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Responses of *Ventenata dubia* and other species seven years after herbicide applications to an improved pasture in Montana

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Research Article

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Abstract

We evaluated herbicides for controlling the annual grass ventenata [*Ventenata dubia* (Leers) Coss.], with particular interest in indaziflam, a preemergence cellulose biosynthesis inhibitor. In 2016, indaziflam was applied postemergence alone and in mixture with glyphosate, imazapic, propoxycarbazone-sodium, or rimsulfuron to an improved pasture in southwestern Montana. A non-sprayed control was included for comparison purposes. Canopy cover of each species was assessed annually for 7 yr; cover was grouped by life-form and longevity, and species richness was calculated. Five years (2021) after treatment, the seedbank was assessed. Our results indicated that treatments including indaziflam reduced *V. dubia* cover 1 to 3 yr and even up to 6 yr after application, with *V. dubia* cover being zero or close to zero. However, at 7 yr (2023) after treatment, *V. dubia* was low across all treatments, including the non-sprayed control. Perennial grasses and forbs and annual forbs were generally unaffected by any treatment and did not increase in cover over the 7 yr, even though *V. dubia* decreased. Two years after treatment, species richness was lowest in treatments that included indaziflam, but at 7 yr, species richness was similar across all treatments. Indaziflam depleted the monocot and dicot seedbank, with fewer than 5 seedlings of any species emerging from treatments that included indaziflam, while other treatments resulted in 60 to 165 seedlings per sample (40 cm^3 of soil). In summary, at our study site, a single application of indaziflam controlled *V. dubia* for 6 yr, appeared to deplete the seedbank at 5 yr, and cover of perennial and annual vegetation and species richness was unaffected. By the end of the study, though, *V. dubia* cover appeared to be influenced by factors other than herbicide treatments, possibly variable precipitation over time, an exclusion of grazing, and competitive perennial grasses dominating the site.

Introduction

Non-native annual grasses, for example, downy brome (*Bromus tectorum* L.) and ventenata [*Ventenata dubia* (Leers) Coss.], are associated with decreases in livestock and wildlife forage (Hart and Mealor 2021) and native species diversity (Jones et al. 2020) on rangeland in the western United States. Herbicides can be used to minimize impacts from annual grass invasions and promote desired vegetation (Monaco et al. 2017). Several herbicides labeled for rangeland use, including the active ingredients glyphosate, imazapic, rimsulfuron, and sulfosulfuron, provide control of annual grasses for 1 and sometimes 2 yr with minor injury to resident, desirable plants (Hirsch et al. 2012; Kyser et al. 2007; Mangold et al. 2013; Sebastian et al. 2016b, 2017c; Wallace and Prather 2016). Indaziflam, a preemergence cellulose biosynthesis–inhibiting herbicide, provides 2 to 3 yr (Clark et al. 2019, 2020; Koby et al. 2019; Sebastian et al. 2016b) and possibly more years (Courkamp et al. 2022a) of annual grass control. This long duration of control and potential depletion of the seedbank (Courkamp et al. 2022b; Meyer-Morey et al. 2021) due to indaziflam's persistence, may improve our ability to mitigate impacts of annual grasses (Sebastian et al. 2017a).

Ventenata dubia was first reported in the 1950s in Washington and Idaho (Scheinost et al. 2008). Since then, it has invaded the intermountain Pacific Northwest and has spread eastward to the Northern Great Plains (Garner and Lakes 2019; Hart and Mealor 2021). It is associated with up to 50% yield loss in croplands across eastern Idaho and Washington (Wallace and Prather 2016) and substantial ecological and economic impacts in perennial grass habitats across the inland Pacific Northwest (Wallace et al. 2015). Its accessibility and palatability as a source of forage for livestock and wildlife is questionable (Brummer et al. 2023), and its removal with herbicide has been shown to improve forage quality and quantity (Hart and Mealor 2021). The shallow root system creates the potential for soil erosion in areas of monotypic invasions, thus decreasing land productivity and value (Scheinost et al. 2008). *Ventenata dubia* is known to

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Management Implications

Ventenata dubia (ventenata) is a nonnative, annual grass that has spread rapidly across the inland Pacific Northwest and eastward to the Northern Great Plains, potentially causing substantial ecological and economic impacts in perennial grasslands. *Ventenata dubia*'s accessibility and palatability as forage for livestock and wildlife is questionable, and controlling it with a herbicide may lead to improved forage quality and quantity. Indaziflam (Rejuvra®, formerly Esplanade®) is a preemergence herbicide that has shown great potential for annual grass control; however, its effect on *V. dubia* is less known than for the annual grass *Bromus tectorum* (downy brome). Furthermore, the herbicide's effect on *V. dubia* and other aspects of vegetation beyond 3 yr is lacking. We applied indaziflam, other annual grass herbicides (glyphosate, imazapic, propoxycarbazone-sodium, rimsulfuron), indaziflam combined with each of those herbicides, and a non-sprayed control to an improved pasture in southwestern Montana in fall 2016. For 7 yr, we sampled canopy cover of *V. dubia* and the rest of the plant community. In the fifth year, we sampled the seedbank. Our results indicated that *V. dubia* cover was zero or close to zero in treatments that included indaziflam, and this trend held for 6 yr. However, by the seventh year, *V. dubia* had decreased to very low cover across the pasture, including the non-sprayed control, suggesting factors other than herbicide application were playing a role in vegetative dynamics. Perennial grasses and forbs were generally unaffected by treatment and did not increase in cover, even though *V. dubia* decreased. Annual forb cover and species richness were lowest in indaziflam treatments at 2 yr after treatment, but were similar in all treatments by 7 yr. Five years after herbicide application, the number of total seedlings (dicot and monocot combined) emerging from the seedbank was 12 to 33 times lower in indaziflam-treated soil than soil treated with other herbicides or not treated at all. Our study suggests that a single application of indaziflam along with abiotic and biotic environmental variables provided long-term (i.e., 7 yr) control of *V. dubia* and appeared to have depleted the seedbank across all species, but did not result in a release in desired vegetation as has been seen in some studies.

invade areas previously inhabited by *B. tectorum* and medusahead [*Taeniatherum caput-medusae* (L.) Nevski] (Jones et al. 2018; Wallace et al. 2015; Wallace and Prather 2016). Seed longevity is estimated to be less than 4 yr, based upon a field study in which seeds of *V. dubia* were buried 2-cm deep and retrieved at various intervals; a small fraction of seeds was germinable at 3 yr but none were germinable at 4 yr (Wallace et al. 2015).

