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# Establishing Wyoming Big Sagebrush in Annual Brome-Invaded Landscapes with Seeding and Herbicides



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## ABSTRACT

Seeding native plants into degraded grasslands presents major challenges. Often, seeded species fail to establish and areas become/remain dominated by unwanted plants. We combined herbicides and seeding in former coal mining fields dominated by exotic winter annual grasses (downy brome [Bromus tectorum L.] and Japanese brome [Bromus arvensis L], hereafter "annual bromes"). The main interest was restoring Wyoming big sagebrush (Artemisia tridentata spp. wyomingensis [Beetle & A. Young] S.L. Welsh, hereafter "big sage"), a very difficult species to restore to North American grasslands. We tested the nonselective herbicide glyphosate and the grass-specific herbicide quizalofop. The summer following herbicide applications and seeding, annual brome cover in controls 22% (Cl<sub>95%</sub> 13%, 36%) was significantly greater (P < 0.03) than in glyphosate 11% (Cl<sub>95%</sub> 5%, 25%) and quizalofop 16% ( $CI_{95\%}$  7%, 35%) treatments. At Decker mine, glyphosate increased seeded big sage density ( $P < 10^{-10}$ 0.04) from 0.76 ( $CI_{95\%}$  0.27, 2.11) to 3.05 ( $CI_{95\%}$  1.42, 6.56) plants  $\cdot$  m<sup>-2</sup> the second summer after seeding. Corresponding increases for Spring Creek mine were from 0.11 ( $CI_{95\%}$  0.03, 0.43) to 0.43 ( $CI_{95\%}$  0.13, 1.40) plants  $\cdot$  m<sup>-2</sup> (P < 0.04). These results were consistent across two experiments initiated in different years. In addition to big sage, our study's seed mixes contained native grasses and forbs, and herbicide treatments tended to promote establishment of these plant groups. In annual brome-dominated areas of the northern Great Plains, conditions amenable to big sage seedling establishment do not appear entirely uncommon, and herbicides can increase establishment.

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## Introduction

Grassland ecosystems have become increasingly degraded (Knapp, 1996; Merritt and Dixon, 2011; O'Mara, 2012), and efforts to restore these systems are underway in many parts of the World (Jones and Schmitz, 2009; James et al., 2013). Grassland restoration typically involves introducing grasses, forbs or shrubs from seed, and these seeding efforts commonly fail (Carrick and Krüger, 2007; Valladares and Gianoli, 2007; Josa et al., 2012). Shrubs have proven particularly difficult to restore (Kulpa et al., 2012; Schellenberg et al., 2012; Rinella et al., 2015), which is troubling given the important role shrubs play in grasslands. Shrubs provide critical habitat for threatened and endangered species (Knick et al., 2003; Rowland et al., 2006; Hess and Beck, 2012; Knick et al., 2013) and forage for wildlife and livestock (Hubbard, 1957; Ngugi et al., 1992; Shipley et al., 2006). In addition, in some systems, shrubs stabilize soils to reduce desertification risks (e.g., Li et al., 2013;

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Linstädter and Baumann, 2013) and increase resistance to exotic plant invasions (Prevéy et al., 2010).

Weed competition is believed to be a key factor preventing seeded shrubs from surviving beyond the seedling stage. As such, several studies have evaluated herbicides as means for controlling weeds to increase seeded shrub establishment. At least three of these studies have occurred in Oregon and Utah in areas dominated by the non-native perennial crested wheatgrass (Agropyron cristatum [L.] Gaertm). Controlling crested wheatgrass with herbicides has generally not allowed shrubs to establish (Fansler and Mangold, 2011; Davies et al., 2013; Cox and Anderson, 2004). The one exception was Cox and Anderson (2004), who found controlling crested wheatgrass with glyphosate in a relatively wet year increased shrub density from about 0 to 2.5 seedlings · m<sup>-2</sup>. However, when they repeated their experiment in a drier year, shrubs failed to establish in both control or herbicide plots. In addition to crested wheatgrass-dominated areas, there have also been efforts to seed shrubs and other plants into areas dominated by invasive annual grasses like downy brome and medusahead (Taeniatherum caput-medusae [L.] Nevski). Herbicides have usually not aided seeded shrubs in these studies (Cox and Anderson, 2004; Cox and Allen, 2008; Johnston and Chapman, 2014; Brabec et al., 2015; Davies et al., 2015; Bell et al.,

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2016). Exceptions include Cione et al. (2002), who found controlling annual grasses with fluazifop increased shrub densities from 0 to between 1 and 16 seedlings  $\cdot$  m<sup>-2</sup> (depending on shrub species) in California and Johnson (2015), who found imazapic increased seeded shrub cover from about 1% to 9% in Colorado.

