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Suppression of non-native and native grass seed germination using mustard seed meal and mulch biofumigation

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Abstract

Non-native annual grasses have invaded western rangelands in the United States, and novel approaches are needed to supplement existing management strategies. The aim of this study was to investigate biofumigation, the use of Brassicaceae products to suppress weeds, as a control for two non-native annual grasses (cheatgrass, *Bromus tectorum* and ventenata, *Ventenata dubia*) and its effects on two dominant native perennial grasses (bluebunch wheatgrass, *Pseudoroegneria spicata* and Idaho fescue, *Festuca idahoensis*). We tested the effect of the biofumigant *Brassica juncea* (brown mustard), applied as seed meal and mulch, on germination metrics of the four grass species in Petri dishes. Germination metrics included emergence and viability, time to cotyledon and radicle emergence and their lengths at 14 days after sowing. Impacts on emergence and viability were assessed using a binomial mixed effects model, while time to cotyledon and radicle emergence and lengths were assessed using linear mixed effects models. Seed meal reduced emergence and viability at lower application rates for *V. dubia* and *F. idahoensis* than the other two species. Mulch did not consistently reduce emergence or viability. *P. spicata* was the least inhibited by the biofumigants. When using seed meal, radicle emergence and length were inhibited *in B. tectorum*, *V. dubia* and *F. idahoensis* and cotyledon emergence and length were inhibited for all. The mulch did not impact these metrics consistently and may not be a viable management tool. The use of seed meal biofumigant to suppress undesired annual grasses in rangelands seems promising, but response is species-specific.

KEYWORDS
Brassica juncea, *Bromus tectorum*, controlled environment, dose–response, *Festuca idahoensis*, glucosinolates, grasslands, invasive annual grasses, isothiocyanates, *Pseudoroegneria spicata*, rangeland

1 | INTRODUCTION

Semi-arid rangelands in western North America are being altered by the invasion of non-native annual grasses (Watkinson & Ormerod, 2001), potentially limiting their value for cattle production and conservation (DiTomaso, 2000). These semi-arid rangelands are dominated by perennial bunch grasses that emerge in the spring, but there is a notable lack of native annual grasses (Hochstrasser et al., 2002), particularly not many that germinate in the fall. This vacant niche is often filled by non-native annual grasses following

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human caused or natural disturbance (D'Antonio & Vitousek, 1992), and shifts community composition by reducing diversity and ecosystem functioning (Brooks et al., 2004). Due to the negative impact that non-native grasses can have on agricultural systems, an array of approaches is needed for management (Monaco et al., 2017). In rangeland, non-native annual species are most frequently controlled using herbicides (DiTomaso et al., 2010; Mangold et al., 2013; Radosevich et al., 2007) and targeted grazing (DiTomaso et al., 2010). However, repetition and consistency in herbicide treatments create an environment conducive to herbicide resistance, and herbicides used in rangelands may cause non-target injuries on desired annual species. Therefore, alternative strategies are necessary (Menalled et al., 2016). Integrated weed management systems combine chemical, cultural, physical and biological strategies to create a system to suppress weeds with a variety of methods, such as biofumigation (Harker & O'Donovan, 2017; Menalled et al., 2016). Such strategies rely on the ecological characteristics of the target species to maximize effectiveness of control while preventing damage to non-target species (Barrett, 1988). Alternative approaches such as biofumigation have not been tested on annual grass control in rangelands, and thus need to be evaluated.

Biofumigation is the use of a fumigant, often bioactive Brassicaceae cover crops and their byproducts, to suppress soilborne pests, pathogens and more recently weeds, in agricultural fields (Gimsing & Kirkegaard, 2009; Lefebvre et al., 2018; Reddy, 2013). Plants within the Brassicaceae family contain the glucosinolate-myrosinase syndrome that produce volatile terpenoids which suppress weeds (Gimsing & Kirkegaard, 2009; Lefebvre et al., 2018; Mithen, 2001). Glucosinolates are sulphur-containing secondary anti-herbivory compounds derived from amino acids found in roots, shoots and seeds, in conjunction with the enzyme myrosinase (Gimsing & Kirkegaard, 2009; Mithen, 2001). The myrosinase enzyme and glucosinolate products are spatially isolated in intact plant tissues, but following tissue damage, glucosinolates undergo hydrolysis, catalysed by myrosinase, producing several products, especially the bioactive allyl-isothiocyanate (hereafter isothiocyanate) (Mithen, 2001). Isothiocyanates are a volatile terpenoid with short-lived presence in soils (Matthiessen & Kirkegaard, 2007; Rice, 1984). The volatiles prevent germination and inhibit growth in some plant species through non-specific reactions with the sulphur-containing groups in proteins, though plant sensitivity is species-specific (Aghajanzadeh et al., 2014; Lefebvre et al., 2018; Matthiessen & Kirkegaard, 2007; Rice, 1984). Due to the response variability, researchers focus on quantifying individual species responses to varied rates of biofumigants and diverse incorporation strategies.

Brassica juncea (L.) Czern. (brown mustard) is frequently used as a biofumigant due to the high rates of glucosinolates in its tissues (Morris et al., 2020). It is generally believed that the use of biofumigants made from the seeds of the plant should be more effective than those from other plant parts, due to higher glucosinolates concentrations in seeds (Brown & Morra, 1997; Carlson et al., 1987).

Biofumigation has yet to be tested on non-native annual grasses, though it has proven to be effective on other species; however, there is high variability across studies and species that indicate the relationship is less clear (Morris et al., 2020). De Cauwer et al. (2019) found that 10 weed species demonstrated varying sensitivities to *B. juncea* powder in a dose–response Petri dish study. The most sensitive and least sensitive species' ED_{90} (dose required for 90% reduction in germination) differed by a factor of 100, and seed mortality was negatively correlated with physical seed parameters and positively correlated with the ratio of seed length to width (De Cauwer et al., 2019). This study, among others, indicated that seeds with more robust tissues and greater size may be more tolerant to biofumigants (De Cauwer et al., 2019; Lefebvre et al., 2018; Petersen et al., 2001). Weed species evaluated to date include those that are problematic in cropping systems such as perennial grasses, and perennial and annual forbs; Lefebvre et al. (2018) selected common weeds of southern Quebec, Canada, that display different types of dormancy, and De Cauwer et al. (2019) selected European weeds that are difficult to manage in conventional and organic systems and are prone to herbicide resistance. However, such biofumigation experiments have not been applied to non-native annual grasses threatening native perennial rangelands in the western United States.

