Spring Seeding Integrated with a Spring Glyphosate Application Promotes Establishment of *Pseudoroegneria spicata* (bluebunch wheatgrass) in *Bromus tectorum* (cheatgrass)-infested Rangelands

Michelle L. Majeski, Stacy C. Simanonok, Zachariah J. Miller, Lisa J. Rew and Jane M. Mangold

ABSTRACT

Bromus tectorum invasion and associated impacts have been documented extensively in the western United States. Integrated approaches have been shown to be effective in restoring rangeland impacted by *B. tectorum*. While integrating herbicide and seeding of native species can be effective, strategic timing of these tools could further improve restoration outcomes. At three *B. tectorum*-infested rangeland sites in western Montana, we tested the effects of glyphosate application and timing (fall or spring) and five *Pseudoroegneria spicata* seeding dates (one fall and four spring dates) on density and cover of *P. spicata*. *Pseudoroegneria spicata* density was nearly ten times higher with glyphosate applied to *B. tectorum* compared to none at one site, and *P. spicata* abundance was generally greater when spring glyphosate application was combined with spring seeding at two other sites where densities ranged from six to 25 plants per m². Overall, *B. tectorum* abundance was minimally affected by treatments and fluctuated between years and across study sites. Our results indicate that spring seeding of *P. spicata* following a spring glyphosate application promoted establishment of *P. spicata*, increasing its density and cover compared to fall glyphosate application and fall seeding, spring glyphosate following fall seeding, or seeding without any glyphosate. Restoration practitioners have an ecologically-based strategy for timing glyphosate application and seeding *P. spicata* based on our results, where spring-seeded *P. spicata* can grow for several months prior to fall emergence of the next *B. tectorum* cohort.

Keywords: downy brome, herbicide timing, invasive grass, revegetation, seeding timing

Bromus tectorum (cheatgrass) is one of the most widespread non-native annual grasses on rangelands in the western United States (Belnap et al. 2005). The impacts on rangeland ecosystems resulting from vegetation changes from native perennial species to *B. tectorum* are considerable and complex. For example, wildlife that once relied on native vegetation for forage and shelter pursue habitat in other areas (DiTomaso 2000); water, nutrient cycling, and soil food webs are altered (Belnap et al. 2005, Sperry et al. 2006, Chambers et al. 2007); and increased soil erosion can occur because of *B. tectorum*'s ephemeral roots (DiTomaso 2000). Additionally, *B. tectorum* senesces earlier in the summer than native perennial grasses, extending wildfire season and increasing the likelihood of rangeland wildfires (Bradley et al. 2018).

Chemical control is the most common method for reducing *B. tectorum* on western rangeland (Monaco et al. 2017, Mangold et al. 2018) but is not always sufficient to improve rangeland functions. On severely degraded rangeland that lacks adequate perennial vegetation to re-occupy open spaces, herbicide application is often integrated with seeding desirable plant species. Seeding, or revegetation, aims to establish desired perennial species that can compete with *B. tectorum* and thereby provide long term control (Whitson and Koch 1998). A systematic review of research

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Restoration Recap

- *Bromus tectorum* is a widespread, non-native, annual grass on rangelands in the western United States, and long-term management requires an integrated approach. Herbicide is often combined with seeding to restore *B. tectorum*-infested rangeland.
- Glyphosate was applied to *B. tectorum* in fall (October 2017) or spring (April 2018) at two sites and applied in fall only (October 2017) at one site in western Montana. Following glyphosate application, one fall (October or November 2017, depending on the site) and four spring (April through May 2018) *Pseudoroegneria spicata* seeding dates were tested to find the optimal herbicide and seeding times for *P. spicata* establishment.
- Optimal combinations of herbicide and seeding treatments varied across sites, but spring seeding of *P. spicata* following a spring glyphosate application improved *P. spicata* establishment, increasing its density and cover compared to fall glyphosate application and fall seeding, spring glyphosate following fall seeding, or seeding without any glyphosate.
- Restoration practitioners can integrate a spring application of glyphosate, a non-selective and non-persistent herbicide, with spring seeding of *P. spicata*. Spraying and seeding could occur days to weeks apart, as long as glyphosate is applied prior to *P. spicata* emergence. This approach may improve rangeland restoration results, compared to the more conventional fall herbicide application followed by fall or spring seeding.

on *B. tectorum* control methods published over a 64-year period found that herbicide application combined with revegetation was the only approach that resulted in long-term (> 2 yrs.) control (Monaco et al. 2017). Revegetation is difficult, however, and measures taken to remove undesired species like *B. tectorum*, among other factors, can contribute to success or failure (Chambers et al. 2007, Nyamai et al. 2011).

Choosing which herbicide to integrate with seeding includes a trade-off between longevity of *B. tectorum* control and potential injury to seeded species. The active ingredient indaziflam (Rejuvra®, Environmental Science US LLC 2020) is a pre-emergent, non-selective herbicide that can provide up to five years of *B. tectorum* control (Courkamp et al. 2022), although three years may be more typical (Sebastian et al. 2016, Clark et al. 2020). Due to its persistence in the soil and its ability to inhibit root growth of germinating seeds, seeding into areas treated with indaziflam should be delayed for at least 24 months (Environmental Sciences US LLC 2020) and perhaps longer (IMAGINE 2023). In contrast, the active ingredient glyphosate is short-lived in the soil and seeding into areas treated with it can occur within days after its application without injury to seeded species. Glyphosate is a post-emergent, foliar herbicide that can be applied to cheatgrass in fall or spring, with spring typically providing better control, and control typically lasting one growing season (Morris et al. 2016, Sebastian et al. 2017).

The timing of seeding is another important factor that can influence revegetation success (James et al. 2011, Boyd and James 2013). During revegetation of *B. tectorum*-infested rangeland in the semi-arid western United States, seeding often occurs in the fall because weather conditions typically afford a longer window of opportunity, and seeds are expected to remain dormant throughout winter and emerge in the spring (Boyd and James 2013, Harvey et al. 2020). In contrast to seeded species, *B. tectorum* is a winter annual species that typically emerges in the fall, overwinters as a seedling, and continues its growth early in the spring (Mack and Pyke 1983). Early emerging species like *B. tectorum* benefit by having first access to soil nutrients and water, which can result in an earlier developed root structure compared with native perennial grass seedlings that tend to germinate in the spring (Weidlich et al. 2017) as well as greater aboveground biomass and cover than later-emerging plants, creating a seasonal priority effect (Zimdahl 2018, Weidlich et al. 2017). When seeded native perennial species emerge in the spring after seeding, they may already be at a competitive disadvantage relative to fall-emerged *B. tectorum* (Harris and Wilson 1970, Humphrey and Schupp 2004).

