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DOI: 10.1017/wsc.2025.4

Short title: PPO inhibitor-resistant kochia

Protoporphyrinogen oxidase (PPO) inhibitor resistance in kochia (*Bassia scoparia*)

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Abstract

Kochia [*Bassia scoparia* (L.) A.J. Scott] is an invasive tumbleweed in the North American Great Plains that is difficult to manage in croplands and ruderal areas due to widespread resistance to up to four herbicide sites-of-action, including auxin mimics (Herbicide Resistance Action Committee (HRAC) Group 4) and inhibitors of acetolactate synthase (HRAC Group 2), photosystem II (HRAC Group 5), and 5-enolpyruvylshikimate-3-phosphate synthase (HRAC Group 9). Poor *B. scoparia* control with protoporphyrinogen oxidase (PPO)-inhibiting (HRAC Group 14) herbicides was noted in a brown mustard [*Brassica juncea* (L.) Czern.] field near Kindersley, Saskatchewan in 2021. Similar observations were made in a sunflower (*Helianthus annuus* L.) field near Mandan, North Dakota, and in research plots near Minot, North Dakota in 2022. Whole-plant dose-response experiments were conducted to determine if these *B. scoparia* accessions were resistant to the PPO-inhibiting herbicides saflufenacil and carfentrazone and the level of resistance observed. All three *B. scoparia* accessions were highly resistant to foliar-applied saflufenacil and carfentrazone compared with two locally-relevant susceptible accessions. The Kindersley accession exhibited 57- to 87-fold resistance to saflufenacil and 97- to 121-fold resistance to carfentrazone based on biomass dry weight 21 days after treatment (DAT). Similarly, the Mandan accession exhibited 204- to 321-fold resistance to saflufenacil and 111- to 330-fold resistance to carfentrazone, while the Minot accession exhibited 45- to 71-fold resistance to saflufenacil and 88- to 264-fold resistance to carfentrazone. Substantial differences in visible control 7 and 21/28 DAT were also observed between the putative-resistant and susceptible accessions. This study represents the first confirmations of PPO inhibitor-resistant *B. scoparia* globally and the fifth herbicide site-of-action to which *B. scoparia* has evolved resistance. It also documents this issue present at three locations in the Northern Great Plains region that occur up to 790 km apart and on both sides of the Canada/United States border.

Keywords: carfentrazone; Group 14; herbicide resistance; integrated weed management; *Kochia scoparia* (L.) Schrad.; Northern Great Plains; saflufenacil; tumbleweed

Introduction

Kochia [*Bassia scoparia* (L.) A.J. Scott] is a tumbleweed that is native to Eurasia and was introduced to North America as an ornamental plant in the mid- to late-1800s (Friesen et al. 2009). *Bassia scoparia* can be an invasive and troublesome weed in cropping systems, pastureland, and ruderal areas due to its summer-annual life cycle, early seedling emergence (Kumar et al. 2018; Schwinghamer and Van Acker 2008), abiotic stress tolerance (Friesen et al. 2009), competitiveness (Geddes and Sharpe 2022), prolific seed production (Beckie et al. 2016), and short-lived seed persistence in both aerial (Geddes and Pittman 2023) and soil seedbanks (Beckie et al. 2018; Dille et al. 2017; Schwinghamer and Van Acker 2008). High genetic diversity (Martin et al. 2020) combined with efficient pollen- and seed-mediated gene flow (Beckie et al. 2016) cause rapid evolution of *B. scoparia* in response to management practices. Herbicides remain the primary method used to manage this weed, and in response, *B. scoparia* populations have evolved resistance to up to four herbicide sites-of-action (Beckie et al. 2019; Varanasi et al. 2015).

Multiple herbicide-resistant *B. scoparia* is a widespread issue throughout the Great Plains of North America (Kumar et al. 2019), where it can cause substantial crop yield losses if left unmanaged (Geddes and Sharpe 2022). *Bassia scoparia* was the most-abundant herbicide-resistant broadleaf weed in a 2019/2020 survey of Saskatchewan where it occupied an estimated 19.5% of annual cropland (Geddes et al. 2024). Herbicide resistance in *B. scoparia* dates back to 1976 where photosystem (PS) II inhibitor [Herbicide Resistance Action Committee (HRAC) Group 5] resistance was reported in Kansas (Heap 2024). Acetolactate synthase (ALS) inhibitor (HRAC Group 2)-resistant *B. scoparia* was reported first in Kansas and North Dakota in 1987. ALS inhibitor-resistant *B. scoparia* is widespread and was present in all survey samples tested in Canada in recent decades (Beckie et al. 2015; Hall et al. 2014). *Bassia scoparia* with resistance to auxin mimics (HRAC Group 4) was reported first in Montana in 1993/1994 and only recently in Canada since 2015 (Beckie et al. 2019; Cranston et al. 2001; Geddes et al. 2022a, 2022b, 2022c, 2023; Heap 2024). Glyphosate (HRAC Group 9)-resistant *B. scoparia* was documented first in Kansas in 2007 and later in multiple states and provinces (Beckie et al. 2013; Hall et al. 2014; Heap 2024). After only a single decade since the first report of glyphosate-resistant *B. scoparia* in Canada (2011), this biotype was present in about three quarters of *B. scoparia* samples tested (n = 889) between 2018 and 2021 (Geddes et al. 2022b, 2023; Sharpe et al. 2023).

