DISSERTATION

TURNING THE TIDE ON THE SAGEBRUSH SEA: LONG-TERM INVASIVE ANNUAL GRASS CONTROL AND RANGELAND RESTORATION WITH INDAZIFLAM

Submitted by

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ABSTRACT

TURNING THE TIDE ON THE SAGEBRUSH SEA: LONG-TERM DOWNY BROME CONTROL AND RANGELAND RESTORATION WITH INDAZIFLAM

The invasive winter annual grass downy brome (*Bromus tectorum* L.) has invaded vast expanses of sagebrush-grassland in western North America, and the fine fuel associated with invasion increases the frequency of burning such that native plants struggle to persist. Recent research suggests that *B. tectorum* invasion may expand across an even larger portion of the US Intermountain West in the absence of effective and proactive management. The herbicide imazapic is widely used to manage *B. tectorum*, but control often declines after one year and reinvasion is typical. Several trials have demonstrated that the newer herbicide, indaziflam, can selectively control annual grasses for three or more years, and past studies indicate that *B. tectorum* seed banks are relatively short-lived in the field (<5 years). This suggests that consecutive years of control with indaziflam may eliminate *B. tectorum* seed banks and increase the duration of control, but it is unclear if this will require multiple applications. In addition, existing studies evaluating the effects of treatment on native rangeland plant communities are limited by small plot sizes, and the potential for impacts to native species seed banks is unclear.

The studies detailed in Chapter 1 evaluated the effectiveness of imazapic and indaziflam for reducing *B. tectorum* density and cover over a period of approximately 5 years (57 months after treatment; MAT) at two invaded sagebrush-grassland sites near Pinedale, Wyoming. Treatments included three different indaziflam rates (51, 73, and 102 g ai ha⁻¹) and imazapic (123 g ai ha⁻¹) and were reapplied to half of each plot 45 MAT to evaluate the effects of two sequential applications. Perennial grass cover was also measured because positive perennial grasss responses were observed after release from *B. tectorum* competition in other studies, and perennial grasses may provide resistance to *B. tectorum* reinvasion. Intermediate and high indaziflam rates (73 and 102 g ai ha⁻¹) resulted in significant reductions in *B. tectorum* cover and density 45 MAT, and perennial grass cover responded positively to some treatments early in the study. Imazapic reduced *B. tectorum* initially, but did not have a

significant effect on density or cover at either site beyond 21 MAT. Reapplication did not substantially improve *B. tectorum* control 57 MAT in plots treated with intermediate and high indaziflam rates, suggesting that long-term control with a single indaziflam treatment may be possible in some cases.

The studies detailed in Chapter 2 assessed the potential for non-target impacts resulting from indaziflam treatment. Modified-Whittaker multiscale vegetation plots were used to compare diversity (species accumulation) in three treatment (73 g ai ha⁻¹ indaziflam) and three control plots in a sagebrush-grassland plant community near Pinedale, Wyoming that is invaded by *B. tectorum*. In addition, a seed bank assay assessed the density and richness of shallow (0-1 cm depth) and deep (1-5 cm depth) germinable seed banks in these same treatment and control plots during a 20-week greenhouse study. Vegetation data and seed bank samples were collected during the third growing season after treatment. Species diversity did not differ between treatment and control plots, but this contrasted with the results of the seed bank assay, which showed that the shallow and deep seed banks had significantly fewer germinable seeds and native species richness was significantly lower in the shallow seed bank of treated areas. While significant non-target impacts to native annual seed banks were observed, all impacted species were detected in the aboveground plant community in treatment plots after treatment occurred, suggesting that reduced native annual abundance may be temporary.

The results presented herein suggest that long-term *B. tectorum* control is possible with a single indaziflam application, and that when indaziflam treatment without associated revegetation is a suitable management intervention (i.e., invaded perennial communities), the benefits of protecting and promoting established perennial plants likely far outweigh the potential for non-target impacts to native species and native species seed banks. The ultimate goal of any weed management program is to reduce the impacts of invasive species to the greatest degree possible with the resources available, whether that is through eradication or conciliation and containment. Our results suggest that in the case of *B. tectorum* invading established sagebrush-grassland plant communities, indaziflam will have a significant role to play in helping managers achieve this objective.

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CHAPTER 1: Indaziflam reduces downy brome (*Bromus tectorum* L.) density and cover five years after treatment in sagebrush-grasslands with no impact on perennial grass cover

SUMMARY¹

The invasive annual grass downy brome (Bromus tectorum L.) is one of the most critical threats to the semi-arid shrublands that characterize western North America. More abundant fine fuel after invasion typically increases fire frequency in plant communities adapted to relatively infrequent burning, reducing the likelihood of native plant persistence. Currently, imazapic is most often used to manage B. tectorum, but reinvasion from the seed bank after treatment is common. Indaziflam is a newer herbicide recently labeled for use in rangelands grazed by livestock, and many research trials have demonstrated its ability to deplete invasive annual grass seed banks. We sought to evaluate the effectiveness of these herbicides for reducing B. tectorum density and cover over a period of approximately 5 years (57 months after treatment; MAT) at two invaded sagebrush-grassland sites near Pinedale, Wyoming. Treatments included three different indaziflam rates (51, 73, and 102 g ai ha⁻¹) and imazapic (123 g ai ha-1) and were reapplied to half of each plot 45 MAT to evaluate the effects of two sequential applications. We also measured perennial grass cover because positive perennial grass responses were observed after release from B. tectorum competition in other studies, and perennial grasses may provide resistance to B. tectorum reinvasion. Intermediate and high indaziflam rates (73 and 102 g ai ha⁻¹) resulted in significant reductions in B. tectorum cover and density 45 MAT, and perennial grass cover responded positively to some treatments early in the study. Imazapic reduced B. tectorum initially, but did not have a significant effect on density or cover at either site after 21 MAT. Reapplication did not substantially improve B. tectorum control 57 MAT in plots treated with intermediate and high indaziflam rates, suggesting that long-term control with a single indaziflam treatment may be possible in some cases.

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INTRODUCTION

Downy brome (*Bromus tectorum* L.) invasion into the sagebrush ecosystems of western North America is one of the most critical threats facing these important rangelands (Clark 2020; DiTomaso et al. 2010; Smith et al. 2021). *Bromus tectorum* opportunistically germinates whenever conditions are favorable, utilizing important soil resources before native plants and exploiting the striking heterogeneity of the rangelands that characterize this region (Beck 2009; Knapp 1996; Mack 1981). Variable germination timing, along with other characteristics including a rapid growth rate (Arredondo et al. 1998), high seed production (Young et al. 1987), and acquisitive root morphology (Aguirre and Johnson 1991; Arredondo and Johnson 1999), increase the likelihood of successful *B. tectorum* establishment. The altered fire regimes that typically follow invasion promote its increasing dominance, continued spread, and severe impacts to native plant communities (Clark 2020; Davies 2011; Davies et al. 2021a; West 1983).

Historical fire regimes are difficult to determine in relatively arid plant communities with few trees, but the long recovery time of big sagebrush (*Artemisia tridentata* Nutt.), the region's most iconic plant, suggests that fires were relatively infrequent in the past and limited in their extent (Miller et al. 2013; Schlaepfer et al. 2014). This is thought to have maintained a mosaic of shrub- and perennial grass-dominated plant communities in different phases of recovery from wildfire (Davies and Bates 2020; McAdoo et al. 2013), and supported a variety of different habitat types for the region's diverse wildlife (Burkhardt 1996; McAdoo et al. 2004). After invasion, more and more continuous fine fuel resulting from *B. tectorum* litter production can substantially increase the likelihood of wildfire ignition and the rate of wildfire spread where and when *B. tectorum* occurs (Balch et al. 2013; Bradley et al. 2018; Davies and Nafus 2013).

More frequent wildfires can be difficult for relatively slow-growing native perennials to cope with, but annual *B. tectorum* can recover rapidly (Humphrey and Schupp 2001; Perryman et al. 2020; Young and Evans 1978, Young et al. 1987), resulting in a destructive grass-wildfire feedback loop that has been identified in a variety of arid and semi-arid ecosystems worldwide (D'Antonio and Vitousek 1992). Across 51 paired burned and unburned sagebrush sites, Swanson et al. (2018) found that native plant cover declined after fire in all cases where pre-fire *B. tectorum* cover exceeded 15%, and Bradley et al. (2018) estimate that *B. tectorum* cover exceedes 15% over approximately 30% of the US Intermountain West. Recent estimates suggest that the current extent of western North America's sagebrush steppe represents roughly half of what it was historically (Miller et al. 2011), in large part due to this destructive fire-invasion feedback loop.

The rate of *B. tectorum* expansion makes the situation even more pressing. Smith et al. (2021) estimated that the area of *B. tectorum*-dominated communities has increased eightfold since 1990 in the Great Basin, with the most rapid expansion occurring in the most recent decade they considered (2011-2020). While relatively cold, high elevation rangelands have long been considered more resistant to *B. tectorum* invasion (Chambers et al. 2014), this may be changing and expansion into higher elevation rangelands would allow *B. tectorum* invasion to continue unabated over an even larger portion of the region (Mealor et al. 2012; Smith et al. 2021). These findings suggest that, unless effective management interventions are developed and deployed, *B. tectorum* is likely to continue severely impacting rangeland ecosystems in western North America, incurring substantial costs associated with wildland firefighting and restoring repeatedly burned landscapes (Davies et al. 2021a; Mack 2011; Perryman et al. 2018).

Existing approaches to manage *B. tectorum* often provide short-term reductions in abundance (<2 years after treatment; YAT), but long-term control is difficult to achieve and reinvasion is common without continued management (Mack 2011; Monaco et al. 2017). Imazapic is a broad-spectrum herbicide that inhibits the enzyme acetolactate synthase. Imazapic is selective against annual grasses at low use rates (Kyser et al. 2013), and until recently, was the most widely used herbicide for managing annual grasses because of its ability to provide both pre- and post-emergent control (Mangold et al. 2013). Imazapic treatment has provided variable results (Applestein et al. 2018; Mangold et al. 2013), often reducing *B. tectorum* in the first year after treatment but having inconsistent long-term effects on *B. tectorum* abundance (Davison and Smith 200; Elseroad and Rudd 2011; Morris et al. 2009; Munson et al. 2015).

Indaziflam is a broad-spectrum, pre-emergent herbicide recently approved for use on rangelands and natural areas, including those grazed by livestock (US EPA, 2020). A cellulose biosynthesis inhibitor with a unique site of action and no reported cases of resistance (Brabham et al. 2014; Tateno et al. 2016), indaziflam treatment often results in multi-year annual grass control (Clark 2020; Sebastian et al. 2016; Sebastian et al. 2017a). This herbicide is uniquely suited to managing *B. tectorum* because of its selectivity and long period of residual activity (Clark 2020; Sebastian et al. 2016). Indaziflam binds tightly to soil organic matter and remains near the soil surface where it can selectively inhibit the root growth of germinating *B. tectorum* seeds without harming established perennials with deeper roots (Clark 2020; Sebastian et al. 2017a). Further, because it typically provides three or more years of control and *B. tectorum* seed banks are generally short-lived in the field (\leq 5 years; Burnside et al. 1996; Sebastian et al. 2017b; Smith et al. 2008), indaziflam could deplete *B. tectorum* seed banks with a single application, or a sequence of applications spaced several years apart. While non-target impacts to native annual plants have been observed (Courkamp and Meiman, unpublished data; Meyer-Morey et al. 2021), field trials across the western US have demonstrated indaziflam's effectiveness for controlling invasive annual grasses with no apparent impacts to established perennial plants (Clark et al. 2019; Clark et al. 2020; Hart and Mealor 2021; Sebastian et al. 2016; Sebastian et al. 2017a).

Perennial bunchgrasses are a key component of rangeland plant communities that increase their resistance and resilience to annual grass invasion and wildfire, respectively (Applestein and Germino 2021; Blank et al. 2020; Chambers et al. 2014; Davies and Johnson 2017). Thus, proactively treating invaded areas that continue to support relatively abundant perennial grasses may represent a highly effective approach to *B. tectorum* management. While sagebrush steppe perennial grasses typically live longer than the expected period of residual activity from indaziflam treatment (Svejcar et al. 2014), they rely on recruitment from seed for long-term persistence (Hamerlynck and Davies 2019), which suggests that they may be sensitive to non-target impacts from repeated indaziflam treatments that extend residual activity over longer periods of time.

The objectives of our study were to: (1) evaluate the effectiveness of indaziflam in high-elevation, cold desert shrublands where *B. tectorum* invaded communities continue to support abundant perennial grasses; and (2) identify an effective herbicide treatment (application rate and number of applications) for land managers in these areas to deplete *B. tectorum* seed banks and achieve long-term control with minimal harm to established perennial grasses. In light of these objectives, we addressed the following questions:

(1) What rates of indaziflam treatment reduce *B. tectorum* cover and density for 3+ years in cold desert sagebrush-grasslands?

(2) Does indaziflam outperform imazapic in terms of reducing *B. tectorum* cover and density in cold desert sagebrush-grasslands?

(3) Is reapplication necessary to prevent long-term (>3 YAT) reinvasion from the *B. tectorum* seed bank? If so, does this change depending on application rate?

(4) Does indaziflam or imazapic treatment, including reapplication, reduce the cover of co-

occurring perennial grasses relative to untreated plots?

Consistent with existing studies (Sebastian et al. 2017a; Sebastian et al. 2016), we predicted that indaziflam treatment would significantly reduce *B. tectorum* cover and density for at least 3 years, particularly at higher application rates, and that both indaziflam and imazapic treatment would reduce *B. tectorum* initially (1-2 YAT), but that *B. tectorum* density and cover would increase over time in imazapic-treated plots while control was maintained or improved over time in indaziflam-treated plots. We also predicted that reapplication would be necessary to extend control beyond 3 years, but acknowledged that it may be possible to deplete *B. tectorum* seed banks more quickly in some environments because a variety of factors including climate, fire history, and the specifics of the soil resource environment can affect the likelihood of seed germination and seed bank longevity in the field (Baskin and Baskin 2014; Bazzaz 1996). We also expected treatment to have no observable impact on the cover of co-occurring perennial grasses based on previous research (Clark et al. 2020; Sebastian et al. 2017).

MATERIALS AND METHODS

Site Description

The experiments were established in 2016 at two sites in Sublette Country, Wyoming. Site 1 (610774 m E, 4746164 m N, UTM zone 12 T, approx. 2250 m elevation) was located near Boulder Lake in the Bridger-Teton National Forest, and Site 2 (602945 m E, 4748832 m N, UTM zone 12 T, approx. 2250m elevation) was located in the Half Moon Habitat Management Unit managed by the Wyoming Game and Fish Department. These study sites were approximately 8 km apart in the Cold Desert region of the North American Deserts ecoregion; Site 1 was characterized by a coarse upland ecological site (R043BY208WY), and Site 2 was characterized by a shallow loamy ecological site (R043BY162WY; USDA-NRCS 2021).

The soil at Site 1 was Pointer-Lateral complex (loamy-skeletal, mixed, superactive, Ustic Haplocryolls) which is characterized by a very cobbly sandy loam surface soil with 3.3% organic matter and 6.8 pH in the top 20 cm (USDA-NRCS 2021). The soil at Site 2 was Blackbear, rubbly-Branham, rubbly-Bobowic complex (loamy-skeletal, mixed, superactive, Pachic Agricryolls) which is characterized by cobbly or gravelly coarse sandy loam surface soil with 5.3% organic matter and 6.6 pH in the top 20 cm (USDA-NRCS 2021). Both sites had south facing aspects but Site 1 was slightly steeper than Site 2.

When treatments were applied, both sites supported plant communities dominated by native perennial bunchgrasses and shrubs, but invaded such that the majority of interspaces between established plants were infested by *B. tectorum*. The most common perennial grasses at both sites were needle and thread [*Hesperostipa comata* (Trin. & Rupr.) Barkworth] and bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh.) Á. Löve], and the most common shrubs included antelope bitterbrush [*Purshia tridentata* (Pursh.) DC], mountain big sagebrush [*Artemisia tridentata* Nutt. ssp. *Vaseyana* (Rydb.) Beetle], and mountain snowberry [*Symphoricarpos oreophilus* A. Gray]. The dominant forb at both sites was arrowleaf balsamroot [*Balsamorbiza sagittata* (Pursh.) Nutt.]. A variety of less common native and non-native plants existed at low abundance at the time of treatment.

Mean annual precipitation based on the 30-yr mean (1981 to 2010) was 294 mm for both sites based on the nearest weather station (located in Pinedale, 16 km from Site 1, 9 km from Site 2; WRCC 2021). Relative to this average, the 2017 and 2019 water years (October-September) were particularly wet (481 mm and 421 mm, respectively) and 2021 was particularly dry (250 mm). All other study years (2016-2021) were within 10% of the 30-year mean (WRCC 2021)

An incidental, human-ignited wildfire (Boulder Lake Fire) burned Site 1 in August 2019. The fire was ignited on August 17, 100% contained on August 26, and declared out September 17 (Teton Interagency Fire 2019). All study plots at this site were completely burned and data from 45 and 57 months after treatment (MAT) at Site 1 were collected approximately 9 and 21 months after the fire, respectively.