To alleviate competition between *B. tectorum* and native perennial grass seedlings and thereby improve the likelihood of revegetation success, the seasonal priority effect granted to *B. tectorum* due to its winter annual life history needs to be addressed. Overcoming a seasonal priority effect has been a common research focus over the last decade (Wainwright et al. 2012, Young et al. 2014, Vaughn and Young 2015), including several studies where *Pseudoroegneria spicata* (bluebunch wheatgrass) has been the focal native, perennial grass (Orloff et al. 2013, Schantz et al. 2016, Harvey et al. 2020). *Pseudoroegneria spicata* is a ubiquitous, long-lived native bunchgrass on western rangeland, well-suited for managed grazing because of its high protein content and palatability to all classes of livestock (Boyd and James 2013, Ogle et al. 2013). In a greenhouse study, Orloff et al. (2013) found that when *P. spicata* had a four-leaf size advantage, which was approximately four weeks of growth, over emerging *B. tectorum*, it more effectively suppressed *B. tectorum* than when it was at the seedling stage. This situation could be created in the field by seeding *P. spicata* in spring where it would have one

Table 1. Site elevation, average temperature and precipitation during the first growing season of the study (NOAA, 2020). Dates of *Pseudoroegneria spicata* seeding, growing degree days and herbicide application dates. Cumulative growing degree days (GDD = sum of average daily temperature °C–temperature base 4.4°C between 1 March 2018 to 31 May 2018) for *Pseudoroegneria spicata* by planting date.

Elevation, Temperature, and Precipitation	Belgrade	Corvallis West	Corvallis East
Elevation (m)	1363	1096	1424
Avg. Temp. °C (Hi / Lo., 2018 Mar–May)	13 / -1	14 / 2	14 / 2
Avg. Temp. °C (Hi / Lo., 2018 June–Aug.)	27 / 8	26 / 8	26 / 8
Avg. Precip., mm (2018 Mar–May)	53.8	32.5	46.0
Avg. Precip., mm (2018 June–Aug.)	42.7	40.1	40.9
Seeding Dates (day-month-year), and Cumulative Growing Degree Days (GDD)			
Fall	15-November-17	30-October-17	30-October-17
Spring 1	21-April-18, 34.3	3-April-18, 20.9	3-April-18, 20.9
Spring 2	3-May-18, 85.7	19-April-18, 47.6	19-April-18, 47.6
Spring 3	17-May-18, 201	4-May-18, 118	4-May-18, 118
Spring 4	31-May-18, 210	15-May-18, 338	15-May-18, 338
Herbicide Application Dates (day-month-year)			
Fall	19-October-17	15-October-17	15-October-17
Spring	Not applicable	11-April-18	11-April-18

season of growth prior to *B. tectorum* emergence in fall. To that end, Harvey et al. (2020) tested *P. spicata* fall dormant seeding and a range of spring seeding dates between early April and mid-May into weed-free, bare ground field sites in southwestern Montana. Fall seeding of *P. spicata* led to fewer, larger plants, while early spring seeding resulted in higher densities of smaller plants. In eastern Oregon rangelands, Schantz et al. (2016) seeded a four-species native grass mix that included *P. spicata* in fall, spring, or fall plus spring. Seeding sequentially in fall and spring or spring only resulted in higher perennial grass density than seeding only in fall (Schantz et al. 2016).

While previous research has demonstrated that integrating herbicide application with revegetation is necessary to provide long-term *B. tectorum* control (Monaco et al. 2017), and that the timing of seeding perennials (Orloff et al. 2013, Schantz et al 2016, Harvey et al. 2020) and applying chemical control (Morris et al. 2016, Sebastian et al. 2017) is critical to restoration outcomes, how the timing of herbicide application and timing of seeding can be integrated in an ecologically-informed manner has been minimally evaluated. Given the seasonal priority effect typically demonstrated by *B. tectorum* relative to native perennial grasses, the aim of our study was to test how different timings of seeding integrated with an application of the herbicide glyphosate, including timing of application, would affect *P. spicata* establishment. Our working hypothesis was that controlling *B. tectorum* with glyphosate the previous fall or prior to seeding in the spring would allow seeding of *P. spicata* without risk of herbicide injury and provide one season of growth for *P. spicata* before the next cohort of *B. tectorum* emerged in the fall. We predicted that: 1) integrating glyphosate with seeding would result in lower abundance (foliar canopy cover) of *B. tectorum* and higher abundance (foliar canopy cover and density) of *P. spicata* compared to not integrating glyphosate application; 2) spring glyphosate application would result in lower abundance of *B. tectorum* than fall application, resulting in higher abundance of *P. spicata* with the exception of spring application combined with fall seeding; and 3) spring seeding would result in higher abundance of *P. spicata* than fall seeding.

Methods

Three field sites were located in western Montana that were primarily dominated by *B. tectorum* and other co-dominate species: Belgrade (45°48′02.3″N, 111°09′10.6″W), with a Beaverell series loam soil classification, co-dominated with *Sisymbrium altissimum* (tall tumblemustard) and *Carduus nutans* (musk thistle); Corvallis West at the Montana State University Western Agricultural Research Center (46°19′40.3″N, 114°05′06.4″W), with a soil classification of coarse-loamy in the Burnt Fork series, co-dominated with *Tragopogon dubius* (western salsify) and *Centaurea stoebe* (spotted knapweed); and Corvallis East, located approximately 5.6 km east of Corvallis West on Soft Rock Road (46°19′34.4″N, 114°00′08.2″W), where soil is classified as coarse-loamy of the Burnt Fork-Wimper-Fairway complex series, co-dominated with *C. nutans* and *Galium aparine* (sticky willy) (United States Department of Agriculture, 2019, Table 1).

We used a randomized block, split-plot design with three blocks at each site to evaluate the effects of seeding timing treatments and glyphosate treatments. The five *P. spicata* seeding treatments were fall seeding and four spring dates starting in April and occurring every two weeks through May. The herbicide treatments were none or glyphosate applied in fall (2017) at Belgrade and fall (2017) or spring (2018) at Corvallis West and East sites. A non-seeded, non-sprayed plot was included in each block at each site to track any temporal changes in the plant community in the absence of treatment. Seeding treatments were applied to whole-plots (6 m × 3.7 m), and herbicide treatments were applied to split-plots (3 m × 3.7 m). Due to variation in climate across the three sites, we applied treatments at Corvallis East and Corvallis West about two weeks earlier than at Belgrade (Table 1).