Knowledge about the effectiveness of indaziflam on *V. dubia* and any impacts on desirable vegetation over an extended period is limited. Indaziflam and other commonly used active ingredients have been tested on *V. dubia* and associated vegetation in the intermountain Pacific Northwest over a 2-yr period (Koby et al. 2019). Additionally, the effect of indaziflam on *V. dubia* and other vegetation, especially perennial grasses, has been evaluated across multiple sites in Wyoming for 1 yr posttreatment (Hart and Mealor 2021). The objective of our study was to compare effectiveness of indaziflam and other commonly used annual grass herbicides for *V. dubia* control, while accounting for effects on other vegetation over a 7-yr period at a site in southwestern Montana. We also assessed the effect of herbicide treatments on the seedbank at 5 yr after application. Response of *V. dubia* and desired vegetation by functional group, species richness, rank abundance, and number of seedlings (total, dicot, and monocot) emerging from the seedbank was assessed across 10 herbicide treatments: a non-sprayed control, indaziflam (Esplanade®; Anonymous 2016), glyphosate (Accord® XRT II; Anonymous 2014), imazapic (Plateau®; Anonymous 2011), propoxycarbazone-sodium (Lambient™; Anonymous 2009), and rimsulfuron (Matrix®SG; Anonymous 2017), and indaziflam combined with each of the other four active ingredients. Based on previous studies (Sebastian et al. 2016a, 2017a), we hypothesized that indaziflam applied alone and in combination with the other herbicides would control *V. dubia* for three or more years, while the other herbicides applied alone would control *V. dubia* for a year, and that a similar effect would be observed in the seedbank (i.e., emergence of fewer seedlings). Further, we sought to quantify any changes in species richness or abundance (i.e., cover) of vegetation other than *V. dubia* (perennial grass, perennial forb, and annual forb), expecting an increase in non-target vegetation as has been seen with other indaziflam studies (Clark et al. 2020; Hart and Mealor 2021; Koby et al. 2019; Sebastian et al. 2017a).

Materials and Methods

The study was carried out in an improved pasture near Bozeman in southwestern Montana (45.8808°N, 111.0567111°W). Mean annual precipitation is 47 cm and temperature is 6 C. Soil (0 to 15 cm) is a Sawicki cobbly loam (41% sand, 38% silt, 21% clay) with 6.2 pH and 2.7% organic matter. The site was dominated by smooth brome (*Bromus inermis* Leyss.) interspersed with western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve] and mountain brome (*Bromus marginatus* Nees ex Steud.), and a uniform infestation of *V. dubia*. The pasture is lightly grazed by sheep and cattle; however, grazing was excluded from the study area while the study was conducted by using an electric fence.

Ten herbicide treatments (Table 1) were replicated three times and arranged in a randomized complete block design using 9.1 m by 3.1 m plots. Herbicide treatments were applied on November 4, 2016, using a CO_2 backpack sprayer that delivered 182 L water ha^{-1} at 290 kPa. The non-ionic surfactant Induce® (Helena Chemical Company, Billings, MT) was applied at a 0.25% v/v rate along with each herbicide treatment. At the time of treatment, *V. dubia* was in the 1- to 2-leaf growth stage and appeared alive and free of any frost injury.

Foliar canopy cover (hereafter referred to as cover) for each species was measured using three randomly located 20 cm by 50 cm Daubenmire (1959) frames per plot in early to mid-July from 2017 through 2023. Cover (%) of all observed species was estimated to the nearest 1%, trace values were replaced with 0.1 for analyses, and total cover could exceed 100. Species other than *V. dubia* were later combined into functional groups of perennial grasses, perennial forbs, or annual forbs for analyses.

Five years posttreatment (August 2021), the germinable seedbank was sampled by compositing five cores (5.2 cm [w] by 5.6 cm [d] by 10.9 cm [l], 40 cm^3 total volume) within each plot. The composited samples were stored for 3 mo at 5 C. Samples were sieved (4-mm and 2-mm sieves) to remove all litter, rocks, and root material, and any visible seeds were removed with tweezers but immediately returned to their respective samples. Each sample was spread over pasteurized (70 C for 60 min) potting soil in a 12 cm (w) by 5.7 (d) by 16.5 cm (l) tray within Montana State University's Plant Growth Center (MSU PGC) (45.6681°N, 111.0533°W). The PGC potting soil is equal parts (by volume) of loam soil, washed

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Table 1. Herbicide treatments applied to *Ventenata dubia*–infested study site near Bozeman in southwestern Montana in November 2016.

Treatment	Common product name	Application rate g ai ha^{-1}	Herbicide manufacturer
Non-sprayed control	Not applicable	Not applicable	Not applicable
Indaziflam	Esplanade® 200 SC	102.4	Bayer Environmental Science, Cary, NC, https://www.bayer.com/en/agriculture/environmental-science
Glyphosate	Accord® XRT II	533.0	Corteva AgriScience, Indianapolis, IN, https://www.corteva.com
Imazapic	Plateau®	122.7	BASF Corporation, Research Triangle Park, NC, https://www.basf.com/us/en
Propoxycarbazone-sodium	Lambient®	60.0	Bayer Environmental Science, Cary, NC, https://www.bayer.com/en/agriculture/environmental-science
Rimsulfuron	Maxtrix® SG	70.1	DUPONT, Wilmington, DE, https://www.dupont.com
Glyphosate + indaziflam	Accord® XRT II + Esplanade® 200 SC	533.0, 102.4	See above
Imazapic + indaziflam	Plateau® + Esplanade® 200 SC	122.7, 102.4	See above
Propoxycarbazone-sodium + indaziflam	Lambient® + Esplanade® 200 SC	60.0, 102.4	See above
Rimsulfuron + indaziflam	Maxtrix® SG + Esplanade® 200 SC	70.1, 102.4	See above

concrete sand, and Canadian sphagnum peat moss with AquaGro 2000 G (Aquatrols, Paulsboro, NJ) wetting agent blended in at 454 g 0.76 cm^{-3} of soil mix. Samples were kept moist by misting with water at least once daily, and trays were monitored twice weekly for seedling emergence from November 3 to 1 December 1, 2021. Seedlings were counted and identified as monocot or dicot.

All analyses and graphical interpretations were conducted using R software v. 4.3.2 (R Core Team 2023). Specifically, we used routines within BiodiversityR, dplyr, emmeans, lme4, lmertest, multcomp, ggpatterns, and ggplot2 packages (Bates et al. 2015; Mike et al. 2022; Hothorn et al. 2008; Kindt and Coe 2005; Kuznetsova et al. 2017; Wickham 2016; Wickham et al. 2018). Ten treatments replicated three times and sampled over 7 yr precluded us from analyzing all treatments over all years due to a lack of statistical power. Therefore, we analyzed all treatments over 4 yr (2017 to 2019, 2023). Years were selected based upon expected persistence of the tested herbicides ranging from 1 to 3 yr and to capitalize on the relatively long-term nature of our data set at 7 yr.

Ventenata dubia, perennial grass, perennial forb, and annual forb cover over 4 yr were evaluated using separate linear mixed-effect models with the interaction between herbicide treatment and year as a fixed effect and sampling plot as a random effect. The interaction was significant and retained in all models except the perennial forb model, where herbicide treatment and year were retained as additive. Due to a violation of the assumption of heteroscedasticity, a square-root transformation was needed on all response variables. A type II ANOVA was used to determine whether each parameter indicated significant impact on the response variable ($\alpha = 0.05$). A post hoc Tukey Kramer HSD pairwise comparison was used to elucidate differences between the herbicide treatment and year combinations using the *glht* function of the multcomp package (Hothorn et al. 2008). A similar method was repeated for species richness; however, this model used a Poisson distribution and did not require transformation. *Ventenata dubia* was not included in species richness calculations. *Bromus tectorum* and houndstongue (*Cynoglossum offinale* L.) were the only other two invasive species found at the site, and they occurred infrequently (Supplementary Table S1).