Bromus tectorum L. (cheatgrass) is a notorious non-native winter annual grass that is weedy in rangeland and winter wheat throughout western United States that has threatened rangelands since the middle of the 19th century (Mack, 1981). *Ventenata dubia* (Leers) Coss (ventenata, North African grass) is a more recent invader; it was first observed in the United States in Washington in the 1950s (Barkworth et al., 1993; Wallace et al., 2015). *V. dubia* is becoming increasingly abundant in the Inland Pacific Northwest and Northern Great Plains (Wallace et al., 2015), particularly in disturbed environments including hay fields, rangelands and along transportation corridors (Adhikari et al., 2022; Fryer, 2017). It has been shown to be associated with a decline in native species richness and diversity in areas it has invaded (Jones et al., 2020). Since these two non-native annuals can cause decline in native species richness and diversity, which are vital for a resilient and profitable rangelands, we should quantify management strategies for their removal, with benefits to desired native forage. *Festuca idahoensis* Elmer (Idaho fescue) and *Pseudoroegneria spicata* (Pursh) Á. Löve (bluebunch wheatgrass) are key perennial bunchgrass in western North American semi-arid rangelands that are desired forage species for cattle, horses and wildlife (Ogle et al., 2010; Ogle et al., 2011). Established stands of *P. spicata* can be competitive against non-native annual grasses, making it an important member of an invasion resistant plant community (Blank & Morgan, 2012; Ogle et al., 2010). These four species' seeds represent a range in sizes, with *F. idahoensis* being the smallest, followed by *V. dubia*, *B. tectorum* and *P. spicata* the largest, which may suggest a range in sensitivities, as other studies have shown smaller seeds being more sensitive than the larger (De Cauwer et al., 2019; Lefebvre et al., 2018; Petersen et al., 2001).

This study evaluated (1) the effect of *B. juncea* rehydrated seed meal powder (hereafter seed meal) and chopped aboveground biomass (hereafter mulch) at increasing rates on the emergence and viability (ED_{50} and LD_{50}), radicle and cotyledon emergence periods and length of four species; non-native *B. tectorum* and *V. dubia* and native

F. idahoensis and *P. spicata*, and (2) if seed size parameters (length, width, surface and mass) impacted sensitivity to the biofumigant. These species were selected for study due to their co-occurrence in rangelands but contrasting life history strategies that may impact sensitivity to biofumigation. Based on seed size we hypothesized that our smallest seeds, *F. idahoensis*, would be the most sensitive and our largest seeds, *P. spicata*, the least. We assessed both emergence and viability because previous studies have found changes in dormancy, where lower rates of biofumigant may induce dormancy but not cause seeds to be non-viable (Lefebvre et al., 2018).

2 | METHODS

2.1 | Materials

All experiments were performed on four species (non-native *B. tectorum* and *V. dubia*, and native *F. idahoensis* var. *nezpurs* and *P. spicata* var. *goldar*). *B. tectorum* seeds were harvested from Red Bluff research ranch near Norris, MT (N 45°33′1.85121″, W 111°39′30.62804″) in 2019. *V. dubia* seeds were harvested from near Missoula, MT (N 46°53′55.6″, W 113°56′58.3″); Gallatin Valley, MT (N 45°45′32.3″, W 111°08′39.3″); and Lodge Grass, MT (N 45°16′17.4″, W 107°35′16.9″) in 2019, and seeds mixed. *P. spicata* and *F. idahoensis* seeds were grown and purchased from Bruce Seed Farm Inc. in Townsend, MT (N 46°17′44.38535″, W 111°28′59.99716″). Thus, all seed grew from plants in the same area. Seeds were rinsed in distilled water then dried prior to experimentation. Seed length, width and surface (length and surface excluding awn) measurements were performed using the ImageJ image measuring software (Schneider et al., 2012). Seed mass was measured on a Mettler Toledo analytical balance. *B. juncea* seed meal was sourced from Farm Fuel Inc. in Watsonville, CA., and mulch was harvested from a *B. juncea* crop at the Montana State University Post Agronomy Farm (N 45°40′16.428″, W 111°8′34.584″) in autumn 2019. Samples of seed meal and mulch were analysed for sinigrin, a precursor of isothiocyanates, using high-performance liquid chromatography at New Mexico State University as a proxy for isothiocyanate levels (Tsao et al., 2002).

Seeds were exposed to six rates of seed meal (0 mg, 5 mg, 10 mg, 15 mg, 20 mg and 25 mg ± 0.001 mg) immediately following dilution with 5 mL of distilled water in a Petri dish to make a leachate (Lefebvre et al., 2018). Seeds were exposed to a leachate from four rates of mulch (0 mg, 50 mg, 125 mg and 250 mg ± 0.001 mg). The mulch was soaked for 40 h in distilled water before solids were filtered from the liquid solution: 5 mL of the mulch leachate was poured into the Petri dish as above. The mulch had fewer application rates due to physical constraints in the application process. Filter paper (9 cm diameter, flow rate 60 mL/min, Fisherbrand) was placed on the leachate to assist in spreading the water-soluble components, and 10 seeds were placed equidistantly across the filter paper. The Petri dishes were sealed with Parafilm® M laboratory film (3.2 cm width, Amcor) and randomly placed in a growth chamber (10/14 h, light/dark at 20°/15°C) with position in chamber changed daily. We conducted daily visual inspections of seeds, and time until radicle and cotyledon emergence was recorded daily for 14 days for each seed. Seeds were considered emerged when the radicle reached 2 mm in length. Cotyledon and radicle length of seeds were measured (mm) after 14 days.

Seed viability was determined for all non-emerged seeds using a tetrazolium test with a 1% solution of 2,3,4-triphenyl-2H-tetrazolium chloride after 14 days (Peters, 2000). If there was evidence of an embryo, the seed was deemed viable but dormant; seeds that showed no response to the tetrazolium test were considered non-viable. Within each trial there were four repetitions of each species. Three full trials were conducted from May 2020 through January of 2021.

2.2 | Data analysis

All statistical analyses were performed using R 4.2.3 (R Core Team, 2023). Emergence of seeds in the control treatment (not exposed to any mustard) was determined to range from 80% to 97%. Total emergence and viability values were adjusted according to each species' control.

The interspecific difference among seed parameters was evaluated using a linear model with predictors: length, width, surface and mass. The effect of seed parameters on total emergence and viability success/failure (success defined as emerged or viable) were evaluated using a binomial linear mixed model with the predictors: length, width, surface and mass, and their interactions with species and mustard rate; and random effects of trial and Petri dish. A type II Anova was used to determine whether each parameter showed significant impact on response variables ($\alpha = 0.05$).

Total emergence (emerged vs. non-emerged) and viability (viable vs. non-viable) were evaluated using binomial linear mixed models of emergence or viability success/failure with the predictors: species and rate, with trial and Petri dish as random effects. All four species demonstrate physiological dormancy, so dormancy type was not used as an explanatory variable (Fryer, 2017; Klemmedson & Smith, 1964; Lapp et al., 2001, 2008).

