Management of annual brome invasion within northern mixed grassland using indaziflam

by

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Abstract

Annual brome grasses are some of the most widespread and problematic weeds in North America and present a challenge in managing rangeland, with control options needed. The herbicide indaziflam has shown promise in long-term annual brome reduction on western U.S. rangeland, though how these results apply to northern temperate grasslands of western Canada remains uncertain.

In chapter 2 we studied the effect of indaziflam rate (0, 37.5, 75, and 150 g ai ha^{-1}) and timing (fall vs spring application) on long-term biomass and density of annual brome at two sites in Canada's northern Mixedgrass Prairie. Reductions in brome were not evident until the second growing season following treatment. During the third growing season, the current recommended rate (75 g ai ha^{-1}) of indaziflam reduced brome biomass and density by at least 90% at both sites. By the fourth season indaziflam continued to reduce brome biomass by 11 and 66%, and brome density by 76 and 95%, at the two sites, respectively. Reductions in brome biomass and density occurred at rates as low as 37.5 g ai ha^{-1} but were not as reliable as higher rates of 75 and 150 g ai ha^{-1}. The timing of indaziflam application had less impact on long-term brome reduction. A single application of indaziflam can reduce annual brome, including corn brome (*Bromus squarrosus* L.), in northern Mixedgrass Prairie grasslands for up to four years.

In Chapter 3, to understand how non-brome northern temperate grassland vegetation responds to indaziflam herbicide treatment, we studied the effect of indaziflam rate (0, 37.5, 75, and 150 g ai ha^{-1}) and timing (fall vs spring application) on plant species cover, richness, diversity, total plant biomass, perennial grass biomass, and forb biomass. Indaziflam application did not affect the cover, richness, or diversity of non-brome plant species until the fourth year after treatment, at which time species richness and diversity were reduced by most indaziflam treatments at both sites. In the fourth year, indaziflam treatment also altered the cover of the three most abundant grasses but did not affect the three most abundant forbs. Total biomass first decreased in the second year, then increased in the third, and stabilized in the fourth year following indaziflam treatment. Perennial grass biomass either increased or remained the same and did not decline under any indaziflam treatment in any year. Forb biomass was not affected by indaziflam treatment. Next, we studied the effect of indaziflam rate (0, 37.5, 75, and 150 g ai ha^{-1}) on seedling emergence from the soil seedbank treated with indaziflam two years prior. Indaziflam reduced forb emergence but did not reduce perennial grass emergence. Finally, we studied the effect of indaziflam rate (0, 75, and 150 g ai ha^{-1}) on root and shoot biomass of four species of actively growing perennial grasses. Indaziflam reduced root and shoot biomass of four-month-old perennial grasses grown in greenhouse conditions.

Our study provides an improved understanding of how indaziflam affects plant community composition and biomass within the northern Mixedgrass Prairie where native grasslands are relatively less invaded by annual brome grasses.

“And if you missed a day, there was always the next,
  and if you missed a year, it didn’t matter,
  the hills weren’t going anywhere,
  the rosemary and thyme kept coming back,
  the sun kept rising, the bushes kept bearing fruit –”

–Louise Glück

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List of Abbreviations

AB: Alberta

ABMI: Alberta Biodiversity Monitoring Institute

ac: Acre

ACIS: Alberta Climate Information Service

AGCM: Agriculture Climate Monitoring Station

ai: Active ingredient

AIXR: Air Induction Extended Range

ANOVA: Analysis of variance

ºC: Celsius

cm: Centimeter

exp: Exponential

g: Gram

g ai ha^{-1}: Grams of active ingredient per hectare

ha: Hectare

hr: Hour

kg: Kilogram

km: Kilometer

Kow: n-octanol-water partition coefficient

kPa: Kilopascal

L: Liter

ln: Natural log

LnRR: Log response ratio

m: Meter

mm: Millimeter

NMDS: Non-metric multidimensional scaling

oz: Ounce

PerMANOVA: Permutational analysis of variance

r: Correlation coefficient

R: R programming language

RTV: Rough terrain vehicle

SE: Standard error

U.S.: United States

USA: United States of America

1 Introduction

1.1 Weeds and rangeland

Rangeland plant communities are dynamic. Their composition and function changes when weeds are introduced to the system. This presents a perpetual challenge in managing rangelands worldwide as land managers aim to preserve the function and services of rangeland ecosystems. Invasive weeds often compete with native plants for limited resources, increasing their abundance at the expense of the natives (Germino et al. 2016). These abundant weeds are often less palatable or nutritious to grazing animals, resulting in negative impacts to livestock production (Haferkamp et al. 2001). The loss of layers of plant community structure that results from invasion of early-seral weed species reduces the efficiency of solar energy capture, resulting in the production of lower biomass, less biodiversity, and loss of habitat and food source for wildlife (Germino et al. 2016). When plant communities are less diverse and one species becomes overabundant, ecosystems are less resilient or resistant to extreme or unexpected events such as drought or fire (Stewart and Young 1939).

Lost ecosystem function has societal and economic cost. Some of the weeds in North American rangeland considered to be of major importance include downy brome (*Bromus tectorum* L.), Canada thistle [*Cirsium arvense* (L.) Scop.], Russian knapweed [*Rhaponticum repens* (L.) Hidalgo], spotted knapweed (*Centaurea stoebe* L.), leafy spurge (*Euphorbia esula* L.), dalmatian toadflax [*Linaria dalmatica* (L.) Miller], and purple loosestrife (*Lythrum salicaria* L.) (Duncan et al. 2004). These weeds invade millions of hectares of land within North America and are estimated to cost millions to billions of dollars in lost ecosystem function and cost of control (Duncan et al. 2004). Therefore, decisions on how to manage invasive weed species have great significance economically and ecologically.

1.2 Annual brome habitat and biology

In contrast to perennial grasses that overwinter and regrow each year, annual bromes are grasses in the genus *Bromus* that complete their life cycle in one year and rely on seed for reproduction. They are some of the most widespread invasive weeds on North American rangeland (Duncan et al. 2004). Perhaps the most prevalent and studied of them is downy brome (*Bromus tectorum*). Another common species in North America is Japanese brome (*Bromus japonicus* Thunb.). Both were introduced to North America from Eurasia in the mid-19th century and have been found to establish in a wide variety of habitats and environments (Beck 2009). They have commonly been found in disturbed areas (Knapp 1996) but can also establish in stands of little-disturbed native vegetation (Hulbert 1955). Both species are found in every contiguous state of the U.S. and nearly every province of Canada (Beck 2009). They are particularly abundant in the Great Basin area of the western U.S. (Knapp 1996), an area spanning across Nevada, Oregon, Utah, Idaho, Wyoming, and California, and are also abundant in the Great Plains. In 2004, it was estimated that 22 million ha of the 17 western U.S. states were invaded by downy brome and that the annual rate of spread was 14% (Duncan et al. 2004).

There is not as much information about the distribution of annual brome within Canada or Alberta. Data from Alberta Environment and Parks range health assessments and Alberta Biodiversity Monitoring Institute (ABMI) surveys from 2003-2018 provide an idea of some reported occurrences of annual brome within the province, where there appear to be a higher density of reported occurrences in the south near the border of Montana (Figure 2.1 and Figure 2.2 in Chapter 2). As for the other provinces of Canada, downy brome is present in grasslands of the interior of British Columbia (Upadhyaya et al. 1986) and is also found in grassland and winter wheat fields in Saskatchewan and Manitoba (Douglas et al. 1990). Japanese brome is also commonly found within the northern mixed prairie (Ogle et al. 2003), and in addition, we have found that the species “corn brome” (*Bromus squarrosus* L.), an annual brome introduced to North America from Russia and central Europe (Pavlick 1993), is abundant at sites in southern Alberta grasslands. In contrast to the abundance of information about downy and Japanese brome within North America, there is limited literature about corn brome. However, the morphology and phenology of these three annual bromes are considered to be very similar (Fenesi et al. 2011; Oja and Paal 2003), although, interestingly, corn brome is found mainly in southern Canada and the northern U.S. in comparison to the more southern distribution of downy brome (Pavlick 1993). In addition to these three species, the Flora of Alberta describes three other annual bromes that occur in Alberta, all introduced species from Eurasia: rye brome (*Bromus secalinus* L.), hairy brome (*Bromus commutatus* Schrader), and soft brome (*Bromus hordeaceus* L.). Annual bromes can be considered an uncommon functional group in the northern grassland ecosystem because of their annual lifecycle in contrast to the perennial lifecycle of most other species.

Historically, annual bromes have not been as common in the northern Great Plains compared to the southern area of the region (Monaco et al. 2017). Reasons for the lack of spread of annual bromes in Canadian rangeland compared to U.S. rangeland up until this time are uncertain, but likely include a combination of limited anthropogenic distribution, climatic factors such as higher moisture, shorter growing season and extended snow cover, and perhaps less degraded rangeland. The most heavily invaded region with downy brome in the U.S. is the sagebrush steppe of the Great Basin where downy brome has nearly entirely displaced perennial grasses and sharply decreased the dominance of native plant species (Knapp 1996). Rangelands of the Great Basin did not evolve with widespread grazing, and as a result, when cattle were introduced much of this area became severely overgrazed (Knapp 1996). With rangeland degradation, invading downy brome was able to exploit unused resources (Knapp 1996). Compounding the degradation of Great Basin rangeland is the limited precipitation in the region (Knapp 1996). Limited summer precipitation in particular affects native plants more severely than downy brome because by summer downy brome has already completed its life cycle. Downy brome relies predominantly on fall/winter/spring moisture in order to first germinate in the fall, successfully overwinter, and then in spring use its established roots to exploit the limited spring soil moisture at the expense of native perennial grasses. A model predicting downy brome distribution indicated that the climatic factors that best explained its distribution were summer precipitation, followed by annual precipitation, spring precipitation, winter maximum temperature, and winter minimum temperature (Bradley 2009). Consequently, areas projected to receive less summer precipitation or more variable precipitation could become increasingly vulnerable to annual brome invasion in the future.

In addition to their winter annual lifecycle resulting in capture of early spring moisture at the expense of perennials, the annual lifecycle strategy relies on greater seed production and germination than perennial grasses. Annual bromes have finely divided, shallow root systems and only a few main roots (Beck 2009; Upadhyaya et al. 1986), suggesting a strong allocation of energy into seed production rather than developing a robust root system. Annual brome species are known to be prolific seed producers (Beck 2009). For example, downy brome plants produce copious quantities of seeds (25 to 5000 seeds per plant) annually, the amount depending on environmental site conditions, but always enough to perpetuate their population – within infested areas, plant density in subsequent years is typically more a function of the conditions for germination and establishment rather than seed availability (Beck 2009). High amounts of litter accommodate the establishment of downy brome seeds through the retention of water, although seeds can germinate even when soil is relatively dry (Beck 2009; McDonough 1975). Seeds typically germinate in the fall when temperatures cool, following a loss of seed dormancy when exposed to high summer temperatures (Hulbert 1955). Plants then overwinter in a vegetative state, resuming growth in the spring with rapid root growth, and reach maturity in late spring (Beck 2009). Seed longevity in the soil is relatively short, rarely lasting longer than five years for downy brome (Wicks et al. 1971; Sebastian et al. 2017), though dry seeds may last 8 (Steinbauer and Grigsby 1957) to 11 years (Hulbert 1955). With proper conditions seeds typically germinate in the first fall following their production and seed cast (Hulbert 1955; Wicks et al. 1971). Most downy and Japanese brome seeds germinate after 1 year, with some germinating after 2 years (Baskin and Baskin 1981; Wicks et al. 1971), and the majority of seeds are found in the top 1.25 inches (3 cm) of soil (Whisenant 1990). Downy brome seeds germinate better when covered in soil than when they are on the soil surface (Wicks et al. 1971), however in mesic areas, like the northern mixedgrass prairies, they can readily establish on bare soil (Young et al. 1972).

1.3 Impacts of annual brome invasion on ecological function

Annual bromes compete with native perennial plant species for soil water and produce large amounts of seed that result in dramatic increases in its cover (Harris 1967; Knapp 1996; Melgoza et al. 1990). Downy brome invaded sites may have less perennial grass cover, less forb cover, and more bare ground (Leger 2008). Additionally, sites invaded by annual grasses frequently have lower diversity and richness of plant species (D'Antonio and Vitousek 1992; Germino et al. 2016; Tortorelli et al. 2020). Further, senesced litter of annual grasses is highly flammable. As a result, their invasion can lead to increased risk of wildfire and even greatly reduce the mean fire return interval, which expands the disturbed area for brome to invade, further perpetuates and exacerbates many ecological impacts, as well as poses a risk to public safety, infrastructure, livestock, and wildlife (D'Antonio and Vitousek 1992). The invasion of annual brome and subsequent shifts in plant community composition from perennial grass to annual grass has many implications for the functioning and use of rangeland/grassland ecosystems.

1.3.1 Forage production

Forage production is an important component of assessing a land's ability to sustain livestock and wildlife. Although downy brome and Japanese brome can be palatable to livestock (Harris 1967), the use of annual brome as forage appears to be limited for several reasons. First, the phenological development of annual brome leads to these species remaining 'green' for only a short period of time early in the season (Stewart and Young 1939). Long awns that cause mouth discomfort to animals reduce palatability once seed set occurs (Upadhyaya et al. 1986). Second, there is evidence that annual bromes and areas invaded by annual bromes yield less forage than stands of native perennial grasses. For example, perennial forage stands in the Intermountain region of Idaho yielded twice as much as downy brome stands in wet years, and much more in dry years (Stewart and Young 1939). In the northern mixed grass prairies of South Dakota, brome removal in fall and again in spring resulted in higher above ground and below ground biomass production of the plant community at the end of the growing season (Ogle et al. 2003). Following brome removal, perennial grasses increased in tiller density and biomass, leading to long-term increases in total biomass, although it should be noted that immediately after brome removal total biomass may be temporarily reduced (Haferkamp et al. 1997; Melgoza et al. 1990). Third, the presence of annual brome can make determining the sustainable grazing capacity of invaded land difficult. It can be risky to base grazing capacity on the use of annual brome because of the uncertainty of its year-to-year production (Stewart and Young 1939). Because annual brome production is so dependent on precipitation and timing of precipitation, forage production in stands invaded by annual brome is more variable year-to-year than perennial stands less invaded or not invaded by brome (Klemmedson and Smith 1964; Stewart and Young 1939). While perennial forage production is also dependent on precipitation, its production generally fluctuates less so than annuals, making it more dependable as a supply of forage for supporting annual grazing (Stewart and Young 1939). Finally, livestock performance can be reduced when grazing on brome invaded rangeland. For example, steers grazing on grassland in Montana where annual brome had been suppressed gained more weight than steers grazing on brome infested sites (Haferkamp et al. 2001).

1.3.2 Wildlife habitat

Annual brome invasion also affects wildlife, with altered vegetation composition affecting their habitat and availability of preferred food sources. For example, a study in the Pacific Northwest showed that local birds had a strong preference for native grass seed over annual brome seed (Goebel and Berry 1976). Another study showed that uniform brome stands may impede the above ground movement of small mammals (Gano and Rickard 1982). In the sagebrush steppe, annual brome invasion changed the structure of vegetation by creating a thick litter layer that reduced the foraging ability of small mammals (Bachen et al. 2018). These impacts can ultimately lead to changes in animal abundance. Downy brome dominated areas in bitterbrush communities of Washington had three times fewer small mammals than shrub dominated areas (Gano and Rickard 1982). Another concerning example within the sagebrush ecosystem is that increased wildfire facilitated by annual brome has substantially changed the structure of native plant communities by removal of sagebrush. These changes, in turn, have resulted in a reduction in hiding, nesting, and foraging cover, leading to population declines in small mammals and birds, including the at-risk species sage grouse (Gano and Rickard 1982; Knick 1999). There is generally a negative correlation between homogenization of habitat and wildlife diversity (Fuhlendorf and Engle 2001).

1.3.3 Carbon storage

Rangelands cover vast areas of North America and are an important terrestrial carbon reserve (Havstad et al. 2007). Patterns of carbon storage on rangelands are affected as shallow rooted annual grasses displace deeper rooted perennials (Bradley et al. 2006). Aboveground biomass carbon may initially increase following invasion in areas like the Great Basin as annual brome fills ‘empty’ spaces within the community (Nagy et al. 2020); however, when annual brome becomes the dominant vegetation, aboveground and belowground biomass carbon is significantly lower compared to native plant communities (Bradley et al. 2006). In varying ecoregions across the Great Basin, downy brome dominance was associated with a consistent loss of above ground and below ground biomass carbon (Nagy et al. 2020). Changes in soil carbon are more nuanced. Downy brome invasion has been associated with no change in soil organic carbon or even increases in soil organic carbon, especially within the topsoil (Bradley et al. 2006; Germino et al. 2016; Nagy et al. 2020). Invasion by brome is more likely associated with a loss of deep soil organic carbon due to the loss of deep rooting perennial plants and loss of soil aggregates in invaded areas (Germino et al. 2016; Nagy et al. 2020). In one study, soil organic carbon below the 60 cm depth was reduced in sites experiencing brome invasion (Rau et al. 2011). In another study, when comparing stages of annual grass invasion, sagebrush ecosystems in Nevada that were heavily invaded had lower soil total carbon than the less invaded category (Mahood et al. 2021). Carbon stocks are also closely associated with fire (Nagy et al. 2020). Fire causes direct combustion of above ground biomass carbon, but can also cause the loss of topsoil organic carbon through erosion (Germino et al. 2016).

1.4 Control of annual brome

Given the ecological impacts of annual brome grass invasion, control of these species is desirable. However, despite much research, reliable control methods are lacking (Lehnhoff et al. 2019; Monaco et al. 2017). Attempts to control annual brome on rangeland have included the use of herbicide, soil amendment, burning, revegetation and grazing, although herbicide is the most commonly used control strategy (Monaco et al. 2017; DiTomaso 2000).

1.4.1 Grazing

Like all control methods, use of grazing requires consideration of how it will impact both the invasive species and the native species. Ideally, for optimal restoration of habitat, annual brome would decrease in abundance while perennial grasses are maintained or even increased in abundance (Monaco et al. 2017). Two variables that affect the way annual brome and native plant species respond to grazing are the timing and intensity of grazing. Grazing in the spring may be the best way to balance brome control via defoliation while minimizing damage to native plants, and this is most beneficial because annual brome is growing in spring and is at its most palatable, while native plants may still be dormant (DiTomaso 2000; Lehnhoff et al. 2019). There are contrasting strategies for intensity of grazing. One strategy is to moderately graze invaded areas to minimize damage to native plants and disturbance to soil (DiTomaso 2000). Another strategy is to intensively graze to reduce selectivity, forcing defoliation of annual brome (DiTomaso 2000). However, grazing does not appear to be a consistent way to control annual brome. Targeted high intensity sheep grazing in spring did not reduce downy brome production or cover in the year of and year after treatment on grassland in western Montana that had around 20% cover of downy brome (Lehnhoff et al. 2019). Similarly, low, moderate, or high intensity rotational grazing of cattle did not reduce downy brome production or cover in the big sagebrush steppe of Oregon that had around 1% cover of downy brome; in this study, downy brome production increased over time with grazing (Bates and Davies 2014). However, cattle intensively grazing in both early and late May decreased production of downy brome in the following year by 10% in the Great Basin of Nevada in which downy brome cover was initially around 60% (Diamond et al. 2012). Overall, the effectiveness of grazing is limited in controlling annual brome. A meta-analysis by Monaco et al. (2017) indicated that grazing does not control downy brome biomass over the long-term. Grazing is limited in its use as a primary control strategy over the long-term because while targeted high intensity grazing can reduce the reproductive potential of downy brome, plants can still set seed and replenish the seed bank (Diamond et al. 2012). High intensity grazing is also spatially and temporally limited in its use over a large scale.

