DISSERTATION

INDAZIFLAM: A NEW CELLULOSE BIOSYNTHESIS INHIBITING HERBICIDE PROVIDES LONG-TERM CONTROL OF INVASIVE WINTER ANNUAL GRASSES

Submitted by

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ABSTRACT

INDAZIFLAM: A NEW CELLULOSE BIOSYNTHESIS INHIBITING HERBICIDE PROVIDES LONG-TERM CONTROL OF INVASIVE WINTER ANNUAL GRASSES

Invasive winter annual grasses such as downy brome (*Bromus tectorum* L.) are a threat to native ecosystems throughout the US. These invasive grasses exploit moisture and nutrients throughout the fall and early spring before native plants break dormancy. This results in decreased native species abundance and development of monotypic stands. Short-term downy brome management has been shown to be effective; however, the soil seed reserve has often been overlooked although it's the mechanism responsible for rapid re-establishment. While glyphosate, imazapic, and rimsulfuron are herbicides commonly recommended to control invasive, annual grasses, their performance is inconsistent, and they can injure desirable perennial grasses. Indaziflam is a recently registered cellulose-biosynthesis inhibiting herbicide, providing broad spectrum control of annual grass and broadleaf weeds. Indaziflam (Esplanade®, Bayer CropScience) is a cellulose biosynthesis inhibiting (CBI) herbicide that is a unique mode of action for resistance management and has broad spectrum activity at low application rates. At three sites, glyphosate and rimsulfuron provided less downy brome control than indaziflam one year after treatment (YAT). Percent downy brome control with imazapic decreased significantly 2 YAT (45-64%), and 3 YAT (10-32%). Across all sites and application timings, indaziflam provided the greatest downy brome control 2 YAT (89-100%) and 3 YAT (83-100%). At two additional sites evaluating five application timings, indaziflam treatments resulted in superior invasive winter annual grass control 2 YAT (84% ± 5.1 to 99% ± 0.5) compared to imazapic

(36% ± 1.2). Indaziflam treatments significantly increased biomass and species richness of co-occurring species, 2 YAT. In a greenhouse bioassay, indaziflam was significantly more active on downy brome, feral rye (*Secale cereale* L.), jointed goatgrass (*Aegilops cylindrica* L.), Japanese brome (*Bromus japonicus* Thunb.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski), and ventenata (*Ventenata dubia* (Leers) Coss) compared to imazapic, with the exception of jointed goatgrass. Comparing all species, the GR_{50} values for imazapic were on average 12 times higher than indaziflam. Indaziflam's increased activity on monocots could provide a new alternative management strategy for long-term control of multiple invasive winter annual grasses that invade >23 million ha of US rangeland. Indaziflam could potentially be used to eliminate the soil seed bank of these invasive grasses (< 5 years), decrease fine fuel accumulation, and ultimately increase the competitiveness of perennial co-occuring species.

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DEDICATION

I dedicate this work to my father, James Sebastian.

Thank you for your constant mentorship, guidance, and support.

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CHAPTER 1: A POTENTIAL NEW HERBICIDE FOR INVASIVE ANNUAL GRASS CONTROL ON RANGELAND[1]

SUMMARY[1]

Downy brome (*Bromus tectorum* L.), a winter annual grass, is considered one of the most invasive non-native rangeland species in the U.S. While glyphosate, imazapic, and rimsulfuron are herbicides commonly recommended to control invasive, annual grasses, their performance is inconsistent, and they can injure desirable perennial grasses. Indaziflam is a recently registered cellulose-biosynthesis inhibiting herbicide, providing broad spectrum control of annual grass and broadleaf weeds. Indaziflam is labeled for winter annual grass control in citrus, grape, and tree nut crops, and could represent a new mode of action for selective winter annual grass control on rangeland. Three field experiments were conducted to compare indaziflam to imazapic, rimsulfuron, and glyphosate, three herbicides commonly used for downy brome control. Multiple herbicide application timings were evaluated. At all three sites, glyphosate and rimsulfuron provided less downy brome control than indaziflam one year after treatment (YAT). Percent downy brome control with imazapic decreased significantly 2 YAT (45-64%), and 3 YAT (10-32%). Across all sites and application timings, indaziflam provided the greatest downy brome control 2 YAT (89-100%) and 3 YAT (83-100%). Indaziflam did not significantly reduce species richness. This study demonstrates that indaziflam can provide extended downy brome control compared to currently used herbicides.

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INTRODUCTION

Downy brome (*Bromus tectorum* L.) is a competitive winter annual grass that has rapidly spread throughout many regions of the U.S. This species favors disturbed areas such as roadsides, overgrazed pastures, and abandoned crop fields [2, 3]. The most recent estimates indicate downy brome infests >22 million ha in the western US and the annual rate of spread is ~14% [4]. One consequence of downy brome invasion is increased fire frequency and intensity [5, 6]. The cost of fighting downy brome fueled fires were estimated to average $10 million per year in the Great Basin alone [7]. The fire return interval is four to six times shorter for downy brome invaded sites (50-78 years) compared to native sites (~294 years)[7, 8].

Shorter fire return intervals further the replacement of native plants by downy brome. For example, increased wildfire frequency has contributed to significant reductions in plant communities dominated by sagebrush [6, 9, 10], which provides essential habitat for sagebrush-dependent wildlife such as sage-grouse (*Centrocercus urophasianus* and *C. minimus*) [9, 10]. Downy brome can decrease species diversity and productivity, increase soil erosion, and decrease abundance of soil biota [11, 12]. Furthermore, downy brome depletes soil moisture and nutrients before perennial grasses break dormancy in the spring [11].

Herbicides are one of the most widely used tools for managing rangeland weeds [13]. Herbicides with residual soil activity are particularly important for controlling downy brome because the seedbank allows for rapid reinvasion [14]. Imazapic has been one of the most-commonly used herbicides on rangeland because of its residual soil activity, and relative selectivity at low use rates [13, 15, 16]. Several other herbicides including glyphosate and rimsulfuron have been used for short-term downy brome control [15]. These herbicides do not provide consistent control of downy brome, and can injure perennial grasses [13-15, 17]. Currently,

there are no herbicides that consistently control winter annual grasses for multiple growing seasons without damaging co-occurring species.

Indaziflam (Esplanade®, Bayer CropScience), a recently registered cellulose-biosynthesis inhibitor (CBI) herbicide, can provide broad-spectrum control of annual grass and broadleaf weeds [18, 19]. There are no reported cases of resistance to this mode of action in turf, ornamentals, citrus, grape, and tree nut crops [18, 20]. Because Indaziflam applied alone has little post-emergence activity, it is commonly applied pre-emergence, or as a tank-mix with foliar applied post-emergence herbicides like glyphosate to provide residual weed control. Labeled application rates of indaziflam range between 51 and 102 $g \cdot ai \cdot ha^{-1}$, and it is fairly persistent in aerobic soils ($t_{1/2}$ >150 days)[21]. Indaziflam is not currently labeled for use on sites grazed by domestic livestock; however, Bayer CropScience is conducting studies to establish a grazing tolerance (David Spak, Bayer CropScience, Research Triangle Park, NC). The EPA establishes a grazing tolerance for herbicides used on any forage crop to determine the potential for the herbicide to appear in the milk or meat of domestic livestock should they consume treated forage [22]. Herbicides without a grazing tolerance should not be used on grazed sites.

Indaziflam's residual activity on annual weeds in established turf [23, 24], demonstrates the potential of indaziflam to control annual weeds such as downy brome on rangeland. The objective of this research was to compare indaziflam to glyphosate, imazapic, and rimsulfuron in terms of downy brome control and damage to co-occurring species.

METHODS

Site Description

Field experiments were established in Colorado at three downy brome-infested sites in 2010. Sites 1 (lat 40°42'40"N, long 104°56'54"W, 1,585 m elevation) and 2 (lat 40°28'0.68"N, long 105°9'13"W, 1,676 m elevation) were 32 km apart. Site 3 (lat 39°28'42"N, long 107°53'0.45"W, 1,768 m elevation) was ~390 km from the other sites. Site 1 was located on an abandoned crop field with 90-100% canopy cover of actively growing downy brome (June 2010), a dense downy brome litter layer (2 to 6 inches), and no other species prior to herbicide application. Site 2 had a mixture of downy brome (60-80% canopy cover at peak standing crop), and other scattered desirable species (20-30% canopy cover) including western wheatgrass (*Pascopyrum smithii*), blue grama (*Bouteloua gracilis*), fringed sage (*Artemisia frigida*) and scarlet globemallow (*Sphaeralcea coccinea*) prior to herbicide application (June 2010). Site 3 was a reclaimed oil pad drilled with western and streambank (*Elymus lanceolatus*) wheatgrass approximately five years prior to our study. Non-native crested wheatgrass (*Agropyron cristatum*) and native forbs were also present including scarlet globemallow, broom groundsel (*Senecio spartioides*), and short's milkvetch (*Astragalus shortianus*). Site 3 burned the year before herbicide treatment, resulting in the removal of all shrubs. Prior to herbicide application, downy brome and native plant canopy cover were approximately 70-90% and 10-20%, respectively (June 2010).

Four 10-cm deep soil cores were taken in each replication, combined into one composite soil sample per site, and analyzed at the Colorado State University Soil Testing Laboratory. Soil series classification for Sites 1, 2, and 3 were: Ascalon sandy loam (fine-loamy, mixed, superactive, mesic Aridic Argiustoll); unclassified sandy loam (sandy loam, haplustoll); and

Ildefonso loam (loamy-skeletal, mixed, mesic Ustollic Calciorthid), respectively. Soil properties were: 1.5% organic matter, pH 7.6, 62% sand, 16% silt, and 22% clay for Site 1, 2.50% organic matter, pH 6.30, 56% sand, 26% silt, and 18% clay for Site 2, and 1.5% organic matter, pH 7.9, 42% sand, 38% silt, and 20% clay for Site 3.

Experimental Design

Herbicides were applied August-September 2010 prior to downy brome emergence (PRE), and November-December 2010 when downy brome had 1 to 3 leaves (EPOST). Additionally, at Sites 1 and 2, applications were made March 2011 at the 2 leaf to 1 tiller stage (LPOST). Treatments were applied to 3 x 9 m plots arranged in a randomized complete block design with four replications. All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat fan nozzles calibrated to deliver at 187 $L \cdot ha^{-1}$ at 207 kPa. At Sites 1 and 2, herbicide treatments applied at all three timings were: rimsulfuron (Matrix®, Bayer CropScience, 53 $g \cdot ai \cdot ha^{-1}$), imazapic (Plateau®, BASF, 105 $g \cdot ai \cdot ha^{-1}$), indaziflam (Esplanade®, Bayer Crop Science, 58 $g \cdot ai \cdot ha^{-1}$), glyphosate (Roundup Weathermax®, Monsanto, 630 $g \cdot ae \cdot ha^{-1}$), imazapic 105 $g \cdot ai \cdot ha^{-1}$ + glyphosate 210 $g \cdot ae \cdot ha^{-1}$, indaziflam 58 $g \cdot ai \cdot ha^{-1}$ + glyphosate 630 $g \cdot ae \cdot ha^{-1}$, indaziflam 58 $g \cdot ai \cdot ha^{-1}$ + rimsulfuron 53 $g \cdot ai \cdot ha^{-1}$, and non-treated. Site 3 treatments were imazapic applied PRE, indaziflam applied PRE, imazapic + glyphosate applied EPOST, rimsulfuron applied EPOST, and non-treated. All treatments included 1% $v \cdot v^{-1}$ methylated seed oil.

Treatment Evaluation and Analysis

Percent control was visually estimated June 2011-2013. Control was determined by comparing visual estimates of downy brome canopy cover in the treated compared to non-treated

plots (downy brome canopy cover estimates prior to herbicide application were previously described).

For sites one and two, all percent control data were arcsine square-root transformed. After failing to reject the null hypothesis of equal variance, the same residual variance was assumed for Sites 1 and 2 ($P = 0.374$). Repeated measures analysis of variance was performed using the PROC MIXED method in SAS 9.3, testing for treatment effects at $\alpha = 0.05$ [25]. Factors included in the repeated measures model statement were site, treatment, year, and interactions, with year as the repeated measure. Using AIC model selection, a Tukey-Kramer adjustment was performed and the heterogeneous variance first-order autoregressive structure (ARH(1)) was chosen. Further analysis of the year by treatment interaction was performed in PROC GLIMMIX using the LINES statement. This statement provided comparisons between all pairs of least squares means across years ($P < 0.05$, Fig. 1.1). For Site 3, the same analysis was performed, but site was dropped from the model and the Tukey-Kramer adjustment was removed.

A separate evaluation in 2013 at Site 3 was conducted to determine native species tolerance to herbicide treatments. Omitting downy brome, numbers of plants per plot were determined for each of the five desirable grass and forb species. Species richness was then calculated by determining the number of species present in each plot. Perennial grass injury was visually estimated for crested, western, and streambank wheatgrass (June 2013). Western and streambank wheatgrass injury data were pooled. PROC GLIMMIX was used to determine differences between least squares richness and frequency means. The richness data were assumed to follow a Poisson distribution.

RESULTS

Indaziflam and imazapic applied PRE provided similar downy brome control 1 YAT, while indaziflam outperformed imazapic 2 and 3 YAT. Indaziflam PRE provided superior downy brome control compared to rimsulfuron PRE (Fig. 1.1). Indaziflam and imazapic at the EPOST and LPOST application timings provided similar downy brome control 1 YAT. Conversely, indaziflam provided greater downy brome control than imazapic and the other herbicides, 2 and 3 YAT (Fig. 1.1).

At Site 3, Indaziflam PRE, rimsulfuron EPOST, and imazapic + glyphosate EPOST provided similar downy brome control 1 YAT. According to point estimates, imazapic PRE resulted in only 32% downy brome control 3 YAT (Fig. 1.2), while indaziflam PRE provided 100% downy brome control 3 YAT. Indaziflam provided a significant improvement over currently recommended treatments (Fig. 1.2).

At Site 3, where herbicide impacts on non-target species were evaluated, there were no significant differences in species richness between the herbicide treatments and the non-treated (Fig. 1.3). Imazapic PRE caused no visual injury to any of the perennial wheatgrass species, while indaziflam PRE, rimsulfuron EPOST, and imazapic + glyphosate EPOST resulted in perennial grass injury of 5% ± 0.3%, 28% ± 2%, and 28% ± 2%, respectively (Fig. 1.3).

DISCUSSION

Indaziflam is the first cellulose biosynthesis inhibiting herbicide that could potentially be used for winter annual grass control on rangeland. Indaziflam inhibits root elongation in seedling grasses and broadleaf species, providing broad-spectrum weed control. In this study, there were only minimal negative impacts on the native perennial plant community (Figure S1;

available online at [insert URL here]). Imazapic and rimsulfuron inhibit the enzyme acetolactate synthase (ALS), an herbicide mode of action prone to resistance evolution. A downy brome biotype identified in Madras, OR in 1997 has confirmed resistance to ALS inhibiting herbicides, thus illustrating the importance of finding new modes of action for winter annual grass control [20, 26].

Indaziflam may provide rangeland managers with another option for managing downy brome and may prove even more effective if integrated with other control methods. In addition, indaziflam provided 80 to 99% control of feral rye (*Secale cereale* L.) 3 YAT [27]. This suggests indaziflam has the potential to control other invasive winter annual grasses such as medusahead (*Taeniatherum caput-medusae* [L.] Nevski), ventenata (*Ventenata dubia* (Leers) Coss), Japanese brome (*Bromus japonicus* Thunb.), and jointed goatgrass (*Aegilops cylindrical* L.).

There is a fundamental need for new downy brome management strategies that provide consistent control without negatively impacting native plants (Fig. S1). The long-term residual downy brome control provided by a single indaziflam application could provide the opportunity to significantly reduce downy brome in the soil seed bank and reduce the amount of fine fuel produced by new downy brome crops. By increasing the fire return interval and reducing downy brome in the soil seed bank, remnant native plant communities would have a much better chance to dominate invaded sites.

IMPLICATIONS

One of the major limitations for downy brome management is the lack of consistent long-term control [13, 14, 28]. In our study, indaziflam provided better downy brome control than currently recommended herbicides 2 and 3 YAT. Indaziflam caused only mild injury to

perennial grasses, and did not negatively impact species richness. Because downy brome seeds remain viable in the soil for ≤5 years, managing downy brome with glyphosate, imazapic, or rimsulfuron would require yearly herbicide applications [29]. Additionally, the repeated use of ALS inhibiting herbicides such as imazapic and rimsulfuron can lead to resistant downy brome populations. Therefore, new herbicide modes of action are increasingly important for winter annual grass control on rangeland. Indaziflam has the potential to have positive long-term impacts on the structure and function of rangeland communities invaded by winter annual grasses. Unfortunately, indaziflam cannot be used on sites grazed by domestic livestock; however, Bayer CropScience is conducting studies to establish a grazing tolerance. Indaziflam is currently labeled for use on open spaces, natural areas, and other non-grazed sites.

1.6 FIGURES

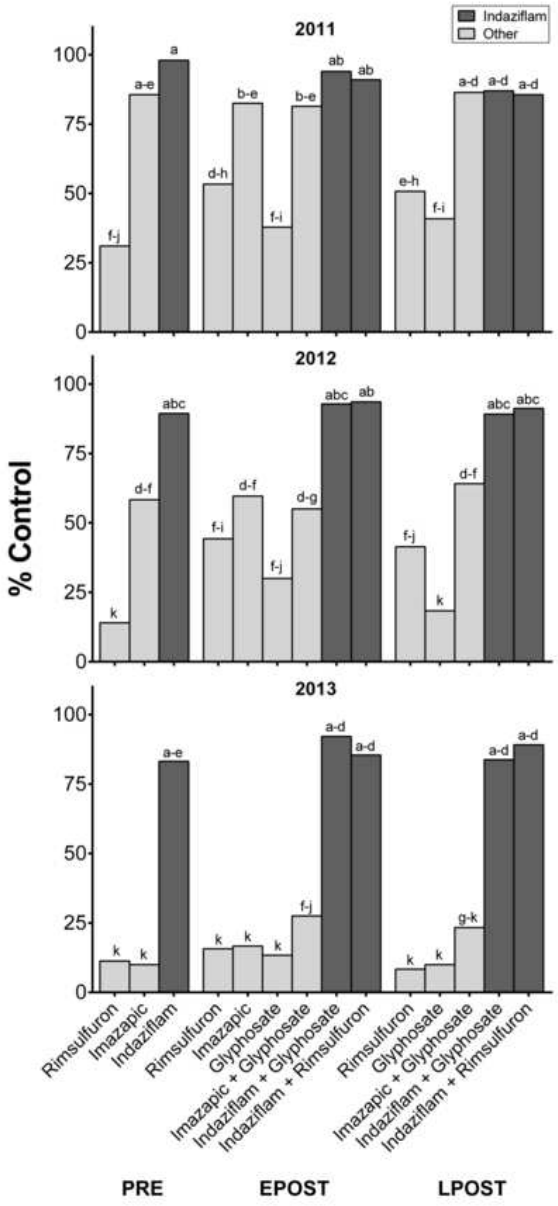

Figure 1.1. Sites 1 and 2 percent downy brome control compared to the non-treated 1, 2, and 3 YAT. Data from sites were combined for analysis of variance. Application timings included: pre-emergence, applied August 2010 (PRE), early post-emergence at the 1 to 2 leaf stage, applied December 2010 (EPOST), and late post-emergence at the 2 leaf to 1 tiller stage, applied March 2011 (LPOST). Letters indicate differences among herbicide treatments across all three timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: rimsulfuron (53 g·ai·ha^{-1}), imazapic (105 g·ai·ha^{-1}), indaziflam (58 g·ai·ha^{-1}), glyphosate (630 g·ae·ha^{-1}) , imazapic (105 g·ai·ha^{-1}) + glyphosate (210 g·ae·ha^{-1}), indaziflam (58 g·ai·ha^{-1}) + glyphosate (630 g·ae·ha^{-1}), indaziflam (58 g·ai·ha^{-1}) + rimsulfuron (53 g·ai·ha^{-1}), non-treated.

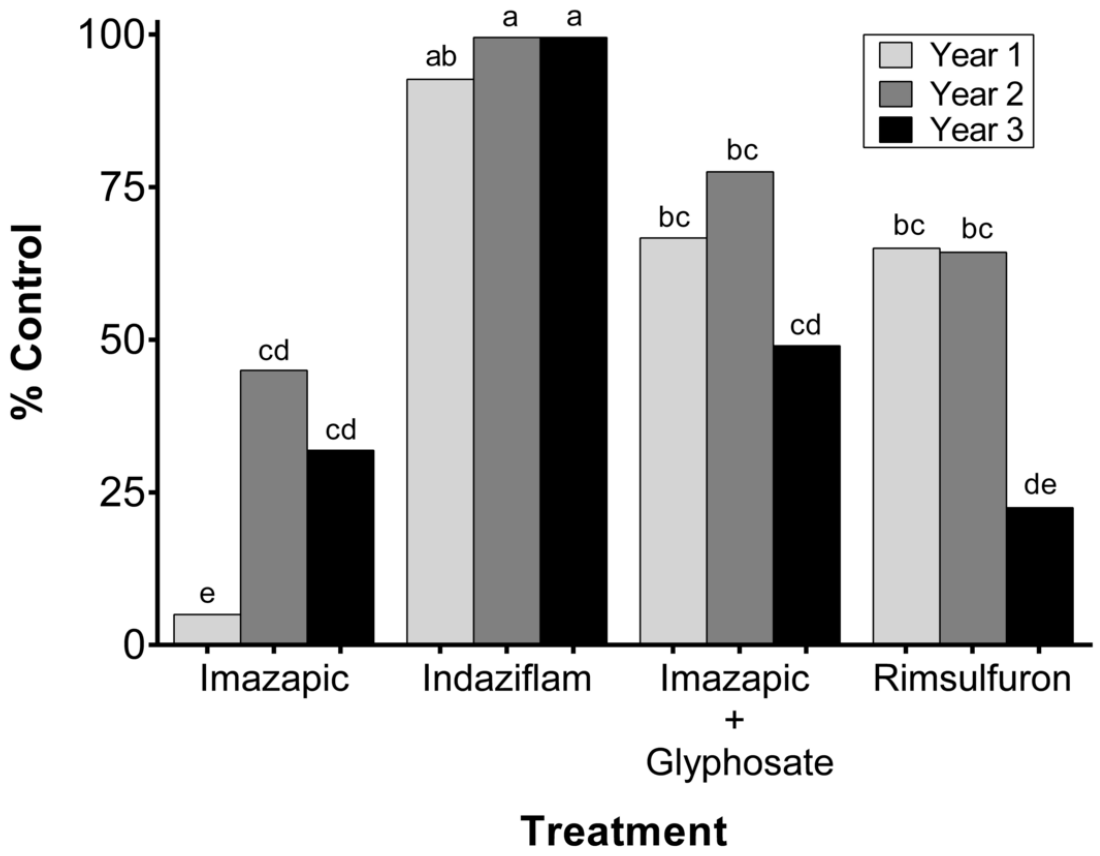

Figure 1.2. Site 3 percent downy brome control compared to the non-treated 1, 2, and 3 YAT. Application timings included: PRE, applied September 2010, and EPOST at the 1 to 3 leaf stage, applied November 2010. LPOST was not studied at Site 3. Letters indicate differences among herbicide treatments across all years, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: imazapic (PRE, 105 g·ai·ha^{-1}), indaziflam (PRE, 58 g·ai·ha^{-1}), imazapic (EPOST, 105 g·ai·ha^{-1}) + glyphosate (210 g·ae·ha^{-1}), rimsulfuron (EPOST, 70 g·ai·ha^{-1}), non-treated.

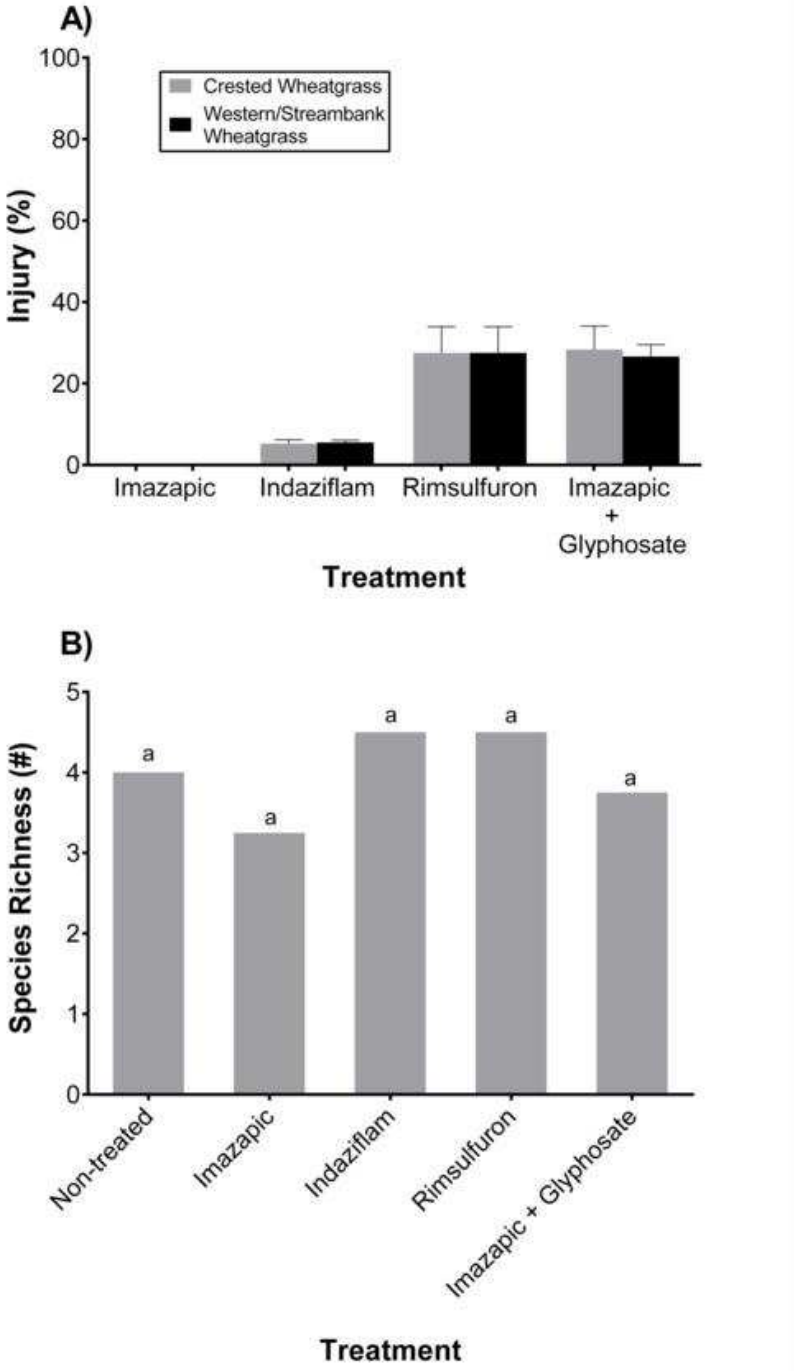

Figure 1.3. At Site 3, (A) perennial grass injury from herbicide treatments compared to the non-treated, and (B) species richness (#) for each treatment. Letters indicate differences among herbicide treatments using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: imazapic (PRE, 105 g·ai·ha^{-1}), indaziflam (PRE, 58 g·ai·ha^{-1}), imazapic (EPOST, 105 g·ai·ha^{-1}) + glyphosate (210 g·ae·ha^{-1}), rimsulfuron (EPOST, 70 g·ai·ha^{-1}), non-treated.