Current stewardship guidelines indicate that indaziflam should be applied before germination of the target invasive annual grass, and if that window is missed, indaziflam should be combined with a postemergence herbicide, like imazapic (Envu Environmental Science US Inc. 2023). Therefore, we also analyzed *V. dubia* cover as affected by indaziflam and imazapic + indaziflam treatments and compared it to the non-sprayed control for all 7 yr of the study (2017 to 2023) using the same methods as described earlier. No other treatments were analyzed for all 7 yr of the study.

Table 2. Herbicide treatment effects on *Ventenata dubia*, perennial grass, perennial forb, and annual forb cover across 4 yr (2017–2019, 2023) for a site near Bozeman in southwestern Montana, using ANOVA.

Main effects and interaction	df	*F*-value	P-value[a]
Ventenata dubia			
Herbicide treatment	9, 312	63.63	**<0.001**
Year	3, 8	15.27	**0.001**
Herbicide treatment*year	27, 312	9.223	**<0.001**
Perennial grass			
Herbicide treatment	9, 312	5.808	**<0.001**
Year	3, 8	14.60	**=0.001**
Herbicide treatment*year	27, 312	2.756	**<0.001**
Perennial forb			
Herbicide treatment	9, 312	3.949	**<0.001**
Year	3, 8	0.208	0.888
Herbicide treatment*year	27, 312	0.682	0.884
Annual forb			
Herbicide treatment	9, 312	7.155	**<0.001**
Year	3, 8	18.35	**<0.001**
Herbicide treatment*year	27, 312	3.899	**<0.001**

[a]Bold numbers are significant at $\alpha = 0.05$.

Rank and relative abundance of all species were also evaluated for the non-sprayed control, indaziflam, and imazapic + indaziflam treatments over 4 yr (2017 to 2019, 2023). For the top five species, abundance and proportion were calculated. Abundance represents the sum of cover estimates for a species across all replications of the treatment, and proportion relativizes the species to all other species found in the treatment.

For the seedbank analysis, a type II ANOVA was used to determine whether herbicide treatment affected total, dicot, and monocot seedlings that emerged ($\alpha = 0.05$). Post hoc Tukey Kramer HSD pairwise comparisons were made to separate means.

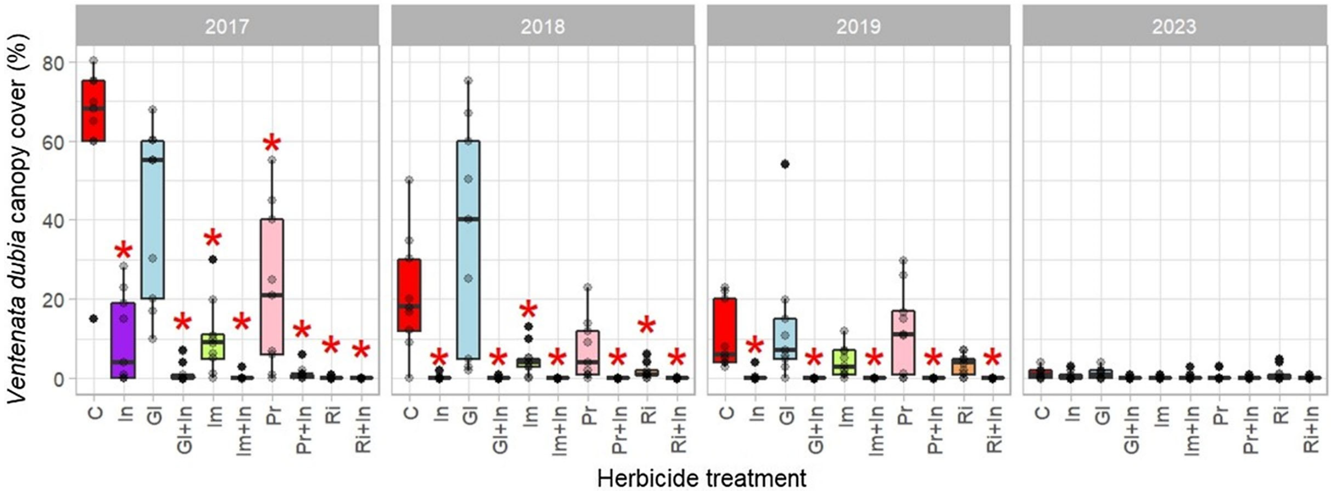

Figure 1. *Ventenata dubia* canopy cover (%) for 10 herbicide treatments (C, non-sprayed control; In, indaziflam; Gl, glyphosate; Gl+In, glyphosate + indaziflam; Im, imazapic; Im+In, imazapic + indaziflam; Pr, propoxycarbazone-sodium; Pr+In, propoxycarbazone-sodium + indaziflam; Ri, rimsulfuron; Ri+In, rimsulfuron + indaziflam) over 4 yr (2017–2019, 2023) applied in 2016 at a site near Bozeman in southwestern Montana. Boxes represent interquartile range, with line at center indicating median; whiskers are 95% range of the data; semi-transparent points are raw data points, with solid points indicating extreme outliers. Red asterisks (*) indicate treatments that were significantly different (P < 0.05) from that year's non-sprayed control (C). *Ventenata dubia* cover ranged from 0% to 80%.

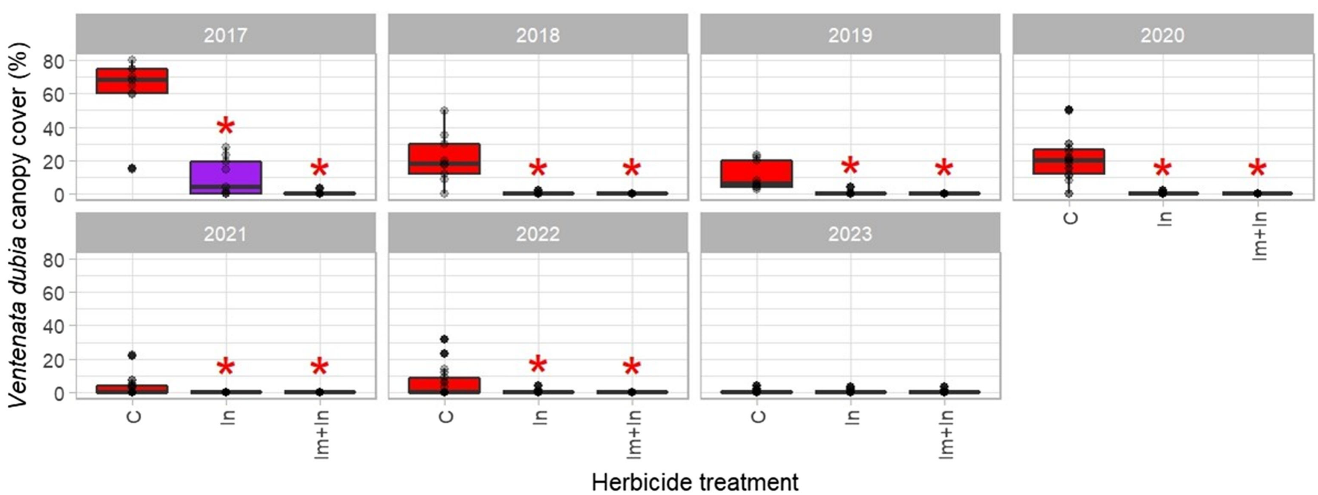

Figure 2. *Ventenata dubia* canopy cover (%) for three herbicide treatments (C, non-sprayed control; In, indaziflam; Im+In, imazapic + indaziflam) over 7 yr (2017–2023) applied in 2016 at a site near Bozeman in southwestern Montana. Boxes represent interquartile range, with line at center indicating median; whiskers are 95% range of the data; semi-transparent points are raw data points, with solid points indicating extreme outliers. Red asterisks (*) indicate treatments that were significantly different (P < 0.05) from that year's non-sprayed control.