1.4.2 Herbicide

Commonly recommended herbicides for downy brome control include glyphosate, rimsulfuron, and imazapic (Lehnhoff et al. 2019; Sebastian et al. 2016b). However, these herbicides provide only temporary residual control of the soil seed bank and are often unsuccessful in preventing reinvasion of annuals (Kyser et al. 2013; Sebastian et al. 2016b). Lack of long-term control is a key issue in annual brome management (Monaco et al. 2017).

Indaziflam (Rejuvra®, Envu) is a herbicide that, as of June 2020, has been registered for annual grass and broadleaf weed control on rangeland in the U.S. It is a pre-emergence alkylazine herbicide that controls weeds through inhibition of cellulose biosynthesis in establishing seedlings. It is non-selective and presumably active on any germinating seed. When seedling cellulose biosynthesis is inhibited, radial swelling and ectopic lignification occur, and roots fail to expand (Brabham et al. 2014). Notably, indaziflam has shown potential in providing long-term control of downy brome due to its residual activity. When compared to glyphosate, rimsulfuron and imazapic, indaziflam emerged as the front runner by providing the highest long-term (2 and 3 years after treatment) control of downy brome (Sebastian et al. 2016b). Compared to non-treated plots, indaziflam had 83-100% control of downy brome three years after treatment. Compared to other commonly used rangeland herbicides, indaziflam has relatively low water solubility of 2.8 mg L^{-1}, high log K_{ow} of 2.8 at pH 7, and long half-life (>150 days), which could explain its residual soil activity (Tompkins 2010); however, a herbicide's persistence in soil is not only a function of its chemical properties, but is also a function of soil properties. Soil organic matter content, soil pH and soil clay content all affect herbicide availability (Alonso et al. 2011). Soil organic matter is the primary soil property that has been found to be correlated with indaziflam efficacy (Sebastian et al. 2016a). Sebastian et al. (2016a) found a negative correlation between soil organic matter and indaziflam efficacy such that when soil organic matter was higher, greater doses of indaziflam were required to reduce weed growth. Additionally, precipitation and soil moisture affect adsorption and leaching of herbicide (Sebastian 2016a). Therefore, a site's agroclimatic properties will also influence the efficacy of indaziflam.

Ecosystem recovery following herbicide application also depends on how the resident plant community of other grasses, forbs, and shrubs are affected. The native plant community will be affected not only by annual brome seed germination inhibition, but also by native seed germination inhibition. Survival of perennial grass species are expected to be less affected because they reproduce largely by tillers and less from seed. Meanwhile, species that rely on seed for reproduction are expected to decline in abundance. In the western US, indaziflam has shown no impact to native perennial grass or forb abundance, nor species richness (Clark et al. 2019). However, the diversity of highly invaded plant communities may already have been compromised by annual brome invasion prior to herbicide application. In less invaded, more intact plant communities, there is higher potential for loss of native plant species (Clark et al. 2019). Nevertheless, there is concern that native species may be affected by herbicide injury. Necrosis or yellowing of leaves may be observed with herbaceous tissues in contact with indaziflam (Bayer Crop Science). Additionally, in greenhouse food crops, indaziflam applied post-emergence has been shown to cause injury and adversely affect shoot and root growth rates even at low rates (Jeffries et al. 2014).

1.5 Study objectives

Here, we use field and greenhouse experiments to better understand the effects of indaziflam use within northern mixed grassland. The objectives of Chapter 2 were to determine the magnitude and length of annual brome reduction, evaluate the U.S. recommended rate within Canadian grasslands, and compare fall and spring seasons of application. The objectives of Chapter 3 were to determine how indaziflam affects northern mixed grassland plant species composition, richness, diversity, and biomass. We also evaluated how indaziflam affects perennial grass and forb recruitment, and how the cellulose biosynthesis inhibiting action of indaziflam affects the biomass of actively growing perennial grasses. Ultimately, the goal was to provide regionally tested information to stakeholders seeking management options for annual brome, and who are interested in the judicious use of herbicide, including the optimal rate and timing of application, how much control can be expected, and what potential drawbacks might be to native plant community richness, diversity, and forage production.

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2 Indaziflam provides long-term reduction of annual brome grass (*Bromus* spp.) in Canada's mixedgrass prairie

2.1 Introduction

Annual brome grasses are prevalent weeds on North American rangeland that affect many ecosystem goods and services, including livestock and wildlife grazing (Duncan et al. 2004). These invasive species compete with perennial plants for soil resources when establishing in fall and spring, and produce large numbers of seed that result in dramatic increases in brome cover (Harris 1967; Melgoza et al. 1990). Increased annual brome abundance is known to lower forage biomass availability and quality (Haferkamp et al. 2001; Ogle et al. 2003). In addition, annual bromes have less stable production between years compared to native perennial grasses, thereby increasing uncertainty in forage supply (Stewart and Young 1939). Where annual brome is reduced or removed, perennial grass production may increase, providing increases in preferable forage (Clark et al. 2020; Haferkamp et al. 2001; Ogle et al. 2003). As a result, control of annual brome is of significant interest to land managers.

Annual bromes are some of the most geographically widespread weeds in North America (DiTomaso 2000). It has been estimated that downy brome, *Bromus tectorum* L., the most abundant and well-studied annual brome in North America, inhabits tens of millions of hectares in the United States (U.S.) and is particularly abundant across the 17 western states (Beck 2009; Duncan et al. 2004). Downy brome is found in all states of the U.S. and every province of Canada except Newfoundland and Labrador (Beck 2009). While its extent on Canadian rangeland has not been documented in as much detail as across the U.S., records indicate that annual bromes are spreading in the northern Great Plains where they have been less abundant historically (Douglas et al. 1990; Monaco et al. 2017; Valliant et al. 2007). Records compiled on confirmed brome locations in southern Alberta, Canada during range health assessments on public grazing leases indicated 530 occurrences of annual brome from 2003 through 2018 (Figure 2.1), leading to concerns about the future status of Alberta's Mixedgrass Prairie rangeland and its conservation. Concurrent surveys by the Alberta Biodiversity Monitoring Institute (ABMI) have also detected annual brome in southern and central Alberta (Figure 2.2). The spread of annual brome has also been identified as a concern in Manitoba (Cici and Van Acker 2009), British Columbia (Lee et al. 2014), and Saskatchewan (Douglas et al. 1990).

Generally, herbicides have provided greater control of annual brome compared to other practices like grazing or burning (Monaco et al. 2017). Despite this, long-term control has been difficult to achieve even with commonly recommended herbicides for controlling invasive weeds such as imazapic, rimsulfuron, and glyphosate (Sebastien et al. 2016b). In Canada, there are currently limited herbicide options for the control of annual brome in perennial grasslands. Imazapic and rimsulfuron are not registered for grassland application in Canada (Corteva; Health Canada 2015). Many herbicides that control annual grasses are registered for use only on cropland and not rangeland (e.g. pyroxsulam, glyphosate). In addition, there has been herbicide resistance reported within downy brome populations to Group 1 acetyl-coenzyme A carboxylase inhibitor (Ball et al. 2007) and Group 2 acetolactate synthase (Zuger and Burke 2020) in the U.S., and most recently group 9 enolpyruvylshikimate-3-phosphate inhibitors (glyphosate) in southern AB winter wheat fields (Geddes and Pittman 2022). Recently, a relatively new herbicide to rangeland called indaziflam (Envu^{TM}, Canada) has shown potential for the long-term control of annual grasses (Clark et al. 2020; Sebastian et al. 2016b). Indaziflam was registered for use on natural grasslands/rangeland across the U.S. in 2020 and is in the process of being registered for rangeland use in Canada. Indaziflam is a group 29 soil applied cellulose biosynthesis inhibiting herbicide that targets establishing seedlings. Cellulose biosynthesis inhibition is non-selective and affects expanding tissues, inhibiting the growth of seedlings, particularly root growth (Brabham et al. 2014). The principle behind the use of indaziflam on rangeland is that the ongoing prevention of seedling establishment over an extended time will deplete the annual weed seed bank. Conversely, while perennial plant species seeds will also be prevented from establishing, previously established perennial plants are thought to be less affected due to their perennial life cycle and prevalence of roots below the treated soil layer. Indaziflam has a water solubility of 2.8 mg L^{-1}, log K_{ow} of 2.8 at pH 7, and a half-life of >150 days (Tompkins, 2010), properties that typically result in extended soil residual activity, potentially providing long-term control of the annual brome seed bank.

Field studies in Colorado (Clark et al. 2020; Sebastian et al. 2016b; Seedorf et al. 2022) and Wyoming (Courkamp et al. 2022) have found that indaziflam provides a high level of reduction of annual brome even at relatively low rates (44, 58, or 73 g ai ha^{-1}) and that single applications of indaziflam can provide multiple years of control. There are agroclimatic differences, however, that are likely to influence the efficacy of indaziflam in the northern Mixedgrass prairie of western Canada. For example, soil organic matter tends to increase as latitude increases from the southern to the northern Great Plains (Epstein et al. 2002). Soil organic matter adsorbs herbicide thereby influencing its availability and is the primary soil property that has been found to be correlated with indaziflam efficacy (Sebastian et al. 2016a). Sebastian et al. (2016a) found a negative correlation between soil organic matter and indaziflam efficacy such that when soil organic matter was high, greater doses of indaziflam were required to reduce the establishment of the weed kochia (*Kochia scoparia* L.). The northern Great Plains also have cooler temperatures and shorter growing seasons. As temperature is a factor moderating microbial activity and therefore herbicide degradation in soil (Torstensson 1988), this could alter the longevity of indaziflam efficacy on annual brome in northern temperate grasslands. Agroclimatic differences also result in plant community differences, as cool season grasses increase in dominance towards northern latitudes (Moser and Hoveland 1996). Finally, the focus of annual brome control in the U.S. has been on their most abundant species, *B. tectorum* and *Bromus japonicus* Thunb (Beck 2009). In Alberta, annual brome populations are also represented by the presence of corn brome, *Bromus squarrosus* L., an introduced annual brome that has been minimally studied.

This study was conducted to evaluate how indaziflam controls annual brome within affected grasslands of western Canada's Mixedgrass Prairie. Specific study objectives were to:

1) Monitor levels of annual brome biomass and density for multiple years post-treatment to evaluate the long-term (4 year) effects of indaziflam within the northern Dry Mixedgrass Prairie of Alberta, Canada.
2) Evaluate whether the U.S. recommended rate of indaziflam is an appropriate rate for reduction of annual brome grass in the northern Mixed prairie through comparison of four application rates.
3) Compare fall and spring seasons of indaziflam application for differential long-term annual brome grass control.
4) Quantify annual brome seedbank viability in relation to different rates of indaziflam application two years post-treatment.

2.2 Materials and Methods

2.2.1 Study Site Description

Two annual brome-invaded grassland study sites were identified near the Alberta-Montana border in the Dry Mixedgrass Natural Subregion of Alberta, Canada in 2018. The Aden site (49.12233, -111.22783) was near Aden, AB on a private ranch, while the Pinhorn site (49.16456, -110.92450) was approximately 20 km away in the Pinhorn Provincial Grazing Reserve, AB. The region has a mean annual temperature of 4.2ºC and mean annual precipitation of 333 mm (Downing and Pettapiece 2006). Based on the Pinhorn AGCM weather station, total accumulated precipitation in 2019, 2020, 2021, 2022, and 2023 was 272, 305, 224, 333, and 283 mm respectively (ACIS 2023). Each site was fenced to exclude livestock for the study duration. In 2019, Aden had 7% annual brome cover (*B. squarrosus* and *B. tectorum*), and the dominant native plant species were western wheatgrass (*Pascopyrum smithii* (Rydberg) Barkworth & D.R. Dewey), blue grama grass (*Bouteloua gracilis* (Kunth) Lagasca ex Griffiths), upland sedges (*Carex* spp. L.), needle and thread (*Hesperostipa comata* (Trinius & Ruprecht) Barkworth) and Sandberg's bluegrass (*Poa secunda* J. Presl). Soil at the Aden site was an Orthic Brown Chernozem with silty clay loam texture (37.1 % clay, 50.9% silt, 12.1% sand), 4.4% organic matter, and pH of 6.9 (0-10 cm). The Pinhorn site had 7% annual brome cover and the dominant native species were western wheatgrass, upland sedge, blue grama grass, junegrass (*Koeleria macrantha* (Ledebour) Schultes) and needle and thread. The soil was a Solonetzic Brown Chernozem with clay loam texture (37.1% clay, 36.4% silt, 26.5% sand), 4.8% organic matter, and pH of 7.2 (0-10 cm).

2.2.2 Experimental Design and Treatments

Treatments were applied using a randomized complete block design with four blocks, each of which contained 6 herbicide treatment plots and two non-treated control plots. Plots were 7 × 3 m in size. Indaziflam was applied at one of two times: fall (October 16, 2019) or spring (April 9, 2020). Indaziflam rates sprayed at each timing were 37.5, 75 (U.S. recommended rate), and 150 g ai ha^{-1}. The recommended rate was based on the 5 oz ac^{-1} U.S. registered rate of Rejuvra^{TM} (Envu^{TM}, USA) for areas grazed by livestock, not the 7 oz ac^{-1} rate with grazing restrictions. Treatments were applied with a Kubota (Canada) RTV 1100 four-by-four vehicle driving 4 km hr^{-1} using TeeJet (USA) AIXR 11003 nozzles at 275 kPa, at a water volume of 251.4 L ha^{-1}.

2.2.3 Brome Assessment

Biomass and density data were both measured at peak biomass during the growing season between June 20 and June 24 in each of 2020, 2021, 2022, and 2023. Biomass was harvested from two randomly placed 0.25m² quadrats (50 × 50 cm) per plot from areas with no previous vegetation harvest in prior years. After harvest all biomass was dried to constant mass at 45ºC for five days and weighed. Annual brome plant density was determined by counting annual brome plants within one permanently marked 0.25m² (50 × 50 cm) quadrat per plot, which remained non-defoliated throughout the monitoring period.

2.2.4 Seedbank Evaluation

Soil cores were collected at both study sites on June 24, 2021 from the non-treated and fall sprayed plots (approximately 20 months after spraying). Eight cores, 10 cm in diameter and 5 cm deep, were taken at intervals along the center line of the 7 × 3 m plots that had previously been treated with indaziflam at 37.5, 75, and 150 g ai ha^{-1} on October 16, 2019, as well as the non-treated control plots (0 g ai ha^{-1}). In total 16 plots (4 plots per rate) were sampled at each site. The eight cores from each plot were combined into composite samples, stored in a cooler, then frozen until use.

After thawing, litter and large roots were removed from soil. Two cm of sterilized sand was spread into trays (28 cm wide × 54 cm long × 6 cm deep) to provide additional rooting depth, onto which the composite samples of extracted soil were spread. Trays were kept in the greenhouse with 16 hr of light at 18-22 ºC and watered regularly to moisten soil and promote germination. Care was taken to not overwater and saturate the soil. As seedlings emerged they were identified and removed. Seedlings that could not be identified were repotted and grown until they could be distinguished. Soil was stirred when germination slowed after 5 months. Seedling emergence was monitored every 2-3 days for a total of 8 months.

2.2.5 Data Analysis

Changes in relative biomass and density abundance of annual brome were calculated as natural log response ratios [ln(treatment/control)], abbreviated as lnRR, for each treatment and compared to the average of the two controls in the repetition block that the treatment was within. This process normalized the distribution and linearized the response ratio so deviations in numerator and denominator have equal effect (Hedges et al. 1999). Log response ratios were then evaluated by year and site combinations using mixed model ANOVA with the R package lmerTest (Kuznetsova et al. 2017) and function lmer, with the three rates and two seasons as fixed effects, and block as random effect. Significance was set at $P < 0.10$ to minimize potential for a type II error. Estimated marginal means and 95% confidence intervals were calculated from the mixed model with the R package emmeans (Lenth 2023). Effects of individual treatments were considered significant if the 95% confidence intervals did not cross zero. Proportional (%) changes in brome abundance were calculated by back-transforming means and confidence intervals using [(exp(x)-1)*100]. Biomass or density values equaling zero at a plot were replaced with 0.1 kg ha^{-1} or 0.1 plants m^{-2} so a response ratio and natural log could be taken.

For the seedbank study, brome seedling emergence was transformed to satisfy assumptions of normality and then tested separately for each site using a linear mixed model ANOVA with indaziflam rate as a fixed effect and plot replicate as random. This was followed with pairwise comparisons of brome emergence among indaziflam rates for each site with significant overall effects ($P < 0.05$).

2.3 Results

2.3.1 Annual Brome Biomass

Reductions of annual brome biomass were not apparent within any treatment the growing season immediately after (2020) indaziflam application (Figure 2.3). By 2021, 20.5 and 15 months after the fall and spring treatments, respectively, several treatments had lower brome biomass compared to the control. At both sites the ANOVA indicated both rate and season effects (Table 2.1). At Pinhorn, all fall treatments reduced brome biomass with the fall 150 g ai ha^{-1} treatment leading to the maximum estimated reduction (Table 2.2). The back transformed lnRR estimated there was 95% confidence the fall 75 g ai ha^{-1} rate reduced brome biomass by 10-97%, while the fall 150 g ai ha^{-1} rate reduced biomass by 84-99%. Of the spring treatments, only the 150 g ai ha^{-1} rate led to significantly lower brome biomass than the non-treated control, with a 35-98% reduction. At Aden, all treatments reduced biomass by at least 31%. The fall 150 g ai ha^{-1} treatment again had the largest reduction of 91-99%, with the spring 37.5 g ai ha^{-1} showing the smallest reduction of 31-92%.

Treatment effects were more pronounced in 2022, 32.5 and 27 months after the fall and spring treatments, with every indaziflam treatment reducing brome biomass at both sites. ANOVA indicated neither rate nor season influenced differences in the treatment effects in 2022. Effect sizes indicated that every treatment at Pinhorn reduced brome biomass between 90-100% (with 95% confidence), except for the fall 37.5 g ai ha^{-1} (73-99%). At Aden, brome biomass was reduced by 80-100% within the spring treatments, and 90-100% within the fall treatments, except for the fall 37.5 g ai ha^{-1} treatment (46-98%).

In the final year of data collection, 2023, ANOVA indicated that indaziflam rate continued to influence brome at both sites. The lowest herbicide rate within the fall and spring treatments (37.5 g ai ha^{-1}) at Aden did not consistently reduce brome biomass, while the 75 g ai ha^{-1} rate reduced biomass by 11-99% when applied in the fall, and 29-99% in the spring. The 150 g ai ha^{-1} treatments reduced biomass at both sites by at least 90%. At Pinhorn, the fall 75 g ai ha^{-1} and spring 75 g ai ha^{-1} treatments reduced biomass by 66-100% and 41-99%, respectively. In contrast with Aden, the lowest indaziflam rate at Pinhorn (fall and spring 37.5 g ai ha^{-1}) markedly reduced brome biomass in the final year by 77-100 and 75-100%, respectively.