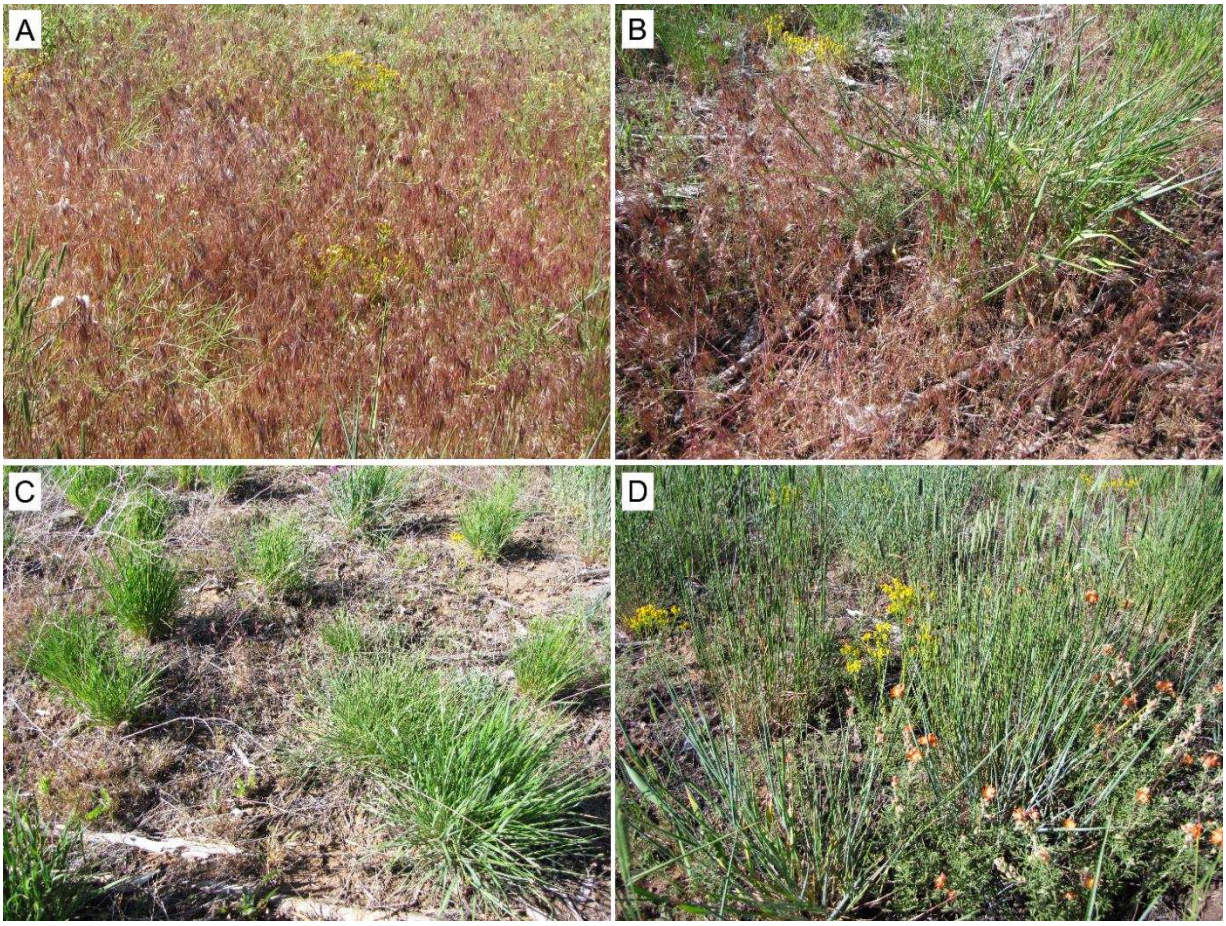

Figure 1.4. Downy brome control and perennial grass response at Site 3, 2 YAT. Herbicide treatment rates are as follows: Non-treated (A), imazapic PRE, 105 g·ai·ha^{-1} (B), imazapic EPOST, 105 g·ai·ha^{-1} + glyphosate 210 g·ae·ha^{-1} (C), indaziflam PRE, 58 g·ai·ha^{-1} (D).

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CHAPTER 2: INDAZIFLAM: A NEW CELLULOSE BIOSYNTHESIS INHIBITING HERBICIDE PROVIDES LONG-TERM CONTROL OF INVASIVE WINTER ANNUAL GRASSES[1]

SUMMARY[†]

Indaziflam (Esplanade™, Bayer CropScience) is a cellulose biosynthesis inhibiting (CBI) herbicide that is a unique mode of action for resistance management and has broad spectrum activity at low application rates. This research further explores indaziflam's activity on monocotyledons and dicotyledons, and evaluates indaziflam's potential for restoring non-crop sites infested with invasive winter annual grasses. Treated Arabidopsis, downy brome, feral rye, and kochia were all susceptible to indaziflam in a dose-dependent manner. We confirmed indaziflam has increased activity on monocots (average GR_{50} = 231 ρM and 0.38 g·ai·ha^{-1}) at reduced concentrations compared to dicots (average GR_{50} = 512 ρM and 0.87 g·ai·ha^{-1}). Fluorescence microscopy confirmed common CBI symptomologies following indaziflam treatments, as well as aberrant root and cell morphology. Across five application timings, indaziflam treatments resulted in superior invasive winter annual grass control 2 YAT (84% ± 5.1 to 99% ± 0.5) compared to imazapic (36% ± 1.2). Indaziflam treatments significantly increased biomass and species richness of co-occurring species, 2 YAT. Indaziflam's increased activity on monocots could provide a new alternative management strategy for long-term control of multiple invasive winter annual grasses that invade >23 million ha of US rangeland. Indaziflam could potentially be used to eliminate the soil seed bank of these invasive grasses, decrease fine fuel accumulation, and ultimately increase the competitiveness of perennial co-occuring species.

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INTRODUCTION

Herbicide discovery has slowed drastically with no new major mode of action introduced in the last 20 years[2]. As herbicide resistance continues to spread[3-5], there is a need for compounds with new target sites[2]. It is more important than ever for land managers to sustain their herbicide tools by incorporating multiple modes of action[6, 7]; however, limited herbicide alternatives can make this difficult. Many herbicides used for cropland weed management are overlooked for use in non-crop markets, providing an opportunity to introduce new herbicide modes of action and weed management solutions for non-cropland weed management. While land managers rely on the chemical industry to provide weed solutions via new chemistries, it is equally important that land managers continually challenge their current weed management strategies and decrease resistance selection pressure by using herbicide alternatives. Indaziflam [N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine], first released in 2010, is a relatively new cellulose biosynthesis-inhibiting (CBI) herbicide that is an underutilized tool for weed control and resistance management in non-crop markets[8-10].

Indaziflam is registered in the US for use in several perennial cropping system including established citrus, grape, and tree nut crops, and was recently registered in Brazil for use in sugar cane, eucalyptus, and pines[9, 11-14]. Labeled non-crop application sites include rights-of-way, turf, and ornamentals[9, 12]. A recently established non-crop label for the release or restoration of desirable vegetation in natural areas, open spaces, wildlife management areas, and fire rehabilitation areas are the focus of this research[9, 12, 15].

Indaziflam represents a resistance management alternative with a unique mode of action and application timing[10, 15, 16]. Indaziflam provides broad spectrum pre-emergence control of several annual grasses and broadleaf weeds[9]. Indaziflam is lipophilic (log K_{ow}= 2.8) and has a low water solubility (3.6 $mg \cdot L^{-1}$), explaining its increased residual soil activity compared to other commonly recommended herbicides[9, 17]. Indaziflam is applied at low use rates and recommended at 73 and 102 $g \cdot ai \cdot ha^{-1}$ for residual winter annual grass control in open spaces and natural areas.

Although indaziflam is classified as a CBI, there is very little known about the actual mechanism of action[10, 18]. Cellulose is a composite polymer of glucan chains[10], synthesized at the plasma membrane by large cellulose synthase (CESA) complexes that directly release the developing cellulose polymers into the cell wall[19, 20]. The cellulose synthase complex (CSC), arranged in a rosette pattern, has recently been shown to consist of 18 to 24 catalytic CESA proteins; however, the number of different CESA gene products required for the assembly of a functioning CSC remains to be clarified[8, 21]. Interestingly, all of these proteins are potential sites of action for CBI herbicides such as indaziflam[22].

CBI herbicides, including indaziflam, isoxaben, and dichlobenil, are a diverse group of compounds with different sites of action directly or indirectly affecting cellulose synthesis[8]. Herbicides in the alkylazine class, such as indaziflam, are unique, resulting in inhibitory activity three orders of magnitude higher than benzonitriles (dichlobenil) or benzamides (isoxaben). The specific mechanism of action of indaziflam, isoxaben, and dichlobenil have been compared. Isoxaben treatments resulted in the depletion of CESA proteins from the plasma membrane and accumulation in cytosolic vesicles[20, 23, 24], while dichlobenil treatments resulted in immobilization of CESA proteins and hyperaccumulation in the plasma membrane[25].

Indaziflam, however, has been shown to increase the density of CESA particles at the plasma membrane and also reduce CESA particle velocity by approximately 65%, inhibiting polymerization [10]. This increase in density has also been shown to decrease the colocalization between the microtubules and the CESA in the region near the root apical hook[10]. Although these studies confirm that indaziflam has a unique interaction with the complex cellulose biosynthesis pathway, there is limited research attempting to explain indaziflam's phytotoxicity on both monocotyledonous (monocots) and dicotyledonous (dicots) plants, which is unusual as other CBI herbicides are more active on dicots[8].

Indaziflam is unique in that is has been shown to provide long-term selective control of the most prevalent invasive winter annual grass in the US, downy brome (*Bromus tectorum* L.)[15, 26, 27]. Currently, there has been one downy brome biotype identified that is highly resistant to acetolactate synthase (ALS) (imazamox, primisulfuron, propoxycarbazone, sulfosulfuron) and photosystem II inhibitors (PSII) (atrazine, metribuzin), and moderately resistant to acetyl CoA carboxylase inhibitors (ACCase) (clethodim, fluazifop)[28, 29]. Imazapic and glyphosate are currently the two most commonly recommended herbicides for invasive winter annual grass control; however, these herbicides provide inconsistent control[30-33], and represent two modes of action that are highly prone to resistance development[2, 4, 34]. This increases the necessity for new modes of action, such as CBIs, for controlling downy brome and other invasive winter annual grasses in non-crop areas.

Indaziflam has also been shown to control other monocot weeds including feral rye, Japanese brome (*Bromus japonicus* Thunb. or *Bromus arvensis* L.), jointed goatgrass (*Aegilops cylindrica* L.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski), and ventenata (*Ventenata dubia* (Leers) Coss). Invasive winter annual grass invasions are increasing at an

alarming rate; displacing native vegetation that is critical habitat for wildlife and livestock and increasing fire frequency and intensity due to the dense accumulation of fine fuel[35-40]. Although land managers have been attempting for decades to recover these sites dominated by invasive winter annual grasses, few have been consistently successful[30]. As these natural ecosystems continue to shift from perennial-grass domination to invasive winter annual grass-domination[41], the necessity for new management tools continues to increase[40].

Better understanding of the mode of action and selectivity of new herbicides such as indaziflam for invasive winter annual grass weed management will minimize potential non-target risks and provide insight into the potential large-scale application of this herbicide in open spaces and natural areas. The objectives of this study were to 1) evaluate the differential response of indaziflam on monocot (downy brome [*Bromus tectorum* L.] and feral rye [*Secale cereale* L.]) and dicot (Arabidopsis [*Arabidopsis thaliana*] and kochia [*Kochia scoparia* L.]) plants using root and greenhouse dose-response bioassays, 2) investigate the inhibitory effect of indaziflam on cellulose biosynthesis using fluorescence microscopy, and 3) compare indaziflam to imazapic, currently the most commonly recommended herbicide, in terms of both invasive winter annual grass control and response of the native plant communities (co-occurring species). Based on previous field research, we hypothesized that the relative potency of indaziflam would be elevated with monocots as compared to dicots, and subsequent microscopy could be a tool used to visualize this differential response. This work also expands on past field research comparing indaziflam invasive winter annual grass control with imazapic by comparing additional species, application timings, and further evaluation of non-target impacts.

MATERIALS AND METHODS

2.1 Chemicals

For the root bioassay and microscopy, we used indaziflam analytic standard provided by Bayer CropScience. Calcofluor white (Fluorescent Brightener 28, MP Biomedicals) was used for cellulose fluorescence. For the greenhouse dose-response and field experiments we used commercial herbicide formulations of indaziflam (Esplanade™; Bayer CropScience, Research Triangle Park, NC), imazapic (Plateau®; BASF, Research Triangle Park, NC), and glyphosate (Accord® XRT II; Dow AgroSciences, Indianapolis, IN).

2.2 Indaziflam Root Bioassay

2.2.2 Experimental Design

For *in vitro* dose response experiments, we used a series of 1.5% agarose plates that contained 0 ρM, 50 ρM, 100 ρM, 200 ρM, 400 ρM, 800 ρM, 1200 ρM, 1600 ρM, and 3200 ρM indaziflam. A series of plates were generated for each species (downy brome, feral rye, Arabidopsis, kochia) and repeated in triplicate. Before planting, seeds were sterilized using a 70% ethanol solution. Seeds (12 Arabidopsis and kochia seeds, and 8 feral rye and downy brome seeds) were placed in a line along one edge of the plates (~1 cm from the top edge). The plates were arranged vertically with the line of seeds on the uppermost edge of the plate and placed in a growth chamber under continuous dark conditions and allowed to germinate.

2.2.3 Data Analysis

Photographs of each plate were taken at a constant distance (25 cm) using a Nikon D3X camera, every 12 hr, up to 84 hr after the seeds were planted. Root length measurements were conducted using ImageJ[42]. Total root length for each treatment were converted to a percentage of the root length of the non-treated control 84 HAT. The means of the three replicates (n = 8 or

12 seeds per plate) were plotted and used for generating the dose-response curves. Graphpad Prism 6 software for Windows (La Jolla, CA USA, www.graphpad.com) was used to determine indaziflam rates required to reduce root length by 50% (GR_{50}) for downy brome, feral rye, Arabidopsis, and kochia. The four parameter log-logistic regression equation regressing root length (as a percent of the non-treated root length) with herbicide concentration is

$$Y = C + \frac{(D - C)}{1+10^{(\mathrm{Log}GR_{50} - X) \cdot b}} \qquad [1]$$

where C is the lower limit of the response, D is the upper limit of the response, b is the slope, and GR_{50} is the herbicide rate resulting in 50% root length reduction. Means were separated for each species to determine significant differences in GR_{50} values using Fisher's Protected LSD test at the 5% level of probability.

Additionally, the average root length for each species, time point, replicate, and concentration were plotted in an X,Y scatterplot and a line of best fit calculated for each growth curve. The slope of this line was calculated and representative of the average rate of root growth from 0 to 72 hours after planting (distance/time). The average rate of growth of the 3 replicates for each species were calculated and then plotted against increasing indaziflam concentrations. The same four parameter log-logistic regression equation shown above was used for regressing average rate of root growth as a percent of the non-treated, with herbicide concentration.

2.3 Root Fluorescence Microscopy

Roots from treated and control plants (Section 2.2) were stained for 1 minute in 1% Calcofluor white (Fluorescent Brightener 28, MP Biomedicals), followed by 1 minute de-staining in deionized water[43-45]. Roots were mounted in water and imaged using a Leica 5500 microscope (Leica Microsystems) running IPLab version 4 software (BD Biosciences) with a C4742-95 camera (Hamamatsu Photonics). Cellulose fluorescence was observed with a DAPI

filter cube (Leica Microsystems). Images were composited for each root using Adobe Photoshop (http://www.photoshop.com/) and Image Composite Editor (http://research.microsoft.com/en-us/um/redmond/groups/ivm/ice/).

2.4 Indaziflam Greenhouse Dose Response

A greenhouse dose-response experiment was conducted to confirm the results from Section 2.2, and to further evaluate the relative sensitivity of the monocot (downy brome, feral rye) and dicot (Arabidopsis, kochia) species to indaziflam in field soil. Arabidopsis was unable to uniformly germinate in this experiment and was omitted from further analysis. Arabidopsis has a very small seed size and growth is affected by many environmental factors[46]; therefore, it was not a surprise to have difficulty generating dose-response curves with this species.

2.4.2 Experimental Design

The study used seven herbicide concentrations and a non-treated control arranged in a completely randomized design with four replications. The study was performed on December 29, 2015 and repeated January 19, 2016. Based on the results from a preliminary experiment, the indaziflam concentrations used for the kochia dose-response were 0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, and 11.7 g ai ha^{-1}. The indaziflam concentrations used for all other species were 0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9, and 5.9 g ai ha^{-1}.

Seeds were planted in square plastic containers (12 x 12 x 6 cm) in an Otero sandy clay loam field soil (Coarse-loamy, mixed, mesic Aridic Ustorthents) with 3.9% OM and pH 7.7. All species were planted at a depth of 0.5 cm, with the exception of Arabidopsis which was planted at the soil surface. Seeding densities were adjusted based on germinability to reach a target density of 30 plants/pot. Indaziflam was applied using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray

nozzle calibrated to deliver 187 $L \cdot ha^{-1}$ at 172 kPa. Treated pots were transferred immediately to a greenhouse with a 15 h photoperiod and 25/20 °C day/night temperature regime. Natural light was supplemented with high-intensity discharge lamps when light was below 25 mW cm^{-2}. Plants were misted daily to reduce soil crusting and subirrigated as needed. Aboveground plant biomass was harvested at the soil surface 3 weeks after treatment and dried for 3 d at 60 C before recording dry weights.

2.4.3 Data Analysis

Total dry weights for each treatment were converted to a percentage of the biomass of the non-treated control and analyzed in Graphpad Prism 6 (Section 2.1.3). Data from repeated studies were combined after the null hypothesis of equal variance was not rejected. The same four parameter log-logistic regression equation from Section 2.1.3 was used to construct the species-specific dose-response curves and determine the indaziflam concentrations required to reduce dry biomass by 50% (GR_{50}). Significant differences in GR_{50} values were evaluated using Fisher's Protected LSD test at the 5% level of probability.

2.5 Invasive Winter Annual Grass Field Efficacy Studies

2.5.1 Site Description

In 2014, field experiments were conducted to expand on previous literature comparing the effectiveness of indaziflam and imazapic for long-term invasive winter annual grass control, and to evaluate the response of the native plant communities. The experiments were established at two sites on the Colorado Front Range dominated by invasive winter annual grasses. Site 1 (lat 40°15'2"N, long 105°12'56"W) was infested with equal amounts of downy brome and Japanese brome, and Site 2 (lat 40°43'23"N, long 104°55'58"W) was infested with feral rye. Sites were approximately 58 km apart. Site 1 was located on Rabbit Mountain Open Space

(Boulder County) and Site 2 was located on a Colorado Parks and Wildlife Area (Larimer County). Before herbicide application (July 2014), we made visual estimates across the entire study area of percentage of living canopy cover for all species present at both sites. Site 1 was characterized by ~80-100% downy brome and Japanese brome canopy cover with a dense fine-fuel layer (2 to 5 cm), and a scattered stand of co-occurring species (~0-10% canopy cover, Table 2.1). Site 2 had >95% canopy cover of actively growing feral rye, a fine fuel layer of 2 to 5 cm, and <5% canopy cover of western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love) and sand dropseed (*Sporobolus cryptandrus* (Torr.) A. Gray).

The soil at Site 1 was Baller sandy loam (loamy-skeletal, mixed, mesic Lithic Haplustolls), with 1.5% organic matter in the top 20 cm[47]. The average elevation was 1,737 m (5,700 ft). The soil at Site 2 was Terry sandy loam (coarse-loamy, mixed, superactive, mesic Ustollic Haplargids), with 1.3% organic matter in the top 20 cm[47]. The average elevation was 1,646 m (5,400 ft). At Sites 1 and 2, mean annual precipitation based on the 30-yr average (1981-2010) was 379 and 363 mm, and the mean annual temperatures were 9.1 and 8.6 C, respectively[48]. Precipitation was close to the 30-yr average in 2014; however, in 2015, both sites received an additional 199 and 212 mm above the 30-yr averages, respectively[49]. A drought occurred in 2016, with an annual precipitation of 235 and 290 mm at Sites 1 and 2, respectively.

2.5.2 Experimental Design

Herbicides were applied at five application timings to evaluate variations in invasive winter annual grass control, potential non-target impacts, and the potential release of co-occurring species after herbicide treatment. Herbicides were applied both before (PRE) and after (POST) winter annual grass emergence. Timings were designated as early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015), and

late POST (LPOST, April 2015). We had four treatments at each application timing: indaziflam (Esplanade™) at three concentrations (44, 73, and 102 $g \cdot ai \cdot ha^{-1}$) and imazapic (Plateau®) at 123 $g \cdot ai \cdot ha^{-1}$. Imazapic and indaziflam have limited to no POST activity; therefore, all POST treatments included 420 $g \cdot ae \cdot ha^{-1}$ glyphosate (Accord® XRT II) as the burndown herbicide. The 21 herbicide treatments (including a non-treated control) were applied to 3 by 9 m plots arranged in a randomized complete block design with four replications. All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat fan nozzles at 187 $L \cdot ha^{-1}$ at 207 kPa. All treatments included 1% $v \cdot v^{-1}$ methylated seed oil.

2.5.3 Treatment Evaluation and Data Analysis

Biomass harvests and species richness evaluations were conducted in August (2015 and 2016) to evaluate invasive winter annual grass control and response of co-occurring species. Above-ground biomass of the winter annual grasses, perennial grasses, and forbs were harvested from randomly placed 1-m^2 quadrats; quadrats were not taken from the same location in consecutive years. Site 1 had an equal distribution of downy brome and Japanese brome (Section 2.5.1), therefore, biomass of both species were combined for analysis. Directly following harvest, the material was dried at 60°C for 5 d to calculate dry biomass. Additionally, at Site 1 species richness was calculated for each treatment as a simple estimate of biological diversity[50]. Species richness was defined as the total number of unique species (grasses and forbs) occurring per unit area (e.g. 27 m^2 plot size). These count data were assumed to follow a Poisson distribution.

Invasive winter annual grass biomass was converted to a percentage of the non-treated control and data were combined across sites after the null hypothesis of equal variance was not rejected. However, due to unequal variances across sites for perennial grass biomass

($P<0.0001$), data from Sites 1 and 2 were analyzed separately. Because Site 2 only had two desirable grass species and no forbs, forb biomass data and richness are only presented for Site 1. All response variables (invasive winter annual grass biomass, perennial grass biomass, forb biomass, and species richness) were first evaluated for significant main effects and interactions by performing an ANOVA using the PROC MIXED method in SAS 9.3[51]. Factors included in the model statement were treatment, site, year after treatment, and all interactions, with year after treatment defined as the repeated measure. The random factor was site nested within replication, and a Tukey-Kramer adjustment was performed. To meet ANOVA assumptions of normality, we used an arcsin square root transformation for invasive winter annual grass biomass (% of non-treated), a square root transformation for perennial grass and forb biomass; however, no transformations were required for forb richness. To evaluate the significant treatment-by-year interaction for all response variables ($P<0.0001$), an ANOVA was conducted using the PROC GLIMMIX method and the LINES statement. This provided comparisons between all pairs of least squares means across years ($P<0.05$). All means presented in figures are non-transformed data.

RESULTS AND DISCUSSION

3.1 Differential Response of Monocotyledons and Dicotyledons to Indaziflam

Currently, there is limited research attempting to further explain the unique phytoxicity of indaziflam on both monocots and dicots. Because CBI herbicides involve a complex mechanism of action and it appears as though different CBIs inhibit different proteins within the cellulose synthase complex, most of the published literature has been constrained to studies of a model organism. These model organisms, such as Arabidopsis, have a fully sequenced genome that

provides the opportunity to identify unique genes in a pathway of interest such as cellulose synthesis. In these studies we expand on previous research with Arabidopsis, and quantify the differential response of indaziflam treated monocot and dicot weeds. Previous research has used CBIs as a tool to better understand cellulose biosynthesis, whereas the focus of these data were to better understand indaziflam's mode of action for practical use in non-crop weed management.