Herbicide treatments consisted of a 2% glyphosate solution applied using a hand-pumped backpack sprayer with a single fan nozzle. The sprayer was pumped at a rate to maintain constant pressure. At Belgrade, glyphosate was applied as Accord® plus Activator 90 surfactant at 0.25% v/v and indicator dye. The water carrier rate was 8.5 l/ha. Since fall was the only glyphosate treatment applied at Belgrade, treatments are hereafter referred to as not treated or treated for this site. At Corvallis West and East, glyphosate was applied in the fall or spring as Roundup® plus liquid ammonia at 0.25% v/v to help with absorption and indicator dye. The water carrier rate was 4.2 l/ha. Specific dates of application are found in Table 1.

Pseudoroegneria spicata seeds, Goldar variety, were acquired from Bruce Seed Farm near Townsend, MT, approximately 90 km from the Belgrade study site and approximately 315 km from the Corvallis study sites. At Belgrade, *P. spicata* was broadcast seeded by hand at 24 kg/ha pure live seed (PLS) rate. Corvallis West and East sites were seeded at 12 kg PLS/ha using a Landpride® no-till drill with a seeding depth of 0.5–1.25 cm. Seeding rates followed those of Orloff et al. (2013) and United States Department of Agriculture (2014).

Since two seeding methods (broadcast and drill-seed) were used across the three sites, we executed two sampling methods. At Belgrade, which was broadcast seeded, we randomly located three 50 cm × 100 cm permanent frames in each split-plot. *Pseudoroegneria spicata* density and foliar canopy cover (hereafter referred to as cover) and *B. tectorum* cover were estimated to the nearest 1% within the frames. Sampling occurred 10 July 2018 and over four days in mid- to late June 2019. For the drill-seeded sites (Corvallis West and East), we estimated *P. spicata* density based on plant counts in three, 50 cm row sections per split-plot. Cover of *P. spicata* and *B. tectorum* was estimated to the nearest 1% using a Daubenmire (1959) frame (50 cm × 20 cm) centered over the row sections where density was estimated. Sampling locations were permanent from 2018 to 2019. Sampling occurred on 26 June 2018 and 2 July 2019 at Corvallis West and 27 June 2018 and 2–4 July 2019 at Corvallis East.

We used nested analysis of variance (ANOVA) to analyze the response variables of *P. spicata* density and cover and *B. tectorum* cover. Poisson distribution was used for *P. spicata* density and Gaussian distribution was used for *P. spicata* and *B. tectorum* cover. Chi-square test statistic was used for density, and F statistic was used for cover. Seeding date was modeled at the whole-plot level, with herbicide treatment as a split-plot treatment and repeated measures (year) as a split-split-plot effect. Fixed effect variables were herbicide application, seeding date and sampling year (year). To meet assumptions of constant variance and normality, a response variable was natural log- or square root-transformed, but non-transformed means are presented for ease of interpretation. Each site was analyzed separately due to different protocols (i.e., seeding, spraying, and sampling methods). A Tukey HSD pairwise comparison was performed for significant factors with $\alpha = 0.05$. Statistical analyses and data visualization were conducted with R Software 3.6.1 with agricolae, dplyr, emmeans, and ggplot2 packages (Wickham 2016, R Core Team 2019, de Mendiburu and Yaseen 2020, Lenth 2020, Wickam et al. 2020). The non-seeded, non-sprayed plots were not included in analyses since split-plot treatments were not applied to these plots. Instead, mean *B. tectorum* cover was calculated and compared to *B. tectorum* cover in treated plots for reference when appropriate.

Results

Belgrade Site

Pseudoroegneria spicata density was affected by herbicide application ($X^2_{1,10} = 84.670$, $p < 0.001$) and the interaction between seeding date and year ($X^2_{4,20} = 8.263$, $p < 0.001$, Table 2). *Pseudoroegneria spicata* density was nearly ten times higher with herbicide applied to *B. tectorum* compared to none (12.6 ± 2.0 plants per m^2 and 1.6 ± 0.6 plants per m^2, respectively). The effect of seeding date on *P. spicata* density differed between years (Figure 1). In 2018 Fall seeding resulted in low *P. spicata* density (2.0 ± 1.1 plants per m^2), and density trended higher with spring seeding compared to fall. Spring 3 and Spring 4 seeding dates resulted in the highest densities at ~18 plants per m^2. Densities of *P. spicata* generally declined from 2018 to 2019 with greater declines in later seeding dates with higher initial densities. In 2019 densities decreased in the last two spring seeding dates so that all seeding dates were similar to each other with the exception of Spring 2 being about three times greater (6.3 ± 3.1 plants per m^2) than Fall (1.9 ± 0.7 plants per m^2).

The effects of treatments on *P. spicata* cover differed slightly from the effects on density. Specifically, seeding date did not affect *P. spicata* cover, but there was an interaction between herbicide application and year ($F_{1,20} = 29.72$, $p < 0.001$, Table 2). *Pseudoroegneria spicata* cover was higher in the herbicide treatments in the second year, and although cover was still somewhat low, it was more than ten times greater (5.7 ± 1.1%) in treated plots than in non-treated plots (0.3 ± 0.3%) (Figure 2). Without herbicide