Results and Discussion

Ventenata dubia Cover

Differences in *V. dubia* cover were evident across treatments for the first 3 yr (2017 to 2019) but not the final year (2023) ($P < 0.001$) (Table 2; Figure 1). In 2017, all treatments, except glyphosate alone, had less *V. dubia* than the control. Indaziflam, imazapic, and propoxycarbazone-sodium applied alone reduced cover to a lesser extent than rimsulfuron alone or indaziflam combined with the other active ingredients. Cover of *V. dubia* in the indaziflam, imazapic, propoxycarbazone-sodium, and rimsulfuron treatments was 5.6 ± 2.0%, 8.0 ± 2.4%, 16.4 ± 3.4%, and 0.1 ± 0.2%, respectively, with the indaziflam, imazapic, and rimsulfuron treatments reducing *V. dubia* by at least 90% compared with the non-sprayed control, arguably a satisfactory outcome from a management perspective. Imazapic and rimsulfuron applied alone continued to reduce *V. dubia* cover in 2018 but were less effective by 2019. Treatments that included indaziflam provided the highest and most persistent reduction in *V. dubia* cover in 2018 and 2019, with *V. dubia* being absent or nearly absent from plots treated with either indaziflam alone or in combination with the other active ingredients. Our results are consistent with other studies that evaluated similar herbicides for *V. dubia* control (Davies and Hamerlynck 2019; Koby et al. 2019; Sebastian et al. 2016a; Wallace and Prather 2016) and suggest multiple options for controlling *V. dubia*.

As stated earlier, current stewardship guidelines indicate that indaziflam should be mixed with a postemergence herbicide like imazapic if applied after annual grass emergence. When we analyzed the indaziflam and imazapic + indaziflam treatments compared with the non-sprayed control for each of 7 yr (2017 to 2023), we saw that these two treatments reduced *V. dubia* cover for 6 yr (through 2022), χ^2 (12, $N = 216$) = 256.2, $P < 0.001$) (Figure 2). The two treatments performed similarly to each other, except in 2017, when *V. dubia* cover in the imazapic + indaziflam treatment was about 4% lower than in the indaziflam treatment ($P < 0.01$). The 2017 result was not surprising, as the herbicide treatments were applied postemergence in 2016, and we expected the combination treatment to outperform the indaziflam-only treatment, as the combination would have had activity on both emerged and yet-to-emerge seedlings. More surprising is that the indaziflam-only application reduced *V. dubia* compared with the non-sprayed control to the extent that it did, and that these treatments influenced *V. dubia* cover for 6 yr. Our study was longer than others (Davies and Hamerlynck 2019; Koby et al. 2019; Sebastian et al. 2016a; Wallace and Prather 2016) and offers encouraging evidence for invasive plant managers regarding how long a single application of indaziflam can control *V. dubia*. Where rangeland and pastures are highly degraded and revegetation is necessary, our study also offers encouraging evidence that less-persistent herbicides like imazapic and rimsulfuron can provide satisfactory reductions in *V. dubia* for a year or two. Invasive plant managers could integrate these herbicides with seeding, allow seeded species a year or two to establish, then follow with an application of indaziflam to control any reinvading *V. dubia* if necessary.

By 2023, *V. dubia* cover was similarly low across all treatments, including the non-sprayed control (Figures 1 and 2); cover in the non-sprayed control decreased from 61% in 2017 to 0.7% in 2023, and there was a decrease in *V. dubia* each year, with levels <10% for the fifth year on. Interannual variability in annual grass abundance, including *V. dubia*, is common (Courkamp et al. 2022a; Orloff et al. 2015; Wallace and Prather 2016) and can be strongly influenced by precipitation and temperature (Adhikari et al. 2023; Bradley et al. 2018; Bradley and Mustard 2005; Clinton et al. 2010). Spring through midsummer (April 1 to July 31) precipitation varied greatly during the study from a low of 12 cm in 2021 to a high of 23 cm in 2018 (NOAA 2023). Years 4 (2020), 5 (2021), and 6 (2022) were generally drier than the first 3 yr or the last year of the study. Year 5 (2021) was very dry, and the entire county where the study site was located (Gallatin County) was in severe to extreme drought by the end of July (NDMC 2023). However, *V. dubia* cover remained low despite the final summer (2023) being wet (22 cm).

Cover of Other Functional Groups

Perennial grass cover was influenced by the interaction of herbicide treatment and year ($P < 0.001$; Table 2) with the effect of glyphosate in 2017 being primarily responsible for this interaction (Figure 3). Cover in the glyphosate treatment was 5.2 ± 1.8%, while mean cover in the other treatments, including the non-sprayed control, ranged from 12.6% to 33.8%. For years other than 2017, there were no differences in perennial grass cover relative to the non-sprayed control and among treatments, and cover remained mostly consistent within each treatment over the duration of the study. Two exceptions to this were the glyphosate treatment, which increased in cover from 2017 to 2023 ($P < 0.01$), and the imazapic + indaziflam treatment, which declined in perennial grasses from 2017 to 2018 ($P < 0.01$) and then increased from 2018 to 2023 ($P < 0.01$).

Some research has shown an increase in perennial grass abundance following herbicide applications to control *V. dubia* or other invasive annual grasses (Clark et al. 2020; Courkamp et al. 2022a; Hart and Mealor 2021; Koby et al. 2019; Sebastian et al. 2017a), and on range and pasture where livestock production is the primary management objective, this increase is touted as an economic benefit of controlling invasive annual grasses that can help to offset the cost of applying herbicide (Hart et al. 2023). We did not see an increase in perennial grass cover as we had hypothesized, which was disappointing, considering grazing was deferred for the duration of the study. We did not measure perennial grass biomass over the 7-yr study, and it is possible that meaningful increases in biomass were not reflected in cover; however, biomass was not higher at 2 yr posttreatment (Harvey 2019). The dominant perennial grass species at our site were *B. inermis*, *B. marginatus*, and *P. smithii*, all species considered competitive, resilient, and stress tolerant (USDA-NRCS 2023a, 2023b, 2023c). *Ventenata dubia* appeared to grow under the canopy of these species, especially the *Bromus* species, and our results suggest that *V. dubia* was not affecting the abundance of these species, as its removal did not coincide with an increase in perennial grass.