One reason herbicides have not consistently aided shrubs is they have not consistently suppressed target weeds (e.g., Cox and Anderson, 2004; Fansler and Mangold, 2011). However, shrubs have often failed to establish in cases where herbicides reduced weed cover as much as 75% to 90% (Cox and Allen, 2008; Johnston and Chapman, 2014; Bell et al., 2016). In at least one case herbicide (i.e., imazapic) directly damaged the shrubs (Johnston and Chapman, 2014), but in other cases factors unrelated to herbicides and weeds presumably prevented establishment, such as unsuitable weather (Mangla et al., 2011), granivory (Suazo et al., 2013), and soil crusting (Wood et al., 1982).

In this study, we tested whether controlling exotic "annual brome" grasses [i.e., downy brome (*Bromus tectorum* L.) and Japanese brome (*Bromus arvensis* L.)] with herbicides increased establishment of "big sage" [i.e., Wyoming big sagebrush (*Artemisia tridentata* spp. *wyomingensis* [Beetle & A. Young] S.L. Welsh)] in mixed grass prairie of the northern Great Plains. When weed abundances are low, successful shrub establishment is not uncommon in this system, particularly on topsoil newly deposited after mining operations (Schuman et al., 2012; Rinella et al., 2016). This led us to believe herbicides might prove more beneficial in this system compared to systems where shrubs usually fail to establish even when weed abundances are low. In addition to big sage, we tested herbicide effects on native grasses and forbs included in our seed mixes.

Our annual brome-dominated sites supported scattered individuals of native forb and shrub species, and we chose herbicide treatments that posed minimal risk to these species. One treatment was the nonselective herbicide glyphosate applied the fall before seeding when annual brome seedlings had recently emerged but most forbs and shrubs were dormant and hence unsusceptible to glyphosate. A second treatment was guizalofop, an herbicide that controls grasses but not broadleaf plants, applied in spring just prior to seeding when annual bromes were at vegetative stages. Quizalofop damage to native grasses was not a concern as they were nearly absent, but quizalofop was applied when nonselective herbicides like glyphosate would have damaged actively growing forbs and shrubs. A third treatment combined the fall glyphosate and spring quizalofop treatments, and a fourth treatment was a no herbicide control. Because annual bromes were highly abundant, we hypothesized establishment of big sage and other seeded species would be negligible in no herbicide controls. We hypothesized the glyphosate treatment and the quizalofop treatment would promote seeded plant establishment by reducing annual brome competition. Finally, by targeting annual bromes twice, we hypothesized the treatment that combined glyphosate and quizalofop would provide the greatest suppression of annual bromes and greatest establishment of seeded species.

### Methods

## Site Description

Sites were in the northern Great Plains, north of Decker, Montana, on Spring Creek mine ( $45^{\circ}12'N$ ,  $106^{\circ}91'W$ ) and Decker mine ( $45^{\circ}06'N$ ,  $106^{\circ}84'W$ ), two surface coal mines separated by about 15 km. Topography is rolling plains fragmented by drainages and rocky outcroppings. Mean elevation and annual temperature, wind speed, and precipitation are about 1070 m, 8.5 C, 11.5 km  $\cdot$  hr<sup>-1</sup>, and 350 mm. Vegetation of nearby unmined areas is perennial grasses including blue grama (*Bouteloua gracilis* [Willd. ex Kunth] Lag. ex Griffiths), prairie Junegrass (*Koeleria macrantha* [Ledeb.] Schult.), alkali sacaton (*Sporobolus airoides* [Torr.] Torr.), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.), sand dropseed (*Sporobolus cryptandrus* [Torr.] A. Gray), sandberg bluegrass (*Poa secunda* J. Presl), and western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Love). Forbs include western yarrow (*Achillea millefolium* L. var. occidentalis DC), prairie coneflower (*Ratibida columnifera* [Nutt.] Wooton & Standl.), white prairie clover (*Dalea candida* Michx. ex Willd.), scarlet globemallow (*Sphaeralcea coccinea* [Nutt.] Rydb.), prairie sagewort (*Artemisia frigida* Willd.), and blacksamson echinacea (*Echinacea angustifolia* DC.). Shrubs include big sage, winterfat (*Krascheninnikovia lanata* [Pursh] A. Meeuse & Smit), and rubber rabbitbrush (*Ericameria nauseosa* [Pall. ex Pursh] G.L. Nesom & Baird). After mining of the sites, topsoil was deposited on top of subsoil and coarse, rocky material generated during the mining process. Then, between 2009 and 2011, sites were seeded with native grasses, forbs and shrubs, but few seeded plants established, and sites became dominated by annual bromes.