Interest in protoporphyrinogen oxidase (PPO)-inhibiting herbicides has grown recently due, in part, to the continued evolution and spread of glyphosate-resistant weeds (Barker et al. 2023; Dayan et al. 2018). Herbicides targeting this site-of-action have been commercialized for over a half-century, despite their mechanism of action being elucidated only in recent decades (Matringe et al. 1989a, 1989b). In susceptible plants, PPO inhibitors cause chlorosis, wilting and necrosis and have been referred to colloquially as bleaching or peroxidizing herbicides. Following plant uptake, the PPO-inhibiting active ingredient enters photosynthetically-active parenchyma cells where it inhibits PPO isoforms, PPO1 and PPO2, located in the chloroplast. Protoporphyrin IX (proto) is then leaked into the cytoplasm where it is converted to photodynamic protoporphyrin IX (proto). Proto generates a flush of reactive oxygen species (ROS) under light which ultimately cause membrane lipid peroxidation (Barker et al. 2023). There are currently 21 unique herbicide active ingredients commercialized that inhibit PPO spanning four different chemical families (HRAC 2024b).

Rapid evolution and spread of multiple herbicide resistance traits in *B. scoparia*, and widespread glyphosate resistance in particular, resulted in greater reliance on PPO-inhibiting herbicides for *B. scoparia* control. Several studies document excellent foliar- and soil-applied activity of PPO inhibitors on *B. scoparia* (Kumar and Jha 2015; Torbiak et al. 2021a, 2021b, 2022, 2024; Yadav et al. 2020). For example, preemergence sulfentrazone (105 g ai ha⁻¹) controlled glyphosate-resistant *B. scoparia* in spring wheat (*Triticum aestivum* L.) by 95–99% 3 weeks after postemergence herbicides were applied (Torbiak et al. 2021b). Carfentrazone + sulfentrazone (9 + 105 g ai ha⁻¹) applied preemergence controlled glyphosate- and ALS inhibitor-resistant *B. scoparia* in field pea (*Pisum sativum* L.) by 94% on average 3 weeks after the postemergence herbicide treatment timing (Torbiak et al. 2022). In chemical fallow, glyphosate (450 g ae ha⁻¹) mixed with saflufenacil (18 or 50 g ai ha⁻¹), carfentrazone (18 g ai ha⁻¹) or carfentrazone + sulfentrazone (9 + 53 or 9 + 105 g ai ha⁻¹) resulted in ≥90% control of glyphosate-resistant *B. scoparia* in Alberta (Torbiak et al. 2021a). In Montana, saflufenacil (25 g ai ha⁻¹) applied postemergence controlled *B. scoparia* by 90% 1 week after treatment (WAT), which decreased to 67% by 5 WAT absent of crop interference (Kumar and Jha 2015). Glyphosate + sulfentrazone (1,261 + 210 g ae/ai ha⁻¹) resulted in near-complete control and 97–100% biomass reduction of *B. scoparia* in glyphosate/dicamba-resistant soybean grown in Montana and Kansas (Yadav et al. 2020). Excellent *B. scoparia* control with PPO-inhibiting

herbicides resulted in extensive adoption of glyphosate and PPO-inhibiting herbicide mixtures to control glyphosate-resistant *B. scoparia* before crop planting in the conservation tillage systems that dominate the Great Plains region. However, due to widespread glyphosate resistance in this species, this resulted in only a single herbicide site-of-action with sufficient activity on *B. scoparia*. When this is combined with widespread ALS inhibitor resistance in *B. scoparia* resulting in no effective postemergence herbicides in many pulse crops grown in the region, and auxinic herbicide resistance limiting postemergence weed control in small-grain cereals, heavy reliance on PPO inhibitors for *B. scoparia* control could increase risk of selection for PPO inhibitor resistance (Sharpe and Novek 2024).