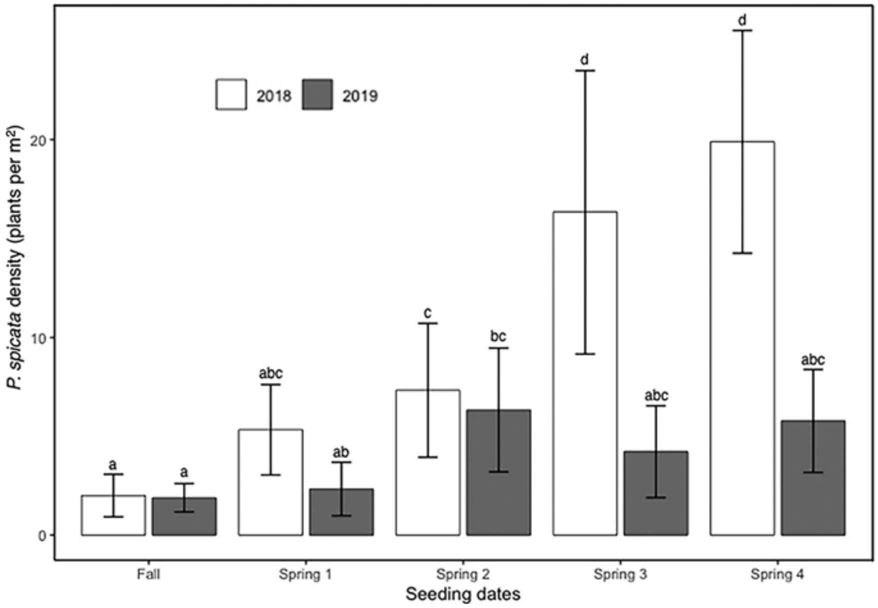

Figure 1. ***Pseudoroegneria spicata*** **density (plants per m²) at Belgrade as affected by seeding date and year. Fall seeding = 15 November 2017, Spring 1 = 21 April 2018, Spring 2 = 3 May 2018, Spring 3 = 17 May 2018, and Spring 4 = 31 May 2018. Similar letters indicate no difference in density across seeding dates and year. Error bars represent ± 1 standard error, α = 0.05.**

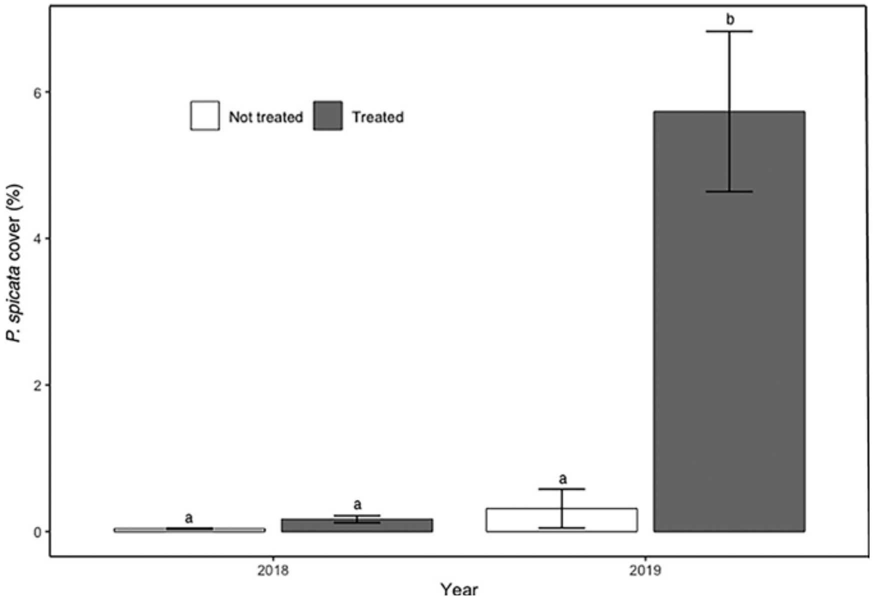

Figure 2. ***Pseudoroegneria spicata*** **cover (%) at Belgrade as affected by year and herbicide application. Similar letters indicate no difference in cover across years and herbicide application. Error bars represent ± 1 standard error, α = 0.05.**

application *P. spicata* cover did not change between the years.

Bromus tectorum cover was affected by the interaction among herbicide application, seeding date and year ($F_{4,20} = 3.476$, $p = 0.026$, Table 2). In 2018, herbicide application eliminated *B. tectorum* across all seeding dates (Figure 3). Cover in non-treated plots was low and did not differ significantly from the sprayed plots except for the Spring 2 seeding date. In 2019 the effect of the glyphosate application disappeared, and *B. tectorum* cover was similar among seeding dates with or without herbicide treatment (~5%). The exception to this was again the Spring 2 seeding date without herbicide application (27.0 ± 11.0%), which increased from 2018 to 2019 and was higher than all other treatment combinations.

Corvallis West Site

Pseudoroegneria spicata density differed among seeding dates ($X^2_{4,8} = 5.09$, $p = 0.025$) as well as the interaction between herbicide application timing and year ($X^2_{1,20} = 6.604$, $p = 0.018$, Table 2). *Pseudoroegneria spicata* density was similarly highest in Spring 1 (38.6 ± 9.0 plants per m²), Spring 2 (32.5 ± 5.46 plants per m²), and Spring 3 (35.6 ± 7.8 plants per m²) seeding dates. These seeding dates were followed by Spring 4 seeding date (22.2 ± 5.1 plants per m²) and the Fall seeding date (10.3 ± 3.0 plants per m²).

The interaction between herbicide application timing and year was driven by differences in density between years within each herbicide treatment (Figure 4). In each year, however, *P. spicata* densities were similar between the two herbicide treatments. *Pseudoroegneria spicata* density decreased between 2018 and 2019 with Fall herbicide treatment but not with Spring herbicide treatment.

Table 2. Analysis of Variance (ANOVA) table for *Pseudoroegneria spicata* (PSSP) density and cover and *Bromus tectorum* (BRTE) cover by site. Year represents sampling year of 2018 and 2019. Values in table represent the following in order: X^2/F, *p*-value, degrees of freedom, with X^2 applying to Density and F applying to Cover.

		Belgrade			Corvallis West			Corvallis East		
	Statistic	**PSSP Density**	**PSSP Cover**	**BRTE Cover**	**PSSP Density**	**PSSP Cover**	**BRTE Cover**	**PSSP Density**	**PSSP Cover**	**BRTE Cover**
Herbicide	X^2/F	84.670	74.325	16.830	0.137	30.152	2.828	0.493	1.572	0.706
	p-value	< 0.001	< 0.001	0.002	0.719	< 0.001	0.124	0.499	0.238	0.420
	df	1, 10	1, 10	1, 10	1, 10	1, 10	1, 10	1, 10	1, 10	1, 10
Seeding	X^2/F	5.392	1.003	1.315	5.09	15.23	0.856	3.151	1.375	0.398
	p-value	0.021	0.459	0.343	0.025	< 0.001	0.529	0.078	0.324	0.805
	df	4, 8	4, 8	4, 8	4, 8	4, 8	4, 8	4, 8	4, 8	4, 8
Year	X^2/F	47.685	4.66	99.230	10.930	11.701	27.52	17.85	0.088	22.60
	p-value	< 0.001	0.043	< 0.001	0.004	0.003	< 0.001	< 0.001	0.770	< 0.001
	df	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20
Herbicide * Year	X^2/F	0.532	29.72	3.069	6.604	5.056	0.151	0.253	0.348	0.083
	p-value	0.474	< 0.001	0.095	0.018	0.036	0.702	0.621	0.562	0.777
	df	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20	1, 20
Seeding * Year	X^2/F	8.263	1.10	1.619	2.124	0.682	0.033	3.005	0.745	1.098
	p-value	< 0.001	0.384	0.209	0.116	0.613	0.998	0.043	0.573	0.385
	df	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20
Herbicide * Seeding	X^2/F	2.573	0.893	2.092	1.811	6.228	1.149	4.003	0.409	1.043
	p-value	0.103	0.503	0.157	0.203	0.009	0.388	0.034	0.799	0.432
	df	4, 10	4, 10	4, 10	4, 10	4, 10	4, 10	4, 10	4, 10	4, 10
Herbicide * Seeding * Year	X^2/F	1.250	0.29	3.476	0.124	0.676	0.792	1.061	0.090	1.040
	p-value	0.322	0.881	0.026	0.972	0.616	0.544	0.401	0.984	0.412
	df	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20	4, 20