3.1.1 Root Bioassay and Microscopy

Downy brome, feral rye, Arabidopsis, and kochia were susceptible to indaziflam and their growth was inhibited in a dose-dependent manner (Fig. 2.1). The indaziflam concentrations resulting in 50% reduction in root length (GR_{50}) compared to the non-treated control for downy brome, feral rye, Arabidopsis, and kochia were 211, 251, 363, and 661 *p*M, respectively. The GR_{50} values between the monocots (downy brome, feral rye) and dicots (Arabidopsis, kochia) were significantly different, which is a unique finding. Downy brome showed the most susceptibility to indaziflam, with a GR_{50} value approximately three times lower than the kochia GR_{50} ($P<0.0001$). Indaziflam GR_{50} values for feral rye ($P=0.0069$) and Arabidopsis ($P=0.0016$) were also significantly lower than the kochia GR_{50}. Indaziflam treated seedlings exhibited common CBI symptomology including radial expansion and inhibition of root and hypocotyl elongation[8, 52] (Fig. 2.1).

Evaluating changes in the average growth rate of indaziflam treated roots (0 to 72 hours) revealed a differential response for monocots and dicots (Fig. 2.2). The herbicide concentration resulting in 50% reduction in root growth rate was on average 2.9 times lower for monocots than dicots. This analagous finding is consistent with the root bioassay (Fig. 2.1), providing additional evidence that while indaziflam inhibits root expansion and elongation, the speed at which this inhibition occurs is faster for monocots than dicots (Fig. 2.2).

Using treated roots from the root bioassay, fluorescent microscopy using Calcofluor white to visualize cell walls by cellulose fluorescence revealed similar and also unique symptomologies from other published indaziflam research. Treated roots were wider and their cells were larger than in non-treated roots, as has been previously reported[8, 10]. Cell walls in monocot roots showed a strikingly different response compared to dicot roots (Fig. 2.3). Treated roots of downy brome and feral rye exhibited large areas of gapped cells (cellulose deficiency); more severe symptomology than what has been previously reported as gapped cell walls[45, 52, 53] (Fig. 2.3A, B). A previous study showed somewhat similar results with *prc1* (CesA6 mutation), or dichlobenil/isoxaben treated wild-type seedlings[45]. Incomplete cell walls were observed, but shown to be connected by a membranous structure that is not stainable by Calcofluor white[45]. However, in our study, these incomplete, non-staining areas spanned large areas of the root and in some cases, the root appeared to be split open (downy brome, 1200 *p*M; feral rye, 800 and 1200 *p*M) (Fig. 2.3A, B). These areas were also missing in the bright field view, suggesting that cells were totally absent rather than being present but lacking cell walls made of cellulose (data not shown).

Although we observed gaps in the root structure of monocots, indaziflam-treated dicot roots had differing phenotypes. In Arabidopsis, an overabundance of root hairs was observed, so that it was difficult to discern the underlying root, while in kochia, some cells acquired a nearly circular shape, but only at higher doses of at least 1200 pM. Although monocot cells also appeared swollen and misshapen, they did not quite reach the circularity of kochia root cells. Perhaps the swollen cells in time lead to the gapped areas observed in the monocots; a time-course of roots growing in indaziflam-treated plates could be useful to reveal how these symptoms arise.

In all species, few cellular deformities (other than enlarged cells) were observed in the zone of division. Symptoms appeared concurrently with root hairs, in the elongation zone, and persisted and grew more dramatic through the zone of elongation. Misshapen cells were also present in the root caps, most prominently in the monocot species. Since the root cap is also composed of mature cells arising from the zone of division, this suggests indaziflam acts during the cell elongation and maturation process.

3.1.2 Indaziflam Greenhouse Dose-Response

Similar results were observed between the root and greenhouse bioassays in terms of the differential response of monocots and dicots to indaziflam (Figs. 2.1 and 2.4). The indaziflam concentrations resulting in 50% reduction in root length (GR_{50}) compared to the non-treated control for downy brome, feral rye, and kochia were 0.25, 0.51, and 0.87 $g{\cdot}ai{\cdot}ha^{-1}$, respectively (Fig. 2.4). It is not unusual for herbicides to be more active in the greenhouse under ideal environmental conditions, so it was not surprising to us that GR_{50} values were much lower than recommended field concentrations (73 and 102 $g{\cdot}ai{\cdot}ha^{-1}$). The indaziflam concentration needed to reduce kochia dry biomass by 50% was approximately two and four times the concentration required for feral rye ($P<0.0001$) and downy brome ($P<0.0001$), respectively (Fig. 2.4).

Indaziflam has a unique mode of action compared to other CBI herbicides because it can control both monocots and dicots; however, our results suggest the relative potency of indaziflam varies across these two plant classes. Increased monocot inhibition at lower use rates as compared to dicots has been confirmed with mitotic disrupter heribicides such as dinitroanilines (i.e. trifulralin, oryzalin, pendimethalin)[54], but this is not the case for CBI herbicides[8, 55]. In particular, isoxaben activity is specific to dicots and primarily used for PRE control of broadleaf weeds[8]. Because the mechanism of action of these chemically diverse CBI herbicides are very

complex and poorly understood, these data provide useful information that could be utilized for further exploration of indaziflam's unique cellulose biosynthesis inhibiting mechanism.

3.2 Invasive Winter Annual Grass Field Efficacy Study

3.2.1 Invasive Winter Annual Grass Control

The significant treatment-by-year ($P<0.0001$) interaction on invasive winter annual grass control was evaluated. The combined data from Sites 1 and 2 showed a similar level of invasive winter annual grass control (downy brome, feral rye, Japanese brome) 1 year after treatment (YAT), except for imazapic at the EPRE timing (~41% control, Fig. 2.5). Across all five application timings, indaziflam at 73 and 102 g·ai·ha^{-1} provided >99% control 1 YAT (2015). These data suggest that 1 YAT, imazapic treatments at the POST timings provided superior control to imazapic applied PRE. This difference in efficacy could be explained by the addition of the glyphosate burndown at the POST timings, or the later application timings had less microbial degradation, and therefore, an increased concentration of imazapic in the soil during peak growth (summer 2015).

Indaziflam treatments across all application timings (except indaziflam applied at the lowest rate of 44 g·ai·ha^{-1}, EPRE and PRE), provided superior invasive winter annual grass control 2 YAT (2016) compared to imazapic (Fig. 5). Indaziflam applied at 102 g·ai·ha^{-1} controlled 97 to 99% ± 0.5 (mean ± SE) of downy brome, feral rye, and Japanese brome, while imazapic provided only 32 to 35% ± 1.5 control, 2 YAT (Fig. 2.5). An additional observation of this study was the impact of herbicide treatments on fine fuel accumulation. Before herbicide treatments were initiated (2014), both sites had accumulated fine fuel layers of ~2 to 5 cm. At both sites, indaziflam treatments eliminated further residue inputs via residual control 2 YAT, resulting in the complete decomposition of these fine fuel layers (~9 to 12 MAT).

Invasive winter annual grass control responded to indaziflam treatments in a dose-dependent manner. The 102 g·ai·ha^{-1} concentration is highly effective and should be strongly considered for management of invasive winter annual grasses with a short seed viability (~3 to 5 years)[56, 57]. To achieve, or increase the success of long-term invasive winter annual grass control, it is imperative to limit the seed rain during this 3- to 5-year period and choose management options that provide close to 100% control. If the soil seed bank is able to re-generate, the invasive winter annual grass is likely to re-establish. This has often been the case for herbicides with limited soil residual activity beyond the initial year of application such as imazapic[30]. These data support previous downy brome research[15]; however, we also provide evidence that indaziflam can provide residual control of multiple invasive winter annual grasses that may coexist at a site (Fig. 2.5).

3.2.2 Perennial Grass Response

The significant treatment-by-year interaction ($P<0.0001$) was evaluated separately at Sites 1 and 2. The increased level of invasive winter annual grass control (Fig. 2.6) 2 YAT, for indaziflam, was evident in the superior re-establishment of co-occurring species compared to imazapic (Fig. 2.6). By providing residual control of the invasive winter annual grasses, this likely made available a surplus of moisture and nutrients resulting in the positive response of co-occurring perennial grasses. Across application timings at Sites 1 and 2, indaziflam at the highest concentration (102 g·ai·ha^{-1}) provided the greatest increase in perennial grass biomass 2 YAT, while biomass in imazapic-treated plots was no different than the non-treated control ($\alpha=0.05$, Fig. 6). Averaged across both sites, indaziflam applied EPRE, PRE, EPOST, POST, or LPOST resulted in a 38-, 35-, 39-, 28-, and 42-fold increase in perennial grass biomass compared to the non-treated control (Fig. 2.6). At both sites, indaziflam treatments provided greater

residual control of invasive winter annual grasses 2 YAT compared to imazapic, allowing for significant increases in biomass and re-establishment of co-occurring species, 1 and 2 YAT (Fig. 2.6).

At Site 1, there was no difference in perennial grass dry biomass for all POST and LPOST treatments compared to the non-treated check, 1 YAT (2015) (α=0.05, Fig. 2.6). At Site 1, western wheatgrass and other cool season grasses were not dormant at these late spring POST and LPOST timings; therefore, reduced perennial grass biomass at these timings (compared to EPRE, PRE, EPOST) was attributed to glyphosate injury. In year 2, biomass significantly increased for all indaziflam treatments applied POST, and the LPOST indaziflam 102 g·ai·ha^{-1} treatment. At Site 1, indaziflam treatments POST and LPOST resulted in a 14- to 20-fold and 10-to 32-fold biomass increase compared to the non-treated control 2 YAT, respectively. Imazapic treatments at the POST and LPOST application timings resulted in a 7- and 3-fold increase in perennial grass biomass 2 YAT, respectively; however, this was not statistically different from the non-treated control (Fig. 2.6). Summarizing these data across years, indaziflam treatments applied EPRE, PRE, or EPOST resulted in the greatest increase in perennial grass biomass across sites, although recovery of co-occurring species was also seen in the POST and LPOST timings, 2 YAT.

3.2.3 Forb Response and Species Richness

There was a similar response of forb biomass compared to perennial grass biomass. Treatments at the EPOST and POST timings resulted in the greatest increase in forb biomass, 1 YAT (Fig. 2.7). With the exception of imazapic PRE, no treatments 1 YAT resulted in a reduction in forb biomass. All imazapic treatments 2 YAT, had similar levels of forb biomass compared to the non-treated control plots (Fig. 2.7). A significant increase in the re-

establishment of forbs in indaziflam treated plots was not seen until 2 YAT (2016). With the exception of the indaziflam 44 g·ai·ha^{-1} EPRE treatment, all other indaziflam treatments resulted in a significant increase in forb biomass compared to the non-treated control plots. Averaged across timings, indaziflam treatments at 44, 73, and 102 g·ai·ha^{-1} resulted in a 3-, 5-, and 5-fold increase in forb biomass, respectively, compared to the non-treated control plots (Fig. 2.7).

The forb biomass data can be used as an estimate of the quantity of forbs in a plot; however, species richness evaluations allowed us to further evaluate the effect of herbicide treatments on species diversity. The list of co-occurring species present at Site 1 can be seen in Table 2.1. Species richness increased 1 YAT for all species, but this increase was not significantly greater compared to the non-treated control (Fig. 2.8). Species richness further increased two years after indaziflam treatments, whereas species richness after imazapic treatments remained fairly constant between 1 (6.0 ± 0.3 species·plot^{-1}) and 2 YAT (6.4 ± 0.4 species·plot^{-1}). All treatments with indaziflam, regardless of application rate, increased species richness compared to the non-treated control, from 4.3 ± 0.6 species·plot^{-1} 1 YAT in the control plot to an average of 7.9 species·plot^{-1} 2 YAT in the treated plots (Fig. 2.8). These data provide strong evidence for the selectivity of indaziflam on perennial co-occurring species, allowing for an increase in establishment as early as 1 YAT (Fig. 2.9). The increase in forb biomass, species composition, and diversity over time is evidence that indaziflam treatments have positive impacts on the perennial native plant communities (Fig. 2.9).

CONCLUSION

Indaziflam represents a new weed management opportunity in non-crop areas with a unique mode of action that currently has no reported cases of herbicide resistance. In this study, we expand on previous work with Arabidopsis[10], providing practical implications for how indaziflam (Esplanade™; Bayer CropScience) could be used to increase weed management success in open spaces and natural areas.

Monocots and dicots diverged appoximately 200 million years ago[58], resulting in significant variations in cellulose synthesis and cell wall architecture between these plant classes. One explanation for the differences in relative potency of indaziflam on monocots and dicots could be the unique cell wall structure between dicots/liliaceous monocots (type 1 cell walls), and Poales/commelinid monocots (type 2 cell walls)[18]. In this study, Arabidopsis and kochia, both dicots, have type 1 cell walls while downy brome and feral rye, both commelinid monocots, have type 2 cell walls. Factors within the two plant classes that could also influence relative potency of indaziflam are seed size, metabolism, sequestration, herbicide absorption and translocation, or genetic differences[18]. Because cellulose synthesis is such a complex process there are likely many contributing factors involved in indaziflam's ability to control both monocots and dicots. We can conclude from the root bioassay, greenhouse dose-response, and fluorescence microscopy that indaziflam does in fact inhibit monocot root elongation and provide control at lower rates compared to dicots. We also observed more severe CBI symptomology in monocot species than dicot species treated with the same herbicide concentration. Understanding the difference between the monocot and dicot response to indaziflam treatment will require further studies to identify the target protein of indaziflam, such as forward and reverse genetic screens in Arabidopsis (a model dicot) and *Brachypodium*

distachyon or rice (both model monocot species). Indaziflam may also prove to be useful in basic research into the still-unresolved complexities of cellulose synthesis.

Root inhibition was noticeable at ρM concentrations. This observable activity at extremely low concentrations explains the increased residual weed control provided by indaziflam compared to other herbicides. Dichlobenil and isoxaben, two other CBI herbicides, are labeled at approximately 40- and 10-times greater herbicide concentrations than indaziflam (73 and 102 $g \cdot ai \cdot ha^{-1}$)[21, 59]. In addition, indaziflam has several other chemical properties that result in enhanced residual weed control: lipophilicity (log K_{ow}= 2.8), low water solubility (3.6 $mg \cdot L^{-1}$), no photodegredation, and a strong positive correlation between sorption and soil organic matter[17, 59]. Therefore, lethal indaziflam concentrations are biologically available at the soil surface with sufficient moisture for plant uptake[17], resulting in extended weed control. This response has been observed under several of indaziflam's labeled use patterns; however, there is limited supporting data in non-crop markets including indaziflam's new open space and natural areas label.

In this study, we provide the first field data showing that indaziflam can provide superior residual control of multiple invasive winter annual grasses (downy brome, feral rye, Japanese brome) compared to the currently recommended herbicide, imazapic. These data directly support the limited field[15, 60] and greenhouse studies that have been conducted evaluating the effectiveness of indaziflam to provide residual control of invasive winter annual grasses in open spaces and natural areas. Overall, indaziflam provided residual control 2 YAT, ultimately decreasing the seed rain back into the soil seed bank. Because invasive winter annual grasses have seed viabilities of approximately 3 to 5 years[56], land managers should consider applying a sequential indaziflam treatment 2 or 3 years after initial treatments to potentially exhaust the seed

bank of these invasive grasses. The sequential treatments could provide the residual control necessary to reach the 3- to 5-year seed longevity period. This management approach could decrease labor and herbicide costs compared to herbicides with limited residual control that require yearly applications (e.g. imazapic), while also minimizing the herbicide's environmental footprint.

An additional observation in this field study associated with indaziflam's long-term residual control, was its utility as a tool for fine-fuels reduction. These fine fuel layers associated with invasive winter annual grasses have resulted in major changes in fire-return intervals, dramatically increasing fire frequency and intensity[38] particularly in sagebrush ecosystems of the Great Basin[40, 61]. Additionally, many open spaces and natural areas infested with invasive winter annual grasses are bordered by houses or other structures, and are at a high fire risk with these dense, highly flammable fine fuel layers. Additional research should be conducted to quantify fine fuel decomposition over time with other common invasive winter annual grasses found in the US including jointed goatgrass (*Aegilops cylindrica* L.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski), and ventenata (*Ventenata dubia* (Leers) Coss). Herbicide efficacy should also be compared between sites with no remaining fine fuel in recently burned areas (natural or prescribed) and non-burned sites.

This field study also provided much needed field tolerance data for the response of co-occurring grasses and forbs to herbicide treatments. Indaziflam promoted the re-establishement of the co-occurring plant community by increasing perennial grass and forb biomass, and plant diversity (richness) over time. Imazapic at all application timings did not provide the necessary residual invasive winter annual grass control for re-establishment of co-occurring species, 2 YAT. Depleting the invasive winter annual grass soil seed bank and decreasing fine fuel

ultimately allowed the invaded sites to be converted from an annual weed-dominated plant community to one that is primarily perennial-dominated by natives. Across both sites evaluated in this study, indaziflam treatments promoted (released) the remnant perennial grass and forb plant communities and these sites are now more resistant and resilient to future invasions[40].

2.6 TABLES

Table 2.1. List of co-occurring species at Site 1.

Common Name	Scientific Name
Blue grama	*Bouteloua gracilis* (Willd. ex Kunth) Lag. Ex Griffiths
Western wheatgrass	*Pascopyrum smithii* (Rydb.) A. Love
Western ragweed	*Ambrosia psilostachya* DC.
Tarragon	*Artemisia dracunculus* L.
Fringed sagebrush	*Artemisia frigida* Willd.
Prairie sage	*Artemisia ludoviciana* Nutt.
Winged buckwheat	*Eriogonum alatum* Torr.
Blanketflower	*Gaillardia aristata* Pursh
Parry's geranium	*Geranium caespitosum* James var. parryi (Engelm.) W.A. Weber
Dotted gayfeather	*Liatris punctata* Hook.
Pricklypear cactus	*Opuntia polyacantha* Haw.
Slender-flowered scurfpea	*Psoralidium tenuiflorum* (Pursh) Rydb.
Prairie coneflower	*Ratibida columnifera* (Nutt.) Wooton & Standl.
Woods' rose	*Rosa woodsii* Lindl.
Scarlet globemallow	*Sphaeralcea coccinea* (Nutt.) Rydb.
Porter's aster	*Symphyotrichum porteri* (A. Gray) G.L. Nesom
Yellow salsify	*Tragopogon dubius* Scop.

2.7 FIGURES

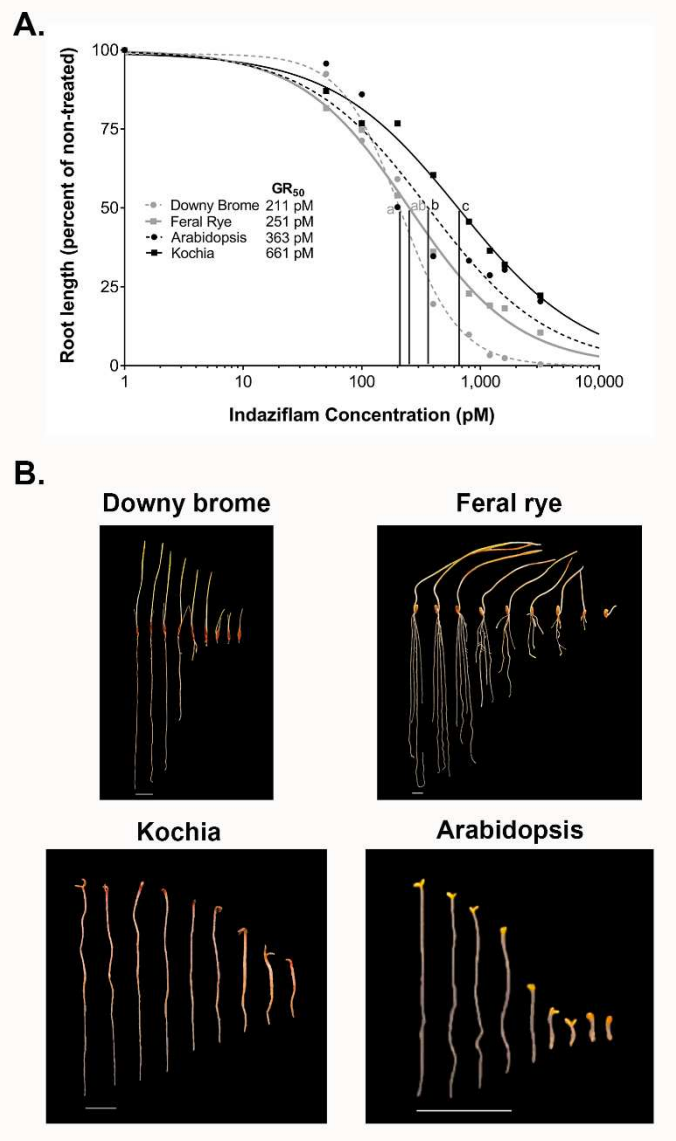

Figure 2.1. (A) Response of root length to increasing herbicide concentrations 84 hours after planting, represented as a percentage of the non-treated control. Dose response curves were fit using four parameter log-logistic regression. Mean values of the 3 replicates (plates) are plotted (n = 8 or 12 seeds per plate) at each indaziflam concentration. Vertical lines represent the indaziflam concentration resulting in 50% reduction in root length (GR_{50}) for each species, and letters signify differences in GR_{50} values using Fisher's Protected LSD test at the 5% level of probability (B) Representative images of the indaziflam root bioassay with 7-d-old seedlings. Indaziflam concentrations used from left to right were 0, 50, 100, 200, 400, 800, 1,200, 1,600, and 3,200 ρM. Scale bar = 1 cm.

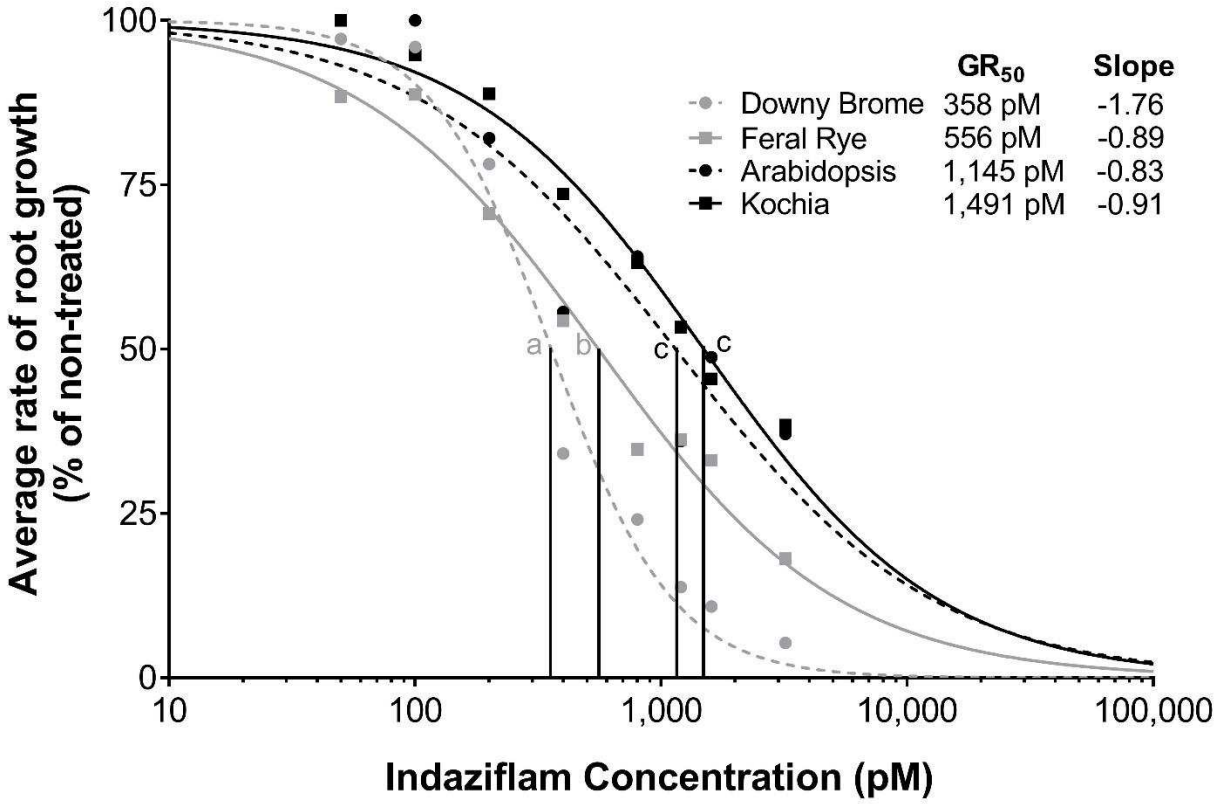

Figure 2.2. Effect of indaziflam on the average rate of root growth from 0 to 72 hrs (12 hr increments) after planting. Dose response curves were fit using four parameter log-logistic regression. Mean values of 3 replicates (plates) are plotted (n = 8 or 12 seeds per plate). Vertical lines represent the indaziflam concentration resulting in 50% reduction in root growth rate (GR_{50}) for each species. Letters signify differences in GR_{50} values using Fisher's Protected LSD test at the 5% level of probability.