Effective dose calculations and subsequent figures were made using a four parameter log logistic model via the DRC package (Ritz et al., 2015). Effective dose calculations were relative absolute dose, where our curves were relativized to the control for each species. A post-hoc Tukey–Kramer test ($\alpha = 0.05$) was performed to make pairwise comparisons of the application rates within each species using the glht function of the multcomp package (Hothorn et al., 2008). We calculated ED_{50} and LD_{50} values in keeping with other studies; other levels were evaluated but did not improve elucidation of the data and are not shown.

Radicle and cotyledon time to emergence and their lengths after 14 days were evaluated using separate linear mixed models with the predictors: species and rate, with random effects controlling for trial and Petri dish. Individual seeds that did not demonstrate radicle or cotyledon emergence at the end of the 14 days were censored from time to emergence data. A post hoc Tukey–Kramer test ($\alpha = 0.05$)

was performed to make pairwise comparisons of the application rates within each species for both seed meal and mulch separately.

3 | RESULTS

3.1 | Biofumigant

Seed meal had 18 times the mean sinigrin of the mulch (Table 1) at 1 g dry weight. Our seed meal experimental rates ranged from 2.12 µmol sinigrin to 10.62 µmol, and our mulch rates ranged from 1.18 µmol to 5.92 µmol sinigrin (Table 1).

3.1.1 | Seed measurements

Seeds showed species level differences in all parameters (length, width, surface and mass), but the pattern of differences was not consistent. *F. idahoensis* had the smallest length, width and surface, *V. dubia* had the smallest mass, while *P. spicata* had the largest mass, and *B. tectorum* had the largest length and surface (Figure 1). The proportion of seeds that emerged was not related to seed parameters for either biofumigant.

3.1.2 | Emergence and viability

Total emergence (when the radicle reached 2 mm in length) was lower when exposed to seed meal for all species. A post-hoc Tukey HSD test showed *F. idahoensis* and *V. dubia* emergence decreased by 41% and 31%, respectively, at the low-medium rate ($p < 0.01$ for both), *B. tectorum* emergence decreased by 21% at the medium rate ($p < 0.01$), and *P. spicata* by 32% in the medium-high rate ($p < 0.01$) (Table 2).

TABLE 1 Chemical analysis of *Brassica juncea* biofumigant using high-performance liquid chromatography.

Mustard product	Mean dry weight (g)[a]	Mean sinigrin (µmol)
Meal	1	424.67
Low	0.005	2.12
Low-medium	0.010	4.25
Medium	0.015	6.37
Medium-high	0.020	8.49
High	0.025	10.62
Mulch	1	23.69
Low	0.050	1.18
Medium	0.125	2.96
High	0.250	5.92

Note: Mean sinigrin was used as a proxy for isothiocyanate levels. Sinigrin levels were extracted from our seed meal and mulch samples and corresponding estimates of sinigrin per treatment rate were determined.
[a]Mean dry weight of *Brassica juncea* biofumigant product, either seed meal or mulch.

The ED_{50} doses differed between the most sensitive (*F. idahoensis*) and least (*P. spicata*) by a factor of 3.7 ($p = 0.01$) (Table 3, Figure 2A). Viability for all four species declined when exposed to the seed meal treatment; *F. idahoensis* declined by 26% at the medium rate ($p < 0.01$), and *B. tectorum* and *V. dubia* declined by 29% and 15%, respectively, at the medium-high rate ($p < 0.01$, $p < 0.01$), *P. spicata* declined by 21% at the high rate ($p = 0.01$), and there was no difference in LD_{50} values among species (Table 3, Figure 2B).

Total emergence declined when exposed to mulch leachate for all four species, with *V. dubia* being the most sensitive, and *P. spicata* the least sensitive (Table 2). *V. dubia* emergence decreased by 55% at the low rate ($p < 0.01$), *B. tectorum* and *F. idahoensis* by 82% and 53%, respectively, at the medium rate ($p < 0.01$ for both), and *P. spicata* by 74% at the high rate ($p < 0.01$) (Table 2) when compared to the control. The ED_{50} differed between the most (*V. dubia*) and least (*P. spicata*) sensitive, by a factor of 3.1 ($p < 0.01$) (Table 3, Figure 2C). Viability for all four species was lower at the high rate of mulch leachate; *B. tectorum* decreased by 80%, followed by *V. dubia* and *F. idahoensis* which decreased by 53% and 51%, respectively, and the least sensitive *P. spicata* decreased by 46% ($p < 0.01$, $p < 0.01$, $p = 0.02$, $p = 0.03$) (Table 2) when compared to the control. The LD_{50} differed between the most (*B. tectorum*) and least (*P. spicata*) sensitive, by a factor of 1.6 ($p = 0.01$) (Table 2; Figure 2D).

3.1.3 | Emergence period and length

The time to radicle emergence was delayed when exposed to seed meal leachate, in three of four species; *B. tectorum* and *V. dubia* by 1.8 and 1.2 days, respectively at the medium rate ($p < 0.01$ for both), and *P. spicata* by 2 days at the medium-high rate ($p < 0.01$), while *F. idahoensis* was unaffected (Figure 3A–D). The length of the radicle was shorter when exposed to seed meal in *F. idahoensis* and *B. tectorum* by 4.3 and 3.6 cm, respectively at the low rate ($p < 0.01$ for both), *P. spicata* by 1.2 cm at the low-medium rate ($p < 0.01$), but *V. dubia* showed no effect (Figure 5A). Cotyledon emergence was delayed for *B. tectorum* by 2.6 days at the low-medium rate ($p = 0.04$), *V. dubia* by 4.1 days at the medium rate ($p = 0.01$), *P. spicata* by 9.9 days at the medium-high rate ($p < 0.01$) and *F. idahoensis* by 4.7 days at the high rate ($p = 0.01$) (Figure 4A–D). Cotyledon length was shorter for all four species: *B. tectorum* by 3.2 cm at the low rate ($p = 0.01$), *F. idahoensis* and *P. spicata* by 1.4 and 0.7 cm, respectively at the low-medium rate ($p < 0.01$ for both) and *V. dubia* by 0.3 cm at the medium rate ($p = 0.02$) (Figure 5B).