#### Experimental Design

To increase chances of evaluating herbicide effects under conditions conducive to shrub establishment, we repeated our experiment beginning in two different years. The first experiment began fall 2014 (2014 Experiment), and the second experiment began fall 2015 (2015 Experiment). Each experiment included six Decker and three Spring Creek sites. At each site,  $10 \ 12 \times 12 \ m$  plots were established (2 experiments  $\times 2 \ mines \times 9 \ sites \times 10 \ plots = 360 \ plots$ ). In eight plots at each site, a dense annual brome litter layer was removed to increase light availability and seed-soil contact and four herbicide treatments (glyphosate, quizalofop, both herbicides, control) were factorially combined with two big sage seed rates (3.4, 5.6 kg  $\cdot \ ha^{-1}$ ) (Table 1). A ninth plot received no treatment and a tenth plot received only litter removal.

Glyphosate (Roundup PRO, Monsanto at 0.877 L  $\cdot$  ha<sup>-1</sup>) was applied on 17 October 2014 (2014 Experiment) and from 13 October to 14 October 2015 (2015 Experiment) when annual bromes had recently emerged and most native plants were dormant. Quizalofop (Assure II, DuPont at 0.950 L  $\cdot$  ha<sup>-1</sup>) was applied from 24 April to 28 April 2015 (2014 Experiment) and on 4 April 2016 (2015 Experiment) when annual bromes and some native plants had recently begun spring growth. Although quizalofop is registered for use in noncrop areas, our study appears to be the first to use quizalofop to aid seeded shrub establishment, though fluazifop, another grass-specific herbicide, has been tested for this purpose in California annual grasslands (Cox and Allen, 2008; Bell et al., 2016). Herbicides were mixed with water and applied with even flat spray tips (TeeJet 8002E) on an all terrain vehicle-mounted sprayer calibrated to deliver 128 liters · ha<sup>-1</sup>. A nonionic surfactant (Brewer 90-10, Brewer International) was added at 0.25% v/v. Litter was removed 3 or more days after quizalofop applications (28 April 2015–18 May 2015 [2014 Experiment] and 7-14 April 2016 [2015 Experiment]) using a chain harrow pulled by a tractor or by accidental burning at three Decker sites on 28 April 2015.

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Pure live see	l rates (kg	<ul> <li>ha<sup>-1</sup>) for</li> </ul>	experiments.
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Functional group	Species	Spring Creek	Decker
Cool-season	Sandberg bluegrass	0.6	0
grasses	Prairie junegrass	1.4	0
Warm-season	Alkali sacaton	0.7	1.7
grasses	Blue grama	1.1	1.1
	Sideoats grama	0	2.2
	Sand dropseed	0.6	1.7
	Prairie coneflower	1.1	0
	Scarlet globemallow	0.2	0.2
	Western yarrow	0.3	0.3
Forbs	Blacksamson		
	echinacea	0.5	0
	White prairie clover	0	0.4
	Prairie sagewort	0.3	0
Shrubs	Big sage	Mix 1: 3.4 Mix 2: 5.6	Mix 1: 3.4 Mix 2: 5.6

Seeding occurred from 18 to 19 May 2015 (2014 Experiment) and 8 to 14 April 2016 (2015 Experiment). Big sage seeds sown at Decker and Spring Creek were collected from wild populations in Utah and Wyoming, respectively. Except for big sage seed rates, seeding treatments differed somewhat between mines, though all seeded species are common to natural grasslands of our study region (Table 1). Seed was deposited on the soil surface with a Flex II Seeder (Truax Company, Inc) at Decker and a Sunflower Seeder (AGCO Corporation) at Spring Creek using cracked corn and rice hulls as carriers.

A year after seeding the 2014 experiment (4 April 2016), half of each plot formerly treated with glyphosate, quizalofop or glyphosate plus quizalofop was retreated with quizalofop to further control annual bromes using methods described above.

#### Vegetation Measurements

Within each plot half, four random points were permanently marked. Prior to applying any treatments (6–7 October 2014 [2014 Experiment] and 5–12 October 2015 [2015 Experiment]), annual brome cover was visually estimated in circular frames (0.10 m<sup>2</sup>) at marked points. Following initial herbicide treatments, litter removal and seeding, cover by species and big sage density were estimated in circular frames (0.25 m<sup>2</sup>) at marked points. Sampling occurred from 7 to 22 July 2015 (2014 Experiment) and 12 to 29 July 2016 (2014 and 2015 Experiments).

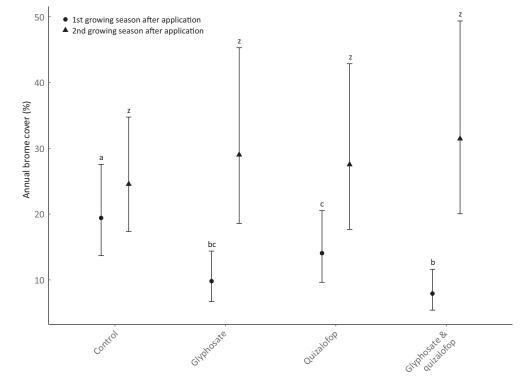
### Data Analysis

Responses were natural log cover of annual bromes and non-native annual forbs, big sage density, and cover of native seeded forbs and grasses. Because 35% of seeded forb and 67% of seeded grass values were 0, these data were analyzed with a nonparametric randomization test (Hjorth, 1994). Other responses were analyzed with linear mixedeffects models fit with R version 3.3.2 (R Core Team, 2016) using the lme4 (Bates et al., 2015), ImerTest (Kuznetsova et al., 2016), and lsmeans (Lenth, 2016) packages. For each response, we used the Akaike Information Criterion (Akaike, 1998) to select among large sets of candidate models containing main effects, interactions, and covariates.

