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Glyphosate-resistant and susceptible downy brome (*Bromus tectorum*) management with soil-applied residual herbicides

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Research Article

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Abstract

Downy brome is a cleistogamous facultative winter-annual grass weed that invades cropland, pastureland, and ruderal areas in western North America. Glyphosate-resistant downy brome, the first known glyphosate-resistant grass weed in Canada, was confirmed in a glyphosate-resistant canola field in southern Alberta in 2021. A controlled-environment study was conducted to determine the impact of preemergence soil-applied residual herbicides on glyphosate-resistant and susceptible downy brome in two field soils. Flumioxazin/pyroxasulfone (70/89 g ai ha^{-1}), carfentrazone/pyroxasulfone (18/150 g ai ha^{-1}), sulfentrazone/pyroxasulfone (100/100 or 150/150 g ai ha^{-1}), and saflufenacil/pyroxasulfone (36/120 g ai ha^{-1}) resulted in excellent (≥90%) visible control and downy brome biomass reduction 8 wk after treatment (WAT). The low rate of carfentrazone/pyroxasulfone (12/100 g ai ha^{-1}) resulted in good (≥80%) visible control and biomass reduction 8 WAT, while the low and medium rates of saflufenacil/pyroxasulfone (18/60 or 25/84 g ai ha^{-1}) resulted in ≥80% biomass reduction but suppression only (66% to 75%) based on visible control. Flumioxazin alone (105 g ai ha^{-1}) resulted in good visible control (81%) 8 WAT in a sandy loam soil, but poor (13%) control in a clay loam soil. Soil type affected plant growth as evidenced by reduced growth in the untreated sandy loam soil compared to clay loam soil. The glyphosate-resistant population emerged and grew more vigorously than the glyphosate-susceptible population resulting in greater plant densities in the untreated control and some less-effective herbicide treatments. These results suggest that mixtures of a protoporphyrinogen oxidase-inhibiting herbicide with the very-long-chain fatty acid elongase inhibitor pyroxasulfone applied preemergence at ≥89 g ai ha^{-1} could be effective components of an herbicide layering strategy targeting glyphosate-resistant and glyphosate-susceptible downy brome.

Introduction

Downy brome is a cleistogamous facultative winter-annual grass weed that was introduced to North America before 1861 (Upadhyaya et al. 1986). It has been described as one of the most successful invasive weeds globally (Revolinski et al. 2023). Downy brome can invade and dominate plant communities (Mack 1981), alter the local nitrogen cycle (Rimer and Evans 2006), deplete surface soil moisture (Mitich 1999), and create substantial wildfire risk in naturalized areas (Bradley et al. 2018). Its invasive potential is aided by early growing season germination, growth, and flowering, which result in early resource capture and a competitive advantage over native vegetation or planted crops (Mitich 1999; Morrow and Stahlman 1984). Recent estimates suggest that downy brome occupies about 21 million ha of the Great Basin in the intermountain western United States (Bradley et al. 2018). Based on future climate scenarios, the habitable area of the Rocky Mountain National Park in Colorado suitable for downy brome was predicted to increase from 5.5% in 2015 to 20.4% by 2050 (West et al. 2015).

While downy brome is perhaps best known for its rapid invasion of naturalized areas and transformation of native plant communities into a weed monoculture, it can also substantially impact arable cropping systems. Annual brome species [including downy brome and Japanese brome (*Bromus japonicus* Houtt.)] were the 13th and 15th most abundant weed species in summer-annual crops grown in the fescue grassland and moist mixed grassland ecoregions of Alberta (Leeson et al. 2019). No recent surveys of weed abundance in winter wheat (*Triticum aestivum* L.) have been conducted in this region, representing a knowledge gap of downy brome abundance in winter wheat where the species tends to thrive. Downy brome infestation of croplands was aided by widespread adoption of conservation tillage (Douglas et al. 1990). Increased soil water retention and reduced temperature fluctuation due to reduced tillage are both factors that promote successful germination of downy brome seeds (Evans and Young 1972; Froud-Williams et al. 1981). Burial of downy brome seeds via tillage results in lower densities of downy brome in subsequent crops (Young et al. 2014). Adoption of winter wheat in southern Saskatchewan also promoted invasion by downy brome (Douglas et al. 1990) due to synchronous phenology of these winter-annual species resulting in less opportunity to disrupt the downy brome life cycle. Downy brome can be problematic particularly in winter wheat, where high densities reduced grain yield by up to 68% in Alberta (Blackshaw 1993) and 92% in Washington (Rydrich and Muzik 1968).

Individual downy brome plants can produce up to 5,000 seeds in the absence of competition, and as few as 25 seeds in very dense stands of about 10,000 plants m^{-2} (Young et al. 1987). Most seeds shatter within a few weeks of maturity in June (Hulbert 1955). While seeds are generally dispersed near the parent plant, dispersal can also occur by wind moving seed along the soil surface, through ectozoochory, contamination of harvested grain, or by farm equipment (Hulbert 1955; Mack 1981; Morrow and Stahlman 1984). Most new seeds germinate and emerge within 1 yr of entering the soil seedbank (Burnside et al. 1996). Short (2 to 5 yr) seedbank longevity represents a population management opportunity where a few years of effective control will substantially reduce downy brome densities (Mack and Pyke 1983; Sebastian et al. 2017; Smith et al. 2008).