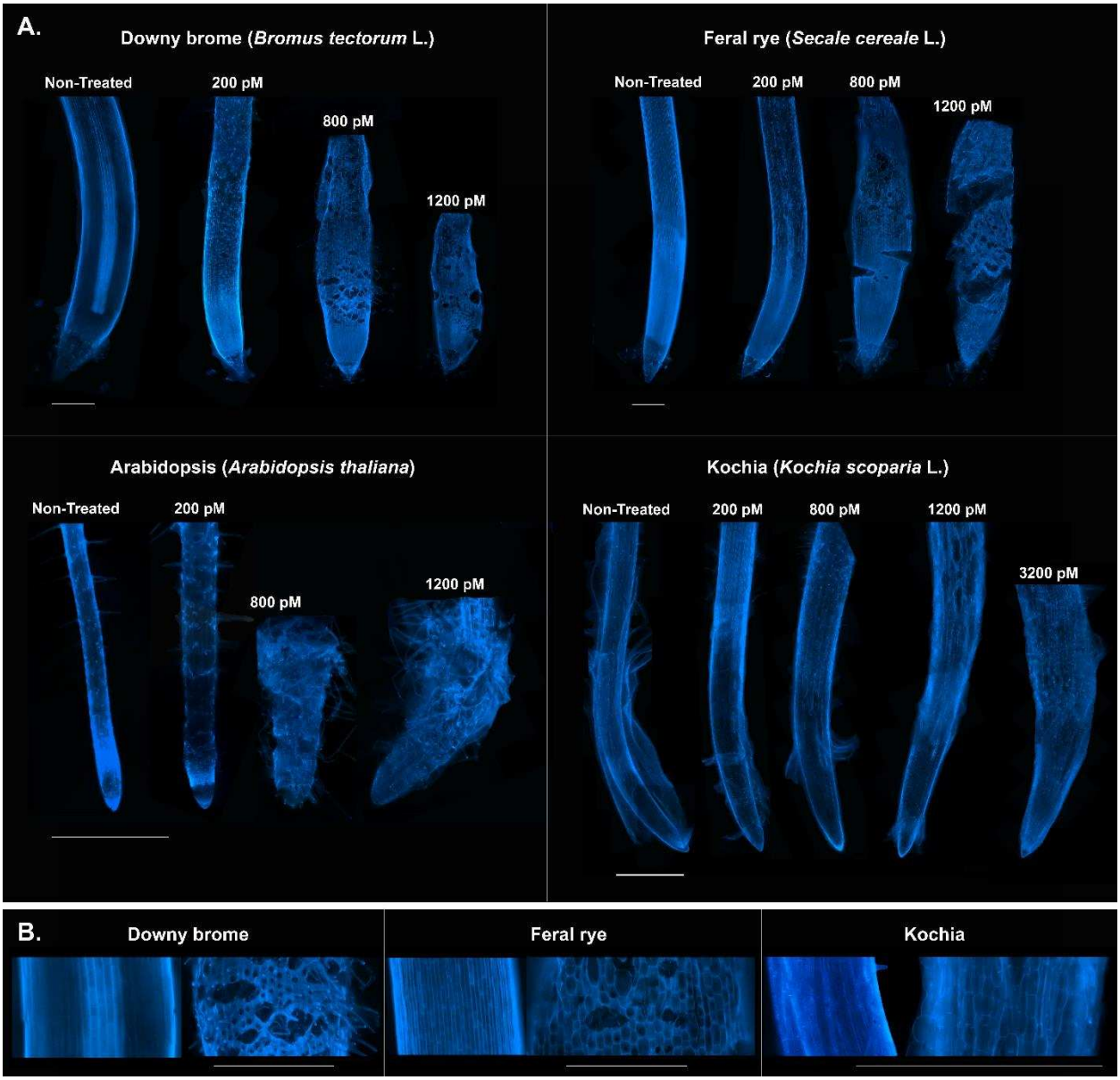

Figure 2.3. (A) Cellulose fluorescence of indaziflam treated monocot (downy brome, feral rye) and dicot (Arabidopsis, kochia) seedlings were examined using a Leica 5500 microscope (DAPI filter cube) and Calcofluor white stain. (B) Indaziflam symptomology of downy brome (non-treated, 800 ρM), feral rye (non-treated, 800 ρM), and kochia (non-treated, 1,200 ρM). Non-treated roots (left) show uniform cellulose synthesis. Indaziflam treated seedlings exhibited radial swelling, cell deformities, large non-staining areas (monocots), split roots, swollen cells (circular), and overabundance of root hairs (dicots). Scale bar = 500 μm.

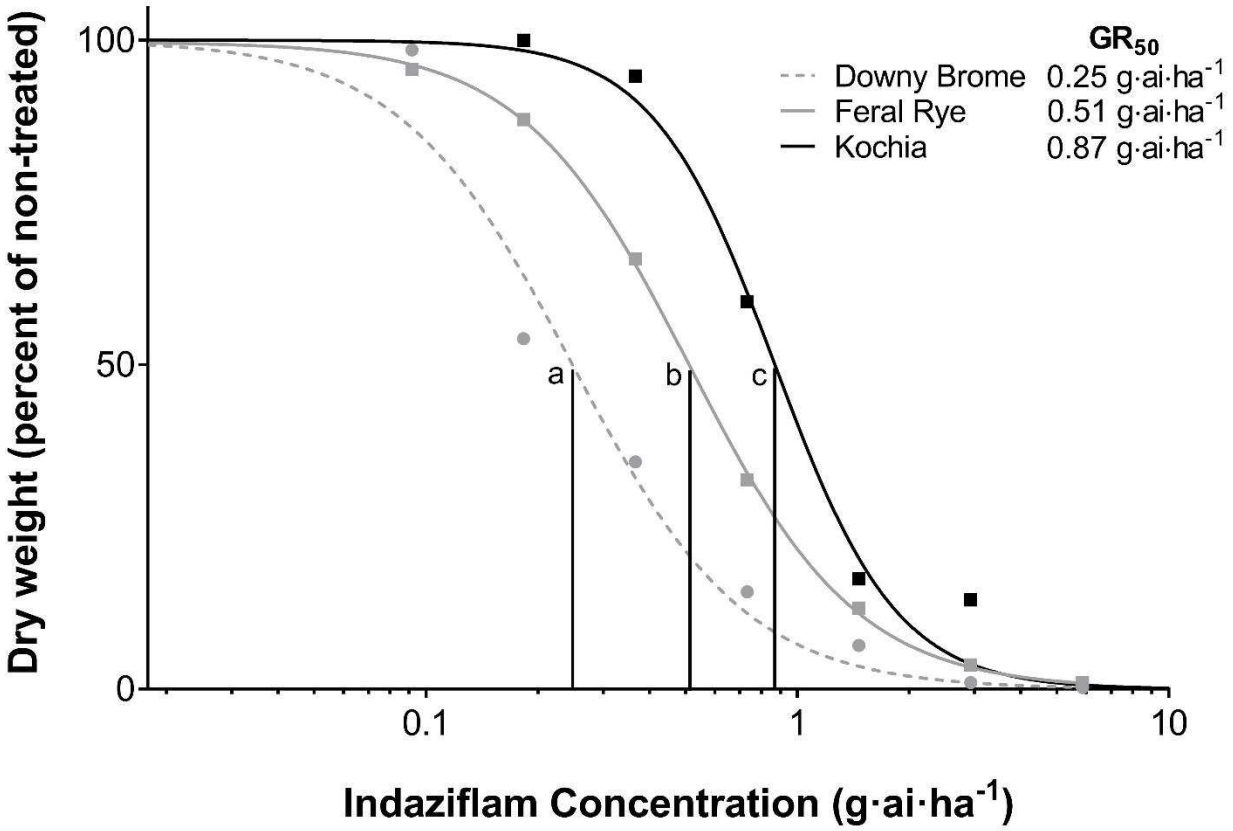

Figure 2.4. Greenhouse dose-response evaluating the reduction in dry weight represented as a percentage of the non-treated control. Herbicide concentrations used for kochia were 0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, and 11.7 g ai ha^{-1} and 0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9, and 5.9 g ai ha^{-1} for downy brome and feral rye. Dose response curves were fit using four parameter log-logistic regression. Mean values of 4 replications are plotted. Vertical lines represent the indaziflam concentration resulting in 50% reduction in dry weight (GR_{50}) for each species. Letters signify differences in GR_{50} values using Fisher's Protected LSD test at the 5% level of probability.

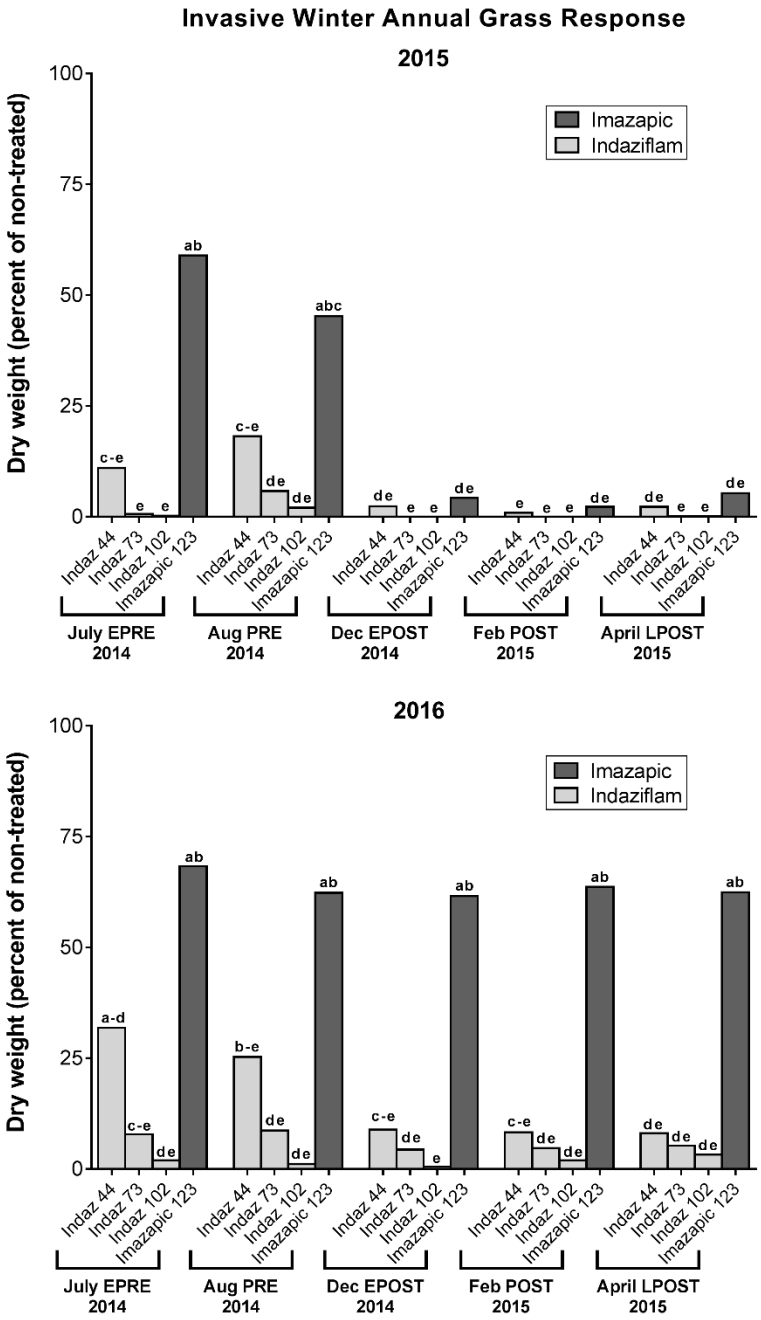

Figure 2.5. Sites 1 and 2 percent invasive winter annual grass control (downy brome, feral rye, Japanese brome) compared with the non-treated 1 (2015) and 2 (2016) YAT. Five application timings were evaluated including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015), and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73, and 102 g·ai·ha^{-1} and imazapic at 123 g·ai·ha^{-1}. All POST treatments included 420 g·ae·ha^{-1} glyphosate as the burndown.

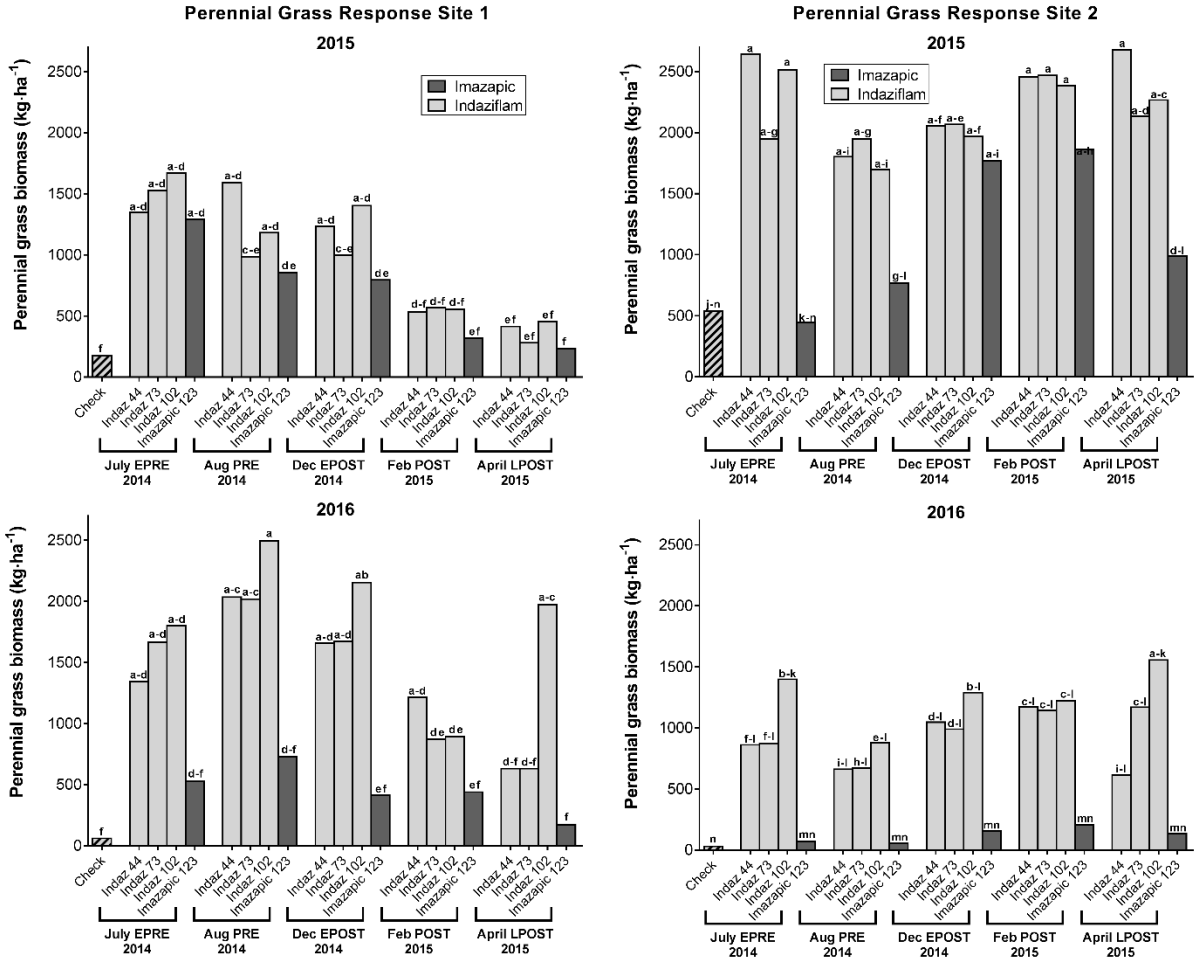

Figure 2.6. Sites 1 and 2 perennial grass biomass response to herbicide treatments, 1 (2015) and 2 (2016) YAT. Five application timings were evaluated including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015), and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73, and 102 g·ai·ha^{-1} and imazapic at 123 g·ai·ha^{-1}. All POST treatments included 420 g·ae·ha^{-1} glyphosate as the burndown.

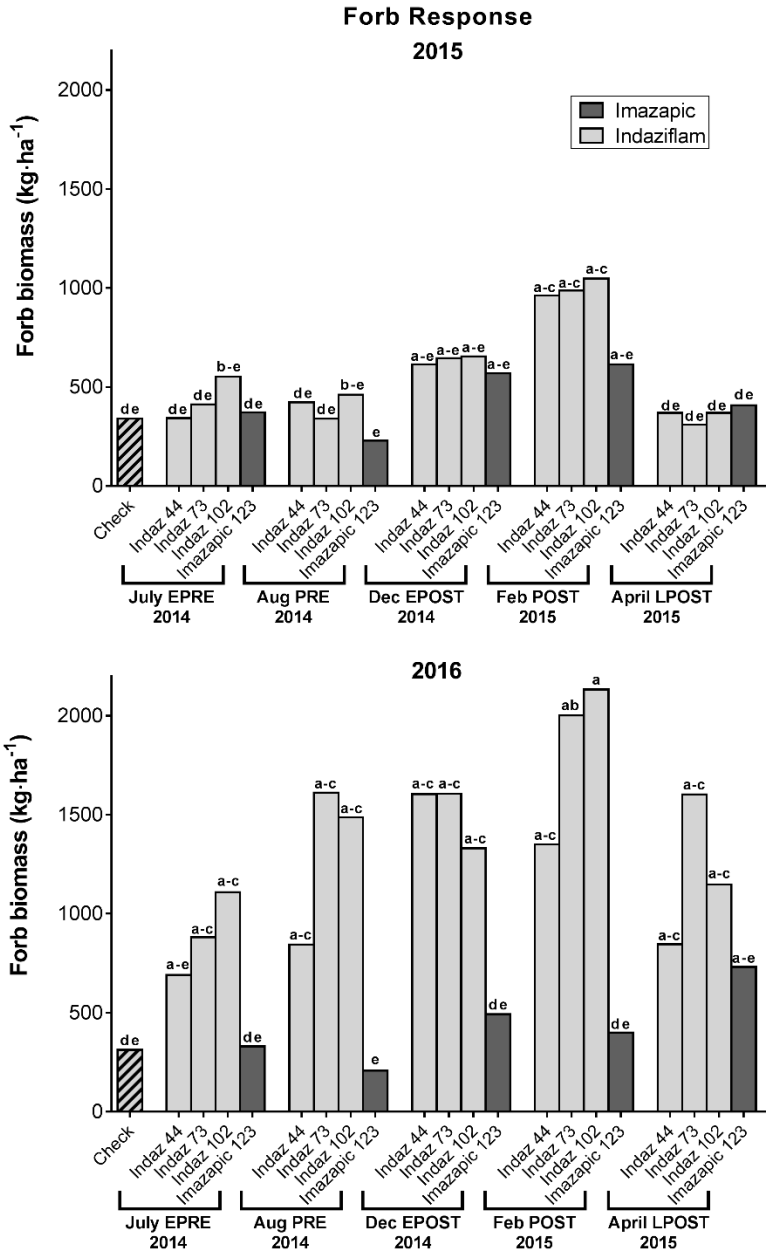

Figure 2.7. At Site 1, forb biomass response to herbicide treatments 1 (2015) and 2 (2016) YAT. Five application timings were evaluated including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015), and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73, and 102 g·ai·ha^{-1} and imazapic at 123 g·ai·ha^{-1}. All POST treatments included 420 g·ae·ha^{-1} glyphosate as the burndown.

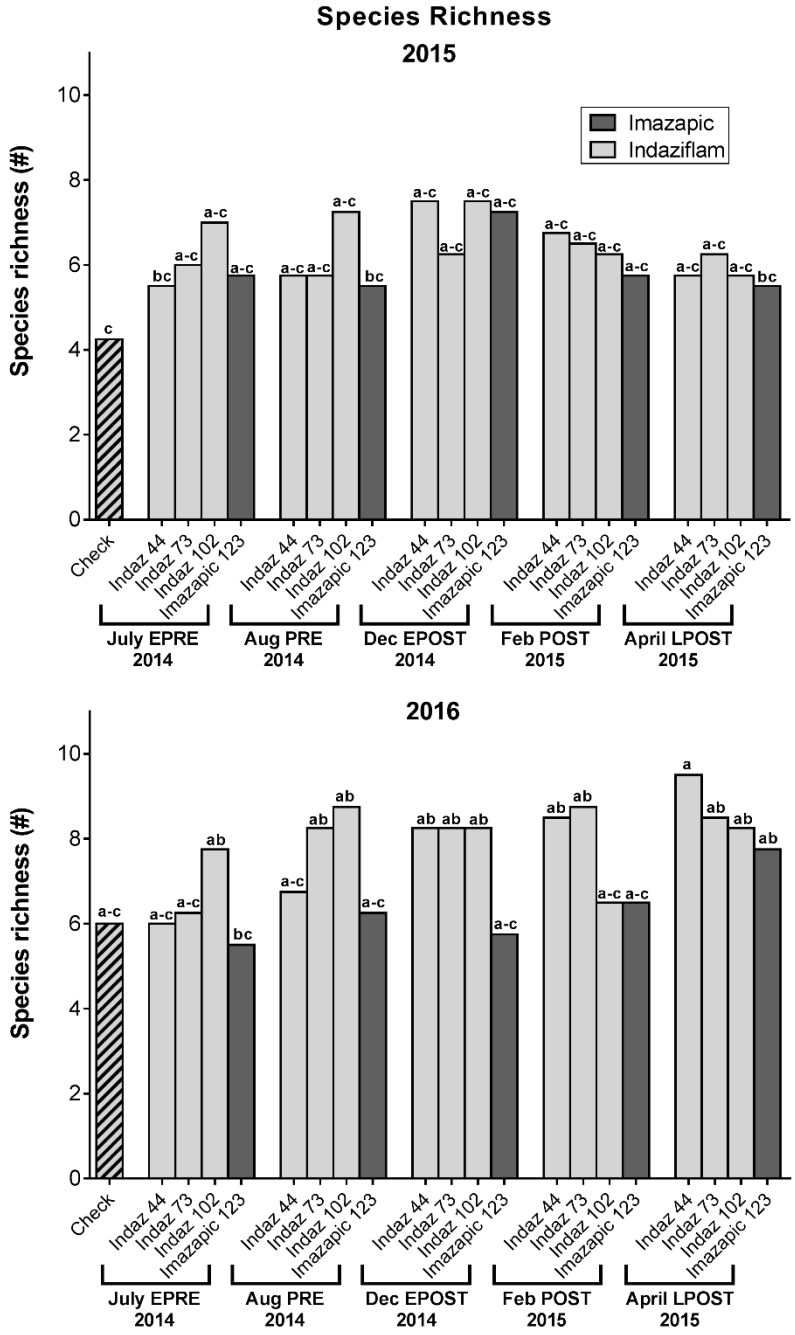

Figure 2.8. At Site 1, species richness defined as the total number of unique co-occurring species (grasses and forbs) occurring per unit area (27 m^2 plot size), 1 (2015) and 2 (2016) YAT. Five application timings were evaluated including early PRE (EPRE, July 2014), PRE (August 2014), early POST (EPOST, December 2014), POST (February 2015), and late POST (LPOST, April 2015). Letters indicate differences among herbicide treatments across all five timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates at each timing are as follows: indaziflam at 44, 73, and 102 $g{\cdot}ai{\cdot}ha^{-1}$ and imazapic at 123 $g{\cdot}ai{\cdot}ha^{-1}$. All POST treatments included 420 $g{\cdot}ae{\cdot}ha^{-1}$ glyphosate as the burndown.

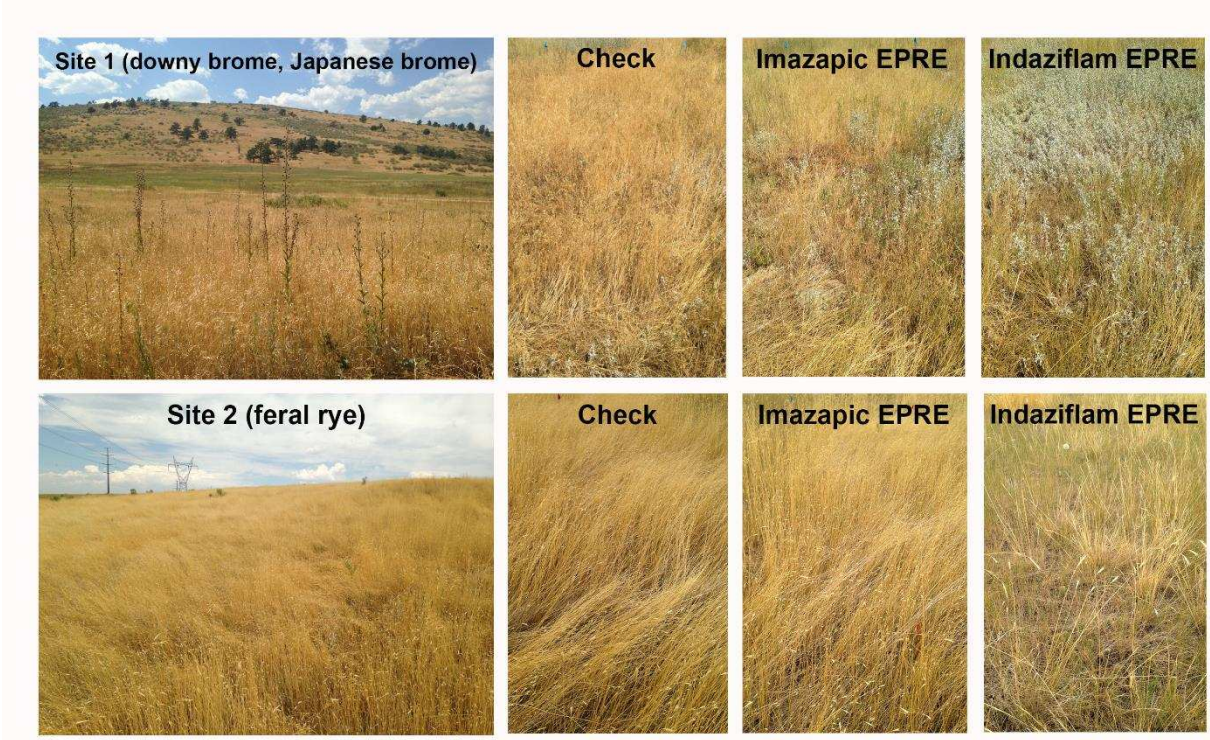

Figure 2.9. Photos of Sites 1 and 2 taken July 2016. Treatment photos include imazapic 144 g·ai·ha^{-1} and indaziflam 102 g·ai·ha^{-1} at the July 2014, EPRE timing (2 YAT). Indaziflam treatments provided the long-term invasive winter annual grass control necessary for the re-establishment of co-occurring perennial grasses and forbs.