2.3.2 Annual Brome Density

In 2020, the first growing season following indaziflam treatment, none of the lnRR 95% confidence intervals showed a treatment effect (Figure 2.4), while ANOVA indicated a season effect at both sites (Table 2.1). In 2021, at both the Pinhorn and Aden sites, all but the lowest rate spring treatments were effective at reducing brome density, and ANOVA indicated rate and season effects among the treatments. Thereafter in 2022, only the rate of indaziflam influenced brome density at both sites. All indaziflam treatments at both sites reduced annual brome density. At Pinhorn, the spring and fall 37.5 g ai ha^{-1} treatments reduced brome density by 90-100%, while the 75 and 150 g ai ha^{-1} treatments in either spring or fall reduced brome density by 99-100% (Table 2.3). At Aden, the fall 37.5, spring 37.5, and spring 75 g ai ha^{-1} treatments reduced brome density by at least 11, 45, and 58% respectively, while the fall 75 g ai ha^{-1}, and both the spring and fall 150 g ai ha^{-1} treatments, reduced annual brome density by at least 95%. In the final year of sampling of 2023, all treatments at Pinhorn continued to reduce brome density and had similar estimates of at least a 95% reduction. At Aden, there was a rate but not season effect of indaziflam. The spring 37.5 g ai ha^{-1} treatment did not exhibit a consistent density reduction, while the fall 37.5 g ai ha^{-1} rate led to a smaller reduction estimate of brome density (56-100%) compared to the 75 and 150 g ai ha^{-1} rates, the latter of which led to at least a 76% reduction in brome density.

2.3.3 Annual Brome Seedbank in 2021

The seedbank results showed an indaziflam rate effect on brome seedling emergence at both Pinhorn ($F=14.5_{3,9}$; $p<0.001$) and Aden ($F=8.0_{3,9}$; $p=0.007$) based on soil samples removed in 2021, 20 months after treatment. Brome seedling emergence from the seedbank at Pinhorn declined as indaziflam rates increased from 0 to 75, and then up to 150 g ai ha^{-1} (Table 2.4). However, emergence at the 37.5 g ai ha^{-1} rate was not significantly different from the non-treated control. At Aden, the 150 g ai ha^{-1} indaziflam rate led to significantly lower brome seedling emergence than the non-treated control, with no other differences.

2.4 Discussion

2.4.1 Initial Brome Responses to Indaziflam Timing

Larger reductions of annual brome biomass and density generally took two years to occur and were not apparent in the growing season immediately after indaziflam application. Annual brome grasses can germinate at any time when conditions are favourable (Upadhyaya et al 1986), but in North America are thought to typically follow a winter annual growth cycle, germinating in the fall following a loss of seed dormancy when exposed to summer heat, then overwintering and resuming their growth the following spring (Hulbert 1955). It may be that by the time our treatments were applied, even in fall, that the upcoming season's brome had already germinated and was therefore less susceptible to indaziflam exposure. This would be particularly likely if the roots of developing annual brome plants were below the location of indaziflam in the soil profile. Similarly, Clark et al. (2020) found no significant reduction of annual brome at three field sites in the first growing season following indaziflam treatment when indaziflam was applied in the spring, presumably due to brome being actively growing at the time of application.

Another possible explanation is that the relatively low precipitation following our application may have resulted in indaziflam remaining adsorbed to vegetation and litter before adequate precipitation washed it into the soil layer, thereby limiting contact between brome seeds and indaziflam during the first growing season. Immediate rainfall events following application and adequate soil moisture are required for indaziflam to be active in the soil (Clark et al. 2019a), thereby increasing the likelihood of seedling to herbicide contact. There is a positive correlation between rainfall amount and indaziflam desorption from litter (Clark et al. 2019a). Rainfall data from the Pinhorn AGCM weather station showed the rainfall events following the fall October 16 indaziflam application in 2019 were 0.9 mm on October 22nd and 6.5 mm on the 25th (ACIS 2023). This delay and low amount of rainfall following indaziflam application could have reduced the short-term efficacy of the fall treatment during the subsequent growing season of 2020. Notably, the spring indaziflam application on April 9th was similarly followed by low amounts of rainfall, which were limited to 1.8 mm on April 10th, and 3.6 mm on the 11th. Finally, greater litter biomass also is known to intercept more herbicide (Clark et al. 2019a). Prior to application in 2019, the average litter biomass was 1193 kg ha^{-1} at the Aden site and 1216 kg ha^{-1} at the Pinhorn site, both of which were more than double the 570 kg ha^{-1} previously reported in this region for native grasslands of the Mixedgrass Prairie (Willms et al. 1986).

In general, the fall applied treatments tended to provide earlier and more consistent brome reduction compared to the spring applied treatments. This response appears to reinforce that in the Dry Mixedgrass prairie brome follows a winter annual life cycle. Fall indaziflam treatments targeted brome prior to germination or shortly after germination, while the spring treatments likely occurred post-germination of brome seedlings. Although brome biomass was not reduced significantly in the season following either timing of application, a greater amount of brome with deeper roots was likely actively growing at the time of spring application compared to the fall application, thereby limiting herbicide uptake and associated efficacy. Considering the large number of seeds an individual brome plant can produce (between 25 and 5000 seeds per plant, depending on plant density) (Beck 2009), even a small amount of brome controlled by the fall treatments in 2020 could explain why the fall applied treatments subsequently achieved lower brome biomass at Pinhorn during 2021. Differences between the fall and spring treatment reduction levels could also be explained by varying time lags in the desorption of indaziflam from litter, which could have been shorter in the fall applied treatment plots following overwinter snowfall and melt. Importantly, the season of application no longer affected treatment efficacy in 2022 or 2023, indicating that in the long term the timing of indaziflam application does not seem to be a critical consideration in ensuring annual brome control. Clark et al. (2020) also found that by the second growing season after treatment, indaziflam applied during the spring after brome emergence led to a similar level of suppression as compared to indaziflam applied in a tank mix with glyphosate intended to control any already growing brome.

2.4.2 Optimal Rate Effects of Indaziflam

A key objective of this study was to evaluate the current U.S. registered (and recommended) rate of indaziflam within Alberta's northern Mixedgrass Prairie where growing seasons are considerably shorter than in the southern prairies of the U.S. Of particular interest is knowing the rate of indaziflam that can provide adequate brome reduction while avoiding unnecessary excess application within these native grasslands where many non-target plants may be present. The current results indicate that the recommended 75 g ai ha^{-1} rate of indaziflam was suitable for achieving substantial annual brome reduction at our study sites for a period of at least four years. The 150 g ai ha^{-1} (double) rate appeared to offer some improvement in brome reduction over the recommended rate, but only during the fourth (and final) year post-treatment. This response is not surprising and is likely the result of a longer-lasting residual effect of the additional herbicide initially applied. Interestingly, during 2023 the fall 37.5 g ai ha^{-1} half rate continued to provide at least some measurable brome biomass suppression/reduction at one site, together with a reduction in brome density at both sites. Clark et al. (2020) and Sebastian et al. (2016b) also found long-term reductions of annual brome with relatively low indaziflam rates (44 and 58 g ai ha^{-1}) in Colorado, USA. However, in another study undertaken in Colorado, reduction of brome cover was not significant two years after treatment when using a 44 g ai ha^{-1} rate of indaziflam (Clark 2019b).

Northern Mixedgrass Prairie soils of western Canada generally tend to have higher organic matter than comparable regions in the midwestern plains (Epstein et al. 2002), which could lead to greater indaziflam adsorption and therefore less control at a given rate. On the other hand, these northern grasslands also have colder climates than those tested in the U.S., which could slow herbicide degradation (Torstensson 1988), potentially resulting in longer lasting suppression/reduction of the target plants within a given indaziflam rate. Four growing seasons after indaziflam treatment, the single applications of indaziflam tested here continued to reduce annual brome at both experimental sites across all treatments with the exception of the lowest herbicide rate. Potential long-term benefits of the higher rate treatment were particularly noticeable in the fourth year of the study but could become even more apparent in future years and were outside the timeline of this study. Potential benefits and drawbacks of using a single application of a higher rate or multiple sequential applications of a single rate of indaziflam are also unknown, and warrant testing.

In our seedbank study where soil was extracted from the field and seedlings were encouraged to germinate under controlled greenhouse conditions, there was a benefit evident from the double indaziflam rate, while the half rate did not consistently reduce annual brome seed abundance. It should be noted that the seedling trays in the greenhouse were regularly watered and this could have enhanced leaching of herbicide as compared to field conditions. Had leaching occurred, this might lower indaziflam efficacy, and partly explain the limited reduction in brome density observed within the greenhouse under the lowest indaziflam rate. While untested, within grasslands having greater precipitation, higher rates of indaziflam may be required to achieve the same duration of annual brome reduction.

2.4.3 Annual Brome Dynamics

Finally, we observed marked fluctuations in the amount of brome between years within the non-treated field plots, a response that was unrelated to indaziflam treatment. The biomass (Table 2.2) and density (Table 2.3) of annual brome in 2022 and 2023 were markedly lower than in 2020 and 2021. Annual brome abundance is known to fluctuate from year to year and be highly dependent on precipitation during the fall of the previous growing season, as well as on current spring precipitation (Stewart and Young 1939), an important reason why annual bromes are regarded as an undesirable forage. Low fall (September to November) precipitation during 2021 may have resulted in less fall germination and subsequent brome growth during the growing season of 2022 (Figure 2.5). Notably, brome seedlings in the greenhouse readily germinated from soil cores collected during the 2021 growing season, indicating there was potential for a high brome population during 2022 (Table 2.4). Perennial grass biomass in our non-treated study plots was also lowest in the growing season of 2022 (data not shown), indicating a strong precipitation effect that year. However, the same was not true during 2023 when grass yields were more normal (data not shown). The limited presence of annual brome in 2022 and associated low seed production may have carried through to 2023 in our study. There is also the possibility that the northern temperate Mixed Prairie plant communities tested here may be more resilient to annual brome invasion than those in the Great Basin of the western U.S. where these species have been particularly widespread and detrimental (Beck 2009). As of yet annual brome grasses have not been as problematic (i.e. well distributed) in northern rangelands of Western Canada, which could be a function of factors such as greater precipitation, shorter growing seasons, longer winters, lower disturbance and reduced bare ground, or endemic plant communities with greater perennial grass cover and resistance to invasion (Beck 2009; Bradley 2009). Alternatively, it could be that annual brome simply has been slower to establish into these regions. Another possibility is that the main annual brome species at our particular sites, corn brome, may act differently as an invasive species than downy brome, although the morphology and phenology of downy, Japanese, and corn brome are considered to be very similar (Fenesi et al. 2011; Oja and Paal 2003). In 2019, 2020, and 2021 the corn brome at our sites was abundant and visibly spreading.

2.5 Conclusion

Our study provides an understanding of how indaziflam affects annual brome within the context of the northern Mixedgrass Prairie where soils are relatively high in organic matter, the climate more cool continental, and the plant communities are unique from those previously tested in the U.S. Additionally, in contrast to well-studied annual brome species like downy brome (*B. tectorum*) and Japanese brome (*B. japonicus*), there is minimal existing literature about corn brome (*B. squarrosus*), which appears to be well distributed and a growing concern to producers in southern Alberta and other northern mixed grasslands. Overall, our results provide evidence that a one-time indaziflam treatment can provide long-lasting reduction of annual brome, including corn brome, in northern Mixedgrass Prairie grasslands for up to four years. Moreover, reductions in brome biomass and density occurred at indaziflam rates as low as 37.5 g ai ha^{-1}, but peaked at higher rates of 75 and 150 g ai ha^{-1}. While grassland managers can expect long-term brome reductions from indaziflam treatment, our findings also indicate that immediate control may be unpredictable depending on agroclimatic factors. In contrast, the seasonal timing of indaziflam application had less impact on brome suppression in the long term, though quicker responses may be possible where indaziflam is sprayed in fall rather than spring.

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Table 2.1. ANOVA summary of rate and season of indaziflam application and their interaction on annual brome biomass and density at Pinhorn and Aden in each of the 2020 through 2023 growing seasons. P-values in bold indicate significance at $P < 0.10$.

Year	Factor	Biomass (kg ha^{-1})				Density (plants m^{-2})			
		Pinhorn		Aden		Pinhorn		Aden	
		F value†	P value	F value†	P value	F value†	P value	F value†	P value
2020	Rate	$1.31_{2,15}$	0.298	$0.46_{2,15}$	0.642	$0.01_{2,15}$	0.989	$0.16_{2,15}$	0.856
	Season	$1.88_{1,15}$	0.190	$1.24_{1,15}$	0.283	$8.02_{1,15}$	**0.013**	$4.67_{1,15}$	**0.047**
	Rate × Season	$0.23_{2,15}$	0.800	$0.25_{2,15}$	0.779	$0.57_{2,15}$	0.576	$2.55_{2,15}$	0.110
2021	Rate	$6.39_{2,15}$	**0.010**	$3.66_{2,15}$	**0.051**	$4.29_{2,15}$	**0.034**	$3.08_{2,15}$	**0.076**
	Season	$6.85_{1,15}$	**0.019**	$3.53_{1,15}$	**0.080**	$10.64_{1,15}$	**0.005**	$6.51_{1,15}$	**0.022**
	Rate × Season	$0.36_{2,15}$	0.705	$2.18_{2,15}$	0.148	$0.04_{2,15}$	0.964	$2.66_{2,15}$	0.103
2022	Rate	$0.57_{2,15}$	0.575	$1.66_{2,15}$	0.223	$6.89_{2,15}$	**0.008**	$3.87_{2,15}$	**0.044**
	Season	$1.20_{1,15}$	0.291	$0.39_{1,15}$	0.544	$0.00_{1,15}$	1.000	$0.90_{1,15}$	0.357
	Rate × Season	$0.53_{2,15}$	0.602	$1.71_{2,15}$	0.214	$0.00_{2,15}$	1.000	$1.00_{2,15}$	0.390
2023	Rate	$3.88_{2,15}$	**0.044**	$4.45_{2,15}$	**0.030**	$0.63_{2,15}$	0.549	$3.65_{2,15}$	**0.051**
	Season	$0.12_{1,15}$	0.731	$0.01_{1,15}$	0.927	$2.50_{1,15}$	0.135	$0.46_{1,15}$	0.506
	Rate × Season	$0.09_{2,15}$	0.913	$0.13_{2,15}$	0.881	$0.63_{2,15}$	0.549	$1.53_{2,15}$	0.248

† F value subscript indicates the numerator and denominator degrees of freedom.

Table 2.2. Annual brome biomass in the non-treated control plots and the percent change in brome biomass[1] compared to the non-treated controls in each of the 2020 through 2023 growing seasons as a result of indaziflam applied at different rates, seasons and locations prior to the 2020 growing season.

Site	**Year**	**Non-treated brome biomass (kg ha^{-1})**	**Treatment [Season of Application × Indaziflam Rate (g ai ha^{-1})]**					
			Fall 37.5	Fall 75	Fall 150	Spring 37.5	Spring 75	Spring 150
			Percent change in brome biomass					
Pinhorn	2020	968.5 (236.3)	-6.2 (-71.8, 211.7)	-24.7 (-77.3, 150.3)	-53.0 (-85.9, 56.1)	38.2 (-58.4, 359.3)	-11.5 (-73.4, 194.1)	-11.4 (-73.3, 194.5)
	2021	1167.7 (368.9)	**-81.1 (-96.4, -1.0)**	**-82.8 (-96.7, -9.5)**	**-96.9 (-99.4, -83.7)**	-31.3 (-86.9, 261.1)	-68.7 (-94.0, 64.2)	**-87.6 (-97.6, -34.6)**
	2022	27.9 (14.7)	**-94.9 (-99.0, -73.1)**	**-98.4 (-99.7, -91.8)**	**-98.7 (-99.7, -93.0)**	**-98.9 (-99.8, -94.4)**	**-98.4 (-99.7, -91.5)**	**-99.2 (-99.9, -96.0)**
	2023	17.8 (3.7)	**-97.6 (-99.8, -76.5)**	**-96.5 (-99.6, -65.8)**	**-99.3 (-99.9, -93.4)**	**-97.5 (-99.7, -75.2)**	**-94.0 (-99.4, -40.8)**	**-99.3 (-99.9, -93.4)**
Aden	2020	617.1 (51.9)	-23.7 (-51.3, 19.6)	-8.8 (-41.8, 42.9)	-35.6 (-58.9, 0.9)	-11.3 (-43.5, 39.1)	-2.4 (-37.7, 53.0)	-7.4 (-40.9, 45.2)
	2021	478.4 (110.6)	**-82.9 (-94.0, -51.3)**	**-94.6 (-98.1, -84.6)**	**-96.8 (-98.9, -91.0)**	**-75.8 (-91.5, -31.2)**	**-94.6 (-98.1, -84.6)**	**-77.7 (-92.2, -36.6)**
	2022	19.9 (8.9)	**-89.2 (-97.8, -45.6)**	**-99.2 (-99.8, -96.0)**	**-98.4 (-99.7, -91.9)**	**-96.4 (-99.3, -82.1)**	**-96.2 (-99.2, -80.9)**	**-96.8 (-99.4, -84.1)**
	2023	50.6 (11.6)	-78.7 (-98.0, 123.5)	**-91.6 (-99.2, -11.3)**	**-99.5 (-100.0, -94.8)**	-88.0 (-98.9, 26.3)	**-93.2 (-99.4, -28.9)**	**-99.1 (-99.9, -91.0)**

[1] Percent change was calculated by back-transforming log response ratio means and confidence intervals using [(exp(x)-1)*100]. Log response ratio was calculated using [ln(treatment/control)].

Brackets indicate 95% confidence intervals for the percent change and standard errors for non-treated brome biomass.

Bolded values have confidence intervals that do not overlap with zero, indicating significant differences from the non-treated control.

Table 2.3. Annual brome density in the non-treated control plots and percent change[1] in brome density compared to the non-treated controls in each of the 2020 through 2023 growing seasons as a result of indaziflam applied at different rates, seasons and locations prior to the 2020 growing season.