Many farmers rely on glyphosate for nonselective control of downy brome following emergence in the fall, before summer-annual crop emergence in the spring, or selective control in glyphosate-resistant (GR) crops. Previous research showed that glyphosate had very good efficacy for control of glyphosate-susceptible (GS) downy brome (Blackshaw 1991). In 2021, GR downy brome was confirmed in a GR canola field in southern Alberta (Geddes and Pittman 2022). This population exhibited 11.9-fold resistance to glyphosate, $\leq$14% control when glyphosate was applied at the field rate (900 g ae ha^{-1}), and was the first confirmed GR grass weed in Canada. This population was found to be susceptible to other postemergence herbicides registered for control of downy brome (Geddes and Pittman 2023), however, its response to preemergence residual herbicides remains unknown. The aim of this study was to determine 1) which preemergence soil-applied residual herbicides control GR downy brome in controlled-environment conditions, 2) whether their efficacy differed between GR and GS downy brome populations, and 3) how their efficacy differed between a sandy loam and a clay loam soil.

Materials and Methods

Seed Accessions

Mature seeds were collected from the GR downy brome population and a GS control population in southern Alberta in 2021. Detailed seed collection and processing procedures were described by Geddes and Pittman (2022, 2023). The seed accessions were tested for viability using a petri dish germination assay. Twenty seeds of each seed accession were placed in separate 90-mm petri dishes (Phoenix Biomedical Products, Murcia, Spain) with two Whatman® No. 1 (VWR International, LLC, Edmonton, AB) filter papers and 6 ml of distilled H_2O. The seeds were imbibed at ambient room temperature (21 C) in the dark for 14 d. Seed germination was evaluated every 2 d, and germinated seeds were removed from the petri dishes. Seeds were considered germinated when the radicle had protruded through the seed coat. After the 14-d germination period, viable ungerminated seeds were determined using a seed crush test (Sawma and Mohler 2002). The total number of viable seeds was the sum of viable germinated and ungerminated seeds. The total number of viable seeds was expressed as a percent of the total number of seeds tested and averaged across all four replicates for each population to determine seed viability.

Soils

The experiment evaluated herbicide treatments in two soil types collected near Lethbridge, AB (hereafter referred to as “clay loam”), and Purple Springs, AB (hereafter referred to as “sandy loam”). The clay loam and sandy loam soils were dark brown and brown Chernozems, respectively, characterized as Calcic Haplocryolls. The soils were chosen because they are representative of field soils present in the region where the first case of GR downy brome in Canada was confirmed (Geddes and Pittman 2022) and because they varied in texture and organic matter, which are known to interact with the efficacy of soil-applied residual herbicides (Westra et al. 2015). Both soils were collected from permanent grassland sites absent of previous treatment with soil-applied residual herbicides. Topsoil to a depth of 15 cm was collected, and then homogenized and analyzed for texture, organic matter, and pH; and for soluble salt, nitrate nitrogen (NO_3-N), phosphorus, potassium, and sulfate sulfur (SO_4-S) content (Table 1) by a local soil testing laboratory (Down to Earth Labs, Lethbridge, AB). Downy brome was not a member of the weed community present at either collection site.

Experimental Design and Treatment Structure

The greenhouse experiment followed a randomized complete block design with a three-way factorial treatment structure and four replications. The three factors included downy brome population (GS vs. GR), soil type (sandy loam vs. clay loam), and herbicide treatment (Table 2). The herbicide treatment factor included 14 herbicide and rate combinations registered (or being considered) for control or suppression of downy brome or Japanese brome in cropping systems in western Canada (Anonymous 2022), in addition to an untreated control. The experiment was repeated in two different greenhouses located at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre. The two repeats took place between October and December 2022 and were separated in time by 1 wk.

Experimental Procedures and Data Collection

Each soil was sieved (1 cm), air dried, and fertilizer containing equal parts of nitrogen, phosphorus, and potassium was mixed in at a rate of 25, 25, and 25 mg kg^{-1} soil, respectively. The soils were homogenized and used to fill 10- by 10- by 12-cm plastic greenhouse pots. Each pot was thoroughly watered to settle the soil before seeding. Downy brome seeds were added at a density of seven viable seeds per pot determined by adjusting the seeding rate for each seed accession based on seed viability. Seeds were buried in the soil at a depth of 1 cm followed by additional watering before applying the herbicide treatments.

The herbicide treatments were applied immediately after seeding using a moving-nozzle cabinet CO_2 sprayer. The sprayer was equipped with a flat-fan 8002VS TeeJet® nozzle (Spraying Systems Co., Wheaton, IL) that delivered 200 L ha^{-1} solution with a pressure of 275 kPa at a speed of 2.4 km h^{-1} and a height of 50 cm above the soil surface. After herbicide treatment, the pots were returned to the greenhouse and withheld from watering for 24 h. The pots were

Table 1. Characteristics of the sandy loam and clay loam soils collected from southern Alberta and used to evaluate soil-applied residual herbicides for glyphosate-resistant and -susceptible downy brome management in a controlled-environment pot study

Texture	Sand	Silt	Clay	OM	pH	Soluble salts	NO_3-N	P	K	SO_4-S
	%					dS m^{-1}	kg ha^{-1}			
Sandy loam	76	12	12	1.5	7.2	1.0	160	30	542	20
Clay loam	26	26	38	2.6	7.4	1.1	110	12	653	210

Table 2. Soil-applied herbicide treatments evaluated for management of glyphosate-resistant and -susceptible downy brome in a controlled-environment pot study