Poor control of *B. scoparia* with PPO-inhibiting herbicides was identified in a brown mustard [*Brassica juncea* (L.) Czern.] field located near Kindersley, Saskatchewan, Canada in 2021. Glyphosate and sulfentrazone either alone or mixed with carfentrazone were applied preemergence during the previous three growing seasons (Table 1). Similarly, poor *B. scoparia* control with carfentrazone + sulfentrazone was noted in a sunflower (*Helianthus annuus* L.) field near Mandan, North Dakota, US and in research plots near Minot, North Dakota, US in 2022 (Table 2). The objectives of this research were to determine (a) if the *B. scoparia* accessions collected from Saskatchewan and North Dakota were resistant to the foliar-applied PPO-inhibiting herbicides saflufenacil and carfentrazone, and if so (b) the level of resistance observed.

Materials and Methods

Plant Material

Mature seeds from at least 20 uncontrolled *B. scoparia* plants were collected at random from the fields of interest (Figure 1). The Saskatchewan fields were sampled in October 2021 and the North Dakota fields were sampled in October 2022. The putative-resistant sample from Saskatchewan was collected from a field planted to brown mustard near Kindersley, Saskatchewan and designated ‘KindersleyR’ (coordinates not provided to protect farmer identity). Two susceptible control accessions were also collected, one from a field near Eastend, Saskatchewan (hereafter ‘EastendS’) and another being a lab-maintained ALS inhibitor-resistant, but glyphosate- and auxin mimic-susceptible control collected near Rosetown, Saskatchewan (hereafter ‘RosetownS’). Two previously collected accessions were used as susceptible controls in the North Dakota experiments. A field near Fargo, North Dakota (hereafter ‘FargoS’) was sampled in 2012, and a field in Minot, North Dakota (hereafter ‘MinotS’) was sampled in

approximately 2010. The putative-resistant accessions collected near Mandan and Minot, North Dakota in 2022 were designated ‘MandanR’ and ‘MinotR’, respectively.

The field-collected samples from Saskatchewan were subjected initially to single-dose screening with saflufenacil (Heat[®] LQ, BASF Canada Inc., Mississauga, ON) at 50 g ai ha⁻¹. The single-dose screening was unreplicated and consisted of three *B. scoparia* accessions (KindersleyR, EastendS, and RosetownS) and two herbicide regimes (treated and untreated). The *B. scoparia* accessions were planted in 24 × 24 × 5 cm greenhouse flats filled with Cornell soilless potting medium (Sheldrake and Boodley 1966) targeting 40 plants flat⁻¹. The flats were placed in the greenhouse at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre where they were watered daily. The greenhouse followed a 20/18°C day/night temperature regime with 16 h photophase and 8 h scotophase. Fluence RAZR 3 light emitting diode bulbs (Fluence, Austin, TX) provided 230 μmol m⁻² s⁻¹ supplemental light. The herbicide was applied at 5–8 cm plant height using a moving-nozzle cabinet sprayer with a TeeJet[®] flat-fan 8002VS nozzle calibrated to deliver 200 L ha⁻¹ spray solution at 275 kPa when traveling at 2.4 km h⁻¹. To limit the potential impact of parental environment on the phenotypic expression of resistance, and to demonstrate transfer of the resistance trait to subsequent generations (HRAC 2024a), survivors from the treated KindersleyR accession and untreated EastendS and RosetownS accessions were transplanted separately into larger containers 21 days after treatment (DAT) and grown for seed under pollination bags created from 10 μm nylon mesh (Miami Aqua-culture, Inc., Boynton Beach, FL). Four surviving plants from each accession were placed under a pollination bag where they were allowed to cross-pollinate within each accession to avoid inbreeding depression. The second generation seeds were hand-harvested, threshed, and the seed was stored at 4°C until used for the dose-response experiments. The North Dakota accessions (MandanR, MinotR, FargoS, and MinotS) were field-collected samples that were not regenerated under controlled-environment.

Dose-Response

Separate dose-response bioassays were conducted for the Saskatchewan (KindersleyR, EastendS, and RosetownS) and North Dakota (MandanR, MinotR, FargoS, and MinotS) accessions in the greenhouses at the Lethbridge Research and Development Centre and North Dakota State University, respectively. Each experiment included either one (Saskatchewan) or two (North Dakota) putative PPO inhibitor-resistant *B. scoparia* accessions and two locally-relevant

