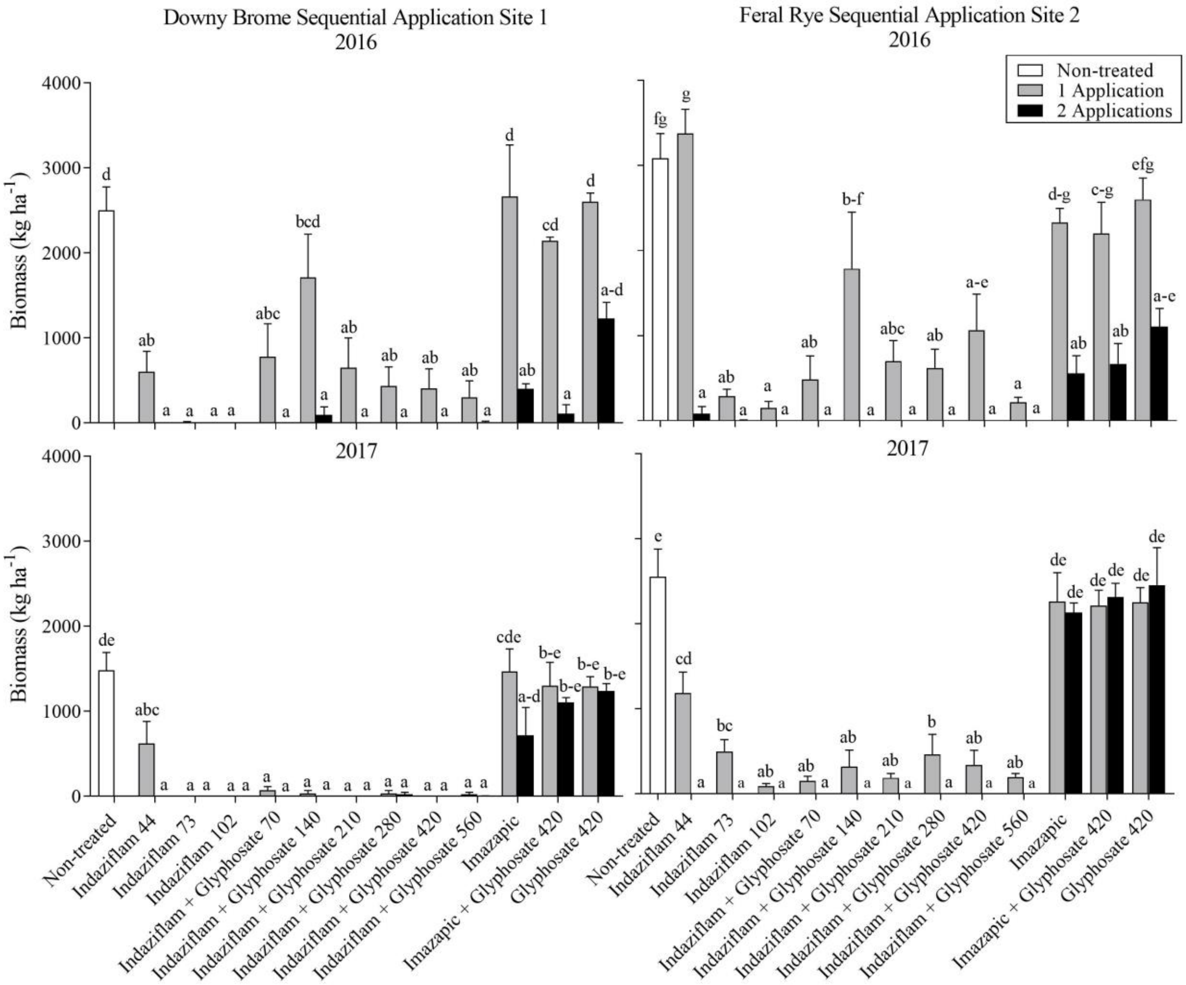
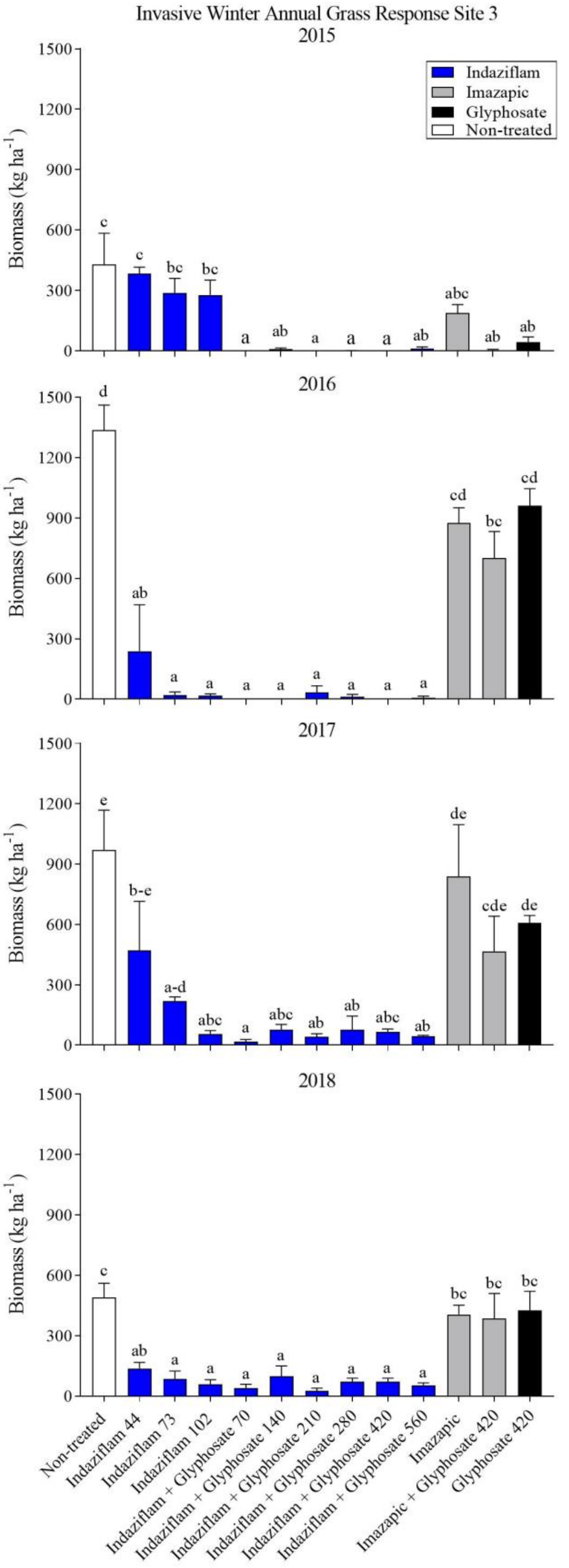


Figure 2.3: Invasive winter annual grass biomass response to 1 or 2 herbicide applications at Site 1 (downy brome) and Site 2 (feral rye), 2 YAT (2016) and 3 YAT (2017). Initial application was POST in March 2014, second application was POST in February 2016. Letters indicate differences among herbicide treatments across application number separated by year and by site, using least-squares means ($P < 0.05$). Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).


Invasive Winter Annual Grass Response Site 3
2015
Indaziflam
Imazapic
Glyphosate
Non-treated
Biomass (kg ha^{-1})
0
300
600
900
1200
1500
c
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2018
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bc
Non-treated
Indaziflam 44
Indaziflam 73
Indaziflam 102
Indaziflam + Glyphosate 70
Indaziflam + Glyphosate 140
Indaziflam + Glyphosate 210
Indaziflam + Glyphosate 280
Indaziflam + Glyphosate 420
Indaziflam + Glyphosate 560
Imazapic
Imazapic + Glyphosate 420
Glyphosate 420

Figure 2.4: Invasive winter annual grass (downy and Japanese brome) biomass response to herbicide treatments at Site 3, year of treatment (2015), 1 YAT (2016), 2 YAT (2017), and 3 YAT (2018). Application timing was after brome emergence (POST) in April 2015. Letters indicate differences among herbicide treatments by year, using least-squares means ($P < 0.05$). Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).

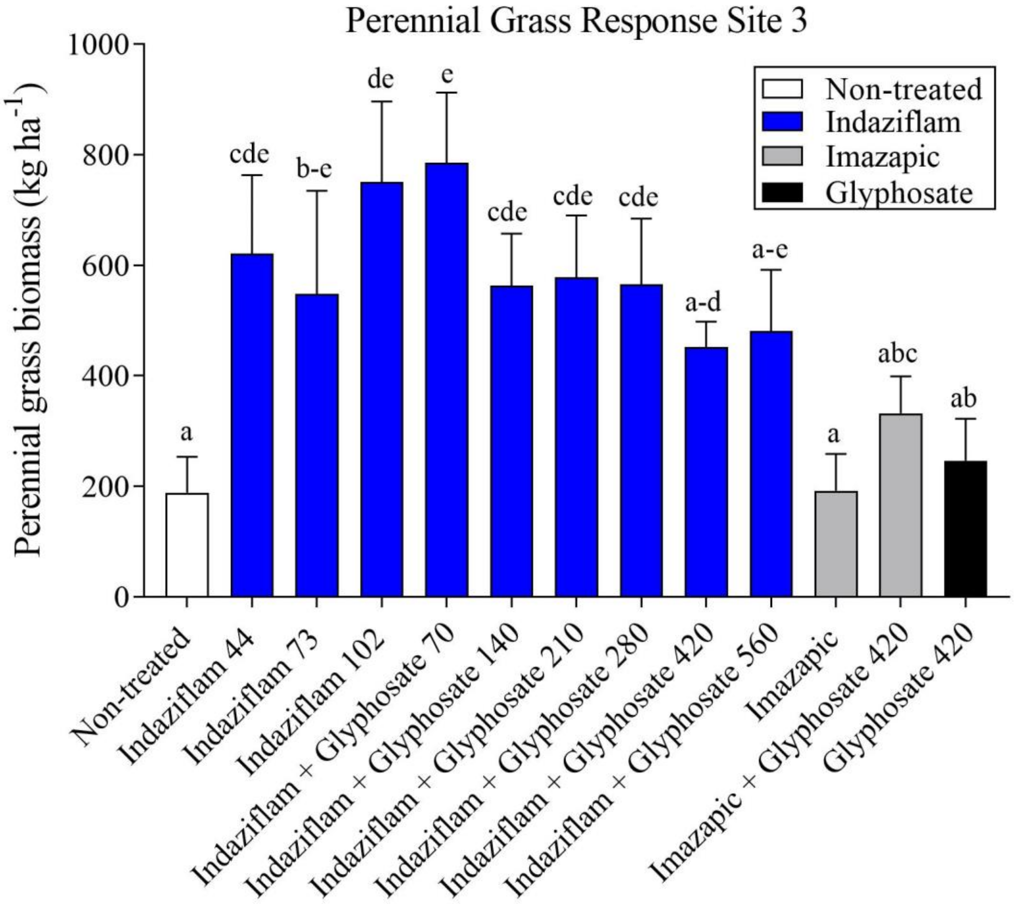

Figure 2.5: Perennial grass biomass response to herbicide treatments at Site 3, all four years combined. Letters indicate differences among herbicide treatments, using least-squares means ($P < 0.05$). Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).

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Chapter 3: Extending the Duration of Biennial and Perennial Weed Seedling Control with Indaziflam Tank-Mixes

INTRODUCTION

Invasive weeds present the greatest challenge for rangeland managers, with an estimated spread rate of 8 to 14% and projected costs of $5 billion in control efforts annually (Pimentel 2009; Duncan et al. 2004; DiTomaso 2000). This estimate does not include losses of ecosystem services such as livestock and wildlife forage, nutrient cycling, or recreational benefits (Chambers et al. 2014; Belnap et al. 2012; Pimentel 2009; Pimentel et al. 2000). Winter annual grasses have completely transformed western US rangelands, decreasing fire intervals to less than five years in many areas of the Great Basin region and causing a precipitous decline in native species (Chambers et al. 2007). The disturbances caused by exotic winter annual grasses on millions of hectares of rangeland have created ample opportunities for broadleaf weeds to invade, although broadleaf weeds have invaded intact native perennial systems as well (DiTomaso 2000; Sheley et al. 1996). Invasive plants can alter rangeland ecosystems by decreasing native species diversity, lowering forage quantity and quality, and depleting critical wildlife habitat (DiTomaso 2000).