Radicle emergence was delayed when using mulch leachate by 2 days at the low rate ($p = 0.04$) for *F. idahoensis* with no difference for the other species. Radicle length was shorter for *F. idahoensis* by 1.5 cm at the medium rate ($p = 0.04$) and *P. spicata* by 2.4 cm at the high rate ($p < 0.01$), but 2.5 cm longer for *B. tectorum* at the low rate ($p < 0.01$) while *V. dubia* had no decline (Figure 6A). Cotyledon emergence was delayed by 4 days in *B. tectorum* at the medium rate ($p < 0.01$), while the other three species were unaffected. Finally, *P. spicata* cotyledon length was shorter (1.8 cm) at the medium rate

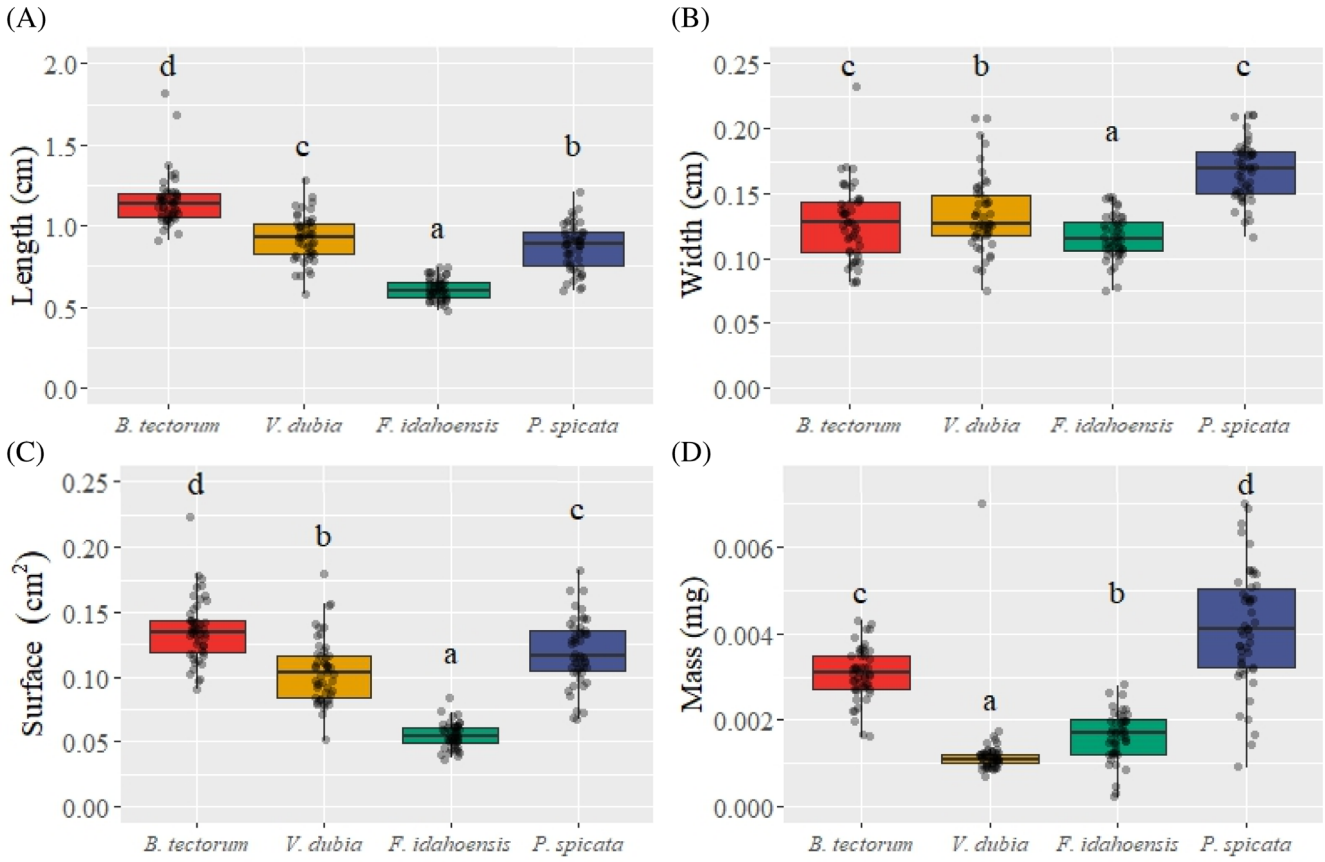


FIGURE 1 Boxplots of seed parameters including (A) length, (B) width, (C) surface and (D) mass of *Bromus tectorum* (*B. tectorum*), *Ventenata dubia* (*V. dubia*), *Festuca idahoensis* (*F. idahoensis*) and *Pseudoroegneria spicata* (*P. spicata*). Line at centre of box is the median, boxes represent interquartile range, whiskers represent range of 95% of the data, semitransparent points are raw data points, with points outside of whiskers being extreme outliers, and letters are the compact letter display of the Tukey–Kramer pairwise comparisons ($p < 0.05$).

($p = 0.01$), while the other three species showed no response (Figure 6B).

4 | DISCUSSION

There were intra- and inter- specific differences in sensitivity to both biofumigation products. While our species' seeds did show interspecific differences in length, mass and surface, we did not find a relationship between emergence following meal or mulch treatment based on any seed measurement parameter. Other research has focused on identifying sensitivities of different plant species through Petri dish dose–response studies, in potted soil in small containers, or in controlled row-cropped fields (De Cauwer et al., 2019; Lefebvre et al., 2018; Morris et al., 2020). In one such Petri dish dose–response study, Lefebvre et al. (2018) exposed eight weed species to increasing rates of dried *B. juncea* powder and recorded dormancy and mortality, along with physical seed parameters including seed mass, length, width, testa (seed coat) hardness and related ratios. It was found that increasing the dose of *B. juncea* increased mortality in all species but one in a Petri dish study. Initial dormancy had a positive relationship with ED_{50} and LD_{50} with an interaction with seed surface and width (Lefebvre et al., 2018). In our study while there was no relationship with specific seed traits and emergence and viability, there was a general pattern; *P. spicata*, the heaviest seed was the least impacted in terms of emergence and viability for both biofumigants, where effects were not obvious until the higher rates. *F. idahoensis*, which had the smallest length, width and surface, was the most sensitive out of all four species to seed meal, with a 3.7-fold difference in ED_{50} value between it and *P. spicata*. However, *V. dubia* was the most sensitive to mulch out of all four species, where there was a 3.1-fold difference between it and *P. spicata*. The differences in sensitivity were not related to the species life history nor native range. Radicle and cotyledon emergence periods and lengths were variable and species specific for both seed meal and mulch. For example, radicle emergence was delayed for all species except *F. idahoensis* with the addition of meal, but only *F. idahoensis* was affected by addition of the mulch. The intraspecific differences in sensitivity aligned with other researchers' findings in that each species demonstrated its own levels of sensitivity to the biofumigant dependent on the type of product (Lefebvre et al., 2018; Matthiessen & Kirkegaard, 2007; Rice, 1984).