Perennial forb cover was affected by herbicide treatment ($P < 0.001$), but not year ($P = 0.888$) or the interaction of herbicide treatment and year ($P = 0.884$; Table 2). Mean cover ranged from 0.3% to 4.1% across herbicide treatments, and only propoxycarbazone-sodium differed from the non-sprayed control (Figure 4). Comparing across treatments other than the non-sprayed control, perennial forb cover was higher in the glyphosate treatment (4.1 ± 1.1%) than in the indaziflam (0.9 ± 0.5%), imazapic (0.6 ± 0.4%), propoxycarbazone-sodium (0.3 ± 0.3%), and rimsulfuron + indaziflam (0.8 ± 0.5%) treatments; and it was higher in the glyphosate + indaziflam treatment (3.4 ± 1.0%) than in the propoxycarbazone-sodium treatment.

Annual forb cover was affected by the interaction between herbicide treatment and year ($P < 0.001$; Table 2), but there was no evidence of a difference in cover between a herbicide treatment and the non-sprayed control within a year (Table 2; Figure 5). Instead, the interaction was due to differences among herbicide treatments (other than the non-sprayed control) in the second year postapplication (2018), when cover levels tended to be higher in the treatments without indaziflam. In 2018, which was 2 yr after herbicide application, imazapic (5.1 ± 1.2%), propoxycarbazone (5.4 ± 1.3%), and rimsulfuron (5.0 ± 1.2%) applied alone had higher annual forb cover than glyphosate (1.8 ± 0.7%) applied alone or indaziflam and any of the active ingredients combined with indaziflam (0.0 to 1.3%). By 2023, annual forbs were rarely found at the study site regardless of herbicide treatment.

Perennial and annual forbs may respond differently to an indaziflam application. Based on previous research, perennial forbs can increase (Sebastian et al. 2017a), decrease (Meyer-Morey et al. 2021), or be unaffected (Clark et al. 2020; Sebastian et al. 2016b), while annual forbs appear to decrease (Courkamp et al. 2022b; Meyer-Morey et al. 2021), which makes sense, given the life histories of the two functional groups and the characteristics of indaziflam as a preemergence herbicide. In our study, there was no response of perennial forbs, but annual forbs during the second year after application decreased when indaziflam was applied alone or in combination with other active ingredients compared with when those active ingredients were applied singly.

Species Richness

Over the course of the study, 51 species were recorded at the site (not including *V. dubia*) (Supplementary Table S1). Species

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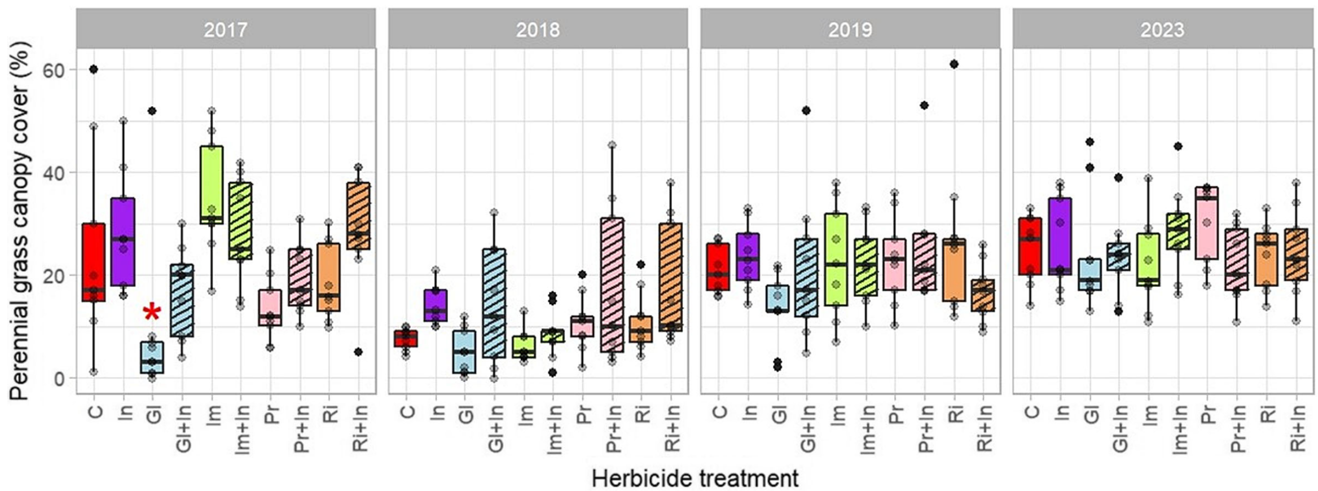

Figure 3. Perennial grass canopy cover (%) for 10 herbicide treatments (C, non-sprayed control; In, indaziflam; Gl, glyphosate; Gl+In, glyphosate + indaziflam; Im, imazapic; Im+In, imazapic + indaziflam; Pr, propoxycarbazone-sodium; Pr+In, propoxycarbazone-sodium + indaziflam; Ri, rimsulfuron; Ri+In, rimsulfuron + indaziflam) over 4 yr (2017–2019, 2023) applied in 2016 at a site near Bozeman in southwestern Montana. Boxes represent interquartile range, with line at center indicating median; whiskers are 95% range of the data; semi-transparent points are raw data points, with solid points indicating extreme outliers. Red asterisks (*) indicate treatments that were significantly different ($P < 0.05$) from that year's non-sprayed control.

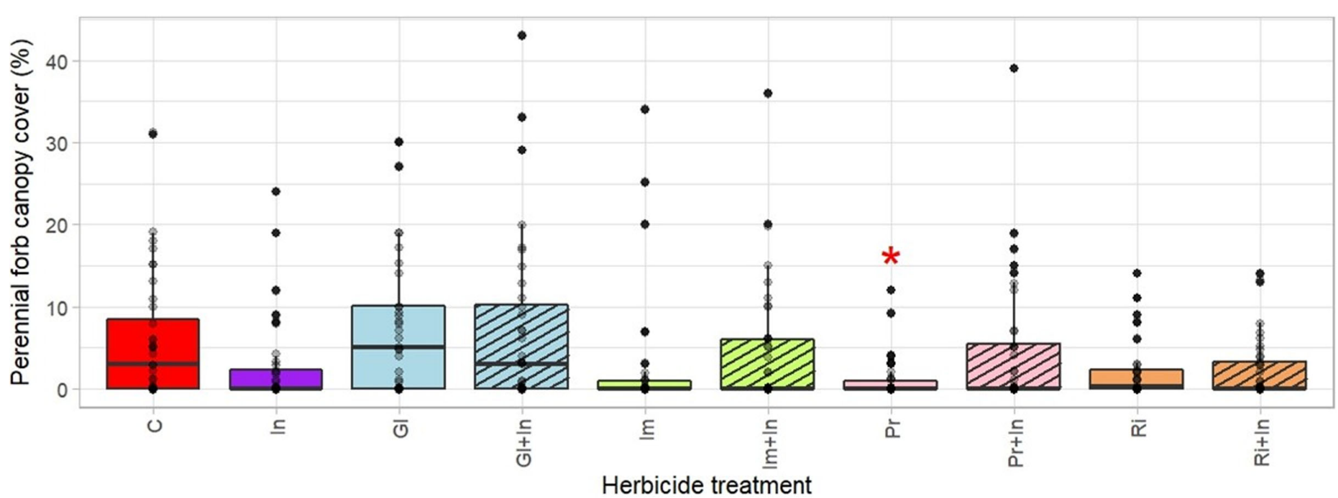

Figure 4. Perennial forb canopy cover (%) for 10 herbicide treatments (C, non-sprayed control; In, indaziflam; Gl, glyphosate; Gl+In, glyphosate + indaziflam; Im, imazapic; Im+In, imazapic + indaziflam; Pr, propoxycarbazone-sodium; Pr+In, propoxycarbazone-sodium + indaziflam; Ri, rimsulfuron; Ri+In, rimsulfuron + indaziflam) combined over 4 yr (2017–2019, 2023) applied in 2016 at a site near Bozeman in southwestern Montana. Boxes represent interquartile range, with line at center indicating median; whiskers are 95% range of the data; semi-transparent points are raw data points, with solid points indicating extreme outliers. There were no significant differences ($P < 0.05$) relative to the control treatment; however, there were differences among treatments.