Herbicide common name	Herbicide trade name	Rate	Herbicide group	Concentration	Formulation^{a}	Manufacturer^{b}
		g ai ha^{-1}		g ai L^{-1}		
Ethalfluralin	Liquid Ethalfluralin	850	3	354	EC	Gowan
Ethalfluralin	Liquid Ethalfluralin	1,100	3	354	EC	Gowan
Trifluralin	Treflan® Liquid EC	850	3	480	EC	Gowan
Trifluralin	Treflan® Liquid EC	1,100	3	480	EC	Gowan
Flumioxazin	Valtera$^{\text{TM}}$ EZ	70	14	479	SC	Valent
Flumioxazin	Valtera$^{\text{TM}}$ EZ	105	14	479	SC	Valent
Flumioxazin/Pyroxasulfone	Fierce® EZ	70/89	14/15	160/203	SC	Valent
Carfentrazone/Pyroxasulfone	Focus®	12/100	14/15	53/447	SE	FMC
Carfentrazone/Pyroxasulfone	Focus®	18/150	14/15	53/447	SE	FMC
Sulfentrazone/Pyroxasulfone	Authority® Supreme	100/100	14/15	250/250	SC	FMC
Sulfentrazone/Pyroxasulfone	Authority® Supreme	150/150	14/15	250/250	SC	FMC
Saflufenacil/Pyroxasulfone	Heat® Complete^{c}	18/60	14/15	342/500	SC	BASF
Saflufenacil/Pyroxasulfone	Heat® Complete^{c}	25/84	14/15	342/500	SC	BASF
Saflufenacil/Pyroxasulfone	Heat® Complete^{c}	36/120	14/15	342/500	SC	BASF

[a] Abbreviations: EC, emusifiable concentrate; SC, suspension concentrate; SE, suspension emulsion.

[b] BASF, BASF Canada Inc., Mississauga, ON; FMC, FMC of Canada Ltd., Mississauga, ON; Gowan, Gowan Company LLC, Yuma, AZ; Valent, Valent Canada Inc., Guelph, ON.

[c] Mixed with Merge® surfactant blend at 0.5% v/v.

watered daily thereafter by misting from above. The herbicides were not mechanically incorporated into the soil to reflect the no-till systems where GR downy brome was confirmed in southern Alberta and also due to potential bias induced by attempting to mimic field incorporation in greenhouse pots absent of crop residue cover characteristic of no-till systems. The greenhouses used for both runs of the experiment were set to an 18-h photoperiod with 22/18 C temperature regime. The greenhouse used for the first run was equipped with Heliospectra MITRA light-emitting diode (LED) bulbs (Heliospectra, Gothenburg, Sweden) that provided 200 μmol m^{-2} s^{-1} supplemental light. The greenhouse used for the second run was equipped with Fluence RAZR 3 LED bulbs (Fluence, Austin, TX) that delivered 230 μmol m^{-2} s^{-1} supplemental light.

The density of downy brome plants was determined at 2, 4 and 8 wk after treatment (WAT) by counting all seedlings present in each experimental unit (greenhouse pot). Visible control was estimated as a percent from 0% (similar to the untreated control) to 100% control (complete necrosis) at 4 and 8 WAT following the procedures outlined by the Canadian Weed Science Society/Société Canadienne de Malherbologie (2018). This rating scale provides a visual estimate of reduction in plant growth compared with the untreated control, which encompasses a qualitative assessment of plant density, biomass, and height. Downy brome biomass was determined at 8 WAT by removing and weighing all living and dead downy brome plant tissue above the soil surface. The biomass samples were then dried for 1 wk at 60 C and dry weight (DW) was determined.

Statistical Analysis

Downy brome density (2, 4, and 8 WAT), visible control (4 and 8 WAT), and biomass DW (8 WAT) data were subjected to ANOVA using the MIXED procedure with SAS Studio 3.81 software (SAS Institute Inc., Cary, NC). The initial model included herbicide treatment, soil type, downy brome population, experimental run, and their interactions as fixed factors, while replicate nested within run was a random factor. Subsequent model diagnostics showed that the run factor accounted for <5% of the total sums of squares for each model. In contrast, visual inspection of the residuals showed homogeneity of variance across both runs (Littell et al. 2006). Therefore, the run factor was removed from the final models, and data were analyzed across both runs. Residual fit to the Gaussian distribution was assessed based on the Shapiro-Wilk statistic using the UNIVARIATE procedure, while homoscedasticity was visually assessed by plotting the residuals and predicted values (Kozak and Piepho 2018). The untreated control treatment was removed from analyses of visible control data due to lack of variability since the visible control assessment was relative to the untreated control (considered 0% control) for each replicate. The arcsine square root transformation was used to meet the assumptions of ANOVA for the visible control data. The repeated group option was used to adjust further for homoscedasticity based on minimization of the Akaike information criterion (Littell et al. 2006). Outlier data points did not warrant removal based on the Lund test (Lund 1975). Tukey's honestly significant difference test ($\alpha = 0.05$) was used for post hoc means comparison. Linear correlations between the response variables were determined using the CORR procedure ($\alpha = 0.05$).

Results and Discussion

Herbicide Treatments

Several soil-applied herbicides controlled downy brome by ≥80% among soils and downy brome populations based on visible control estimates and biomass at 8 WAT (Figure 1). A commonality was observed where any of the treatments that included pyroxasulfone applied at or above 89 g ai ha^{-1} controlled downy brome by ≥80%