Although annual grasses are considered the most detrimental, there are several biennial and perennial weed species that greatly impact western rangelands (DiTomaso et al. 2010; DiTomaso 2000). Diffuse knapweed (*Centaurea diffusa* Lam.) has a variable life-cycle as a biennial or short-lived perennial, bolting and setting seed only when growing conditions are favorable (Sheley et al. 1998). Infesting over 1.3 million hectares in the western US, diffuse knapweed plants break off at the ground during winter, tumbling and distributing seed over several miles (Duncan et al. 2004; Sheley et al. 1998). DiTomaso (2000) lists *Centaurea* species, including diffuse knapweed, as the largest threat to rangelands in the intermountain region. Dalmatian toadflax [*Linaria dalmatica* (L.) P. Mill] is a short-lived herbaceous perennial that favors disturbed areas such as roadsides, recent burns, abandoned crop fields, and overgrazed sites ((Robocker 1970; Alex 1962). With an estimated annual spread rate of 8 to 29%, Dalmatian toadflax is considered a major rangeland weed in the western US (Duncan et al. 2004). Unlike many perennial weed species which reproduce primarily by vegetative means, research has shown that the spread of toadflax species is driven more by sexual reproduction through seed (Ward et al. 2009; Ward et al. 2008). Another problematic rangeland weed, common mullein (*Verbascum thapsus* L.), was introduced to the US over 300 years ago and is now considered naturalized in most areas (Gross and Werner 1978). This biennial weed species is a prolific seed producer that thrives in bare ground and disturbed sites, although plants can move from these areas into intact, perennial rangeland (Semenza et al. 1978). The wooly leaves of common mullein deter grazers and reduce foliar herbicide uptake (Pitcairn 2000). Populations can spread rapidly and form dense stands, overtaking native species, reducing available forage for livestock, and re-establishing after management due to a persistent soil seed bank (Pitcairn 2000).

Herbicides are the most widespread and viable method for controlling weeds in natural areas and rangeland (DiTomaso et al. 2006; Jacobs et al. 2006; Sheley et al. 1996). Synthetic auxins are arguably the most important herbicides for broadleaf weed control on these sites, many providing both PRE and POST control (DiTomaso et al. 2010; Sheley et al. 1996). Picloram is one of the oldest and most effective auxinic herbicides used on rangeland, while aminocycopyrachlor (AMCP) is a newer auxin herbicide and the first pyrimidine carboxylic acid to become commercially available (Finkelstein et al. 2008; Arnold and Santelmann 1966). Both herbicides control several invasive rangeland weeds with minimal injury to desirable grasses (Conklin 2012; Huffman and Jacoby Jr. 1984; Arnold and Santelmann 1966). Chlorsulfuron, a sulfonylurea, is another commonly used rangeland herbicide, often applied in combination with picloram or AMCP and especially active on toadflax species (Kyser and DiTomaso 2013; Jacobs and Sheley 2005; Ray 1984). All three herbicides have both PRE and POST activity and provide extended residual weed control (Grossmann 2010; Ray 1984). In cooler and/or drier climates, picloram and AMCP can remain active in the soil for over 2 yr (Conklin and Lym 2013; Herr et al. 1966).

Broadleaf weed management on rangelands remains a constant challenge faced by land managers. Herbicides often fail to provide long-term control of invasive broadleaves, even when adequate first-year control is achieved, due to weeds reinvading from the soil seedbank (Sebastian et al. 2017b; Sebastian et al. 2012; Davies and Johnson 2011; DiTomaso et al. 2010). The weed seedbank consists of viable weed seeds that exist on the soil surface and throughout the soil profile (Fenner 1985). The continued survival of annuals, biennials and short-lived perennials especially depends on prolific seed production and persistence in the weed seedbank (Davis 2006; Sheley et al. 1998; Robocker 1970). Species such as Dalmatian toadflax can produce half a million seeds in a season under ideal growing conditions and remain viable in the soil for up to 10 yr, while common mullein seeds may remain viable in the seedbank for over 100 yr (Kivilaan and Bandurski 1981; Robocker 1970).Therefore, in order to manage the weed seedbank, herbicides are needed that provide long-term residual control of broadleaf weed seeds (DiTomaso et al. 2010).

Indaziflam is a cellulose biosynthesis inhibitor with PRE activity on both monocots and dicots (Brabham et al. 2014; Tateno et al. 2016). The original labelled use for this herbicide was in perennial tree crops to provide broad-spectrum control of grass and broadleaf seedlings in tree rows where bareground is desired (Grey et al. 2018). Shortly after its release, researchers began investigating indaziflam for use in perennial rangeland systems, and a supplemental label was established for natural areas specifically for selective invasive winter annual grass control (Anonymous 2016; Sebastian et al. 2017a; Sebastian et al. 2016). Indaziflam has been shown to provide three or more yr of winter annual grass control, yet there is limited information regarding the use of this herbicide for PRE control of broadleaf weeds on rangeland sites (Sebastian et al. 2017a; Sebastian et al. 2016). A previous greenhouse study by Sebastian et al. (2017c) showed that indaziflam was more active than AMCP and aminopyralid in providing PRE control of several invasive rangeland weeds. The same researchers also indicated extended Dalmatian toadflax control with indaziflam combinations in the field, although the research was conducted at only one field site (Sebastian et al. 2017c). Therefore, the objective of this research was to evaluate the performance of currently recommended broadleaf herbicides with and without indaziflam to control several broadleaf weed species on rangeland.

MATERIALS AND METHODS

Site Description

The experiment was established in 2016 at two sites in Boulder County, CO. The Hilltop site (latitude 39° 56' 20" N, longitude 105° 9' 59" W) and the Hillside site (latitude 39° 55' 52" N, longitude 105° 10' 31" W) were located along the Coalton Trail on Boulder County Open Space, approximately 1 km apart. The plant community at these sites is shortgrass prairie and is in the High Plains subregion of the Great Plains ecoregion. Both sites were infested with the invasive winter annual grasses downy brome (*Bromus tectorum* L.) and Japanese brome (*Bromus japonicus*), plus invasive broadleaves, diffuse knapweed (*Centaurea diffusa* Lam.) and

Dalmatian toadflax [*Linaria dalmatica* (L.) P. Mill]. The Hilltop site was also invaded by common mullein (*Verbascum thapsus* L.). Both sites also had several co-occurring native grasses, forbs, and shrubs which are listed in Table 3.1.

The soil at both sites was a Valmont cobbly clay loam (Clayey over loamy-skeletal, montmorillonitic, mesic Aridic Argiustolls); the Hilltop site had 3.0% organic matter and 7.0 pH in the top 20 cm and the Hillside site had 2.6% organic matter and 7.1 pH in the top 20 cm. The Hilltop site was very flat with an average elevation of 1740 m (5708 ft) and the Hillside site had a slight incline of approximately 5 to 15 degrees and an average elevation of 1711 m (5614 ft) (Natural Resources Conservation Service 2017).

Mean annual precipitation based on the 30-yr average (1981-2010) was 364 mm at both sites based on the closest weather station (approximately 14 km from both sites) (Western Regional Climate Center 2018). In 2016, the year the sites were established, total precipitation was 57 mm below the 30-yr average while 2017 had near average precipitation (Western Regional Climate Center 2018). In 2018, the sites again experienced decreased precipitation similar to the 2016 levels. During the years of the study, the average temperature for the sites was ~1°C higher than the 30-year average of 11.3°C (Western Regional Climate Center 2018).

Experimental Design

Herbicide applications were made March 2016 at both sites to evaluate long-term control of two *Bromus* species and subsequent biennial and perennial weed seedling control with indaziflam combinations. Downy brome and Japanese brome were actively growing approximately 5 to 8 cm tall with 3 to 4 tillers when herbicide applications were made. Second year diffuse knapweed and common mullein plants were in the rosette stage and actively growing, and new seedlings had emerged. Dalmatian toadflax seedlings had also emerged, and mature plants had broken dormancy. Nine herbicide treatments and one non-treated control were applied to 0.8-hectare plots as a POST treatment at both sites and are listed in Table 3.2. Due to space constraints, there was only one large-scale plot for each treatment at each site, although the order in which treatments occurred were randomized between the two sites. Treatments were applied with a John Deere 6420 tractor (Deere & Company, Moline, IL) equipped with a 1135 liter boom sprayer (FIMCO industries, North Sioux City, SD) using TeeJet XR8003-VS flat-fan nozzles (TeeJet® Technologies, Wheaton, IL) at 281 L ha^{-1} at 331 kPa. All treatments included 630 g ae ha^{-1} glyphosate and 0.25% v/v non-ionic surfactant.

Treatment Evaluations and Data Analysis

Each 0.8 hectare plot was separated into quadrants and subsamples were collected from each quadrant for data analysis. Downy brome and Japanese brome, perennial grass, and forb biomass were harvested at both sites in August 2017 and 2018 using randomly placed 1 m^2 quadrats in each quadrant of the plots; quadrats were not placed in the same location in subsequent years. The two brome species were combined, so total biomass of both species together was recorded. To determine canopy cover, visual percent cover estimates of all weeds were conducted in September 2017 and 2018 (1 and 2 YAT). Estimates were made over each quadrant, resulting in four subsamples of cover estimates per 0.8 hectare plot. Species richness was determined by counting the number of species occurring in each quadrant.

Weed cover data for all three species were arcsine square root transformed to meet the assumptions of normality. After failing to reject the null hypothesis of equal variance, the same residual variance was assumed at both sites for diffuse knapweed ($P = 0.7095$) and Dalmatian toadflax ($P = 0.5127$) cover. Common mullein cover was only analyzed for the Hilltop site. To test the effect of herbicide treatment on weed cover, a linear mixed-effects model was created using the 'lme4' package in R version 3.4.3, testing for treatment effects at $\alpha = 0.05$ (R Core Team 2017). The fixed factors included in the model were treatment, year, and their interaction, with year as the repeated measure; block was included as a random factor. Further analysis of the treatment and year interaction was performed using the 'emmeans' package in R (R Core Team, 2017) to obtain comparisons between all pairs of least squares means by year with a Tukey adjustment ($P < 0.05$). All grass and brome biomass data were square root transformed to meet the assumptions of normality. For the C_3 and C_4 grass biomass the same analysis was performed, although sites were separated after rejecting the null hypothesis of equal variance (C_3, $P < 0.001$; C_4, $P = 0.0031$).

For the brome biomass equal variances were assumed ($P = 0.2891$) and data were analyzed using the linear mixed effects model used for the weed cover data. Several treatments had a mean brome biomass of zero which created problems in normality of these data, even when transformations were applied. During the analysis, all treatments with a mean of zero were excluded from the model. Confidence intervals were then estimated for all treatments with a non-zero mean. Further analyses of the treatment effect were performed using the 'emmeans' package in R (R Core Team, 2017) to obtain comparisons between all pairs of least squares means for each year with a Tukey adjustment ($P < 0.05$). Treatments with a mean of zero were then grouped with treatments in which the confidence interval contained zero, in order to have a full set of treatment comparisons. Any treatment with a confidence interval not containing zero was considered significantly different from treatments in which the mean was zero.

To analyze treatment effects on species richness, a generalized linear mixed-effects model was created with treatment, year and their interaction as fixed factors. Block was included as the random factor. After failing to reject the hypothesis that count data of species richness were from a Poisson distribution ($P = 0.9363$) based on a Chi-squared Goodness of Fit Test, a Poisson distribution was assumed in the model. Significant treatment and year effects were determined post-hoc using Tukey's method for multiple comparisons ($P < 0.05$) ('lme4' and 'emmeans' packages, R Core Team 2017).

RESULTS AND DISCUSSION

Biennial and Perennial Weed Control

The significant treatment by year interaction ($P < 0.001$) was evaluated for each of the three broadleaf weed species. Every herbicide treatment reduced biennial and perennial weed cover 1 YAT, while only treatment combinations which included indaziflam continued to control all three species 2 YAT (Table 3.3). All herbicide treatments reduced diffuse knapweed cover (0.4% to 23% cover) compared to the nontreated (46% cover) 1 YAT. Herbicide treatments which included picloram or AMCP with indaziflam outperformed AMCP treatments without indaziflam and indaziflam alone (Table 3.3). By 2 YAT only treatments with indaziflam and the picloram plus chlorsulfuron treatment were still reducing diffuse knapweed cover, although treatments including AMCP or picloram with indaziflam performed better (0.3% to 1.8% cover) than picloram without indaziflam and indaziflam alone (15% to 20% cover) (Table 3.3). All herbicide treatments performed similarly in reducing Dalmatian toadflax cover 1 YAT (Table 3.3). Dalmatian toadflax cover averaged 2.3% in plots treated with herbicide compared to 19.4% cover in the nontreated. By year two, only treatments containing indaziflam were still providing reductions in Dalmatian toadflax (Table 3.3). Common mullein cover was only consistent at the Hilltop site; therefore, data analyses were based on one site. With the exception of picloram alone, all herbicide treatments reduced common mullein abundance (0 to 11% cover) 1 YAT compared to the nontreated (28% cover) (Table 3.3). By 2 YAT, treatments including indaziflam and AMCP alone were the only treatments still providing reductions in common mullein abundance, although combinations containing indaziflam reduced common mullein significantly more than AMCP alone (Table 3.3).