richness was affected by the interaction of herbicide treatment and year, χ^2 (27, $N = 360$) 41.01, $P = 0.041$), which was due largely to the second year after herbicide application (2018). In this year, there were differences between the non-sprayed control (6.5 ± 0.9 species) and any treatment that included indaziflam (2.4 to 3.7 species) (Figure 6). In that same year, all treatments that included indaziflam resulted in lower species richness than any treatment that was a singularly applied active ingredient. Species richness increased from 2017 to 2018 in all treatments that did not include indaziflam, which may have been due to higher precipitation in 2018, for example, June precipitation in that year was two times what it was in 2017 (8.3 cm vs. 4.0 cm) (NOAA 2023). By 2019, species richness declined in those treatments that peaked in 2018 so that all treatments had similar species richness, a result that held into 2023.

Previous research has indicated increases (Clark et al. 2019; Sebastian et al. 2017a), decreases (Meyer-Morey et al. 2021), and no change (Hart and Mealor 2021) in species richness in response to applications of indaziflam for annual grass control. These studies and our own occurred in different geographic locations that ranged from sites with low disturbance and high richness and diversity (e.g., Meyer-Morey et al. 2021) to more disturbed and depauperate sites (e.g., Sebastian et al. 2017a)—our current site was an improved pasture dominated by perennial grasses. We saw a short-term decrease in species richness, possibly due to loss of annual forbs, but there was no effect on species richness by the end of the study. Our results indicate indaziflam controlled *V. dubia* with minimal impacts to non-target vegetation. However, there is still limited knowledge of the effects of cellulose biosynthesis–inhibiting herbicides like indaziflam on monocot and dicot proteins (Brabham et al. 2014;

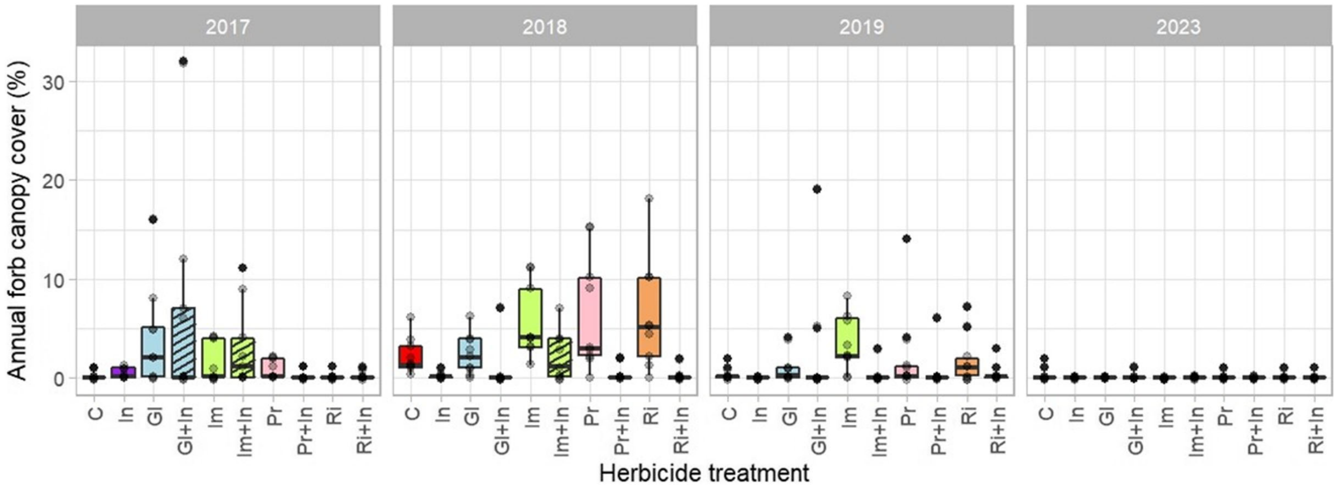

Figure 5. Annual forb canopy cover (%) for 10 herbicide treatments (C, non-sprayed control; In, indaziflam; Gl, glyphosate; Gl+In, glyphosate + indaziflam; Im, imazapic; Im+In, imazapic + indaziflam; Pr, propoxycarbazone-sodium; Pr+In, propoxycarbazone-sodium + indaziflam; Ri, rimsulfuron; Ri+In, rimsulfuron + indaziflam) combined over 4 yr (2017–2019, 2023) applied in 2016 at a site near Bozeman in southwestern Montana. Boxes represent interquartile range, with line at center indicating median; whiskers are 95% range of the data; semi-transparent points are raw data points, with solid points indicating extreme outliers. There were no significant differences (P < 0.05) relative to the non-sprayed control.

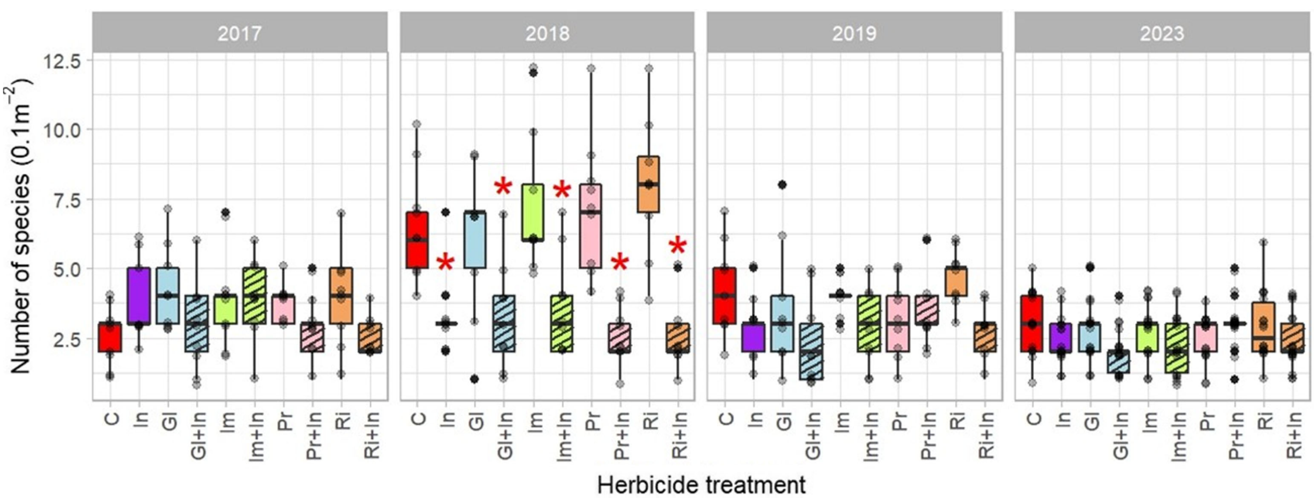

Figure 6. Species richness (species per 0.1 m^2) for 10 herbicide treatments (C, non-sprayed control; In, indaziflam; Gl, glyphosate; Gl+In, glyphosate + indaziflam; Im, imazapic; Im+In, imazapic + indaziflam; Pr, propoxycarbazone-sodium; Pr+In, propoxycarbazone-sodium + indaziflam; Ri, rimsulfuron; Ri+In, rimsulfuron + indaziflam) over 4 yr (2017–2019, 2023) applied in 2016 at a site near Bozeman in southwestern Montana. Boxes represent interquartile range, with line at center indicating median; whiskers are 95% range of the data; semi-transparent points are raw data points, with solid points indicating extreme outliers. Red asterisks (*) indicate treatments that were significantly different (P < 0.05) from that year's control treatment.