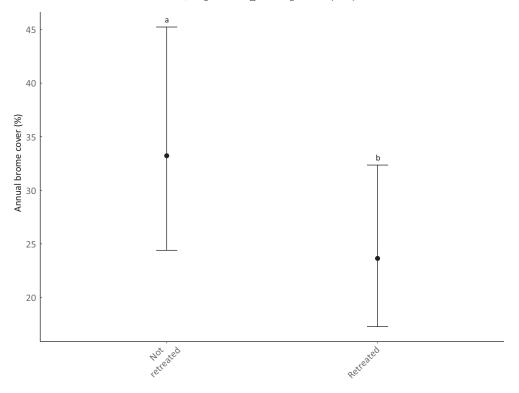
The annual brome cover model and the non-native annual forb model had mine, experiment, mine by experiment, and herbicide treatment as fixed effects, site and site by herbicide as random effects, and pretreatment brome cover standardized to mean 0, standard deviation 1 as a covariate (see Appendix A for model details). Our Poisson model for big sage density was the same except seeding (seeded versus not) was included as a fixed effect, site by seeding was included as a random effect and the brome cover covariate was excluded. The 2014 experiment was measured a second growing season after seeding, and these annual brome, annual forb, and big sage density data were analyzed with separate models having fixed effects for mine, herbicide, and seeding and random effects for site, site by herbicide, and site by seeding. In addition, to account for retreating half of each herbicidetreated plot with quizalofop in spring of the second growing season, the models included a fixed effect for quizalofop retreatment and a random effect for plot.

## Results

In both experiments, first growing season (1 April–31 August) precipitation exceeded the 30-year, 200-mm average, and this precipitation did not vary widely between 2014 (250 mm) and 2015 (230 mm) experiments (National Oceanic and Atmospheric Administration station within 10 km of sites, http://www.noaa.gov/). Consequently, annual brome cover did not vary by experiment (P = 0.58), nor did it vary by mine (Fig. 1; P = 0.54). The summer after seeding, annual brome cover was 19% (Cl<sub>95%</sub> 14%, 28%) in the control, which was significantly greater than in glyphosate 10% (Cl<sub>95%</sub> 7%, 14%), quizalofop 14% (Cl<sub>95%</sub> 10%, 21%), and glyphosate plus quizalofop 8% (Cl<sub>95%</sub> 5%, 12%) treatments (P < 0.04). In contrast with our hypothesis, glyphosate followed by quizalofop was not more effective than glyphosate alone (P = 0.60). No herbicide treatments affected annual bromes beyond the first



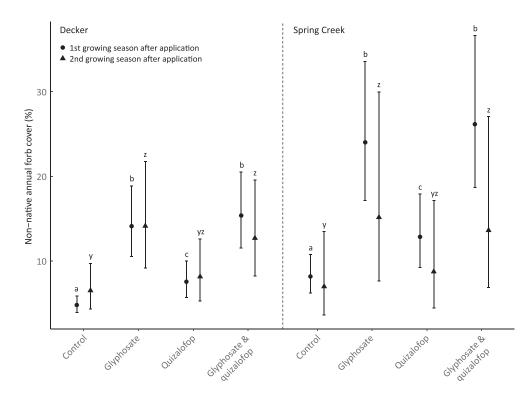
**Figure 1.** Point estimates (dots, triangles) and 95% confidence intervals (bars) estimating annual brome cover the first summer after herbicide treatments at Decker and Spring Creek mines. Results did not differ by mine or between experiments initiated 2014 and 2015. Within a summer, estimates with different letters significantly differ (*P* < 0.05).



**Figure 2.** Point estimates (dots) and 95% confidence intervals (bars) estimating effect of retreating annual bromes with quizalofop a year after initial herbicide treatments. Results did not differ between Decker and Spring Creek mines or between initial herbicide treatments. Estimates with different letters significantly differ (*P* < 0.05).

summer after application (Fig. 1). Retreating with quizalofop a year after initial herbicide treatments reduced annual brome cover from 33% ( $CI_{95\%}$  24%, 45%) to 24% ( $CI_{95\%}$  17%, 32%) (Fig. 2; P < 0.001). Seeding did not impact cover of annual bromes and annual forbs (P > 0.54).