We found differences in efficacy rates between seed meal and mulch, in that far less seed meal was required to inhibit germination metrics than mulch, likely due to the greater levels of sinigrin

TABLE 2 Proportional seed emergence and viability (proportion of total seeds emerged or viable for each treatment rate relative to the proportion of seeds emerged or viable in the control) responses of the four test species to different rates of exposure to seed meal and mulch biofumigant in a controlled environment study.

		Seed response		
Species	**Treatment**	**Rate[a]**	**Emerged[b]**	**Viable[b]**
Bromus tectorum	Seed meal	Low	$0.91 \pm 0.03_{ACD}$	$0.94 \pm 0.03_{BC}$
		Low-medium	$0.90 \pm 0.04_{ACD}$	$0.93 \pm 0.03_{BC}$
		Medium	$0.88 \pm 0.05_{CE}$	$0.95 \pm 0.03_{BC}$
		Medium-high	$0.54 \pm 0.06_{GH}$	$0.71 \pm 0.05_{EFGH}$
		High	$0.53 \pm 0.06_{GI}$	$0.70 \pm 0.05_{EFGH}$
	Mulch	Low	$0.99 \pm 0.09_{ABC}$	$1.03 \pm 0.03_{B}$
		Medium	$0.22 \pm 0.10_{E}$	$0.80 \pm 0.10_{BCD}$
		High	$0.00 \pm 0.00_{AE}$	$0.20 \pm 0.10_{A}$
Ventenata dubia	Seed meal	Low	$0.92 \pm 0.03_{DEF}$	$1.11 \pm 0.02_{B}$
		Low-medium	$0.77 \pm 0.05_{FGH}$	$0.94 \pm 0.03_{BCE}$
		Medium	$0.55 \pm 0.06_{HIJK}$	$0.99 \pm 0.04_{CG}$
		Medium-high	$0.33 \pm 0.06_{K}$	$0.77 \pm 0.06_{FGH}$
		High	$0.44 \pm 0.06_{IJK}$	$0.61 \pm 0.06_{H}$
	Mulch	Low	$0.55 \pm 0.12_{CE}$	$0.71 \pm 0.11_{BCD}$
		Medium	$<0.10 \pm 0.04_{E}$	$0.88 \pm 0.11_{BCD}$
		High	$0.11 \pm 0.06_{E}$	$0.55 \pm 0.12_{AD}$
Festuca idahoensis	Seed meal	Low	$0.93 \pm 0.03_{CE}$	$0.99 \pm 0.01_{B}$
		Low-medium	$0.66 \pm 0.05_{GJ}$	$0.96 \pm 0.02_{BC}$
		Medium	$0.28 \pm 0.05_{IJK}$	$0.74 \pm 0.05_{EFGH}$
		Medium-high	$<0.10 \pm 0.02_{L}$	$0.88 \pm 0.05_{DEFGH}$
		High	$0.34 \pm 0.05_{JK}$	$0.59 \pm 0.05_{GH}$
	Mulch	Low	$0.61 \pm 0.12_{ABCD}$	$1.03 \pm 0.03_{AB}$
		Medium	$0.47 \pm 0.12_{BE}$	$0.97 \pm 0.06_{B}$
		High	$0.00 \pm 0.00_{AE}$	$0.49 \pm 0.12_{AD}$
Pseudoroegneria spicata	Seed meal	Low	$0.98 \pm 0.01_{AC}$	$1.02 \pm 0.01_{AB}$
		Low-medium	$0.89 \pm 0.03_{AE}$	$0.98 \pm 0.01_{B}$
		Medium	$0.84 \pm 0.04_{ACD}$	$0.97 \pm 0.02_{B}$
		Medium-high	$0.68 \pm 0.05_{EG}$	$0.97 \pm 0.02_{B}$
		High	$0.70 \pm 0.05_{EG}$	$0.79 \pm 0.04_{ACF}$
	Mulch	Low	$0.94 \pm 0.06_{A}$	$0.97 \pm 0.04_{B}$
		Medium	$0.71 \pm 0.11_{ABC}$	$0.90 \pm 0.08_{BC}$
		High	$0.26 \pm 0.11_{DE}$	$0.54 \pm 0.12_{AC}$

[a]Rates for seed meal corresponding to low 5 mg, low-medium 10 mg, medium 15 mg, medium-high 20 mg, and high 25 mg ± 0.001 mg. Rates for mulch corresponding to low 50 mg, medium 125 mg, and high 250 mg ± 0.001 mg.

[b]Mean proportion emerged (germinated vs. dormant or dead) or viable (viability defined as seeds that emerged or were dormant) ± SE. Dead seeds not included in this analysis. Means within a treatment (seed meal or mulch) followed by different upper case alphabets are different given a post-hoc Tukey–Kramer HSD test with $p < 0.05$.

(Morra & Kirkegaard, 2002). While the seeds of Brassicaceae plants do have higher concentrations of glucosinolates and should thereby be a more effective biofumigant (Brown & Morra, 1997; Carlson et al., 1987), a meta-analysis of 46 publications on the use of biofumigants on pests, disease and plants found specific plant parts did not impact the efficacy of the biofumigant due to extremely high variation from study to study (Morris et al., 2020). However, analysis of our biofumigants found an 18-fold higher concentration of sinigrin in our seed meal than in our mulch, with our medium meal and high mulch having similar sinigrin levels. Regardless, lower doses of seed meal were required to inhibit total emergence, time to emergence and length, further indicating that seed-based products may be more effective biofumigants than aboveground biomass-based products (De Cauwer et al., 2019; Reddy, 2013).

The four species we studied are rangeland grass species which have not previously been tested with these treatments. Species tested

Species	Treatment	ED50[a]	LD50[a]
Bromus tectorum	Seed meal	$21.6 \pm 0.99_{B}$	$26.1 \pm 1.54_{A}$
	Mulch	$82.0 \pm 14.55_{B}$	$168.5 \pm 23.37_{A}$
Ventenata dubia	Seed meal	$13.3 \pm 1.48_{A}$	$27.0 \pm 3.00_{A}$
	Mulch	$44.6 \pm 10.58_{A}$	$258.0 \pm 214.79_{AB}$
Festuca idahoensis	Seed meal	$10.3 \pm 0.87_{A}$	$30.0 \pm 4.25_{A}$
	Mulch	$88.7 \pm 21.18_{AB}$	$247.3 \pm 19.56_{B}$
Pseudoroegneria spicata	Seed meal	$37.6 \pm 11.32_{B}$	$30.1 \pm 5.32_{A}$
	Mulch	$140.0 \pm 25.23_{B}$	$277.1 \pm 94.73_{AB}$

[a]Effective dose defined as dose required to cause seeds to not emerge, whether that be due to dormancy or non-viability. Lethal dose defined as the dose required to cause the seed to no longer be viable. Values within a treatment (seed meal or mulch) followed by different upper case alphabets are different given a post-hoc Tukey–Kramer HSD test with $p < 0.05$.