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CHAPTER 3: SEED BANK DEPLETION: THE KEY TO LONG-TERM DOWNY BROME (*BROMUS TECTORUM* L.) MANAGEMENT[1]

SUMMARY[‡]

Invasive winter annual grasses such as downy brome (*Bromus tectorum* L.) are a threat to native ecosystems throughout the US. Downy brome is able to exploit moisture and nutrients throughout the fall and early spring before native plants break dormancy. This results in decreased native species abundance and development of monotypic downy brome stands. Short-term downy brome management has been shown to be effective; however, the soil seed reserve has often been overlooked although it's the mechanism responsible for rapid re-establishment. This field study was conducted at two sites in Colorado to evaluate the longevity of the downy brome soil seed reserve and its implications on long-term downy brome control. Glyphosate plus adjuvant applications were made for 0, 1, 2, 3, 4, or 5 consecutive years. Downy brome and perennial grass biomass harvests were conducted yearly to determine changes in species composition. In addition, soil cores were collected to evaluate the yearly variation and depletion of the downy brome soil seed bank in response to consecutive glyphosate applications. We found that 1 to 3 years of consecutive glyphosate treatments were insufficient to deplete the downy brome soil seed bank. Downy brome biomass and the soil seed bank recovered within 1 to 2 years after glyphosate treatments were terminated; however, 4 and 5 consecutive years of glyphosate applications were sufficient to control downy brome through depletion of the soil seed bank. Managing downy brome for 4 to 5 consecutive years resulted in a 4- to 9-fold increase in perennial grass biomass. These data suggest that long-term management of downy brome is dependent on eliminating the soil seed bank using a multi-year approach.

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INTRODUCTION

Downy brome (*Bromus tectorum* L.) is one of the most researched invasive weed species on rangeland. A Web of Science search identified 1,057 citations containing the words "downy brome" or "*Bromus tectorum*" since 1990, with 79% of the citations occurring between 2000 and 2016. This suggests that concerns about downy brome's many ecological and economic impacts are increasing [2-4]. There is evidence that some of these impacts could be approaching the point where they are no longer reversible [5-9].

There is limited research on the implications of managing the downy brome soil seed bank on long-term control. This is a crucial aspect for managing invasive species that reproduce only by seed such as downy brome; however, re-establishment via the soil seed bank is often overlooked or not well understood. Downy brome is a winter annual grass species that commonly germinates in the fall; however, downy brome can behave more like a spring annual at higher elevations [10], limiting recruits to more favorable weather conditions in the spring. Downy brome that germinates in the fall through early spring occupies an open-niche, exploiting moisture and nutrients throughout the winter and early spring when most other desirable co-occurring species are dormant. Early season utilization of soil moisture and nutrients allows downy brome to displace native grass, forb, and shrub species [10-12]. If land managers fail to manage the downy brome soil seed bank, further invasions and re-establishment are likely to occur.

Long-term downy brome control might seem nearly impossible, but a number of researchers have identified a key aspect of downy brome biology that could provide the basis for long-term management: seed viability and seed longevity. Studies have shown a very high percentage (96-99%) of first year downy brome seeds germinate the fall following addition to the

soil seed bank [13], with very few persisting more than 2 years in the soil [14, 15]. Others studies have found that there was no persistence in the soil seed bank after 5 years [11, 13]. Studies conducted by Andersen et al. [16] and Hewlett et al. [17] showed that downy brome management of greater than 2 years is necessary to deplete the soil seed bank. Manipulating the soil seed bank may hold considerable promise for long-term downy brome management.

Managing downy brome with herbicides to enhance native grass establishment is not a new concept. Many of the same concerns about the loss of sagebrush ecosystems were articulated in the 1960's and 70's, surprisingly for the same reasons described in 2014 [8]. Previous reports described the use of atrazine and paraquat to manage downy brome infestations and enhance native grass establishment [18, 19]. Newer herbicides are available but provide limited residual downy brome control. Integrating prescribed burning with herbicides [20-24] and targeted grazing [25] have provided some increase in the length of downy brome control, but not to the extent necessary to deplete the soil seed bank [26].

A recent publication describing a new herbicide for winter annual grass control suggested if downy brome was controlled for 4 to 5 years the soil seed bank could be depleted [27]. Multiple reports suggest the longevity of downy brome seed in the soil is less than 5 years [10, 13, 14]. Therefore, it may be possible to eliminate downy brome by managing seed production with herbicides alone or in combination with prescribed burning or other management practices [14, 22, 25, 27].

The objective of this research was to test the hypothesis that eliminating downy brome seed production for multiple seasons could deplete the soil seed bank. This research was conducted at two locations in Colorado that were severely impacted by downy brome, but still retained some native vegetation.

METHODS

Site Description

In 2010, field experiments were established at two downy brome infested sites that were approximately 40 km apart. Site 1 (lat 40°28'2.58"N, long 105°9'13.40"W, 1,670 m elevation) was located near Loveland, Colorado on Devil's Backbone Open Space property (~890 ha), and is designated as a priority conservation area. Site 2 (lat 40°42'38.12"N, long 104°51'53.02"W, 1,640 m elevation) was located near Nunn, Colorado on a State Wildlife area that had previously been taken out of crop production. Both sites are located on the western edge of the central shortgrass prairie and are dominated by western wheatgrass (*Pascopyrum smithii*), green needlegrass (*Stipa viridula*), blue grama (*Bouteloua gracilis*), and sand dropseed (*Sporobolus cryptandrus*).

To determine soil characteristics at each site, three, 10-cm-deep soil cores were taken in each of the four replications. These soil cores were combined into a composite soil sample, and analyzed at the Colorado State University Soil Testing Laboratory. Site 1 has shallow, well-drained soils in the Ratake series (Sandy loam, loamy-skeletal, micaceous, frigid, shallow Typic Haplustolls) with 2.5% organic matter, and Site 2 has deep, well-drained soils in the Nunn series (Sandy clay loam, fine, smectitic, mesic Aridic Argiustolls) with 2.0% organic matter [28].

Mean annual precipitation based on the 30-yr average (1981-2010) was 420 mm at Site 1 and 361 mm at Site 2 [29]. Precipitation across both sites was close to the 30-yr average in 2010 and 2011. A statewide-drought occurred in 2012 with average total precipitation for both sites decreasing 160 mm below their 30-yr averages. In 2013, Site 1 received an additional 174 mm above the 30-yr average, while Site 2 had average precipitation. Both Sites received an additional 58 and 76 mm of precipitation above their 30-yr averages in 2014 and 2015,

respectively [30]. The mean annual temperatures ranged from 8.7 to 8.9°C, and during the years of this study temperatures were close to average.

Before herbicide applications, visual percent canopy cover was estimated by a team of experienced rangeland specialists, across the entire study area for all species present at both locations. Site 1 was characterized by ~90% downy brome canopy cover with a dense litter layer (2 to 7 cm), and scattered perennial grasses including western wheatgrass, blue grama, and sand dropseed (8% ± 3% (mean ± SE),15% ± 4%, and 9% ± 4% canopy cover, respectively). Site 2 had less downy brome canopy cover before herbicide application (~70% cover) and several desirable species, including western wheatgrass, sand dropseed, and green needlegrass (13% ± 5%, 6 ± 1%, and 3% ± 1% canopy cover, respectively).

Experimental Design and Evaluations

Field Study

We applied glyphosate to 6 x 9 m plots in late spring (between March 15 and 29) after annual grass emergence, to eliminate downy brome seed production for periods ranging from 0 to 5 consecutive years (2011-2015). At the time of application all perennial grasses were considered dormant. Six herbicide treatments, including a non-treated control, were arranged in a randomized complete block design with four replications. All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat fan nozzles calibrated to deliver 187 $L \cdot ha^{-1}$. Glyphosate (Roundup Weathermax, Monsanto, 1.26 $kg \cdot ae \cdot ha^{-1}$) plus adjuvant (methylated seed oil, MSO Concentrate with LECI-TECH®, Loveland Products, 1.17 $L \cdot ha^{-1}$) was applied for 0, 1, 2, 3, 4, or 5 consecutive years. The high glyphosate rate in this study was used to ensure complete downy brome control at this late spring timing.

Biomass Harvest

Biomass harvests were conducted in August (2011-2015) to evaluate compositional changes in the plant community in response to sequential glyphosate applications. Above-ground biomass of the downy brome and perennial grasses were harvested from randomly placed 1-m^2 quadrats. One quadrat was harvested per plot per year at each site (n = 24 per site). Harvested quadrats were not taken from the same location in the plot in consecutive years. Perennial grasses were separated by species during harvest. The material was dried at 60°C for 7 d to determine species dry biomass for each quadrat.

Greenhouse Soil Cores

To evaluate the yearly variation and depletion of the downy brome soil seed bank in response to consecutive glyphosate applications (0 to 5 years), soil cores were obtained annually in March prior to herbicide application. Baseline cores were taken March 2011 at initiation of the study and final cores were taken January 2016. Soils were collected from random locations within each plot (6 total cores per plot) using 3.8 cm deep x 5.1 cm diameter soil cores. Downy brome seedlings that had already emerged in the field during soil core collection were counted and added to the final downy brome total for the entire plot. The six soil cores from each plot were combined into one composite sample and immediately frozen at -20 °C until greenhouse planting. Approximately 5 mo after collection, composite soil samples were spread uniformly over 25 x 25 x 6 cm flats arranged in a completely randomized designed. Flats were kept at field capacity with a 15-hr photoperiod to promote germination of all viable seeds. We allowed ~3 wks for all seedlings to germinate before conducting downy brome and perennial grass seedling counts to determine germination across sequential glyphosate treatments as compared to the non-treated controls. Downy brome seedlings counted in March and greenhouse germinated

seedlings from soil cores were pooled into a single value representing the viable downy brome seed in each treatment.

Statistical Analysis

Biomass Harvest

We utilized a repeated measures (PROC GLIMMIX) in SAS 9.3 to analyze downy brome field biomass harvest data [31]. Factors included in the repeated measures model were experiment, treatment, year, and all possible interactions, with year as the repeated measure. Dry biomass data were converted to a percentage by comparing treated to non-treated plots to normalize data variations in overall downy brome and perennial grass biomass across sites and years. These percentages were arcsine square root transformed and a Tukey-Kramer adjustment was applied. After failing to reject the null hypothesis of equal variance for the repeated experiment ($P=0.452$), the same residual variance was assumed and data were combined across sites for analysis. Differences among least squares means were analyzed across all 5 years to evaluate the significant treatment-by-year interaction ($P<0.0001$).

The biomass harvest conducted the last year of the study (August 6, 2015) provided a final downy brome and perennial grass evaluation. Four-parameter logistic regression of dry biomass was conducted in Graphpad Prism 6 using the model:

$$Y = C + \frac{(D - C)}{1+10^{(\mathrm{Log}GR_{50} - X) \cdot b}}$$

Where C is the lower limit of response, D is the upper limit of response, b the slope, and GR_{50} is the herbicide rate resulting in 50% reduction in biomass. Analysis was performed separately at each site for downy brome and perennial grass biomass because of unequal variances ($P<0.0001$ and $P=0.0063$, respectively).

Canopy Cover Estimates

Following the final treatment year percent canopy cover estimates were also conducted in August 2015 for all perennial grasses. Canopy cover was determined by comparing visual estimates of downy brome canopy cover in the treated compared with non-treated plots using the whole 6 x 9 m plot area. All warm and cool season species were evaluated separately at each site. After failing to reject the null hypothesis of equal variance for the repeated experiment, the same residual variance was assumed and data were combined across sites for analysis of variance (ANOVA).

Greenhouse Soil Cores

Soil cores were analyzed to estimate the longevity of the downy brome soil seed bank. Because soil cores were collected in March (2011-2016) before treatments were applied, emerged seedlings were included in the total seedling counts for each treatment. Seedling counts were summed for each plot by combining emerged downy brome seedling counts made during collection of soil cores from the field (6 cores/plot), with seedling counts from the soil core greenhouse bioassay. These total counts were representative of the downy brome emerging as seedlings before the yearly glyphosate treatments and those remaining in the soil seed reserve after treatment. Total seedling counts were converted to a percent of the non-treated controls and analyzed in SAS 9.3. Data were arcsine square root transformed and least squares means were analyzed using repeated measures as previously described. After failing to reject the null hypothesis of equal variance for the repeated experiment, the same residual variance was assumed and data were combined for analysis.

RESULTS

Field Biomass

Based on the evaluation of the significant treatment-by-year interaction ($P<0.0001$) and pairwise comparisons of least squares means ($\alpha = 0.05$), 1 to 3 years of consecutive glyphosate applications were insufficient to deplete the downy brome soil seed bank (Fig. 3.1). Although treatment comparisons showed downy brome biomass was significantly reduced after glyphosate applications up to 3 consecutive years, downy brome biomass and the soil seed bank recovered within 1 to 2 years after applications were terminated ($P>0.05$) (Fig. 3.1). Treatments with 4 and 5 consecutive years of glyphosate were necessary to eliminate the downy brome seed rain, while also depleting all viable downy brome seed in the soil seed bank (Fig. 3.1). In year 5, downy brome re-established completely in treatments of 1 to 3 years of glyphosate applications as compared to 4 and 5 years of soil seed bank management ($P<0.0001$).

The biomass harvest in the final year of our study (2015) showed a similar trend in downy brome biomass reduction compared to the yearly biomass harvests. Applying glyphosate to control downy brome biomass and seed production for 1, 2, and 3 consecutive years resulted in similar downy brome biomass to the control (no herbicide treatment) ($P=0.285$ to 0.700); however, eliminating downy brome seed production for 4 and 5 years using glyphosate was effective in managing the downy brome soil seed bank as reflected by downy brome biomass (Fig. 3.2) ($P<0.0001$). Compared to the non-treated control plots, perennial grass biomass remained fairly stable with 1, 2, and 3 years of consecutive glyphosate applications compared to the non-treated ($P= 0.145$ to 0.850) (Fig. 3.2). Eliminating downy brome competition with 4 consecutive years of glyphosate resulted in a significant 4-fold increase in perennial grass biomass for Sites 1 and 2, respectively ($P=0.040$ and 0.019, respectively), while 5 years of

consecutive glyphosate applications resulted in a significant 7-fold and 9-fold increase in perennial grass biomass at Sites 1 and 2 compared to the non-treated, respectively (P=0.001 and 0.0002, respectively) (Fig. 3.2).

Eliminating downy brome competition and seed production for 5 years using glyphosate significantly increased perennial grass canopy cover approximately 2.9- and 1.6-fold as compared to the non-treated at Sites 1 and 2, respectively (Table 3.1) (P=0.0011 and P=0.0004, respectively). Although perennial grass biomass increased significantly with 4 years of consecutive glyphosate applications at Sites 1 and 2 (Table 3.1, P=0.006 and 0.001, respectively), percent canopy cover estimates of all perennial grass (August 2015) showed a shift in the native plant community (Fig. 3.3). The plant community shifted from a cool season to primarily a warm season grass-dominated plant community (Fig. 3.3). In order to control all the emerged downy brome with a single herbicide application it was necessary to wait as long as possible in the spring. It is very possible that the cool season grasses were not completely dormant when glyphosate was applied and the stress association with the herbicide treatments were responsible for shifting the plant community to one dominated by warm season grasses.

Applying high rates of glyphosate in the late spring poses a risk and would not be a recommended practice; however, it represented the best option for complete downy brome control with a single herbicide treatment. This project was intended to explore the importance of the soil seed bank as a key component in maintaining downy brome populations at levels that cause significant ecological impacts.

Greenhouse Soil Core Bioassay

Seedling counts made in the field and seedlings that established from soil cores in the greenhouse showed a similar trend to the yearly biomass harvests (Figs. 3.1 and 3.4). Baseline

soil cores collected in 2011 before herbicide treatments were initiated showed no difference among downy brome seedling counts across the sites (Fig. 3.4, $P>0.05$). 1 year of glyphosate resulted in a 60% reduction in seedling germination from the soil seed bank compared to the non-treated; however, if glyphosate treatments were terminated downy brome seedling counts recovered to baseline levels within 2 years (2014) ($P=0.355$). This same trend was consistent with 2 and 3 consecutive years of glyphosate treatments. After glyphosate treatments were terminated it took approximately 2 to 3 years for the downy brome soil seed bank to recover to the level of the non-treated plots (Fig. 3.4) ($P>0.416$).

In 2015, plots where downy brome biomass and seed production were eliminated for 4 and 5 years using glyphosate, downy brome seedling counts were 1% and 0% compared to the non-treated plots, and in 2016 seedling counts were 4% and 0% compared to the non-treated plots, respectively (Fig. 3.4). By 2016, the soil seed bank for all other treatments had recovered to levels similar to the non-treated controls ($P>0.979$), suggesting that greater than 3 years of effective management is required to exhaust the downy brome soil seed bank (Fig. 3.5). Final soil core results in 2016 suggest that compared to 1, 2, and 3 years of glyphosate, 4 and 5 years of consecutive glyphosate application were critical to prevent downy brome re-establishment via the soil seed bank (Fig. 3.5) ($P<0.0001$). Interestingly, downy brome emergence from soil cores in the greenhouse showed no perennial grass seedling emergence in the treatments with 0 to 3 years of glyphosate; however, soil cores taken from Sites 1 and 2 with four years of consecutive applications had on average 1,584 ± 336 (mean ± SE) and 1,120 ± 480 perennial grass seedlings per m^2, respectively. Perennial grass seedling counts further increased with 5 years of glyphosate applications at both sites with an average of 2,528 ± 1,072 and 1,616 ± 848 seedlings per m^2, respectively.

DISCUSSION

Our study provides evidence to support the hypothesis that the downy brome soil seed bank can be managed to a point of full control. Yearly field biomass harvests showed that at least 4 years of consecutive control were required to maintain downy brome control, while at the same time depleting the soil seed bank. Management strategies that only provide 1 to 3 years of control are susceptible to re-establishment from the soil seed bank. It is crucial when managing invasive winter annual grasses such as downy brome to consider the longevity of the seed in the soil seed bank. This may represent a trait that can be exploited to reduce the potential for re-establishment and it is a trait shared by a number of other invasive winter annual grasses [13, 32-35].

Our data provide a framework for managing downy brome with a multi-year approach. It has been common for land managers to use herbicides, prescribed burning, or targeted grazing for a single growing season, where follow up treatments or sequential herbicide applications are not made. Commonly recommended herbicides such as imazapic, glyphosate, or rimsulfuron provide limited or no residual downy brome control past the initial application year and can injure co-occurring species [27, 36-43]. Without long-term management of the soil seed bank the site with downy brome will be rapidly re-established and return to non-treated plant densities within 1 to 2 years (Fig. 1) [44, 45].

The results from the current study suggest that land managers have two main herbicide approaches for depleting the soil seed bank in an attempt to restore downy brome invaded rangeland. These include (1) annual applications of an herbicide such as glyphosate with limited residual downy brome control or, (2) apply an herbicide with residual control every other year. An herbicide that provides extended downy brome control is necessary to exhaust the soil seed bank; however, there are limited herbicides that can provide this residual control. Land

managers could use this framework to plan sequential applications like the methods used in this study, to control the downy brome crop for the 4 and 5 years necessary to deplete the downy brome seed bank.

Indaziflam (Esplanade, Bayer CropScience) offers a new mode of action to non-cropland weed management that provides up to 3 years of residual downy brome and feral rye (*Secale cereale* L.) control with a single application [27, 46]. Using an indaziflam treatment the first year with our approach has the potential to provide residual control for 2 to 3 years, requiring only one additional treatment to exceed the three-year downy brome seed bank threshold. Reducing herbicide applications from annual to once every 2 to 3 years may minimize non-target impacts to the desirable plant community, decrease labor costs, and decrease selection pressure for herbicide resistance. In contrast, the application of sequential glyphosate in late spring may also result in shifts in native species compositions over time [47, 48]. Indaziflam could provide an alternative strategy for land managers to treat downy brome for long-term control while also minimizing negative impacts to the desirable plant community [27, 49].

Long-term management of downy brome and the soil seed bank could be an important strategy to restore rangeland infested with downy brome and other annual grasses particularly within the sage-steppe ecosystem [4, 50]. Among the 350 species that call the sage-steppe ecosystem home, the greater sage-grouse is one species in particular that has been directly impacted by large scale downy brome invasions [4, 43, 50]. According to a Department of the Interior news release, Secretarial Order 3336 (January 5, 2015), reducing downy brome impacts is vital to sagebrush landscapes and productive rangelands [50]. Managing downy brome and its soil seed bank is imperative to create large scale fire breaks and large blocks of high-quality sagebrush habitat needed for the many species that utilize the sage-steppe [8]. Collaboration

between federal and state agencies (70% of sagebrush habitat) will be critical to address annual grass invasions [50].

IMPLICATIONS

Downy brome invasions are rapidly transforming perennial plant communities into annual grass-dominated communities [51], with an average annual spread rate of 14% [3]. Restoring the structure and function of these invaded ecosystems can be accomplished by targeting these invasive annual grasses; however, long-term control options are limited. There are many factors that can lead to the success or failure of downy brome control and our research suggests that one major factor to consider is the longevity of the downy brome seeds in the soil seed bank. Managing the downy brome seed bank targets a fundamental biological and ecological survival mechanism of this invasive weed. Our study provides much needed evidence for why re-establishment via the soil seed bank occurs when using short-term downy brome control methods such as herbicides (glyphosate, imazapic, or rimsulfuron), prescribed burning, or targeted grazing. These control methods are commonly recommended, yet they have provided limited residual activity [36-39] and inconsistent long-term control [25]. We suggest eliminating downy brome seed production for more than 3 years provides the time needed to deplete the downy brome soil seed bank and significantly increase desirable perennial grass biomass and cover.

We recommend land managers recognize the importance of managing the downy brome soil bank and develop a multi-year plan to combat invasive winter annual grasses. Products such as indaziflam with residual control may provide an additional effective tool for invasive winter annual grass control that could be used in alternate years reducing the amount of total herbicide applied. Otherwise, managers could choose to apply herbicides with shorter residual control

(e.g., glyphosate, imazapic, rimsulfuron) yearly until the soil seed bank is depleted (~3 years). We caution managers to evaluate potential impacts to native seed banks and existing desirable flora before any application.

3.7 TABLES

Table 3.1. Total perennial grass canopy cover in reponse to sequential glyphosate applications at sites 1 and 2. Visual percent canopy cover estimates (mean ± SE) were conducted August 2015 after the final year of herbicide applications.

Site	Sequential Glyphosate Applications (No.)	% Total Perennial Grass Cover (Mean ± SE)
1	0	28.3 ± 14.1
1	1	17.3 ± 4.2
1	2	12.0 ± 2.6
1	3	21.0 ± 4.9
1	4	62.3 ± 8.3
1	5	80.8 ± 10.6
2	0	62.3 ± 10.0
2	1	60.6 ± 2.8
2	2	54.1 ± 5.3
2	3	69.3 ± 6.4
2	4	92.3 ± 3.8
2	5	98.8 ± 1.3

3.8 FIGURES

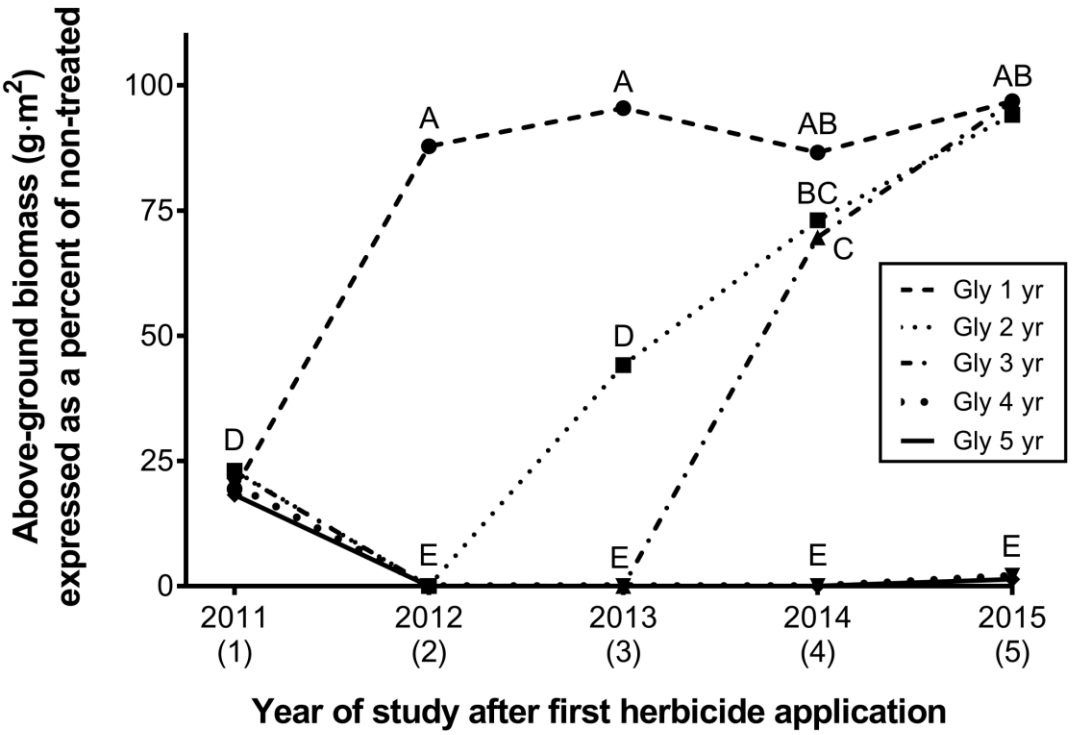

Figure 3.1. Effects of sequential annual glyphosate applications at Sites 1 and 2 on downy brome biomass represented as a percent of the non-treated. Lines signify treatments with different levels of sequential glyphosate applications (Gly, 1.26 kg·ae·ha^{-1}). Letters indicate differences in least squares means across years ($P < 0.05$).