Experimental Design

Initial herbicide treatments were applied at both sites on September 9, 2016 using a CO₂-pressurized custom-built backpack sprayer with 11002LP flat-fan nozzles (TeeJet® Spraying Systems, P.O. Box 7900, Wheaton, Il 60187) delivering 187 L ha⁻¹ at 207 kPa. Along with an untreated control, four herbicide treatments were applied to 3 by 9 m plots in a randomized complete block design with four replications at each site. Herbicide treatments included three different indaziflam rates (51, 73, 102 g ai ha⁻¹; henceforth low, intermediate, and high rates, respectively) and imazapic applied at 123 g ai ha⁻¹. At the time of application, native plants were dormant, *B. tectorum* was 100% post seed set, and no *B. tectorum* fall germination was observed.

The same herbicide treatments were reapplied to half of each treated plot approximately 45 months after initial herbicide application to evaluate long-term reductions in *B. tectorum* abundance with one and two applications of each treatment. This resulted in eight different herbicide treatments (one and two applications of four treatments, 3 by 4.5 m plots) along with an undivided control plot. Reapplication occurred on June 26, 2020 at Site 1 and June 27, 2020 at Site 2 using a CO2-pressurized handheld research sprayer with 8002VS flat-fan nozzles (TeeJet® Spraying Systems, P.O. Box 7900, Wheaton, IL 60187) delivering 187 L ha⁻¹ at 207 kPa. At the time of reapplication, native plants were still actively growing and *B. tectorum* was nearing 100% post seed-set. All treatments (initial and reapplication) included a 0.25% v/v nonionic surfactant.

Treatment Evaluations and Data Analysis

To quantify herbicide treatment effects, we used 0.5-m² frames (Bonham et al. 2004) to measure *B. tectorum* density and perennial grass and *B. tectorum* absolute canopy cover (henceforth cover). We counted individual *B. tectorum* plants and recorded ocular estimates of cover to the nearest 1% in these frames. When *B. tectorum* was especially dense, plants were counted in only a portion of the larger frame to estimate density. Prior to reapplication, data were collected from five randomly located frames (subsamples) in each plot and after reapplication, data were collected from three randomly located frames in each divided treatment plot. Data were collected 9, 21, 33, 45 and 57 MAT (June 2017-2021), with data collection 57 MAT occurring 12 months after reapplication.

To test treatment effects on *B. tectorum* density and *B. tectorum* and perennial grass cover prior to reapplication, we used the 'lme4' package in R. v. 3.6.2 (R Core Team 2019) to create linear mixed effects models and ANOVA to test for treatment effects at α =0.05. Due to substantial interannual variability and environmental differences between sites, site and year were analyzed independently in all cases with block included as a random factor. Visual inspection of quantile-quantile and fitted vs. residual plots was used to verify that data met the assumptions of ANOVA. Cover data were arcsine square-root transformed and density data were square-root transformed (n + 0.5) as necessary to meet these assumptions. When ANOVA indicated that significant differences existed between treatments, we used the 'emmeans' package (R Core Team 2019) to obtain pairwise comparisons between treatment groups using a Tukey adjustment (α =0.05).

After reapplication, our experiment did not represent a true split-plot experimental design because no sequential treatment was applied to the control plots and these plots remained undivided. Thus, to analyze data from 57 MAT, each herbicide treatment and sequence (one or two applications) was considered a single experimental treatment; data included eight herbicide treatments and an untreated control 57 MAT. Because *B. tectorum* was nearly absent from all plots at Site 2 at this time, including non-treated controls, we only compared *B. tectorum* cover and density 57 MAT at Site 1. Otherwise, data were analyzed using the same procedure used to compare treatments prior to reapplication. Results presented in the manuscript are in their original dimensions and means are reported with standard errors.

RESULTS AND DISCUSSION

Bromus tectorum Density and Cover

ANOVA results for *B. tectorum* density and cover are shown in Table 1.1. At Site 1, all treatments significantly reduced *B. tectorum* density 9 MAT except the low indaziflam rate (imazapic P<0.001; low P=0.11; intermediate P=0.041; high P=0.017; Figure 1.1a), and all treatments significantly reduced *B. tectorum* density 21 MAT (P<0.01; Figure 1.1a). At Site 2, responses to treatments 9 MAT were more variable; *B. tectorum* density was only significantly reduced compared to the non-treated control in plots treated with the low and high indaziflam rates (low P=0.020; high P<0.01; Figure 1.2a). However, similar to Site 1, all treatments significantly

reduced *B. tectorum* density 21 MAT (imazapic P=0.027; low P<0.01; intermediate P=0.032; high P=0.014; Figure 1.2a). The more variable results at Site 2 may be the result of unexpected fall *B. tectorum* germination occurring prior to herbicide application in September 2016. Indaziflam has no post-emergence activity, so *B. tectorum* plants that emerged prior to application would not be controlled the following spring. While no fall emergence was noted at the time of treatment, dense *B. tectorum* litter was present at both sites and this may have made fall emergence difficult to observe.

Contrary to the variability observed 9 and 21 MAT, the intermediate and high indaziflam rates reduced *B. tectorum* density compared to the non-treated control at both sites 33 MAT and 45 MAT (P<0.01; Figure 1.1a; Figure 1.2a). The effects of imazapic and the low indaziflam rate were less consistent as the study progressed (Figure 1.1a; Figure 1.2a). At Site 1, imazapic and the low indaziflam rate did not reduce *B. tectorum* density compared to the non-treated control 33 MAT (imazapic P=0.82; low P=0.11) and 45 MAT (imazapic P=0.85; low P=0.13; Figure 1.1a). The effects of imazapic at Site 2 mirrored those of Site 1, with no significant effects on *B. tectorum* density 33 MAT (P=0.10) and 45 MAT (P=0.11; Figure 1.2a). However, the low indaziflam rate was comparable to the intermediate and high rates at Site 2, with significant reductions in *B. tectorum* density observed 33 and 45 MAT (P<0.01; Figure 1.2a).

The difference in the effectiveness of the low indaziflam rate between sites may be related to the overall difference in the density of *B. tectorum*. Mean *B. tectorum* density in non-treated control plots ranged from 592-1,441 m⁻² at Site 1 and 381-594 m⁻² at Site 2 over the first three years of the study (Figure 1.1a; Figure 1.2a; 9, 21 and 33 MAT), suggesting that the density of the infestation may have been greater at Site 1 when treatment occurred and in the first few years after treatment. If there were more *B. tectorum* seeds present at Site 1 when treatment occurred, the low indaziflam rate may have been enough to achieve longer term reductions (33 and 45 MAT) in the relatively less invaded community at Site 2, but not the relatively more invaded and propagule-rich community at Site 1.

The effects of treatment on *B. tectorum* cover were less consistent (Figure 1.1b; Figure 1.2b). At Site 1, only imazapic treatment significantly reduced *B. tectorum* cover compared to the non-treated control 9 MAT (P<0.001); all treatments except the low indaziflam rate (P=0.66) significantly reduced *B. tectorum* cover 21 MAT

(imazapic P=0.030; intermediate P=0.033; high P<0.001); and only the intermediate and high indaziflam rates reduced *B. tectorum* cover 33 MAT (P<0.001) and 45 MAT (intermediate P=0.013; high P<0.01; Figure 1.1b). At Site 2, all treatments except the intermediate rate of indaziflam (P=0.62) reduced *B. tectorum* cover compared to the non-treated control 9 MAT (P<0.01); only the low indaziflam rate reduced *B. tectorum* cover 21 MAT (P=0.023); the low and high indaziflam rates reduced *B. tectorum* cover 33 MAT (low P=0.025; high P=0.018), and all indaziflam treatments reduced *B. tectorum* cover 45 MAT (P<0.01; Figure 1.2b).

We predicted that indaziflam treatment would significantly reduce *B. tectorum* cover and density for at least three years, particularly at higher application rates, and that both indaziflam and imazapic treatment would reduce *B. tectorum* initially (1-2 YAT), but that *B. tectorum* density and cover would increase over time in imazapic-treated plots while control was maintained or improved over time in indaziflam-treated plots. Consistent with this prediction, indaziflam treatments at the intermediate and high rates consistently reduced *B. tectorum* density and cover to very low levels 33 and 45 MAT at both sites, and imazapic was generally effective in the near-term, but *B. tectorum* density and cover were not significantly reduced in imazapic-treated plots beyond 21 MAT (Figure 1.1a & b; Figure 1.2a & b).

We observed an overall decline in *B. tectorum* abundance at our sites during the study. This decline was more pronounced at Site 2 and is best reflected by our *B. tectorum* cover data, which shows a steady decline in *B. tectorum* cover in non-treated control plots at both sites over the course of the study (Figure 1.1b; Figure 1.2b). While *B. tectorum* density remained high in non-treated controls through 33 MAT at both sites (592-1,441 m⁻² at Site 1 and 381-594 m⁻² at Site 2), it was much lower 45 MAT (78 m⁻² at Site 1 and 76 m⁻² at Site 2; Figure 1.1a; Figure 1.2a), and *B. tectorum* was nearly absent from all plots 57 MAT at Site 2 (not shown).

This may reflect the relative unsuitability of the region for *B. tectorum*. Higher elevation sagebrush communities are thought to present greater challenges to *B. tectorum* establishment due to colder soil temperatures and more abundant perennial grasses (Chambers et al. 2014), and these same challenges could result in *B. tectorum* extirpation under the right circumstances. One well-studied montane *B. tectorum* population located at a similar elevation (2,328 m) is known to have disappeared between 2005 and 2012 at Strawberry Mountain in Utah (Merrill et al. 2012), and other studies have observed temporary declines in *B. tectorum*

abundance at high elevations related to periodic spring drought (Smith et al. 2021). We observed natural declines in *B. tectorum* at both our sites which are located at the same elevation (approx. 2250 m), but the decline was more pronounced at Site 2. This may be related to differences in the degree of slope between our two sites. Both sites have south-facing aspects, but Site 1 may be slightly warmer due to its steeper slope and this may favor *B. tectorum* by allowing it to begin growing earlier in the season, when resources are abundant and competition with native plants is minimal.

Due to the near absence of *B. tectorum* 57 MAT at Site 2, we only analyzed *B. tectorum* cover and density after reapplication at Site 1 (Table 1.1). We predicted that reapplication would be necessary to maintain reductions in *B. tectorum* density and cover 3+ years after initial treatment, but also acknowledged that it may be possible to deplete *B. tectorum* seed banks more quickly in some environments because a variety of factors including climate, fire history, and the specifics of the soil resource environment can affect the likelihood of seed germination and seed bank longevity in the field (Baskin and Baskin 2014; Bazzaz 1996).

Our results were mostly inconsistent with our prediction because all treatments except single applications of imazapic (P>0.9) and the low indaziflam rate (P>0.9) resulted in significant reductions in *B. tectorum* density compared to the non-treated control 57 MAT (P<0.001; Figure 1.3a), and there was no difference in *B. tectorum* density between treatment groups that received one or two applications of the intermediate and high indaziflam rates (P>0.9; Figure 1.3a). Treatment effects on *B. tectorum* cover after reapplication were similar to those on density; the differences between treatment groups that received one or two applications of the intermediate and high rates of indaziflam were non-significant (P>0.9; Figure 1.3b). However, *B. tectorum* cover was only significantly reduced compared to the non-treated control 57 MAT by two indaziflam applications at these same rates (intermediate P=0.028, high P=0.027; Figure 1.3b). *Bromus tectorum* cover was still very low 57 MAT in plots that received only one application of the intermediate and high rates of indaziflam (Figure 1.3b), but the differences between these plots and the non-treated control plots were only significant at the α =0.10 level (intermediate P=0.071; high P=0.092).

The differences between results for *B. tectorum* density and *B. tectorum* cover may reflect the environmental conditions that escaped *B. tectorum* individuals can expect to experience after indaziflam

treatment. Assuming that herbicide treatment was otherwise effective, an escaped *B. tectorum* plant would have few intraspecific competitors. *Bromus tectorum* plants in dense monocultures often have only one or a few tillers, while solitary individuals in resource-rich environments can produce many tillers (Young et al. 1987; Zouhar 2003). Over the course of our study we regularly observed solitary *B. tectorum* individuals with 25+ tillers in indaziflam-treated plots. These plants can have large impacts on cover with minimal effects on density (Elzinga et al. 1998). They may also increase the likelihood of reinvasion because a solitary escaped *B. tectorum* plant with many tillers can produce hundreds, and perhaps thousands, of seeds (Young et al. 1987; Zouhar 2003); one can assume seeds that matured after treatment will be more likely to outlast indaziflam's residual activity. In some cases, overall seed production has increased with relatively modest reductions in *B. tectorum* density (Hulbert et al. 1955). This is unlikely to occur in situations where *B. tectorum* is reduced to very low levels by herbicide, but our results may illustrate how a relatively small number of escapes can impact cover and, potentially, the likelihood of persistence and reinvasion.

A single indaziflam application can significantly reduce *B. tectorum* density to very low levels 57 MAT demonstrating the potential for managing annual grasses and suggesting that it may be possible to deplete *B. tectorum* seed banks with only one herbicide application at some sites. It is unlikely that indaziflam is still actively inhibiting *B. tectorum* 45 and 57 MAT (Courkamp and Meiman, unpublished data), but if near-complete seed bank depletion occurs in the years immediately following application, control may continue until *B. tectorum* seeds disperse into treated areas from elsewhere. Relatively more precocious seed germination has been associated with *B. tectorum* populations collected in higher-elevation montane environments similar to our study sites (Allen and Meyer 2002; Meyer and Allen 1999), and this may promote more rapid seed bank depletion in these areas because one would expect a greater fraction of the *B. tectorum* seeds present at the time of treatment to germinate in the period of residual activity following a single application. Many factors can influence both *B. tectorum* seed longevity in field conditions (Baskin and Baskin 2014; Bazzaz 1996) and the likelihood of seed dispersal (e.g., Monty et al. 2013), thus we caution against assuming that this outcome will be typical and emphasize the importance of site-specific approaches to annual grass management with indaziflam (Seedorf et al. 2021).

While our results may tempt land managers to consider only one treatment when it appears that the annual grass seed bank is depleted, there are reasons to think that follow-up treatments will be advisable in most circumstances. The large-scale reliability of herbicide treatments in agronomic settings is unlikely to translate directly to rangelands, where underlying heterogeneity dictates that treatments occurring at meaningful scales (e.g., wildfire prevention) will include substantial microsite variation with the potential to influence treatment outcomes (Applestein et al. 2018; Applestein and Germino 2021; Boyd and Svejcar 2009; Heady 1974; Svejcar et al. 2017). For example, if a large tract of rangeland contains relatively more and less densely infested areas, a single treatment applied to the entire area may struggle to deplete the annual grass seed bank in areas with high initial densities, while successfully depleting areas with low initial densities. This may explain the difference we observed between our two sites in the duration of control in plots treated with the low indaziflam rate (Figure 1.1a & b; Figure 1.2a & b).

When treating large areas of rangeland aerially, patch-scale variation in treatment effectiveness is likely even under ideal conditions (e.g., Applestein et al. 2018), and the risk of forgoing a second treatment and allowing *B. tectorum* to reestablish is worth considering. *Bromus tectorum* is known for its rapid population growth (Humphrey and Schupp 2001; Perryman et al. 2020; Young and Evans 1978), and if enough individuals survive until residual herbicide activity has waned, they may be able to rapidly replenish the seed bank and force land managers to start the process of depleting the seed bank for a second time. Compared to a single application, a sequence of two treatments may prevent this by increasing the consistency of treatment outcomes and the likelihood of near-complete seed bank depletion at meaningful spatial scales.

Forgoing a second treatment may also increase the risks of herbicide avoidance over time. While studies suggest that *B. tectorum* seed banks are relatively short-lived (\leq 5 years; Burnside et al. 1996; Sebastian et al. 2017b; Smith et al. 2008), only a small number of populations have been directly investigated and other studies have identified ecologically-relevant heritable variation in *B. tectorum* germination traits across different environments in North America (Allen and Meyer 2002; Meyer and Allen 1999). The offspring of plants that produce relatively longer lasting seeds at the time of treatment may have a better chance of escaping the effects of indaziflam treatment by outlasting the period of residual activity that follows application. Assuming this

variation is heritable, allowing these longer-lasting seeds to germinate and establish after residual activity has waned may encourage the development of a form of temporal herbicide avoidance wherein *B. tectorum* is able to prevent seed bank depletion with longer lasting seeds. Variable seed dormancy has made achieving long-term control very difficult for similar annual weeds (e.g., wild oats; Miller et al. 1982; Seeley 1977), and rapid adaptation in response to selection from management has been observed in some cases (e.g., wild radish; Ashworth et al. 2016). A sequence of at least two treatments is likely to reduce these risks by extending the period of residual herbicide activity and preventing *B. tectorum* reestablishment.