Downy and Japanese Brome Response

There was a significant year by treatment interaction ($P < 0.001$) for brome biomass. Every treatment containing indaziflam provided excellent downy and Japanese brome control over the two years the study was conducted (Figure 3.1). The year following treatment (2017), the nontreated had an average of 360 kg ha^{-1} of brome biomass while indaziflam treatments averaged less than 1 kg ha^{-1} (Figure 3.1). Two years after herbicide application (2018), indaziflam treatments still averaged less than 1 kg ha^{-1} of brome biomass (Figure 3.1). Treatments containing AMCP without indaziflam also had less brome biomass than the nontreated 1 YAT, with an average of 110 kg ha^{-1}, although by 2 YAT there was no longer a reduction in biomass (Figure 3.1). Multi-year brome control observed among indaziflam treatments corroborates previous findings by Sebastian et al. (2017a, 2016). It has also been noted that AMCP has some PRE activity on brome germination, although any observed control generally does not persist for multiple years (Sebastian et al. 2017c; Ball 2014).

Native Perennial Grass and Forb Response

There was not a significant year effect or year-by-treatment interaction for C_3 grass response at the Hilltop site although there was a treatment effect ($P < 0.001$); therefore, grass abundance was averaged across year by treatment. There were increases in C_3 grass biomass in the AMCP plus chlorsulfuron, picloram plus indaziflam, and picloram plus chlorsulfuron and indaziflam treatments (Figure 3.2). At the Hillside site the significant treatment-by-year interaction ($P < 0.001$) was analyzed. The year following herbicide application (2017), picloram plus indaziflam and picloram plus chlorsulfuron and indaziflam had increases in C_3 grass biomass compared to the nontreated (Figure 3.3). By 2 YAT (2018), picloram plus chlorsulfuron and indaziflam and AMCP plus indaziflam had greater cool season grass biomass than the non-treated (Figure 3.3).

At the Hilltop site all treatments, with the exception of picloram plus chlorsulfuron and indaziflam, resulted in increases in C_4 grass biomass 1 YAT (Figure 3.4). By 2 YAT only AMCP plus indaziflam and AMCP plus chlorsulfuron and indaziflam still had an increased abundance of warm season grass (Figure 3.4). At the Hillside site 1 YAT, only AMCP plus chlorsulfuron and indaziflam resulted in greater C_4 grass biomass (Figure 3.4). By 2 YAT indaziflam alone, AMCP plus indaziflam, and AMCP plus chlorsulfuron and indaziflam had greater C_4 grass abundance than nontreated plots (Figure 3.4). The increased abundance of C_4 grasses in AMCP plus indaziflam treatments is likely due to a combination of broad-spectrum weed control and AMCP injury to the western wheatgrass, the dominant C_3 grass at both sites (Hergert et al. 2015; Conklin 2012). Previous research by Conklin (2012) showed reductions in western wheatgrass biomass following AMCP applications, while there was minimal injury observed between C_4 species, big bluestem and sideoats grama (both present in our sites).

Species richness was evaluated to assess herbicide effects on species diversity. The list of co-occurring native species can be seen in Table 1. Overall, increases in species richness were only observed in this study among treatments which also provided downy and Japanese brome control. There was an increase in species richness 1 YAT in the indaziflam alone treatment and both treatments which included AMCP plus indaziflam (Figure 3.5). These treatments averaged 13.6 species compared to the 8.7 species observed in the nontreated. Similar trends were observed 2 YAT, with increased species richness for indaziflam alone and AMCP plus chlorsulfuron and indaziflam (average 13.8 species) compared to the nontreated (average 4.8 species) (Figure 3.5). Picloram plus chlorsulfuron and indaziflam also had increased species richness (average 9.5 species) 2 YAT (Figure 3.5).

Under greenhouse conditions, indaziflam was 11, 19, and 45 times more active than AMCP in controlling diffuse knapweed, Dalmatian toadflax, and common mullein seedlings, respectively (Sebastian et al. 2017c). This observation was replicated under field conditions when Sebastian et al. (2017c) observed that Dalmatian toadflax control was extended with indaziflam combinations. Our results provide further evidence that combining indaziflam with POST broadleaf herbicides can extend the control of several biennial and perennial broadleaf weeds. Because indaziflam has almost no POST activity, these data suggest that indaziflam provides extended control by inhibiting reinvasion from the weed seedbank (Tateno et al. 2016; Brabham et al. 2014). Even though picloram and AMCP can remain active in the soil for over 2 yr, these herbicides failed to provide long-term broadleaf weed control unless they were combined with indaziflam. A possible explanation for this performance difference is an increased relative potency of indaziflam to control weed seedlings (Sebastian et al. 2017c), as well as less dilution through the soil profile due to much lower water solubility as compared with the other herbicides (Sebastian et al. 2017c; Sebastian et al. 2017d).

To achieve long-term weed management beyond the residual control provided by herbicides, land managers must establish a plant community resistant to invasion (Chambers et al. 2014; Chambers et al. 2007; Sheley et al. 1996). Weed-resistant communities consist of diverse native plant species that can allocate resources throughout the growing season, mitigating the effects of seasonal variances and becoming more competitive for limited resources (Sheley et al. 1996; Pyke and Archer 1991). Results from our study showed that treatments which included

AMCP with indaziflam increased the abundance of C_4 grasses, while also increasing species richness, whereas picloram treatments tended to be dominated by C_3 grasses. In areas such as the Great Plains which were historically abundant in C_4 grasses, land managers could potentially use AMCP as a tool to control broadleaf weeds, while selecting for a more diverse grass community that can better utilize limited resources. Overall, perennial grass increases only persisted in indaziflam treated plots that provided broad-spectrum, multi-year weed control. These data suggest that both winter annual grass and broadleaf weed control is critical for a sustainable response of the native plant community.

Managing the weed seedbank is the key to long-term control of invasive rangeland weeds (Sebastian et al. 2017b; DiTomaso et al. 2010; Sheley et al. 1996). In sites dominated by native perennials, indaziflam can be a tool to help manage the seedbank of both grass and broadleaf weeds without negatively impacting native species (Sebastian et al. 2017a; Sebastian et al. 2017c; Sebastian et al. 2016). Using indaziflam in combination with broadleaf herbicides has the potential to provide multi-season weed control, possibly allowing enough time to increase the resistance and resilience of the native plant community. Management objectives should be considered when selecting broadleaf herbicides, as there is potential to influence a more balanced and diverse plant community that is better able to capture resources throughout interannual variations in climate. Future studies are needed to evaluate the potential for indaziflam to control other biennial and perennial weed seedlings under field conditions and evaluate its performance in more arid climates across the western United States.

Table 3.1: List of co-occurring grass, forb and shrub species at the Hillside and Hilltop sites.

Scientific name	Common name[a]	Hilltop	Hillside
Cool season grasses (C_3)			
Nassella viridula	green needlegrass	X	
Pascopyrum smithii	western wheatgrass	X	X
Warm season grasses (C_4)			
Aristida purpurea	purple threeawn	X	X
Andropogon gerardii	big bluestem	X	X
Bouteloua curtipendula	sideoats grama	X	X
Bouteloua dactyloides	buffalograss	X	X
Bouteloua gracilis	blue grama	X	X
Perennial forbs			
Psoralidium tenuiflorum	slimflower scurfpea	X	X
Dalea purpurea	purple prairie clover	X	X
Oenothera suffrutescens	scarlet beeblossom	X	X
Pterogonum alatum	winged buckwheat	X	X
Helianthus pumilus	little sunflower	X	X
Liatris punctata	Dotted blazing star	X	X
Sphaeralcea coccinea	scarlet globemallow	X	X
Ratibida columnifera	ppright prairie coneflower	X	X
Cirsium undulatum	wavyleaf thistle	X	X
Phacelia heterophylla	varileaf phacelia	X	
Senecio spartioides	broom-like ragwort	X	X
Hymenopappus filifolius	fineleaf hymenopappus	X	
Oxytropis sericea	white locoweed	X	
Argemone polyanthemos	crested pricklypoppy	X	X
Physalis heterophylla	clammy groundcherry	X	X
Allium textile	textile onion	X	
Penstemon virens	Front Range beardtongue	X	X
Linum lewisii	Lewis flax	X	X
Leucocrinum montanum	common starlily	X	
Asclepias speciosa	showy milkweed		X
Oenothera caespitosa	tufted evening primrose		X
Ambrosia psilostachya	western ragweed	X	X
Plantago patagonica	woolly plantain	X	
Subshrubs/Shrubs			
Artemisia dracunculus	tarragon	X	X
Artemisia frigida	prairie sagewort	X	X
Artemisia ludoviciana	white sagebrush	X	X
Opuntia polyacantha	Plains pricklypear	X	X
Yucca glauca	soapweed yucca	X	X
Heterotheca villosa	hairy false goldenaster	X	X
Gutierrezia sarothrae	broom snakeweed	X	X
Symphyotrichum porteri	smooth white aster		X

[a] Common names based on United States Department of Agriculture PLANTS database: https://plants.sc.egov.usda.gov/.

Table 3.2: Herbicides and rates applied in evaluating the response of annual, biennial and perennial weed species.

Common name	Rates applied[a] (g ai ha^{-1})
Indaziflam	102
Aminocyclopyrachlor	140
Aminocyclopyrachlor + chlorsulfuron	140 + 52
Aminocyclopyrachlor + indaziflam	140 + 102
Aminocyclopyrachlor + chlorsulfuron + indaziflam	140 + 52 + 102
Picloram	560
Picloram + chlorsulfuron	560 + 52
Picloram + indaziflam	560 + 102
Picloram + chlorsulfuron + indaziflam	560 + 52 + 102

[a] All treatments included glyphosate at 630 g ae ha^{-1} and 0.25% v/v non-ionic surfactant.

Table 3.3: Response of diffuse knapweed, Dalmatian toadflax, and common mullein cover to herbicide treatments.

Herbicide treatments[a]	Diffuse knapweed[b]	Dalmatian toadflax[b]	Common mullein[b,c]
	---------------------	% cover	---------------------
1 YAT			
Non-treated	45.6d	19.4b	27.5c
Indaziflam	18.1c	3.4a	0.3a
AMCP	22.5c	4.6a	11.3b
AMCP + chlorsulfuron	20bc	5a	5.3ab
AMCP + indaziflam	0.9a	0.8a	0a
AMCP + chlorsulfuron + indaziflam	0.4a	0.6a	0.5a
Picloram	0.8a	3.4a	27.5c
Picloram + chlorsulfuron	3.5ab	1.6a	1.8a
Picloram + indaziflam	1.9a	1a	0.5a
Picloram + chlorsulfuron + indaziflam	1.1a	0.4a	0.3a
2 YAT			
Non-treated	51.9de	15.6bc	23.8c
Indaziflam	15c	0.9a	0a
AMCP	50.6de	18.8c	10.3b
AMCP + chlorsulfuron	57.5e	14.4bc	43.8d
AMCP + indaziflam	1.8ab	0.3a	2.5a
AMCP + chlorsulfuron + indaziflam	0.8a	0.3a	0a
Picloram	28.8cd	21.4bc	61.3d
Picloram + chlorsulfuron	20bc	7.9ab	21.3bc
Picloram + indaziflam	0.5a	0.3a	0.5a
Picloram + chlorsulfuron + indaziflam	0.3a	0.5a	1.5a

[a] Herbicide treatment rates are as follows: indaziflam (102 g ai ha^{-1}), aminocyclopyrachlor (AMCP, 140 g ai ha^{-1}), chlorsulfuron (52 g ai ha^{-1}), picloram (560 g ai ha^{-1}), and non-treated.

[b] Means followed by the same letter are not significantly different at the $P \leq 0.05$ level within year by species as determined by Tukey's multiple comparison test.

[c] Common mullein evaluations are based on one site (Hilltop site).