Site	**Year**	**Non-treated brome density (plants m^{-2})**	**Treatment [Season of Application × Indaziflam Rate (g ai ha^{-1})]**					
			Fall 37.5	Fall 75	Fall 150	Spring 37.5	Spring 75	Spring 150
			Percent change in brome density					
Pinhorn	2020	505.5 (90.0)	-42.9 (-74.9, 28.3)	-33.5 (-70.4, 49.3)	-12.9 (-61.2, 95.6)	79.6 (-20.0, 303.3)	60.1 (-28.7, 259.5)	30.6 (-41.8, 193.4)
	2021	1755.0 (537.6)	**-91.2 (-98.9, -27.3)**	**-98.9 (-99.9, -90.8)**	**-99.0 (-99.9, -92.0)**	-44.9 (-93.3, 353.1)	**-89.6 (-98.7, -14.2)**	**-91.8 (-99.0, -32.6)**
	2022	51.5 (23.7)	**-97.7 (-99.5, -89.5)**	**-99.8 (-99.9, -98.9)**	**-99.8 (-99.9, -98.9)**	**-97.7 (-99.5, -89.5)**	**-99.8 (-99.9, -98.9)**	**-99.8 (-99.9, -98.9)**
	2023	32.5 (12.4)	**-99.0 (-99.8, -95.3)**	**-99.0 (-99.8, -95.3)**	**-99.6 (-99.9, -98.1)**	**-99.6 (-99.9, -98.1)**	**-99.6 (-99.9, -98.1)**	**-99.6 (-99.9, -98.1)**
Aden	2020	678.0 (70.7)	-45.6 (-73.0, 9.5)	4.1 (-48.3, 109.5)	-12.2 (-56.4, 76.7)	55.1 (-22.9, 212.2)	6.1 (-47.3, 113.8)	8.4 (-46.1, 118.2)
	2021	823.5 (49.3)	**-85.8 (-96.8, -38.1)**	**-93.1 (-98.4, -69.9)**	**-99.3 (-99.8, -97.1)**	-71.3 (-93.4, 25.4)	**-90.1 (-97.7, -56.8)**	**-81.4 (-95.8, -18.9)**
	2022	169.5 (30.9)	**-95.7 (-99.8, -10.8)**	**-99.8 (-100.0, -96.1)**	**-99.8 (-100.0, -96.7)**	**-97.3 (-99.9, -44.6)**	**-98.0 (-99.9, -58.4)**	**-99.7 (-100.0, -94.6)**
	2023	87.5 (16.2)	**-95.5 (-99.5, -55.5)**	**-97.6 (-99.8, -76.1)**	**-99.9 (-100.0, -98.7)**	-87.8 (-98.8, 20.4)	**-99.4 (-99.9, -94.5)**	**-98.7 (-99.9, -87.5)**

[1] Percent change was calculated by back-transforming log response ratio means and confidence intervals using [(exp(x)-1)*100]. Log response ratio was calculated using [ln(treatment/control)].

Brackets indicate 95% confidence intervals for the percent change and standard errors for non-treated brome biomass.

Bolded values have confidence intervals that do not overlap with zero, indicating significant differences from the non-treated control.

Table 2.4. Annual brome seedling emergence in the greenhouse as occurred from soil collected in June 2021, further stratified by site and indaziflam rate. Soil was collected from treatments where indaziflam was applied in October 2019 to a depth of 5 cm. Within a column, means lacking the same letter indicate a difference between treatments ($P < 0.05$).

Rate (g ai ha^{-1})	Brome seedling emergence (seedlings per 3141 cm^{-3} soil) ±1 standard error	
	Pinhorn	Aden
0	412 (85) a	212 (43) a
37.5	226 (36) ab	133 (27) ab
75	199 (27) bc	168 (33) a
150	93 (40) c	55 (6) b

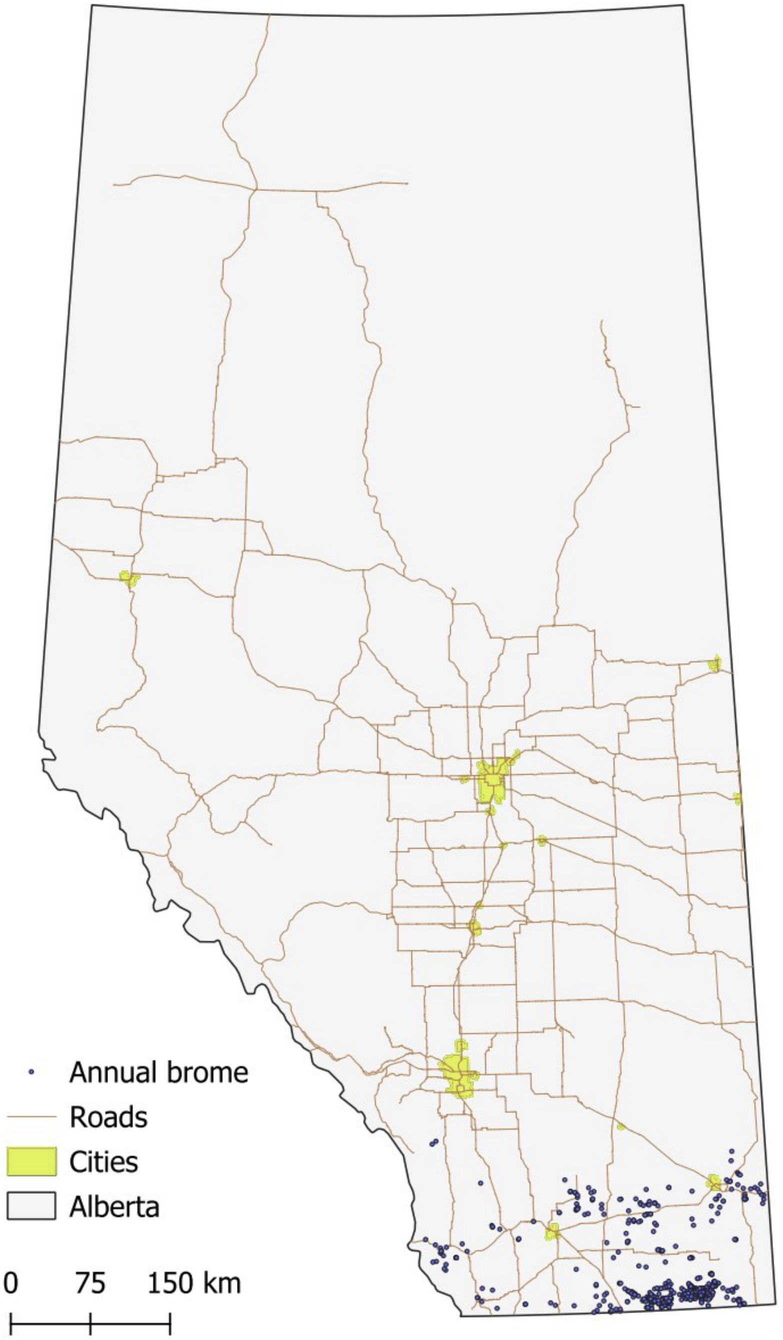

Figure 2.1 Data from Alberta Environment and Parks, Range Management Branch in 2018 indicating 530 observations of annual brome grasses (species included *Bromus tectorum* and *Bromus japonicus*) in southern Alberta during range health assessments on grazing leases/reserves from 2003 to 2018.

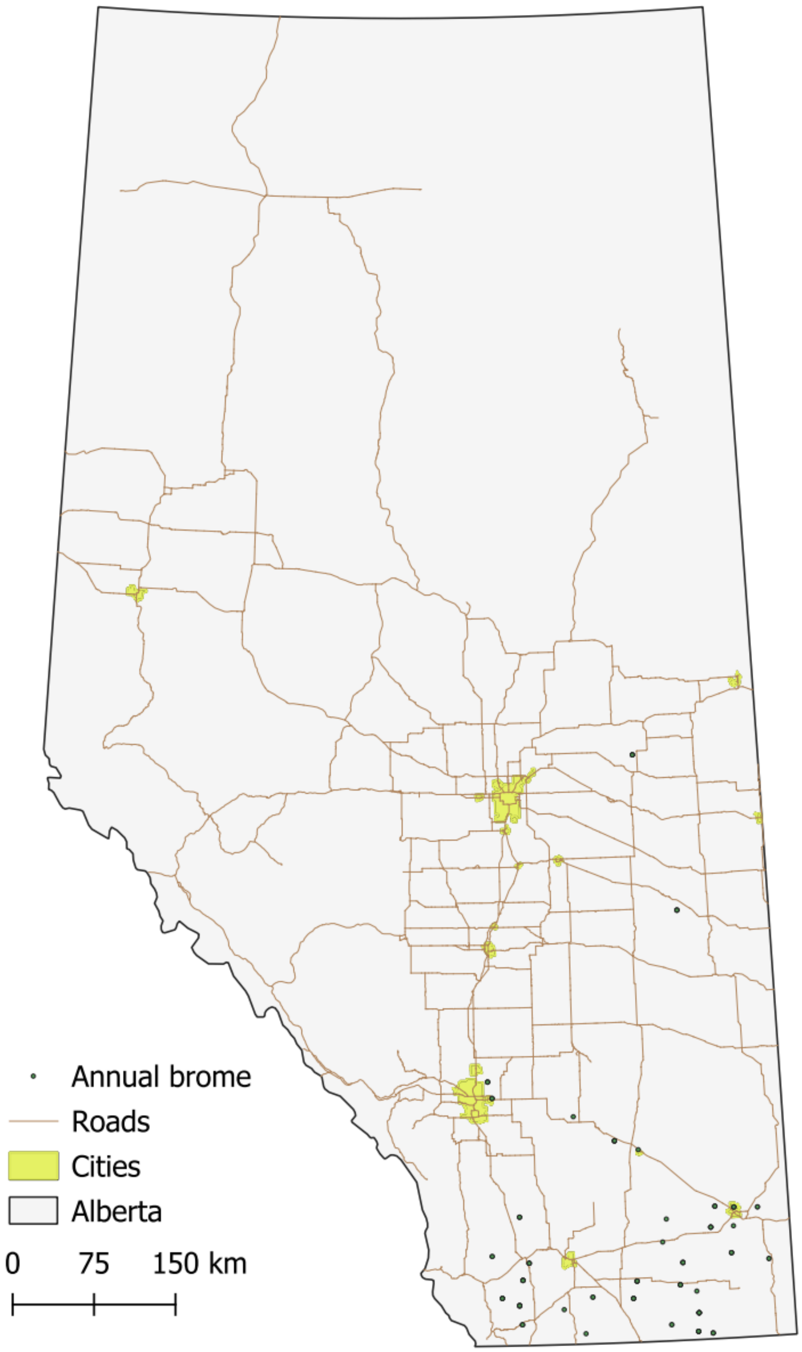

Figure 2.2. Annual brome (2003-2018) presence detected by Alberta Biodiversity Monitoring Institute surveys at over 1000 sites systematically distributed across Alberta. Species included *Bromus tectorum, Bromus japonicus, Bromus squarrosus, and Bromus commutatus*. Data are from the Alberta Biodiversity Monitoring Institute and only sites with publicly available coordinate information were used. More information on the ABMI can be found at: www.abmi.ca.

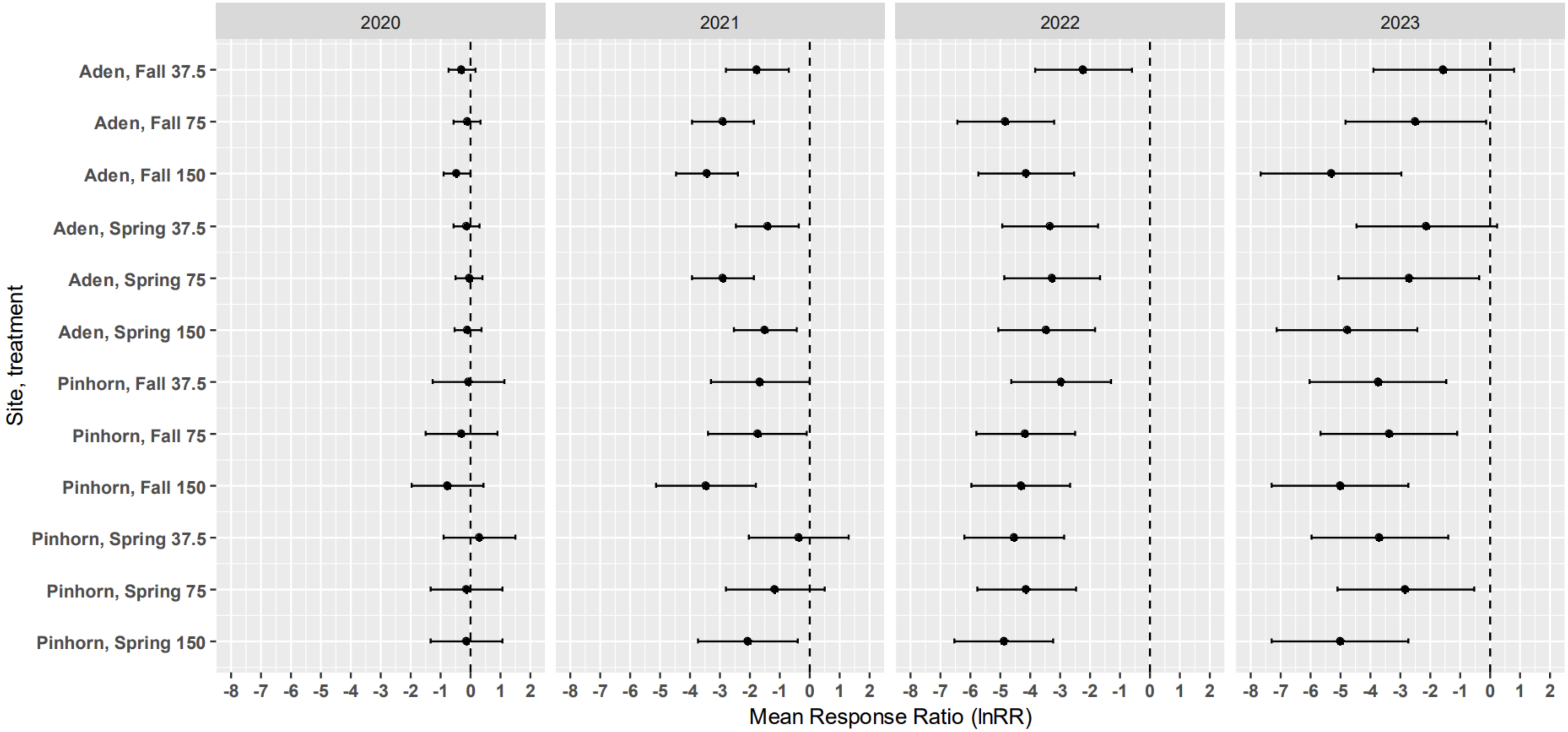

Figure 2.3. Log response ratio of annual brome biomass in each post-indaziflam treatment year. Treatments are shown for all combinations of rate, season and location, with treatments occurring in October 2019 (fall) and April 2020 (spring). Log response ratio was calculated using [ln(treatment/control)]. Error bars represent 95% confidence intervals.

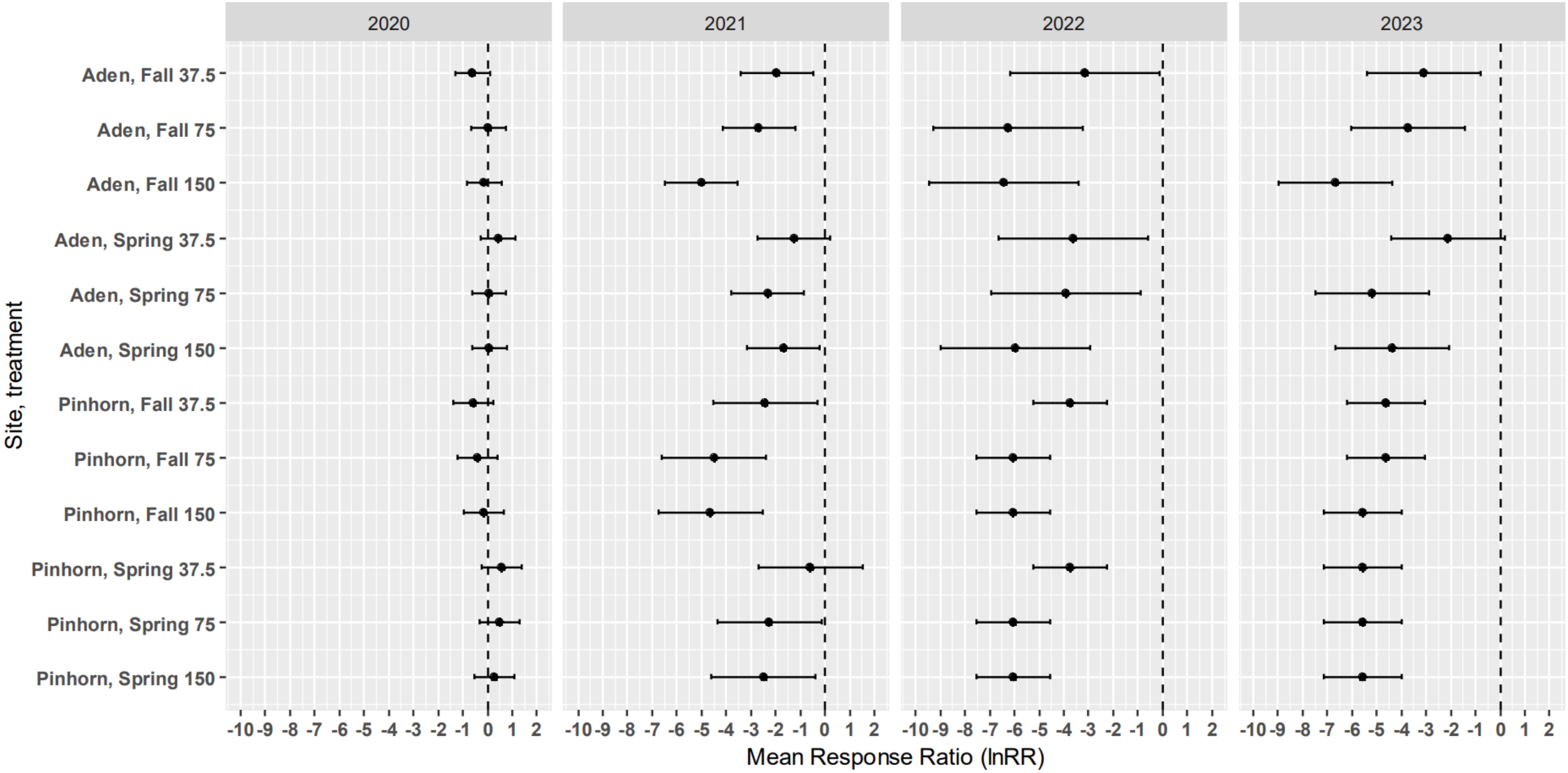

Figure 2.4. Log response ratio of annual brome density in each post-indaziflam treatment year. Treatments are shown for all combinations of rate, season and location, with treatments occurring in October 2019 (fall) and April 2020 (spring). Log response ratio was calculated using [ln(treatment/control)]. Error bars represent 95% confidence intervals.