At Corvallis West cover of *P. spicata* was affected by interactions of seeding date and herbicide application timing ($F_{4,10} = 6.228$, $p = 0.009$) and herbicide application timing and year ($F_{1,20} = 5.056$, $p = 0.036$, Table 2). In the interaction of seeding date and herbicide application timing, *P. spicata* cover was generally highest with spring herbicide application combined with spring seeding dates (Figure 5). *Pseudoroegneria spicata* cover was low (< 4%) and not affected by seeding date when herbicide was applied in the fall. With spring herbicide application, seeding date had large effects on *P. spicata* cover. Spring 1, Spring 2, and Spring 3 seeding dates had greater cover than fall seeded *P. spicata* when combined with spring herbicide application, and the Spring 1 seeding date resulted in higher *P. spicata* cover (14.3 ± 2.4%) than Spring 2 and Spring 4 seeding dates.

The interaction between year and timing of herbicide application was driven by spring application of glyphosate resulting in increased *P. spicata* cover compared to fall application, and the magnitude of the difference between these was larger in the second year than in the first (Figure 6). *Pseudoroegneria spicata* cover increased in the Spring herbicide application from 5.2 ± 1.2% in 2018 to 8.8 ± 1.8% in 2019, while cover remained the same in the Fall herbicide treatment from one year to the next, at about 1.5%.

Bromus tectorum cover was not affected by seeding date or herbicide treatments but was lower in 2018 (1.9 ± 0.8%) than 2019 (10.7 ± 2.9%) across all treatments. Non-seeded, non-sprayed plots followed this trend as well with 0.2 ± 0.2% *B. tectorum* in 2018 and 18.0 ± 17.9% in 2019.

Corvallis East Site

Pseudoroegneria spicata density responded to the interactions between seeding date and herbicide application timing ($X^2_{4,10} = 4.003$, $p = 0.034$) and seeding date and year ($X^2_{4,20} = 3.005$, $p = 0.043$, Table 2). Overall, *P. spicata* establishment was low at this site, with most treatments producing between four and nine plants per m² (Figure 7). Spring 4 seeding date with Spring herbicide application produced the highest *P. spicata* density in 2019 at 21.1 ± 8.1 plants per m².

Pseudoroegneria spicata density was similar between years with most seeding dates, except for Spring 4 (Figure 8). Density was highest with Spring 4 seeding date in 2018 at 23.3 ± 8.0 plants per m² and declined to 3.9 ± 3.3 plants per m² in 2019. Second highest *P. spicata* density occurred with Spring 2 seeding in 2018 at 11.1 ± 4.0 plants per m² with the remaining combinations resulting in fewer than five plants per m². *Pseudoroegneria spicata* cover was very low at this site (< 1%) in both years and was not affected by treatments.

Bromus tectorum cover differed between years in the treated plots ($F_{1,20} = 22.60$, $p < 0.001$, Table 2). Though *B. tectorum* cover was low (< 3%) in both years, cover in 2018 (0.4 ± 0.1%) was lower than in 2019 (2.2 ± 0.5%). In the non-seeded, non-sprayed plots, *B. tectorum* cover

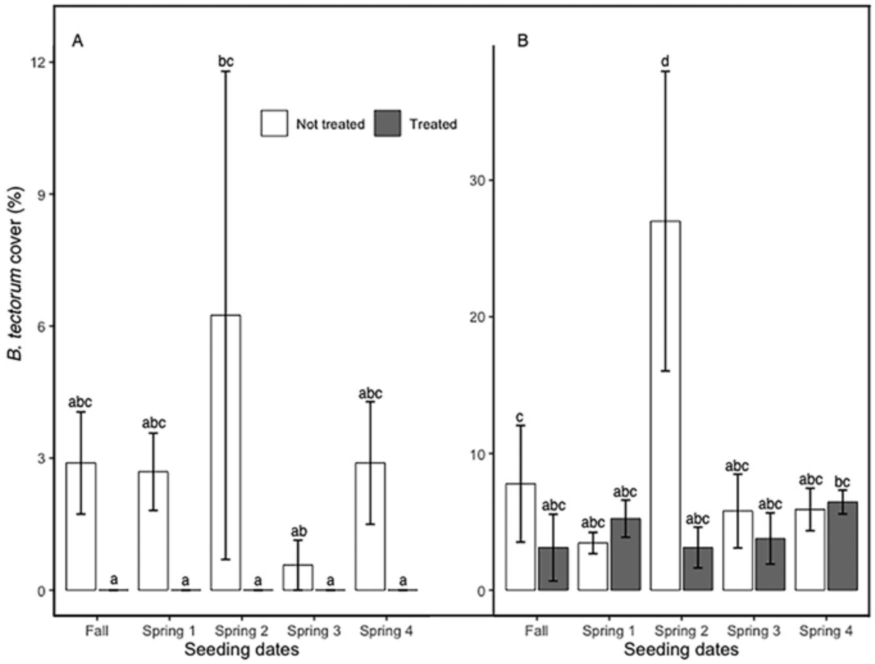

Figure 3. ***Bromus tectorum*** **cover (%) at Belgrade as affected by seeding date and herbicide application in A) 2018 and B) 2019. Fall seeding = 15 November 2017, Spring 1 = 21 April 2018, Spring 2 = 3 May 2018, Spring 3 = 17 May 2018, and Spring 4 = 31 May 2018. Herbicide application occurred 19 October 2017. Similar letters indicate no difference in cover across seeding date, herbicide application and year. Error bars represent ± 1 standard error, α = 0.05. Note that y-axes values are different between A) 2018 and B) 2019.**