Controlling annual bromes often increased the non-native annual forbs kochia (*Kochia scoparia* [L.]) and Russian thistle (*Salsola tragus* [L.]) (Fig. 3). For example, the summer following application, glyphosate increased non-native forb cover from 5% (Cl<sub>95%</sub> 4%, 6%) to 14% (Cl<sub>95%</sub>



**Figure 3.** Point estimates (dots and triangles) and 95% confidence intervals (bars) estimating non-native annual forb (kochia and Russian thistle) cover the first and second summer after herbicide applications at Decker and Spring Creek mines. Results did not differ between experiments initiated 2014 and 2015. Within mine and summer, estimates with different letters significantly differ (*P* < 0.05).

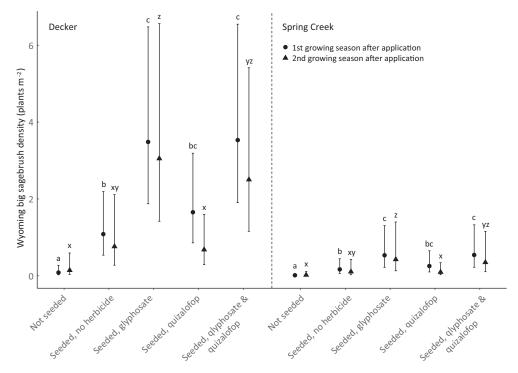


Figure 4. Point estimates (dots, triangles) and 95% CIs (bars) estimating big sage densities the first and second summer after seeding and herbicide treatments at Decker and Spring Creek mines. Results did not differ between experiments initiated 2014 and 2015. Within mine and summer, estimates with different letters significantly differ (*P* < 0.05).

11%, 19%) at Decker and from 8% ( $CI_{95\%}$  6%, 11%) to 24% ( $CI_{95\%}$  17%, 34%) at Spring Creek (P < 0.001). Applying quizalofop a year after initial herbicide treatments did not further increase non-native forbs.

Big sage height was 1 to 7 cm and 1 to 15 cm the first and second summer after seeding, respectively. Big sage densities were greater at Decker than Spring Creek (Fig. 4). In contrast with our hypothesis. seeding increased big sage density even without herbicides: the summer after seeding, seeding alone increased big sage density from 0.08 (Cl<sub>95%</sub> 0.03, 0.27) to 1.08 (Cl<sub>95%</sub> 0.54, 2.19) plants  $\cdot$  m<sup>-2</sup> at Decker (P < 0.001) and from 0.01 (Cl<sub>95%</sub> 0.00, 0.05) to 0.17 (Cl<sub>95%</sub> 0.06, 0.44) plants  $\cdot$  m<sup>-2</sup> at Spring Creek (P < 0.001) (Fig. 5). In no case did quizalofop increase big sage densities (Fig. 4). At Decker, glyphosate increased big sage density from 1.08 (CI<sub>95%</sub> 0.54, 2.19) to 3.48 (CI<sub>95%</sub> 1.87, 6.48) plants  $\cdot$  m<sup>-2</sup> and from 0.76 (Cl<sub>95%</sub> 0.27, 2.11) to 3.05 (Cl<sub>95%</sub> 1.42, 6.56) plants  $\cdot$  m<sup>-2</sup> the first and second summers after seeding, respectively (P < 0.04). Corresponding increases for glyphosate at Spring Creek are from 0.17 ( $CI_{95\%}$  0.06, 0.44) to 0.53 ( $CI_{95\%}$  0.22, 1.31) plants  $\cdot$  m<sup>-2</sup> and from 0.11 ( $CI_{95\%}$  0.03, 0.43) to 0.43 (Cl<sub>95%</sub> 0.13, 1.40) plants  $\cdot$  m<sup>-2</sup> (*P*<0.04). Because glyphosate alone and glyphosate followed by quizalofop similarly controlled annual bromes, both treatments had similar effects on big sage density (P >0.98). Increasing big sage seed rate from 3.4 to 5.6 kg  $\cdot$  ha<sup>-1</sup> did not increase big sage densities (P > 0.34).

Consistent with our hypothesis, seeding increased seeded forb cover only when combined with herbicides (Fig. 5). At Decker, seeding combined with any of the three herbicides treatments led to seeded forb cover of 0.7% to 2.3%, which was significantly greater than the ~0.4% observed without herbicides and seeding (P < 0.05). At Spring Creek, the most effective treatment for establishing seeded forbs appeared to be quizalofop in the 2014 experiment and glyphosate followed by quizalofop in the 2015 experiment (Fig. 5).