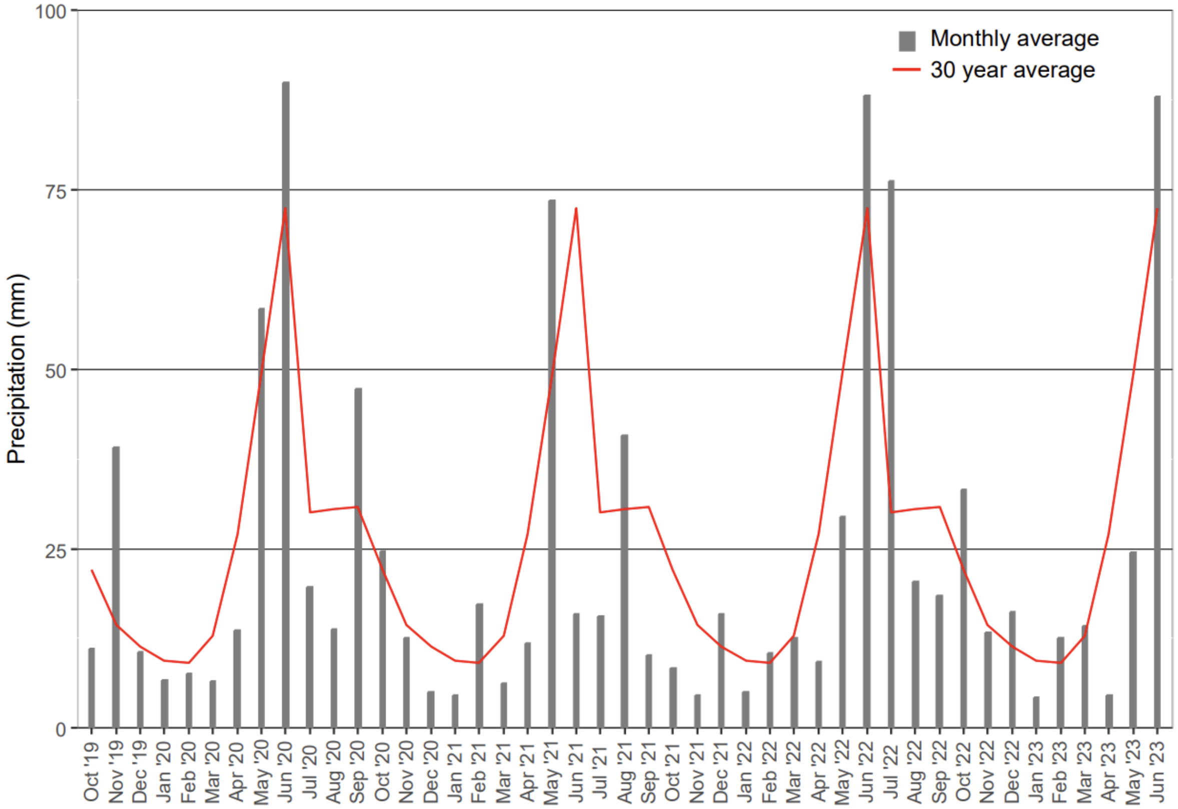

Figure 2.5. Monthly precipitation at the Pinhorn AGCM weather station during the field study period, starting in October 2019 when the fall indaziflam treatment was applied, and average precipitation over the last 30 years for each month (1994-2023). Data provided by Agriculture and Irrigation, Alberta Environment and Parks and Environment Canada, retrieved from the Alberta Climate Information Service: https://acis.alberta.ca/acis/.

3 Plant community and forage responses in mixedgrass prairie treated with indaziflam for annual brome control

3.1 Introduction

Despite being widespread globally, temperate grasslands are among the least protected habitats around the world and Canadian grasslands are among the world's most endangered ecosystems (Petermann and Buzhdygan 2021). Over the last century, cultivation for cropping agriculture has reduced the original 60 M ha of Canada's prairie grasslands to 11 M ha (Bailey et al. 2010). Of this area, 49% is in southern Alberta, 41% in Saskatchewan, and 10% in Manitoba. In Alberta, the 5.7 M ha of natural grasslands that remain provide habitat for up to 80% of the province's species-at-risk (Wilkinson et al. 2014), thereby demonstrating the consequences of ongoing global change across the region.

Invasive species, along with land use change, fragmentation of habitat, woody plant encroachment, overgrazing, and fire suppression, have threatened the function and biodiversity of the grassland biome (Petermann and Buzhdygan 2021). Invasive species, including their ability to displace native plant species (Bennett et al. 2014), are a challenge to maintaining functional and resilient rangeland ecosystems. One notable example of this is the invasion of the annual grass downy brome (*Bromus tectorum* L.) within the Great Basin and Great Plains of the United States, where it has caused significant changes to ecosystem function and structure (Germino et al. 2016). Annual brome abundance often exhibits an inverse relationship with the cover of native perennial species (Germino et al. 2016; Leger 2008) and changes in plant community structure, in turn, have negative impacts on the habitat, movement, and food sources of mammals and birds (Bachen et al. 2018; Gano and Rickard 1982; Goebel and Berry 1976). In addition, litter build-up of annual grasses is highly flammable, and is thought to have led to an increase in the frequency of fires across the intermountain west of the U.S. (D'Antonio and Vitousek 1992). Increased wildfire within the sagebrush ecosystem has substantially changed the structure of native plant communities through replacement of native shrub vegetation by annual dominated grasslands and led to population declines in several small mammals and birds, including sage grouse (*Centrocercus urophasianus* Bonaparte) (Gano and Rickard 1982; Knick 1999).

While herbicides have generally been more effective at long-term annual brome control in grasslands compared to cultural strategies such as grazing or burning (Monaco et al. 2017), there are limited herbicide options for controlling annual grasses within native grasslands of Alberta and western Canada. Many brome-controlling herbicides are not registered for use on natural areas in Alberta (e.g. florasulam; glyphosate; imazapic; rimsulfuron; pyroxsulam). An additional concern is of reported herbicide resistance of downy brome populations to group 1 acetyl-coenzyme A carboxylase (Ball et al. 2007), group 2 acetolactate synthase (Zuger and Burke 2020), and, in particular, group 9 enolpyruvylshikimate-3-phosphate inhibitors as resistant populations have been found in fall seeded cereals in southern Alberta in close geographic proximity to native grasslands (Geddes and Pittman 2022).

Indaziflam is a herbicide that was recently registered for use on rangeland in the U.S. and is in the process of being registered for use on rangeland in Canada. Within field studies conducted in Colorado, indaziflam has been more effective at long-term annual brome control compared to other commonly used herbicides such as imazapic, glyphosate, and rimsulfuron (Sebastian et al 2016a; Sebastian et al. 2016b). Indaziflam is a soil-applied group 29 herbicide that targets establishing seedlings by inhibiting cellulose biosynthesis within expanding tissues of the root and hypocotyl (Brabham et al. 2014). This effect is non-selective and occurs in both monocot and dicot seedlings (Brabham et al. 2014). While the objective of indaziflam is to prevent undesirable annual species establishment to diminish the annual weed seed bank, desirable non-target species seeds are also presumably prevented from establishing during the time indaziflam is active in soil (Courkamp et al. 2022b). Indaziflam has a water solubility of 2.8 mg/L at 20°C and pH of 9, log K_{ow} of 2.8 at pH of 7, and a half-life >150 days (Tompkins 2010), properties which could result in considerable long-term soil residual activity.

How indaziflam's cellulose biosynthesis inhibition affects the existing native grassland communities of the region is less known. Maintaining resilient, functional ecosystems, including those that provide ample forage for the predominant land use of grazing, requires that annual brome be reduced while native plant species be simultaneously maintained or their abundance and diversity improved. Simply removing brome is not the only objective as the remaining plant community following control will also determine recovery (DiTomaso 2000). For example, having higher perennial grass cover following brome control can lead to greater ecosystem recovery and future invasion resistance (Monaco et al. 2017). Therefore, native plant community responses to indaziflam must be considered.

Multiple field studies in Colorado and Wyoming, U.S.A., have shown that following indaziflam treatment on annual brome invaded sites, reductions in annual brome have corresponded with either an increase or no change in the abundance and/or richness of above ground native plants (Clark et al. 2019; Clark et al. 2020; Seedorf et al. 2022; Courkamp et al. 2022a; Courkamp et al. 2022b; Arathi and Hardin 2021). Indaziflam also did not impact biological soil crusts (moss and lichen) in a Montana field study (Slate et al. 2023). On the other hand, in a mountain ecosystem in Montana, indaziflam treatment reduced aboveground plant species richness and diversity (Meyer-Morey 2021). This site had a diverse initial plant community and was relatively undisturbed prior to treatment. Given Canada's less invaded and degraded prairie ecosystems, as well as unique agroclimatic conditions, a key consideration in the use of indaziflam across this region is that of the pre-existing native plant community. For example, Canadian grasslands subject to indaziflam treatment might have a higher initial remnant native plant presence than areas examined in previous U.S. studies. As a result, the diversity of these highly invaded plant communities tested in the U.S. may have already been compromised prior to herbicide treatment. Canadian prairies also are at the northern extent of the Great Plains and therefore contain different plant communities than those further south, a result of differences in climatic and soil conditions. The northern Mixedgrass prairie plant communities of Alberta are heavily dominated by cool-season (C3) perennial grasses, including in their contribution to total forage productivity (Willms et al. 1986).

An additional difference between the southern and northern Great Plains is that soil organic matter tends to increase with increasing latitude across the region (Epstein et al. 2002). Organic matter is the main soil property that affects indaziflam efficacy (Sebastian et al. 2016c). Indaziflam may have greater adsorption in soils with higher organic matter, resulting in the need for greater indaziflam doses to achieve the same weed control. Higher doses of indaziflam, in turn, might have unintended consequences to non-target native plant species. Further, the rate of microbial degradation in northern regions may be slower due to cooler conditions resulting in a longer residence time of herbicide in soil (Torstensson 1988).

Based on the need to better understand how northern temperate grassland vegetation responds to indaziflam treatment, the goal of this study was to test indaziflam effects on native plant communities in treated mixedgrass prairie. Specific study objectives were to:

1) Determine how different rates and seasons of indaziflam application affect northern Mixedgrass prairie plant species composition, richness, and diversity.
2) Quantify the initial and longer-term effects of indaziflam on native grassland biomass in Canada's mixed prairie.
3) Evaluate how indaziflam affects the recruitment of perennial grasses and forbs from the seedbank.
4) Determine how the cellulose biosynthesis inhibition action of indaziflam affects the root and shoot biomass of actively growing perennial grasses.

3.2 Materials and Methods

3.2.1 Study Site Description

In 2018 a field experiment was established within each of two annual brome invaded sites that were approximately 20 km apart, near the Alberta-Montana border at the southern edge of the Dry Mixedgrass Prairie Natural Subregion. This subregion has a mean annual temperature of 4.2ºC and mean annual precipitation of 333 mm (Downing and Pettapiece 2006). The sites were near Aden, Alberta on a private ranch (49°07'20.4''N, 111°13'40.2''W) and at the Pinhorn Provincial Grazing Reserve, Alberta (49°09'52.4''N, 110°55'28.2''W). Total accumulated precipitation at the Pinhorn AGCM weather station in 2019, 2020, 2021, 2022, and 2023 was 272, 305, 224, 333, and 283 mm respectively (ACIS 2023). Prior to treatment in 2019, both the Aden and Pinhorn sites had 7% annual brome cover which was comprised of a mix of corn brome (*Bromus squarrosus* L.) and downy brome (*Bromus tectorum* L.). Dominant native species at Aden were western wheatgrass (*Pascopyrum smithii* (Rydberg) Barkworth & D.R. Dewey), blue grama grass (*Bouteloua gracilis* (Kunth) Lagasca ex Griffiths), upland sedges

(*Carex* spp. L.), needle and thread grass (*Hesperostipa comata* (Trinius & Ruprecht) Barkworth) and Sandberg's bluegrass (*Poa secunda* J. Presl). The soil was an Orthic Brown Chernozem with silty clay loam texture (37.1 % clay, 50.9% silt, 12.1% sand), 4.4% organic matter, and pH of 6.9 (0-10 cm). Dominant native species at Pinhorn were western wheatgrass, sedge, blue grama grass, junegrass (*Koeleria macrantha* (Ledebour) Schultes) and needle and thread grass. The soil was a Solonetzic Brown Chernozem with clay loam texture (37.1% clay, 36.4% silt, 26.5% sand), 4.8% organic matter, and pH of 7.2 (0-10 cm).

3.2.2 Experimental Design and Treatments

3.2.2.1 Field Experiment

At each field site a randomized complete block design was established with four blocks. Each block contained 6 different indaziflam treatment plots (fall or spring treatment, at each of 37.5, 75, and 150 g ai ha^{-1}), and two non-treated control plots; all plots were 7 × 3 m in size. Fall treatments occurred on October 16, 2019, and spring treatments on April 9, 2020. The 75 g ai ha^{-1} rate is the U.S. registered rate of Rejuvra® (Envu) for areas grazed by livestock. Treatments were applied using a Kubota RTV 1100 four-by-four with TeeJet AIXR 11003 nozzles at 275 kPa, at a water volume of 251.4 L ha^{-1}. Sites were fenced to exclude livestock for the study duration.

Plant community composition and biomass were evaluated at peak growth (late June) during the growing seasons of 2020, 2021, 2022, and 2023. Vegetation biomass in two 0.25 m^2 quadrats (50 × 50 cm) per plot was harvested each year in a location where biomass had previously not been cut, separated into annual brome, perennial grass (including sedges), forb, and litter, dried at 45ºC for five days, then weighed. Total plant biomass was the sum of annual brome, perennial grass, and forb biomass. Plant community composition was evaluated through foliar estimates of cover collected using three permanent 0.25 m^2 quadrats (50 × 50 cm) per plot in areas where biomass had not been harvested.

3.2.2.2 Seed Bank Evaluation

Eight soil cores, 10 cm in diameter and 5 cm deep, were collected in June 2021 from one non-treated plot per rep and all fall treatment plots (37.5, 75, and 150 g ai ha^{-1}) at both field sites. Cores were stored in a cooler for one day, then frozen until use. Soil was spread into 28 cm wide × 54 cm long × 6 cm deep trays over two centimeters of sterile sand after litter and large roots were removed. Trays were kept in the greenhouse at 18-22ºC with 16 hr of light and were watered regularly to promote germination without saturating the soil. Seedling emergence was monitored every 2-3 days for 8 months; soil was stirred once at 5 months when initial germination slowed.

3.2.2.3 Greenhouse Experiment

Non-treated topsoil (top 20 cm) from the Pinhorn site was collected and stored at room temperature for 10 months, then mixed before use. In the greenhouse, seeds of four species of the most common native perennial grasses in Alberta (*Hesperostipa comata*, *Koeleria macrantha, Pascopyrum smithii,* and *Poa secunda*) were planted into 12 containers for each species (8 cm diameter × 17.5 cm tall) filled with 1 gallon of Pinhorn soil, which were subsequently thinned to one plant per pot following successful establishment. Grasses grew in the greenhouse for two months with 16 hr of light at 18-22 ºC and were watered when dry. Care was taken to distribute water equally among containers and not overwater and saturate soil. After two months of growth, indaziflam was applied using a sprayer cabinet with air bubble jet nozzles, water volume of 200

L ha^{-1}, pressure of 207 kPa, and speed of 1.49 km hr^{-1}. Indaziflam was applied at either 0, 75, or 150 g ai ha^{-1} equivalence in a randomized complete block pattern with 4 replications of 3 containers of each grass species. Two months after indaziflam treatment, root and shoot biomass of each plant were measured. Due to the texture of the soil and delicate roots, roots were first washed whole and then laid flat to separate into the shallow (0-4 cm), middle (4-8 cm), and deep (8+ cm) depths. Root and shoot biomass were dried at 45ºC for three days and weighed.

3.2.3 Data Analysis

3.2.3.1 Field experiment analysis

A permutational multivariate analysis of variance (PerMANOVA) was used to test whether unique combinations of rate and season of indaziflam treatment affected non-brome collective plant community composition at each site and each year. When significant, an NMDS ordination was done to gain further insight into cover composition changes (distance = Bray-Curtis, dimensions = 2). Plant species vectors and additional vectors of perennial forb, annual forb, forb, perennial grass, richness, Shannon's diversity index, and inverse Simpson's diversity index were added to the plot with a cut off value of r = 0.5.

Species richness was calculated by counting every unique non-brome species in a plot. Inverse Simpson's and Shannon-Wiener indices were calculated using the diversity function in R software. Shannon-Wiener index was calculated by $\mathrm{H}' = -\Sigma_i\, \rho_i\, log_b\, \rho_i$ and inverse Simpson's index by $1/\,(D = \Sigma\, \rho_i^2)$ where ρ_i is the proportional abundance of species i. Richness and diversity indices were evaluated by year and site using mixed model ANOVA with treatment (unique rate and season of indaziflam application) as a fixed effect, and replication block as a random effect. Richness and diversity indices did not require transformation before ANOVA because histograms and residuals satisfied assumptions of normality based on visual inspection. Post-hoc treatment vs non-treated control comparisons were done when ANOVA was significant.

Due to differences in species diversity in 2023, the cover of select individual dominant plant species was analyzed; this was done for three perennial grasses (*Pascopyrum smithii, Bouteloua gracilis,* and *Hesperostipa comata*) and three forb species (*Selaginella densa, Achillea millefolium,* and *Artemisia frigida*), which had the highest sum cover over the four years of data collection. The cover of each of these individual species in 2023 was transformed to meet assumptions of normality, and then evaluated using linear mixed model ANOVA for each site; treatment (unique rate and season indaziflam treatment) was considered a fixed effect and replication block as random. When significant ($p < 0.05$), treatment vs non-treated control comparisons were done.

Changes in relative biomass of total herbage (including annual brome grass) and that of perennial grasses were calculated as natural log response ratios [ln(treatment/control), abbreviated as lnRR], for each treatment compared to the average of the two controls in the repetition block. This normalized the distribution of the response ratio and generated deviations in numerator and denominator of equal effect (Hedges et al. 1999). Estimated marginal means and 95% confidence intervals were calculated using the R package emmeans. Treatments were considered to have a significant effect when the 95% confidence intervals of the LnRR did not cross zero. Log response ratio means and 95% confidence intervals were back-transformed using [(exp(x)-1)*100] to derive proportional (%) changes in biomass.

Forb biomass was low at both sites, including many treatments with zero forb biomass, resulting in a highly skewed distribution and an inability to calculate LnRR. Instead Kruskal-Wallis tests were used to determine whether combinations of unique rates and seasons of indaziflam treatment affected forb biomass. Post-hoc tests were done using a Dunn's test.

3.2.3.2 Greenhouse analysis

ANOVA was used to test whether the root and shoot biomass of greenhouse grown perennial grasses were affected by rate of indaziflam and species of grass. Root biomass was square root transformed to meet assumptions of normality. Estimated marginal means were generated using the emmeans package and post-hoc contrasts were used to determined differences in biomass within each grass species between the non-treated control plants and those sprayed with the two rates of indaziflam ($p<0.05$). To evaluate how indaziflam rate affected root biomass at different depths, the four species were pooled and ANOVA was done for each of the shallow (0-4 cm), middle (4-8 cm), and deep (8+ cm) root biomass layers. Shallow root biomass was log transformed, and middle and deep layers were square root transformed to meet assumptions of normality. Post-hoc contrasts were used to determine differences in root biomass within each depth between the non-treated control plants and those sprayed with the two rates of indaziflam ($p<0.05$).

Finally, we used linear mixed model ANOVA to evaluate how rates of indaziflam affected forb and perennial grass seedling emergence from the soil seedbank treated by indaziflam two years prior. Seedling emergence was evaluated by site, with rate as a fixed effect and replication block as a random effect. Forb and grass emergence were log transformed to better meet assumptions of normality. Post-hoc treatment vs non-treated control comparisons were done when ANOVA was significant ($p<0.05$).