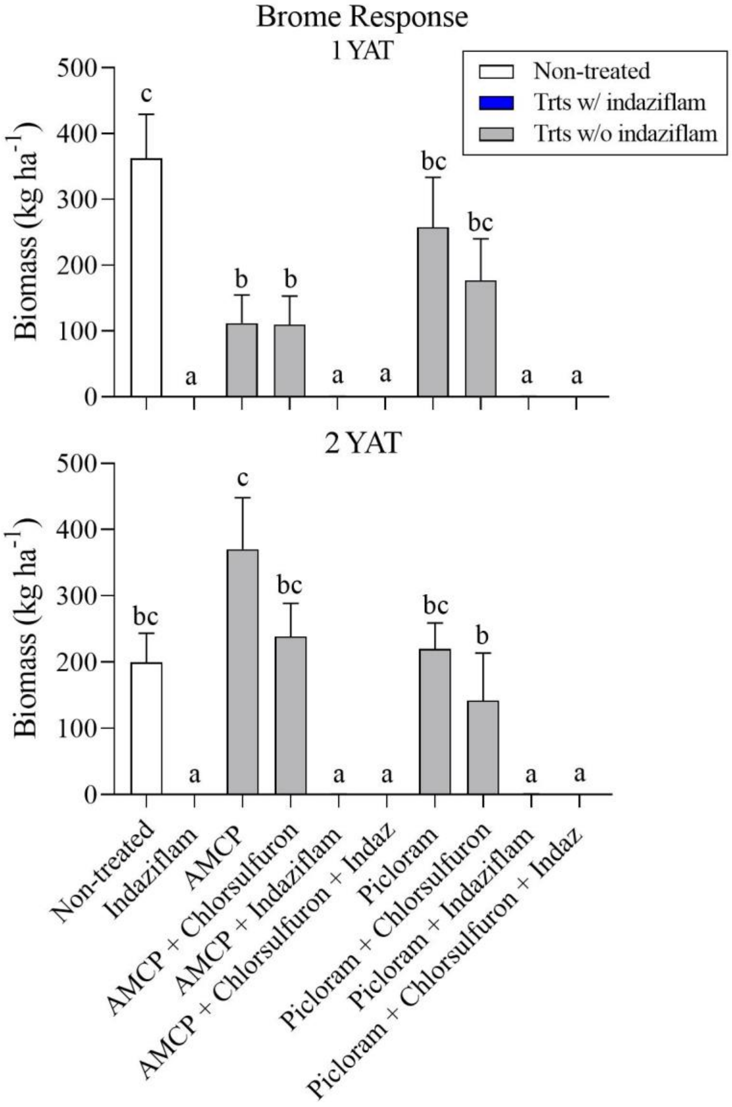

Figure 3.1: Downy and Japanese brome biomass at the Hilltop and Hillside sites 1 and 2 years after treatment (YAT). Data from sites were combined for analysis of variance. Letters indicate differences among herbicide treatments separated by year, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: indaziflam (Indaz, 102 g ai ha^{-1}), aminocyclopyrachlor (AMCP, 140 g ai ha^{-1}), chlorsulfuron (52 g ai ha^{-1}), picloram (560 g ai ha^{-1}), and non-treated.

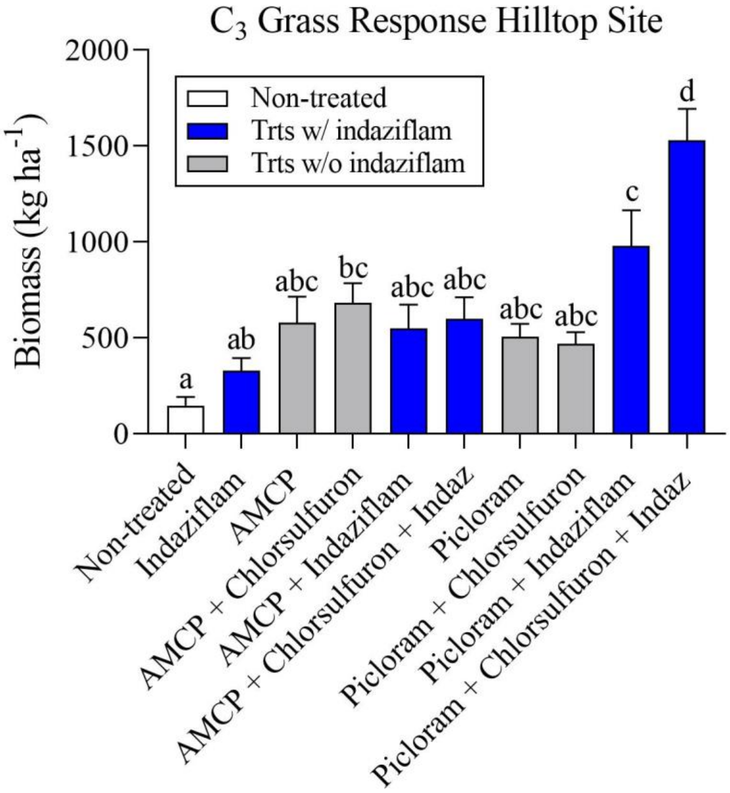

Figure 3.2: Cool season (C_3) grass biomass at the Hilltop site. Data was combined across years for analysis of variance. Letters indicate differences among herbicide treatments using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: indaziflam (Indaz, 102 g ai ha^{-1}), aminocyclopyrachlor (AMCP, 140 g ai ha^{-1}), chlorsulfuron (52 g ai ha^{-1}), picloram (560 g ai ha^{-1}), and non-treated.

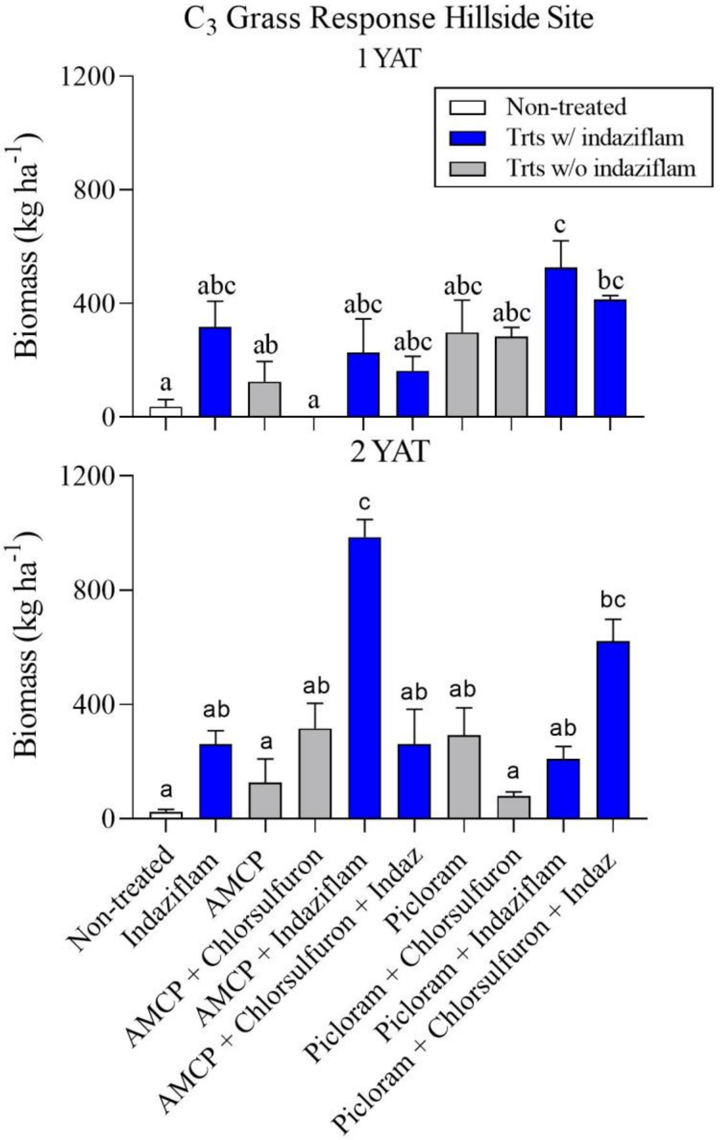

Figure 3.3: Cool season (C_3) grass biomass at the Hillside site 1 and 2 years after treatment (YAT). Letters indicate differences among herbicide treatments separated by year, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: indaziflam (Indaz, 102 g ai ha^{-1}), aminocyclopyrachlor (AMCP, 140 g ai ha^{-1}), chlorsulfuron (52 g ai ha^{-1}), picloram (560 g ai ha^{-1}), and non-treated.

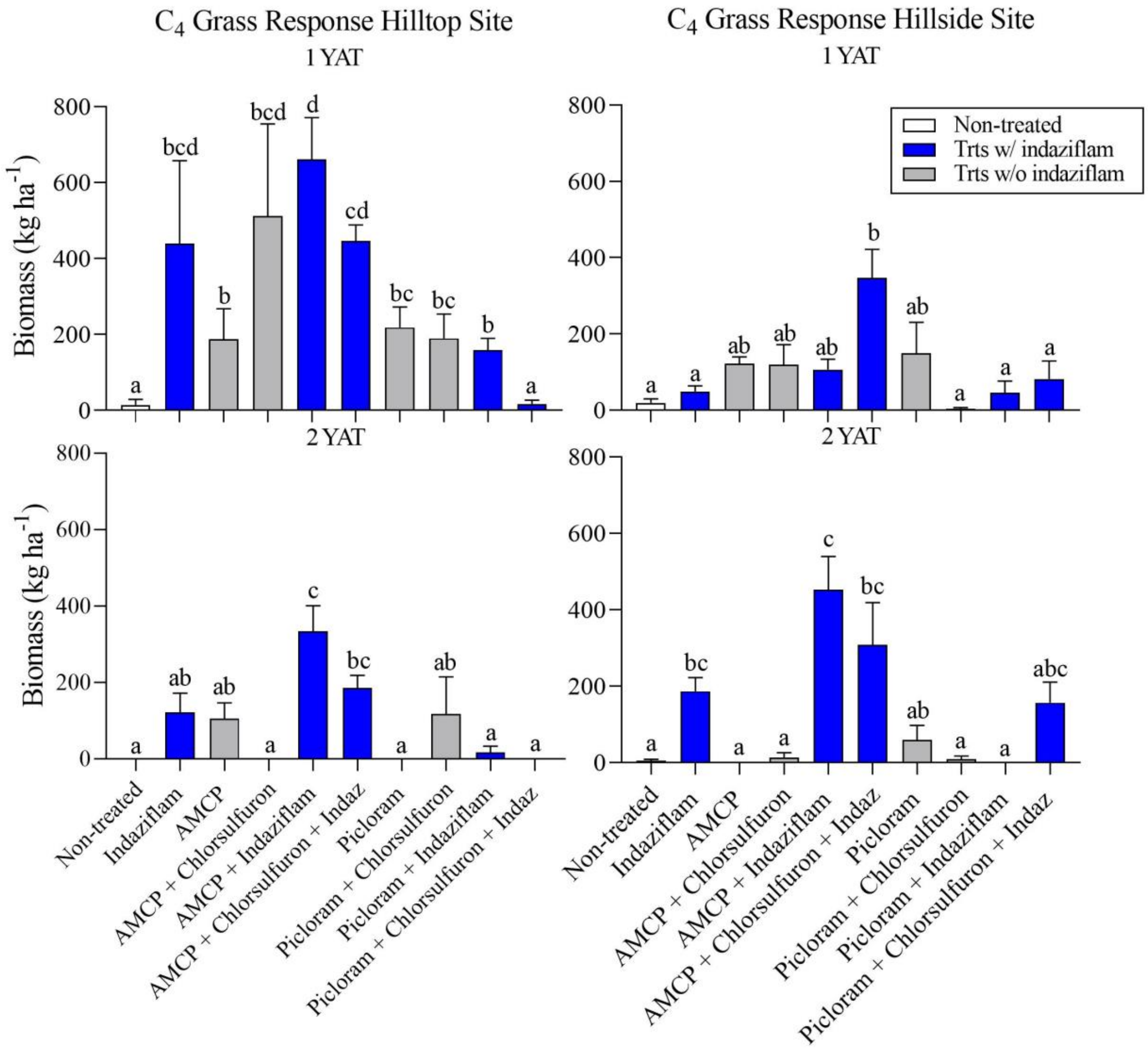

Figure 3.4: Warm season (C_4) grass biomass at the Hilltop and Hillside sites 1 and 2 years after treatment (YAT). Data from sites were combined for analysis of variance. Letters indicate differences among herbicide treatments separated by year and by site, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: indaziflam (Indaz, 102 g ai ha^{-1}), aminocyclopyrachlor (AMCP, 140 g ai ha^{-1}), chlorsulfuron (52 g ai ha^{-1}), picloram (560 g ai ha^{-1}), and non-treated.