In the 2014 experiment at Decker, it was clear by the 2nd growing season after seeding that seeding increased grass cover (Fig. 6). Treatment means for glyphosate (2.8%) and glyphosate followed by quizalofop (2.6%) suggest these were the most effective treatments, but these treatments were not significantly different than seeding without herbicide (0.9%; P > 0.06). Likewise, in the 2015 experiment at

Decker, treatment means suggest glyphosate treatments were most effective, but differences between glyphosate treatments and seeding without herbicide were again not significant (Fig. 6; P > 0.08). In the 2014 experiment at Spring Creek, seeded grasses did not establish. In the 2015 experiment at Spring Creek, no seeded grasses were observed without herbicide, and herbicide treatments provided seeded grass cover of 0.03% to 0.08%. Reapplying quizalofop a year after original applications did not significantly increase seeded species cover (P > 0.19).

## Discussion

Our finding that herbicides increased shrub seedling establishment (Fig. 4) accords with a few studies (Cione et al., 2002; Cox and Anderson, 2004; Johnson, 2015) but conflicts with most studies (see Introduction). Big sage established both seeding years (Fig. 4), suggesting the difference between ours and most studies is our study environment is more consistently conducive to shrub establishment. Supporting this hypothesis are other studies of our coal mining environment conducted 200 to 250 km to the southeast. Here, seeding generated big sage densities even greater than ours: about 5 (Williams et al., 2002) and 12 seedlings  $\cdot$  m<sup>-2</sup> (Schuman et al., 1998). However, in these studies and ours, seeding year precipitation was above average, so instead of our system being more consistently amenable to shrub establishment, studies of our system may have spanned atypically favorable growing seasons. Yet, previous research at Decker and Spring Creek mines suggests shrub cover declines with increasing first growing season precipitation because precipitation increases weed and grass competition (Rinella et al., 2015), so shrub densities might have been even greater had ours and other studies spanned drier years. In any case, growing conditions allowing shrub establishment do not appear uncommon to our system, and this points to a role for herbicides, because herbicides can increase shrub establishment only in systems like ours where factors unrelated to weeds (e.g., granivory, disease, herbivory) do not consistently prevent establishment.

In addition to increasing big sage densities, glyphosate combined with seeding tended to increase seeded forb cover at Decker and seeded grass cover at both mines, except for the 2014 experiment at Spring

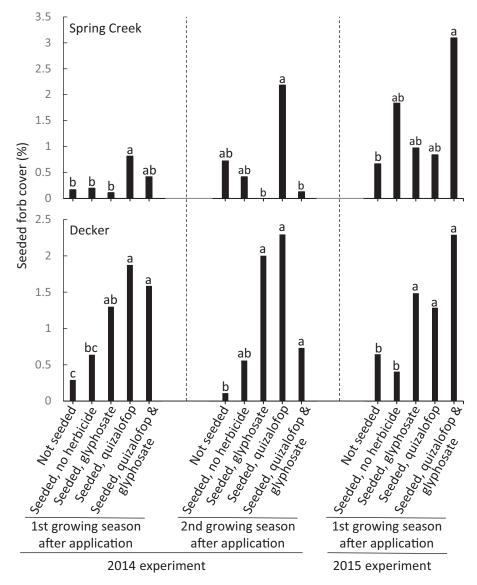


Figure 5. Seeded forb cover at Decker and Spring Creek mines. Within mine and growing season, estimates with different letters significantly differ (P < 0.05).

Creek (Figs. 5 and 6). The finding that controlling grassland weeds with herbicides increased seeded grasses is consistent with some studies (e.g., Masters et al., 2001; Endress et al., 2012), though there are likely just as many or more studies where herbicides have not increased seeded grasses or forbs (e.g., Fansler and Mangold, 2011; Johnston, 2014). Although annual bromes remained dominant and perennial grass cover remained low over our study, we believe perennial grasses could gradually increase on our study sites. Long-term monitoring at one of our study mines (i.e., Decker) has shown initially extremely sparse stands of the perennial western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Love) can gradually increase and displace annual bromes (Prodgers, 2013).

Controlling winter annual bromes with glyphosate in fall and/or grass-specific herbicide in spring caused a flush of weedy summer annual forbs (Fig. 3), but this is unlikely to persist beyond a few growing seasons. Like herbicide use, topsoil placement is another disturbance that causes dramatic increases in annual forbs on Decker and Spring Creek mine, and the forbs become gradually displaced by seeded species and/or annual bromes following topsoil placement (Prodgers, 2013). Annual forb cover differences between herbicide and no herbicide treatments shrank considerably over the study period (Fig. 3).

Big sage seedling densities did not vary between 3.4 and 5.6 kg  $\cdot$  ha<sup>-1</sup> seed rates. Likewise, Rinella et al. (2015) observed no relationship

between shrub seed rate and cover in a previous study at Decker and Spring Creek. Alternatively, at a nearby coal mine, Williams et al. (2002) found big sage densities increased as seed rates increased from 1 to 2 to 4 kg  $\cdot$  ha<sup>-1</sup>, indicating 1 and 2 kg  $\cdot$  ha<sup>-1</sup> rates did not deliver seed to all safe sites (Fowler, 1988). Conversely, our 3.4 kg  $\cdot$  ha<sup>-1</sup> rate apparently saturated safe sites.