3.3 Results

3.3.1 Non-Brome Plant Community Cover and Diversity

PerMANOVA indicated that indaziflam application did not affect the cover of non-brome plant species during the post-treatment years of 2020, 2021, or 2022, at either site ($F \leq 1.19_{6,25}$, $P \geq 0.226$). During 2023, prior application of indaziflam affected the plant community, but only at the Aden site ($F = 1.73_{6,25}$, $P = 0.012$), and not the Pinhorn location ($F = 1.21_{6,25}$, $P = 0.199$). An NMDS of the plant community data at the Aden site from 2023 showed that non-treated control plots were associated with greater cover of select perennial grasses such as *Bouteloua gracilis* and *Nassella viridula*, as well as the native forbs *Arnica sororia, Artemisia ludoviciana, and Solidago missouriensis* (Figure 3.1) The NMDS also indicated the control plots were associated with greater richness, Shannon's diversity and Simpson's diversity in 2023. In contrast, indaziflam treated plots were associated with greater cover of *Pascopyrum smithii*, and perennial grasses as a group in general.

When analyzed with ANOVA, species richness and diversity were likewise not affected by treatment in 2020, 2021, or 2022; however, in 2023 there were effects on plant species richness, Simpson's diversity, and Shannon's diversity at both study sites (Table 3.1). The fall 75 g ai ha^{-1}, spring 75 g ai ha^{-1}, and spring 150 g ai ha^{-1} indaziflam treatments all reduced species richness at Pinhorn, while the fall 75 g ai ha^{-1}, fall 150 g ai ha^{-1}, spring 37.5 g ai ha^{-1} and spring 75 g ai ha^{-1} reduced richness at Aden (Table 3.2). All indaziflam treatments at both sites reduced inverse Simpson's diversity. All herbicide treatments at Aden reduced Shannon's diversity, while Shannon's diversity at Pinhorn was lower within the fall 75 g ai ha^{-1}, spring 75 g ai ha^{-1} and spring 150 g ai ha^{-1} treatments.

Indaziflam treatment altered the cover of the three most abundant grass species in 2023, but did not affect the three most abundant forbs (Table 3.3). At Aden, all indaziflam treatments except fall 37.5 g ai ha^{-1} increased *P. smithii* cover, while *B. gracilis* cover declined within the fall 37.5, fall 150, and spring 75 g ai ha^{-1} treatments; *H. comata* was not altered by indaziflam at this location (Table 3.4). At Pinhorn, the fall 75, fall 150, and spring 37.5 g ai ha^{-1} indaziflam treatments reduced *B. gracilis*, while the fall 75 g ai ha^{-1} treatment and all three rates applied in spring reduced *H. comata* cover, with *P. smithii* being non-responsive at this location.

3.3.2 Biomass

<u>3.3.2.1 Total Biomass</u>

Total plant biomass was not affected by the indaziflam treatments at either site in 2020, the growing season immediately following herbicide application (Figure 3.2; Table 3.5). During 2021, total biomass at Pinhorn was reduced by the fall 37.5 g ai ha^{-1} (-35.5%), fall 75 g ai ha^{-1} (-34.7%), fall 150 g ai ha^{-1} (-44.2%), spring 75 g ai ha^{-1} (-37.5%), and spring 150 g ai ha^{-1} (-49.4%) treatments (Table 3.5). The spring 37.5 g ai ha^{-1} treatment did not affect total biomass. Total biomass at Aden was reduced during 2021 only by the fall 75 g ai ha^{-1} (-45.1%) and spring 75 g ai ha^{-1} (-44.9%) treatments. By 2022, total herbage biomass increased with select 150 g ai ha^{-1} treatments at both sites; total biomass increased within the Pinhorn fall 150 g ai ha^{-1} treatment (75.5%), the Pinhorn spring 150 g ai ha^{-1} treatment (55.6%), and the Aden fall 150 g ai ha^{-1} treatment (44.9%). There were no other differences. In 2023, total biomass at both sites was not affected by any treatment.

3.3.2.2 Perennial Grass Biomass

Similar to total biomass, perennial grass biomass was not affected by indaziflam treatment in 2020 (Figure 3.3; Table 3.6). During 2021, all fall treatments at Pinhorn led to increased perennial grass biomass (33.5-42.3%), while confidence intervals at Aden all crossed zero suggesting no net change in grass biomass (Table 3.6). During 2022, only the highest indaziflam rate led to greater perennial grass biomass: at Pinhorn, the fall and spring 150 g ai ha^{-1} treatments increased grass biomass by 60.1% and 65.0%, respectively; at Aden, the fall 150 g ai ha^{-1} treatment similarly increased grass biomass (59.4%). In 2023, only the spring 150 g ai ha^{-1} treatment led to greater perennial grass biomass at Aden (33.5%).

3.3.2.3 Forb Biomass

Indaziflam treatment did not affect forb biomass except at Aden during 2022 when the spring 37.5 g ai ha $^{-1}$ treatment led to higher forb biomass than the spring 75 and spring 150 g ai ha^{-1} treatments ($\chi^2 = 14.33$, p = 0.026) (Data not shown).

3.3.3 Greenhouse Study

Two months after indaziflam treatment, indaziflam rate ($F = 15.5_{2,36}$, p <0.001) and grass species ($F = 33.6_{3,36}$, p <0.001), but not their interaction ($F = 1.32_{6,36}$, p = 0.272), affected total root biomass of the four-month-old grasses. The 150 g ai ha^{-1} indaziflam treatment reduced *P. smithii*, *H. comata,* and *K. macrantha* root biomass (Table 3.7). *H. comata* root biomass was also reduced by the 75 g ai ha^{-1} treatment. There were no differences in root biomass between indaziflam rates for *P. secunda*. Overall, *P. secunda* grew poorly in the greenhouse.

Total aboveground (shoot) biomass of the grasses was affected by indaziflam rate (F = $36.9_{2,36}$, p <0.001), grass species (F = $39.1_{3,36}$, p <0.001), and their interaction (F = $6.92_{6,36}$, p <0.001). The 150 g ai ha^{-1} indaziflam treatment reduced aboveground biomass of *P. smithii*, *H. comata, and K. macrantha* (Table 3.7). *P. smithii* and *H. comata* aboveground biomass were also reduced by the 75 g ai ha^{-1} treatment. There were no differences in aboveground biomass between indaziflam rates for *P. secunda*.

Rates of indaziflam also affected shallow (F = $4.37_{2,45}$, p = 0.018), middle (F = $5.60_{2,45}$, p = 0.007), and deep (F = $2.94_{2,45}$; p = 0.063) root biomass. The 150 g ai ha^{-1} indaziflam treatment reduced root biomass of all layers, including shallow, middle, and deep roots (Table 3.8). In contrast, the 75 g ai ha^{-1} treatment reduced biomass of the middle root layer, with no affect to either shallow or deep roots.

3.3.4 Seedbank Study

Rate of indaziflam applied in the field and evaluated in soil removed during June 2021 to a depth of 5 cm affected forb seedling emergence at both Aden (F = $6.98_{3,9}$, p=0.010) and Pinhorn (F = $7.04_{3,9}$, p<0.001), but did not affect perennial grass seedling emergence at Aden ($2.34_{3,9}$, p=0.141) or Pinhorn ($1.57_{3,9}$, p=0.263). Forb seedling emergence was reduced by all three rates of indaziflam at Pinhorn (Table 3.9). Forb and perennial grass emergence were low overall. Emergence by individual species is shown in Figure 3.4.

3.4 Discussion

3.4.1 Non-Brome Plant Cover Composition and Diversity

It took four years to manifest shifts in the non-brome plant species composition, richness, and diversity of these mixedgrass prairie grasslands after being treated with indaziflam. There did not appear to be differences in diversity responses based on the spring or fall timing of indaziflam application, likely due to the long-term soil residual nature of the herbicide. Notably, there were reductions in species richness and diversity even at low rates of indaziflam (37.5 g ai ha^{-1}). Reductions in diversity were most apparent in the fourth year of the study, which could be a result of select perennial plants reaching the end of their life cycle in the prior three years, coupled with insufficient regeneration of these species from seed during the interim due to indaziflam. Even without the addition of indaziflam, native forb and perennial grass germination from seed is generally low in semiarid grasslands (Coffin and Lauenroth 1989), and this is further supported by our seedbank study. Our seedbank results further indicate that the reduction in diversity may have been caused more by the inhibition of forb establishment rather than the establishment of perennial grasses. It could also be that particularly dry weather conditions during the prior (third) growing season caused plants more stress, resulting in greater susceptibility to indaziflam of remaining plants. This, in turn, possibly led to shifts in composition towards more tolerant species by the fourth year.

A reduction in plant species richness has typically not been found in previous indaziflam studies. Sites with high levels of brome invasion (ranging from 70-90% cover) and remnant native vegetation (10-30% cover) have exhibited either increases in species richness or stable richness when treated with indaziflam at lower (44 or 58 g ai ha^{-1}) and higher (73 or 102 g ai ha^{-}

^{1}) rates (Clark et al. 2019; Clark et al. 2020; Arathi and Hardin 2021; Sebastian et al. 2016a). Notably, these sites were more degraded (i.e., had more annual brome) than our sites, and therefore started with less native species cover. It is possible that when sites are highly degraded an increase in species richness is more likely following annual brome removal and resultant reduction in competition. On the other hand, when 63 g ai ha^{-1} indaziflam was applied to control invasive *Alyssum* species at less invaded, relatively undisturbed sites across an elevational gradient in Yellowstone National Park, Montana, mean species richness and Shannon's diversity of the plant community were reduced within indaziflam treated plots relative to non-treated controls (Meyer-Morey et al. 2021). Perennial grass richness was not impacted but perennial forb and annual forb richness were reduced, which is consistent with our seedbank study results.

Indaziflam effects on species richness and diversity therefore appear closely linked to the amount of prior disturbance at a site. Considering that even the low indaziflam rates used in our study (37.5 and 75 g ai ha^{-1}) and in other studies (63 g ai ha^{-1}) could reduce richness and diversity, the rate of indaziflam did not appear as important to affecting diversity as how disturbed the site was prior to application. An additional factor contributing to detecting diversity responses could be the length of time following treatment that the site was sampled. All the forementioned examples reported richness or diversity between one and three years following indaziflam treatment. In our study, richness and diversity were stable until the fourth year after treatment. Our results provide evidence of the benefits of long-term monitoring as changes in community composition were primarily evident only during the fourth growing season after treatment.

Graminoids appeared to be more affected than forbs when evaluating the cover of dominant plant species among sites. Grasses were present in higher abundance than forbs, and occupied every treatment plot, and therefore may have been easier to detect cover-based changes within. Grasses are also more mobile than forbs as they are consistently producing new tillers or rhizomes, and this vegetative propagation allows them to spread out spatially in response to environmental conditions. Indaziflam treatment resulted in greater *P. smithii* cover but lower cover of both *B. gracilis* and *H. comata*. Although not always statistically significant, these respective increases and decreases were evident under all treatments at both sites, indicating that these compositional changes were independent of study site conditions. The deep nature of *P. smithii* roots (down to 140+ cm; Coupland and Johnson 1965), coupled with its extensive rhizomes, may allow this species to both tolerate application of indaziflam and rapidly cover new niche space following brome removal. When growing in loamy soils together, *P. smithii* averages greater rooting depths than *H. comata* (Coupland and Johnson 1965). In contrast, *B. gracilis* has much shallower roots than the other dominant grasses and has large amounts of root mass in the topsoil (Coupland and Johnson 1965; Henderson et al. 2004), which may explain the increased sensitivity of this species to indaziflam. Unlike the other species, *H. comata* has moderately deep roots, and a higher density of lateral roots compared to *P. smithii* (Coupland and Johnson 1965). These rooting characteristics could result in greater susceptibility to indaziflam, as indaziflam is expected to be adsorbed and remain in the top layer of soil. These results were further supported by the greenhouse study where *H. comata* showed greater sensitivity to indaziflam than *P. smithii*. In the greenhouse *H. comata* was the only grass where both root and shoot biomass declined from the 75 g ai ha^{-1} indaziflam rate. The NMDS ordination from the Aden site also showed that indaziflam treated plots were associated with greater cover of *P. smithii* while non-treated plots were associated with *B. gracilis*, thereby further corroborating the greenhouse findings.

3.4.2 Biomass Responses

There were no changes in biomass during the first growing season following indaziflam application, potentially due to a lack of precipitation to carry herbicide into the soil, thereby delaying herbicide impacts on the annual brome. After the first year, total plant biomass initially decreased under select indaziflam treatments during the second growing season post treatment, then increased during the third year. By the fourth growing season there were no differences in total biomass among treatments. Land managers could initially expect a decline in total biomass as annual brome is reduced by indaziflam, with this lost biomass subsequently replaced by perennial grasses the following year. The absence of differences in total biomass during the final year suggests that other factors may have constrained plant growth within both treated and non-treated plots; 2023 was particularly dry across the study area, which may have limited both annual brome growth, and its impact on the native vegetation.

Perennial grass biomass did not decline under any treatment in any year, indicating that either established graminoids were not negatively impacted by indaziflam, or if they were, the resultant reduction in competition from annual brome control led to a net benefit in perennial grass growth and resulting biomass. These perennial grass biomass responses are similar to other studies done in the U.S. reporting no decrease in biomass, although we did not see as large an increase as other studies (Sebastian et al. 2017; Clark et al. 2020). Increases in perennial grass biomass were also temporary and there was no pattern obvious across rates or seasons of indaziflam application. It should be noted that in the third and fourth year annual brome cover within non-treated areas was generally low at our sites, at levels substantially lower than in other studies. As a result, there may have been less opportunity for perennial grass biomass to increase because perennial grass abundance remained relatively high.

Indaziflam generally did not affect forb biomass within these mixed grasslands, with the exception of the Aden site during the third growing season where the lowest herbicide rate applied in spring led to higher forb biomass. This finding parallels Sebastian et al. (2017) who reported an increase in forb biomass following indaziflam application. It could be that more forb seedlings germinated and survived at our lower rate of indaziflam, although this response was not evident following the fall treatments or within the other study site. Coupled with rather low initial forb cover and biomass within these grasslands, it is difficult to draw conclusions about how indaziflam affected forbs in this investigation. Forbs were distributed unevenly and sparsely across the sites, and most forb species were not consistently observed within every treatment plot.

3.4.3 Greenhouse Experiment

Indaziflam reduced root and shoot biomass of four-month-old perennial grasses. This indicates that the cellulose biosynthesis inhibiting mode of action of indaziflam can reduce the growth of recently established grasses, and not only seedlings, albeit under greenhouse conditions. Notably, this finding is in contrast to the field study wherein indaziflam did not reduce perennial grass biomass under any treatment in any year, and even increased it within select treatments. We postulate that following the reduction of brome in the field, lower competition for nutrients and water led to increased perennial grass growth, thereby negating any negative effect on observed grass biomass. Moreover, this may have occurred even despite small negative effects to perennial grass growth, but due to reduced annual brome competition these effects were not apparent. Future research should try to isolate these effects by evaluating indaziflam in the field when there is no brome present, thereby avoiding confoundment of direct responses to herbicide from associated changes in weed competition. Finally, herbicide effects in greenhouse trials may be exaggerated since plants are not limited by light, water, or nutrients, and their root mass is likely to be restricted (e.g., in depth) compared to field conditions, thereby facilitating increased uptake of herbicide (Führ and Mittelstaedt 1983).

In contrast, there were no effects to perennial grass biomass in the field during the growing season directly following treatment. This may indicate that well established perennial grasses are inherently less susceptible to indaziflam relative to four-month-old grasses. Older plants would not be growing as rapidly, and much of their root biomass would be below the topsoil layer where indaziflam is situated. Another explanation for this result is that the indaziflam may have taken longer to enter the soil in the field due to low precipitation immediately following application. Regular watering in the greenhouse could have promoted herbicide traveling deeper into the soil, which could also help explain the extended depth of reductions in root mass, as discussed further below.

The 150 g ai ha^{-1} rate of indaziflam reduced root biomass of three of the four grass species tested in the greenhouse (*H. comata, K. macrantha, P. smithii)*, while only one species was negatively affected by 75 g ai ha^{-1} indaziflam (*H. comata*). Notably, the 150 g ai ha^{-1} rate also reduced root biomass at all three rooting depths, while the 75 g ai ha^{-1} rate reduced biomass only at the middle depth, indicating increased potential drawbacks to grass fitness from using higher indaziflam rates, as well as a heightened risk where field applications of herbicide may accidentally overlap. Under greenhouse conditions the application of higher rates of indaziflam appeared to translocate deeper into the soil, with parallel reductions in root development, although it is unclear how this may translate into grass responses under field conditions, as discussed earlier. For example, we observed one case of a reduction in the growth of middle roots from the 75 g ai ha^{-1} rate despite no impact to shallow roots, which may be a result of regular watering in the greenhouse moving indaziflam to the middle root layer.

3.5 Conclusion

Our study provides an improved understanding of how the herbicide indaziflam affects native plant community composition and biomass within the northern Mixedgrass Prairie where remaining native grasslands are relatively less invaded by annual brome grasses. Overall, aboveground herbage biomass did not respond until the second year after treatment in the field study, after which indaziflam led to stable or increased herbage biomass, particularly that of perennial grasses. Despite this, our greenhouse results showed that the cellulose biosynthesis inhibiting mode of action of indaziflam can negatively affect roots and shoots of already growing juvenile grasses, with higher rates having more negative impacts at deeper soil depths. In the field indaziflam caused limited reductions in plant species richness and diversity, and only four years after application, although it is unknown how long these decreases will last. To contain the spread of annual brome in the early stages of invasion would require the application of indaziflam onto intact prairie. However, if preservation of biodiversity and perennial grass production is the primary goal when considering the use of indaziflam in Mixed Prairie, land managers will have to weigh small reductions in native diversity and potential declines in perennial grass fitness against the beneficial control of annual brome, the invasion of which can also reduce plant community diversity if allowed to spread.

3.6 References

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Table 3.1. ANOVA results of (non-brome) plant species richness, Shannon’s diversity index, and Simpson’s diversity index in response to various indaziflam treatments (six combinations of rate × season and non-treated control).

Year	Factor	Richness				Shannon’s				Simpson’s			
		Aden		Pinhorn		Aden		Pinhorn		Aden		Pinhorn	
		F-value†	P-value	F-value†	P-value	F-value†	P-value	F-value†	P-value	F-value†	P-value	F-value†	P-value
2020	Treatment	$1.79_{6, 22}$	0.149	$0.69_{6, 22}$	0.658	$1.01_{6, 22}$	0.444	$0.26_{6, 22}$	0.950	$1.00_{6, 22}$	0.453	$0.25_{6, 22}$	0.954
2021	Treatment	$0.64_{6, 22}$	0.697	$0.46_{6, 22}$	0.832	$0.68_{6, 22}$	0.664	$0.37_{6, 22}$	0.888	$0.85_{6, 22}$	0.547	$0.30_{6, 22}$	0.928
2022	Treatment	$1.58_{6, 22}$	0.199	$0.39_{6, 22}$	0.876	$1.53_{6, 22}$	0.216	$1.04_{6, 22}$	0.429	$1.29_{6, 22}$	0.301	$1.29_{6, 22}$	0.302
2023	Treatment	$5.11_{6, 22}$	**0.002**	$2.82_{6, 22}$	**0.035**	$7.98_{6, 22}$	**<0.001**	$3.67_{6, 22}$	**0.011**	$7.00_{6, 22}$	**<0.001**	$5.62_{6, 22}$	**0.001**

† F value subscript indicates the numerator and denominator degrees of freedom.