susceptible control accessions. At each location, separate dose-response experiments were conducted for saflufenacil and carfentrazone (Aim[®] EC, FMC of Canada Ltd., Mississauga, ON) and were repeated once. Each experiment followed a factorial randomized complete block design where the first factor consisted of *B. scoparia* accession and the second factor was herbicide rate. The rate structure followed 0 (untreated), 0.01, 0.1, 1, 3.16, 10, 31.6, and 100× the US field rate for each active ingredient [25 g ai ha⁻¹ for saflufenacil and 17.5 g ai ha⁻¹ for carfentrazone (Ikley et al. 2024)]. The Saskatchewan accession experiments (Figure 2) included nine blocks consisting of one *B. scoparia* plant in each 10 × 10 cm plastic greenhouse pot. The Saskatchewan experiments used the same potting medium, growth environment, and herbicide treatment methodology described previously. The North Dakota accession experiments (Figure 3) included ten blocks consisting of one *B. scoparia* plant in each 4 cm dia. × 21 cm deep cone-tainer (Stuewe & Sons, Inc., Tangent, OR) filled with four parts potting soil (Promix BX, Premier Horticulture Inc., Quakertown, PA) and one part sandy loam soil. In the North Dakota experiments, plants were treated when they reached 2–3 cm in height using a moving-nozzle cabinet sprayer equipped with a TeeJet XR 8002E nozzle calibrated to deliver 140 L ha⁻¹ spray solution at 207 kPa when traveling 5.4 km h⁻¹. Ammonium sulfate and methylated seed oil were added to the spray solution at 10 g L⁻¹ and 1% v/v, respectively. Greenhouse temperatures were maintained between 24 and 27°C with a 16 h photophase and 8 h scotophase supplemented with light from 1000-watt high pressure sodium lamps (P.L. Light Systems, Inc., Beamsville, ON). All experiments were watered from above daily and did not receive fertilization outside of that provided by the potting medium.

The *B. scoparia* measurements included visible control at 7 and 21 DAT and shoot biomass fresh weight (FW) and dry weight (DW) 21 DAT; save for the North Dakota saflufenacil experiments that included visible control, FW, and DW measurements 28 DAT. *Bassia scoparia* visible control was estimated as a percentage from 0% to 100% control relative to the untreated control within each accession and block following the rating scale reported by the Canadian Weed Science Society (2018). *Bassia scoparia* shoot biomass was determined by harvesting all living and dead tissue above the soil surface and weighing (FW), followed by drying in an oven at 60°C until constant weight and weighing again (DW). Both biomass FW and DW were included as response variables to account for the impacts of dead *B. scoparia*

tissue at high herbicide rates on the dose-response relationship due to differential moisture retention between living and dead plant tissue.

Statistical Analyses

The experiments using the Saskatchewan and North Dakota *B. scoparia* accessions were analyzed separately following the same two-stage procedure including analysis of variance (ANOVA) and nonlinear regression. Visible control (7 and 21/28 DAT) and biomass (FW and DW 21/28 DAT) data were subjected to ANOVA using PROC MIXED of SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA). *Bassia scoparia* accession, herbicide rate, experimental run, and their interactions were considered fixed factors while block nested within run was considered a random factor. The model assumptions were assessed using PROC UNIVARIATE based on the Shapiro-Wilk statistic and by plotting the residuals and fitted values (Littell et al. 2006). Variance component analyses were used to determine the percentage of total model sums of squares allocated to each factor. All main and interaction effects including experimental run accounted for <5% of the total sums of squares and therefore this factor was removed from the final analysis after confirming homogeneous variance across runs.

The *B. scoparia* visible control (7 and 21/28 DAT) and biomass (FW and DW 21/28 DAT) data were analyzed using nonlinear regression in the ‘drc’ package of R v. 4.3.1 (R Core Team 2023). The analysis used the three-parameter log-logistic function (Equation 1)

$$y = \frac{d}{1 + \exp\{b[\log(x) - \log(e)]\}} \quad [1]$$

where: y is the response variable, d is the upper asymptote, b is the slope of the regression line at dose e , e is the regression line inflection point, and x is the herbicide rate in g ai ha⁻¹ (Ritz et al. 2015). A four-parameter log-logistic function was fit initially but the lower asymptote did not differ from zero ($\alpha = 0.05$) and so a common lower asymptote was fit based on model parsimony. A similar approach was taken to fit a common upper asymptote when modelling visible control data only when the upper asymptote for each regression curve did not differ from 100% control, in which case the d parameter was fit individually for each *B. scoparia* accession. The ‘ED’ and ‘EDcomp’ functions were used to determine herbicide effective doses resulting in 50% and 80% visible control (ED₅₀ and ED₈₀, respectively) or biomass reduction (GR₅₀ and GR₈₀, respectively) and compare among them ($\alpha = 0.05$). The effective doses for visible control were determined relative to the limits of 0% and 100% control while the effective doses for biomass were determined relative to the predicted biomass for the untreated control within each *B. scoparia*

accession. The resistance index was calculated by dividing the ED₅₀ or GR₅₀ value for each putative-resistant accession by that for each corresponding susceptible control accession. The putative PPO inhibitor-resistant *B. scoparia* accessions were considered to be highly resistant if the resistance index was ≥ 10 (HRAC 2024a).