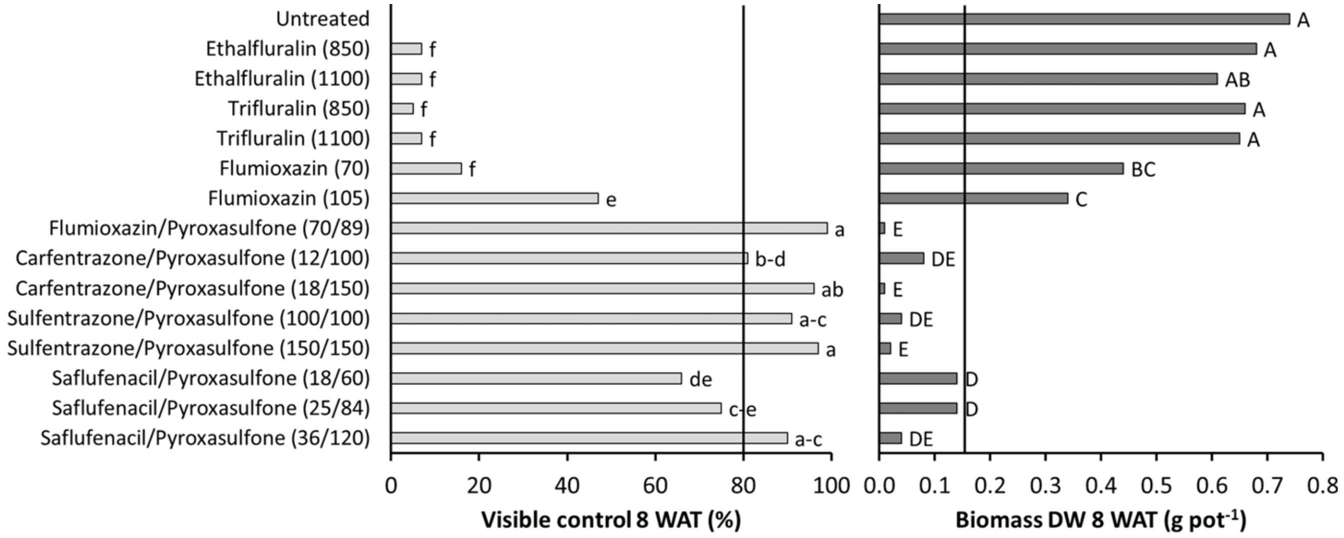

Figure 1. Visible control ($P < 0.0001$) and biomass dry weight (DW; $P < 0.0001$) of downy brome 8 wk after treatment (WAT) with a range of preemergence soil-applied herbicides in combined analyses among sandy loam and clay loam soils and glyphosate-resistant and glyphosate-susceptible downy brome populations. Numbers in parentheses indicate the amount of each active ingredient in g ai ha^{-1}. Within subfigures, different letters indicate significant differences based on Tukey's honestly significant difference test ($\alpha = 0.05$). Vertical lines indicate the threshold for 80% visible control or 80% reduction in biomass DW relative to the untreated control.

when averaged among soils and populations. These results suggest that flumioxazin/pyroxasulfone (70/89 g ai ha^{-1}), carfentrazone/pyroxasulfone (12/100 or 18/150 g ai ha^{-1}), sulfentrazone/pyroxasulfone (100/100 or 150/150 g ai ha^{-1}), and the high rate of saflufenacil/pyroxasulfone (36/120 g ai ha^{-1}) were all options for residual control of downy brome. Health Canada's Pest Management Regulatory Agency (2023) considers a rating of weed "control" as visible efficacy estimates ≥80%, while ≥90% is considered "excellent control" and 60% to 79% is considered "suppression". Of the herbicide treatments that controlled downy brome among soils and populations 8 WAT, flumioxazin/pyroxasulfone (70/89 g ai ha^{-1}) resulted in 99% visible control and biomass reduction, while the high rate of carfentrazone/pyroxasulfone (18/150 g ai ha^{-1}), high rate of saflufenacil/pyroxasulfone (36/120 g ai ha^{-1}), and both rates of sulfentrazone/pyroxasulfone (100/100 or 150/150 g ai ha^{-1}) all resulted in excellent control of downy brome 8 WAT (Figure 1). These results correspond with those reported by Johnson et al. (2018) who found that preemergence-applied pyroxasulfone alone (112 or 150 g ai ha^{-1}) and flumioxazin + pyroxasulfone (88 + 112 g ai ha^{-1}) controlled downy brome by >80% in winter wheat when assessed up to 50 d after spring postemergence herbicide timing in western Canada. Pyroxasulfone applied preemergence reduced downy brome biomass and seed-producing culms by 67% to 87% in winter wheat (Johnson et al. 2018). In Montana, pyroxasulfone alone (89 or 178 g ai ha^{-1}) applied preemergence controlled downy brome by 81% on average 8 wk after the postemergence treatment timing (Kumar et al. 2017). Layering fall-applied pyroxasulfone (89 g ai ha^{-1}) with spring postemergence-applied imazamox (44 g ai ha^{-1}) controlled downy brome by 99% in imidazolinone-resistant winter wheat in their study. The herbicides ethalfluralin and trifluralin, which inhibit microtubule assembly [Herbicide Resistance Action Committee (HRAC) Group 3] did not control or suppress downy brome in the current study (Figure 1) despite trifluralin being registered for this purpose (Anonymous 2022). Reduced efficacy of these dinitroaniline herbicides could be due to adsorption to the soil surface followed by photodecomposition and volatilization (Hollingsworth 1980; Savage and Jordan 1980), which can cause reduced weed control in conservation tillage systems (Ahrens and Endres 1996). Indeed, preplant incorporation is a recommended practice for dinitroaniline herbicides in western Canada (Anonymous 2022), although this practice can be undesirable in no-till systems at risk for soil erosion when crop residue is buried (Anderson et al. 1996). In the current study, we did not mechanically incorporate these herbicides to maintain consistency in application among the herbicide treatments evaluated, and because no-till is the predominant production practice where GR downy brome occurs in Alberta (Geddes and Pittman 2022). However, efficacy of these dinitroaniline herbicides for downy brome control may be improved through soil incorporation. In addition, the experimental units remained absent of overhead misting for the first 24 h after treatment in the current study, which could have limited movement of these herbicides into the soil profile during this time frame. The high rate of the protoporphyrinogen oxidase (PPO)-inhibiting herbicide flumioxazin alone (105 g ai ha^{-1}) controlled downy brome by 82% based on visible ratings 8 WAT in the sandy loam soil but not the clay loam soil (Table 3). Among nine field environments in western Canada, preemergence-applied flumioxazin alone suppressed downy brome early (21 to 28 d after the spring postemergence herbicide timing) but its effect declined over time resulting in similar biomass and seed-producing culms as the untreated control (Johnson et al. 2018). However, flumioxazin + pyroxasulfone had the lowest mean and variation in downy brome biomass among the herbicide treatments evaluated in their study. The additive efficacy of flumioxazin and pyroxasulfone has been well-documented for other weed species (Tidemann et al. 2014b). In the current study, flumioxazin/pyroxasulfone (70/89 g ai ha^{-1}) resulted in the greatest (99%) downy brome control and biomass reduction on a numerical basis, albeit this treatment was statistically similar to that of carfentrazone/pyroxasulfone (18/150 g ai ha^{-1}), sulfentrazone/pyroxasulfone (100/100 or 150/150 g ai ha^{-1}), and saflufenacil/pyroxasulfone (36/120 g ai ha^{-1}) (Figure 1).