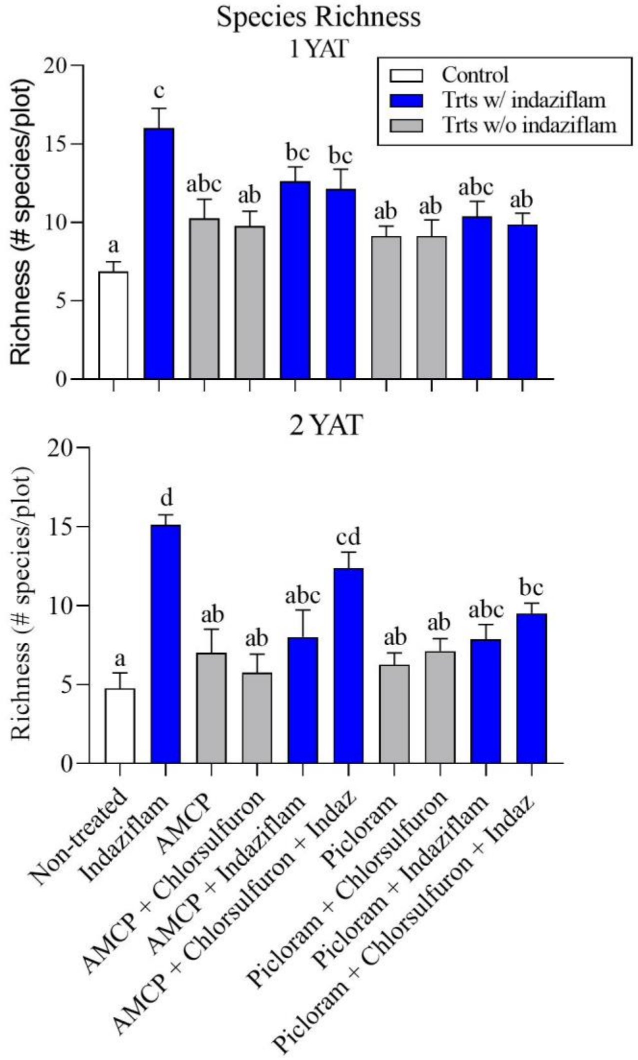

Figure 3.5: Species richness at the Hilltop and Hillside sites 1 and 2 years after treatment (YAT). Data from sites were combined for analysis of variance. Letters indicate differences among herbicide treatments separated by year, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: indaziflam (Indaz, 102 g ai ha^{-1}), aminocyclopyrachlor (AMCP, 140 g ai ha^{-1}), chlorsulfuron (Chlor, 52 g ai ha^{-1}), picloram (Pic, 560 g ai ha^{-1}), and non-treated.

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Chapter 4: Interception, adsorption and desorption of herbicides applied to winter annual grass litter

INTRODUCTION

Exotic winter annual grasses present the largest threat to the arid and semi-arid ecosystems of western North America (DiTomaso 2000). The ecological impacts of winter annual grass invasions include increased fire frequency and intensity, altered nutrient cycling, decreased species diversity, and diminished wildlife habitat (Corbin and D'Antonio 2004; Knapp 1996; Thill et al. 1984). With an estimated annual spread rate of 14%, downy brome (*Bromus tectorum* L.) is the most widespread winter annual grass, invading an estimated 22 million ha of rangeland in the United States (Duncan et al. 2004). Since its introduction into North America in the mid 1800s, downy brome has undergone rapid range expansion, becoming the most dominant and impactful weed in the intermountain West region (Johnson and Davies 2012; Mack 1981). It has been projected that an additional 31 million ha in the western United States are susceptible to invasion by exotic winter annual grasses (Pellant and Hall 1994).

While downy brome receives a majority of the attention in the literature, two other winter annual grasses, medusahead (*Taeniatherum caput-medusae* [L.] Nevski) and ventenata (*Ventenata dubia* [Leers] Coss.), have become major threats to rangelands in recent decades (Wallace et al. 2015; Young 1992). Medusahead was discovered in the United States in 1887, although populations appear to have remained relatively static until around the 1950’s, when it started to become a major concern for livestock producers (Bovey et al. 1961). Infesting over 2 million ha of rangeland, medusahead has low palatability and can reduce grazing capacity by 80% (Dahl and Tisdale 1975; Hironaka 1961). The exotic annual grass ventenata was first reported in Washington in 1952 and spread throughout the Intermountain Pacific Northwest, becoming a major invader in Conservation Reserve Program (CRP) lands and Palouse grasslands (Wallace et al. 2015). As nearly 100% of this geographical area has been converted to farmland, the remnant Palouse Prairie grasslands are critical for providing wildlife habitat and maintaining rare native plant communities (Looney and Eigenbrode 2012). Ventenata is a strong competitor with the ability to thrive in wet or arid conditions. Its competitiveness has allowed it to invade areas previously dominated by downy brome and medusahead, including the Sagebrush Steppe (Wallace et al. 2015).

Winter annual grass infestations accumulate large quantities of litter, or thatch, on the soil surface as plants senesce yearly and decompose slowly (Evans and Young 1970). Litter facilitates invasions by promoting winter annual grass germination and suppressing native plants (Evans and Young 1970). Litter accumulation also contributes to increased fire frequency and intensity in the western United States by providing a continuous layer of fine fuels, especially in the Great Basin where historically there was a significant amount of bare soil between plants (Abatzoglou and Kolden 2011; DiTomaso 2000; Knapp 1996).

It has been widely speculated that winter annual grass litter, which can exceed 15 cm in thickness, can adsorb soil applied herbicides and reduce their performance (Kessler et al. 2015; Mangold et al. 2013; DiTomaso et al. 2006; Monaco et al. 2005; Evans and Young 1970). Past studies have reported improved performance with herbicides when litter has been eliminated, although many of these studies have been confounded by the fact that fire was used to remove the litter layer (Kessler et al. 2015; Davies and Sheley 2011; Kyser et al. 2007; Sheley et al. 2007; Monaco et al. 2005). The impacts of winter annual grass litter on soil applied herbicides has only been empirically evaluated in one published study (Kessler et al. 2015). In that study, up to 74.6 ± 1.8% (Mean ± SE) of imazapic and tebuthiuron were intercepted when applied over high amounts of downy brome litter, and only 69% of the intercepted herbicide could be desorbed from the litter with 15 mm of rainfall 7 days after treatment (DAT) (Kesler et al. 2015). This study and several others evaluating crop residues have hypothesized that some irreversible binding to the litter may be occurring. This hypothesis is based on the fact that rainfall is not able to recover 100% of the applied herbicide (da Silva 2018; Carbonari et al. 2016; Cavenaghi et al. 2007; Ghadiri et al. 1984; Banks and Robinson 1982).

Herbicide sorption increases with the lipophilic components of litter such as lignin, which increases as a % of the dry weight as litter decays (Van Beinum et al. 2006; Dao 1991; Barak et al. 1983). An herbicide's chemical properties also influences sorption, with its adsorption to organic matter dependent on its lipophilic properties (Gennari et al. 1998; Stevenson 1972). Therefore, lipophilic herbicides may adsorb more readily to litter; unfortunately, little is known about differences in adsorption and subsequent desorption from litter between water soluble and insoluble herbicides (Dao 1991; Barak et al. 1983).

Imazapic and rimsulfuron, hydrophilic group 2 herbicides, are industry standards for winter annual grass control on rangeland (Kyser et al. 2007; Kyser et al. 2013; Mangold et al. 2013; Morris et al. 2016; Shaner 2014a, 2014b). Both herbicides have pre-emergent (PRE) residual activity and can provide post-emergent (POST) winter annual grass control when applied at the seedling stage (Kyser et al. 2013; Sebastian et al. 2016; Wallace and Prather 2016). Winter annual grass control can be highly variable with imazapic and rimsulfuron, and although selective at low use rates, perennial grass injury can occur (Kyser et al. 2013; Mangold et al. 2013; Sebastian et al. 2016; Sebastian et al. 2017).

Indaziflam, a lipophilic group 21 herbicide, controls winter annual grasses in rangeland and natural areas by inhibiting seedling establishment (Brabham et al. 2014; Sebastian et al. 2017a; Tompkins, 2010). Indaziflam has no significant POST activity (Brabham et al. 2014) but provides much longer (3+ yr) soil residual control compared to imazapic and rimsulfuron (Sebastian et al. 2017a; Sebastian et al. 2016). This long-term residual control may provide an opportunity to eliminate winter annual grass seeds from the soil seedbank (Sebastian et al. 2017b). Indaziflam's low water solubility suggests that its interaction with winter annual grass litter would be significantly different compared to the two industry standards, imazapic and rimsulfuron. There are also indications that ventanata and medusahead litter have different physical and chemical properties compared to downy brome litter (Bovey et al. 1961; Wallace et al. 2015), therefore, the ability to remove herbicide from litter with rainfall could vary between litter types. For these reasons, the objectives of this research were to 1) quantify imazapic, rimsulfuron, and indaziflam intercepted at two levels of downy brome, medusahead, and ventenata litter, 2) determine the efficiency of various simulated rainfall events to remove the intercepted herbicide from litter and 3) determine if time dependent binding decreases the amount of herbicide that can be removed from litter by rainfall.

MATERIALS AND METHODS

Litter Collection and Description of Experimental Units

Three separate experiments were conducted to evaluate interception and subsequent desorption with rainfall of herbicides applied to winter annual grass litter. Three winter annual grass litter types were collected in August 2016. Downy brome litter was collected from Colorado Parks and Wildlife's Wellington State Wildlife Area in Wellington, CO; ventenata litter was collected from a Conservation Reserve Program field in Latah County, ID; and medusahead litter was collected from a natural area in Cache County, UT. Litter was allowed to dry at room temperature (25 to 28 C) for a minimum of one week before the experiments were conducted. Litter was sieved to remove soil and shoot segments were then cut to 14 cm in length. Each experimental unit consisted of a 150 X 150 X 50 mm Pyrex® dish with a 150 X 150 X 50 mm stainless steel mesh basket placed on top. The baskets consisted of 0.5 mm stainless steel mesh with 6.35 mm openings. All experiments were conducted in a complete randomized design with three replicates and each of the 3 experiments described below was repeated.

Herbicide Interception

To determine the amount of herbicide intercepted by litter, 2.82 g and 5.64 g of each litter type, corresponding to 1300 and 2600 kg ha^{-1}, were spread evenly in the metal baskets. The high litter amount (2600 kg ha^{-1}) corresponded to the field rate where the downy brome litter was collected. Imazapic, rimsulfuron and indaziflam were applied at 122, 70, and 105 g ai ha^{-1}, respectively, using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray nozzle (TeeJet Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L $ha\text{-}^{1}$ (20 gal ac^{-1}) at 172 kPa (25 lb in^{-2}). Immediately after herbicide application, dishes were washed with methanol for imazapic and rimsulfuron applications and acetonitrile for indaziflam applications. The methanol and acetonitrile volumes were recorded, and samples were transferred to 15 ml glass tubes and stored at 0 C for analysis. A dish with an empty metal basket was included for all experiments as a control to determine the total quantity of herbicide applied.

Desorption by Litter Types

To determine herbicide desorption from the three litter types, the largest amount of litter (5.64 g) was spread in the metal baskets and placed over the Pyrex® dishes. All three herbicides were applied as previously described. After herbicide application, baskets were removed and the Pyrex® dishes were washed. Baskets were then placed back on the dishes and 12 mm of simulated rainfall were immediately applied to half of the experimental units, using the same overhead sprayer with an 8004E nozzle traveling at 0.45 m s^{-1}. The other half of the experimental units were removed from the dishes and stored at 25 to 28 C under laboratory inflorescent light conditions. After 1 d, the treated litter was placed over clean dishes and 12 mm of simulated rainfall were applied. After the simulated rainfall, the total water volume was recorded for each dish and an aliquot was taken and stored in a 15 mL glass tube at 0 C.

Herbicide Desorption of Downy Brome

The last experiment was conducted to determine herbicide desorption from downy brome litter at different wait periods and rainfall amounts. The three herbicides were applied to the highest downy brome litter amount (5.64 g) and rainfall was simulated using the previously described procedures. Rainfall was applied in amounts of 3, 6, 12 and 24 mm after periods of 0 d, 1 d, and 7 d. For the 1 d and 7 d periods, experimental units were stored at 25 to 28 C under laboratory inflorescent light conditions. Aliquots were collected and stored in the same manner as previously described until further analysis with liquid chromatography mass spectrometry (LC-MS). The samples from the dishes without litter were diluted 20x with methanol for imazapic and rimsulfuron samples, and acetonitrile for indaziflam samples before filtration.