Perennial Grass Cover

We expected indaziflam treatments to have no observable impact on the cover of co-occurring perennial grasses based on previous research (Clark et al. 2020; Hart and Mealor 2021; Sebastian et al. 2017a; Sebastian et al. 2016). Consistent with our expectation, we did not observe any negative effects on perennial grass cover (Figure 1.1c; Figure 1.2c; Figure 1.4a & b). We did observe positive effects on perennial grass cover early in the study, with perennial grass cover increasing relative to the non-treated control 9 MAT in plots treated with imazapic and the high indaziflam rate at Site 1 (imazapic P=0.014; high P=0.039; Figure 1.1c), and plots treated with the low and high indaziflam rates at Site 2 (low P<0.01; high P=0.024; Figure 1.2c). Beyond 9 MAT, we observed no significant treatment effects on perennial grass cover at Site 2 (P>0.09; Figure 1.2c); Figure 1.4b). At Site 1, perennial grass cover was significantly higher than the non-treated control 21 MAT in plots treated with the low indaziflam rate (P=0.026), and the same was true 33 MAT in plots treated with the low indaziflam rates (intermediate P<0.01; high P=0.015; Figure 1.1c). Beyond 33 MAT, we observed no significant treatment effects on perennial grass cover at Site 1 (P>0.22; Figure 1.1c; Figure 1.4a).

Positive effects on perennial grass biomass following indaziflam treatment have been observed in some studies (Clark et al. 2020; Hart and Mealor 2021; Sebastian et al. 2017a; Sebastian et al. 2016), and resource preemption by *B. tectorum* is relatively well-understood and can have substantial negative effects on perennial grass growth and vigor (Ploughe et al. 2020; Nasri and Doescher 1995). Perennial grass cover 33 MAT at Site 1 showed a positive response at intermediate and high indaziflam rates, where *B. tectorum* density and cover were also lowest at this time (Figure 1.1a & b). These data support previous studies demonstrating that the positive effects we observed probably resulted from reduced *B. tectorum* competition.

If positive perennial grass cover responses were the result of reduced annual grass competition, it is not surprising that differences between treatments diminished over time at Site 2, where the natural decline in *B. tectorum* abundance that occurred during our study was most pronounced. At Site 1, the wildfire that occurred in August 2019 likely precluded our ability to detect significant treatment effects on perennial grass cover 45 and 57 MAT. Our findings add to a growing number of studies demonstrating that indaziflam can selectively control annual grasses with minimal risk to established perennial plants (Clark et al. 2019; Clark et al. 2020; Fowers and Mealor 2020; Hart and Mealor 2021; Sebastian et al. 2016; Sebastian et al. 2017a).

Future research should evaluate the potential for non-target impacts to native perennials with repeated indaziflam treatments; impacts to *P. spicata* and *A. tridentata* seedlings have been observed in a grow room study (Clenet et al. 2019). Compared to annuals, longer-lived plants are less likely to rely on seed banks because producing seeds in multiple years is an alternative strategy for coping with unpredictable environments (Saatkamp et al. 2013; Venable and Brown 1988). For example, *P. spicata* does not produce a persistent seed bank (Kitchen and Monsen 1994), and *A. tridentata* typically produces many seeds in even average years (Schlaepfer et al. 2014). Thus, forgone recruitment resulting from a single treatment is unlikely to have major impacts on communities that support abundant long-lived perennials. However, grazing managers have long understood the importance of allowing grasses to complete their reproductive cycles in at least some years (Burkhardt and Sanders 2012), which suggests that the impacts of repeated treatments may be different.

For bunchgrasses specifically, most research suggests that some individual plants can live for several decades, but average life expectancy is typically much lower (Lauenroth and Adler 2008; Svejcar et al. 2014; West et al. 1979). Consistent with this, Hamerlynck and Davies (2019) reassessed perennial bunchgrasses occurrence in plot cells where known bunchgrasses were planted and found that roughly 80% of individuals present after 13 years had established from seed after initial planting. If indaziflam treatments were repeated every few years, residual activity may extend over a period of time more comparable to perennial bunchgrasses

lifespans, and non-target impacts resulting from reduced recruitment may become more apparent as the population shifts towards a preponderance of older individuals.

The implications of this are unclear because little is known about the effects of aging in plants, which, due to their modularity, may not age in the same way as animals (Anderegg et al. 2012; Thomas 2002). Perennial bunchgrasses appear to become more stress tolerant and likely to survive with increasing age up to some physiological maximum (James et al. 2011; West et al. 1979), but the difficulty of accurately determining individual plant ages makes investigating aging in older grasses exceedingly difficult (Svejcar et al. 2014). Future research should address this knowledge gap to guard against impacts to these important rangeland plants. Multiple treatments will likely increase the effectiveness of indaziflam in the near-term, but the effects of repeated application become more difficult to predict as the number of treatments increases and residual herbicide activity extends over longer periods of time.

We know of no published studies documenting annual grass reductions resulting from indaziflam treatment over a comparably long period of time (57 MAT), and our results reflect the variability that managers can expect to face when treating *B. tectorum* in the notoriously heterogeneous and unpredictable rangeland ecosystems of western North America (Boyd and Svejcar 2009; Svejcar et al. 2017). Land managers should be aware that short-term treatment outcomes may be inconsistent, and control may improve over time when using indaziflam alone, as was observed in our study. Tank mixing indaziflam with a post-emergent herbicide is likely the most straightforward way to minimize inconsistent short-term control. Imazapic consistently reduced *B. tectorum* abundance 9 MAT in our study (Figure 1.1a & b; Figure 1.2a & b), and was also highly effective 57 MAT in plots where herbicide was reapplied (Figure 1.3a & b). Coupled with its selectivity against annual grasses at low use rates (Kyser et al. 2013), the short-term effectiveness of imazapic makes it an ideal tank mix partner that can provide reliable short-term control.

Developing and implementing tools with the capacity to effectively manage annual grasses is critical to prevent the annual grass-fueled "downward spiral" of which researchers have long been aware (West 1983; Young and Evans 1978), and recent research suggesting that *B. tectorum* may be able to expand its dominance in higher elevation sagebrush plant communities only makes this more pressing (Mealor et al. 2012; Smith et al.

2021). The unexplained decline in *B. tectorum* abundance highlighted by our study is likely best understood as a temporary reduction in the density and cover of adult plants and not a durable transition back to a less-invaded state (Davies et al. 2021a; Smith et al. 2021). Our study suggests that indaziflam may provide land managers with a tool that can proactively reduce *B. tectorum* where and when it co-occurs with perennial grasses, and this may be able to preserve some of the resistance to invasion and resilience to wildfire derived from these important rangeland plants.

While the intermediate and high indaziflam rates reduced density and cover to very low levels, no treatment completely eliminated B. tectorum, which is notorious for its ability to rapidly recover from disturbance (Humphrey and Schupp 2001; Perryman et al. 2020; Young and Evans 1978, Young et al. 1987). The scale of B. tectorum invasion and the reality of limited resources for management make it clear that B. tectorum needs to be managed as a permanent component of rangeland plant communities in western North America (Davies et al. 2021a; Perryman et al. 2018). In light of this, future research should consider how best to prevent or delay B. tectorum reinvasion in treated areas and reduce the need for additional treatments after initial seed bank depletion (e.g., Davies and Sheley 2007). Researchers and land managers should also work together to determine how to best deploy indaziflam at landscape scales and combine indaziflam treatment with other practices (e.g., grazing to reduce fine fuels and safe sites for *B. tectorum* germination; Davies et al. 2016; Davies et al. 2021b; Perryman et al. 2020;) and emerging restoration technologies (e.g., seed coatings and herbicide protection pods; Clenet et al. 2019; Holfus et al. 2021; Svejcar et al. 2021). Along with other important studies (Clark et al. 2019; Clark et al. 2020; Fowers and Mealor 2020; Hart and Mealor 2021; Sebastian et al. 2016; Sebastian et al. 2017a), our findings lend support to the notion that indaziflam may allow land managers to achieve objectives that were not feasible with other management tools and suggest that indaziflam will have a significant role to play in the effort to mitigate the devastating impacts of annual grass invasion.

TABLES

	MAT ^a	Response ^b	F (numerator df, denominator df)	$P > F^c$
Site 1	9 MAT	B. tectorum density	8.65 (4,12)	<0.01
		B. tectorum cover	19.41 (4, 12)	<0.0001
		Perennial grass cover	4.55 (4, 15) ^d	0.013
	21 MAT	B. tectorum density	10.87 (4, 15) ^d	<0.001
		B. tectorum cover	10.20 (4, 12)	< 0.001
		Perennial grass cover	3.75 (4, 12)	0.033
	33 MAT	B. tectorum density	17.12 (4, 15) ^d	<0.0001
		B. tectorum cover	16.79 (4, 12)	<0.0001
		Perennial grass cover	7.82 (4, 12)	<0.01
	45 MAT	B. tectorum density	12.60 (4, 12)	<0.001
		B. tectorum cover	12.18 (4, 12)	< 0.001
		Perennial grass cover	2.64 (4, 12)	0.086
	57 MAT	B. tectorum density	17.6 (8, 24)	<0.0001
		B. tectorum cover	6.16 (8, 24)	< 0.001
		Perennial grass cover	1.48 (8, 24)	0.22
Site 2	9 MAT	B. tectorum density	6.2 (4, 12)	<0.01
		B. tectorum cover	8.83 (4, 12)	<0.01
		Perennial grass cover	7.16 (4, 12)	<0.01
	21 MAT	B. tectorum density	5.85 (4, 12)	<0.01
		B. tectorum cover	3.66 (4, 12)	0.036
		Perennial grass cover	0.75 (4, 12)	0.58
	33 MAT	B. tectorum density	12.27 (4, 12)	< 0.001
		B. tectorum cover	5.67 (4, 12)	<0.01
		Perennial grass cover	1.26 (4, 12)	0.34
	45 MAT	B. tectorum density	50.25 (4, 12)	<0.0001
		B. tectorum cover	10.79 (4, 15) ^d	<0.001
		Perennial grass cover	2.59 (4, 12)	0.091
	57 MAT ^e	Perennial grass cover	0.64 (8, 27) ^d	0.74

Table 1.1. Results of ANOVA (α =0.05) for treatment effects on *B. tectorum* density, *B. tectorum* cover, and perennial grass cover at Site 1 (n=4) and Site 2 (n=4).

 $^{a}MAT = months after treatment.$

^bDensity = number of individuals m⁻²; cover = absolute canopy cover.

cSignificant effects (P < 0.05) shown in bold.

^dRandom effect (block) was estimated as zero and removed from the model.

^eTreatment effects on *B. tectorum* density and cover were not assessed 57 MAT at Site 2 due to the near complete absence of *B. tectorum*.

FIGURES



Figure 1.1. Mean (+1 SE) *B. tectorum* density (a), *B. tectorum* cover (b) and perennial grass cover (c) at Site 1 (Boulder Lake) 9, 21, 33 and 45 months after treatment (MAT; cover=absolute canopy cover). Herbicide treatments were applied September 2016 when native plants were dormant and *B. tectorum* was 100% post seed-set. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: imazapic (123 g ai ha⁻¹) and indaziflam (44, 73, and 102 g ai ha⁻¹). All treatments included a 0.25% v/v nonionic surfactant. Note that y-axis scale is consistent across panel rows in all cases except for *B. tectorum* density 45 MAT (*).



Figure 1.2. Mean (+1 SE) *B. tectorum* density (a), *B. tectorum* cover (b) and perennial grass cover (c) at Site 2 (Half Moon) 9, 21, 33 and 45 months after treatment (MAT; cover=absolute canopy cover). Herbicide treatments were applied September 2016 when native plants were dormant and *B. tectorum* was 100% post seed-set. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: imazapic (123 g ai ha⁻¹) and indaziflam (44, 73, and 102 g ai ha⁻¹). All treatments included a 0.25% v/v nonionic surfactant. Note that y-axis scale is consistent across panel rows in all cases except for *B. tectorum* density 45 MAT (*).



Figure 1.3. Mean (+1 SE) *B. tectorum* density (a) and *B. tectorum* cover (b) at Site 1 (Boulder Lake) 57 months after treatment (MAT; cover=absolute canopy cover). Treatment groups followed by a (1) received only one herbicide application and treatment groups followed by a (2) received a sequence of two herbicide applications (diagonal line pattern). Initial herbicide treatments were applied September 2016 and reapplications of the same treatments were made approximately 45 months later in June 2020. Native plants were dormant and *B. tectorum* was 100% post seed-set when initial treatments were applied, and native plants were actively growing and *B. tectorum* was near 100% post seed-set when reapplication occurred. Letters indicate significant differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: imazapic (123 g ai ha⁻¹) and indaziflam (44, 73, and 102 g ai ha⁻¹). All treatments (initial and reapplication) included a 0.25% v/v nonionic surfactant.



Figure 1.4. Mean (+1 SE) perennial grass cover at Site 1 (a; Boulder Lake) and Site 2 (b; Half Moon) 57 months after treatment (MAT; cover=absolute canopy cover). Treatment groups followed by a (1) received only one herbicide application and treatment groups followed by a (2) received a sequence of two herbicide applications (diagonal line pattern). Initial herbicide treatments were applied September 2016 and reapplications of the same treatments were made approximately 45 months later in June 2020. Native plants were dormant and *B. tectorum* was 100% post seed-set when initial treatments were applied, and native plants were actively growing and *B. tectorum* was near 100% post seed-set when reapplication occurred. No significant differences were observed (ANOVA, α =0.05, n=4). Herbicide treatments are as follows: imazapic (123 g ai ha⁻¹), indaziflam (44, 73, and 102 g ai ha⁻¹). All treatments (initial and reapplication) included a 0.25% v/v nonionic surfactant.

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CHAPTER 2: Indaziflam reduces seed bank richness and density but not sagebrush-grassland plant diversity

SUMMARY²

Mitigation of annual grass invasion is critical to halting the conversion of native rangelands to fireprone, annual grass-dominated communities and maintaining ecosystem services provided by perennialdominated plant communities in western North America. The herbicide indaziflam (Rejuvra®, Bayer) may allow managers to selectively deplete annual grass seed banks in plant communities that continue to support desirable perennial vegetation. Non-target impacts are difficult to assess in the small plots often used in herbicide trials and the potential for impacts to the seed bank is not well-understood. To assess the potential for non-target impacts resulting from indaziflam treatment, we used modified-Whittaker multiscale vegetation plots to compare diversity (species accumulation) in three treatment (73 g ai ha-1 indaziflam) and three control plots in a sagebrush-grassland plant community near Pinedale, Wyoming that is invaded by downy brome (Bromus tectorum L.). We also assessed the density and richness of shallow (0-1 cm depth) and deep (1-5 cm depth) germinable seed banks in treatment and control plots by tracking the emergence of seedlings from seed bank samples during a 20-week greenhouse study. Vegetation data and seed bank samples were collected during the third growing season after treatment. Species diversity did not differ between treatment and control plots, but this contrasted with the results of the seed bank assay, which showed that the shallow and deep seed banks had significantly fewer germinable seeds and native species richness was significantly lower in the shallow seed bank of treated areas. While we observed significant non-target impacts to native annual seed banks, all impacted species were detected in the aboveground plant community in treatment plots after treatment occurred, suggesting that reduced native annual abundance may be temporary. Considering the potential for non-target impacts to seed banks will help land managers accurately assess trade-offs when making treatment decisions.

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INTRODUCTION

The invasive annual grass downy brome (*Bromus tectorum* L.) is one of the greatest obstacles land managers face when seeking to conserve the semi-arid sagebrush ecosystems that typify much of western North America (Davies et al. 2011). Downy brome litter increases the amount and continuity of fine fuel in invaded shrublands (Davies and Nafus 2013), and this can increase wildfire frequency and size by increasing the likelihood of ignitions and the rate of wildfire spread (Brooks et al. 2004; D'Antonio and Vitousek 1992; Whisenant 1989). Historic fire frequency is difficult to determine in sagebrush ecosystems with few trees (McAdoo et al. 2013), but given the long recovery time of sagebrush (Schlaepfer et al. 2014; Miller et al. 2013) and increases in the likelihood of burning after cheatgrass invasion (Barker et al. 2019; Bradley et al. 2018; Balch et al. 2013; Whisenant 1989), it is reasonable to conclude that downy brome invasion has increased fire frequency in infested sagebrush ecosystems relative to historic conditions.

Bradley et al. (2018) used remote sensing to estimate that downy brome achieves greater than 15% cover on more than a quarter of the US Intermountain West, and that these areas were four times as likely to have burned multiple times between 2000 and 2015 compared to areas with lower cheatgrass abundance. Swanson et al. (2018) found that across 51 paired burned and unburned sagebrush sites distributed throughout the western US, perennial dominance declined after fire in all cases where downy brome cover exceeded 15% in the pre-fire community. Interpreted together, these two findings suggest that the situation is likely to get worse across much of the sagebrush biome (Davies et al. 2021), especially if effective management interventions cannot be identified and deployed (Monaco et al. 2017; Mack 2011).