The magnitude of reduction in downy brome densities relative to the untreated control for the soil-applied herbicides tended to be less than the visible control estimates or biomass reduction (Table 4; Figure 1). For example, flumioxazin/pyroxasulfone

Table 3. Downy brome plant density 8 WAT, visible control 4 and 8 WAT, and biomass dry weight 8 WAT in response to a range of soil-applied herbicides applied to sandy loam and clay loam soils in a combined analysis across downy brome populations.[a,b]

		Plant density			Visible control						Biomass DW		
		8 WAT			4 WAT			8 WAT			8 WAT		
Herbicide treatment	Rate	Sandy loam	Clay loam	SL vs. CL[c]	Sandy loam	Clay loam	SL vs. CL[c]	Sandy loam	Clay loam	SL vs. CL[c]	Sandy loam	Clay loam	SL vs. CL[c]
	g ai ha^{-1}	—— plants pot^{-1} ——			——— % ———			——— % ———			——— g pot^{-1} ———		
Untreated		5.6 ab	4.3 ab								0.95 a	0.52 ab	***
Ethalfluralin	850	3.2 a-d	3.6 ab		3 d	15 ef		5 d	9 cd		0.82 a	0.53 a	**
Ethalfluralin	1,100	3.4 a-c	2.3 a-d		1 d	26 d-f	*	2 d	15 cd		0.78 a	0.44 a-c	***
Trifluralin	850	4.1 a-c	3.8 ab		1 d	7 f		7 d	3 d		0.75 a	0.58 a	*
Trifluralin	1,100	4.4 a	2.4 a-c	**	0 d	6 f		2 d	13 cd		0.83 a	0.47 a	***
Flumioxazin	70	2.6 a-d	3.6 a		63 c	20 ef	***	33 cd	4 d	**	0.37 b	0.51 a	
Flumioxazin	105	1.9 b-e	2.6 a-c		77 bc	43 ef	**	82 bc	13 cd	***	0.26 bc	0.42 a-c	
Flumioxazin/Pyroxasulfone	70/89	0.4 e	0.6 d		99 a	99 a		100 a	98 a		0.01 d	0.02 e	
Carfentrazone/Pyroxasulfone	12/100	2.2 a-e	3.1 a-d		83 a-c	79 a-d		83 bc	79 ab		0.07 cd	0.09 de	
Carfentrazone/Pyroxasulfone	18/150	1.5 c-e	1.1 b-d		94 ab	95 a-c		97 ab	95 a		0.01 d	0.02 e	
Sulfentrazone/Pyroxasulfone	100/100	1.9 a-e	1.8 a-d		89 a-c	86 a-d		92 ab	89 ab		0.02 cd	0.06 de	
Sulfentrazone/Pyroxasulfone	150/150	1.1 de	0.6 cd		95 ab	97 ab		95 ab	98 a		0.02 d	0.02 e	
Saflufenacil/Pyroxasulfone	18/60	1.9 a-e	3.7 ab		86 a-c	60 de	**	80 bc	51 bc	*	0.10 cd	0.19 b-d	
Saflufenacil/Pyroxasulfone	25/84	1.7 a-e	3.2 a-d		82 bc	64 c-e		82 bc	67 a-c		0.11 b-d	0.17 c-e	
Saflufenacil/Pyroxasulfone	36/120	1.9 a-e	1.9 a-d		89 a-c	85 a-d		94 ab	86 ab		0.02 cd	0.07 de	
P-value		0.0348			< 0.0001			0.0001			< 0.0001		

[a] Abbreviations: CL, clay loam soil; DW, dry weight; SL, sandy loam soil; WAT, weeks after treatment.

[b] Within columns, different letters indicate significant differences based on Tukey's honestly significant difference test ($\alpha = 0.05$).

[c] For each response variable and herbicide treatment combination, asterisks (*, **, and ***) indicate significant differences between soils at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 4. Glyphosate-resistant and glyphosate-susceptible downy brome plant density at 2, 4, and 8 WAT in response to a range of soil-applied herbicides in a greenhouse pot study.[a,b,c]