TABLE 3 Estimated effective dose 50% (ED50) and lethal dose 50% (LD50) ± SE for the four test species in response to exposure to seed meal and mulch biofumigant in a controlled environment study.

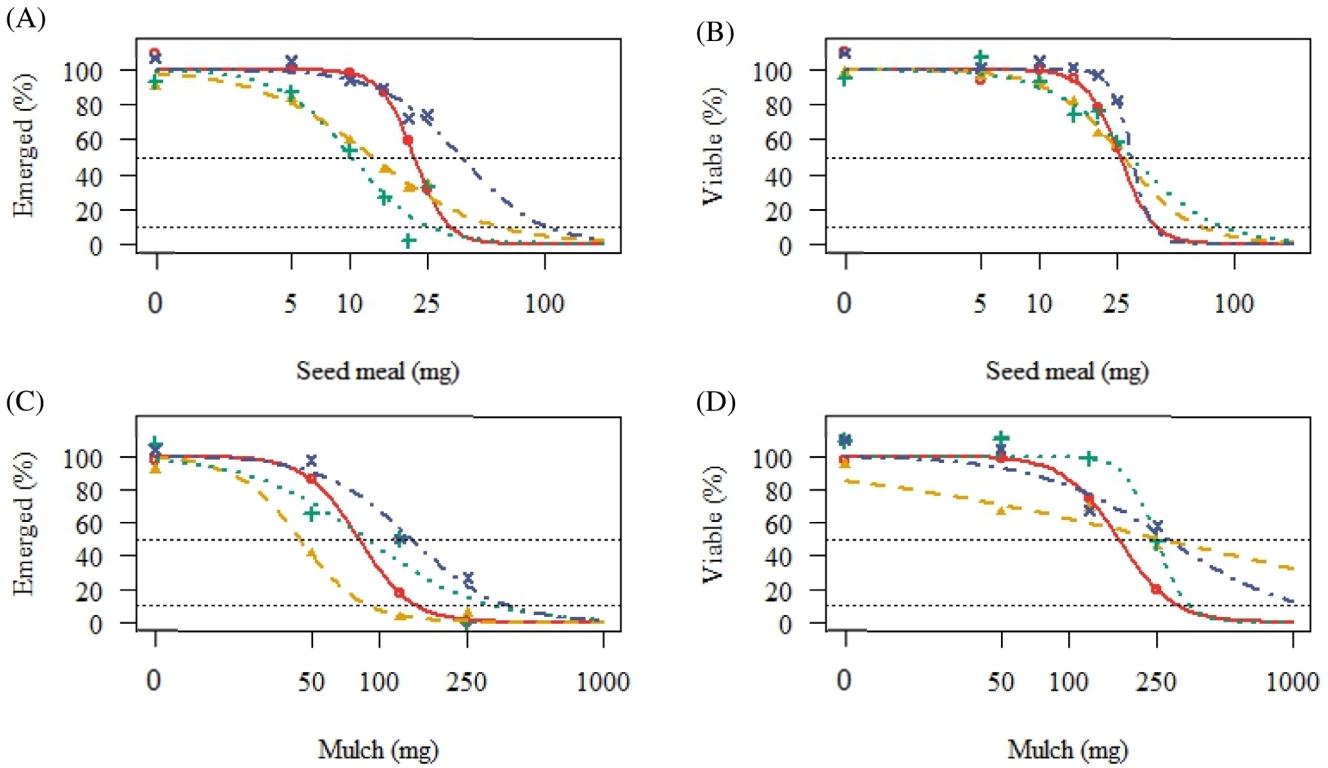


FIGURE 2 Dose response curves for the percentage of seeds (A) emerged (emerged versus non-emerged) and (B) viable (viable vs. non-viable) using seed meal, or (C) emerged and (D) viable with mulch. *Bromus tectorum*, *Ventenata dubia*, *Festuca idahoensis* and *Pseudoroegneria spicata*.

among similar studies are more aligned with cropping system broadleaf weeds. However, Lefebvre et al. (2018) tested *Setaria viridis* (L.) (green foxtail), and De Cauwer et al. (2019) tested *Poa annua* (L.) (annual bluegrass), both species that are in the same family (Poaceae) as our four species. Lefebvre et al. (2018) found dormancy rate was reduced and non-viability increased at 17.41 mg dried mustard powder for *S. viridis*, which is higher than in our *F. idahoensis* (15 mg), but lower than in *B. tectorum* (20 mg), *V. dubia* (20 mg) and *P. spicata* (25 mg). De Cauwer et al. (2019) found the ED_{50} for *P. annua* was 12.1 mg dried mustard powder, which falls between the ED_{50} values we found for our two most sensitive species *V. dubia* (12.26 mg) and *F. idahoensis* (10.28 mg). Lefebvre et al. (2018) did not find a relationship between seed size parameters and LD_{50}, for *S. viridis*, while we also did not find a relationship between seed size and emergence, viability, time to cotyledon or radicle emergence, nor length for our four species.

Seed viability has been negatively correlated with seed mass (De Cauwer et al., 2019; Petersen et al., 2001) and thicker seed testa (Petersen et al., 2001), with some research finding that smaller seeds are more susceptible to isothiocyanates (Petersen et al., 2001),