Unimpeded downy brome invasion can be expected to have ecological consequences. For example, wildfire has been identified as a key threat to greater sage-grouse (*Centrocerus urophasianus*; Smith and Beck 2018; Coates et al. 2016; Brooks et al. 2015), the region's most iconic wildlife species. Sage-grouse are intimately associated with intact sagebrush habitats and rely on sagebrush for nesting and brood rearing, winter forage, thermal cover, and escape from predators (Anthony and Willis 2009; Connelly et al. 2000; Wallestad and Pyrah 1974). Sage-grouse populations typically decline following wildfires that cause landscape-scale sagebrush mortality (Dudley et al. 2021; Coates et al. 2016), and sage-grouse adult and nest survival was markedly reduced

after wildfire in the northern Great Basin (Foster et al. 2019; Lockyer et al. 2015). Given that sagebrush mortality is generally high following wildfire and sagebrush recovery often takes decades (Schlaepfer et al. 2014; Miller et al. 2013), sagebrush habitats that are eliminated by repeated downy brome-fueled wildfires may represent permanent habitat losses for sagebrush-associated wildlife.

Restoring invaded shrublands after wildfire is extremely difficult (Shriver et al. 2019; Pilliod et al. 2017), and this has led some to suggest that downy brome is an ecosystem transformer in sagebrush ecosystems (Pysek et al. 2004). The best path forward for these transformed areas may involve recognizing the state change that has occurred across much of downy brome's invaded range (Davies et al. 2021, Perryman et al. 2018). However, recognizing these landscapes as non-native annual grasslands implies serious alteration of the resources provided by them in the past, and that managers should consider proactive interventions that reduce the likelihood of state change where at-risk perennial-dominated plant communities remain.

Herbicide application represents one of few management tools that can reliably reduce downy brome abundance (Monaco et al. 2017), but in many cases the period of residual control is not sufficiently long to prevent eventual reinvasion from the annual grass seed bank (Courkamp and Meiman 2020; Sebastian et al. 2017a; Sebastian et al. 2016; Mangold et al. 2013). Indaziflam (Rejuvra®, Bayer) is a broad-spectrum, preemergent herbicide recently approved by the Environmental Protection Agency (EPA) for use on rangelands and natural areas, including those grazed by livestock (US EPA 2020). A cellulose biosynthesis inhibitor with a unique site of action and no reported cases of resistance in the field (Tateno et al. 2016; Brabham et al. 2014), indaziflam binds tightly to soil organic matter and remains near the surface after application. This allows for selective activity against annual grasses with minimal impacts to established plants with deeper roots (Clark et al. 2019; Sebastian et al. 2017a; Sebastian et al. 2016). Indaziflam also has a longer period of residual activity, typically providing three or more years of suppression and potentially depleting downy brome seed banks (Clark et al. 2020; Clark 2020; Courkamp and Meiman 2020; Sebastian et al. 2017b; Sebastian et al. 2016).

Indaziflam may represent a new approach to annual grass management with herbicides. The depletion of annual grass seed banks is not a realistic management objective when using other herbicides, except perhaps when small scales permit close observation and repeated treatment (Sebastian et al. 2017b). Indaziflam application may allow managers to deplete annual grass seed banks at meaningful spatial scales with one or two applications (Clark et al. 2020; Sebastian et al. 2017a), and the unique selectivity may provide a new option in areas where downy brome infestations co-occur with existing communities of perennial plants. The resource use patterns of perennial bunchgrasses in particular are known to overlap with downy brome (James et al. 2008), and because these plants typically survive most wildfires (Davies and Bates 2020; Miller et al. 2013), proactive interventions that reduce downy brome abundance relatively early in the invasion process and favor established perennial bunchgrasses may represent a highly effective strategy to increase resistance to annual grass invasion and resilience to the effects of wildfire in sagebrush-grassland ecosystems. Encouragingly, downy brome reduction with indaziflam was recently implicated in halting the spread of an incidental wildfire that burned through a research site in Wyoming (Courkamp and Meiman 2020). The restoration potential of indaziflam treatment in areas already transformed into annual grasslands remains to be evaluated, but researchers and land managers have long been aware of the downward spiral associated with more frequent wildfire and increasing annual grass dominance in sagebrush rangelands (West 1983; Young and Evans 1978). Indaziflam may provide an opportunity for managers to interrupt this downward spiral and prevent conversion to annual grasslands where and when downy brome co-occurs with established perennial plants.

Limited research suggests that indaziflam treatment has little effect on the diversity of established native plant communities in treated areas (Clark et al. 2019), but there is often little overlap between aboveground plant communities and soil seed banks (Pekas and Schupp 2013; Hopfensberger 2007; Bazzaz 1996). Soil seed banks (henceforth seed banks) consist of the viable germinable seeds in the soil of a site and represent a pool of propagules available for future colonization (Faist et al. 2013). Seed bank communities can play important roles in community assembly and succession after disturbance (Saatkamp et al. 2013; Faist et al. 2013; Bossuyt and Honnay 2008). Given that indaziflam directly impacts the downy brome seed bank, and that seed bank communities are often much different than co-occurring communities of established plants, it will be important for managers to understand the potential for non-target impacts to the seed banks of desirable native plants with increased indaziflam use. In addition to the difficulties of observing impacts to seed banks, most herbicide trials use relatively small plot sizes. For example, Clark et al. (2019) assessed the diversity of native plant communities in indaziflamtreated plots measuring 3 by 6 m. These small plots limit the degree to which non-target impacts can be detected when conducting research with a relatively new herbicide that is intended to be applied at much larger spatial scales. There is clearly a need to increase the scale of efforts to evaluate the potential for non-target impacts to better account for the heterogeneity of rangeland plant communities (Svejcar and Havstad 2009; Stohlgren 2002). Species-area models are considered an ideal method for measuring biodiversity across scales (Stohlgren 2007; Magurran 2004), and they permit statistical comparisons of the rate at which species accumulate with increments in area (species accumulation; Connor and McCoy 1979).

Given the prospect of and need for increasing indaziflam use to protect sagebrush ecosystems from repeated wildfire, we sought to build on and replicate existing research on non-target impacts at larger scales and directly address the question of impacts to the seed bank. To accomplish this objective, we addressed three questions:

(1) Does indaziflam treatment alter species accumulation in sagebrush-grassland plant communities?

(2) Does indaziflam treatment affect the density and richness of native species seed banks in sagebrush-grassland plant communities?

(3) Do potential impacts of indaziflam treatment to soil seed banks vary with soil depth and/or by plant growth form?

For the purposes of comparison, we used species accumulation as a measure of species diversity, and based on prior research we predicted that no observable impacts would occur to aboveground communities of native plants. However, we predicted that indaziflam treatment would impact the richness and density of the seed bank near the soil surface, mostly by reducing the abundance of other annuals with germination characteristics similar to downy brome.

MATERIALS AND METHODS

Site Description

Our study was conducted near Boulder Lake, a recreational site in Sublette County, Wyoming managed by the US Forest Service (USFS) in the Bridger-Teton National Forest (610774 m E, 4746164 m N, UTM zone 12 T, approx. 2250 m elevation). The site is located in the Cold Desert region of the North American Deserts ecoregion and is characterized by a Coarse Upland ecological site (R043BY208WY; USDA-NRCS 2021a). The soil is the Pointer-Lateral complex, which is characterized by a very cobbly sandy loam surface soil with 2.85% organic matter and 6.8 pH in the top 20 cm (USDA-NRCS 2021b). Long-term (1981-2010) average annual precipitation at the site was 386 mm, and precipitation in the 12-months prior to seed bank sampling was 451 mm (PRISM 2021).

The site is in the Burnt Lake allotment of the Pinedale Ranger District, on the ridge northeast of Boulder Lake. This allotment has been regularly grazed since the early 20th century with the type of livestock (cattle, sheep, and horses) and the details of grazing (frequency, intensity, and timing) varying. A prescribed fire was conducted in the area in 1997 for the purposes of reducing woody vegetation and increasing grass production. The prescribed burn was followed by a wildfire on a portion of the allotment in 2001; after these fires, downy brome invaded south-facing hillslopes in the area and has remained abundant on the ridge adjacent to Boulder Lake ever since (D. Cottle, personal communication). While the allotment has been regularly grazed during the study, the relatively steep portion where the study is located has likely experienced only limited livestock grazing and signs of recent cattle presence are rarely observed near the study plots.

The study site supports communities of established perennial plants invaded by downy brome. Common plants include bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] Á. Löve), needle and thread (*Hesperostipa comata* [Trin. & Rupr.] Barkworth), mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana* [Rydb.] Beetle), antelope bitterbrush (*Purshia tridentata* [Pursh] DC.), arrowleaf balsamroot (*Balsamorhiza sagittata* [Pursh] Nutt.), and a variety of small native and introduced forbs. Vegetation and seed bank samples were collected from six experimental plots (henceforth plots; approx. 2 ha each) on the south-facing hillslope northeast of Boulder Lake. Slope in the control plots ranges from 14-58 % and slope in the treatment plots range from 12-58 %. Three of these plots were treated aerially (helicopter) with indaziflam in September 2016. Indaziflam was applied at 73 g ai ha⁻¹ to the treatment plots with 47 L ha⁻¹ of water as the carrier.

An incidental, human-ignited wildfire (Boulder Lake Fire) that burned nearly all of the study location occurred after vegetation sampling in 2019, but approximately five weeks prior to seed bank sampling. Significant portions of two treatment plots were left unburned, likely because of herbicide treatment (Courkamp and Meiman 2020). The effects of the fire were inconsistent throughout the burned area, leaving some small patches unaffected while burning other areas more completely. We are confident that the fire did not preclude our ability to determine the effects of herbicide on the seed bank.

Experimental Design

To quantify indaziflam treatment effects on downy brome, we used 0.5 m² frames (Bonham et al. 2004) to measure downy brome cover and density in the treatment and control plots. We counted individual downy brome plants and recorded canopy cover to the nearest one percent in these frames. When downy brome was especially dense, plants were counted in only a portion of the larger frame to estimate density. Data were collected from five frames (subsamples) located near the approximate center and the four corners of each plot. These data were collected in late June in 2017, 2018, and 2019 (10, 22, and 34 MAT respectively).

In each large treatment or control plot, one 1000-m² modified-Whittaker (MW) nested multi-scale vegetation sampling plot (Stohlgren et al. 1995) was established in early-June 2019. These nested vegetation plots include 1-, 10-, and 100-m² subplots and are useful for generating species-area models (Stohlgren 2007; Barnett and Stohlgren 2003; Stohlgren et al. 1995). To generate species-area relationships for treatment and control plots, we recorded plant species richness in each of the MW subplots and then exhaustively searched the MW main plot for any additional species. Plant species richness was sampled on June 6-7, 2019, 33 months after treatment (MAT).

Soil sampling occurred 37 MAT in late-September 2019 (September 23 and 24), and MW main plots (henceforth MW plots) were also used to guide seed bank sampling. To locate seed bank samples, three transects parallel to the long edge of the 20-x 50-m plot were established. A 1-m buffer was applied to the vegetation plot so that no samples were collected within 1 m of a plot boundary. This left 864 m² (18 x 48 m)

available for sampling. Three transects were established at random locations along the 18-m edge of each plot by selecting a random number (1-6) for the start of the first transect and then establishing the following transects at 6-m intervals. Twelve samples were collected along each transect for a total of 36 samples per plot. The location of the first sample was determined by selecting a random number (1-4), after which samples were collected at 4-m intervals.

At each sampling location, soil was collected as close to the transect as possible. If the location was obstructed by rocks or established vegetation, the sampling location was randomly moved either east or west perpendicular to the transect until an unobstructed area was encountered. To collect samples, an 8-cm diameter steel ring was driven into the soil surface to a depth of 1 cm; soil was cleared away along the side of the ring; and a modified putty knife was inserted under the ring while maintaining upward pressure to ensure that soil underneath the ring was not included in the shallow sample. The ring and shallow soil sample was then lifted off the soil surface and placed in a bucket, and a marked 5-cm diameter bulb corer was used to collect the soil immediately below the 1 cm surface sample to a depth of 4 cm (1-5 cm depth from original surface). Litter and soil were collected together, but large woody litter that could potentially obstruct the ring was removed from the soil surface when encountered. This subsurface sample was placed in a bucket separate from the shallow sample. All samples collected in each plot were aggregated into shallow and deep samples yielding a 0-1 cm and 1-5 cm composite soil sample for each of the 6 study plots.

The samples were immediately transported from the study site back to Colorado State University (CSU) and sieved to remove all particles greater than 12.5 mm. Samples were then stored at 2° C for approximately 2 months starting September 26, 2019 until greenhouse germination trials began. During this time, small amounts of all treatment samples were tested for the presence of indaziflam using liquid-chromatograph mass spectrometry (LC-MS). LC-MS detected trace amounts of indaziflam in all treatment soil samples, but these were much lower than levels expected to impact germination. To confirm this, we planted field collected downy brome seeds into subsamples of each treatment sample in the greenhouse. These seeds readily germinated and seedlings grew normally in all cases. At low herbicide concentrations, monocots like downy brome are known to be more sensitive to indaziflam than dicots (Sebastian et al., 2017a), thus we are confident that seedlings in

our samples are unlikely to have been affected by herbicide activity in the greenhouse and that the differences observed in our study result from past herbicide activity in the field.

Germination trials were initiated on November 24, 2019 and terminated on April 11, 2020 (20 weeks). Soil (2 kg) from each sample was spread evenly over a 3-cm base of potting mix (Pro-mix BX General Purpose) in a standard greenhouse flat (25.4-x 52-cm). In total, the study consisted of 12 experimental flats and five greenhouse control flats filled with only potting mix. These greenhouse control flats were intermixed with the experimental flats to account for possible seed dispersal in the greenhouse, but no seedlings were observed in these flats during the study. Flats were placed in a heated greenhouse at CSU (23° C) where they received supplemental light for the duration of the study (16 hrs, 300 µmol m⁻² sec⁻¹). Additionally, flats were placed on propagation mats (Redi-Heat[™], Model # RHM2015, Phytotronics Inc) set to keep soil in the trays at a constant minimum temperature of 23° C (Rawlins et al., 2012). Flats were watered as needed daily to keep the surface of the soil moist in each flat and their positions on the bench were randomized weekly to account for environmental heterogeneity in the greenhouse.

The study consisted of four phases (Table 2.1) wherein soil was periodically turned over using a modified putty knife and fertilized to promote germination (Walck et al. 2011). Germination was assessed weekly for the duration of the study. Seedlings were marked on a rolling basis using color coded toothpicks to prevent counting any seedlings multiple times. Seedlings were removed as soon as a distinct morphotype could be identified and no seedling was left in a tray for longer than 3 weeks after emergence. Representatives of each distinct morphotype were repotted and raised to maturity for identification. Seed bank density was standardized to the area sampled in each plot and the total mass of the sample after sieving and richness was quantified as the number of distinct taxa that emerged from each sample.

Treatment Evaluations and Data Analysis

All statistical analyses were conducted in R v. 3.6.2 (R Core Team 2019). To compare species accumulation, we used multiscale richness data from each MW main plot and subplots to fit species-area models using the power function (Preston 1960; Arrhenius 1921). The power model is represented by an equation of the form:

$$S = cA^{z}$$

where *S* represents species richness, *A* represents area, and *c* and *z* are fitted constants. For statistical purposes, the power model was linearized using a log-log transformation (Connor and McCoy 1979; Preston 1960), then taking the form:

$$\log(S) = \chi \log(A) + \log(a)$$

We used the "sars" package in R (Matthews et al. 2019) to fit transformed power species-area models to the data from each MW plot and its associated subplots. We used the power model because: (1) it fits a wide variety of species-area relationships relatively well (Triantis et al. 2012; Gotelli and Graves 1996; May 1975); (2) it permits comparison of species accumulation between species-area datasets, particularly when communities are relatively similar and area is standardized (Matthews et al. 2016; Rosenzweig 1995; Connor and McCoy 1979); and (3) it has been used successfully in the past with the same multi-scale plot design we employed in our study (Barnett and Stohlgren 2003). We fit models for each MW plot using both total species richness and native species richness data. Native status was determined based on the USDA Plants Database (USDA-NRCS 2021c).

When the power model is fit in log-log form, the z-value is often interpreted as the slope of the speciesarea model (Rosenzweig 1995; henceforth slope and z-value are used interchangeably), with z = 1 indicating that the relationship between species richness and area is isometric, and values between 0 and 1 indicating that species accumulation diminishes with increasing area (Abele and Connor 1978). A variety of z-values have been detected in natural communities, with higher z-values suggesting a relatively high rate of species accumulation compared to lower z-values (Connor and McCoy 1979). Given that species richness typically varies across scales (Gaston 2000), comparisons of model slopes (z-values) can be used to detect differences in species accumulation between species-area datasets (Barnett and Stohlgren 2003; Connor and McCoy 1979; Rosenzweig 1995). After fitting species-area models to the data from each plot, we extracted z-values from each model for statistical comparison.