		Plant density								
		2 WAT			4 WAT			8 WAT		
Herbicide treatment	Rate	GS	GR	GS vs. GR[d]	GS	GR	GS vs. GR[d]	GS	GR	GS vs. GR[d]
	g ai ha^{-1}	— plants pot^{-1} —			— plants pot^{-1} —			— plants pot^{-1} —		
Untreated		3.8 a-d	5.6 a		3.8 a-c	6.1 a		3.7 a-c	6.2 a	*
Ethalfluralin	850	3.6 a-d	3.5 a-c		3.8 a	3.6 a-c		3.4 ab	3.4 a-c	
Ethalfluralin	1,100	3.4 a-c	2.8 a-c		3.6 a	2.7 a-d		3.2 ab	2.6 a-c	
Trifluralin	850	4.0 ab	4.2 a		4.0 a	4.2 a-c		3.8 ab	4.1 ab	
Trifluralin	1,100	4.1 a	2.9 a-c		4.1 a	2.8 a-c	*	4.1 a	2.7 a-c	*
Flumioxazin	70	3.5 ab	4.2 a		2.8 a-c	3.8 ab		2.5 a-c	3.8 ab	*
Flumioxazin	105	4.2 a	3.8 a-c		3.1 ab	2.7 a-d		2.6 a-c	2.0 b-d	
Flumioxazin/Pyroxasulfone	70/89	1.2 cd	1.1 c		0.8 c	0.5 d		0.6 c	0.4 d	
Carfentrazone/Pyroxasulfone	12/100	1.5 a-d	5.3 a		1.6 a-c	4.2 a-c		1.5 a-c	3.8 a-c	*
Carfentrazone/Pyroxasulfone	18/150	0.9 b-d	2.3 a-c		1.3 a-c	2.2 a-d		1.1 a-c	1.5 b-d	
Sulfentrazone/Pyroxasulfone	100/100	1.9 a-d	2.5 a-c		2.2 a-c	2.2 b-d		1.6 a-c	2.0 b-d	
Sulfentrazone/Pyroxasulfone	150/150	0.9 d	1.5 bc		0.8 bc	1.5 cd		0.4 c	1.3 cd	
Saflufenacil/Pyroxasulfone	18/60	3.3 a-d	4.3 a-c		2.4 a-c	4.1 a-c		1.9 a-c	3.6 a-c	
Saflufenacil/Pyroxasulfone	25/84	1.8 a-d	4.7 ab	*	1.6 a-c	3.8 a-c	*	1.6 a-c	3.3 a-d	
Saflufenacil/Pyroxasulfone	36/120	2.3 a-d	2.9 a-c		2.2 a-c	2.2 b-d		2.1 a-c	1.8 b-d	
P-value		0.0462			0.0251			0.0201		

[a] Abbreviations: GR, glyphosate-resistant; GS, glyphosate-susceptible; WAT, weeks after treatment.

[b] Data presented are combined across sandy loam and clay loam soil types.

[c] Within columns, different letters indicate significant differences based on Tukey's honestly significant difference test ($\alpha = 0.05$).

[d] For each assessment timing and herbicide treatment combination an asterisk (*) indicates significant differences between GS ad GR populations at $P < 0.05$.

(70/89 g ai ha^{-1}) and the high rate of sulfentrazone/pyroxasulfone (150/150 g ai ha^{-1}) were the only two herbicide treatments that reduced downy brome density by >80% on average relative to the untreated control by 8 WAT (90% and 84% reduction in plant density 8 WAT). This compared with a reduction in biomass by 99% for flumioxazin/pyroxasulfone (70/89 g ai ha^{-1}) and by 97% for sulfentrazone/pyroxasulfone (150/150 g ai ha^{-1}). However, given the low density and observed variability in downy brome emergence among treatments (Table 4), we suggest that the visible control ratings and biomass data (Table 3) are more representative of herbicide efficacy than plant densities in the current study. Nevertheless, downy brome plant densities tended to remain similar or decline as the measurements progressed from 2 to 8 WAT, suggesting that the residual activity of the herbicides that reduced downy brome density did not diminish over the 8-wk study timeframe and that little to no emergence was observed after 2 wk (Table 4). Downy brome density was correlated only weakly with visible control and biomass 8 WAT (Pearson $R = -0.49$ and 0.51, respectively; $P < 0.0001$). Together, the downy brome density, visible control, and biomass data suggest that the biomass reduction in response to preemergence herbicides was not entirely explained by a reduction in plant density, but also a reduction in growth of the emerged plants.

Downy Brome Population

Despite overall trends indicating that the GR downy brome population was more vigorous than the GS population, a population by herbicide treatment interaction was observed for downy brome plant densities ($P \leq 0.0462$) but not visible control or biomass (Table 5; Supplementary Table S1). Directional trends in plant density between the two downy brome populations were consistent at 2, 4 and 8 WAT within each herbicide treatment, but statistical differences between the two populations varied among assessment timings (Table 4). For example, saflufenacil/pyroxasulfone applied at 25/84 g ai ha^{-1} resulted in a greater density of the GR than the GS population at 2 and 4 WAT, while a similar trend observed at 8 WAT was not significant ($P = 0.0871$). Carfentrazone/pyroxasulfone (12/100 g ai ha^{-1}), flumioxazin alone (70 g ai ha^{-1}), and the untreated control all had greater GR than GS plant density at 8 WAT, but not at the earlier assessment timings. In contrast, however, the high rate of trifluralin (1,100 g ai ha^{-1}) resulted in 33% lower density of the GR than the GS population at 4 and 8 WAT but not 2 WAT. It remains unclear why GR downy brome was present at higher density than GS for some herbicide treatments and the untreated control while the opposite was observed for trifluralin (1,100 g ai ha^{-1}). The herbicide treatments did not result in differential visible control or biomass between GR and GS downy brome ($P \geq 0.1059$; Table 5; Supplementary Table S1) unless data were analyzed across all herbicide treatments and the untreated control (Tables 5 and 6). Among herbicide treatments (including the untreated control) and soil types, the GR downy brome population resulted in about 24% greater plant density (2, 4, and 8 WAT; $P \leq 0.0093$), 16% less visible control (4 and 8 WAT; $P < 0.0001$), and 18% greater biomass ($P = 0.0015$) than the GS population (Table 6). Greater vigor of the GR than the GS downy brome population across herbicide treatments was driven largely by an interaction with soil type where, in the clay loam soil only, the GR downy brome had 67% greater density on average than the GS population across the three assessment timings ($P \leq 0.0006$) (Table 6). This corresponded with less visible control of the GR (46%) than the GS (69%) downy brome in the clay loam soil at 4 WAT ($P = 0.0206$), but not the sandy loam soil.