Results and Discussion

The putative PPO inhibitor-resistant *B. scoparia* accessions from Saskatchewan (KindersleyR) and North Dakota (MandanR and MinotR) were highly resistant to foliar-applied saflufenacil and carfentrazone. These represent the first reports of PPO inhibitor-resistant *B. scoparia* globally, and that the issue was present in the Northern Great Plains region at sites located up to 790 km apart and on either side of the Canada/US border (Figure 1).

Saflufenacil Resistance

Saskatchewan

The *B. scoparia* accession collected near Kindersley, Saskatchewan in 2021 was highly resistant to foliar-applied saflufenacil. KindersleyR exhibited 57.0- to 87.2-fold resistance to saflufenacil based on biomass DW (Table 3; Figures 2 and 4). The differential response of KindersleyR to saflufenacil compared with the susceptible control accessions was evident visually by 7 DAT and extended to at least 21 DAT (Table 3; Figure 4). Visible control resistance indices ranged from 46.9- to 47.4-fold resistance at 7 DAT and increased to 56.5- to 101.1-fold resistance by 21 DAT (Table 3; Figure 4). The estimated rate of saflufenacil causing 80% reduction in biomass DW was 126.9 g ai ha⁻¹ (Table 4). This was well-above the high field rate registered in western Canada (50 g ai ha⁻¹) (Anonymous 2024a, 2024b). This too was >125-fold greater than the saflufenacil rate causing 80% decline in biomass DW for the susceptible control accessions, EastendS and RosetownS (0.8 and 1.0 g ai ha⁻¹, respectively).

North Dakota

The *B. scoparia* accessions collected near Mandan and Minot, North Dakota in 2022 were highly resistant to saflufenacil, similar to the KindersleyR accession. The saflufenacil resistance indices for MinotR were about one-quarter that of MandanR. For example, MandanR exhibited 204.0- to 320.5-fold resistance while MinotR exhibited 45.4- to 71.3-fold resistance to foliar-applied saflufenacil based on biomass DW (Table 3, Figures 3 and 5). While both accessions were highly-resistant based on guidelines recommended by the Global HRAC (HRAC 2024a), the difference in resistance indices between these two accessions was due to very low GR₅₀ values

for the susceptible control accessions that were ≤ 0.7 g ai ha⁻¹ of saflufenacil (Table 5). Like the Saskatchewan accessions, differential response of the resistant from the susceptible North Dakota accessions was evident by 7 DAT and extended to at least 28 DAT (Table 3; Figure 5). However, resistance indices based on visible control ratings were not statistically different from 1 despite R/S ratios that were ≥ 292.8 (Table 3). This was due, in part, to variability around the dose-response model inflection point (Table 5), which could reflect the variable nature of the North Dakota field-collected samples absent of subsequent regeneration under controlled-environment or incomplete (70–72%) visible control of the resistant accessions at the highest saflufenacil rate (2500 g ai ha⁻¹) (Figure 5). Mean visible control at 28 DAT for the susceptible accessions increased from 3% at 0.25 g ha⁻¹ of saflufenacil to 88% at 2.5 g ai ha⁻¹, also contributing to inaccurate estimation of the ED₅₀ values and no statistical difference when determining the visible control resistance indices. The estimated saflufenacil rate causing 80% reduction in biomass DW was 485.6 and 387.8 g ai ha⁻¹ for MandanR and MinotR compared with 0.9 and 0.4 g ai ha⁻¹ for FargoS and MinotS (Table 5), about 16–18 times the typical US burndown rate of 25 g ai ha⁻¹ (Ikley et al. 2024).

Carfentrazone Resistance

Saskatchewan

The KindersleyR accession was highly-resistant to foliar-applied carfentrazone. KindersleyR exhibited 97.0- to 120.9-fold resistance to carfentrazone based on biomass DW, compared with the two susceptible control accessions (Table 3; Figures 2 and 6). Like the response to saflufenacil, differential response to carfentrazone was obvious by 7 DAT and extended to 21 DAT (Table 3; Figure 6). The estimated carfentrazone rate causing 80% reduction in biomass DW of KindersleyR was 157.9 g ai ha⁻¹, which was well above that for the susceptible accessions (2.0–3.0 g ai ha⁻¹) (Table 4) and 6–18 times the registered burndown field rates (9–28 g ai ha⁻¹) for carfentrazone in western Canada (Anonymous 2024a, 2024b).