We used t-tests to evaluate differences in species accumulation (z-values), the density and richness of shallow and deep seed banks, and downy brome cover and density between control and treatment plots. Downy brome cover and density data were analyzed independently in each year due to interannual variation, and visual inspection of quantile-quantile plots was used to verify that data met the assumptions of our statistical analyses. In some cases, seed bank data were square-root transformed prior to analysis in order to satisfy these assumptions. Low occurrence in the treatment seed bank did not permit species-level comparisons of seed bank density for many of the species we observed. In all, we compared germinable seed bank density of combined native species, native annuals, native perennials, downy brome, and mountain big sagebrush. Species-area models are presented with transformed dimensions. Other results presented in the manuscript are in their original dimensions and means are reported with standard errors. Means were considered different when $p \leq 0.05$, but p-values ≤ 0.10 are noted in the text and identified in figures for reference.

RESULTS

Treatment significantly reduced downy brome canopy cover and density in all cases except downy brome cover 22 MAT (t=2.12, p=0.10; Fig. 2.1a; Table 2.2), indicating that treatment had successfully reduced downy brome in our treatment plots for approximately three years prior to sampling.

Species-Area Models

A total of 44 species were detected in the control and treatment plots and mean species richness at the MW plot scale (1000 m²) was 30.3±1.8 and 27±3.2 in the control and treatment plots, respectively. Four of these 44 species were non-native (Table B.1), and mean native species richness at the MW plot scale was 27.3±1.2 in the control plots and 25.3±2.9 in the treatment plots. Species richness and native species richness did not differ significantly between control and treatment plots at the MW plot scale ($p \ge 0.40$; not shown) or any of the MW subplot scales (1, 10, and 100 m²; $p \ge 0.25$; not shown). Four taxa (3 native, 1 non-native) were found exclusively in the control plots: lobeleaf groundsel (*Packera multilobata* [Torr. & A. Gray ex A. Gray] W.A.

Weber & Á. Löve), milkvetch (*Astragalus* sp.), desert parsley (*Lomatium* sp.), and common dandelion (*Taraxacum* officinale F.H. Wigg); and three taxa (all native) were found exclusively in treatment plots: alumroot (*Heuchera* sp.), spiny phlox (*Phlox hoodii* Richardson), and flowery phlox (*Phlox multiflora* A. Nelson).

Transformed power species-area models for total species accumulation represented our data well based on model fit ($R^2=0.67-0.86$; Fig. 2.2a-f), and a comparison of the z-values extracted from the models indicated that model slopes did not differ between treatment and control plots (t=0.063, p=0.95; Fig. 2.2g). Native species accumulation was also represented well by power models ($R^2=0.63-0.85$; Fig. 2.2a-f), and a comparison of zvalues extracted from the models also indicated that model slopes did not differ between treatment and control plots (t=0.52, p=0.63; Fig. 2.2h), suggesting that species accumulation was not significantly different between treatment and control plots in either case.

Seed Bank Assay

A total of 11 different species were observed emerging from the seed bank samples (Table 2.2). These included two introduced annuals and nine native species (Table 2.2). Native species represented in the seed bank included an annual grass, annual forbs, and perennial grasses, forbs, and shrubs (Table 2.2). *Descurainia* spp. that emerged from the seed bank included both the native western tansymustard (*Descurainia pinnata* [Walter] Britton) and the non-native tansymustard (*Descurainia sophia* [L.] Webb ex Prantl.). Both were identified among the subset of individuals replanted and grown to maturity, but due to the high abundance of seedlings of this morphotype (Table 2.2), growing all individuals to maturity for identification was not possible. In order to be conservative, we classified *Descurainia* spp. as native knowing that we likely overestimated impacts to the seed bank density of this species. One species of perennial forb was present only in the deep seed bank; and one perennial grass and one annual forb were present exclusively in the shallow seed bank (Table 2.2). In all plots, most seedlings emerged from the shallow seed bank while the deep seed bank was relatively depauperate (Table 2.2; Fig. 2.3b).

Native species richness was significantly lower in treatment plots in the shallow seed bank (t=7.78, p<0.01; Fig. 2.3a), but the difference in native species richness was only significant at the $p\leq0.10$ level in the deep seed bank (t=2.71, p=0.053; Fig. 2.3a). Among native species, three annuals and one perennial were found

exclusively in control plots in the shallow seed bank; and two annuals and two perennials were found exclusively in control plots in the deep seed bank (Table 2.2).

Across all plots, the seed bank was dominated by annual species, and downy brome was abundant in the shallow seed bank of control plots (Table 2.2; Fig. 2.4b). Treatment reduced downy brome seed bank density significantly in the shallow seed bank (t=4.04, p=0.016; Fig. 2.4b), but not the deep seed bank (t=2.00, p=0.12; Fig. 2.4b) where it was much less abundant overall (Table 2.2). Native species seed bank density was significantly lower in treatment plots for both the shallow (t=8.04, p=0.013; Fig. 2.3b) and deep seed banks (t=4.35, p=0.012; Fig. 2.3b), suggesting that herbicide impacted native species seed bank density in treated areas 37 MAT. Native annual species accounted for a large majority of the reductions in native species seed bank density between treatment and control plots (Table 2.2; Fig. 2.4a). Native annual seed bank density was significantly lower in treatment plots compared to control plots in both shallow (t=5.95, p<0.01; Fig. 2.4a) and deep (t=4.92, p=0.030; Fig. 2.4a) seed banks.

Native perennials were observed infrequently in all plots (Table 2.2), but there were no significant treatment differences (shallow: t=1.99, p=0.12; deep: t=2.25, p=0.10; Fig. 2.4c). Mountain big sagebrush (sagebrush) was the most abundant native perennial observed, and most sagebrush seedlings were observed in the shallow seed bank (Table 2.2). Sagebrush seed bank density in the shallow seed bank was lower in the treatment plots compared to the control, but this comparison was only significant at the p \leq 0.10 level (t=2.46, p=0.070; Fig. 2.4d). We did not compare big sagebrush seed bank density in the deep seed bank because sagebrush seedlings were not encountered in deep seed bank samples collected from the treatment plots (Table 2.2; Fig. 2.4d).

DISCUSSION

Indaziflam treatment may have little effect on the aboveground plant community in areas dominated by perennial plants (Fig. 2.2), but has the potential to reduce the density of the native species seed bank and overall native seed bank richness near the soil surface (Fig. 2.3). Further, impacts to the shallow seed bank are unlikely to be offset by depauperate deeper seed banks at our study site (Figs. 2.3 & 2.4). Our findings support our prediction that species diversity would not be significantly affected by indaziflam treatment and that seed bank impacts would be concentrated near the soil surface and mostly limited to annual species.

Even though the z-value, or model slope, is the preferred parameter when comparing species-area models (Gotelli and Graves 1996; Rosenzweig 1995), its biological relevance has been the subject of considerable debate (e.g., Matthews et al. 2016; Tjørve and Tjørve 2008). However, that the z-value is a relative measure of species accumulation that permits comparison of diversity across scales is widely accepted (Tjørve and Tjørve 2008; Barnett and Stohlgren 2003; Connor and McCoy 1979). Given that we are not interested in the biological processes responsible for discrepancies in z-values between ecosystems with different characteristics (e.g., island biogeography), and are instead interested in evaluating the effects of an experimental treatment within a single ecosystem type, the similar z-values derived from data collected in treatment and control plots (Fig. 2.2) are strong evidence that species diversity has not been significantly altered by indaziflam treatment at our study site. Our results are similar to those of Clark et al. (2019), who found no reduction in established perennial species richness in small plots in the two years following indaziflam treatment. However, our seed bank assay provides evidence that impacts to the seed bank in treated areas may occur.

The wildfire that occurred at our study site five weeks prior to seed bank sampling may have affected our results by either consuming seeds (Allen et al. 2008) or increasing the germinability of seeds near the soil surface, which can occur via several mechanisms associated with fire (Baskin and Baskin 2014; Nelson et al. 2009; van Staden et al. 2000). At the scale of our seed bank sampling, the effects of the fire were difficult to determine and it was often difficult to tell if the soil surface at a given sampling location had "burned". The high downy brome seed bank density we observed in the shallow seed bank in our control plots (Fig. 2.4b) suggests seed consumption was not a major factor. The fire affected treatment plots to a lesser degree than control plots (Courkamp and Meiman 2020), but it also affected each treatment plot differently; one treatment plot was entirely burned, one was mostly burned with patches that resisted burning, and one was mostly unburned. A total of 7, 12, and 4 native species seedlings emerged from the seed bank samples from each of these plots respectively (combined shallow and deep). The consistently low-abundance of the native seed bank across this gradient of fire behavior suggests that the differences we observed were primarily the result of herbicide treatment, but we cannot completely rule out effects related to fire.

The difference in native seed bank density we observed could be exaggerated by the effects of fire if exposure to fire or smoke increased the germination of seeds in our seed bank samples. Native annuals comprised the majority of the native seed bank at our site (Table 2.2), and they often increase after fire (Allen et al. 2008; Humphrey and Schupp 2001; Bazzaz 1996), potentially because smoke can induce germination (Nelson et al. 2009; van Staden et al. 2000), or because of changes in soil resources or light availability in the post-fire environment (Rau et al. 2008; Bazzaz 1996). Exposure to fire or smoke in the field may have increased native annual germination in our seed bank samples. Allen et al. (2008) saw a large increase in the germinable seed bank of western tansymustard (*D. pinnata*) relative to pre-fire conditions after a spring prescribed burn in a juniper-encroached sagebrush community in the central Great Basin. This result was limited to tree canopy microsites and the mechanism was unclear, but the large increase in the germinable seed bank after burning suggests that the same effect could have increased germination in our seed bank assay because *Desenrainia* spp. were a major component of the seed bank at our site. However, the same study also observed a post-fire decline in the germinable seed bank of stickseed (*Lappula occidentalis* [S. Watson] Greene), one of the other abundant annuals in our study, suggesting that the effects of fire on seed banks may vary by species and lead to declines in abundance as well as increases.

Many of the most abundant winter annuals observed in our study would be expected to readily germinate in the fall regardless of exposure to smoke or fire (Forbis 2010; Pake and Venable 1996), especially with the regular watering, soil heating, supplemental light, and fertilizer we supplied in our greenhouse study (Table 2.1). These factors suggest that while fire certainly has the potential to alter the dynamics of seed bank communities, differences we observed are likely to have resulted primarily from herbicide treatment. Indaziflam is a broad-spectrum herbicide (Brabham et al. 2014), useful because it depletes the seed banks of annual plants (US EPA 2020). Thus, the consistently low densities of native annual seed banks in treatment plots (Table 2.2; Fig. 2.4a) are unsurprising. Assessing impacts to the germinable seed bank is an important step in evaluating the potential for unforeseen outcomes related to expanded indaziflam use and providing land managers the

information they need to accurately assess trade-offs when making treatment decisions. However, non-target impacts are unlikely to straightforwardly translate to ecological outcomes in all cases because there are key differences between downy brome and many native annual species.

Downy brome is particularly vulnerable to seed bank depletion due to its very high germinating fraction and relatively short-lived seed bank (Sebastian et al. 2017b; Smith et al. 2008; Burnside et al. 1996). These factors increase the likelihood that indaziflam contacts the root tips of most seedlings present at a site relatively soon after application. Annuals with lower germinating fractions and/or longer-lived seed banks may be resilient to non-target impacts from indaziflam because some of their seeds may carryover beyond the residual period of herbicide activity. Further, short-lived weedy annuals often produce many seeds, which may allow their abundance to recover quickly from relatively few individuals (Grime 2006; Bazzaz 1996).

Germination can also be triggered by environmental conditions that do not arise every year (Bazzaz 1996; Harper 1977). This suggests that the unpredictable nature of much of downy brome's invaded range may reduce the potential for impacts to species with longer-lived seed banks because conditions that favor germination may not always arise during the period of herbicide activity (Svejcar et al. 2017). In our study, only trace amounts of herbicide were present 37 MAT, while some desert annuals are known to have seeds that last much longer (Adonakis and Venable 2004; Epling et al. 1960).

In the context of indaziflam treatment, the similarity of native species to downy brome may increase the likelihood of large reductions in abundance. Several of the impacted native annuals at our site have germination characteristics that are similar to downy brome (Forbis 2010; Pake and Venable 1996), and this may increase the likelihood that they are impacted by indaziflam treatment. At our site, it appears that conditions favoring native annual germination, and thus contact between growing native annual root tips and indaziflam, materialized sometime between application and the cessation of residual herbicide activity, before we collected our seed bank samples.

Many land managers acknowledge the importance of plant community diversity for a variety of reasons (Isbell et al. 2017; Meyer et al. 2017), but it is likely that most would trade reduced native annual abundance for the obvious benefits of long-term invasive annual grass control. However, the potential for non-target impacts to native annuals should be considered alongside the reality that their role in community ecology has been underappreciated in the past largely because they are not often studied or used in restoration (de Queiroz et al. 2021; Forbis 2010). We limit our discussion to the role of native annuals in plant community assembly, but, depending on the species, they may also provide important habitat resources for a variety of animal species (e.g., seed predators, pollinators). The question of their potential ecological role in vegetation communities is important to weighing the costs and benefits of treatment because they may play important roles in recovery from disturbance by: (1) increasing resistance to invasion by annual grasses and facilitating the establishment of native perennials (Leger et al. 2013; Abella et al. 2012; Perry et al. 2009), and; (2) providing hosts for colonizing arbuscular mycorrhizal fungi that promote the recruitment and dominance of perennial plants (Hovland et al. 2019; Lin et al. 2015; Davidson 2015; Busby et al. 2011). In these cases, the ecological value of native annuals is derived from their indirect positive effects on native perennials and the resistance to invasion and resilience to wildfire that the presence of these perennials confers (Davies and Bates 2020; Ellsworth et al. 2020; Davies and Johnson 2017; Chambers et al. 2007).

Native annual forbs may increase a site's resistance to invasion after disturbance because the functional similarity of these species to annual grasses makes them better competitors than slower growing perennial plants at the seedling stage (Abella et al. 2012; Forbis et al. 2010). In a field experiment conducted by Leger et al. (2013), downy brome seed production was reduced by more than 75% when grown in a plot where western tansymustard (*D. pinnata*), one of the most abundant and impacted forbs we observed, was sown at a rate of 400 seeds m⁻². However, this is substantially higher than the mean *Descurainia* spp. seed bank density we observed in the shallow seed bank of our control plots (104.3 \pm 26.9 seedlings m⁻²), which also included the non-native *D. sophia*. Further, not all native annuals are expected to be good competitors with annual grasses. For example, six-seeks fescue (*V. octoflora*) is known to be a poor competitor that is readily displaced by a variety of non-native annual plants (Allen and Knight 1984; Mack 1981), but because these plants typically do not persist in the aboveground plant community over time and are largely relegated to the seed bank (Boyd and Svejcar 2011; Goergen and Chambers 2009; Bazzaz 1996), their abundance following disturbance may prevent near-term downy brome invasion and allow sites to gradually transition to perennial dominance (Leger et al.

2013; Perry et al. 2009). A community depleted of native annuals may be more vulnerable to reinvasion after severe disturbance than one that is not, but this is likely to be less of a concern in areas treated with indaziflam for the time that it remains active; in our study plots, indaziflam treatment was associated with large significant declines in downy brome canopy cover, density, and seed bank density (Fig. 2.1; Fig. 2.4b).

Facilitation by AMF is highly complex in rangeland plant communities and the effects of AMF are difficult to disentangle (Hovland et al. 2019), but these organisms have been linked to plant community composition and succession in other ecosystems (Koziol et al. 2017; van der Heijden et al. 1998). Downy brome is a facultative AMF host (Hovland et al. 2019), and responds negatively to colonization in at least some cases (Reinhart et al. 2017; Owen et al. 2013). In general, it is thought to be a relatively poor host (Al-Qarawi 2002), and invasion may result in depleted, low-richness AMF communities with lower inoculation potential in invaded soils (Busby et al. 2013; Busby et al. 2011; Vogelsang and Bever 2009). In contrast, big sagebrush is considered AMF dependent and exhibits positive responses to specific AMF species (Lindsey 1984). A recent meta-analysis found a 42% increase in survival in inoculated big sagebrush seedlings (Davidson et al. 2015), perhaps due to an increased ability to survive drought stress (Stahl et al. 1998), which has been identified as a benefit of AMF colonization in other plants (Augé et al. 2015; Worchel et al. 2013). Given that early seral plant-AMF relationships are highly variable (Busby et al. 2011; Pendleton and Smith 1983), some native annual species may condition soil AMF communities in ways that promote the recruitment and increasing dominance of perennial plants through time. Lin et al. (2015) showed in a meta-analysis of 304 studies that AMF tend to increase the competitive ability of perennial species when they are competing with annuals, further suggesting that this may be happening in some ecosystems.