Sebastian et al. 2017a). Given that there are thousands of species present on rangeland in the western United States (FNA 1993+), more investigation of non-target species' tolerances and their long-term responses to indaziflam is needed.

Rank and Relative Abundance

With few exceptions, *B. inermis*, *B. marginatus*, and *P. smithii* were among the top five most abundant species in the non-sprayed control, indaziflam, and indaziflam + imazapic treatments over the duration of the study (Table 3). Thickspike wheatgrass [*Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould], prairie Junegrass [*Koeleria macrantha* (Ledeb.) Schult], and intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] were three additional perennial grasses that appeared in the top five most abundant as the study progressed. Common sheep sorrel (*Rumex acetosella* L.) was the most consistently abundant forb, appearing in the non-sprayed control 2017 to 2019 and 2023; it did not appear in the indaziflam, imazapic, or indaziflam + imazapic treatments, except for the indaziflam treatment in 2023.

Seedbank

In addition to herbicides affecting aboveground vegetation dynamics for up to 6 yr, herbicide treatments affected the total (all species) (P < 0.001), dicot (P < 0.001), and monocot (P = 0.047) seedlings emerging from the seedbank 5 yr after application (Table 4). For total seedlings, glyphosate alone had more seedlings than any other treatment at 165 ± 7.4 seedlings per sample (Table 4). Glyphosate was followed by propoxycarbazone-

Table 3. Relative abundance of the top five species in the non-sprayed control (C), indaziflam (In), and imazapic + indaziflam (Im+In) treatments for the first 3 yr (2017–2019) and 7 yr (2023) after treatment application at a study site near Bozeman in southwestern Montana.

Treatment	Species	Rank	Abundance[a]	Proportion[b]
	2017			
C	*Ventenata dubia*	1	568.0	69.4
	Bromus inermis	2	194.0	23.7
	Rumex acetosella	3	27.0	3.3
	Pascopyrum smithii	4	17.0	2.1
	Bromus marginatus	5	8.1	1.0
In	*Bromus inermis*	1	139.0	36.5
	Ventenata dubia	2	90.0	23.7
	Bromus marginatus	3	82.0	21.6
	Pascopyrum smithii	4	34.0	8.9
	Liatris punctata, Hook.	5	17.0	4.5
Im+In	*Bromus inermis*	1	147.0	46.3
	Bromus marginatus	2	72.0	22.7
	Pascopyrum smithii	3	36.0	11.3
	Achillea millefolium, L.	4	23.0	7.2
	Tragopogon dubius, Scop.	5	19.0	6.0
	2018			
C	*Ventenata dubia*	1	191.1	56.2
	Rumex acetosella	2	47.0	13.8
	Bromus marginatus	3	28.1	8.3
	Bromus inermis	4	22.0	6.5
	Pascopyrum smithii	5	12.0	3.5
In	*Bromus inermis*	1	60.0	40.6
	Bromus marginatus	2	40.0	27.0
	Elymus lanceolatus	3	20.0	13.5
	Taraxacum officinale, F.H. Wigg.	4	9.0	6.1
	Pascopyrum smithii	5	7.1	4.8
Im+In	*Pascopyrum smithii*	1	31.0	23.9
	Bromus marginatus	2	22.0	17.0
	Artemisia ludoviciana, Nutt.	3	18.0	13.9
	Bromus inermis	4	16.0	12.3
	Tragopogon dubius	5	14.1	10.9
	2019			
C	*Ventenata dubia*	1	95.0	28.1
	Bromus inermis	2	89.0	26.4
	Bromus marginatus	3	73.0	21.6
	Rumex acetosella	4	30.0	8.9
	Liatris punctata	5	16.0	4.7
In	*Bromus inermis*	1	118.0	44.6
	Bromus marginatus	2	44.0	16.6
	Achillea millefolium	3	32.0	12.1
	Pascopyrum smithii	4	24.0	9.1
	Koeleria macrantha	5	15.0	5.7
Im+In	*Bromus inermis*	1	89.0	31.8
	Bromus marginatus	2	47.0	16.8
	Elymus lanceolatus	3	38.0	13.6
	Balsamorhiza sagittata, (Pursh) Nutt.	4	36.0	12.9
	Pascopyrum smithii	5	29.0	10.4
	2023			
C	*Bromus inermis*	1	251.0	38.1
	Bromus marginatus	2	138.0	21.0
	Rumex acetosella	3	124.0	18.8
	Thinopyrum intermedium	4	64.0	9.7
	Pascopyrum smithii	5	28.0	4.3
In	*Bromus inermis*	1	259.0	49.5
	Bromus marginatus	2	182.0	34.8
	Rumex acetosella	3	25.0	4.8
	Pascopyrum smithii	4	23.0	4.4
	Thinopyrum intermedium	5	14.0	2.7
Im+In	*Bromus inermis*	1	226.0	40.7
	Elymus lanceolatus	2	185.0	33.3
	Bromus marginatus	3	69.0	12.4
	Pascopyrum smithii	4	47.0	8.5
	Artemisia ludoviciana	5	8.0	1.4

[a]Abundance represents the sum of cover estimates for a species across all replications of the treatment.
[b]Proportion relativizes the species to all other species found in the treatment.

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Table 4. Number of total, dicot, and monocot seedlings (mean ± SE) of any species emerging from the seedbank (per 40-cm^3 sample) 5 yr after herbicide treatments were applied at a site near Bozeman in southwestern Montana[a].