Quizalofop applied just before seeding reduced annual bromes, but not enough to aid big sage (Figs. 1 and 4). The same is true of quizalofop applied a year after seeding (Fig. 2), but we nevertheless wonder if repeating herbicide treatments over multiple growing seasons could allow big sage and other seeded species to survive and grow large enough to suppress annual bromes. The challenge is designing treatments to more completely control annual bromes and minimally damage seeded species. Fall glyphosate is an option, but our data illustrate this treatment is not without risks (Fig. 5, Spring Creek panel). Another choice deserving testing is quizalofop or other grass-specific herbicides applied in fall instead of our spring timing. Winter annual weeds are smaller and sometimes better controlled by herbicides in fall than spring (Hasty et al., 2004; Lake and Hager, 2009).

Alternatively, additional herbicide treatments may prove unneeded. A common big sage density goal for our system is  $\geq$  1.0 plants  $\cdot$  m<sup>-2</sup> 6 years after seeding (Schuman et al., 2005, Hild et al., 2006). Two summers after glyphosate and seeding, mean big sage densities were 3.05

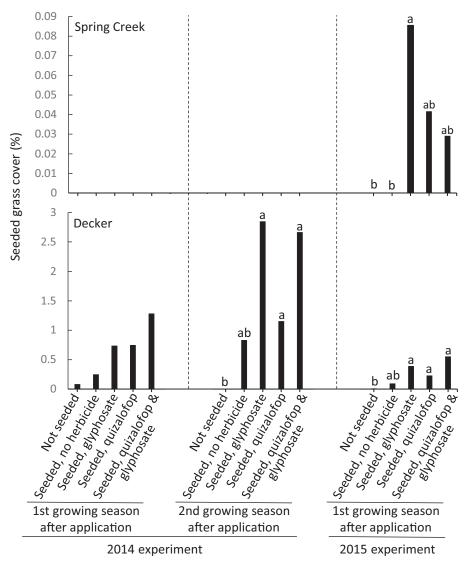


Figure 6. Seeded grass cover at Decker and Spring Creek mines. Within mine and growing season, estimates with different letters significantly differ (P < 0.05).

(Cl<sub>95%</sub> 1.42, 6.56) and 0.43 (Cl<sub>95%</sub> 0.13, 1.40) plants  $\cdot$  m<sup>-2</sup> at Decker and Spring Creek, respectively, so our best treatment may meet this goal at average Decker, and perhaps Spring Creek, sites. Big sage seeds can germinate up to 4 years after seeding (Schuman et al., 1998), and this staggered germination could allow densities to remain stable. In another northern Great Plains coal mine study, seeded big sage densities did not decline dramatically between 2 and 10 years after seeding (i.e., from 7.3  $\pm$  0.9 to 4.5  $\pm$  0.7 plants  $\cdot$  m<sup>-2</sup>), and surviving plants grew appreciably (Hild et al., 2006; Schuman et al., 2012). This provides at least some assurance our study's seedlings may survive, grow, and eventually reproduce.

#### Implications

While establishing big sage from seed has proven very difficult in most North American grasslands, conditions allowing big sage establishment do not appear entirely uncommon in mixed grass prairie of the northern Great Plains. Our study illustrates herbicides can be used to increase big sage seedling survival in downy brome-infested parts of this region, provided the herbicides sufficiently control downy bromes without damaging big sage. More research is needed to determine if big sage seedlings can persist without repeated intervention in our invaded grassland system or if additional herbicide use or other management is needed to ensure survival, growth, and reproduction.

#### Acknowledgments

This research was funded by the Montana Department of Environmental Quality.

## Appendix A

First summer annu	First summer annual brome cover							
	Fixed effe	ects	Random effects					
Variable	Estimate	SE	df	t	р	Variable	Variance	
Intercept	3.090	0.275	20.1	11.3	<0.001	Site Site by	0.316	
Glyphosate	-0.683	0.153	65.8	-4.5	<0.001	herbicide	0.109	
Quizalofop	-0.323	0.149	65.4	-2.2	0.034	Residual	0.288	
Glyphosate &								
quizalofop	-0.897	0.151	64.4	-5.9	<0.001			
2015 Experiment	0.200	0.352	13.7	0.6	0.579			
Spring Creek	-0.274	0.432	13.8	-0.6	0.535			
Pre-treatment								
brome cover	0.148	0.135	153.4	1.1	0.274			
2015 Experiment								
by Spring Creek	-0.626	0.639	16.4	-1.0	0.342			