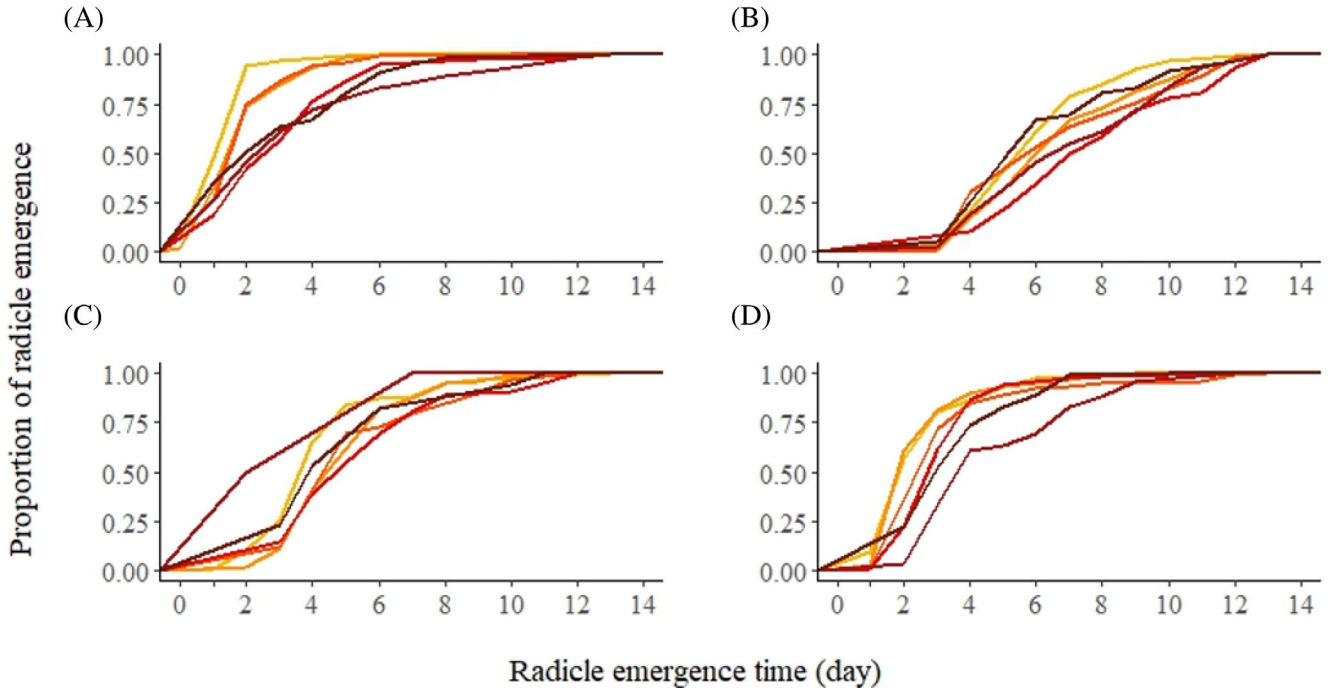


FIGURE 3 Time of radicle emergence for (A) *Bromus tectorum*, (B) *Ventenata dubia*, (C) *Festuca idahoensis* and (D) *Pseudoroegneria spicata* when seeds dosed with seed meal in a controlled environment. Lines are empirical cumulative distributions and indicate the proportion of radicles emerged on each day for the increasing seed meal rates. 0 mg—, 5 mg —, 10 mg—, 15 mg—, 20 mg— and 25 mg —.

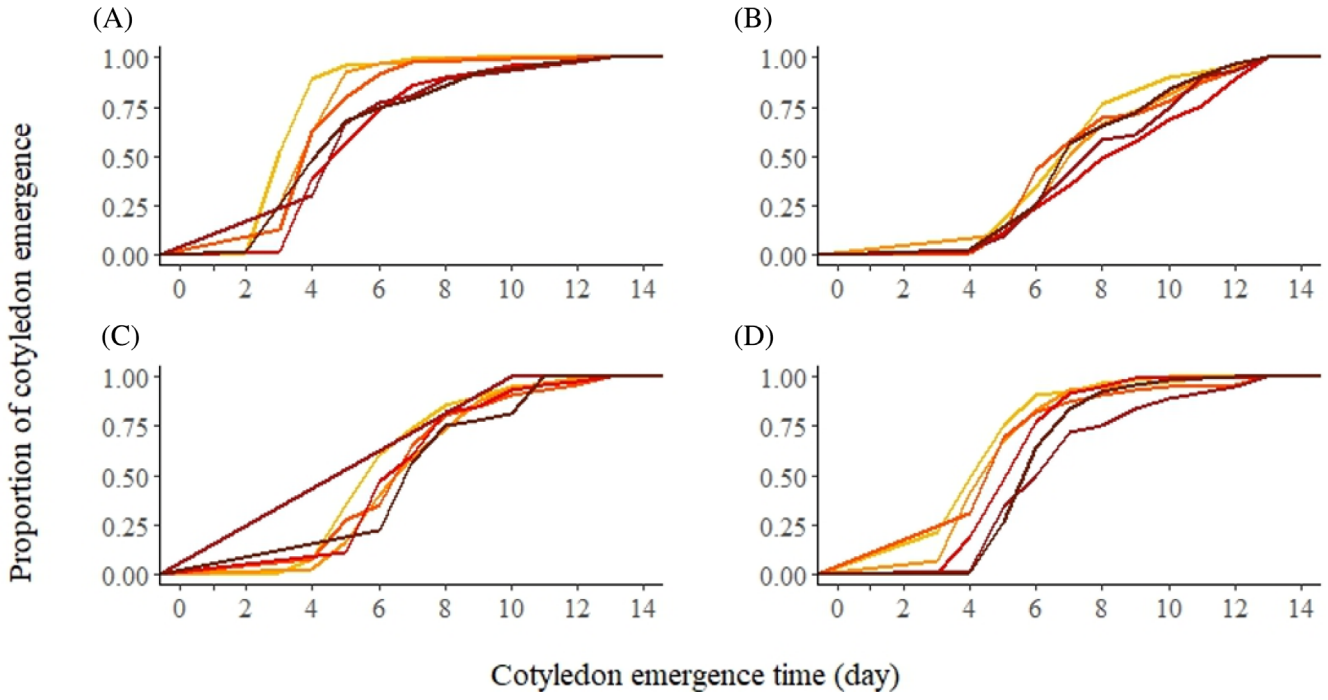


FIGURE 4 Time of cotyledon emergence for (A) *Bromus tectorum*, (B) *Ventenata dubia*, (C) *Festuca idahoensis* and (D) *Pseudoroegneria spicata* when seeds dosed with seed meal in a controlled environment. Lines are empirical cumulative distributions and indicate the proportion of radicles emerged on each day for the increasing seed meal rates. 0 mg—, 5 mg —, 10 mg—, 15 mg—, 20 mg— and 25 mg —.

however, this was not consistent across all studies (Haramoto & Gallandt, 2005; Lefebvre et al., 2018). Similarly, we did not find a relationship in response to biofumigants given our seed parameters, but we also did not investigate seed testa thickness where others found negative correlation between ED_{50} and testa thickness (De Cauwer et al., 2019) or ED_{50} and testa thickness to weight ratio (Lefebvre et al., 2018).