The current study suggests that the GR downy brome population was more vigorous than the GS population, resulting in reduced control overall among the herbicide treatments. However, since the current study tested only two downy brome populations that differed in the glyphosate resistance trait but also genetic background and parental environment (Galloway 2001), we cannot unequivocally conclude that reduced control of the GR downy brome was due to a pleiotropic effect of the glyphosate resistance trait (Vila-Aiub et al. 2015). These observed differences could also be

Table 5. ANOVA table showing the main and interaction effects of soil type, downy brome population, and herbicide treatment on downy brome plant density 2, 4, and 8 WAT; visible control 4 and 8 WAT; and biomass dry weight 8 WAT.[a,b]

	P-values					
	Plant density			Visible control		Biomass DW
Factor	2 WAT	4 WAT	8 WAT	4 WAT	8 WAT	8 WAT
Soil	0.0746	0.6513	0.7638	0.3575	**0.0010**	**0.0001**
Population	**0.0013**	**0.0093**	**0.0072**	< **0.0001**	< **0.0001**	**0.0015**
S × P	< **0.0001**	**0.0006**	**0.0001**	**0.0206**	0.1782	0.9689
Treatment	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**	< **0.0001**
S × T	0.0523	0.0819	**0.0348**	< **0.0001**	**0.0001**	< **0.0001**
P × T	**0.0462**	**0.0251**	**0.0201**	0.1475	0.2197	0.1059
S × P × T	0.9939	0.7499	0.7126	0.9666	0.4478	0.8113

[a] Abbreviations: DW, dry weight; P, downy brome population; S, soil type; T, herbicide treatment; WAT, weeks after treatment.

[b] Bold P-values indicate significant differences at $P < 0.05$.

Table 6. The main and interaction effects of downy brome population (glyphosate-resistant vs. glyphosate-susceptible) and soil type on downy brome plant density 2, 4, and 8 WAT; visible control 4 and 8 WAT; and biomass dry weight 8 WAT in a combined analysis across soil-applied herbicide treatments.[a,b,c,d]

		Plant density			Visible control		Biomass DW
Soil	Population	2 WAT	4 WAT	8 WAT	4 WAT	8 WAT	8 WAT
		plants pot^{-1}			%		g pot^{-1}
Sandy loam	GS	3.0 b	2.9 ab	2.6 ab	65 ab	68	0.31
Sandy loam	GR	2.8 b	2.7 b	2.4 b	56 b	58	0.37
Clay loam	GS	2.4 b	2.2 b	1.9 b	69 a	62	0.25
Clay loam	GR	4.1 a	3.5 a	3.3 a	46 c	42	0.30
P-value		< **0.0001**	**0.0006**	**0.0001**	**0.0206**	0.1782	0.9689
Sandy loam		2.9	2.8	2.5	61	63 a	0.34 a
Clay loam		3.3	2.9	2.6	58	52 b	0.27 b
P-value		0.0746	0.6513	0.7638	0.3575	**0.0010**	**0.0001**
	GS	2.7 b	2.5 b	2.3 b	67 a	65 a	0.28 b
	GR	3.4 a	3.1 a	2.8 a	51 b	50 b	0.33 a
P-value		**0.0013**	**0.0093**	**0.0072**	< **0.0001**	< **0.0001**	**0.0015**

[a] Abbreviations: DW, dry weight; GR, glyphosate-resistant; GS, glyphosate-susceptible; WAT, weeks after treatment.

[b] Analyses of plant density and biomass included all herbicide treatments and the untreated control, while analyses of visible control included all herbicide treatments excluding the untreated control.

[c] Within columns and effect groupings, different letters indicate significant differences based on Tukey's honestly significant difference test ($\alpha = 0.05$).

[d] Bold P-values indicate significant differences at $P < 0.05$.

associated with the genetic background of these two populations or the parental environment under which the seed was produced (Galloway 2001) conferring greater vigor of the GR population in our study. Future research assessing potential fitness differences conferred by the glyphosate resistance trait in downy brome should aim to separate the confounding effects of genetic background, parental environment, and the glyphosate resistance trait through the generation of near isogenic lines that differ only in the trait of interest. Indeed, differential fitness between GR and GS accessions has been observed in other weed species, such as kochia [*Bassia scoparia* (L.) A.J. Scott] in western Canada (Martin et al. 2017).

Soil Type

A marked difference in visible control between the two soils was apparent for flumioxazin applied alone, in that the high rate (105 g ai ha^{-1}) resulted in 13% visible control 8 WAT in the clay loam soil and 82% control in the sandy loam soil (Table 3). However, this did not correspond with quantitative biomass or plant density measurements. A similar observation was present for the low rate of saflufenacil/pyroxasulfone (18/60 g ai ha^{-1}; $P = 0.0110$) for which soil type also made the difference between poor (51% control in clay loam) and adequate (80% control in sandy loam) visible control 8 WAT but did not manifest as different downy brome plant density or biomass (Table 3). The only difference in downy brome plant density between soils was observed for trifluralin (1,100 g ai ha^{-1}) from which plant densities were almost double from the sandy loam compared with the clay loam soil. All differences in downy brome biomass between soils were present only for the dinitroaniline herbicides that did not control or suppress downy brome based on visible ratings (Table 3). This was attributed to almost double the growth of downy brome in the sandy loam than the clay loam soil in the absence of herbicide treatment since all of these herbicide treatments did not result in biomass reduction compared with the untreated control. Together, these data suggest that downy brome was better suited to growth and development, but also more prone to herbicidal control, in the sandy loam compared with the clay loam soil. Indeed, better visible control was observed among herbicide treatments 8 WAT from the sandy loam (63%) compared with the clay loam (52%) soil (Table 6). In contrast, however, overall downy brome biomass (including the untreated control) was 23% greater from the sandy loam compared with the clay loam soil despite better herbicidal control.