Whether competing with invasive annual grasses or conditioning soil microbial communities, it is likely that in some circumstances some native annuals function as "nexus species" that have a strong influence on the outcome of plant community succession despite their low abundance in later seral plant communities (Lockwood and Samuels 2004). These species may become important in cases where severe wildfire follows indaziflam treatment and seed banks of native annuals are depleted. Treatment is expected to reduce the likelihood of burning by reducing fine fuels, but with enough time even uninvaded sagebrush-grassland plant communities will inevitably burn (McAdoo et al. 2013). Natural fire is also likely to occur more frequently in more productive higher elevation sagebrush-grasslands (Miller and Rose 1999); i.e., those that may be excellent candidates for indaziflam treatment due to more abundant established perennials (Chambers et al. 2014; Davies et al. 2011). In cases where severe fire follows treatment, managers should monitor recovery and consider actively seeding native annuals if recovery proceeds more slowly than expected. The potential of native annual seed increase programs and their application in restoration is a subject of recent research interest (de Queiroz et al. 2021; Landeen et al. 2021; Abella et al. 2012).

This is less likely to be necessary in cases where the pre-fire community contains robust perennial grasses that survive burning and recover quickly (Ellsworth et al. 2020; Barker et al. 2019; Chambers et al. 2014), as was the case following the wildfire at our site (J. Kraft, personal communication). Further, vegetation sampling the growing season following seed bank collection at our site identified all 4 of the heavily impacted annuals in the aboveground plant community in the treatment plots (Table B.1). In rare cases when conditions during burning are conducive to high perennial mortality (Bates and Davies 2016; Davies et al. 2016; McAdoo et al. 2013; Miller et al. 2013) and the residual activity of indaziflam has waned, the addition of native annuals may be necessary to facilitate desired plant community development from an earlier seral stage.

Our finding of a seed bank community dominated by annuals and much different than the perennialdominated aboveground plant community is not unique in sagebrush ecosystems (Barga and Leger 2018; Martyn et al. 2016; Pekas and Schupp 2013; Humphrey and Schupp 2001). Because there was little overlap between the established vegetation community and the seed bank, we did not explicitly test their similarity, but some of the most widespread species at the site were not represented in the seed bank (bluebunch wheatgrass, arrowleaf balsamroot). Assuming that the established plant community is desirable aside from being infested with downy brome, a striking difference relative to the seed bank is good news for a manager that can use indaziflam to target only the latter of the two.

The low occurrence of perennial plants in the seed bank at our site limits our ability to draw conclusions about the potential for impacts where the seeds of these species are more abundant. Big sagebrush was by far the most abundant perennial observed in our seed bank assay (Table 2.2), but there were only marginal differences between treatment and control seed banks ($p \le 0.10$; Fig. 2.4d). Big sagebrush seed viability declines rapidly except in rare cases when seeds are buried (Hassan and West, 1986; Young and Evans, 1989; Wijayratne and Pyke, 2012). This suggests that impacts observed 37 MAT would primarily result from relatively recent herbicide activity, which is unlikely because we only detected trace amounts of herbicide in the soil at this time, and these concentrations were insufficient to impact planted downy brome in the greenhouse (see Methods). Additionally, the variation in big sagebrush seed density between plots was substantial (Fig. 4b; Table 2), with 23 of the 32 total big sagebrush seedlings observed emerging from samples collected in a single control plot. The distribution of sagebrush in our plots is patchy (Courkamp and Meiman, Unpublished results), likely as a result of historical wildfires (D. Cottle, Personal communication), and the characteristics of big sagebrush seeds (Hassan and West, 1986; Young and Evans, 1989; Wijayratne and Pyke, 2012), the lack of meaningful concentrations of herbicide in our soil samples, and the variation in big sagebrush seed density we observed between plots, suggests that our results likely reflect this patchiness. Future research may be necessary to assess the potential for non-target impacts to sagebrush recruitment in the field, which is known to be episodic (Perryman et al. 2001), sensitive to unpredictable short-term fluctuations in water availability and temperature (O'Connor et al. 2020), and likely not limited by the abundance of short-lived seeds but rather by seed dispersal (Miller et al. 2013) or the number of juvenile plants that successfully transition to mature adults (Germain et al. 2018; Schlaepfer et al. 2014).

Relative to our study, others have found more abundant perennial seed banks in sagebrush communities, but perennials are nearly always a relatively small component of an overall seed bank that is dominated by annual plants (Hosna 2020; Barga and Leger 2018; Martyn et al. 2016; Pekas and Schupp 2013; Humphrey and Schupp 2001). Longer adult lifespan represents an alternative strategy to seed banks for coping with environmental uncertainty, and while there are exceptions, longer-lived species are generally less likely to produce persistent seed banks (Saatkamp et al. 2013; Venable and Brown 1988). For example, big sagebrush seeds are typically non-dormant unless buried (Wijayratne and Pyke 2013), and bluebunch wheatgrass does not produce a persistent seed bank (Humphrey and Schupp 2001; Kitchen and Monsen 1994). While native perennial plants in sagebrush-grassland ecosystems depend on recruitment from seed for reproduction

(Hamerlynck and Davies 2019; Svejcar et al. 2014), one of the advantages of their longer lifespan is the ability to dampen environmental unpredictability by producing seeds over a span of several years. This is reflected by the demographic characteristics of sagebrush, which produces seeds in most years after reaching maturity and often produces many seeds even in average moisture conditions (Schaepfer et al. 2014). When established perennials exist in treated communities, they may also experience increased growth and reproduction after release from downy brome competition (Clark 2020; Fowers and Mealor 2020; Sebastian et al. 2017a). Interpreted alongside knowledge of the life-histories of perennial plants and the period of residual activity associated with indaziflam treatment, these findings suggest that the persistence of perennial species is more likely influenced by the presence of established adult plants and that the benefits of competitive release after treatment likely far outweigh the potential for non-target impacts to seed banks of perennial species, which may or may not exist at the time of treatment and during the resulting period of residual activity.

When considering the potential for non-target impacts from indaziflam treatment, it is critical that managers and stakeholders remember that downy brome invasion and repeated wildfire in established sagebrush plant communities is associated with striking declines in native species richness and abundance (Mahood and Balch 2019; Davies 2011). Indaziflam may allow managers to achieve objectives that they could not achieve with other management tools (Monaco et al. 2017), and ameliorate the unprecedented impacts of what some have justifiably called the most significant plant invasion in the history of North America (Davies et al. 2021; Chambers et al. 2007; D'Antonio and Vitousek 1992). However, we also recognize that downy brome invasion is a complex problem (Boyd and Svejcar 2009), and unintended consequences are not uncommon in rangeland management (Sayre et al. 2012). This reality is demonstrated by the site of this research, where downy brome invasion was likely facilitated by well-intentioned attempts to increase forage production with prescribed fire. Our research is intended to prevent similar unintended outcomes related to deploying an herbicide that seems likely to be a useful management tool for reducing the abundance of annual grasses, but none would argue has a long track record in rangeland management.

In cases where indaziflam treatment without associated revegetation is a suitable management intervention (i.e., invaded perennial communities), the benefits of protecting and promoting established perennial plants likely far outweigh the potential for non-target impacts to native species and native species seed banks. The difficulty of restoration in the harsh and unpredictable environments that characterize downy brome's invaded range (O'Connor et al. 2020; Shriver et al. 2019; Pilliod et al. 2017; Svejcar et al. 2017) suggests that where desirable perennial plants already exist, their maintenance is of the utmost importance. Managers should be aware, however, that impacts to native annuals may occur in some cases. The ecological role of these species is poorly understood (Forbis 2010), but they may be important in specific circumstances, particularly in the early stages of recovery from disturbance (Lockwood and Samuels 2004). The heterogeneity of seed banks is such that the potential for these impacts may be difficult to generalize across sites, and this will increase the importance of site-specific approaches to monitoring and adapting treatment strategies (Seedorf et al. 2022).

Regardless of how they occur, the likelihood of all non-target impacts increases along with the number of treatments applied. Thus, the ability of managers to predict the sequence of treatments necessary to prevent reinvasion from the annual grass seed bank will be critical, and future research should focus on this topic. While this research considered larger scales than previous studies, examining the potential for non-target impacts in different locations is still necessary. Indaziflam has recently been approved for use in areas grazed by livestock, and relative to the extent of downy brome invasion and similar to existing indaziflam research, the scale of our experiments and findings is still limited. Now that use has been approved in grazed areas, researchers should work with managers to identify opportunities to integrate science and management and expand the spatiotemporal scales considered by indaziflam research (Courkamp et al. 2019; Boyd and Svejcar 2009). The ultimate goal of any weed management program is to reduce the impacts of invasive species to the greatest degree possible with the resources available, whether that is through eradication or conciliation and containment. In the case of downy brome invading established sagebrush-grassland plant communities, indaziflam will have a significant role to play in helping managers achieve this objective.

TABLES

Table 2.1. Schedule of seed bank treatments over the course of the 20-week seed bank assay. Watering occurred as necessary to keep the surface of the soil moist except during the dry period during weeks 15 and 16. A modified putty knife was used to turn soil over and fertilizer was applied as a series of two soil soakings on consecutive days (21-18-18, 200 ppm Nitrogen).

Date of Onset	Study Phase	Duration (weeks)	Methods Applied
24-Nov-2019	1	10	Watering
2-Feb-2020	2	4	Soil Mixing, Watering
2-Mar-2020	3	2	Dry
16-Mar-2020	4	4	Soil Turned Over, Fertilizer, Watering

Table 2.2. Mean (\pm SE) seed density (seedlings m⁻²) for each species and growth form observed in the shallow and deep seed bank 37 months after treatment (n=3). Treatment consisted of indaziflam (73 g ai ha⁻¹) applied via helicopter in September 2016.

	Shallow (0-1 cm)		Deep (1-5 cm)	
Species (Common Name)	Control	Treatment	Control	Treatment
Introduced Annuals				
Bromus tectorum (downy brome)	586.1±93.5	84.9±81.4	32.1±8.9	12.1±6.4
Alyssum desertorum (desert madwort)	1.5±1.5	-	-	-
Native Annuals				
Draba nemorosa (woodland draba)	137.5±117.3	-	2.3±2.3	-
Descurainia pinnata (tansymustard)	104.3±26.9	2.5 ± 2.5	54.6±4.7	6.2±6.2
Vulpia octoflora (sixweeks fescue)	65.9±53.3	-	6.9±3.9	-
Lappula occidentalis (stickseed)	64.6±32.7	9.5±6.1	8.9 ± 5.8	11.7±5.9
Gayophytum diffusum (groundsmoke)	1.5±1.5	-	-	-
Native Perennials				
Artemisia ludoviciana (white sagebrush)	1.3±1.3	-	6.9±3.9	-
Phacelia sericea (silky phacelia)	-	-	2.2±2.2	2.1±2.1
Hesperostipa comata (needle-and-thread)	1.3±1.3	2.1±2.1	-	-
A. tridentata ssp. vaseyana (mtn. big sagebrush)	30.5±17.3	2.1±2.1	15.9±12.5	-
Totals				
Introduced Annuals	587.6±93.5	84.9±81.4	32.1±8.9	12.1±6.4
Native Annuals	374.0±92.4	12.0±6.2	72.6±2.9	17.9±10.8
Native Perennials	33.1±19.8	4.2±4.2	24.9±14.7	2.1±2.1
Totals Seedlings	994.7±104.9	101.1±83.8	129.6±15.3	32.1±18.7



Figure 2.1. Mean (+SE) downy brome cover (a) and density (b) in the control and treatment plots (n=3) at 10, 22 and 34 months after treatment (MAT). Treatment occurred via helicopter in September 2016 (indaziflam 73 g ai ha⁻¹). Asterisks indicate a significant difference at that sampling time based on t-tests ($p \le 0.05$).



Figure 2.2. (Top) Linearized power species-area models (log-log transformed) for control (a-c) and treatment (d-f) plots. Models that include all species are represented by black points and solid black lines, and models that include only native species are represented by grey points and dashed grey lines. The position of the points has been randomly adjusted slightly to prevent overlap. (Bottom) Comparison of model slopes (z-values) in control and treatment plots (n=3) for all species encountered (g) and native species only (h). Mean model slope did not differ between treatment and control plots for either all species or native species based on the results of t-tests ($p \ge 0.05$). Treatment consisted of indaziflam (73 g ai ha⁻¹) applied via helicopter in September 2016 and data were collected 33 months after treatment.



Figure 2.3. Mean (+SE) native species seed bank richness (a) and seed bank density (b) in the shallow and deep seed banks 37 months after treatment (n=3). Treatment consisted of indaziflam (73 g ai ha⁻¹) applied via helicopter in September 2016. Asterisks indicate p-value significance based on the results of t-tests (** $p \le 0.05$, * $p \le 0.10$).



Figure 2.4. Mean (+SE) seed bank density of native annuals (a), downy brome (b), native perennial (c), and mountain big sagebrush (d) in the shallow and deep seed banks 37 months after treatment (n=3). Treatment consisted of indaziflam (73 g ai ha⁻¹) applied via helicopter in September 2016. Asterisks indicate p-value significance based on the results of t-tests (*** $p \le 0.01$, ** $p \le 0.05$, * $p \le 0.10$). Note that the y-axis scale of the upper and lower panels differ.

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CHAPTER 3: Management Implications

Rangeland managers and ecologists have long been aware of the threat posed by downy brome (*B. tectorum*) and the downward spiral of annual grass invasion and repeated wildfire (Stewart and Hull 1949; West 1983), yet tools to effectively manage annual grasses have lagged behind (Mack 2011; Monaco et al. 2017). Indaziflam may help accomplish management goals that were not realistic with other tools (i.e., seed bank depletion), and its selectivity provides a new opportunity for managers when annual grasses co-occur with established perennial plants. Proactive treatments that occur before a community transitions to annual grass dominance can maintain some of the resistance to invasion and resilience to wildfire afforded by established perennials (Melgoza et al. 1990; Chambers et al. 2014), and hopefully help managers prevent annual grass invasion from becoming an even greater challenge than it already is.

Chapter 1 suggests that long-term downy brome control is possible with a single indaziflam application; however, managers should avoid assuming that this outcome will be typical when indaziflam is aerially applied at much larger scales. The treatments evaluated in Chapter 1 were ground applied to small plots using a handheld sprayer, and uncertainty increases when herbicide is applied aerially. Skilled applicators minimize the risk of misses and escapes, but a sequence of two treatments may also help. In addition, any operational treatment that is large enough to be meaningful will include substantial microsite variation with the potential to influence treatment outcomes (Heady 1974; Applestein et al. 2018). Given these uncertainties, a sequence of two applications seems likely to increase the consistency of treatment outcomes and the likelihood of achieving near-complete seed bank depletion at meaningful spatial scales, but future research should evaluate the need for multiple treatments at larger scales to address this uncertainty.

It is important to recognize that completely eliminating downy brome may not be necessary to achieve durable, long-term control. It is widely understood that most plant invasions fail (Richardson and Pysek 2004), and this may still apply to individual plants even after a species establishes and becomes invasive. The immense propagule pressure generated by a dense infestation of downy brome dramatically increases the likelihood of persistence, but just a few downy brome plants may be more likely to fail than otherwise. Given this, it is unclear

if continuing treatment will be necessary after initial seed bank depletion is achieved, and how the need for continued treatment will vary from site to site. If continued treatment is necessary after initial depletion, the appropriate interval between treatments also remains an open question. Less frequent treatment may be necessary after near-complete depletion is achieved, and managers may be able to treat smaller infestations as they arise. Refining and better integrating remote sensing into monitoring and management may allow managers to be more targeted and efficient in the future (Saher et al. 2022).

Chapter 2 suggests that indaziflam is unlikely to impact communities of established perennial plants, but it may negatively affect native species seed banks, mostly by impacting native annual plants with germination characteristics similar to downy brome. Impacts to these plants should be interpreted in light of the fact that they are understudied, and may be more important than is obvious (de Queiroz et al. 2021; Forbis 2010). These species are likely important in the immediate stages of recovery from disturbance because this is when they are most abundant (Bazzaz 1996; Bossuyt and Honnay 2008; Boyd and Svejcar 2011). If a severe disturbance occurs after indaziflam treatment, managers should monitor recovery, and consider adding native annuals to revegetation seed mixes if recovery proceeds more slowly than expected. Native annual seed increase programs are the subject of recent research (de Queiroz et al. 2021; Landeen et al. 2021).

Native perennials are less likely than annuals to rely on seed banks for persistence and because of this they are likely less susceptible to non-target impacts from indaziflam treatment. Compared to persistent seed banks, a relatively long lifespan and the ability to produce seeds in multiple years represents an alternative strategy for coping with unpredictable environments, and, in general, longer lived species are less likely to produce persistent seeds (Venable and Brown 1988; Saatkamp et al. 2013). While it is unlikely that one or two indaziflam treatments will have major effects on communities dominated by established perennial plants, future researchers may wish to consider impacts from repeated treatments that result in longer periods of residual herbicide activity.

It is important to emphasize that different seeds have different requirements for germination; thus, the likelihood of germination depends on a variety of interacting and overlapping factors, including the weather (Bazzaz 1996; Baskin and Baskin 2014). Given that the amount of time between indaziflam treatment and conditions that favor germination is likely to vary from treatment to treatment and species to species, it may be exceedingly difficult to make strong predictions about non-target impacts to native seed banks, and some degree of variability in treatment outcomes should be expected. Land managers will have to consider uncertainties and weigh the tradeoffs of treatment in each case, but it may be possible to sidestep most concerns related to nontarget impacts by clearly defining the circumstances wherein indaziflam treatment is the most suitable management tool.