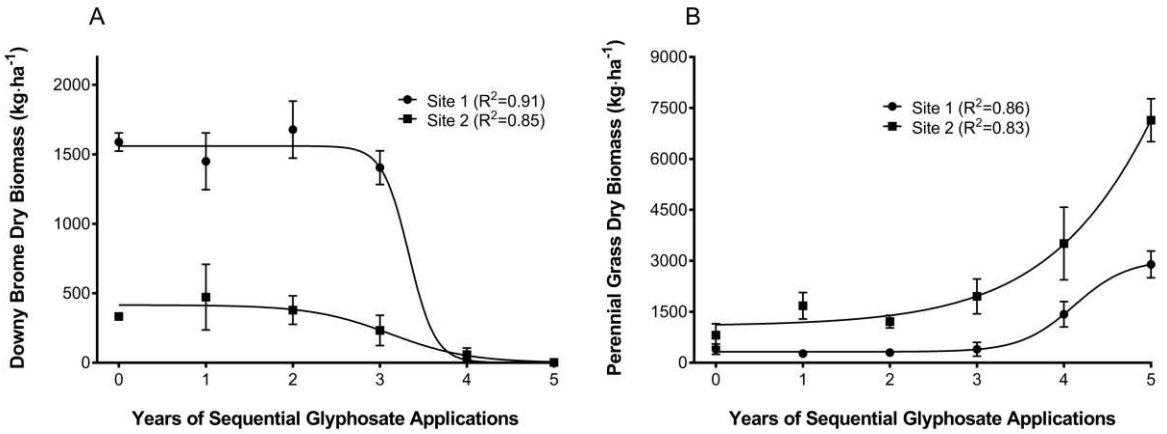

Figure 3.2. Four-parameter logistic regression evaluating the effects of sequential glyphosate applications on (A) downy brome and (B) perennial grass biomass. Data presented are from the August 2015 final biomass harvest. Point estimates ± SE represent differences in biomass across treatments.

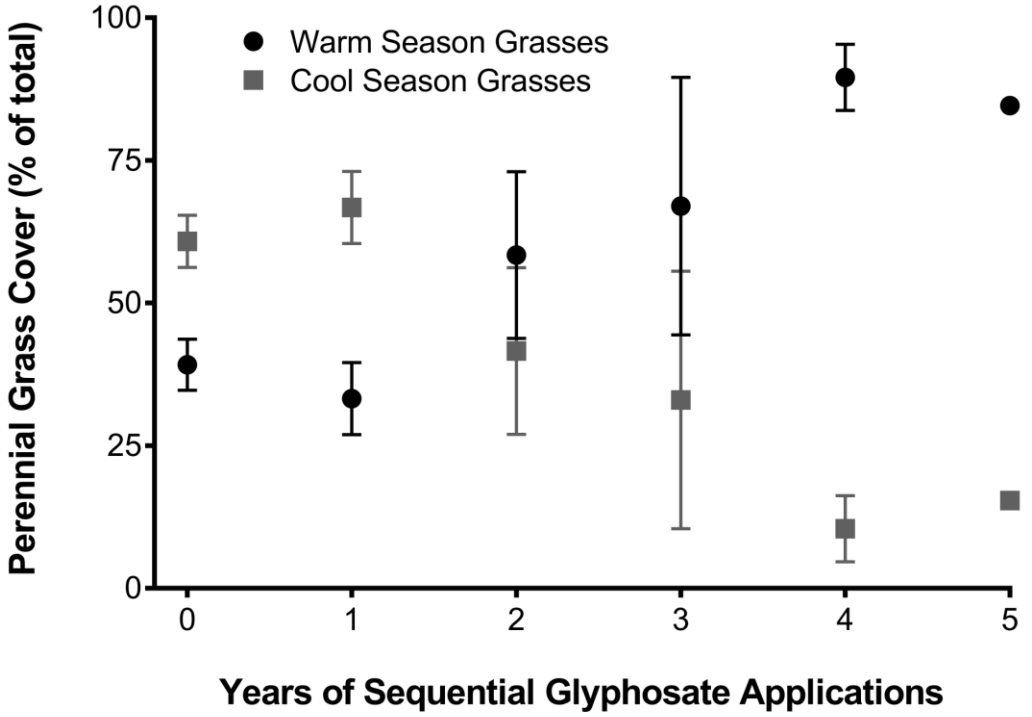

Figure 3.3. Perennial grass response (cool and warm season) to sequential glyphosate applications at two sites. Visual percent canopy cover estimates (mean ± SE) were conducted August 2015 after the final year of herbicide applications.

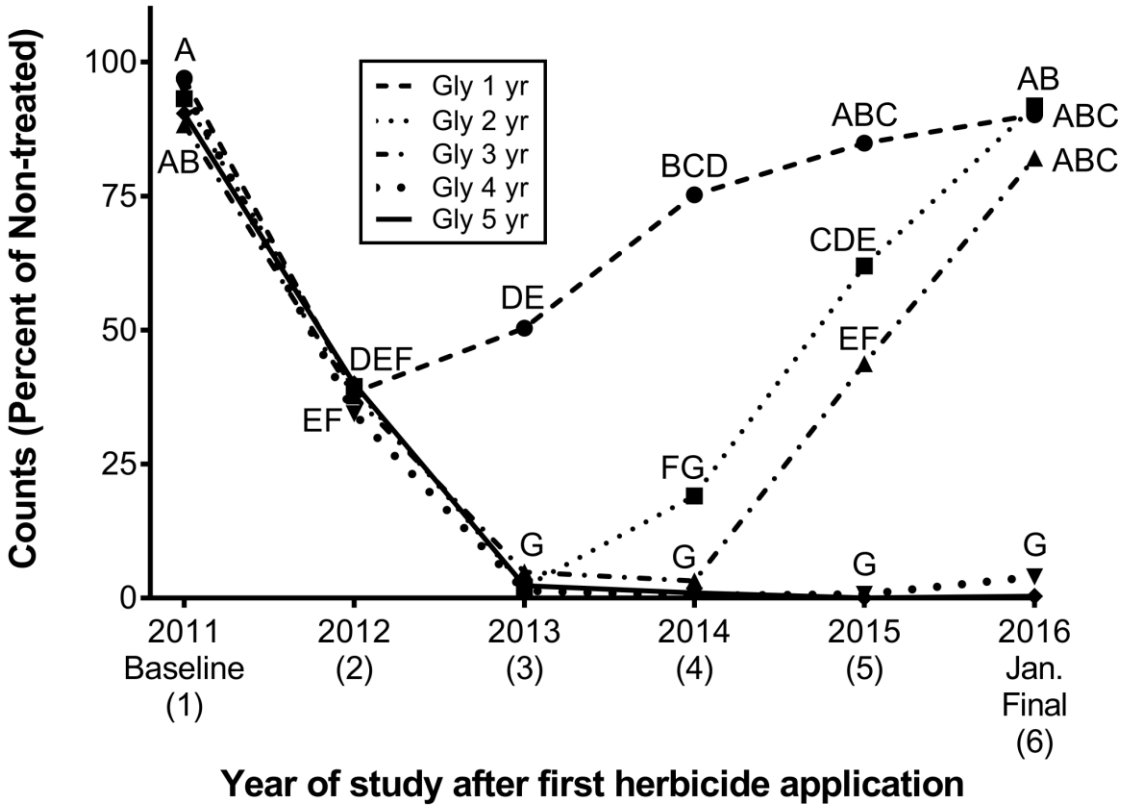

Figure 3.4. Determining the longevity of the downy brome soil seed bank using downy brome seedling emergence (counts) from soil cores taken in the field and germinated under optimum growing conditions in the greenhouse. Seedling counts were represented as a percentage compared to the non-treated. Lines signify treatments with different levels of sequential glyphosate applications. Letters indicate differences at $P<0.05$.

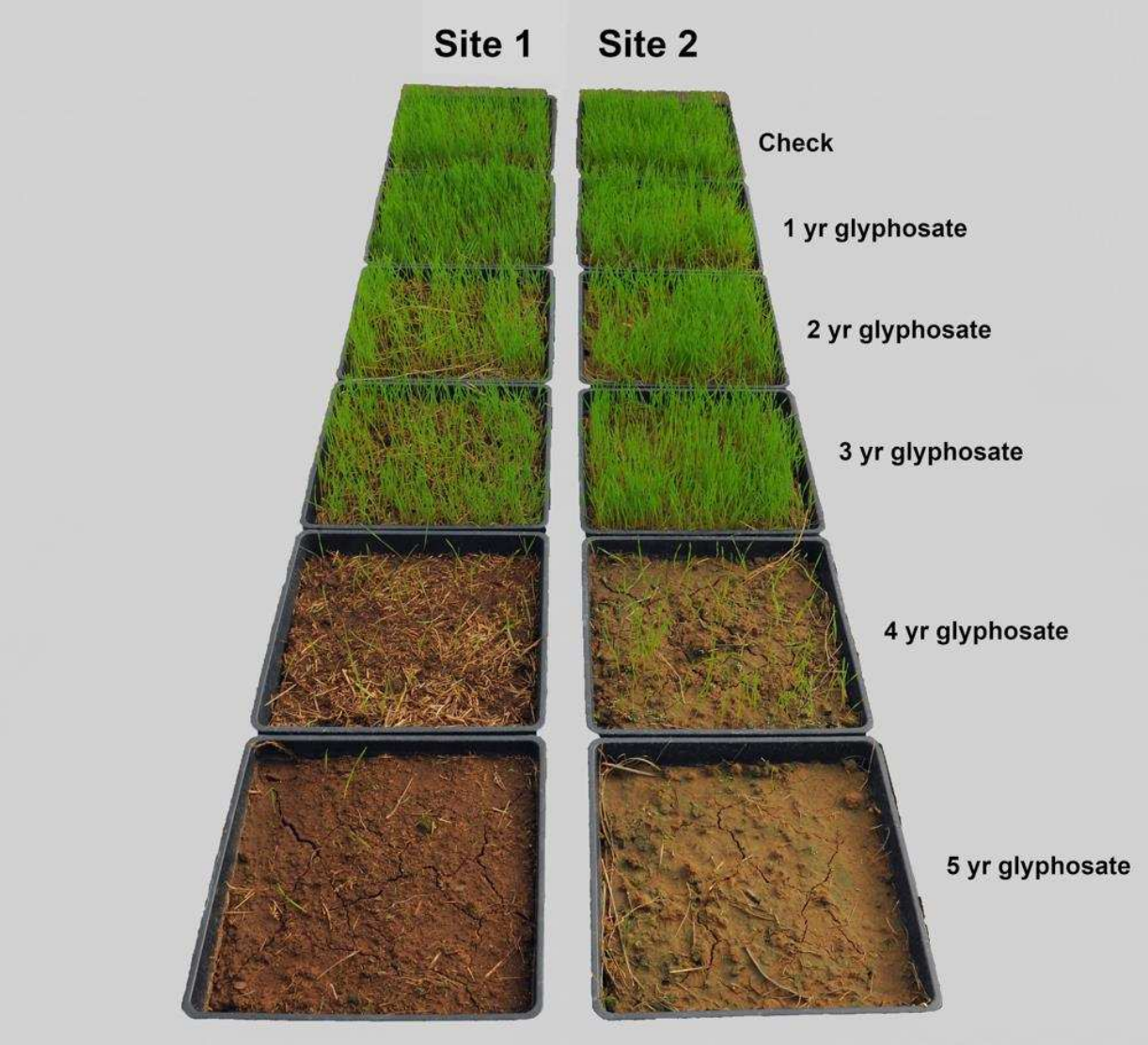

Figure 3.5. Soil cores collected January 2016 at Sites 1 and 2, demonstrating the longevity of the downy brome soil seed bank in response to sequential glyphosate applications.

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CHAPTER 4: PRE-EMERGENCE CONTROL OF SIX INVASIVE WINTER ANNUAL GRASSES WITH IMAZAPIC AND INDAZIFLAM[1]

SUMMARY[§]

Managing invasive winter annual grasses on non-crop and rangeland remains a constant challenge throughout many regions of the US. Currently, there are limited management options for controlling winter annual grasses that work consistently, provide multiple years of control, and do not injure desirable plant communities. Imazapic has been one of the most-widely used herbicides for downy brome control on rangeland; however, control with imazapic has been inconsistent beyond the application year and perennial grass injury is not uncommon. Indaziflam, a new herbicide mode of action for rangeland weed management, has shown promise in providing long-term downy brome (*Bromus tectorum* L.) control. A greenhouse study was conducted to compare pre-emergence activity of imazapic and indaziflam on six invasive winter annual grasses: downy brome, feral rye (*Secale cereale* L.), jointed goatgrass (*Aegilops cylindrica* L.), Japanese brome (*Bromus japonicus* Thunb.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski), and ventenata (*Ventenata dubia* (Leers) Coss). For both herbicides, seven rates were used to develop dose-response curves for each species. Log-logistic regression was conducted to determine the herbicide dose required to reduce biomass by 50% (GR_{50} values). Indaziflam was significantly more active across all species compared to imazapic, with the exception of jointed goatgrass. Comparing all species, the GR_{50} values for imazapic were on average 12 times higher than indaziflam. Japanese brome was the most sensitive to both herbicides, while jointed goatgrass and feral rye were the most difficult winter annual grasses to control with indaziflam and imazapic, respectively. This research provides evidence of a

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potential new mode of action for land managers to control the major invasive winter annual grasses.

MANAGEMENT IMPLICATIONS

Invasive winter annual grasses pose a major threat to native plant communities in the US. The lifecycle of these species increases their invasiveness because few native species behave as winter annuals, providing a niche for invasive annual grasses to exploit moisture and nutrients when most desirable perennial plants are dormant. While downy brome alone infests over 22 million ha of US rangeland, there are five other invasive winter annual grasses that cause significant economic and ecological impacts: feral rye, Japanese brome, jointed goatgrass, medusahead, and ventenata.

Currently, acetolactate synthase (ALS) inhibiting herbicides such as imazapic and rimsulfuron are used for selective winter annual grass control, while non-selective herbicides like glyphosate are also recommended for dormant season applications (late fall or early spring). Unfortunately, none of these herbicides provide consistent control beyond 1 year after treatment (YAT), resulting in rapid reinvasion of treated areas via the soil seed bank. Indaziflam (Bayer CropScience), a cellulose-biosynthesis inhibiting herbicide, is a new mode of action for invasive winter annual grass management. Previous field research demonstrated that indaziflam provided excellent downy brome and feral rye control two and three years after treatment compared to imazapic. Two applications of indaziflam over a five-year period could substantially reduce or possibly eliminate the winter annual grass seed from the soil seed bank. The objective of this study was to evaluate indaziflam's potential to control other problematic invasive winter annual grasses found in the US and compare its activity to the most commonly used herbicide, imazapic. The herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) was calculated for each

invasive winter annual grass. In the greenhouse, indaziflam was significantly more active against all winter annual grasses compared to imazapic, with jointed goatgrass as an exception. Averaged across all invasive winter annual grasses, imazapic GR_{50} values were 12 times greater compared to indaziflam.

The potential for long-term downy brome management is very encouraging; however, downy brome is only one species in a suite of winter annual grasses that threaten native ecosystems from the Great Plains to the Pacific Coast. This research indicates that indaziflam is active in controlling a range of winter annual grasses, and based on what we know about the soil seed bank of these species, indaziflam could be a key component in providing long-term management. Our findings provide evidence that indaziflam could be an alternative strategy for controlling invasive winter annual grasses, including relatively new invaders such as medusahead and ventenata. Additional field research is needed to determine if indaziflam provides the long-term control of ventenata, medusahead, jointed goatgrass, and Japanese brome that has been previously reported with downy brome and feral rye.

INTRODUCTION

Invasive winter annual grasses are a serious concern in the western US and continue to spread rapidly across non-crop and rangeland areas displacing native vegetation. Great Basin sagebrush ecosystems that were once primarily perennial plant dominated are being transformed to annual grass-dominated plant communities [2]. Exotic winter annual grasses are highly competitive with native perennial grasses and greatly reduce above- and belowground biomass, deplete soil moisture, and reduce native plant diversity [3-9]. This can drastically influence the

structure and function of these ecosystems [10, 11], while at the same time decrease their resistance and resilience to invasion [2].

As invasive annual grasses continue to increase, effective management becomes critical for restoring and maintaining native rangeland ecosystems. This is particularly true for the over 23 million hectares of public land in the Great Basin and western US currently infested by annual grasses such as downy brome (*Bromus tectorum* L.) and medusahead (*Taeniatherum caput-medusae* [L.] Nevski) [12, 13]. While downy brome is the most widespread invasive plant in the US [13], medusahead is the most problematic invasive annual grass found on California rangelands and has been found as far east as Nevada and Utah [9, 14, 15] (Figure 1). Other invasive annual grasses that represent substantial threats to natural ecosystems include: feral rye (*Secale cereale* L.)[16-18], jointed goatgrass (*Aegilops cylindrica* L.) [19, 20], Japanese or field brome (*Bromus japonicus* Thunb. or *Bromus arvensis* L.) [3, 21], and ventenata (*Ventenata dubia* (Leers) Coss) [6, 22-24] (Figure 1).

Japanese brome is widespread throughout the US, but is more prolific in the western US and northern Great Plains [3]. Feral rye and jointed goatgrass are two distinctive invasive winter annual grasses that result in high wheat yield losses and also infest areas surrounding these cropping systems. Populations continue to spread to non-cropland areas such as roadsides and overgrazed pastures [18-20]. Ventenata, commonly referred to as wiregrass or North Africa grass, currently invades areas mainly in the Intermountain Pacific Northwest [6, 24, 25]. Ventenata is an increasing threat to recently disturbed perennial grass systems and has even been shown to displace other invasive annual grasses such as downy brome and medusahead [6]. Effective, long-term control strategies are crucial to proactively manage this localized species in order to decrease further spread [26].

Disturbed soils provide conditions for invasive winter annual grasses to establish and spread efficiently; however, it is common for species such as downy brome and medusahead to spread into non-disturbed rangeland via seed dispersal mechanisms [27, 28]. Species evaluated in this study rapidly accumulate dense thatch layers that provide microhabitats that help to perpetuate the invasive species [6, 10]. Downy brome and medusahead thatch layers are highly susceptible to fires and suppress germination and establishment of native rangeland species [10, 15, 29, 30]. The accumulation of these fine fuels shortens fire return intervals resulting in the displacement of sage-brush ecosystems that are habitat to species such as the greater sage-grouse [2, 4, 5, 30, 31].

Among the currently available management strategies, herbicides are the most common method used to control invasive winter annual grasses [7]. Three commonly recommended herbicide treatments and application rates for invasive winter annual grass control in the US include imazapic (Plateau, BASF, 105 g·ai·ha^{-1} with 201 g·ai·ha^{-1} annual maximum) [9, 26, 32, 33], rimsulfuron (Matrix, Bayer CropScience, 53 g·ai·ha^{-1}) [26, 32], and glyphosate (Roundup Weathermax, Monsanto, 420 g·ae·ha^{-1}) [29]. Imazapic and rimsulfuron provided limited residual control and lack consistency beyond the initial application year [9, 14, 29, 30, 34-36]. These herbicides, including glyphosate, can also injure co-occurring species depending on application timing [26, 37-39]. Efforts to restore native plant communities impacted by invasive winter annual grasses are frequently unsuccessful due to rapid reinvasion from the soil seed bank [40], therefore, new management strategies that address the soil seed bank are needed.

Indaziflam (Esplanade, Bayer CropScience), a new pre-emergence herbicide registered in the US for the control of annual grass and broadleaf weeds in citrus, grape, and tree nut crops, could provide the residual weed control necessary to limit reinvasion. This herbicide belongs to

the alkylazine class and is the first cellulose-biosynthesis inhibitor (CBI) that could potentially be used for controlling invasive winter annual grasses found on non-cropland in the US. Bayer CropScience has developed a supplemental label for the release or restoration of desirable vegetation on non-crop areas such as parks and open space, wildlife management areas, fire rehabilitation areas, and other non-grazed sites (May 2016). Studies are currently being conducted to support a grazing tolerance; therefore, current indaziflam treatments are limited to sites not grazed by domestic livestock. Indaziflam has a relatively long half-life (>150 days) in the soil. Application rates of indaziflam range between 51 and 102 g·ai·ha^{-1} with a yearly maximum of 146 g·ai·ha^{-1} [41, 42], while the recommended rates for residual winter annual grass control are 73 and 102 g·ai·ha^{-1}. In field experiments conducted in Colorado, established native perennial grasses, forbs, and shrubs were tolerant to indaziflam [43]. Field studies have shown that indaziflam provides superior downy brome and feral rye control compared to imazapic [32, 44]. Imazapic and indaziflam applied PRE provided similar downy brome control one year after treatment (YAT); however, indaziflam provided 83 to 100% downy brome control 2 and 3 YAT [32]. This level of residual control may help to manage the soil seed bank of invasive winter annual grasses thus limiting re-invasion. There is currently no published literature evaluating indaziflam's activity on invasive winter annual grasses other than downy brome.

The main objective of this research was to compare imazapic and indaziflam activity on invasive winter annual grasses found in the western US using greenhouse dose-response experiments. We hypothesized that indaziflam could provide increased winter annual grass control across all species compared to imazapic. These greenhouse experiments represent the most comprehensive analysis comparing the currently recommended herbicide, imazapic, with indaziflam.

MATERIALS AND METHODS

Study Species. A greenhouse dose-response was conducted to compare the sensitivity of six invasive winter annual grasses to imazapic and indaziflam (Figure 1). All species were collected from their invaded range: downy brome and feral rye (Larimer County, CO), Japanese brome (Jefferson County, CO), jointed goatgrass (Phillips County, Colorado), medusahead (Yuba County, California), and ventenata (Latah County, Idaho). Seeds were collected from senesced plants the year prior to this study and stored at -4 C until planting in 2015.

Seeds were planted in plastic containers (17-cm by 12-cm by 6-cm) filled with field soil. The field soil was an Otero sandy clay loam (Coarse-loamy, mixed (calcareous), mesic Aridic Ustorthents) with 3.9% OM and pH 7.7. Seeding densities were adjusted based on germinability to reach a target density of 40 plants/pot. All species were planted at a depth of 0.5 cm.

Experimental Design. The experimental design was a factorial with six herbicide rates and a non-treated arranged in a completely randomized design with three replicates. The study was repeated 27 July 2015 and 29 September 2015. A preliminary study was conducted to approximate a range of doses that would best fit a logistic regression model for each herbicide and species. It is not unusual for both pre-emergence and post-emergence herbicides to be more active (provide control at lower than labeled rates) in the greenhouse with ideal environmental conditions, so it was not surprising to us that herbicide doses for the regression analysis were much lower than recommended field use rates. Imazapic was applied at rates of 0, 2.2, 4.4, 8.8, 17.5, 35.0, and 70.1 g ai ha^{-1} for downy brome, Japanese brome, medusahead, and ventenata; while, for feral rye rates were 0, 8.8, 13.1, 17.5, 35.0, 70.1, and 140.2 g ai ha^{-1} and for jointed goatgrass rates were 0, 4.4, 8.8, 17.5, 35.0, 70.1, 140.2, and 280.4 g ai ha^{-1}. Indaziflam was applied at rates of 0, 0.2, 0.4, 0.7, 1.5, 2.9, and 5.9 g ai ha^{-1} for all species except jointed

goatgrass where rates of 0, 0.7, 1.5, 2.9, 5.9, 11.7, and 23.4 g ai ha^{-1} were used. Herbicides were applied using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray nozzle (TeeJet Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 $L \cdot ha^{-1}$ at 172 kPa.

Following herbicide treatments, plants were maintained in a greenhouse with a 25/20°C day/night temperature regime at an approximate 60% relative humidity. Natural light was supplemented with high-intensity discharge lamps to give a 15-h photoperiod. Plants were sub-irrigated weekly and misted daily to reduce soil crusting. Aboveground plant biomass was harvested at the soil surface 4 weeks after treatment (WAT) and dried for 5 d at 60 C before recording dry weights.

Data Analysis. Total dry weights for each treatment were converted to a percentage of the biomass in the non-treated. Data were first analyzed using the PROC MIXED method in SAS 9.3 with treatment as a fixed effect and experiment and replicate as random effects [45]. After failing to reject the null hypothesis of equal variance the repeated studies were combined for analysis. Graphpad Prism 6 was used to determine imazapic and indaziflam rates required to reduce plant dry biomass by 50% (GR_{50}) for each invasive winter annual grass. The four parameter log-logistic regression equation regressing biomass as a percent of the non-treated with herbicide concentration is

$$Y = C + \frac{(D - C)}{1+10^{(\mathrm{Log}GR_{50} - X) \cdot b}} \qquad [1]$$

where C is the lower limit of response, D is the upper limit of response, b the slope, and GR_{50} is the herbicide rate resulting in 50% reduction in biomass. Means were separated for each invasive winter annual grass to determine significant differences in GR_{50} values, using Fisher's Protected LSD test at the 5% level of probability. The recommended use rates for indaziflam

range from 70 to 97% (73 and 102 g·ai·ha^{-1}) of the commonly recommended imazapic use rate (105 g·ai·ha^{-1}); therefore, pre-emergence control was compared directly using GR_{50} estimates.

RESULTS AND DISCUSSION

Indaziflam was significantly more active against all winter annual grasses compared to imazapic (Figure 4.2), with the exception of jointed goatgrass. Although indaziflam's GR_{50} value for jointed goatgrass was approximately half that of imazapic, this was the only species where the GR_{50} values were not significantly different (P=0.6447) (Table 4.1). We used these data to confirm results from previous field experiments comparing these two herbicides [32, 43] and make inferences about how these data can be applied to other invasive winter annual grasses that have not been evaluated under field conditions (Table 4.1).