LC-MS/MS Analysis

For imazapic and rimsulfuron the herbicide/methanol and herbicide/water samples were prepared for LC-MS/MS analysis by filtering 1 mL aliquot of all samples through a 0.24 µm membrane syringe filter into 1.5 mL autosampler vials. For indaziflam, the herbicide/acetonitrile samples from the interception experiment were prepared in the same manner. For the indaziflam/water samples, 80% of the original solution was diluted with 20% acetonitrile and a 1 mL aliquot was filtered through a 0.24 µm membrane syringe filter into 1.5 mL autosampler vials. The samples collected from the dishes without litter were prepared by diluting 20x with methanol for imazapic and rimsulfuron applications or acetonitrile for indaziflam applications and using the same filtration process as stated above. The herbicide concentration in each sample was determined by liquid chromatograph coupled to a mass spectrometer (LC/MS; Shimadzu LCMS-8040, Shimadzu Corporation, Kyoto, Japan). The samples were separated on a Kinetex F5 100 Å column (100x4.6 mm; 2.6 µm; Phenomenex, Torrance, CA) maintained at 40°C. For all herbicides, the mobile phase consisted of (A) distilled water and (B) acetonitrile (Millipore Sigma, St. Louis, MO, USA), both acidified with 0.1% of formic acid (Thermo Fisher Scientific Co., Waltham, MA, USA) with an injection volume of 1 µL. For rimsulfuron, the flow rate was 0.4 ml min^{-1}, and the solvent ratio increased gradually from 50% (B) to 100% (B) at min 4 and then returned to the initial condition at min 5.1. The total run time was 8 min, and rimsulfuron retention time was 3.26 min. For imazapic, the flow rate was 0.4 ml min^{-1}, and the solvent ratio increased gradually from 30% (B) to 90% (B) at min 4 and then returned to the initial condition at min 6.1. The total run time was 8 min, and the imazapic retention time was 3.57 min. For indaziflam, the flow rate was 0.4 ml min^{-1}, and the solvent was isocratic with 25% A and 75% B. The total run time was 4 min, and the indaziflam retention time was 2.88. The MS utilized an electrospray ionization source in positive mode (ESI+) for all herbicides. Five concentrations ranging from 0.01 and 1 µg mL^{-1} were created from analytical standards and included as the calibration curve (Rimsulfuron and imazapic 99.9% purity, Sigma-Aldrich, St. Louis, MO, USA; Indaziflam 99.3% purity, Bayer CropScience, Research Triangle Park, NC, USA).

The herbicide concentration from the samples based on the LC-MS analysis was transformed into mass (µg) by adjusting for the volume recorded from the simulated rainfall. For the interception experiment, the measured herbicide concentrations that passed through the litter were subtracted from the total herbicide applied to the empty dishes to determine the amount of herbicide intercepted by the litter. These data were then transformed to a percentage of the total applied herbicide. For the desorption experiments, the concentrations from the simulated rainfall samples were compared against the total herbicide intercepted by the litter in order to calculate percent desorption.

Statistical Analysis

After failing to reject the null hypothesis of a Levene's test that experimental variances are equal, repeated studies for all three experiments were combined for analysis. Interception data were analyzed in R v. 3.4.3 (R Core Team 2017) using analysis of variance with herbicide, litter type and litter amount as the factors. Post-hoc analysis was performed using the 'emmeans' package in R v. 3.4.3 (R Core Team 2017) to obtain comparisons between all pairs of least squares means with a Tukey-Kramer adjustment ($P < 0.05$). To compare desorption among the three litter types, data were analyzed by herbicide and wait period (0 h and 24 h) using analysis of variance with litter type as the factor in R v. 3.4.3 (R Core Team 2017). For the desorption of downy brome, a model comparison procedure was conducted to determine the best fit for these data. Data were subjected to an asymptotic regression (AR) model, a rectangular hyperbolic (RHB) model, and linear regression. For each herbicide, best fit was determined by using the procedure outlined by Kniss et al. (2011) which chooses models with lowest bias-corrected Akaike information criterion corrected for small sample size (AIC*c*) value while also considering residual standard errors and AIC*c* ratios between models. The analysis was conducted using R v.

3.4.4 (R Core Team 2017) using the 'drm' (Ritz and Streibig 2005) and 'qpcR' (Ritz and Spiess 2008) packages. For rimsulfuron and imazapic as well as indaziflam at 1 d and 7 d wait periods, the AR model was chosen as the best fit. The AR equation used to regress rain amount (mm) with percent desorption of intercepted herbicide was:

$$Desorption = A_{max}\,X\,\{1 - \exp\left[(log0.1)X\left(\frac{r}{r_{80}}\right)\right]\}$$

where desorption is a percentage of the total intercepted herbicide, A_{max} is the maximum desorption at large values of r, r is rainfall amount, and r_{80} is the rainfall amount required for 80% of maximum desorption to occur. The AR procedure was performed, and parameter estimates of A_{max} and r_{80} values with standard errors were established. A likelihood ratio test was conducted to compare the parameters statistically among time periods and herbicides. Linear regression was chosen as the best fit for indaziflam data from the 0 d time point, and regression analysis and ANOVA were performed in R v. 3.4.3 (R Core Team 2017).

RESULTS AND DISCUSSION

Interception by Litter

Herbicide and herbicide by litter type were not significant ($P = 0.807$ and $P = 0.1631$, respectively), although there was a difference in interception among the litter types ($P < 0.001$). Therefore, an ANOVA was conducted for all herbicides combined with litter type and litter amount included as main effects. More herbicide was intercepted by the high litter amount (2,600 kg ha^{-1}) compared to the low litter amount (1,300 kg ha^{-1}) ($P < 0.001$) across all three litter types (Figure 4.1). Downy brome litter also intercepted a higher percent of the herbicide than medusahead and ventenata litter at both the low and high litter amounts ($P < 0.001$) (Figure 4.1). At the low litter amount (1,300 kg ha^{-1}), downy brome intercepted 69.9 ± 1.1% (mean ± SE) of the herbicide, while ventenata and medusahead intercepted 52.5 to 54.0 ± 1.3%. Downy brome at the high litter amount (2,600 kg ha^{-1}) intercepted 84.3 ± 1.0% of the herbicide, while ventenata and medusahead averaged 75.5 to 76.4 ± 1.0% interception (Figure 4.1). We were not expecting to observe different interception rates for the three litter types at the same weights, which indicates there may be a difference in surface area between the litter types.

Several field studies reported better control with annual grass herbicides in sites where litter has been removed compared to sites with litter (Kessler et al. 2015; Kyser et al. 2007; Sheley et al. 2007; Monaco et al. 2005). Herbicide interception by the litter layer could account for the inconsistent control observed in the field. In high litter situations, less than 25% of soil active herbicides may be available immediately after application. The high litter amount used in our study (2,600 kg ha^{-1}) is based on the biomass present in the site where the downy brome litter was collected, although past studies have reported litter amounts of over 8,000 kg ha^{-1} (Ogle et al. 2003; Evans and Young 1970). Kessler et al. (2015) demonstrated herbicide interception increased in a linear relationship as downy brome litter increased. In the current study, 14 to 24% more herbicide was intercepted by the litter as the amount increased from 1,300 to 2,600 kg ha^{-1} (Figure 4.1); therefore, at very high litter sites, herbicide interception could approach 100%.

Desorption Comparison Among Litter Types

For all three herbicides, total desorption from the three litter types yielded no differences with 12 mm of simulated rainfall at both 0 d and 1 d after application (Table A3.1). These data suggest that herbicide desorption is not dependent on litter type. Because downy brome is more widespread than medusahead and ventenata, we conducted a more in-depth desorption study using only downy brome litter. This comparison study demonstrates that the desorption study with downy brome should be applicable to medusahead and ventenata litter.

Herbicide Desorption from Downy Brome Litter

For herbicide desorption from downy brome litter, differences were observed among herbicides and wait periods. Overall, rimsulfuron and imazapic behaved similarly in the amount that could be recovered from the litter for each time point. For rimsulfuron, parameter estimates indicated that all the herbicide could be desorbed with rainfall at 0 d, while only 64.6 to 72.3% could be desorbed at 1 d and 7 d (Table 4.1, Figure 4.2). Based on the A_{max} parameters for imazapic, rainfall is estimated to desorb a maximum of 101.2, 69.5 and 66.2% of the herbicide on the litter at 0 d, 1 d, and 7 d, respectively (Table 4.1, Figure 4.3). A comparison of the A_{max} parameter indicates the higher desorption at 0 d was significant compared to 1 d and 7 d for both rimsulfuron and imazapic ($P < 0.001$), while there was not a difference between the A_{max} at 1 d and 7 d (rimsulfuron, $P = 0.2478$; imazapic, $P = 0.3972$). The model estimated that for rimsulfuron only 8.3, 10.9, and 13.1 mm of rainfall would be required to achieve 80% of the total desorption (r_{80}) realized at 0 d, 1 d, and 7 d, respectively (Table 4.1). For imazapic, the r_{80} parameters estimated that between 6 to 9 mm of rainfall was needed to achieve 80% of the total desorption at any time period (Table 4.1).

Indaziflam desorption at 0 d was linear and there was a positive correlation between rainfall amount and desorption ($R^2 = 0.96$, $P < 0.001$). At the lowest rainfall amount (3 mm), 9.3 ± 1.1% of the intercepted herbicide was removed from the litter, while 53.7 ± 1.9% was desorbed with the highest rainfall amount (24 mm) (Figure 4.4). Although this relationship was linear, it would eventually become asymptotic as it reaches the maximum amount than can be desorbed from the litter. The 1 d and 7 d wait periods fit an asymptotic curve, and the AR model estimated an A_{max} of 37.7 and 40.9% desorption, respectively. Comparison of the A_{max} indicated no difference between the two time points ($P = 0.54$) (Table 4.1, Figure 4.4). The r_{80} parameters indicated that 19 and 24 mm of rainfall is required to achieve 80% of the maximum desorption at

1 d and 7 d, respectively. Although 0 d data could not be directly compared to 1 d and 7 d using the A_{max} value, the fact that these data presented as linear instead of asymptotic demonstrates there were differences in desorption with rainfall at 0 d compared to the other wait periods.

For rimsulfuron and imazapic, 12 mm of rainfall was sufficient to achieve maximum desorption at all time points. Although nearly all the herbicide was recovered when rainfall was received immediately after application (0 d), over 30% of the intercepted herbicide could not be desorbed from the litter with 24 mm of rainfall 1 d and 7 d after application (Table 4.1; Figures 4.2 and 4.3). For indaziflam, desorption continued to increase between the 12 and 24 mm rainfall amounts, although only 53.7 ± 1.9% could be desorbed from the litter even with immediate rainfall (0 d). Recovery rates went down to an average of 32.6 ± 1.1% by 1 d and 7 d after application (Table 4.1, Figure 4.4). Interestingly, for all three herbicides there were no differences in maximum desorption if rainfall was received 1 d or 7 d after application.

The amount of herbicide recovered from the litter decreases as time without rainfall increases, implying irreversible binding to litter may be occurring (da Silva 2018; Carbonari et al. 2016; Kessler et al. 2015; Tofoli et al. 2009). Johnson et al. (2000) determined adsorption of imazethapyr to soil was time-dependent, with a rapid, initial adsorption phase occurring in the first 1 to 4 d following application, and adsorption becoming stronger over time. This same time-dependent binding may be occurring as herbicides bind to the lipophilic litter components. Kessler et al. (2015) found that when imazapic and tebuthiuron were allowed to interact with downy brome litter for 7 d, only 69.5% of imazapic and 59.5% of tebuthiuron could be recovered with 15 mm of rainfall. Further litter extractions found that between 15% and 25% of the imazapic and tebuthiuron could not be desorbed from the litter even with methanol (Kessler et al. 2015). There was a 30 to 40% decrease in herbicide recovery when rainfall was delayed for 1 d after application, although there was no additional decrease between 1 DAT and 7 DAT. Other studies have demonstrated that herbicide recovery continues to decrease as time without rainfall increases from 1 to 60 DAT (Carbonari et al. 2016; Tofoli et al. 2009; Cavenaghi et al. 2007).

In our study, the recovery rate for indaziflam was approximately half that for rimsulfuron and imazapic at all three time points, suggesting that the amount of herbicide that can be desorbed from the litter with rainfall is dependent on the physical and chemical characteristics of the herbicide. Rimsulfuron and imazapic are both highly water-soluble herbicides, with log K_{ow} of -1.47 and 0.393, respectively, while indaziflam is lipophilic, with a log K_{ow} of 2.8 (Shaner 2014a; Shaner 2014b; Tompkins 2010). Since lipophilicity increases adsorption to organic matter for most herbicides, the lipophilic nature of indaziflam appears to increase its adsorption to litter (Cox et al. 2000; Barak et al. 1983; Hance 1965).