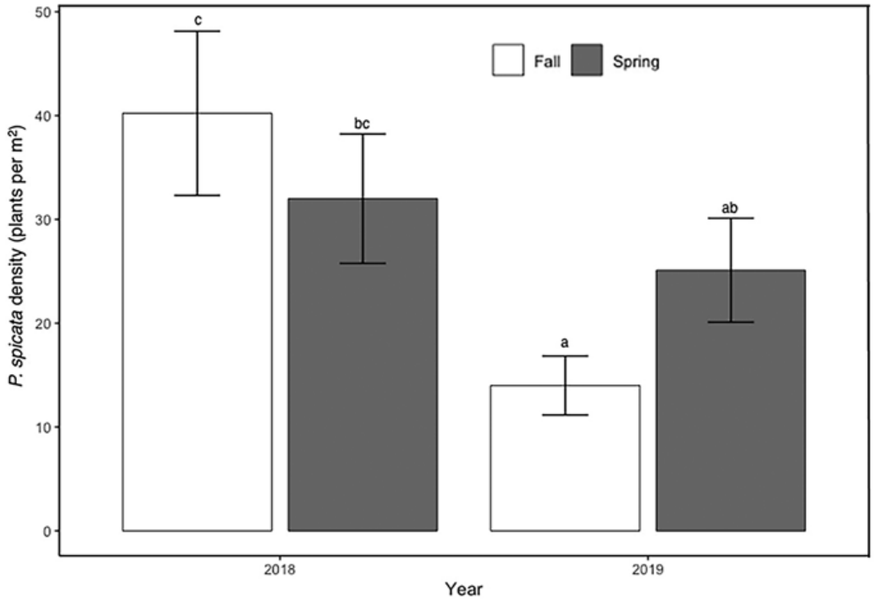

Figure 4. ***Pseudoroegneria spicata*** **density (plants per m²) at Corvallis West as affected by year and herbicide application. Similar letters indicate no difference in density across herbicide application and year. Error bars represent ± 1 standard error, α = 0.05.**

increased between years from ~2% in 2018 to ~12% in 2019.

Discussion

Given the extent of and challenges associated with *B. tectorum* invasion, managers need better approaches to effectively restore invaded rangelands. While seeding and herbicide are effective tools (Monaco et al. 2017), strategic timing of their use could further benefit degraded rangelands. We tested whether timing of seeding and glyphosate application would affect *P. spicata* establishment in *B. tectorum*-infested rangeland, theoretically by overcoming the seasonal priority effect typically demonstrated by *B. tectorum*. We found that an application of glyphosate resulted in at least a short-term decline in *B. tectorum*, and *P. spicata* establishment was improved when glyphosate was applied. These results support our first prediction that integrating herbicide would reduce *B. tectorum* abundance in favor of *P. spicata* establishment and align with the concept that once *B. tectorum* no longer accessed essential plant resources like soil nutrients and water, *P. spicata* was able to take advantage of the available resources and grow without competition from *B. tectorum* for a single growing season; that is, until the next cohort of *B. tectorum* emerged in the fall (Weidlich et al. 2017, Young et al. 2014, Vaughn and Young 2015).

Consistent with our second prediction, spring glyphosate application promoted *P. spicata* establishment better than fall, especially at the Corvallis West site where spring

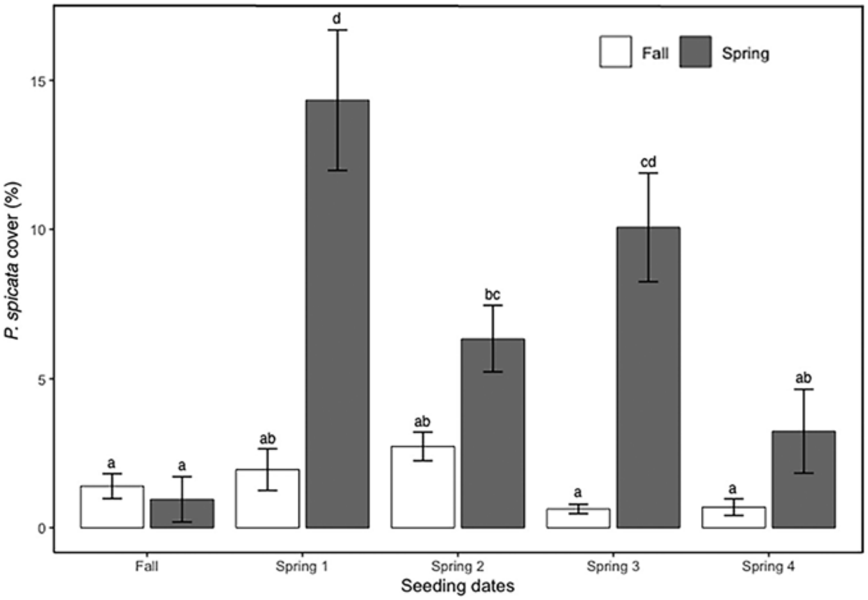

Figure 5. ***Pseudoroegneria spicata*** **cover (%) at Corvallis West as affected by seeding date and herbicide application. Fall seeding = 30 October 2017, Spring 1 = 3 April 2018, Spring 2 = 19 April 2018, Spring 3 = 4 May 2018, and Spring 4 = 15 May 2018. Herbicide application occurred 15 October 2017 (Fall) and 11 April 2018 (Spring). Similar letters indicate no difference in cover across seeding date and herbicide application. Error bars represent ±1 standard error, α = 0.05.**