Table 3.2. Non-brome plant species richness, Simpson’s diversity, and Shannon’s diversity means and standard errors as measured during the 2023 growing season as a result of indaziflam treatment four years prior.

Plant Diversity Response	**Site**	**Treatment [Season of Application × Indaziflam Rate (g ai ha^{-1})]**						
		Non-treated control	Fall 37.5	Fall 75	Fall 150	Spring 37.5	Spring 75	Spring 150
Richness	Pinhorn	9.0 (0.7)	7.0 (0.9)	5.3 (0.9) **	6.8 (0.9)	6.3 (0.9)	6.0 (0.9) *	5.5 (0.9) **
	Aden	9.9 (0.6)	8.3 (0.8)	6.8 (0.8) ***	7.5 (0.8) **	7.8 (0.8) *	5.6 (0.8) ****	7.5 (0.8) **
Inverse Simpson’s Index	Pinhorn	5.12 (0.33)	3.32 (0.47) **	2.58 (0.47) ***	3.14 (0.47) **	3.42 (0.47) **	3.03 (0.47) ***	2.47 (0.47) ****
	Aden	5.00 (0.31)	3.11 (0.44) **	2.49 (0.44) ***	2.75 (0.44) ***	2.92 (0.44) ***	2.41 (0.44) ****	2.74 (0.44) ***
Shannon Index	Pinhorn	1.80 (0.11)	1.42 (0.16)	1.09 (0.16) ***	1.34 (0.16)	1.42 (0.16)	1.25 (0.16) **	1.17 (0.16) ***
	Aden	1.83 (0.10)	1.44 (0.07) **	1.22 (0.10) ****	1.30 (0.10) ***	1.38 (0.10) ***	1.15 (0.10) ****	1.27 (0.10) ****

Asterisks indicate differences between treatments compared to the non-treated control (* p = 0.05-0.1, ** p = 0.05-0.01, *** p = 0.01-0.001, **** p <0.001).

Table 3.3. ANOVA results of the cover of the top 3 most abundant grasses and forbs in response to indaziflam treatment in 2023 ($p < 0.05$).

Group	**Species**	**Aden**		**Pinhorn**	
		F-value†	**P-value**	**F-value†**	**P-value**
Grass	*Pascopyrum smithii*	$3.98_{6,22}$	**0.008**	$2.04_{6,22}$	0.103
	Bouteloua gracilis	$3.67_{6,22}$	**0.011**	$2.43_{6,22}$	**0.059**
	Hesperostipa comata	$0.92_{6,22}$	0.499	$3.87_{6,22}$	**0.009**
Forb	*Selaginella densa*	$0.44_{6,22}$	0.847	$1.68_{6,22}$	0.174
	Achillea millefolium	$0.96_{6,22}$	0.472	$1.08_{6,22}$	0.406
	Artemisia frigida	$0.57_{6,22}$	0.748	$0.70_{6,22}$	0.660

† F value subscript indicates the numerator and denominator degrees of freedom.

Table 3.4. Individual cover (%) of grass species means (SE) in the 2023 growing season as a response to indaziflam treatment.

Site	**Species**	**Treatment [Season of Application × Indaziflam Rate (g ai ha^{-1})]**						
		Non-treated control	Fall 37.5	Fall 75	Fall 150	Spring 37.5	Spring 75	Spring 150
		Cover (%)						
Aden	*Bouteloua gracilis*	8.7 (1.3)	3.4 (1.7)**	6.2 (1.7)	1.4 (1.7)**	5.7 (1.7)	2.7 (2.7)**	5.8 (1.7)
	Hesperostipa comata	6.2 (1.4)	1.7 (2.0)	3.3 (2.0)	3.3 (3.0)	1.8 (2.0)	4.8 (2.0)	4.8 (2.0)
	Pascopyrum smithii	20.2 (3.7)	28.2 (4.3)	33.7 (4.3)**	33.8 (4.3)**	32.5 (4.3)**	35.4 (4.3)**	32.7 (4.3)**
Pinhorn	*Bouteloua gracilis*	5.6 (0.9)	3.7 (1.3)	1.6 (1.3)*	1.3 (1.3)*	1.2 (1.3)*	1.9 (1.3)	1.9 (1.3)
	Hesperostipa comata	6.5 (1.1)	5.9 (1.5)	1.3 (1.5)**	2.5 (1.5)	1.7 (1.5)*	0.8 (1.5)**	0.8 (1.5)**
	Pascopyrum smithii	15.9 (3.5)	17.4 (3.9)	21.2 (3.9)	14.7 (3.9)	19.1 (3.9)	22.1 (3.9)	24.3 (3.9)

Asterisks indicate differences between treatments compared to the non-treated control (* $p = 0.05$-0.1, ** $p < 0.05$).

Table 3.5. Total plant shoot biomass in the non-treated control plots and the percent change[1] in total biomass as a result of indaziflam applied at different rates, seasons and locations prior to the 2020 growing season.

Site	**Year**	**Non-treated total biomass (kg ha^{-1})**	**Treatment [Season of Application × Indaziflam Rate (g ai ha^{-1})]**					
			Fall 37.5	Fall 75	Fall 150	Spring 37.5	Spring 75	Spring 150
			Percent change in total plant shoot biomass					
Pinhorn	2020	1834.9 (265.2)	-4.5 (-53.5, +96.0)	-6.2 (-54.3, +92.6)	-26.0 (-64.0, +51.9)	+12.3 (-45.3, +130.6)	-0.5 (-51.5, +104.3)	-3.6 (-53.0, +97.9)
	2021	1845.3 (346.4)	**-35.5 (-58.1, -0.6)**	**-34.7 (-57.6, +0.6)**	**-44.2 (-63.8, -14.1)**	-17.1 (-46.2, +27.7)	**-37.5 (-59.4, -3.7)**	**-49.4 (-67.2, -22.1)**
	2022	402.7 (54.0)	+12.2 (-27.4, +73.4)	+25.4 (-18.9, +93.9)	**+75.5 (+13.6, +171.4)**	+14.0 (-26.2, +76.3)	+0.5 (-35.0, +55.4)	**+55.6 (+0.6, +140.5)**
	2023	1491.6 (120.9)	-3.2 (-35.0, +44.0)	-14.8 (-42.7, +26.8)	+7.2 (-28.0, +59.5)	+13.0 (-24.1, +68.1)	-0.9 (-33.4, +47.5)	+14.7 (-22.9, +70.6)
Aden	2020	1619.9 (83.6)	-6.8 (-29.0, +22.4)	-18.6 (-38.0, +6.9)	-12.5 (-33.4, +14.9)	-3.8 (-26.7, +26.4)	-3.7 (-26.7, +26.5)	-4.2 (-27.1, +25.8)
	2021	1239.1 (84.2)	-25.1 (-53.4, +20.3)	**-45.1 (-65.9, -11.8)**	-2.0 (-39.1, +57.5)	-32.3 (-57.9, +8.9)	**-44.9 (-65.7, -11.4)**	-20.0 (-50.2, +28.6)
	2022	509.8 (60.1)	+10.8 (-15.7, +45.6)	-3.0 (-26.2, +27.5)	**+47.2 (+12.0, +93.5)**	+30.5 (-0.7, +71.5)	+11.1 (-15.5, +46.0)	+23.1 (-6.3, +61.8)
	2023	1251.6 (84.2)	-16.4 (-32.7, +3.9)	-9.8 (-27.4, +12.1)	+9.0 (-12.3, +35.4)	-11.4 (-28.7, +10.1)	+1.8 (-18.1, +26.5)	+20.4 (-3.1, +49.7)

[1] Percent change was calculated by back-transforming log response ratio means and confidence intervals using [(exp(x)-1)*100]. Log response ratio was calculated using [ln(treatment/control)].

Brackets indicate 95% confidence intervals for the percent change and standard errors for total biomass.

Bolded values have confidence intervals that do not overlap with zero, indicating significant differences from the non-treated control.

Table 3.6. Perennial grass shoot biomass in the non-treated control plots and the percent change[1] in perennial grass biomass as a result of indaziflam applied at different rates, seasons and locations prior to the 2020 growing season.

Site	**Year**	**Non-treated perennial grass biomass (kg ha^{-1})**	**Treatment [Season of Application × Indaziflam Rate (g ai ha^{-1})]**					
			Fall 37.5	Fall 75	Fall 150	Spring 37.5	Spring 75	Spring 150
			Percent change in perennial grass biomass					
Pinhorn	2020	815.4 (91.6)	-3.0 (-33.2, +40.9)	+14.5 (-21.2, +66.3)	-16.2 (-42.4, +21.7)	-16.6 (-42.6, +21.2)	+1.5 (-30.2, +47.4)	-1.2 (-32.0, +43.6)
	2021	637.5 (47.9)	**+33.5 (0.0, +78.1)**	**+37.1 (+2.8, +83.0)**	**+42.3 (+6.7, +90.0)**	+12.0 (-16.1, +49.5)	+17.3 (-12.1, +56.6)	+7.7 (-19.3, +43.7)
	2022	359.4 (51.2)	+20.5 (-22.3, +86.9)	+36.4 (-12.1, +111.5)	**+60.1 (+3.2, +148.2)**	+23.2 (-20.6, +91.0)	+7.8 (-30.5, +67.2)	**+65.0 (+6.4, +155.8)**
	2023	1393.2 (144.5)	+0.1 (-33.0, +49.6)	-12.9 (-41.7, +30.2)	+12.5 (-24.8, +68.1)	+17.2 (-21.6, +75.2)	+5.6 (-29.3, +57.9)	+17.1 (-21.6, +75.1)
Aden	2020	914.9 (91.8)	+6.6 (-22.7, +46.9)	-25.7 (-46.1, +2.4)	+4.5 (-24.2, +44.0)	-10.8 (-35.3, +23.0)	-1.9 (-28.8, +35.2)	+6.4 (-22.8, +46.7)
	2021	740.0 (72.8)	-1.3 (-46.1, +80.8)	-12.8 (-52.4, +59.9)	+62.3 (-11.5, +197.3)	-21.2 (-57.0, +44.5)	-14.2 (-53.1, +57.3)	+17.3 (-36.0, +114.9)
	2022	452.7 (61.0)	+4.2 (-29.4, +53.9)	+6.9 (-27.6, +57.9)	**+59.4 (+8.0, +135.4)**	-0.8 (-32.8, +46.5)	+25.2 (-15.2, +84.9)	+38.3 (-6.4, +104.2)
	2023	1114.5 (83.0)	-14.2 (-34.6, +12.7)	-29 (-26.0, +27.5)	+12.8 (-14.1, +48.0)	-12.9 (-33.6, +14.4)	+13.7 (-13.4, +49.3)	**+33.5 (+1.7, +75.2)**

[1] Percent change was calculated by back-transforming log response ratio means and confidence intervals using [(exp(x)-1)*100]. Log response ratio was calculated using [ln(treatment/control)].

Brackets indicate 95% confidence intervals for the percent change and standard errors for perennial grass biomass.

Bolded values have confidence intervals that do not overlap with zero, indicating significant differences from the non-treated control.

Table 3.7. Mean (SE) of four-month-old perennial grasses root biomass and shoot biomass in response to indaziflam treatment applied at two months age.

Species	**Root biomass (g)**			**Shoot biomass (g)**		
	Rate of indaziflam (g ai ha^{-1})			Rate of indaziflam (g ai ha^{-1})		
	0	75	150	0	75	150
Pascopyrum smithii	14.5 (1.9)	9.9 (0.1)	7.8 (2.1) *	4.7 (0.2)	3.1 (0.4) *	2.0 (0.3) *
Hesperostipa comata	11.4 (2.2)	4.0 (1.2) *	2.5 (0.7) *	6.1 (0.3)	3.7 (1.0) *	1.0 (0.1) *
Koeleria macrantha	6.7 (1.6)	3.9 (1.5)	2.5 (0.5) *	3.3 (0.4)	3.4 (0.4)	1.4 (0.4) *
Poa secunda	1.5 (0.6)	1.2 (0.5)	0.6 (0.20)	0.4 (0.1)	0.4 (0.1)	0.3 (0.1)

Asterisks indicate differences between indaziflam treatments compared to the non-treated control ($p < 0.05$).

Table 3.8. Mean (SE) four-month-old perennial grass root biomass harvested at three successive depths in response to indaziflam applied at two months of age.

Depth (cm)	Rate of indaziflam (g ai ha^{-1})		
	0	75	150
	Root biomass (g)		
Shallow (0-4)	1.90 (0.54)	0.78 (0.22)	0.42 (0.12) *
Middle (4-8)	2.48 (0.50)	1.11 (0.28) *	0.98 (0.36) *
Deep (8+)	4.14 (0.73)	2.85 (0.55)	1.96 (0.44) *

Asterisks indicate differences between indaziflam treatment and the non-treated control ($p < 0.05$).

Table 3.9. Mean (SE) seedling emergence of perennial grass and forb seedlings in response to different indaziflam rates applied two years earlier.

Plant Group	Site	Indaziflam Rate (g ai ha^{-1})			
		Non-treated control	37.5	75	150
		No. seedlings 3141 cm^{-3} soil			
Perennial Grass	Pinhorn	3.0 (0.7)	2.3 (1.3)	5 (3.8)	0.3 (0.3)
	Aden	4.5 (2.9)	3.5 (2.2)	0.8 (0.8)	0.0 (0.0)
Forb	Pinhorn	4.5 (2.1)	0.8 (0.5) *	0.0 (0.0) *	0.0 (0.0) *
	Aden	2.3 (1.0)	5.0 (1.3)	0.5 (0.3)	0.3 (0.3)

Asterisks indicate differences between treatments and the non-treated control ($p < 0.05$).

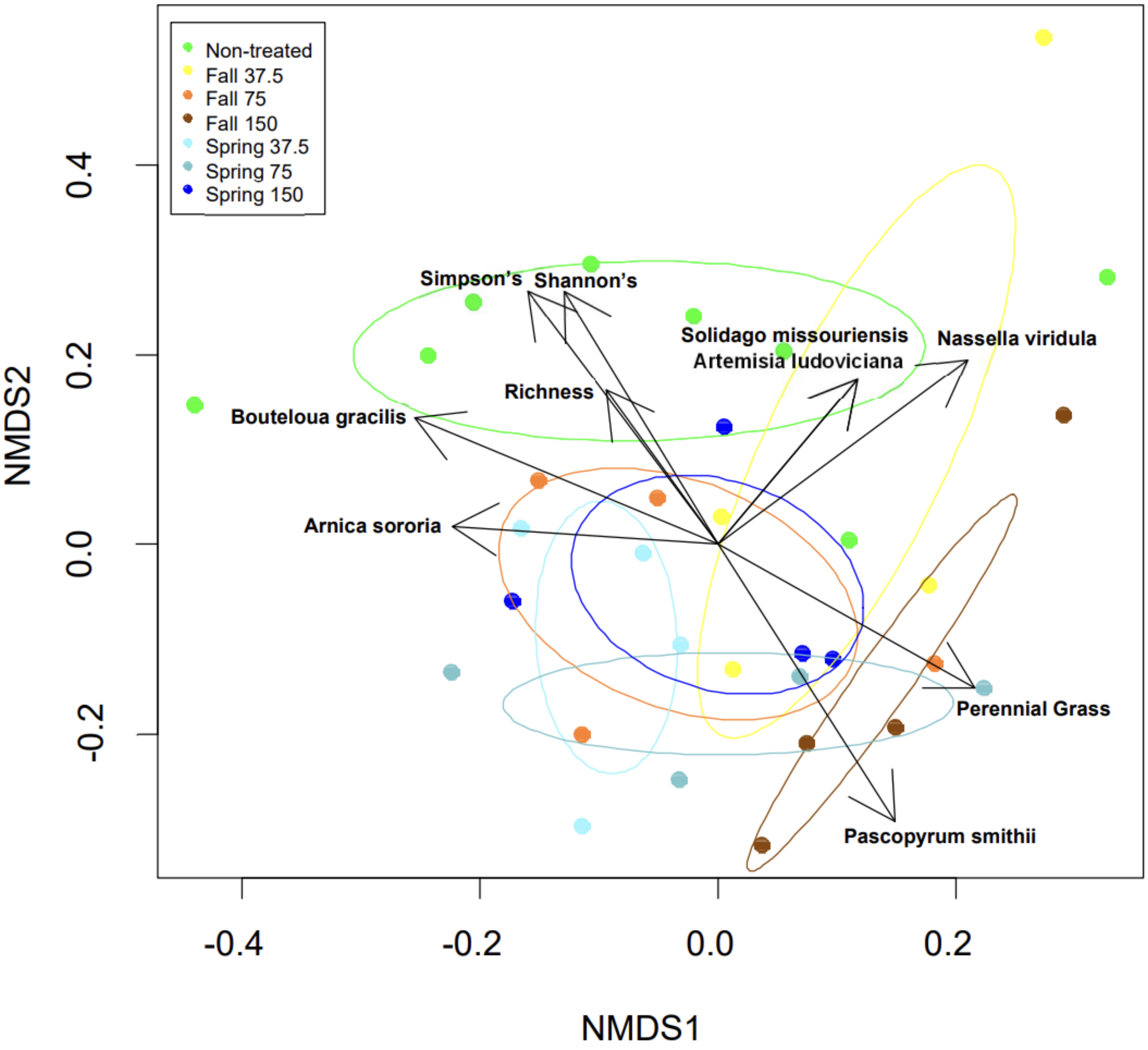

Figure 3.1 NMDS ordination of non-brome plant species composition at Aden during 2023, four years after indaziflam application (distance = Bray-Curtis, dimensions = 2, stress = 0.18). Fall and spring represent the timing of application, and values different herbicide rates (g ai ha^{-1}).