Inconsistencies in winter annual grass control provided by soil applied herbicides have been reported (Kyser et al. 2013; Mangold et al. 2013; Sebastian et al. 2017a; Sebastian et al. 2016; Shinn and Thill 2004). These inconsistencies may be due to the amount of litter present at a site and how soon rainfall occurs after herbicide application. This information is critical for land managers using soil applied herbicides, especially in high litter sites. Applying herbicides before forecasted rain could potentially improve their performance, as maximum desorption from the litter may be attained with as little as 12 mm of rainfall. Because lipophilic herbicides such as indaziflam may bind more readily to litter, tank mixing these herbicides with a more water-soluble partner could potentially improve PRE control, especially in low rainfall areas. Another option for land managers is to combine soil applied herbicides with glyphosate and apply during native species dormancy, while winter annual grasses are in a semi-dormant state (Sebastian et al. 2017a). This combination would provide immediate, POST winter annual grass control from the glyphosate and allow time for precipitation events to desorb the soil applied herbicide from the residue, providing PRE control of seeds germinating from the soil seed bank.

Additionally, land managers could consider using the higher end of application rates, as defined on the label, in high litter situations. This may increase the amount of active ingredient reaching the soil immediately after application and with subsequent rainfall events. Even though a large percentage of the herbicide may be bound to the litter layer, long-term control with indaziflam has been achieved with very low rates (44 to 102 g ai ha^{-1}) in high litter sites, outperforming hydrophilic herbicides, imazapic and rimsulfuron (Sebastian et al. 2017a; Sebastian et al. 2016). Research has suggested that herbicides may be released from the litter in active form as it decays, providing a slow release of the herbicide back to the soil and extending control (Dao 1991), although additional research is needed to determine impacts of litter decay for indaziflam.

Table 4.1: Model parameters (with standard errors) for the asymptotic regression model fit to rimsulfuron, imazapic, and indaziflam desorption data sets.

Treatment	A_{max}	r_{80}	Observed maximum[a]
	-------No. (SE)-------		
Rimsulfuron			
0 d	103 (2.7)	8 (0.6)	103
1 d	65 (3.3)	11 (1.4)	77
7 d	72 (5.7)	13 (2.5)	71
Imazapic			
0 d	101 (2.3)	6 (0.4)	101
1 d	70 (3.0)	8.5 (1.1)	72
7 d	66 (2.4)	8 (0.8)	67
Indaziflam[b]			
0 d	n/a	n/a	60
1 d	38 (2.3)	19 (2.4)	36
7 d	41 (4.6)	24 (4.8)	36

[a] Observed maximum indicates the maximum value obtained by any single observation in the study.

[b] Indaziflam at 0 d was fit with linear regression, therefore only the observed maximum is reported.

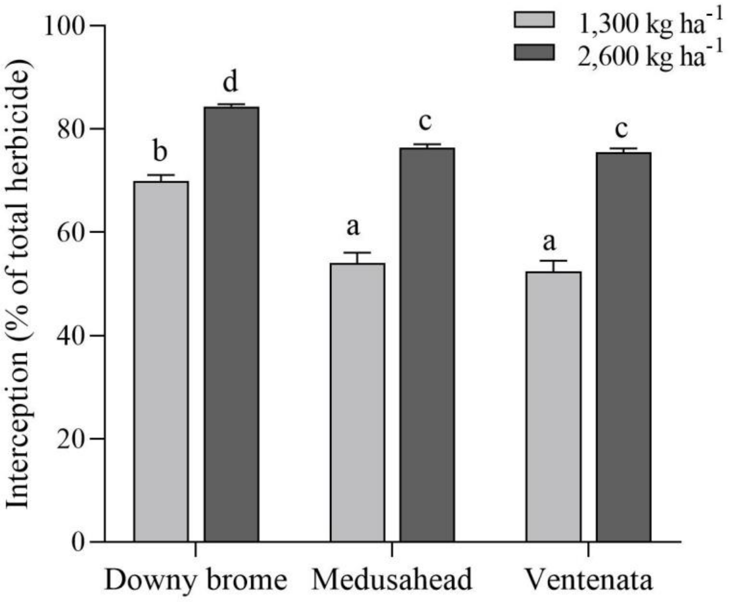

Figure 4.1: Amount of herbicide intercepted by downy brome (*Bromus tectorum*), medusahead (*Taeniatherum caput-medusae*), and ventenata (*Ventenata dubia*) litter, as a percentage of total herbicide applied, at two litter amounts (1,300 kg ha^{-1} and 2,600 kg ha^{-1}). The data for rimsulfuron, imazapic, and indaziflam were combined for analysis of variance. Letters indicate differences among litter types and litter amount, using least squares means ($P < 0.05$).

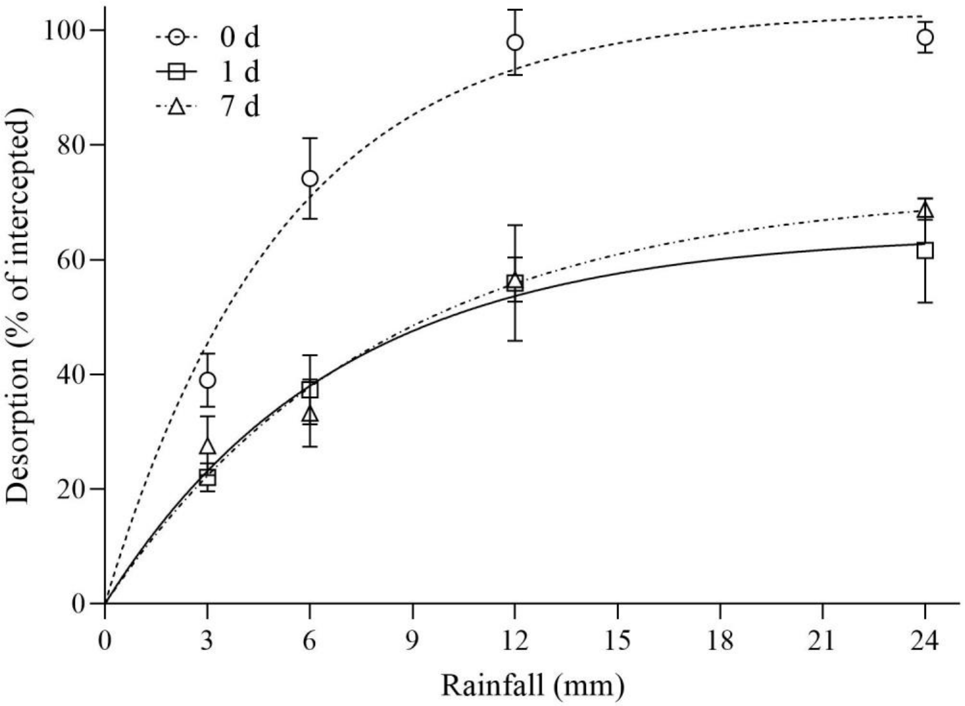

Figure 4.2: Rimsulfuron desorption from downy brome (*Bromus tectorum*) litter as a function of the amount of simulated rainfall after 0 days (0 d), 1 day (1 d), and 7 days (7 d) expressed as a percentage of total herbicide intercepted. Data points are the means of replications with bars indicating the standard error of the mean (n=6): 0 d: y=103.45*{1-exp[(log0.1)*(24/8.35)]}; 1 d: y=64.58*{1-exp[(log0.1)*(24/10.87)]}; 7 d: y=72.31*{1-exp[(log0.1)*(24/13.06)]}.

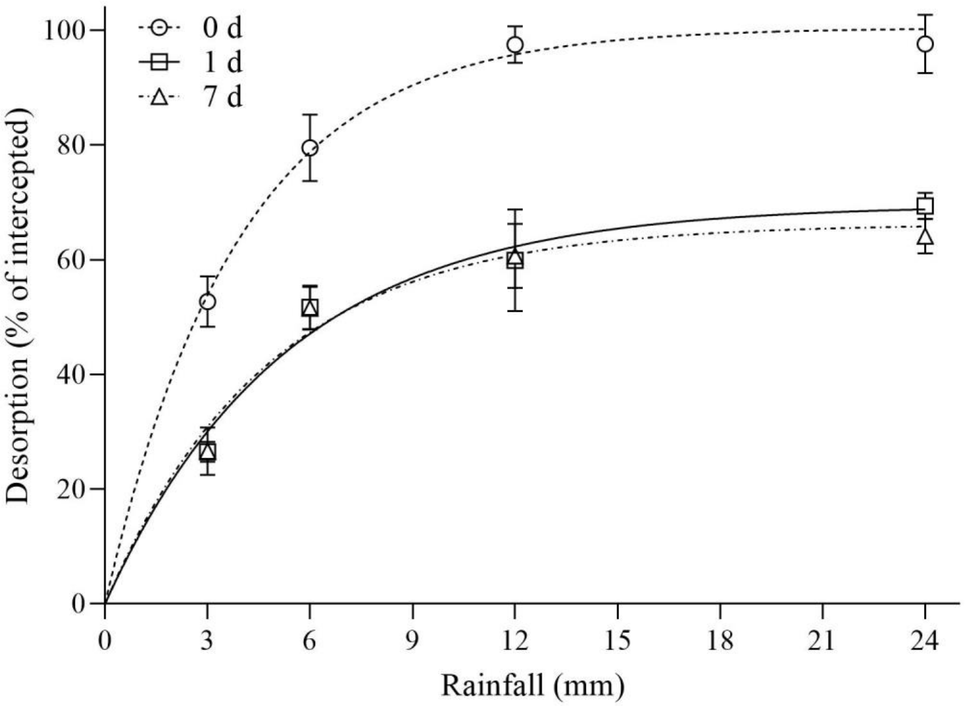

Figure 4.3: Imazapic desorption from downy brome (*Bromus tectorum*) litter as a function of the amount of simulated rainfall after 0 days (0 d), 1 day (1 d), and 7 days (7 d) expressed as a percentage of total herbicide intercepted. Data points are the means of replications with bars indicating the standard error of the mean (n=6): 0 d: y=101.19*{1-exp[(log0.1)*(24/6.39)]}; 1 d: y=69.53*{1-exp[(log0.1)*(24/8.54)]}; 7 d: y=66.22*{1-exp[(log0.1)*(24/7.69)]}.

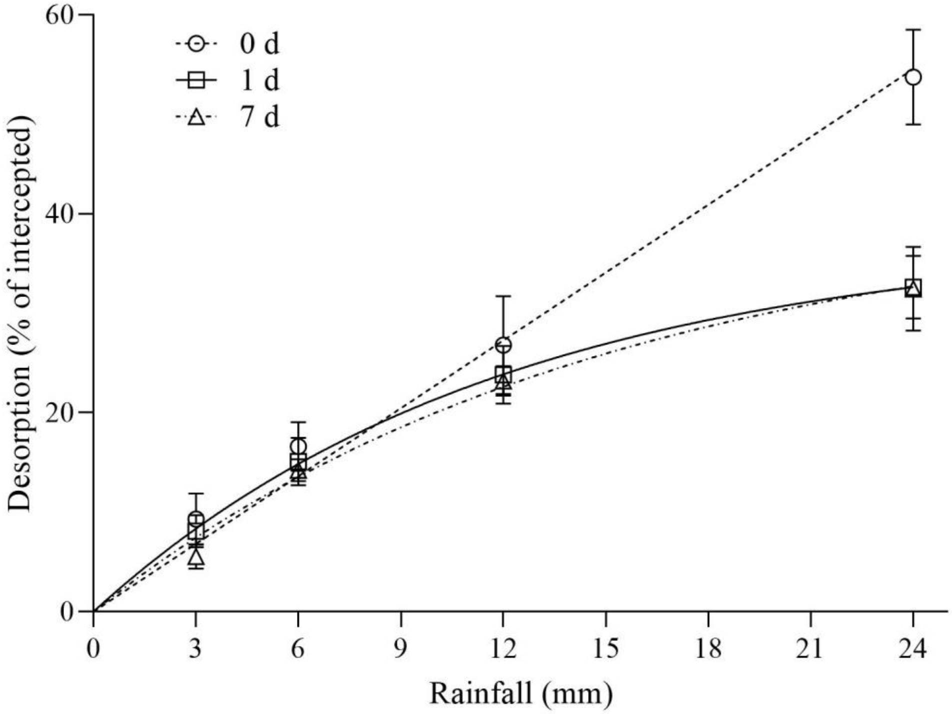

Figure 4.4: Indaziflam desorption from downy brome (*Bromus tectorum*) litter as a function of the amount of simulated rainfall after 0 days (0 d), 1 day (1 d), and 7 days (7 d) expressed as a percentage of total herbicide intercepted. Data points are the means of replications with bars indicating the standard error of the mean (n=6): 0 d: y=2.27x, $R^2 = 0.94$; 1 d: y=37.72*{1-exp[(log0.1)* (24/19.29)]}; 7 d: y=40.89*{1-exp[(log0.1)*(24/23.99)]}.