North Dakota

The MandanR and MinotR accessions were also highly-resistant to carfentrazone. For example, resistance indices based on biomass DW ranged from 110.5- to 330.1-fold for MandanR and from 88.4- to 264.1-fold for MinotR (Table 3; Figures 3 and 7). However, resistance indices based on GR₅₀ values were not significantly different from 1 ($\alpha = 0.05$) for these North Dakota accessions (Table 3). Despite this, resistance indices based on visible control 21 DAT were

significantly different from 1 and ranged from 515.7- to 1008.1-fold resistance for MandanR and from 107.0- to 210.9-fold resistance for MinotR. Like the response of these accessions to saflufenacil, high resistance indices but lack of significant differences from unity for some response variables and not others were caused by a combination of incomplete (50–76%) control of the resistant accessions at the highest carfentrazone rate (1750 g ai ha⁻¹), high variability around the model inflection point, and natural variability in the first generation field-collected samples (Table 5; Figure 7). Nevertheless, taken together, observations across response variables, accessions and locations suggest that these putative PPO inhibitor-resistant *B. scoparia* accessions were highly resistant to carfentrazone (HRAC 2024a).

Untreated *B. scoparia* plants in the Saskatchewan experiments grew approximately 5–10 times larger than the North Dakota experiments (Figures 2–7), which may have contributed to the larger resistance indices observed in North Dakota than Saskatchewan (Table 3). These differences in biomass were caused by earlier herbicide treatment (2–3 cm height) and more restricted *B. scoparia* growth in the 4 cm dia. cone-tainers used in North Dakota compared with slightly later treatment (5–8 cm height) and larger (10 × 10 cm) pots used in Saskatchewan. The smaller size of the cone-tainers may have restricted growth of the North Dakota plants and potentially also led to nutrient deficiency by 21/28 DAT. Indeed, the untreated plants grown in Saskatchewan appeared visually healthy at 21 DAT (Figure 2) while those at 21/28DAT in North Dakota appeared less so (Figure 3). Despite this, apparent stress to the *B. scoparia* plants in the North Dakota experiments did not seem to influence herbicide efficacy since the effective doses for control of the susceptible accessions remained similar between experiments conducted at both locations (Tables 4 and 5; Figures 4–7). Interestingly, untreated plants from both susceptible accessions accumulated less biomass than the resistant accessions in the carfentrazone but not the saflufenacil experiments in North Dakota (Figures 5 and 7). This difference could be explained, in part, by the difference in timing of the biomass measurements in North Dakota which took place at 28 DAT for saflufenacil and 21 DAT for carfentrazone, while greater heterogeneity of these field-collected samples likely also played a role.

To-date, 17 different weed species have evolved resistance to PPO-inhibiting herbicides globally, and the majority of cases reporting PPO inhibitor resistance in the international database also report resistance to other herbicide sites of action (i.e., cross- or multiple resistance) (Barker et al. 2023; Heap 2024). PPO inhibitor-resistant waterhemp [*Amaranthus*

tuberculatus (Moq.) J.D. Sauer] was documented in Kansas in 2001, representing the first case of resistance to this site-of-action among weed species (Shoup et al. 2003). Since then, PPO inhibitor-resistant weeds have been documented in ten countries worldwide and in a range of crop species (Heap 2024). *Bassia scoparia* represents the fourth and seventh weed species to evolve PPO inhibitor resistance in Canada and the US, respectively. It remains unknown whether the three PPO inhibitor-resistant *B. scoparia* accessions identified in the current study also exhibit resistance to other herbicide sites-of-action. This knowledge gap is one focal point, of several new questions regarding PPO inhibitor-resistant *B. scoparia*, that warrant further investigation.

Similar to cases of PPO inhibitor resistance in some other weed species (Dayan et al. 2018), the resistant *B. scoparia* plants exhibited initial necrosis after foliar treatment with saflufenacil or carfentrazone fb healthy new regrowth shortly thereafter (CM Geddes and QD Law, personal obs.). The initial symptomology typical of foliar treatment with PPO-inhibiting herbicides could make field diagnostics difficult if scouting is conducted shortly after application. However, differential control of the resistant and susceptible accessions was evident visually by 7 DAT under controlled-environment (Figures 4–7), which may also translate to a field scenario. Results from the current study suggest that field scouting efforts to identify PPO inhibitor-resistant *B. scoparia* should be effective when conducted between one and three weeks after foliar treatment, but later may be more obvious given the initial necrosis injury observed after treatment of resistant plants.

The current study showed that the PPO inhibitor-resistant *B. scoparia* accessions exhibited cross-resistance to two chemical families of PPO-inhibiting herbicides (Table 3; Figures 2–7); saflufenacil belonging to the N-phenylimides and carfentrazone belonging to the N-phenyltriazolinones (HRAC 2024b). Cross-resistance in *B. scoparia* among the other families of PPO inhibitors warrants further research. Indeed, variable cross-resistance to PPO-inhibiting chemical families has been noted in other weed species, and depends on herbicide application method and timing, the weed species, and the resistance mechanism (Barker et al. 2023). Further research aimed at understanding the mechanism conferring PPO inhibitor resistance in *B. scoparia* may help elucidate more-fully the associated pattern of cross-resistance.