The sandy loam and clay loam soils differed mainly in organic matter (1.5% vs. 2.6%), clay content (12% vs. 38%), sand content (76% vs. 26%), and sulfate sulfur (20 vs. 210 kg ha^{-1}) (Table 1). Pyroxasulfone is an inhibitor of very-long-chain fatty acid elongases (VLCFAEs) resulting in reduced shoot elongation of germinated seeds (Tanetani et al. 2009). Its half-life (DT_{50}) ranged from 47 d to 134 d in clay loam and sandy loam soils in Colorado (Westra et al. 2014), and 8 d to >71 d in a loam soil in Tennessee (Mueller and Steckel 2011). Pyroxasulfone has low water solubility (3.5 mg L^{-1} at 20 C) and also lower soil binding (K_d of 1.7 L kg^{-1}) compared with other VLCFAE-inhibiting herbicides. Reduced soil binding suggests that pyroxasulfone is more available for plant uptake from the soil water solution than other herbicides in this site of action. Among 25 soils that varied in physical and chemical properties, pyroxasulfone adsorption was correlated positively with soil organic matter (Pearson $R = 0.943$) and silt content (Pearson $R = 0.669$) and negatively with sand content (Pearson $R = -0.660$) (Westra et al. 2015). Therefore, pyroxasulfone efficacy tends to decline as soil organic matter increases. Among five locations in Saskatchewan, Tidemann et al. (2014a) observed a positive linear relationship between soil organic matter and the rate of pyroxasulfone required for 50% biomass reduction (GR_{50}) of wild oat (*Avena fatua* L.) and false cleavers (*Galium spurium* L.). The pyroxasulfone GR_{50} for wild oat increased by 34 g ai ha^{-1} for every 1% increase in soil organic matter. This was likely due to a greater proportion of pyroxasulfone binding to soil with greater organic matter, making it less available for plant uptake (Westra et al. 2015). In the current study, downy brome control was similar in both soils for all treatments containing pyroxasulfone with the exception of the low rate of saflufenacil/pyroxasulfone (18/60 g ai ha^{-1}) for which visible control was 33% lower in the clay loam than the sandy loam soil (Table 3). This was likely due to this treatment containing the lowest rate of pyroxasulfone among the herbicides tested (Table 2) and the known interaction of effective pyroxasulfone rate with soil organic matter (Tidemann et al. 2014a; Westra et al. 2015). Higher amounts of organic matter in the clay loam soil could also explain, in part, the lower visible control of downy brome 8 WAT when averaged among herbicide treatments (Table 6).

Effective postemergence herbicides targeting GR downy brome include quizalofop alone or mixed with imazamox, imazamox/imazethapyr, or imazamox + bentazon, and glufosinate mixed with clethodim or tiafenacil (Geddes and Pittman 2023). Downy brome populations that are resistant to herbicides that inhibit acetyl-CoA carboxylase (HRAC Group 1) or acetolactate synthase (HRAC Group 2) have been found in the Pacific Northwest and in Montana, which borders Alberta to the south (Ball et al. 2007; Kumar and Jha 2017; Park and Mallory-Smith 2004; Zurger and Burke 2020). While these biotypes have yet to be documented in Canada to date, continued monitoring will be essential to inform herbicide layering strategies that use multiple effective herbicide sites of action targeting downy brome. However, herbicide strategies alone will not prevent selection for herbicide resistance in downy brome, and therefore, integration of nonchemical tactics will be necessary to mitigate further spread of herbicide resistance in this species. Since downy brome is cleistogamous and predominantly self-pollinated (Mitich 1999; Upadhyaya et al. 1986), the spread of resistance in this species is primarily seed-limited. This suggests that nonchemical tactics that reduce downy brome seed production and return to the soil seedbank will mitigate the spread of glyphosate resistance in this species. Rotating crops with diverse life cycles (Blackshaw 1994b), growing competitive cultivars (Blackshaw 1994a), judicious tillage (Blackshaw et al. 2001; Young et al. 2014), livestock integration (Blackshaw and Rode 1991), and sanitation of harvesting equipment between fields (Geddes and Pittman 2022) are all important components that may be used to augment herbicide layering in an integrated weed management program targeting GR and GS downy brome.

Practical Implications

The current study suggests that mixtures of PPO-inhibiting herbicides with the VLCFAE inhibitor pyroxasulfone at a rate ≥89 g ai ha^{-1} could be an effective preemergence component of an herbicide layering strategy targeting GR and GS downy brome. Lack of difference between GR and GS downy brome densities for the effective preemergence herbicide treatments among soils (Table 4; except the low rate of carfentrazone/pyroxasulfone 8 WAT), and an otherwise similar directional response of visible control and biomass among herbicide treatments (Tables 5 and 6), suggests that this strategy would be consistent regardless of whether the glyphosate resistance trait is present in the targeted downy brome population. Therefore, optimal herbicidal control of GR and GS downy brome may be achieved by layering pyroxasulfone (≥89 g ai ha^{-1}) plus a PPO-inhibiting herbicide preemergence with effective herbicides from alternative sites of action postemergence.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2024.22

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