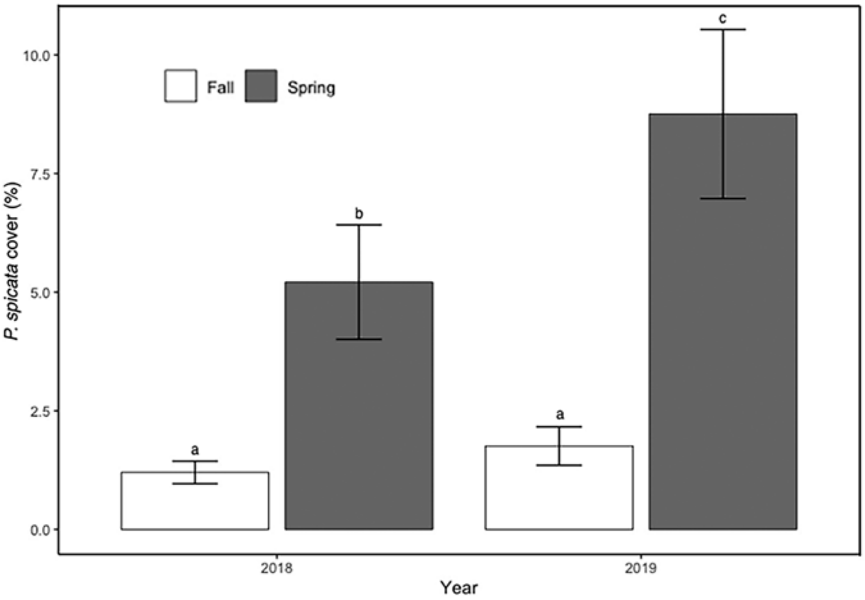

Figure 6. ***Pseudoroegneria spicata*** **cover (%) at Corvallis West as affected by year and herbicide application. Similar letters indicate no difference in cover across year and herbicide application. Error bars represent ±1 standard error, α = 0.05.**

applications decidedly increased *P. spicata* cover over multiple seeding dates and by the second year after treatment. *Bromus tectorum* generally germinates and emerges in fall but can also do so throughout late winter and into spring (Mack and Pyke 1983). Spring herbicide application was likely more effective because it controlled both overwintering and spring-emerging seedlings, not only of *B. tectorum* but also other weedy species that were present at the sites. The effectiveness of spring glyphosate application for controlling *B. tectorum* has been shown in other studies as well (Kyser et al. 2013, Morris et al. 2016, Sebastian et al. 2017). Glyphosate effectiveness can also be affected by how actively growing the target weeds are, which can be influenced by environmental factors like soil moisture and temperature (Patterson 1995, de Ruiter and Meinen 1998, Bastiani et al. 2021). We did not account for this in our study, but we encourage practitioners to consider such factors when deciding the optimum timing for glyphosate application.

We had also predicted that spring herbicide application combined with fall seeding would be an exception to spring glyphosate application working better than fall application. This prediction was based upon the assumption that fall-seeded *P. spicata* could emerge prior to glyphosate application in the spring and thus be injured or killed by the non-selective herbicide. Our results did not, however, show a difference in *P. spicata* abundance between fall and spring herbicide application when combined with fall seeding as we predicted. We did not search for nor casually observe any *P. spicata* seedlings at the time of the spring glyphosate application, so it is possible that emergence had not yet occurred when we applied glyphosate. *Pseudoroegneria spicata* cover and density were generally low with the fall seeding date regardless of glyphosate application timing.

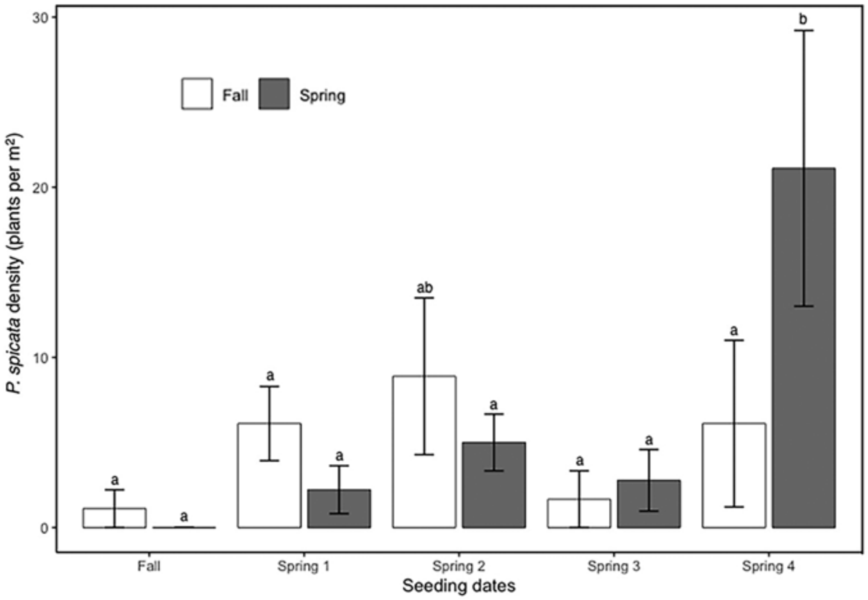

Figure 7. ***Pseudoroegneria spicata*** **density (plants per m²) at Corvallis East as affected by seeding date and herbicide application. Fall seeding = 30 October 2017, Spring 1 = 3 April 2018, Spring 2 = 19 April 2018, Spring 3 = 4 May 2018, and Spring 4 = 15 May 2018. Herbicide application occurred 15 October 2017 (Fall) and 11 April 2018 (Spring). Similar letters indicate no difference in density across seeding dates and herbicide application. Error bars represent ± 1 standard error, α = 0.05.**

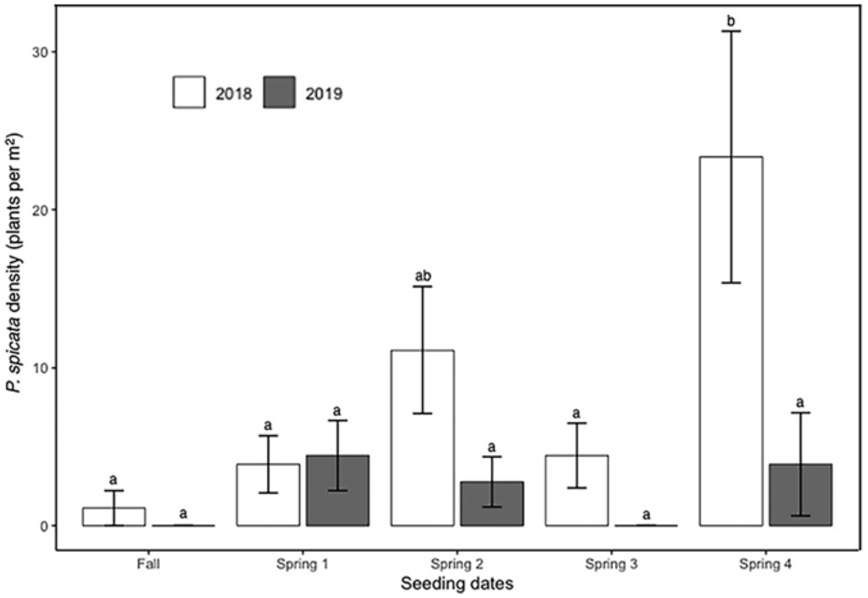

Figure 8. ***Pseudoroegneria spicata*** **density (plants per m²) at Corvallis East as affected by seeding date and year. Fall seeding = 30 October 2017, Spring 1 = 3 April 2018, Spring 2 = 19 April 2018, Spring 3 = 4 May 2018, and Spring 4 = 15 May 2018. Similar letters indicate no difference in density across seeding dates and year. Error bars represent ± 1 standard error, α = 0.05.**

If spring herbicide application is to be combined with fall seeding, restoration practitioners should be careful to spray prior to emergence of seeded species.