The downy brome GR_{50} values were significantly higher for imazapic (2.71 ± 0.10 g·ai·ha^{-1}) as compared to indaziflam (0.23 ± 0.07 g·ai·ha^{-1}) (Figure 2). Furthermore, Japanese brome showed the greatest sensitivity (GR_{50} = 0.19 g·ai·ha^{-1}) to indaziflam, while jointed goatgrass (GR_{50} = 7.37 g·ai·ha^{-1}) was the least sensitive (Table 4.1). For imazapic, Japanese brome showed the greatest sensitivity (GR_{50} = 1.86 g·ai·ha^{-1}), and feral rye (GR_{50} = 24.37 g·ai·ha^{-1}) was the least sensitive (Table 4.1). The indaziflam GR_{50} values for medusahead and ventenata were 6 and 16 times lower compared to imazapic, respectively (P<0.0001, Figure 2).

Ventenata and medusahead are relatively new invaders to the western US [6], increasing the importance of reducing further spread of these species to highly susceptible areas such as the Great Basin. In these areas, productive wildlife habitat, including intact sagebrush landscapes, are crucial for species such as the sage-grouse (*Centrocercus urophasianus and C. minimus*) [2, 5]. Ventenata in particular poses a major threat to the native grassland ecosystems of the Palouse

Prairie of eastern Washington and northern Idaho [6]. Indaziflam appears to be an alternative control option for managing these two invasive annual grasses.

Indaziflam's significantly lower GR_{50} values compared to imazapic provides some evidence to support the idea that several years of residual control could be possible with indaziflam for these other winter annual grasses in a manner similar to what has been reported for downy brome [32]. Previous studies have shown differences in relative potency when comparing indaziflam and flumioxazin for kochia (*Kochia scoparia* L.) control; differences were attributed to variances in herbicide absorption and mode of action [46]. Indaziflam controls weeds as the primary root emerges from the seed, while ALS inhibitors must be absorbed by plant roots, translocated to meristematic regions, and then inhibit fatty acid production in the chloroplast.

Some of the tested winter annual grasses have shown differential responses to other herbicides. Downy brome, feral rye, and jointed goatgrass responded differently to imazamox [47]. The differential response of these species to imazamox was a result of differences in translocation, metabolism, or absorption. Jointed goatgrass was found to be the most susceptible to imazamox, while downy brome control was intermediate, and feral rye was the most tolerant [48]. Similarly, differences in herbicide absorption and mode of action between imazapic and indaziflam could be responsible for the difference in relative potency. Other contributing factors could be the herbicides water solubility and degradation by soil microbes (longer half-life in the soil). Indaziflam has a longer average soil half-life (>150 days) and lower water solubility (4.4 mg/L at pH=4 and 2.8 mg/L at pH=9) than imazapic (120 days, 2,200 mg/L). These characteristics in combination with different modes of action could be the major contributing

factors resulting in indaziflam's long-term residual winter annual grass control and increased phytotoxicity compared to imazapic [49].

It is well documented that invasive winter annual grasses continue to invade sagebrush and grassland ecosystems in the US , resulting in the displacement of native vegetation, reduction in quality wildlife habitat [2, 29, 37], decreased fire-return intervals [2, 4, 50, 51], and altered resistance and resilience of these native ecosystems [2]. Due to the magnitude of invasive winter annual grass infestations and the potential for further spread, new herbicidal modes of action should be considered. Indaziflam showed increased phytotoxicity compared to imazapic across all six species (Table 4.1, Figure 2). These data suggest that indaziflam is more biologically active than imazapic on these species and supports results from field studies (Sebastian et al. 2016).

It is possible that plants evaluated in the greenhouse are more susceptible to herbicide injury; therefore, further research is necessary to determine if these findings are reproducible under field conditions. Imazapic and indaziflam bioavailability have been shown to be affected by differences in soil properties and soil moisture [46, 52-54], so field studies should be conducted across the western US.

Additional studies should also evaluate indaziflam's impacts on annual grassland systems in regions such as California. Over the last few centuries, native perennial vegetation has significantly declined due to invasive species such as downy brome, medusahead, and yellow starthistle (*Centaurea solstitialis* L.)[55]. In California's coastal ranges, central valley, and Sierra Nevada foothills over 73% of the major invasive non-native species are winter annuals[55]. The current study showed that indaziflam controls a wide range of winter annual grasses; therefore,

studies should be conducted to evaluate the potential utility of indaziflam to convert these sites to native perennial bunchgrasses[56, 57].

The information presented in this study will be beneficial to land managers throughout the western US who are seeking new herbicides to control invasive winter annual grasses. These data suggest that indaziflam provides increased winter annual grass control at field application rates comparable to imazapic, and may provide residual control similar to previous studies conducted on downy brome[32, 43]. Additional field-scale research is necessary to evaluate indaziflam's potential for long-term control of other invasive winter annual grass. Areas infested by these invasive grasses are large and are continuing to spread (Figure 4.1). Land managers remain in need of better tools that can control multiple species, while still having the option to re-establish or protect native plant communities. This study provides the first evidence that indaziflam could control a suite of invasive winter annual grasses.

4.6 TABLES

Table 4.1. Imazapic and indaziflam rates resulting in 50 percent reduction in growth of six invasive winter annual grasses. Values were calculated using log-logistic regression. ($GR_{50} \pm SE$).

Invasive Winter Annual Grass	Imazapic GR_{50}[a] ($g \cdot ai \cdot ha^{-1}$)	Indaziflam GR_{50}[a] ($g \cdot ai \cdot ha^{-1}$)	Imazapic/ Indaziflam GR_{50} Ratio	P-value[b]
Downy Brome	2.71 ± 0.10	0.23 ± 0.07	11.78	<0.0001*
Feral Rye	24.37 ± 0.07	0.56 ± 0.06	43.52	<0.0001*
Japanese Brome	1.86 ± 0.08	0.19 ± 0.05	9.80	0.0004*
Jointed Goatgrass	13.96 ± 4.70	7.37 ± 3.58	1.89	0.6447
Medusahead	2.07 ± 0.12	0.36 ± 0.09	5.75	<0.0001*
Ventenata	7.08 ± 0.13	0.44 + 0.09	16.10	<0.0001*

[a]Herbicide dose resulting in 50% biomass reduction.

[b]Within each row, p-values comparing imazapic and indaziflam GR_{50} values (*significance according to Fisher's Protected LSD at the 5% level of probability).

4.7 FIGURES

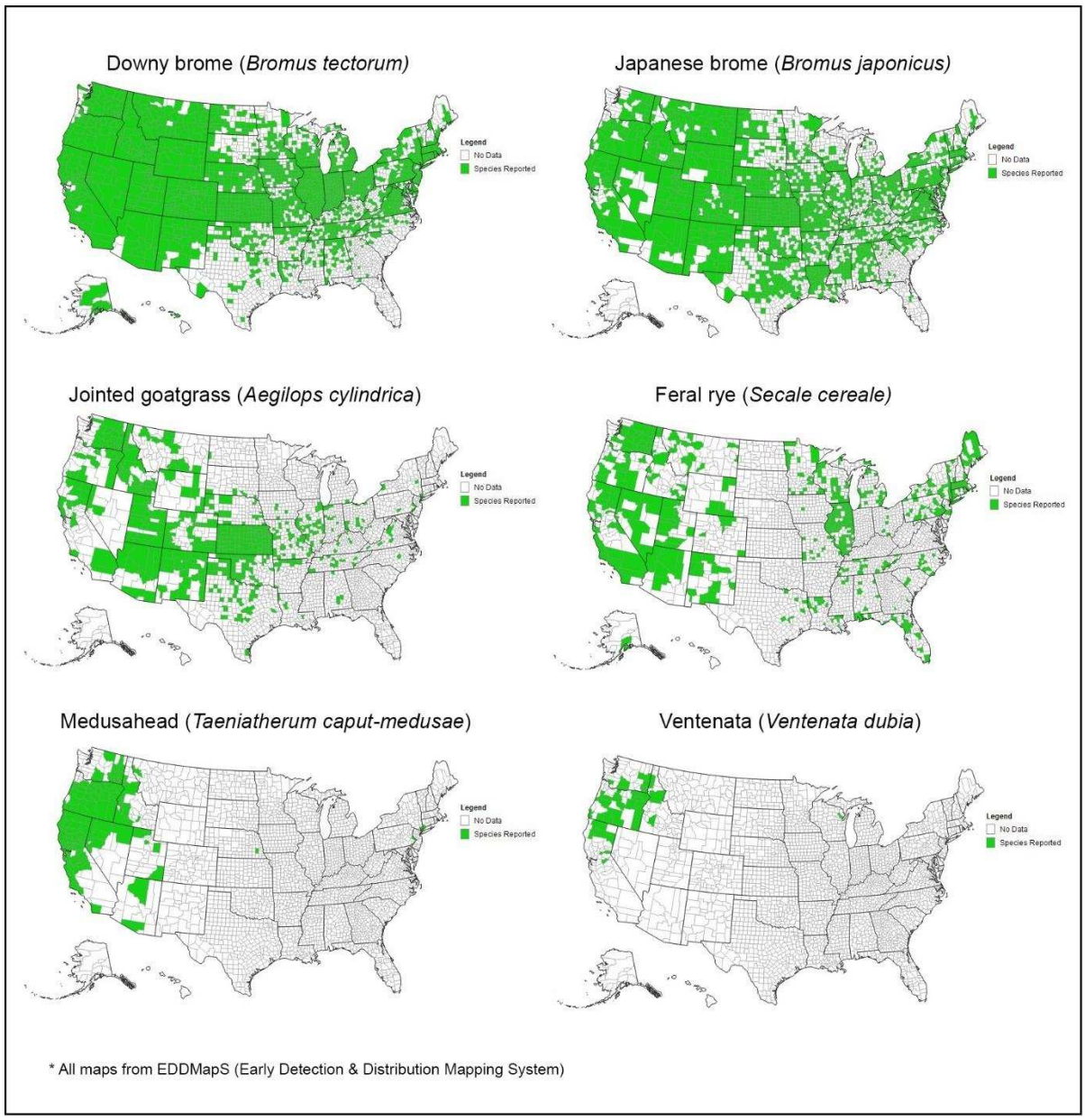

Figure 4.1. US Distribution of the six invasive winter annual grasses evaluated in this study. Maps were taken from the EDDMapS (Early Detection and Distribution Mapping System, https://www.eddmaps.org/distribution/).

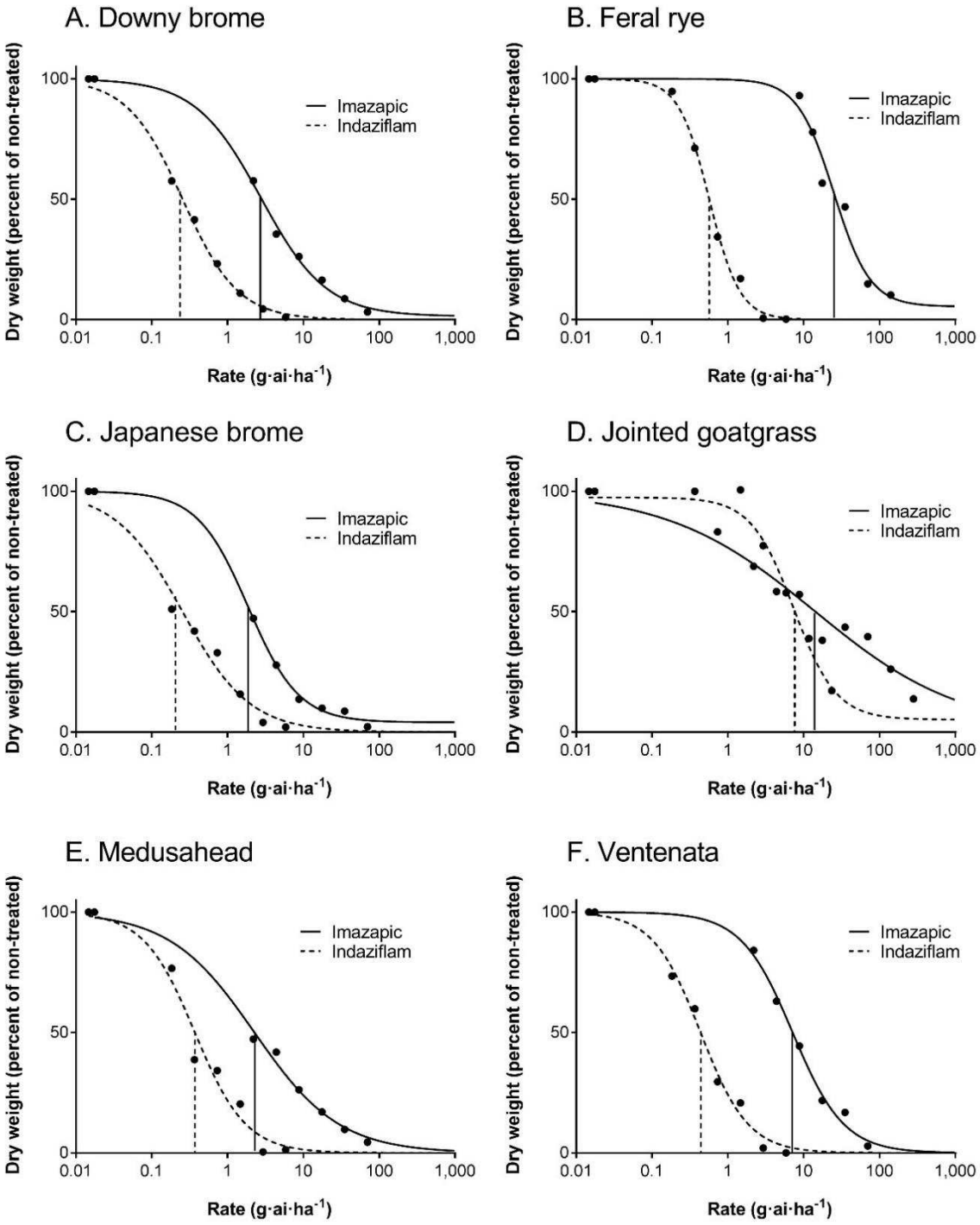

Figure 4.2. Response of (A) downy brome, (B) feral rye, (C) Japanese brome, (D) jointed goatgrass, (E) medusahead, and (F) ventenata to imazapic and indaziflam. Dose response curves were fit using four parameter log-logistic regression. Mean values of six replications are plotted. Vertical lines represent the herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) for each species and herbicide.

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CHAPTER 5: PRE-EMERGENCE CONTROL OF NINE INVASIVE WEEDS WITH AMINOCYCLOPYRACHLOR, AMINOPYRALID, AND INDAZIFLAM[1]

SUMMARY[**]

There are an estimated 400 million ha of non-cropland in the US primarily designated as rangeland and pastureland and there are over 300 invasive weeds found on these sites causing an estimated annual loss of $5 billion. Among the most invasive and problematic weeds are Dalmatian toadflax, diffuse knapweed, downy brome, and musk thistle. Currently, herbicides are the most common management strategy for broadleaf weeds and invasive winter annual grasses. Indaziflam, a new herbicide for invasive plant management in non-crop areas, is a cellulose-biosynthesis inhibitor capable of providing residual invasive winter annual grass control up to 3 years after treatment (YAT). A field experiment was conducted to determine if indaziflam tank-mix-treatments applied at two preemergence (PRE) timings provided longer residual Dalmatian toadflax and downy brome control than previously recommended herbicides (aminocyclopyrachlor, imazapic, picloram) applied without indaziflam. Indaziflam tank-mix treatments provided increased Dalmatian toadflax (84 to 91%) and downy brome (89 to 94%) control 4 YAT. Treatments without indaziflam controlled 50 to 68% of Dalmatian toadflax and <25% downy brome 4 YAT. Based on these results, a greenhouse dose-response experiment was conducted with aminocyclopyrachlor, aminopyralid, and indaziflam to compare the preemergence control of nine invasive weeds commonly found in non-crop areas. Averaged across species, indaziflam was 29- and 52-times more active compared to aminocyclopyrachlor and aminopyralid, respectively. These data suggest that indaziflam could be used for residual

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control of invasive weeds in non-crop areas, as a tank-mix partner with other foliar applied broadleaf herbicides.

MANAGEMENT IMPLICATIONS

Native plant communities that provide wildlife habitat and important ecosystem services are negatively impacted by invasive weeds. Many of these invasive weeds are prolific seed producers, which makes the soil seed bank the primary mechanism responsible for rapid re-establishment. Long-term control of many weed species has been difficult due to limited management options and budget constraints. Short-term control does not provide the time necessary for the re-establishment of the native plant community so there is often an open niche for re-establishment or secondary invasions to occur. Although herbicides are a commonly used management strategy, there are limited herbicide options that provide the long-term control necessary to deplete the soil seed bank of invasive weed seed and allow recovery of co-occurring desired species. An herbicide with residual activity would be desirable for control of germinating seedlings, and while aminocyclopyrachlor, aminopyralid, and picloram have residual activity, their residual activity is less than indaziflam. The results presented here provide evidence that indaziflam could be used alone or in combination with broadleaf herbicides to potentially extend control up to 4 YAT. For invasive winter annual grasses such as downy brome, indaziflam could be applied alone preemergence; however, having limited post-emergence activity, indaziflam would need to be used in combination with other broadleaf herbicides to control actively growing rosettes in the fall or spring. Indaziflam's residual activity could provide the necessary time for desired co-occuring species to re-establish. Indaziflam represents an interesting opportunity to influence rangeland plant community assembly in areas affected by invasive species that take over native rangelands primarily by their high propagule

pressure. Indaziflam could be used in conjunction with other methods to shift the advantage from exotic invaders with high propagule pressure back toward the natives and other desirable vegetation. Because indaziflam is a unique mode of action (cellulose biosynthesis inhibitor) for non-crop weed management, combining indaziflam with other modes of action in a single treatment could also be used for resistance management. Although additional research is necessary to verify these findings under field conditions, results from these studies directly support our previous indaziflam work with downy brome [2].

INTRODUCTION

Invasive weed management in non-crop areas (primarily rangeland and pastureland) remains a significant challenge throughout the US [3-7]. Rangeland and pastures comprise about 42% (400 million ha) of the total land area in the US and in these areas, invasive plants can cause an estimated loss of $5 billion annually [8]. Cultural practices contributing to the establishment and spread of these invasive plants include over grazing by domestic livestock, purposeful introduction for agriculture and horticulture, unintentional introduction via contaminated seed, and climate change [9, 10].

Invasive weeds that infest rangeland and other non-crop areas can have significant negative ecological impacts including depleting soil moisture and nutrients, reducing forage production, reducing plant diversity and community productivity, altering fire frequency, and reducing the value of recreational land [9, 11-14]. Invasive weeds are frequently designated as noxious because of these impacts. Many of these invasive plants are prolific seed producers and exert very high propagule pressures on invaded sites. Propagules can spread by multiple dispersal mechanisms including mechanical (vehicles and contaminated machinery), wildlife and

livestock (ingested or coat hair entanglement), and human recreation [15]. Once established, several noxious weeds have extensive taproot systems allowing them to extract moisture and nutrients from deep within the soil profile [16, 17]. This can result in rapid shifts in the dominant native plant communities [18].

Of the over 300 rangeland weeds in the US, downy brome (*Bromus tectorum* L.) and Dalmatian toadflax (*Linaria dalmatica*) have emerged as two of the most wide-spread and problematic, with average annual spread rates of 14% and 19%, respectively [7, 9, 16]. Disturbance favors these particular invasive plants so they commonly invade degraded areas such as roadsides, abandoned lots and crop fields, gravel pits, clearings, and overgrazed rangeland [19]. Downy brome, an invasive winter annual grass, has rapidly spread throughout many regions of the US displacing native vegetation and altering fire frequency and intensity [13, 14, 20]. Duncan et al. [7] estimated that over 22 million hectares of the western United States are infested with downy brome. Unlike downy brome, Dalmatian toadflax is a short-lived herbaceous perennial plant [21]. This species has escaped cultivation and is most commonly found in semi-arid areas, on course textured, gravelly soils [21, 22]. It is a self-incompatible species contributing to its high level of genetic variability [23, 24]. Dalmatian toadflax produces large amounts of seed that can remain viable in the soil for approximately 10 years [22]. Once established, this high seed production along with aggressive vegetative propagation enables Dalmatian toadflax to spread rapidly and to dominate and persist [23]. Other invasive broadleaf weeds in non-crop areas resulting in major economic and ecological impacts include diffuse knapweed (*Centaurea diffusa* Lam), musk thistle (*Carduus nutans* L.), curly dock (*Rumex crispus* L.), common mullein (*Verbascum thapsus* L.), halogeton (*Halogeton glomeratus* (M. Bieb.) C.A. Mey.), marestail (*Conyza*

canadensis (L.) Cronquist), and common teasel (*Dipsacus fullonum* L.) [7, 16, 25]. There are currently limited management options that provide long-term control of these weeds.

Among the available control strategies for invasive weed control in non-crop areas (mechanical, cultural, biological, and chemical), herbicides are the primary method [4, 16]. Synthetic auxin or growth regulator herbicides such as aminocyclopyrachlor (Method, Bayer CropScience), aminopyralid (Milestone, Dow AgroSciences), and picloram (Tordon, Dow AgroSciences) are commonly recommended residual broadleaf herbicides, while imazapic (Plateau, BASF) has been the primary herbicide for downy brome control because it has some residual activity, and is relatively selective at low use rates [4, 5, 26]. Several other herbicides including glyphosate and rimsulfuron have been used for short-term downy brome control [5]. None of these herbicides have provided long-term control of invasive weeds when used alone, resulting in rapid re-infestations [9, 27, 28].

Lack of residual control and resulting seedling recruitment could be attributed to the chemical properties of these herbicides [28]. The average water solubility and Log K_{ow} (pH 7) of aminocyclopyrachlor, aminopyralid, imazapic, and picloram are 4,200 mg L^{-1} (-2.48), 207,000 mg·L^{-1} (-2.87), 2,200 mg L^{-1} (0.01), and 200,000 mg L^{-1} (1.18), respectively. Because these herbicides are highly water soluble, their leaching potential is high, ultimately decreasing the herbicide concentration available in the soil solution for plant uptake beyond the initial year of application [29]. A study conducted by Oliveira et al. [29] also showed desorption hysteresis with aminocyclopyrachlor and picloram, suggesting that the small amount of herbicide sorbed is resistant to desorption and irreversibly bound to soils.

Another factor to consider for long-term control of invasive plants is the soil seed bank. The longevity of weed seeds in the soil for the species listed above are all >2 years [22, 30]. Therefore, new herbicides should be evaluated that have a decreased leaching potential, and provide the soil residual control necessary to deplete the soil seed bank. Residual control for multiple growing seasons would also provide native perennial plants a competitive advantage for re-establishment [9, 25, 31].

Indaziflam (Esplanade, Bayer CropScience) is a new herbicide with the potential to provide residual control of germinating seeds of annual, biennial, and perennial weeds. Previously, indaziflam has been used primarily for total vegetation management, weed control in turf, established citrus, grape, and tree nut crops [32-35]. Indaziflam is a cellulose-biosynthesis inhibitor (CBI) [36, 37], representing a unique mode of action for non-crop areas with residual soil activity and broad spectrum preemergence (PRE) control [2, 38, 39]. As previously mentioned, the range of water solubility (2,200 to 207,000 mg L^{-1}) and log K_{ow} (-2.87 to 1.18) values of aminocyclopyrachlor, aminopyralid, imazapic, and picloram results in herbicide dilution in the soil profile and short-term soil residual activity; however, indaziflam is more lipophilic with a water solubility of 3.6 mg L^{-1} and log K_{ow} of 2.8 (pH7). The recommended non-crop use rates are relatively low for indaziflam (73 to 102 g ai ha^{-1}), and comparable with imazapic (70 to 123 g ai ha^{-1}), aminocyclopyrachlor (70 to 140 g ae ha^{-1}), and aminopyralid (53 to 123 g·ae·ha^{-1}); however, picloram is recommended at higher use rates (140 to 1,121 g·ae·ha^{-1}). Indaziflam's residual downy brome (*Bromus tectorum* L.) control was evaluated by Sebastian *et al.* [2] and indaziflam treatments provided better residual downy brome control 2 and 3 YAT compared to imazapic, glyphosate, and rimsulfuron. Indaziflam has not previously been evaluated for PRE control of other noxious weeds for use in non-crop areas. Indaziflam is currently restricted to

sites not grazed by domestic livestock; however, Bayer CropScience is currently conducting the studies necessary to establish the grazing tolerance (personal communication; David Spak, Bayer CropScience, Research Triangle Park, NC.).

Based on previous field and greenhouse research, indaziflam appears to have several attributes that could be used to enhance invasive plant management; therefore, a field study was established to determine if tank-mix treatments combined with indaziflam provided longer residual Dalmatian toadflax and downy brome control than aminocyclopyrachlor, imazapic, and picloram applied alone. This would corroborate results presented by Sebastian et al. [2] that indaziflam applied alone increased residual downy brome control, while further evaluating the residual control on the seedlings of an additional invasive weed, Dalmatian toadflax. The second objective of this study was to conduct a greenhouse bioassay to compare the pre-emergence control of nine additional weeds found on rangeland and other non-crop areas with aminocyclopyrachlor, aminopyralid, and indaziflam. These three herbicides all have relatively low recommended field use rates; therefore, this experiment allowed us to directly compare pre-emergence control of the nine species evaluated.