Indaziflam treatment is likely to be most effective in situations where invasive annual grasses and established perennials co-occur, and the plant community is expected to recover on its own without active revegetation. In these cases, the benefits that accrue to established perennials are likely to far outweigh the potential for impacts to native seed banks. In different circumstances the tradeoffs associated with indaziflam treatment may be more substantial (e.g., when revegetation is necessary), but there is no shortage of rangeland in western North America where invasive annual grasses and the prospect of frequent wildfires threatens communities of established perennial plants. Our research, along with other studies (Sebastian et al. 2016; Sebastian et al. 2017; Clark et al. 2019; Clark et al. 2020; Fowers and Mealor 2020; Hart and Mealor 2021), suggests that indaziflam can favor native perennials and reduce the risk of catastrophic, annual-grass fueled wildfire in these communities.

The unplanned wildfire that occurred at one of our study sites also highlights the promise of indaziflam. That large portions of two aerial treatment plots were left as unburned islands completely surrounded by the fire suggests that indaziflam may be an effective tool to mitigate wildfire risk in the future (Courkamp and Meiman 2020). This also highlights an opportunity to demonstrate the value of weed management, applied research, and collaboration involving academia, industry, and on the ground stakeholders. The benefits of natural resource management are often difficult to directly observe, but indaziflam is different. Clear lines between treated and untreated, invaded and uninvaded, and, in some cases, burned and unburned are typical when using this promising new tool. Researchers and managers should work together to take advantage of this opportunity to effectively communicate the value of natural resource management to stakeholders and the public.

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APPENDIX A: Effects of indaziflam treatment on forage quality and quantity in Colorado

SUMMARY

This dissertation originally included another proposed chapter related to evaluating the effects of indaziflam treatment on forage quality and quantity at two sites in Colorado. These studies were unsuccessful due to unforeseen circumstances. At Site 1 (Douglas County), logistical issues substantially delayed the application of some herbicide treatments (Table A.1); at Site 2 (Little Bijou Ranch), initial treatment released Russian thistle (*Salsola kali* L.), which completely dominated the plant community at this site (Figure A.1) and was only reduced after a follow-up treatment that likely impacted all broadleaf species (Table A.2). In light of the realization that these studies do not warrant full consideration in this dissertation, this appendix is intended to document and summarize data collected for this proposed chapter and detail the methods used in their collection and analysis. Herbicide treatments and timings are shown in Table A.1 and Table A.2, the results of statistical analyses are shown in Tables A.3-7. Maps showing the layout of study blocks at Site 1 and Site 2 are shown in Figures A.1 and A.2 and the randomized plot layout (same for both sites) is shown in Figure A.3. Data are displayed in Figures A.5-A.11.

The purpose of the project was to quantify the effects of downy brome (*B. tectorum*) management with indaziflam on forage quality and quantity in central and eastern Colorado rangelands that are invaded by downy brome but still support communities of desirable grasses and forbs. We also sought to identify an effective treatment timing and post-emergent tank mix partner for land managers to improve forage quality and quantity in these ecosystems. Based on other research, we predicted that an early post-emergence (fall-early winter) treatment timing would be most effective at reducing downy brome and that forage quality and quantity would increase most in plots where downy brome reductions were the greatest. We also predicted that impacts to desirable plants may occur when treatments included a post-emergence tank mix partner, and that impacts would be greatest for treatments applied at the pre-emergence (summer) treatment timing because native perennials are still active at this time.

MATERIALS AND METHODS

Site Description

Site 1 was located in Douglas County near the town of Louviers, Colorado (499967 m E, 4370603 m N,610774 m E, UTM zone 13 S, approx. 1720 m elevation), and Site 2 was located at Little Bijou Ranch near Wiggins, Colorado (586872 m E, 4458231 m N, approx. 1364 m elevation). Site 1 was characterized by a sandy foothill ecological site (R049XB210CO) and the soil was Blakeland-Orsa association (sandy, mixed, mesic Torriorthentic Haplustolls). Site 2 was characterized by a sands ecological site (R067BY015CO; USDA-NRCS 2021) and the soil was Valent sand (mixed, mesic Ustic Torripsamments). Mean annual precipitation was 465 mm for Site 1 and 382 mm for Site 2 based on the 30-year average (1991-2020; PRISM 2021).

Before treatments were applied, the plant community at Site 1 was dominated by needle and thread [*Hesperostipa comata* (Trin. & Rupr.) Barkworth] and sand dropseed [*Sporobolus cryptandrus* (Torr.) A. Gray], with an understory dominated by downy brome (*Bromus tectorum* L.) and red-stem fillaree [*Erodium circutarium* (L.) L'Hér ex Aiton]. The pre-treatment plant community at Site 2 was more diverse, primarily due to an abundance of native forbs; the community was dominated by sand dropseed and downy brome, but native forbs including scarlet globernallow [*Spheralcea coccinea* (Nutt.) Rydb.], Hopi tea [*Thelesperma megapotamicum* (Spreng.) Kuntze], slimflower scurfpea [*Psoralidium tenuiflorum* (Pursh) Rydb.], scarlet gaura (*Oenothera suffrutescens* (Ser.) W.L. Wagner & Hoch], and Texas croton [*Croton texensis* (Klotzsch) Müll. Arg.] were also relatively abundant. The introduced forb Russian thistle (*Salsola kali* L.) was also common at Site 2 prior to treatment.

Experimental Design and Measurements

Treatments were applied to 6 by 9 m plots in a randomized complete block design with four replications at each site. The study included six treatments and an untreated control. The treatments occurred at three different application timings related to downy brome's life cycle (early post-emergence, late post-emergence, and pre-emergence; Table A.1). At each timing, two different herbicide treatments were applied (2 herbicide treatments x 3 applications timings = 6 experimental treatments). Herbicide treatments included indaziflam (73 g ai ha⁻¹), alone and in combination with either glyphosate (354 g ae ha⁻¹) or rimsulfuron (53 g ai⁻¹ ha; Table A.2). Glyphosate was not included in pre-emergence treatments because it is a post-emergent

herbicide that would be expected to have no activity against downy brome at this timing (Table A.2). All herbicide treatments included a 0.25% v/v nonionic surfactant and were applied using a CO₂-pressurized custom-built backpack sprayer with 11002LP flat-fan nozzles (TeeJet® Spraying Systems, P.O. Box 7900, Wheaton, Il 60187) delivering 187 L ha⁻¹ at 207 kPa.

Early post-emergence treatments were applied in December 2017 at Site 2, but logistical issues delayed application until March 2018 at Site 1; pre-emergence treatments were applied in June 2018 at both sites; late post-emergence treatments were applied in January 2019 at Site 2, but snow delayed treatment until February 2019 at Site 1 (Table A.1). The logistical issues resulted in the early post-emergence treatments at Site 1 being applied later in the calendar year than the late post-emergence treatments. However, one of the goals of the study was to evaluate the effectiveness of different post-emergent tank mix partners (glyphosate and rimsulfuron) before and after downy brome seedlings penetrated the dense litter that characterizes most invaded sites, and no seedlings were observed above the litter layer when the early post-emergence treatments were applied at Site 1. While this suggests that the delay may have a limited effect on the results of the study, differences between treatments applied at different timings at Site 1 should be interpreted with caution.

At Site 2, initial herbicide treatment unexpectedly released Russian thistle (*Salsola kali*), resulting in its near complete dominance of the plant community at this site (Figure A.4). A follow-up treatment was applied to all plots at Site 2 to control thistle in March 2021 using a UTV-mounted boom sprayer (Table A.2). The treatment included fluroxypyr (245 g ae ha⁻¹), 2,4-D (578 g ae ha⁻¹), and dicamba (280 g ae ha⁻¹). This treatment likely impacted all broadleaf species present at the time of application.

Treatment Evaluations and Data Analysis

To quantify herbicide treatment effects, we used 0.5-m² frames (Bonham et al. 2004) to measure downy brome density and absolute canopy cover (henceforth cover) by functional group (downy brome, C3 perennial grasses, C4 perennial grasses, forbs). We counted individual downy brome plants and recorded ocular estimates of cover to the nearest 1% in these frames. When downy brome was especially dense, plants were counted in only a portion of the larger frame to estimate density. Data were collected from four randomly located frames (subsamples) in each plot. The frame was also used to collect four, 0.25-m² biomass samples from each plot. After cover and density measurements were recorded, the frame was rotated 90, 180, or 270 degrees and all vegetation in the outside half of the frame was clipped at ground level. Care was taken to ensure that biomass samples were not collected in the same location in multiple years. Sampling occurred in mid-July 2018-2021 to approximate peak standing crop. Data were not collected from Site 2 in 2020 due to the dominance of Russian thistle, thus the study was extended until 2021 at Site 2, but no data were collected from Site 1 at this time (Table A.1).

Biomass samples were sorted by functional group (downy brome, perennial grasses, forbs, and shrubs) and oven-dried (55° C) to a constant weight. After weighing, biomass samples were shipped in their entirety to Ward Labs (Ward Laboratories, Inc., Kearney, NE) for forage quality testing. All samples were tested using near infrared spectroscopy (NIRS), but due to concerns about the accuracy of this method for rangeland plants, a subset of samples (approximately 10%) were also tested with wet chemistry methods in 2018 and 2019. Both NIR and wet chemistry testing occurred at Ward Labs. Initial forage quality results using the two different methods were very similar, thus NIRS was used after 2019.

To test for treatment effects, we used the 'lme4' package in R. v. 3.6.2 (R Core Team 2019) to create linear mixed effects models and ANOVA to test for treatment effects at α =0.05. Due to different numbers of treatments across years (Table A.1), substantial interannual variability, and environmental differences between sites, site and year were analyzed independently in all cases with block included as a random factor. Downy brome density, downy brome cover, C3 perennial grass cover, C4 perennial grass cover, forb cover, downy brome biomass, perennial grass biomass (C3 and C4 combined), forb biomass, total biomass (downy brome excluded), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), relative forage value (RFV), total digestible nutrients (TDN), in vitro dry matter digestibility (IVTDMD), and relative forage quality (RFQ) were compared. No C3 perennial grasses were encountered at Site 2 and forage quality responses were not compared in 2019 at Site 2 because of the near-complete dominance of Russian thistle.

Visual inspection of quantile-quantile and fitted vs. residual plots was used to verify that data met the assumptions of ANOVA. Cover data were arcsine square-root transformed and density data were square-root transformed (n + 0.5) as necessary to meet these assumptions. When ANOVA indicated that significant

differences existed between treatments (Tables A.3-A.5), we used the 'emmeans' package (R Core Team 2019) to obtain pairwise comparisons between treatment groups using a Tukey adjustment (α =0.05). When data did not meet the assumptions of ANOVA regardless of transformation, a non-parametric Kruskal-Wallis test was used to test for differences between treatment groups (Table A.6), and a post-hoc Dunn's test was used to obtain pairwise comparisons using a Holm adjustment (α =0.05). Results presented in the appendix are in their original dimensions and means are reported with standard errors (Figures A.5-A.14).

TABLES

			Appro	Approximate MAT at					
	Timing ^a	Application date	2018 ^c	2019	2020 ^d	2021 ^d			
Site 1	EP	8-Mar-2018 ^e	4	16	28	no data			
	LP	16-Feb-2019	no data	5	17	no data			
	Pre	14-Jun-2018	no data	13	25	no data			
Site 2 ^f	EP	20-Dec-2017	7	19	no data	43			
	LP	17-Jan-2019	no data	6	no data	30			
	Pre	14-Jun-2018	no data	13	no data	37			

Table A.1. Dates of treatment for the different application timings at Site 1 (Douglas County) and Site 2 (Little Bijou Ranch).

^aEP = early post-emergence; LP = late post emergence; Pre = pre-emergence.

^bMAT = months after treatment; sampling occurred in mid-July each year.

^cLP and Pre treatments had yet to be applied when 2018 sampling occurred.

^dNo data were collected from Site 2 in 2020 due to the near complete dominance of Russian thistle (*Salsola kali*). Due to the lack of data from 2020, The study at Site 2 was extended to 2021, but no data were collected from Site 1 at this time. ^eLogistical issues delayed the application of EP treatments at Site 1.

^fAll plots at Site 2 received a follow-up treatment on 21-Mar-2021 to control Russian thistle (*Salsola kali*). Treatment was applied to all plots using a UTV-mounted boom sprayer and consisted of fluroxypyr 245g ae ha⁻¹, 2,4-D 578g ae ha⁻¹, and dicamba 280g ae ha⁻¹.

Table A.2. Herbicide treatments applied at each timing at Site 1 (Douglas County) and Site 2 (Little Bijou Ranch).

	Timing ^a	Treatments Applied ^b					
Site 1 & 2	EP	indaziflam + glyphosate 354 g ae ha-1					
		indaziflam + rimsulfuron 53 g ai ha-1					
	LP	indaziflam + glyphosate 354 g ae ha-1					
	LP indaziflam + glyphosate 354 g ae h indaziflam + rimsulfuron 53 g ai ha						
	Prec	indaziflam					
		indaziflam + rimsulfuron 53 g ai ha-1					
Site 2 Only ^d	21-Mar-2021	fluroxypyr 245g ae ha ⁻¹ + 2,4-D 578g ae ha ⁻¹ +					
		dicamba 280g ae ha-1					

^aEP = early post-emergence; LP = late post emergence; Pre = pre-emergence.

 $^{\rm b}$ Indaziflam was applied at 73 g ai ha⁻¹ in all treatments. All herbicide treatments included a 0.25% v/v nonionic surfactant.

^cGlyphosate was not included in pre-emergence treatments because it would have no activity against *B. tectorum* at this timing.

^dAll plots at Site 2 received a follow-up treatment to control Russian thistle (*Salsola kali*). Treatment was applied to all plots using a UTV-mounted boom sprayer.

	Year	Response ^a	F (numerator df, denominator df)	$P > F^{b}$
Site 1	2018 ^d	B. tectorum biomass	23.62 (2, 6)	<0.01
		B. tectorum density	23.78 (2, 9) ^e	<0.001
		B. tectorum cover	27.15 (2, 6)	<0.001
	2019	B. tectorum biomass	9.09 (6, 18)	<0.001
		B. tectorum cover	6.08 (6, 21) ^e	<0.001
	2020	B. tectorum biomass	2.65 (6, 18)	0.051
		B. tectorum density	11.07 (6, 18)	<0.0001
		B. tectorum cover	8.88 (6, 18)	<0.001
Site 2 ^c	2018 ^d	B. tectorum biomass	6.51 (2, 9)°	0.018
		B. tectorum density	14.81 (2, 9) ^e	<0.01
		B. tectorum cover	52.8 (2, 6)	<0.001
	2021	B. tectorum biomass	40.28 (6, 18)	< 0.00001
		B. tectorum density	192.5 (6, 18)	<0.00001
		B. tectorum cover	106.8 (6, 18)	<0.00001

Table A.3. Results of ANOVA (α =0.05) for treatment effects on downy brome (*B. tectorum*) biomass, density, and cover (absolute) at Site 1 (Douglas County; n=4) and Site 2 (Little Bijou Ranch; n=4).

^aSee Table A.7 for responses analyzed with non-parametric Kruskal-Wallis tests. ^bSignificant effects (P < 0.05) shown in bold.

^cNo data were collected from Site 2 in 2020 due to the near complete dominance of Russian thistle (*Salsola kali*); see Table A.7 for 2019 results (all non-parametric).

^dOnly early post-emergence treatments were applied prior to 2018 sampling (Table A.1).

eRandom effect (block) was estimated as zero and removed from the model.

	Year	Response ^a	F (numerator df, denominator df)	$P > F^{b}$
Site 1	2018 ^d	C4 Per. Gr. cover	0.36 (2, 6)	0.71
		C3 Per. Gr. cover	0.34 (2, 9) ^e	0.72
		Per. Gr. biomass	3.73 (2, 9) ^e	0.066
		Forb cover	3.27 (2, 6)	0.11
		Forb biomass	1.23 (2, 9) ^e	0.34
		Total biomass	4.81 (2, 9) ^e	0.038
	2019	C4 Per. Gr. cover	0.60 (6, 18)	0.73
		C3 Per. Gr. cover	0.63 (6, 21) ^e	0.70
		Per. Gr. biomass	3.46 (6, 21) ^e	0.016
		Forb cover	5.40 (6, 18)	<0.01
		Total biomass	3.72 (6, 21) ^e	0.011
	2020	C4 Per. Gr. cover	0.98 (6, 18)	0.47
		C3 Per. Gr. cover	0.50 (6, 18)	0.80
		Per. Gr. biomass	2.53 (6, 18)	0.060
		Forb cover	1.18 (6, 21) ^e	0.36
		Total biomass	2.91 (6, 18)	0.036
Site 2 ^c	2018 ^d	C4 Per. Gr. cover	0.37 (2, 9) ^e	0.70
		Per. Gr. biomass	0.53 (2, 9) ^e	0.61
		Forb cover	1.82 (2, 6)	0.24
		Forb biomass	0.21 (2, 9) ^e	0.82
		Total biomass	0.76 (2, 9) ^e	0.50
	2019	C4 Per. Gr. cover	0.83 (6, 21) ^e	0.56
		Per. Gr. biomass	0.52 (6, 21) ^e	0.79
		Forb cover	1.73 (6, 21) ^e	0.16
		Total biomass	0.83 (6, 21) ^e	0.56
	2021	C4 Per. Gr. cover	3.81 (6, 18)	0.013
		Per. Gr. biomass	2.82 (6, 21) ^e	0.036
		Forb cover	3.28 (6, 18)	0.023
		Total biomass	3.43 (6, 21) ^e	0.016

Table A.4. Results of ANOVA (α =0.05) for treatment effects on C4 perennial grass (Per. Gr.) cover, C3 perennial grass cover, perennial grass biomass, forb cover, forb biomass, and total biomass (*B. tectorum* excluded), at Site 1 (Douglas County; n=4) and Site 2 (Little Bijou Ranch; n=4). All cover measurements are absolute cover.