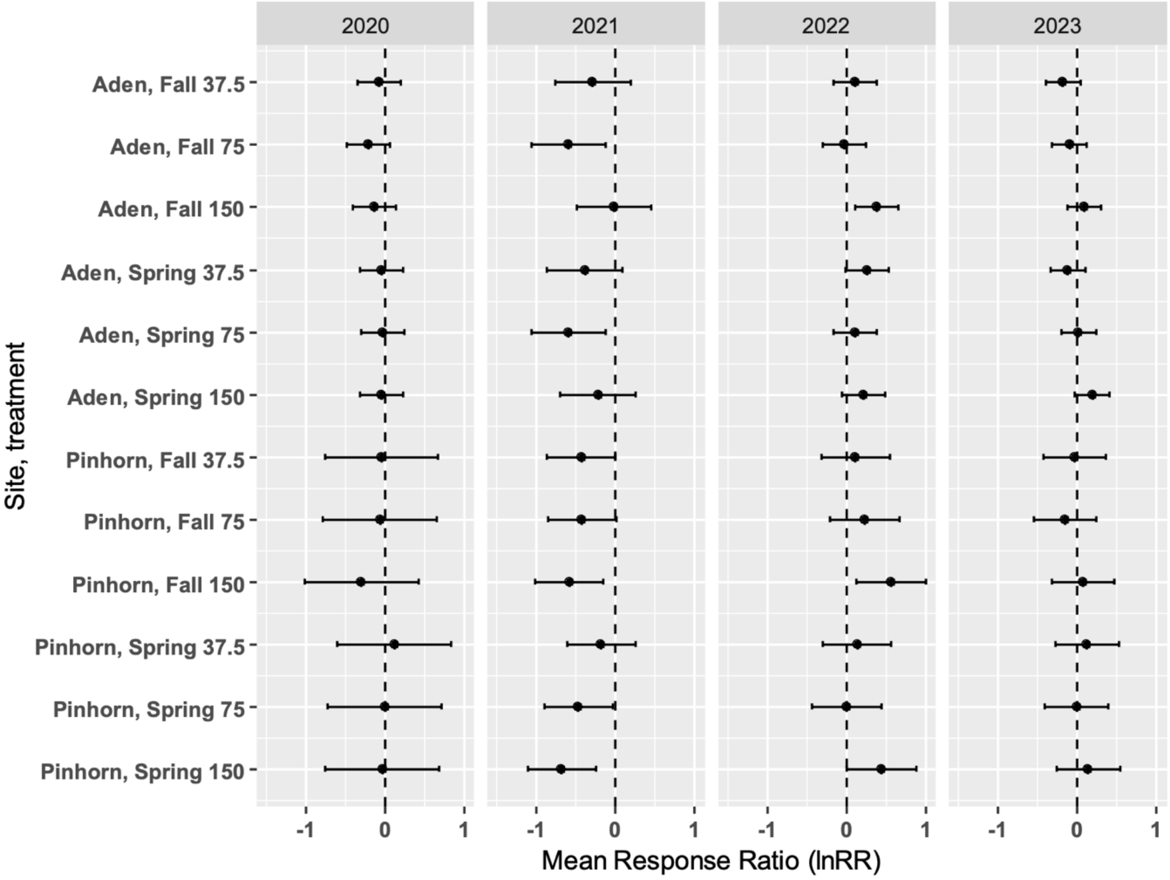

Figure 3.2. Log response ratio of total plant biomass in each post-indaziflam treatment year within the field study. Treatments are shown for all combinations of rate (g ai ha^{-1}), season of application, and location, with treatments occurring in October 2019 (fall) and April 2020 (spring). Log response ratio was calculated using [ln(treatment/control)]. Error bars represent 95% confidence intervals.

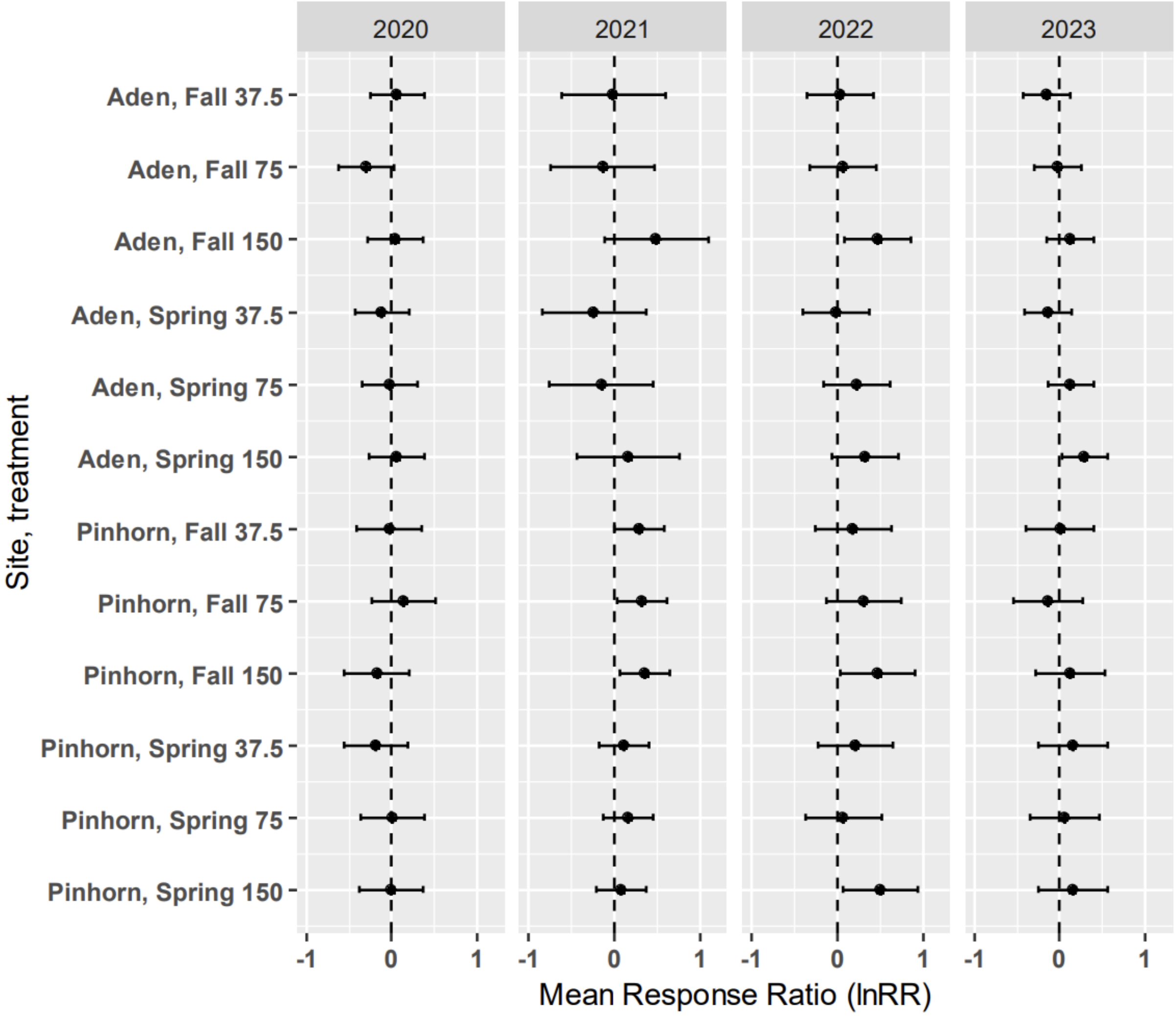

Figure 3.3. Log response ratio of perennial grass biomass in each post-indaziflam treatment year within the field study. Treatments are shown for all combinations of rate (g ai ha^{-1}), season of application, and location, with treatments occurring in October 2019 (fall) and April 2020 (spring). Log response ratio was calculated using [ln(treatment/control)]. Error bars represent 95% confidence intervals.

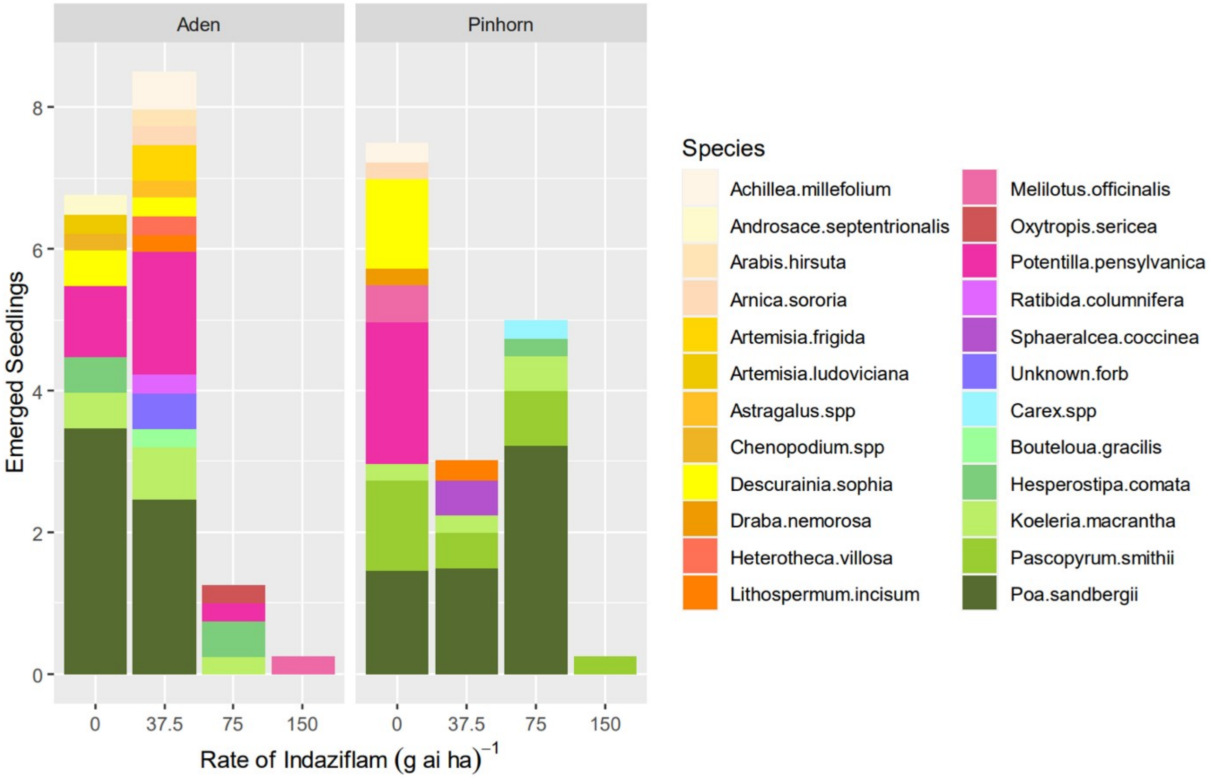

Figure 3.4. Native seedling emergence (seedlings 3141 cm^{-3} of soil) determined over 8 months in the greenhouse. Soils were removed in June 2021 to 5 cm of depth following October 2019 indaziflam treatment in the field. Forb species are shown in shades of yellow to red, and grasses in shades of green.

4 Synthesis

4.1 Introduction

Annual brome grasses represent a significant threat to the environmental and economic sustainability of rangelands in North America (Germino et al. 2016). While invasion by annual brome into western Canadian grasslands has been considered to be relatively minor in the past, there is mounting evidence that they are becoming more common, and where established, may be impacting the grasslands they are invading. In southern Alberta for example, grasslands of the Dry Mixedgrass Prairie along the Montana border are experiencing increasing outbreaks of annual brome, including *Bromus tectorum* (cheatgrass), *B. squarrosus* (corn brome), and *B. japonicus* (Japanese brome).

Out of the tools available to control annual brome, herbicide is one of the most promising long-term approaches (Monaco et al. 2017), although there are limited herbicide control options within Alberta. Indaziflam is a novel herbicide that is expected to become registered for use within Canada in the near future. Indaziflam interferes with the reproduction of annual bromes by inhibiting cellulose biosynthesis within establishing seedlings, controlling annual brome *in-situ* with relatively little impact on the existing native perennial grassland (Sebastian et al. 2016). Perennial plants are less affected by indaziflam since they do not rely solely on seedling establishment for reproduction and yearly regrowth.

This research project aimed to generate and share regionally tested information with stakeholders about the practical use of the herbicide indaziflam on western Canadian grassland. This included insights that could guide land managers, ranchers, conservationists, and range agrologists seeking management options for annual brome within Alberta.

4.2 Summary of Key Research Findings

The objective of Chapter 2 was to determine the expected quantity of brome biomass and density reductions over four years following indaziflam treatment at different rates and seasons of application. We found that land managers can expect high levels of brome reduction, though not in the first growing season. Instead, reductions in brome abundance occurred primarily during the second growing season after indaziflam, with fall applied treatments having slightly greater reductions than those spring applied. This result highlighted that to maximize the efficacy of a single application of indaziflam, it is likely best to apply the herbicide in the late summer/early fall, preferably immediately prior to a rainfall event to ensure herbicide entry into the topsoil. The >80% reductions in brome biomass within all indaziflam treatments (except the lowest 37.5 ai ha^{-1} rate treatments) during the third season demonstrated that if the fall timing of herbicide is not possible, waiting until the following spring can also lead to favorable levels of annual brome control. Overall, the moderate to high level of reductions of annual brome that were observed during the fourth year following treatment showed that indaziflam can be a useful tool for containing the spread and abundance of annual brome within the Canadian prairies in the long-term.

Along with the extent of weed control, however, the successful management of an invasive species is also determined by the response of the remaining native plant community, and specifically in how the herbicide affects forage production and biodiversity management goals. The objective of Chapter 3 was to determine how different rates and seasons of indaziflam affect northern mixed prairie plant species abundance (cover and biomass), composition, richness, and diversity. This was accomplished using a field study and complementary greenhouse experiments. We found that perennial grass biomass was not negatively affected by indaziflam in any year of assessment, and sometimes even increased, presumably as competition from the declining brome population was reduced. Total above ground biomass (i.e. total forage biomass) increased or remained stable during the third and fourth year of assessment. This demonstrated that indaziflam can be a tool to help maintain or increase the amount of palatable forage available for livestock and wildlife. This benefit may be amplified if animals are avoiding brome invaded patches, resulting in the loss of non-brome forage mixed within the invaded area. For example, at the Pinhorn field site we observed clumps of brome and perennial grass that looked like they had been chewed and spat out, resulting in cattle not only avoiding the brome, but also the native plants that normally would have been targeted by grazing cattle.

Changes in native plant community composition were initially not evident, but then eventually manifested during the fourth year after indaziflam treatment. This included a shift from the bunchgrass *Hesperostipa comata* to the deeper rooted rhizomatous grass *Pascopyrum smithii*. There were also reductions in plant species richness and diversity, possibly a result of low regeneration of forbs from seed, as also observed in the parallel greenhouse seedbank experiment. In the greenhouse we also found that the impacts of the cellulose biosynthesis inhibition mode of action of indaziflam was not limited exclusively to germinating seedlings, but reduced root and shoot growth (biomass) of 2-4 month old established perennial grasses. Therefore, continuously applying indaziflam every four years could (in theory) result in long-term changes in native grassland plant diversity if treatments were repeated over an extended period. If treatments are not repeated, or are spaced out at lengthy intervals, it is expected that once indaziflam sufficiently degrades within the soil, native grasses and forbs from the seedbank will once again germinate and add to the population.

4.3 Management Implications and Future Research Needs

Indaziflam application to mixed prairie grasslands that had been invaded by annual brome provided an important tool to help reduce the weed outbreak, while also maintaining forage production and availability. Moreover, these benefits were evident for up to four years after application, providing an opportunity for the existing native grassland vegetation to more effectively compete against any future weed outbreak. Despite this, results from other locations in North America where annual bromes have been problematic suggest that once established, the complete reversal of annual brome invasion (i.e., eradication) is unlikely (Monaco et al. 2017), meaning that great care will be needed to manage these grasslands in spite of the presence of annual brome. For example, due to the viability of annual brome seed (3-5 years), it is possible, and even likely, that repeat applications of herbicide may be needed to achieve longer-term, and sustaining reductions in the abundance of the weed (Sebastian et al. 2017). Moreover, cessation of treatment may also lead to reinvasion of annual brome, particularly from adjacent areas that were not treated, and therefore provide a key source of propagules for invasion. Additionally, conservative livestock stocking rates may be warranted to help maintain the vigor of native grassland species, which could help minimize annual brome abundance via competitive suppression (Brooks et al. 2016). Similarly, managers should be aware of the increased wildfire risk that affected grasslands in the region pose due to the large accumulation of fine fuel, which has altered fire regimes in many regions of the western U.S. (D'Antonio and Vitousek 1992).

Given the limited changes in plant diversity that occurred to the mixedgrass communities treated with indaziflam, while considering that the diversity of native plant communities in the U.S. are known to be compromised by annual brome invasion (Germino et al. 2016), and considering that indaziflam helped maintain the forage supply of native grasses, I support the use of a one-time application of indaziflam at 75 g ai ha^{-1} to attempt to contain the brome seedbank. Treatment with indaziflam would be especially beneficial if applied to small patches during the initial stages of brome invasion into Alberta, as this could both reduce the amount of herbicide applied, while also containing the weed before becoming more widely established. There would not be great harm in applying indaziflam onto a small patch of annual brome but a great benefit in proactively containing the spread of the brome across the rest of the land/region. In contrast to early proactive management, attempting to control problematic weeds like annual brome at a later stage requires more herbicide, greater expense, and possibly a risk of longer-term loss of diversity (DiTomaso 2000). The resulting ecological and economical costs would be much larger across a larger scale application area.

While reductions in diversity from a single application were minor, and it is expected that native forbs and grasses will resume regeneration from seed once indaziflam degrades within the soil, sequential applications of indaziflam could compromise diversity by preventing ongoing perennial forb and grass regeneration. Future research could examine the effects of indaziflam treatment every 5 - 10 years and the optimal intervals between applications to best balance brome control with the maintenance of native plant diversity.

While the extent and severity of annual brome invasion in the U.S. has been well documented, it remains unknown just how widespread and severe annual brome will become in the grasslands of western Canada. If annual brome invasion within Alberta is not as aggressive as in the U.S. there may be less reason to implement widespread use of indaziflam. Notably, the dramatic reduction in annual brome cover/biomass/density within our sites during the growing seasons of 2022 and 2023 occurred without the assistance of herbicide treatment. This occurred within the downy brome and corn brome present within our two sites, although there is greater evidence that this reduction affected corn brome since it was the dominant brome species within our sites. During field tours with Alberta range agrologists and an Alberta Conservation Association biologist, we noticed that many sites in the Forty Mile County and Warner County region of southern Alberta were dominated by corn brome. For future research it would be beneficial to understand more about the distribution of different species of annual brome, particularly corn brome, within Alberta and how invasive/detrimental to diversity different species of annual bromes may be.

It would also be beneficial to understand how invasive these species might be within the more mesic ecosystems of central and northern Alberta. The productive plant communities with relatively higher summer precipitation in these regions may be more resistant to brome invasion (Chambers et al. 2016). Management recommendations for brome control using indaziflam would be different within these northern areas, as the increased precipitation could infiltrate herbicide deeper into the soil, and leach it from the soil. On the other hand, drought may become more prevalent across Alberta. Drought, climate change, changes in timing of precipitation, and longer/warmer growing seasons could result in greater invasibility and brome migration across Alberta (Chambers et al. 2016).

Should annual brome become established across Alberta, understanding how it alters forage use by cattle will be of immediate interest to ranchers. Though annual bromes are known to be unpalatable to cattle once they mature, there is less information on whether cattle avoid invaded areas of brome on the landscape. Annual brome typically forms patches with a high density of plants within the field (an indicator that at a local scale their invasion is microclimate or soil determined) (Belnap et al. 2016). If animals avoid patches of brome, this could result in a significant loss of forage within pastures and ultimately less weight gain for animals. For example, steers grazing within brome invaded pastures had lower daily gain than steers grazing in pastures where brome had been suppressed (Haferkamp et al. 2001).

While indaziflam is one of the tools that helps manage the problem of annual brome invasion within Albertan grassland ecosystems, an integrated system management approach improves the chance of comprehensive containment of invasion in the long-term. Grazing can be simultaneously used along with indaziflam to further suppress brome. For example, grazing in the spring when plants are young and palatable prevents establishment of brome seedheads (Lehnhoff et al. 2019). Overall, the greatest chance of preventing and controlling brome invasion is with the establishment of robust and healthy plant communities (Beck 2009). This can be achieved by avoiding overgrazing of pastures and providing pasture enough rest. Using indaziflam together with careful grazing management will help encourage a healthy rangeland that will sustain grazing and biodiversity for the future.

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