MATERIALS AND METHODS

Herbicide Efficacy Field Trial and Experimental Design. In 2010, a field trial was conducted to evaluate the effectiveness of herbicides for long-term downy brome and Dalmatian toadflax control. The experiment was conducted at only one site; however, the results provide the framework for the subsequent greenhouse experiment. The field experiment was located in Longmont, CO (lat 40°14'57.53"N, long 105°12'35.46"W) on Rabbit Mountain Open Space, the easternmost point of the foothills in Boulder County. The canopy cover of actively growing

downy brome and Dalmatian toadflax at peak standing crop was approximately 85% and 30%, respectively. Before herbicide application (June and August 2010) perennial grasses (<10% canopy cover) included primarily western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love), and native forbs and sub-shrubs (~20% canopy cover) included Louisiana sage (*Artemisia ludoviciana* Nutt.), fringed sage (*Artemisia frigida* Willd.), common sunflower (*Helianthus annuus* L.), sulphur-flower buckwheat (*Eriogonum umbellatum* Torr.), and hairy goldenaster (*Heterotheca villosa* (Pursh) Shinners). The soil at the study site was Baller sandy loam (loamy-skeletal, mixed, superactive, mesic Lithic Haplustolls), with 1.5% organic matter in the top 20 cm [40]. The average elevation was 1,725 m (5,660 ft). Mean annual precipitation based on the 30-yr average (1981-2010) was 363 mm and the mean annual temperature was 9.1 C [41]. Precipitation was close to the 30-yr average in 2010, 2011, and 2014. A statewide-drought occurred in 2012 and average total precipitation decreased 134 mm; however, in 2013, the site received an additional 110 mm above the 30-yr average [42].

Herbicides were applied in the summer at two application timings; June 20, 2010 when Dalmatian toadflax was in the flowering growth stage and August 11, 2010 during Dalmatian toadflax regrowth. These two application timings (June and August 2010) were both before downy brome emergence (PRE). The 13 herbicide treatments (including a non-treated control) were applied to 3 by 9 m plots arranged in a randomized complete block design with four replications, and are listed in Table 1. All treatments were applied with a CO_2-pressurized backpack sprayer using 11002LP flat fan nozzles at 187 $L \cdot ha^{-1}$ at 207 kPa. All treatments included 1% $v \cdot v^{-1}$ methylated seed oil.

Visual percent control evaluations were conducted in June of each year (2011-2014). Control evaluations were estimated by comparing visual estimates of Dalmatian toadflax and downy brome cover in the treated plots (using the entire 3 by 9 m plot area) compared with the non-treated plots. Plots with 0% canopy cover received a 100% control rating, while conversely, plots with 100% canopy cover received a 0% control rating.

Greenhouse Experiment: Comparing Aminocyclopyrachlor, Aminopyralid, and Indaziflam Preemergence Weed Control. Based on the results of the field experiment, we designed a greenhouse experiment to determine if the extended control of Dalmatian toadflax and downy brome provided by indaziflam in the field was due to increased residual seedling control. This experiment was designed to compare indaziflam's pre-emergence efficacy to the currently recommended herbicides (aminocyclopyrachlor and aminopyralid) for annual, biennial, and perennial weed control in non-crop areas. Aminopyralid was used in this greenhouse bioassay in place of picloram because the average recommended use rate for indaziflam is comparable to the average aminopyralid use rate. This allowed for direct comparisons between herbicides on an active ingredient basis for aminopyralid, aminocyclopyrachlor, and indaziflam.

For the greenhouse bioassay, seeds were planted at a constant depth of 0.5 cm in 13- by 9- by 6-cm plastic containers, filled with an Otero sandy clay loam field soil (Coarse-loamy, mixed (calcareous), mesic Aridic Ustorthents) with 3.9% OM and pH 7.7. Seeding densities were adjusted based on germinability to reach a target density of 40 plants/pot. Plants were maintained in a greenhouse with a 25/20°C day/night temperature with natural light supplemented with high-intensity discharge lamps to give a 15-h photoperiod. Plants were sub-irrigated as needed and misted overhead daily to reduce soil crusting.

The greenhouse experiment was a completely randomized design with a factorial of seven herbicide rates and a non-treated control with three replicates per treatment. The experiment was conducted 10-December 2016 and repeated 16-February 2016. A preliminary greenhouse study was conducted for each herbicide and species to determine a range of doses that would best fit a logistic regression. It is not unusual for both preemergence and postemergence herbicides to provide control at lower than labeled rates in the greenhouse with ideal environmental conditions, so it was not surprising to us that herbicide doses for the regression analysis were much lower than recommended field use rates. Rates used in the dose-response are listed in Table 2. Herbicides were applied preemergence using a Generation III research track sprayer (DeVries Manufacturing, Hollandale, MN) equipped with a TeeJet 8002 EVS flat-fan spray nozzle (TeeJet Spraying Systems Co., Wheaton, IL) at 187 $L \cdot ha^{-1}$ at 172 kPa.

Plants were harvested at the soil surface approximately 4 to 5 WAT depending on the growth stage of each species. Weights were recorded after samples were dried for 5 d at 60 C. Percent dry weight reduction was calculated relative to the non-treated control plants for each treatment.

Data Analysis. For the herbicide efficacy field experiment, repeated measures analysis of variance (ANOVA) was used to determine the effects of herbicide treatments on long-term Dalmatian toadflax and downy brome control. Percent control data were first analyzed in SAS 9.3 using Proc MIXED, with year after treatment defined as the repeated measure [43]. A Tukey-Kramer adjustment was performed and factors included in the model were treatment, timing, year, and all possible interactions. Dalmatian toadflax and downy brome control response variables were analyzed separately, and main effects and interactions were tested at the $\alpha = 0.05$ significance level. Before analysis, all response variables were arcsine square root-transformed

to meet the assumption of normality. To determine herbicide impacts on residual Dalmatian toadflax and downy brome control, the significant treatment-by-year interaction was evaluated using the Proc GLIMMIX method and the LINES statement. This provided comparisons of least squares means across years ($P \leq 0.05$). Non-transformed means are presented in all figures.

Data from the greenhouse dose-response experiment were first analyzed using the PROC MIXED method in SAS 9.3 with treatment as a fixed effect and experiment and replicate as random effects [43]. Based on a non-significant homogeneity of variance (ANOVA) and experiment-by-herbicide rate interaction, results from the repeated experiments were pooled. The treatment effect was significant, therefore, nonlinear regression in Graphpad Prism 7.00 (GraphPad Software, La Jolla California USA, www.graphpad.com) was used to describe the response of the nine weed species to aminocyclopyrachlor, aminopyralid, and indaziflam. The herbicide concentrations resulting in 50% reduction in plant biomass (GR_{50}) compared to the non-treated control were determined for each invasive weed species using four-parameter log-logistic regression. The equation used to regress herbicide concentration with percent reduction in plant dry biomass as compared to the non-treated control was:

$$Y = C + \left[\frac{(D - C)}{1+10^{(\mathrm{Log}GR_{50} - X)\cdot b}}\right] \quad [1]$$

where *C* and *D* represent the lower and upper limits of the dose-response curve, respectively, and *b* represents the slope of the best-fitting curve through the GR_{50} value. For curve fitting and GR_{50} estimation, the model was constrained to a maximum of 100 and minimum of 0. Mean separation of herbicide GR_{50} values were analyzed by Fisher's Protected LSD test at the 5% level of probability. The average recommended use rate for indaziflam ranges from 83 to 94% (73 and 102 g ai ha^{-1}) of the average recommended aminocyclopyrachlor (70 to 140 g ae ha^{-1}) and

aminopyralid (53 to 123 g·ae·ha^{-1}); therefore, pre-emergence control was compared directly using GR_{50} estimates.

RESULTS AND DISCUSSION

Field Experiment.

Dalmatian Toadflax Control. At both application timings (June and August), the significant treatment-by-year interaction (P<0.001) was evaluated (Figure 5.1). All herbicide treatments except imazapic provided similar Dalmatian toadflax control 1, 2, and 3 YAT. The only treatments providing residual Dalmatian toadflax control above 80% 4 YAT were treatments including indaziflam (Figure 5.1). At the June and August application timings, aminocyclopyrachlor alone provided 50% and 55% Dalmatian toadflax control, while control with picloram was 68% and 64% 4 YAT, respectively. These same treatments tank-mixed with indaziflam resulted in 84 to 91% Dalmatian toadflax control 4 YAT. A previous study conducted by Sebastian et al. [28] illustrated the importance of residual weed seedling control following the initial year of application. Dalmatian toadflax control with aminocyclopyrachlor was 90 to 97% 1 YAT; however, seedlings appeared in plots as early as 15 MAT, and there was limited control of those individuals (4 to 26%) 2 YAT. Without residual weed seedling control invasive weeds such as Dalmatian toadflax are able to re-establish via the soil seed bank.

Downy Brome Control. The treatment-by-year interaction (P<0.001) was more pronounced for downy brome than with Dalmatian toadflax, and there was no effect of application timing on herbicide efficacy (P=0.830). Compared to the non-treated plots, downy brome control with imazapic and indaziflam treatments were statistically similar at P<0.05 (84 to 99%) 1 YAT; however, residual downy brome control was greatly reduced for imazapic alone 2

YAT (61 to 64%). By 2014 (4 YAT), the downy brome population had recovered via the soil seed bank and imazapic control was less than 25% (Figure 5.1). Indaziflam treatments, however, provided significantly greater residual downy brome control 3 (91 to 96%) and 4 YAT (89 to 94%), compared to treatments not including indaziflam.

Indaziflam's soil residual properties combined with the results from this and other similar field experiments [2, 39] provide evidence that indaziflam used in combination with commonly recommended broadleaf herbicides (e.g. aminocyclopyrachlor and picloram), could significantly decrease the soil seed bank of annual and biennial species such as downy brome and Dalmatian toadflax. This could greatly decrease weed seedling pressure in the years following initial treatments, providing the time necessary to facilitate the recovery of co-occurring species [44, 45]. Reducing yearly applications to potentially every 4 years as these data suggest, would decrease herbicide costs, reduce the total amount of herbicide applied, minimize non-target impacts, and reduce the potential of artificially shifting the native plant community with annual herbicide treatments [16].

Results from our field experiment established that indaziflam's control of germinating seeds provided residual Dalmatian toadflax and downy brome control 4 YAT. Based on these data, we hypothesized that indaziflam may also provide residual control of many other invasive weeds found in non-crop areas. This field experiment was used as a foundation for the subsequent greenhouse bioassay comparing the pre-emergence control of aminocyclopyrachlor, aminopyralid, and indaziflam.

Greenhouse Experiment. Dalmatian toadflax and downy brome control with aminocyclopyrachlor, aminopyralid, and indaziflam are presented in Figure 5.2. The GR_{50} estimates for downy brome showed that indaziflam was 125- and 99-times more active compared

to aminocyclopyrachlor and aminopyralid, respectively (P<0.0001, Table 5.3). Similarly, indaziflam was 19- and 247-times more active on Dalmatian toadflax pre-emergence compared to aminocyclopyrachlor and aminopyralid, respectively (P<0.0001, Table 5.3). This is conformational evidence for the cause of extended weed control with indaziflam under field conditions for Dalmatian toadflax and downy brome compared to treatments without indaziflam (Figure 5.1).

The response of the seven remaining weed species to aminocyclopyrachlor, aminopyralid, and indaziflam are presented in Figure 2, and GR_{50} estimates are found in Table 5.3. Indaziflam was 106- (P<0.0001), 4- (P<0.0001), 9- (P=0.0012), and 5-times (P<0.0001) more active than aminopyralid on common mullein, diffuse knapweed, halogeton, and marestail, respectively; however, these two herbicides had similar activity on curly dock (P=0.3421) and musk thistle (P=0.8674) (Table 5.3). Aminopyralid was 2- and 9-times more active (lower GR_{50}) on common teasel compared to indaziflam and aminocyclopyrachlor, respectively (P<0.0001) (Table 5.3). Compared to aminocyclopyrachlor across all nine species, indaziflam was 3- to 145-times more active (P<0.0001, Table 5.3).

Averaging across all nine species, indaziflam was 29- and 52-times more active then aminocyclopyrachlor and aminopyralid, respectively. This indicates that indaziflam appears to provide increased seedling control of these invasive species compared to commonly recommended broadleaf herbicides. These data are consistent with the idea that the long-term residual control by indaziflam observed in the field (Figure 5.1) could be due to less dilution in the soil profile and increased relative potency [46-48] as compared to other broadleaf herbicides such as aminocyclopyrachlor and aminopyralid. Indaziflam could be tank-mixed with other herbicides commonly used for non-crop weed management (2,4-D, chlorsulfuron, clopyralid,

dicamba, glyphosate, imazapyr, metsulfuron, triclopyr). This could extend weed control beyond the initial year of application, and provide multiple modes of action in a single application as a tool for resistance management [49]. Indaziflam has limited postemergence activity so, tank-mixing with herbicides evaluated in this study and those listed above would be needed to control established weeds. Indaziflam could then provide the residual activity necessary to control germinating seedlings that appear as early as the year after initial herbicide application [28].

Tank-mixing indaziflam with the suite of primarily broadleaf herbicides provides land managers with an opportunity to consider managing the soil seed bank of invasive weeds in non-crop areas. This would likely provide the necessary time for co-occuring species to respond with increased abundance, increasing the overall resistance and resilience of the dominant native plant community [50]. Integrating indaziflam with other mechanical, cultural, and biological tools could also greatly increase the success of long-term management programs [16]. Further tolerance studies should be conducted to determine any potential non-target impacts. In addition, the impact of indaziflam on long-term control of these key invasive weeds needs to be evaluated under field conditions and compared to treatments without indaziflam.

5.6 TABLES

Table 5.1. Herbicides and rates applied in evaluating the dose-response of eight annual, biennial, and perennial weed species.

Common name	Trade name	Rates applied[a] (g ai ha^{-1})	Application timing[b]
Aminocyclopyrachlor	Method	57	June 2010
Imazapic	Plateau	105	June 2010
Picloram	Tordon	227	June 2010
Aminocyclopyrachlor + Indaziflam	Method + Esplanade	57 + 58	June 2010
Picloram + Indaziflam	Tordon + Esplanade	227 + 58	June 2010
Aminocyclopyrachlor	Method	57	August 2010
Imazapic	Plateau	105	August 2010
Picloram	Tordon	227	August 2010
Aminocyclopyrachlor + Indaziflam	Method + Esplanade	57 + 58	August 2010
Picloram + Indaziflam	Tordon + Esplanade	227 + 58	August 2010
Aminocyclopyrachlor + Imazapic	Method + Plateau	57 + 105	August 2010
Picloram + Imazapic	Tordon + Plateau	227 + 105	August 2010

[a] All treatments included 1% v v^{-1} methylated seed oil.
[b] At the June 2010 and August 2010 application timings, Dalmatian toadflax was in the flowering and re-growth stages, respectively, while both application timings were preemergence for downy brome.

Table 5.2. Species, herbicides, and rates applied in greenhouse studies evaluating the dose-response of nine annual, biennial, and perennial weed species.

Common name	Scientific name	Rates applied (g ai ha^{-1})		
		Aminocyclopyrachlor	Aminopyralid	Indaziflam
Common mullein	*Verbascum thapsus*	0, 9, 18, 35, 70, 140, 210, 280	0, 1.8, 3.5, 7, 14, 28, 56, 112	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Common teasel	*Dipsacus fullonum*	0, 1, 2, 4, 9, 18, 35, 70	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Curly dock	*Rumex crispus*	0, 2, 4, 9, 18, 35, 70, 140	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Dalmatian toadflax	*Linaria dalmatica*	0, 1, 2, 4, 9, 18, 35, 70	0, 1.8, 3.5, 7, 14, 28, 56, 112	0, 0.05, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9
Diffuse knapweed	*Centaurea diffusa*	0, 4, 9, 18, 35, 70, 140, 280	0, 1.8, 3.5, 7, 14, 28, 56, 112	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Downy brome	*Bromus tectorum*	0, 9, 18, 35, 70, 140, 280, 560	0, 3.5, 7, 14, 28, 56, 112, 224	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7
Halogeton	*Halogeton glomeratus*	0, 2, 4, 9, 18, 35, 70, 140	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9
Marestail	*Conyza Canadensis*	0, 0.5, 1, 2, 4, 9, 18, 35	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.1, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9
Musk thistle	*Carduus nutans*	0, 1, 2, 4, 9, 18, 35, 70	0, 0.9, 1.8, 3.5, 7, 14, 28, 56	0, 0.2, 0.4, 0.7, 1.5, 2.9, 5.9, 11.7

[a] All treatments were applied pre-emergence.

Table 5.3. Aminocyclopyrachlor, aminopyralid, and indaziflam rates resulting in 50 percent growth reduction of nine common invasive weeds found on non-cropland. Values were calculated using log-logistic regression[b]

Weed (common name)	GR_{50}[a] (g ai ha^{-1})			GR_{50} ratio	
	Aminocyclopyrachlor ($g \cdot ai \cdot ha^{-1}$)	Aminopyralid ($g \cdot ai \cdot ha^{-1}$)	Indaziflam ($g \cdot ai \cdot ha^{-1}$)	Aminocyclopyrachlor/ Indaziflam	Aminopyralid/ Indaziflam
Common mullein	3.05 b	7.45 c	0.07 a	44.57	106.43
Common teasel	6.89 c	0.75 a	1.33 b	5.18	0.56
Curly dock	21.3 b	1.25 a	1.10 a	19.36	1.14
Dalmatian toadflax	1.16 b	14.8 c	0.06 a	19.33	246.67
Diffuse knapweed	6.20 c	2.50 b	0.58 a	10.69	4.31
Downy brome	56.4 b	38.5 b	0.39 a	144.62	98.72
Halogeton	1.04 b	3.11 c	0.36 a	2.89	8.64
Marestail	2.09 c	0.80 b	0.17 a	12.29	4.71
Musk thistle	1.25 b	0.31 a	0.33 a	3.79	0.94

[a] Herbicide dose resulting in 50% dry biomass reduction.
[b] GR_{50} values within each weed (row) followed by the same lower case letter are not significantly different at the 5% level of probability.

5.7 FIGURES

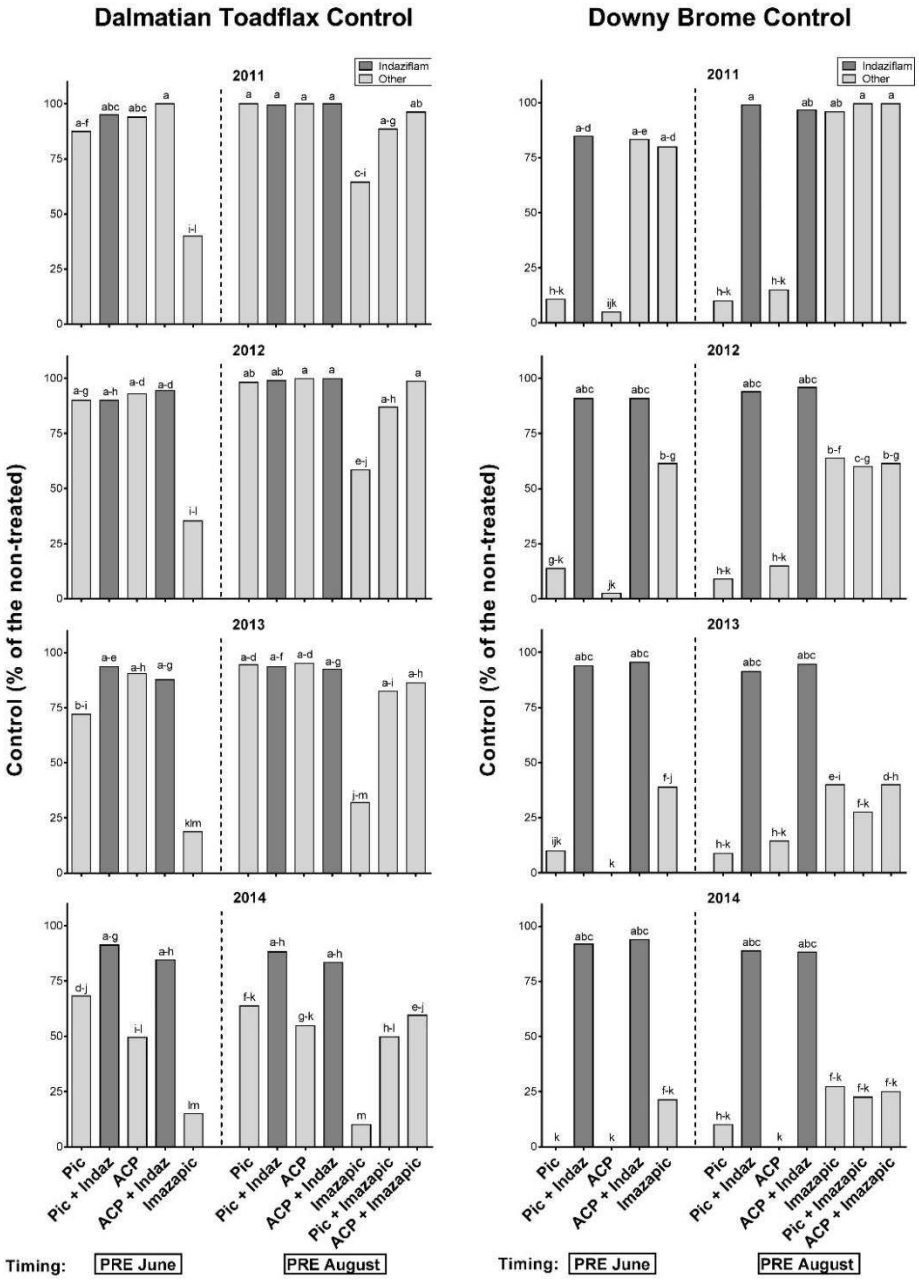

Figure 5.1. Dalmatian toadflax and downy brome control represented as a percent of non-treated plots 1, 2, 3, and 4 YAT. Application timings were June and August 2010. At the June and August application timings, Dalmatian toadflax were in the flowering and re-growth stages, respectively; however, both timings were prior to downy brome emergence (PRE). Letters indicate differences among herbicide treatments across both timings and years, using least squares means ($P < 0.05$). Herbicide treatment rates are as follows: aminocyclopyrachlor (ACP, 57 g·ai·ha^{-1}), imazapic (105 g·ai·ha^{-1}), indaziflam (Indaz, 58 g·ai·ha^{-1}), picloram (Pic, 227 g·ai·ha^{-1}), non-treated.

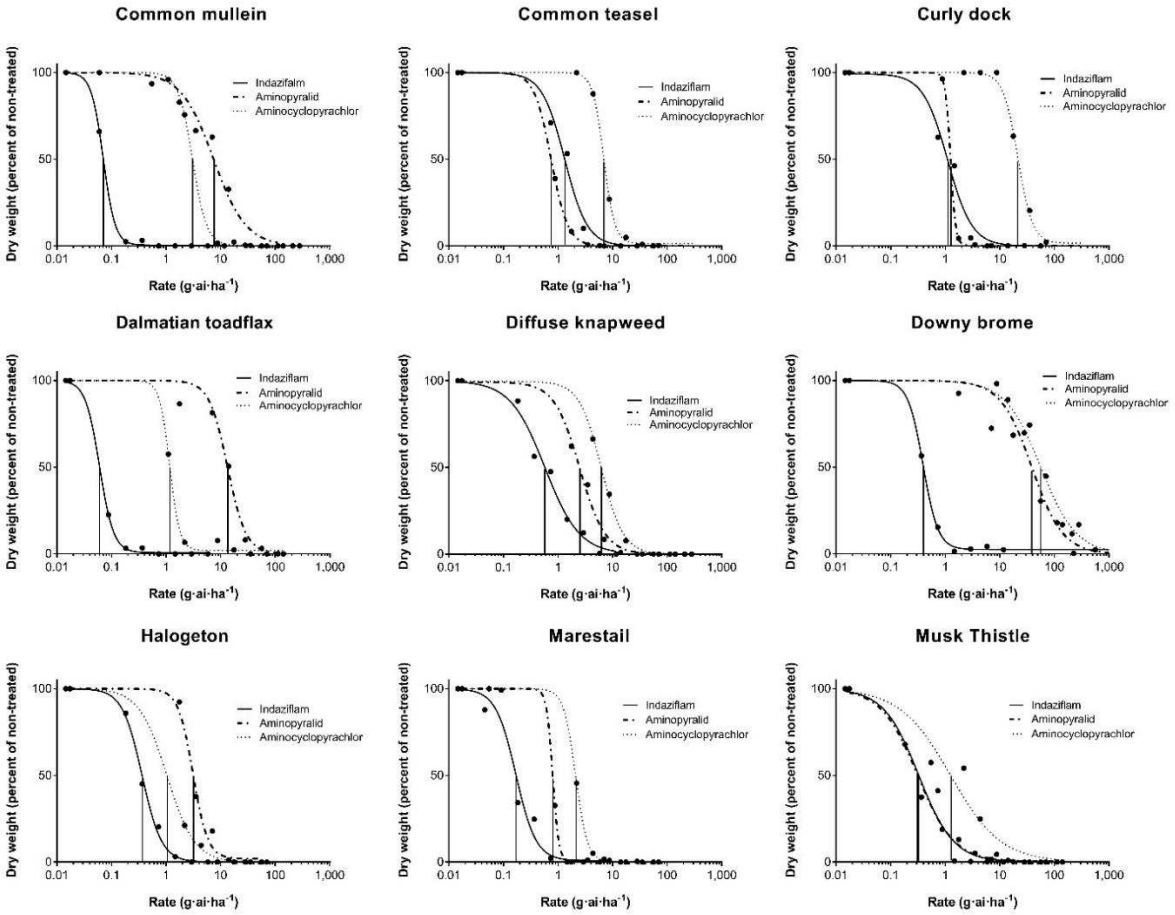

Figure 5.2. Response of nine invasive species found in non-crop areas to aminocyclopyrachlor, aminopyralid, and indaziflam. Dose response curves were fit using four parameter log-logistic regression. Mean values of six replications are plotted. Vertical lines represent the herbicide dose resulting in 50% reduction in dry biomass (GR_{50}) for each species and herbicide.

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