Practical Implications

Spread of PPO inhibitor-resistant *B. scoparia* could limit options for herbicidal control, especially given the likely stacking of multiple resistance traits in this species resulting in resistance across a wide range of herbicide sites-of-action. Herbicide resistance traits can evolve and spread efficiently in *B. scoparia* as demonstrated by the rapid increase in frequency of populations resistant to glyphosate and ALS-inhibiting herbicides in recent decades (Geddes et al. 2022b, 2023; Kumar et al. 2019; Sharpe et al. 2023; Westra et al. 2019). In *B. scoparia*, rapid evolution and spread of these traits is due to a combination of large selection pressure, high genetic diversity (Martin et al. 2020), and efficient seed- and pollen-mediated gene flow (Beckie et al. 2016). PPO inhibitor resistance in *B. scoparia* will create a gap particularly during the preplant/ preemergence weed control window in several field crops grown in the Northern Great Plains (Tables 6 and 7). As multiple herbicide resistance traits continue to stack in this species, the available options for herbicidal control become limited, causing reliance on contact-type herbicides like glufosinate (HRAC Group 10) postemergence in crops engineered to resist this glutamine synthetase inhibitor or the PSII inhibitor bromoxynil (HRAC Group 6) alone or mixed with an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HRAC Group 27). One key difference between Canada and the US, among others, is the commercial availability of the PSI-inhibiting herbicide paraquat (HRAC Group 22) in the US (Ikley et al. 2024) but not Canada (Anonymous 2024a, 2024b); which further limits herbicidal control options north of the Canada/US border.

Careful stewardship of herbicides that remain effective on multiple herbicide-resistant *B. scoparia* is warranted through further and more-targeted implementation of integrated weed management programs. Past research has shown that *B. scoparia* responds to competitive crop scenarios by substantially reducing seed production (Mosqueda et al. 2020). Management practices targeting *B. scoparia* seed production and return to the soil seedbank represent a key choking point in the life cycle of this weed (Geddes and Davis 2021) due to short seed longevity once it enters the soil seedbank (Beckie et al. 2018; Dille et al. 2017; Geddes 2021; Schwinghamer and Van Acker 2008). Integrating non-chemical practices, such as competitive crops (Mosqueda et al. 2020), alternative crop life cycles (Geddes and Davis 2021), higher crop seeding rates (Geddes and Kimmins 2021), strategic yet judicious tillage (Obour et al. 2021), or cutting *B. scoparia* for animal feed (Nair et al. 2021), may serve to reduce *B. scoparia* seedbank replenishment. In addition, cutting or mowing *B. scoparia* plants could help mitigate the globe-

shaped growth structure characteristic of tumbleweeds by physically disrupting unfettered growth and development thereby preventing *B. scoparia* movement beyond its source location. Physical barriers like fence lines or shelterbelts may also serve to mitigate seed-mediated gene flow by catching *B. scoparia* plants that move beyond field boundaries (Beckie et al. 2016; Geddes and Sharpe 2022). Mitigation efforts should employ the core foundational principles of integrated weed management where multiple cultural, physical, and biological tactics are implemented along with strategic herbicide use to limit *B. scoparia* proliferation. Of utmost importance will be continued investment in the design and understanding of sustainable integrated weed management strategies that target the unique biology of this troublesome and highly-elastic species.

Acknowledgements

We thank Bailey Maurstad for assisting with the research at North Dakota State University. We also thank the Saskatchewan agronomist who brought the initial observation of lack of control to our attention and the greenhouse staff at the Lethbridge Research and Development Centre for maintaining the growth environment.

Funding

Funding for the research conducted at the Lethbridge Research and Development Centre was provided by the Prairie Oat Growers Association, Manitoba Crop Alliance, Saskatchewan Canola Development Commission, Saskatchewan Pulse Growers, Saskatchewan Wheat Development Commission, Western Grains Research Foundation, ADAMA Agricultural Solutions Canada Ltd., BASF Canada Inc., Bayer CropScience Inc., FMC of Canada Ltd., Gowan Agro Canada, Nufarm Agriculture Inc., Valent Canada Inc., and the Saskatchewan Ministry of Agriculture – Agriculture Development Fund through project #ADF20230188. Research conducted at North Dakota State University was partially funded by the North Dakota Agricultural Experiment Station and USDA-NIFA Hatch Project #ND01532.

Competing Interests

Authors IM and AP are employees of BASF. Author CMG received grant funding for this research from the crop protection companies: ADAMA Agricultural Solutions Canada Ltd., BASF Canada Inc., Bayer CropScience Inc., FMC of Canada Ltd., Gowan Agro Canada, Nufarm Agriculture Inc., and Valent Canada Inc.