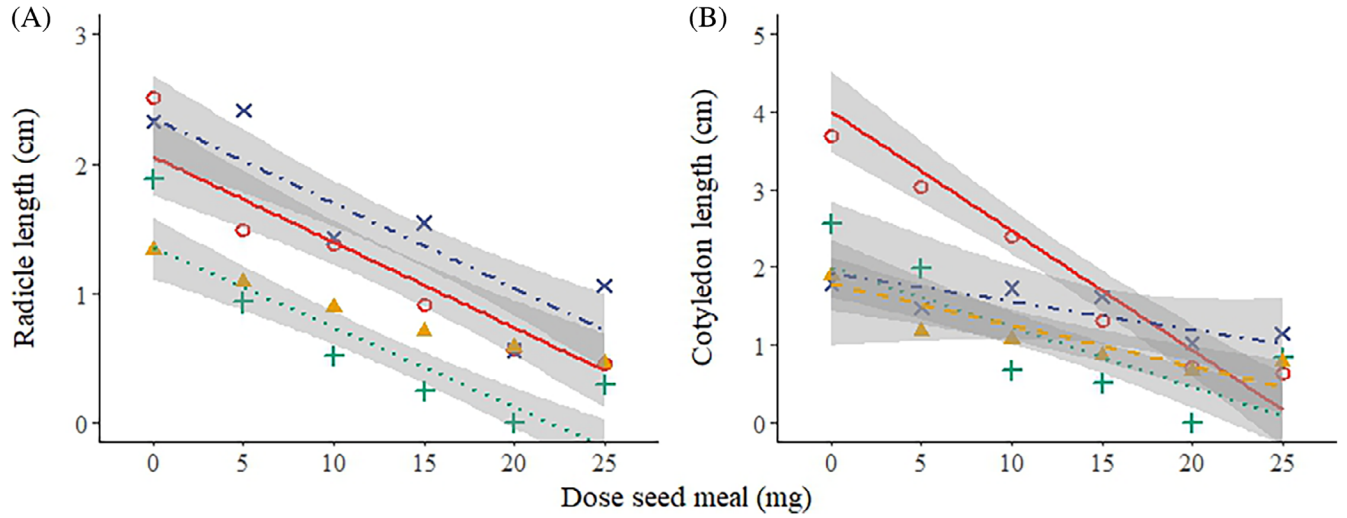


FIGURE 5 Length of (A) radicle and (B) cotyledon when seeds dosed with seed meal in a controlled environment. Lines indicate predicted mean with the band indicating a 95% confidence interval. Lines shown only when relationship significant ($p < 0.05$). *Bromus tectorum*, *Ventenata dubia*, *Festuca idahoensis* and *Pseudoroegneria spicata*.

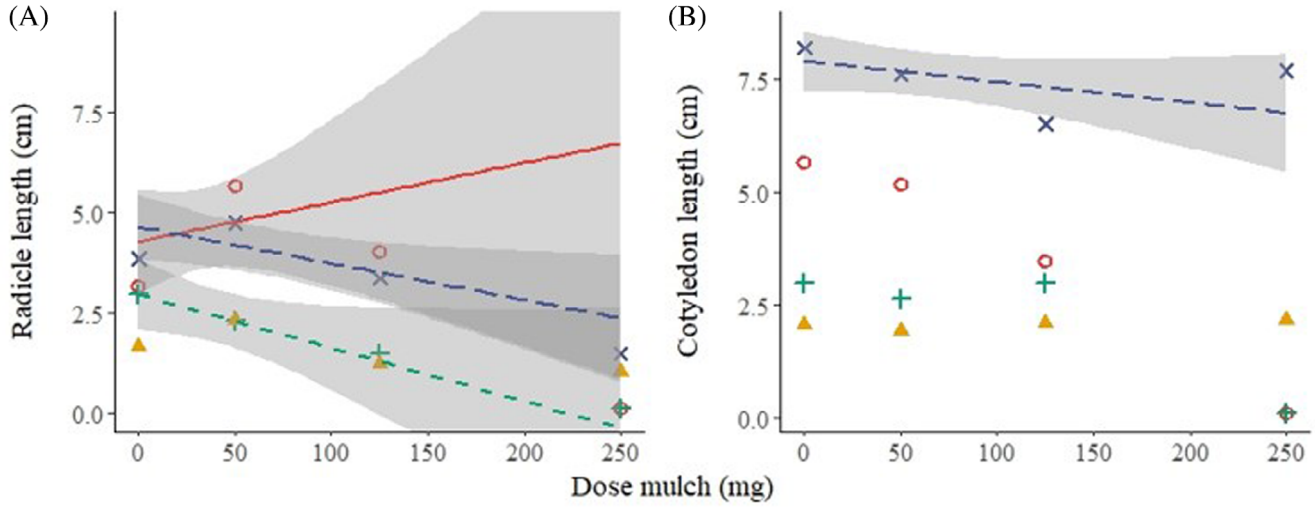


FIGURE 6 Length of (A) radicle and (B) cotyledon when seeds dosed with mulch in a controlled environment. Lines indicate predicted mean with the band indicating a 95% confidence interval. Lines shown only when relationship significant ($p < 0.05$). *Bromus tectorum*, *Ventenata dubia*, *Festuca idahoensis* and *Pseudoroegneria spicata*.

Delays in emergence caused by the biofumigant products could be vital in managing non-native winter annual grasses in perennial dominated environments. The physiological dormancy demonstrated by our two non-native species responds to environmental factors of temperature and moisture, as suitable conditions break dormancy and cause germination (Fryer, 2017; Klemmedson & Smith, 1964). If a biofumigant were applied in the autumn delaying emergence and inducing dormancy past when physiological dormancy would normally end, the seeds may not be able to germinate and would be delayed until the following spring, forcing them to compete with native perennials emerging at that time (Ogle et al., 2010, 2011). This would remove the priority effect giving non-native species an advantage over the native (Wolkovich & Cleland, 2011). Further, isothiocyanate volatiles are rather short-lived in the soil, with their detection rapidly declining after only a few days (Gimsing & Kirkegaard, 2009; Petersen et al., 2001), meaning they would be wholly absent by the time it was spring and the perennials seedlings are germinating and established plants re-growing. This balance of timing may prove useful in removing non-native annuals while not harming native perennials and facilitating their spread.

5 | CONCLUSION

Several studies have been conducted on the use of biofumigants in agricultural settings with a variety of agricultural species, however

there have yet to be any studies in rangelands on rangeland species. This controlled setting study showed that our four target species were impacted by biofumigants, with one of the natives being impacted at similar dose rates to the non-natives. However, there is potential in targeting annual grasses by identifying ideal application timing, likely in the autumn, when only the seeds of non-native annual grasses are likely to be germinating. Therefore, the goal of future biofumigant research with rangeland species should be to improve understanding of how seeds that become dormant due to biofumigants break dormancy, and the response of seeds of these species to biofumigants in soil media, first within controlled environments and then potentially in the field. Such studies will determine if dose and timing specificity could make biofumigation an effective rangeland weed management tool in an integrated weed management toolbox.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/wre.12612.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, LS, upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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