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Appendix 1: Effect of Indaziflam on Native Species in Natural Areas and Rangeland

Table A1.1: Results of the factorial ANOVA to evaluate the effects of ten herbicide treatments. Analysis is shown for percentage cover of downy brome, cool season grasses, and warm season grasses.

	Percentage cover				
	Downy brome	C3 grass		C4 grass	
Source of variation	Site 2	Site 1	Site 2	Site 1	Site 2
Treatment	<0.001	< 0.001	0.6324	0.4656	0.0381
Year	0.1975	0.0217	0.0074	0.6975	0.4358
Treatment by year	0.1007	0.7632	0.2686	0.3899	0.7792

Table A1.2: Mean percentage cover of perennial warm season (C_4) grasses at both sites 1 and 2 YAT. Means followed by the same letter do not differ significantly at $P < 0.05$.

	Perennial C4 grass cover			
	Site 1		Site 2	
	1 YAT	2 YAT	1 YAT	2 YAT
	%		%	
Non-treated control	19 a	24 a	46 a	48 a
Picloram	27 a	15 a	43 a	39 a
Aminocyclopyrachlor	8 a	17 a	58 a	45 a
Imazapic	14 a	12 a	38 a	41 a
Indaziflam 44	19 a	26 a	65 a	61 a
Indaziflam 73	13 a	27 a	60 a	61 a
Indaziflam 102	19 a	21 a	48 a	55 a
Aminocyclopyrachlor + indaziflam	28 a	26 a	63 a	59 a
Aminocyclopyrachlor + imazapic	20 a	14 a	41 a	45 a
Picloram + indaziflam	25 a	17 a	52 a	70 a
Picloram + imazapic	23 a	15 a	52 a	60 a

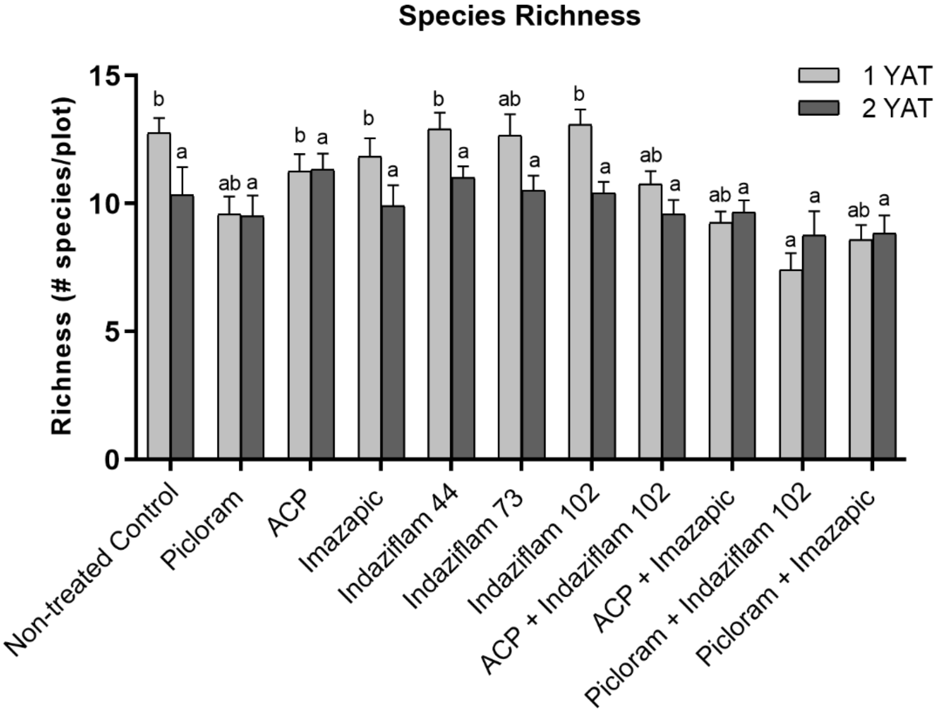


Figure A1.1: Species richness (#) for each treatment combined across sites, 1 YAT (2016) and 2 YAT (2017). Letters indicate significant differences among herbicide treatments across years, using least-squares means ($P < 0.05$). Herbicide treatment rates are as follows: picloram (227 g ai ha^{-1}), aminocyclopyrachlor (ACP, 57 g ai ha^{-1}), imazapic (105 g ai ha^{-1}), indaziflam (I, 44, 73 and 102 g ai ha^{-1}), and non-treated control.

Appendix 2: Evaluating Winter Annual Grass Control and Native Species Establishment Following Applications of Indaziflam on Rangeland

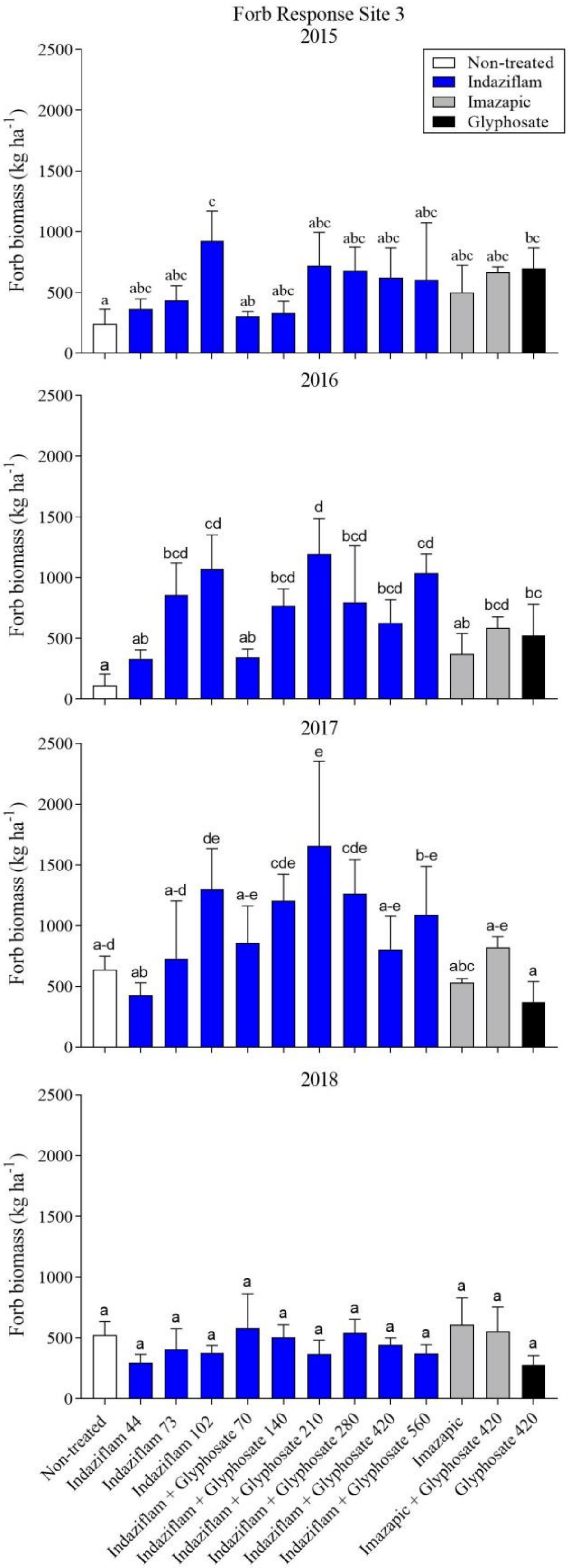
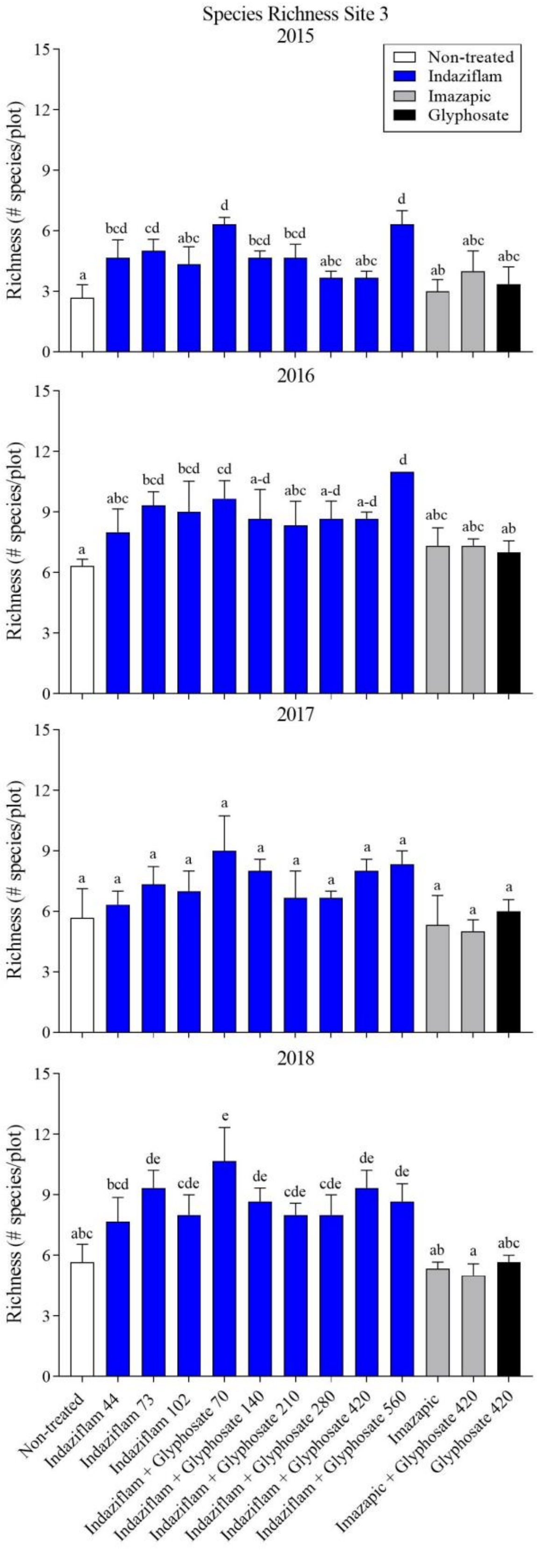

Forb Response Site 3
2015
Non-treated
Indaziflam
Imazapic
Glyphosate
Forb biomass (kg ha^{-1})
0
500
1000
1500
2000
2500
a
abc
abc
c
ab
abc
abc
abc
abc
abc
abc
abc
bc
2016
a
ab
bcd
cd
ab
bcd
d
bcd
bcd
cd
ab
bcd
bc
2017
a-d
ab
a-d
de
a-e
cde
e
cde
a-e
b-e
abc
a-e
a
2018
a
a
a
a
a
a
a
a
a
a
a
a
a
Non-treated
Indaziflam 44
Indaziflam 73
Indaziflam 102
Indaziflam + Glyphosate 70
Indaziflam + Glyphosate 140
Indaziflam + Glyphosate 210
Indaziflam + Glyphosate 280
Indaziflam + Glyphosate 420
Indaziflam + Glyphosate 560
Imazapic
Imazapic + Glyphosate 420
Glyphosate 420

Figure A2.1: Forb biomass response to herbicide treatments at Site 3, year of treatment (2015), 1 YAT (2016), 2 YAT (2017), and 3 YAT (2018). Letters indicate differences among herbicide treatments by year, using least-squares means ($P < 0.05$). Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).



Species Richness Site 3

2015

2016

2017

2018

Figure A2.2: Species richness at Site 3, year of treatment (2015), 1 YAT (2016), 2 YAT (2017) and 3 YAT (2018). Letters indicate differences among herbicide treatments by year, using least-squares means ($P < 0.05$). Herbicide treatments are as follows: Indaziflam (Indaz, 44, 73, 102 g ai ha^{-1}), indaziflam (Indaz, 73 g ai ha^{-1}) plus glyphosate (Glyph, 70, 140, 210, 280, 420, 560 g ae ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}), imazapic (Imaz, 122 g ai ha^{-1}) plus glyphosate (Glyph, 420 g ae ha^{-1}), and glyphosate (Glyph, 420 g ae ha^{-1}).

Appendix 3: Interception, adsorption and desorption of herbicides applied to winter annual grass litter

Table A3.1: ANOVA results for comparison of herbicide desorption from downy brome (*Bromus tectorum*), ventenata (*Ventenata dubia*) and medusahead (*Taeniatherum caput-medusae*) litter with 12 mm rainfall at 0 days (0 d) and 1 day (1 d) after treatment.

Treatment	*df*	F	P value
Rimsulfuron			
0 d	2	3.7094	0.051
1 d	2	0.1972	0.8245
Imazapic			
0 d	2	1.7652	0.2495
1 d	2	1.7426	0.2531
Indaziflam			
0 d	2	0.349	0.7188
1 d	2	1.2548	0.3153