^aPer. Gr. = perennial grass; no C3 perennial grass were encountered at Site 2; *B. tectorum* was excluded from Total biomass; see Table A.7 for responses analyzed with non-parametric Kruskal-Wallis tests

^bSignificant effects (P < 0.05) shown in bold.

^cNo data were collected from Site 2 in 2020 due to the near complete dominance of Russian thistle (*Salsola kali*).

^dOnly early post-emergence treatments were applied prior to 2018 sampling (Table A.1). ^eRandom effect (block) was estimated as zero and removed from the model.

	Year	Response ^a	F (numerator df, denominator df)	$P > F^{b}$
Site 1	2018c	СР	4.07 (2, 6)	0.077
		ADF	6.29 (2, 6)	0.034
		NDF	1.51 (2, 6)	0.30
		RFV	2.19 (2, 6)	0.19
		TDN	6.30 (2, 6)	0.034
		IVTDMD	2.11 (2, 6)	0.20
		RFQ	1.88 (2, 6)	0.23
	2019	СР	4.01 (6, 18)	0.010
		ADF	1.92 (6, 18)	0.13
		NDF	0.78 (6, 18)	0.60
		RFV	0.70 (6, 18)	0.66
		TDN	1.92 (6, 18)	0.13
		IVTDMD	0.73 (6, 18)	0.63
		RFQ	1.60 (6, 18)	0.21
	2020	СР	0.90 (6, 21) ^d	0.51
		NDF	1.10 (6, 21) ^d	0.39
		RFV	1.34 (6, 21) ^d	0.28
		TDN	1.00 (6, 18)	0.45
		IVTDMD	0.94 (6, 18)	0.49
		RFQ	$0.86 (6, 21)^{d}$	0.54

Table A.5. Results of ANOVA (α =0.05) for treatment effects on crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), relative forage value (RFV), total digestible nutrients (TDN), in vitro dry matter digestibility (IVTDMD), and relative forage quality (RFQ), at Site 1 (Douglas County; n=4).

^aCP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; RFV = relative forage value; TDN = total digestible nutrients; IVTDMD = in vitro dry matter digestibility; RFQ = relative forage quality; see Table A.7 for responses analyzed with non-parametric Kruskal-Wallis tests.

^bSignificant effects (P < 0.05) shown in bold.

^cOnly early post-emergence treatments were applied prior to 2018 sampling (Table A.1).

^dRandom effect (block) was estimated as zero and removed from the model.

Table A.6. Results of ANOVA (α =0.05) for treatment effects on crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), relative forage value (RFV), total digestible nutrients (TDN), in vitro dry matter digestibility (IVTDMD), and relative forage quality (RFQ), at Site 2 (Little Bijou Ranch; n=4). Forage quality was not compared at Site 2 in 2019 or 2020 because Russian thistle (*Salsola kali*) comprised nearly all of the biomass collected in 2019 and no data were collected in 2020 due to its near complete dominance of the vegetation community.

	Year	Response ^a	F (numerator df, denominator df)	$P > F^{b}$
Site 2	2018c	СР	0.0067 (2, 9)°	0.99
		ADF	0.23 (2, 9) ^c	0.80
		NDF	0.16 (2, 6)	0.86
		RFV	0.091 (2, 6)	0.91
		TDN	0.22 (2, 9) ^c	0.81
		IVTDMD	0.90 (2 , 9) ^c	0.44
		RFQ	0.74 (2, 9)°	0.51
	2021	СР	6.22 (6, 18)	<0.01
		ADF	7.00 (6, 18)	<0.001
		NDF	6.43 (6, 18)	<0.001
		RFV	3.63 (6, 18)	0.015
		TDN	7.04 (6, 18)	<0.001
		IVTDMD	15.75 (6, 18)	<0.00001
		RFQ	18.01 (6, 21) ^c	<0.00001

^aCP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; RFV = relative forage value; TDN = total digestible nutrients; IVTDMD = in vitro dry matter digestibility; RFQ = relative forage quality.

^bSignificant effects (P < 0.05) shown in bold.

^cOnly early post-emergence treatments were applied prior to 2018 sampling (Table A.1).

^dRandom effect (block) was estimated as zero and removed from the model.

Table A.7. Results of non-parametric Kruskal-Wallis tests (α =0.05) for treatment effects on responses for which data did not meet the assumptions of ANOVA at Site 1 (Douglas County; n=4) and Site 2 (Little Bijou Ranch; n=4).

	Year	Response ^a	Kruskal-Wallis H ^b	$P > H^c$
Site 1	2019	B. tectorum density	22.11	<0.01
		Forb biomass	10.41	0.11
	2020	Forb biomass	7.68	0.26
		$\mathrm{ADF}^{\mathrm{d}}$	6.47	0.37
Site 2	2019	B. tectorum density	2.89	0.82
		B. tectorum cover	3.58	0.73
		B. tectorum biomass	8.15	0.23
		Forb biomass	4.80	0.57
	2021	Forb biomass	7.43	0.28

^aSee Tables A.3-A.6 for responses analyzed with ANOVA.

 $^{b}df = 6$ for all tests.

^cSignificant effects (P < 0.05) shown in bold.

 ^{d}ADF = acid detergent fiber.

FIGURES



Figure A.1. Map showing the layout of study blocks at Site 1 (Douglas County).



Figure A.2. Map showing the layout of study blocks at Site 2 (Little Bijou Ranch).

101	102	103	104	105	106	107
Untreated Control	Pre Indaz	Pre Indaz + Rim	Early Post Indaz + Gly	Early Post Indaz + Rim	Late Post Indaz + Gly	Late Post Indaz + Rim
201	202	203	204	205	206	207
Pre Indaz	Pre Indaz + Rim	Untreated Control	Late Post Indaz + Rim	Late Post Indaz + Gly	Early Post Indaz + Rim	Early Post Indaz + Gly
301	302	303	304	305	306	307
301 Untreated Control	302 Late Post Indaz + Gly	303 Early Post Indaz + Gly	304 Pre Indaz + Rim	305 Pre Indaz	306 Late Post Indaz + Rim	307 Early Post Indaz + Rim
301 Untreated Control 401	302 Late Post Indaz + Gly 402	303 Early Post Indaz + Gly 403	304 Pre Indaz + Rim 404	305 Pre Indaz 405	306 Late Post Indaz + Rim	307 Early Post Indaz + Rim

Figure A.3. Randomized Layout of study treatment plots at Site 1 (Douglas County) and Site 2 (Little Bijou Ranch). The same randomization was used in both cases. Information about treatment timings can be found in Table A.1. and Table A.2. Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.4. Example of Russian thistle (*Salsola kali*) dominance at Site 2 (photo: J. Courkamp, 18-July-2019). This plot was treated with the indaziflam (73 g ai ha⁻¹) and glyphosate (354 g ae ha⁻¹) at the early post-emergence timing. This level of Russian thistle dominance was typical of all treated plots in 2019 and 2020 at Site 2.



Figure A.5. Mean (+1 SE) downy brome (*B. tectorum*) density (a), absolute cover (cover; b), and biomass (c) at Site 1 (Douglas County) in 2018, 2019, and 2020. Only early post-emergence treatments were applied before 2018 sampling. See Table A.1 for application dates and Tables A.3-A7 for ANOVA and Kruskal-Wallis statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD or Dunn's Test, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.6. Mean (+1 SE) C3 perennial grass absolute cover (cover; a), C4 perennial grass cover (b), and perennial grass biomass (combined C3 and C4; b) at Site 1 (Douglas County) in 2018, 2019, and 2020. Only early post-emergence treatments were applied before 2018 sampling. See Table A.1 for application dates and Tables A.3-A7 for ANOVA statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.7. Mean (+1 SE) forb absolute cover (cover; a), and biomass (b) at Site 1 (Douglas County) in 2018, 2019, and 2020. Only early post-emergence treatments were applied before 2018 sampling. See Table A.1 for application dates and Tables A.3-A7 for ANOVA and Kruskal-Wallis statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.8. Mean (+1 SE) total biomass (*B. tectorum* excluded; a), crude protein (b), acid detergent fiber (ADF; c), and neutral detergent fiber (NDF; d) at Site 1 (Douglas County) in 2018, 2019, and 2020. Only early postemergence treatments were applied before 2018 sampling. See Table A.1 for application dates and Tables A.3-A7 for ANOVA and Kruskal-Wallis statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.9. Mean (+1 SE) relative forage value (RFV; a), total digestible nutrients (TDN; b), in vitro dry matter digestibility (IVTDMD; c), and relative forage quality (RFQ; d) at Site 1 (Douglas County) in 2018, 2019, and 2020. Only early post-emergence treatments were applied before 2018 sampling. See Table A.1 for application dates and Tables A.3-A7 for ANOVA and Kruskal-Wallis statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.10. Mean (+1 SE) downy brome (*B. tectorum*) density (a), absolute cover (cover; b), and biomass (c) at Site 2 (Little Bijou Ranch) in 2018, 2019, and 2021. Only early post-emergence treatments were applied before 2018 sampling and no data were collected in 2020 due to the near complete dominance of Russian thistle (*Salsola kali*). See Table A.1 for application dates and Tables A.3-A7 for ANOVA and Kruskal-Wallis statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.11. Mean (+1 SE) C4 perennial grass absolute cover (cover; a), and perennial grass biomass (b) at Site 2 (Little Bijou Ranch) in 2018, 2019, and 2021 (no C3 perennial grasses were encountered at Site 2). Only early post-emergence treatments were applied before 2018 sampling and no data were collected in 2020 due to the near complete dominance of Russian thistle (*Salsola kali*). See Table A.1 for application dates and Tables A.3-A7 for ANOVA statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.12. Mean (+1 SE) forb absolute cover (cover; a), and biomass (b) at Site 2 (Little Bijou Ranch) in 2018, 2019, and 2021. Only early post-emergence treatments were applied before 2018 sampling and no data were collected in 2020 due to the near complete dominance of Russian thistle (*Salsola kali*). See Table A.1 for application dates and Tables A.3-A7 for ANOVA and Kruskal-Wallis statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.13. Mean (+1 SE) total biomass (*B. tectorum* excluded; a), crude protein (b), acid detergent fiber (ADF; c), and neutral detergent fiber (NDF; d) at Site 2 (Little Bijou Ranch) in 2018, 2019, and 2021. Only early postemergence treatments were applied before 2018 sampling. Forage quality (b-d) was not compared at Site 2 in 2019 or 2020 because Russian thistle (*Salsola kali*) comprised nearly all of the biomass collected in 2019 and no data were collected in 2020 due to its near complete dominance of the vegetation community. See Table A.1 for application dates and Tables A.3-A7 for ANOVA statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.



Figure A.14. Mean (+1 SE) relative forage value (RFV; a), total digestible nutrients (TDN; b), in vitro dry matter digestibility (IVTDMD; c), and relative forage quality (RFQ; d) at Site 2 (Little Bijou Ranch) in 2018, 2019, and 2020. Only early post-emergence treatments were applied before 2018 sampling. Forage quality was not compared at Site 2 in 2019 or 2020 because Russian thistle (*Salsola kali*) comprised nearly all of the biomass collected in 2019 and no data were collected in 2020 due to its near complete dominance of the vegetation community. See Table A.1 for application dates and Tables A.3-A7 for ANOVA statistics. Letters indicate significant within year differences among treatment means (Tukey's HSD, α =0.05, n=4). Herbicide treatments are as follows: Indaz = indaziflam 73 g ai ha⁻¹; Indaz + Gly = indaziflam 73 g ai ha⁻¹ + glyphosate 354 g ae ha⁻¹; Indaz + Rim = indaziflam 73 g ai ha⁻¹ + rimsulfuron 53 g ai ha⁻¹. All herbicide treatments included a 0.25% v/v nonionic surfactant.

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APPENDIX B: Supplementary Tables

Table B.1. Species presence in aerial control and treatment plots (Chapter 1; Modified-Whittaker main plots) in early June 2019 and 2020 (33 and 45 months after treatment, respectively). Plant codes and native status are based on the USDA plants database³ and non-native species are highlighted in yellow.

	2019						2020									
	Со	ntrol P	lots	Trea	tment	Plots	Com	bined	Со	ntrol P	lots	Trea	tment	Plots	Combined	
Species ³	C1	C2	C3	T1	T2	Т3	Con	Trt	C1	C2	C3	T1	T2	Т3	Con	Trt
ACHY	Х		Х			Х	Х	X			Х	Х	Х	Х	Х	Х
ACNE9									х	х	Х	х	Х		X	X
AGGL	х	х		х	х		x	X	х			х	Х		X	X
ALDE	Х	Х	Х	Х			Х	х	Х	Х	Х	Х			Х	х
ANRO2	х	Х	Х	х	х	х	x	X		х	Х	х	Х	Х	X	X
ARLU	х	Х	Х		Х	Х	X	X	х	х	Х		Х	х	X	X
ARTRV	х	х	Х	х	х	х	x	X			х		Х	х	X	X
ASCO12		Х	Х			Х	х	X		Х	Х			Х	X	X
ASTRA		Х					X									
BASA3	х	х	Х	х	х	х	х	X	х	х	х	х	Х	х	x	Х
BRTE	Х	Х	Х	Х	Х	Х	Х	х	х	х	Х	Х	Х	Х	Х	х
CANU3											Х	х		х	X	X
CAREX	х	Х	Х	х	Х	Х	X	X	х	х	Х	х	Х	х	X	X
CHVI8	х	Х		х	х	х	x	X	х	х		х	Х	Х	X	X
COLLI			Х	х			x	X								
соим	х		Х	х	х		x	X	х		Х	х	Х		X	X
CRAC2	х	х		х			x	X								
CRFE3	х	Х	Х	Х	х	х	х	X	х						x	
CRYPT3		х	Х		х		х	X			Х		Х		x	X
Cryptantha sp. (perennial)	х	Х	х	х	х	х	х	X	х	х		х	х		х	х
DEPI									х	х	Х	х	Х		x	X
DRABA	х	Х	Х		х		х	X	х	х			Х		X	X
ERIGE2	х			х			х	x								
ERUM		х		х	х	х	х	X				х	Х	х		х
GADI2	х	х	Х	х		х	х	X								
HAPAS	х	Х	Х	х	х		х	X	х	х	х	х	Х	х	X	х
HECO26	х	х	х	х	х	х	х	X	х	х	х	х	х	х	x	X
HEUCH				х				X								
LAOC3	х	Х	Х		х		х	X	х	х	х	х	Х		X	х
LIRU4	х	х	х	х	х	х	х	x	х	х	х	х	х	х	x	X
LOMAT	х						х									
MARE11	х				х		х	x	х				х		x	X
MEBU											х	х		х	x	x
OPPO	х	х	х	х	х	х	х	X	х	х	х	х	х	х	x	X
PAMU11		х					х									
рнно				х				x								
PHLO2	х			х	х	х	х	X	х			х		х	x	X
PHMU3				х				x								
POFE											х	х	х		x	х
POSE	х	х	х	х	х	х	х	X	х	х	х	х	х	х	x	X
PSSP6	х	х	х	х	х	х	x	x	х	х	х	х	х	х	x	X
PUTR2		х	х	х	х	х	х	x			х	х	х	х	x	X
ROWO											х				x	
SCLI	х	х	х	х	х		x	x	х	х	х	х	х	х	x	x
SYMPH	х	х		х	х	х	х	x	х	х	х	х	х	х	x	X
TAOF	х	Х					х									
TRDU	х				х		х	х	х			Х	Х		х	х
VIOLA	х	х	х	х	х		х	х					х			х
νυος			Х	х			х	X	х	х	х	х	х		x	X
ZIPA2	х			х			х	x	х			х			x	X
Total	33	31	27	32	28	21	41	40	27	22	28	30	31	22	37	37
Total Native	29	28	25	30	26	20	37	37	24	20	26	27	29	21	34	34

³USDA-NRCS (2021c) Plants Database. Available at: https://plants.usda.gov/home. Accessed 1 June 2021