	Total	Dicot	Monocot
Non-sprayed	89.3 ± 18.1 bc	52.0 ± 9.0 abc	37.3 ± 11.9 ab
Indaziflam	2.33 ± 1.5 e	1.33 ± 0.9 c	1.00 ± 0.6 b
Glyphosate	165 ± 46.8 a	95.3 ± 13.7 a	69.3 ± 38.9 a
Glyphosate + indaziflam	4.67 ± 1.2 e	3.67 ± 0.3 c	1.00 ± 1.0 b
Imazapic	60.0 ± 27.1 d	42.7 ± 22.1 abc	17.3 ± 7.6 ab
Imazapic + indaziflam	5.33 ± 3.9 e	5.00 ± 3.6 bc	0.33 ± 0.3 b
Propoxycarbazone-sodium	101 ± 19.6 b	69.7 ± 12.0 ab	31.7 ± 16.8 ab
Propoxycarbazone-sodium + indaziflam	4.00 ± 2.5 e	2.33 ± 1.9 c	1.67 ± 0.7 ab
Rimsulfuron	70.0 ± 36.1 cd	56.3 ± 28.2 abc	13.7 ± 11.7 ab
Rimsulfuron + indaziflam	1.33 ± 0.7 e	0.33 ± 0.3 c	1.00 ± 0.6 b

[a]The same letter following means indicates no difference between treatments within total seedlings (α = 0.05), dicot seedlings (α = 0.05), and monocot seedlings (α = 0.10).

sodium, the non-sprayed control, rimsulfuron, and imazapic, with seedlings per sample ranging from 60 to 90. Indaziflam applied alone and in combination with the other herbicides resulted in five or fewer total seedlings per sample. Dicot and monocot seedlings trended similarly to total seedlings, but differences among treatments were not as distinct, and the effects of indaziflam were not as clearly evident as they were with total seedlings. For dicot and monocot seedlings separately, none of the treatments reduced seedlings compared with the non-sprayed control (Table 4).

Seedbank depletion of target species is critical for invasive annual grass management (Sebastian et al. 2017b), and indaziflam remains active at low concentrations in the upper couple of centimeters of soil for at least 2 to 3 yr (Sebastian et al. 2017a). *Ventenata dubia* seed longevity is estimated to be less than 4 yr (Wallace et al. 2015), and our results provide evidence of seedbank depletion up to 5 yr after a single indaziflam application. At a site in Wyoming, a single application of indaziflam provided 5 yr of *B. tectorum* control (Courkamp et al. 2022a). Courkamp et al. (2022a) speculated that the maximum single-use application rate of indaziflam (Indaziflam GRZ; USEPA 2023), the rate used in our study, could have depleted the seedbank of *B. tectorum*, which is estimated to be up to a year longer than that of *V. dubia* (Thill et al. 1984).

We did not identify seedlings to species in the seedbank analysis, so we are unable to say whether the indaziflam treatments were selecting for *V. dubia* over other species in the seedbank. However, we differentiated between monocots and dicots, and dicots generally made up a greater proportion of total seedlings emerging from the seedbank than monocots. Additionally, indaziflam treatments tended to result in much lower dicot or monocot seedling emergence when compared with the treatments that yielded the highest number of seedlings (e.g., glyphosate). Indaziflam is reported to have more activity on monocots than dicots (Sebastian et al. 2017a), contributing to its value for invasive annual grass management. At the same time, there is increasing evidence that indaziflam reduces native annual forbs (dicots) in the seedbank (Courkamp et al. 2022b; Meyer-Morey et al. 2021). Whether the reduction of the annual forb seedbank is reflected in the aboveground plant community has been mixed. Meyer-Morey et al. (2021) observed an absence of native annual forbs emerging from the seedbank ex situ and in situ from high elevation, diverse sagebrush steppe communities 2 yr following indaziflam application. In contrast, Courkamp et al. (2022b) observed native annual forbs at their field site 3 yr after an indaziflam application, even though those same species were absent from the seedbank.

Our study, which analyzed the seedbank 5 yr after indaziflam application, provides longer-term evidence relative to other studies (Courkamp et al. 2022b; Meyer-Morey et al. 2021) of indaziflam's depauperating effect on the seedbank. We saw parallels between the seedbank and aboveground vegetation in terms of annual species, which is consistent with other seedbank studies where annual species are typically well-represented using a greenhouse germination method (Ball and Miller 1989; Chiquoine and Abella 2018). The methods used for our seedbank analysis were similar to those of Sebastian et al. (2017b) but shorter in duration (about 1 mo) than most studies (e.g., 4 to 17 mo) (Ball and Miller 1989; De Villiers et al. 2003; Meyer-Morey et al. 2021; Plue et al. 2017; Price et al. 2010). Furthermore, our seedbank analysis did not employ methods such as stirring (Courkamp et al. 2022b; Price et al. 2010), stratification (Meyer-Morey et al. 2021; Plue et al. 2017), or addition of gibberellic acid (Chiquoine and Abella 2018) to stimulate additional germination following the initial germination period and therefore may have underrepresented the total number of seeds. However, our seedbank analysis offers a cursory evaluation of the effects of indaziflam on the seedbank 5 yr after application; further and more rigorous examination of how impacts to the seedbank influence aboveground plant community composition over time is warranted.

We observed variability in *V. dubia* cover over time. Cover was highest in the non-sprayed control in 2017, and by the seventh year after treatments were applied, *V. dubia* was nearly absent from the study site, regardless of treatment. Interannual variability in annual grass abundance is common (Courkamp et al. 2022a; Ehlert et al. 2015; Orloff et al. 2015; Wallace and Prather 2016) and can be strongly influenced by precipitation and temperature (Adhikari et al. 2023; Bradley et al. 2018; Bradley and Mustard 2005; Clinton et al. 2010). Spring through midsummer precipitation varied during our study with the middle years (2020 and 2021) being drier than average. These variations in precipitation appear to have been particularly detrimental to annual species at our site, as both *V. dubia* and annual forb cover declined to near zero, while perennial grasses and forbs maintained their presence. Perennial grasses like *B. inermis* and *B. marginatus*, generally regarded for their competitive vigor (USDA-NRCS 2023a, 2023b), became proportionately more dominant as the study progressed. Our results support the view that plant communities change in response to invasive annual grass management but, importantly, also in response to abiotic and biotic interactions, and monitoring beyond a year or two after management may reveal unexpected vegetation dynamics.

Given the decline in *V. dubia* at our improved pasture site regardless of herbicide treatment, a rangeland manager may question the need to invest in *V. dubia* management. Variability in precipitation coupled with competition dynamics among species within a plant community adds to the challenge of making management decisions regarding *V. dubia*. While there is still much to learn about *V. dubia*, we argue that its control is advised, given evidence for its recent (Jones et al. 2020; Ridder et al. 2022) and predicted future (Adhikari et al. 2023) success. For example, *V. dubia* cover increased by 30% and frequency by 50% in a 15-yr period on undisturbed (no fire, no grazing) grasslands in the inland Pacific Northwest (Ridder et al. 2022); furthermore, suitable habitat for *V. dubia* is predicted to increase under future climate scenarios in the same county where this study occurred, especially along roads and in agricultural lands (Adhikari et al. 2023). *Ventenata dubia*'s lack of value as forage for livestock or wildlife (Brummer et al. 2023) is yet another reason to control it. On sites like ours, with perennial grass cover approximately 20%, monitoring before management would be a viable option, with particular focus on the abundance of perennial species that can reproduce vegetatively to help ensure satisfactory aboveground cover. Our study occurred at a single site, which can limit its applicability to other areas where *V. dubia* is problematic.

Rather than confounding decision making, we hope our results are encouraging to rangeland managers in that a single application of indaziflam along with abiotic and biotic environmental variables, like variable precipitation over time, an exclusion of grazing, and competitive perennial grasses dominating the site, provided numerous years of *V. dubia* control and appear to have depleted the seedbank. Treatment did not result in an increase of any other desired species, though, and this potential outcome should be considered when making management decisions.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2024.20

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Competing interests. No conflicts of interest have been declared.

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