Our third prediction that spring versus fall seeding would result in higher abundance of *P. spicata* and lower abundance of *B. tectorum* was partially supported. *Pseudoroegneria spicata* density and cover were generally higher with spring seeding dates when compared to fall seeding, but optimal spring timing when combined with glyphosate application varied across sites. At Belgrade this occurred with Spring 2 (3 May), Spring 3 (17 May) and Spring 4 (31 May) seeding dates; at Corvallis West this included Spring 1 (3 April), Spring 2 (19 April) and Spring 3 (4 May) seeding dates; and at Corvallis East this included Spring 4 (15 May) seeding date. In a related study, Harvey et al. (2020) advised that spring seeding of *P. spicata* should occur prior to the accumulation of 206 growing degree days (GDD), which corresponded to 5 May at two sites in southwestern Montana. Therefore, we explored growing degree days (GDD) for Belgrade and Corvallis sites as a possible explanation to the differences among spring seeding dates (Table 1). At Belgrade and Corvallis West, the optimal seeding dates occurred prior to or near 206 GDD; at Corvallis East, the optimal seeding date was beyond 206 GDD. This result at Corvallis East is difficult to explain but could be due to the site being at higher elevation than Corvallis West, slightly different soil, and the presence of different weedy species in addition to *B. tectorum*. Nevertheless, consistent results at two of three sites and across six of eight spring seeding dates supports spring seeding integrated with glyphosate application prior to or close to 206 GDD.

Seeded species density commonly decreases in the initial years following seeding (Fansler and Mangold 2011, Boyd and Lemos 2015, Schantz et al. 2016), and this held true at our sites with *P. spicata* density declining between 2018 and 2019. Seeded species' mortality between years could be a result of competition between *P. spicata* and other vegetation at the sites. For example, other perennial grasses increased from 2018 to 2019 at Belgrade (data not shown), and at all sites *B. tectorum* increased across the two years. Glyphosate, the herbicide used in this study, does not persist in the soil to provide residual weed control (Henderson et al. 2010) and was only expected to control weedy vegetation for a short period of time. The decline in *P. spicata* density also could have occurred because of mortality of seedlings during the winter following their emergence, as has been documented in some studies (James et al. 2011, Boyd and James 2013). Even with the decline in *P. spicata* from 2018 to 2019, results of this study are promising. In the second year *P. spicata* density at Belgrade was six plants per m^2 with Spring 2 and Spring 4 seeding dates, and at Corvallis West several spring seeding dates averaged about 25 plants per m^2. Revegetation may be considered successful when desired vegetation is > 5 plants per m^2 in rangelands (Valentine 1989). At Belgrade and Corvallis West, not only was revegetation successful, but the plants that survived to 2019 were bigger, as evidenced by the increase in *P. spicata* cover from 2018 to 2019. This is encouraging, especially considering that some revegetation studies have shown that initial, sometimes less-promising results can improve over the long term, e.g., four and up to 15 years after treatment (Rinella et al. 2012, Rinella et al. 2020).

Glyphosate has been shown to effectively reduce weedy plant competition prior to seeding (Morris et al. 2016, Sebastian et al. 2017). We found, however, that *B. tectorum* abundance was minimally affected by our treatments and appeared to fluctuate between years and across study sites. Other studies focused on restoring rangelands impacted by *B. tectorum* have seen similar fluctuations in *B. tectorum* abundance, seemingly unrelated to treatments (Orloff et al. 2015, Ehlert et al. 2019). Longer term changes in *B. tectorum* abundance in response to our seeding treatments may occur (Rinella et al. 2012, Monaco et al. 2016, Rinella et al. 2020) but were beyond the scope of this study.

The objective of this study was to test whether timing of seeding integrated with a glyphosate application would affect *P. spicata* establishment. Our results generally indicate that spring seeding following a spring glyphosate application is an ecologically-based strategy worth considering for re-establishing the ubiquitous, long-lived native bunchgrass *P. spicata* on rangeland impacted by *B. tectorum* in the western United States. Our results suggest an application of the non-persistent herbicide glyphosate can be conducted prior to seeding (if spring-seeded) or desired species emergence (if fall-seeded), with the added benefit of improving efficiency and reducing restoration costs (Sheley et al. 2012). Due to the non-selective nature of glyphosate, however, this approach should be reserved for severely degraded rangeland where there is little to no perennial vegetation remaining that could re-occupy open spaces following a selective herbicide application.

Our results are encouraging because even though recent advances in chemical control show multi-year *B. tectorum* control from a single application of indaziflam (Sebastian et al. 2016, Clark et al. 2020, Courkamp et al. 2022), areas treated with it cannot be seeded into for at least 24 months (Environmental Sciences US LLC 2020, IMAGINE 2023), which could leave bare ground in areas heavily infested with annuals prior to its application. Instead, seeding could be integrated with an herbicide like glyphosate that has no soil residual, then, once perennial species have established a robust root system, indaziflam could be applied to control any re-invading *B. tectorum*.

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Michelle L. Majeski, Montana State University, College of Agriculture, Department of Land Resources and Environmental Sciences, Bozeman, MT

Stacy C. Simanonok, Montana State University, College of Agriculture, Department of Land Resources and Environmental Sciences, Bozeman, MT

Zachariah J. Miller, Western Agriculture Research Center, Montana State University, Corvallis, MT

Lisa J. Rew, Professor, Montana State University, College of Agriculture, Department of Land Resources and Environmental Sciences, Bozeman, MT

Jane M. Mangold (corresponding author), Montana State University, College of Agriculture, Department of Land Resources and Environmental Sciences, Bozeman, MT 59717, jane.mangold@montana.edu

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