Second summer annual brome cover

	Fixed effects					Random e	ffects
Variable	Estimate	SE	df	t	р	Variable	Variance
Intercept	3.682	0.174	12.5	21.1	<0.001	Plot	0.005
Glyphosate	0.167	0.240	39.6	0.7	0.491	Site Site by	0.066
Quizalofop	0.114	0.233	35.7	0.5	0.627	herbicide	0.289
Glyphosate &							
quizalofop	0.248	0.244	42.4	1.0	0.316	Residual	0.162
Quizalofop							
retreatment	-0.340	0.080	21.7	-4.2	<0.001		
Spring Creek	-0.318	0.258	6.9	-1.2	0.258		
<b>Pre-treatment</b>							
brome cover	0.188	0.079	111.1	2.4	0.020		

Model results assessing (natural log) annual brome cover. Bolded predictors are significant (p < 0.05). Terms were included for differences between a control and three herbicide treatments (i.e. glyphosate, quizalofop, glyphosate & quizalofop), between experiments initiated 2014 and 2015 (i.e. 2015 Experiment) and between Decker and Spring Creek (i.e. Spring Creek).

First summer annu	First summer annual weed cover							
	Fixed effe	cts				Random effects		
Variable	Estimate	SE	df	t	р	Variable	Variance	
Intercept	1.413	0.142	112.6	10.0	<0.001	Site Site by	0.000	
Glyphosate	1.078	0.169	84.4	6.4	<0.001	herbicide	0.237	
Quizalofop	0.453	0.164	82.8	2.8	0.007	Residual	0.177	
Glyphosate &								
quizalofop	1.163	0.168	82.4	6.9	<0.001			
2015 Experiment	0.260	0.146	84.2	1.8	0.079			
Spring Creek	0.425	0.207	75.8	2.1	0.043			
Pre-treatment								
brome cover	0.048	0.077	149.7	0.6	0.535			
2015 Experiment								
by Spring Creek	0.212	0.295	108.6	0.7	0.474			

Second summer annual weed cover

	Fixed effe	cts				Random ef	fects
Variable	Estimate	SE	df	t	р	Variable	Variance
Intercept Glyphosate	1.779 0.774	0.173 0.191	9.3 35.7	10.3 4.1	<0.001 <0.001	Plot Site Site by	0.000 0.108
Quizalofop Glyphosate &	0.224	0.183	30.5	1.2	0.228	herbicide	0.136
<b>quizalofop</b> Quizalofop	0.667	0.195	38.5	3.4	0.001	Residual	0.139
retreatment	-0.003	0.076	72.1	0.0	0.966		
Spring Creek Pre-treatment	0.070	0.315	6.5	0.2	0.831		
brome cover	-0.116	0.076	107.5	-1.5	0.130		

Model results assessing (natural log) non-native forb cover. Bolded predictors are significant (p < 0.05). Terms were included for differences between a control and three herbicide treatments (i.e. glyphosate, quizalofop, glyphosate & quizalofop), between experiments initiated 2014 and 2015 (i.e. 2015 Experiment) and between Decker and Spring Creek (i.e. Spring Creek).

First summer Wyoming big sagebrush density							
	Fixed effe	ects		Random effe	cts		
Variable	Estimate SE z p Variable Variance						
Intercept Glyphosate	-2.457 1.168			<0.001 <0.001		0.615 0.497	

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	Fixed effe	cts			Random effects		
Variable	Estimate	SE	Z	р	Variable	Variance	
					herbicide		
					Site by		
Quizalofop	0.422	0.342	1.2	0.216	seeding	0.100	
Glyphosate &							
quizalofop	1.181	0.330	3.6	<0.001			
Seeded	2.564	0.602	4.3	<0.001			
2015 Experiment	-0.054	0.529	-0.1	0.918			
Spring Creek	-2.313	0.707	-3.3	0.001			
2015 Experiment by							
Spring Creek	0.875	0.993	0.9	0.379			

Second	summer	Wyoming	big sage	brush (	densitv

	Fixed effe	Fixed effects			Random effe	cts
Variable	Estimate	SE	Z	р	Variable	Variance
Intercept	-1.933	0.715	-2.7	0.007	Plot	0.362
Glyphosate	1.388	0.434	3.2	0.001	Site Site by	0.628
Quizalofop Glyphosate &	-0.115	0.473	-0.2	0.808	herbicide Site by	0.000
quizalofop	1.190	0.437	2.7	0.006	seeding	0.059
Seeding Quizalofop	1.674	0.728	2.3	0.022		
retreatment Spring Creek	-0.027 <b>-1.970</b>	0.246 <b>0.685</b>	-0.1 <b>-2.9</b>	0.912 <b>0.004</b>		

Model results assessing Wyoming big sagebrush density (plants m<sup>-2</sup>). Bolded predictors are significant (p < 0.05). Terms were included for differences between a control and herbicide treatments (i.e. glyphosate, quizalofop, glyphosate & quizalofop), between experiments initiated 2014 and 2015 (i.e. 2015 Experiment) and between Decker and Spring Creek (i.e. Spring Creek).

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