References

- Anonymous (2024a) The Blue Book: Alberta's Crop Protection Guide 2024. Alberta Grains, Calgary, AB. 740 p
- Anonymous (2024b) Guide to Crop Protection 2024. Saskatchewan Ministry of Agriculture, Regina, SK. 834 p
- Barker AL, Pawlak J, Duke SO, Beffa R, Tranel PJ, Wuerffel J, Young B, Porri A, Liebl R, Aponte R, Findley D, Betz M, Lerchl J, Culpepper S, Bradley K, Dayan FE (2023) Discovery, mode of action, resistance mechanisms, and plan of action for sustainable use of Group 14 herbicides. *Weed Sci* 71:173-188
- Beckie HJ, Blackshaw RE, Low R, Hall LM, Sauder CA, Martin S, Brandt RN, Shirriff SW (2013) Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Kochia scoparia*) in western Canada. *Weed Sci* 61:310-318
- Beckie HJ, Gulden RH, Shaikh N, Johnson EN, Willenborg CJ, Brenzil CA, Shirriff SW, Lozinski C, Ford G (2015) Glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Saskatchewan and Manitoba. *Can J Plant Sci* 95:345-349
- Beckie HJ, Blackshaw RE, Hall LM, Johnson EN (2016) Pollen- and seed-mediated gene flow in kochia (*Kochia scoparia*). *Weed Sci* 64:624-633
- Beckie HJ, Blackshaw RE, Leeson JY, Stahlman PW, Gaines TA, Johnson EN (2018) Seedbank persistence, germination and early growth of glyphosate-resistant *Kochia scoparia*. *Weed Res* 58:177-187
- Beckie HJ, Hall LM, Shirriff SW, Martin E, Leeson JY (2019) Triple-resistant kochia [*Kochia scoparia* (L.) Schrad.] in Alberta. *Can J Plant Sci* 99:281-285
- Canadian Weed Science Society (2018) Description of 0-100 rating scale for herbicide efficacy and crop phytotoxicity. https://weedsociety.ca/cwss_scm-rating-scale/ Accessed: June 24, 2024
- Cranston HJ, Kern AJ, Hackett JL, Miller EK, Maxwell BD, Dyer WE (2001) Dicamba resistance in kochia. *Weed Sci* 49:164-170
- Dayan FE, Barker A, Tranel PJ (2018) Origins and structure of chloroplastic and mitochondrial plant protoporphyrinogen oxidases: implications for the evolution of herbicide resistance. *Pest Manag Sci* 74:2226-2234

- Dille JA, Stahlman PW, Du J, Geier PW, Riffel JD, Currie RS, Wilson RG, Sbatella GM, Westra P, Kniss AR, Moechnig MJ, Cole RM (2017) Kochia (*Kochia scoparia*) emergence profiles and seed persistence across the central Great Plains. *Weed Sci* 65:614-625
- Friesen LF, Beckie HJ, Warwick SI, Van Acker RC (2009) The biology of Canadian weeds. 138. *Kochia scoparia* (L.) Schrad. *Can J Plant Sci* 89:141-167
- Geddes CM (2021) Burial environment drives seed mortality of kochia (*Bassia scoparia*), wild oat (*Avena fatua*), and volunteer canola (*Brassica napus*) irrespective of crop species. *Plants* 10:1961
- Geddes CM, Davis AS (2021) The critical period for weed seed control: A proposed framework to limit weed seed return. *Weed Res* 61:282-287
- Geddes CM, Kimmins MT (2021) Wheat density alters but does not repress the expression of a fluroxypyr-resistant kochia (*Bassia scoparia*) phenotype. *Agronomy* 11:2160
- Geddes CM, Pittman MM (2023) Serotiny facilitates kochia (*Bassia scoparia*) persistence via aerial seedbanks. *Can J Plant Sci* 103:324-328
- Geddes CM, Sharpe SM (2022) Crop yield losses due to kochia (*Bassia scoparia*) interference. *Crop Prot* 157:105981
- Geddes CM, Ostendorf TE, Owen ML, Leeson JY, Sharpe SM, Shirriff SW, Beckie HJ (2022a) Fluroxypyr-resistant kochia [*Bassia scoparia* (L.) A.J. Scott] confirmed in Alberta. *Can J Plant Sci* 102:437-441
- Geddes CM, Pittman MM, Gulden RH, Jones T, Leeson JY, Sharpe SM, Shirriff SW, Beckie HJ (2022b) Rapid increase in glyphosate resistance and confirmation of dicamba-resistant kochia (*Bassia scoparia*) in Manitoba. *Can J Plant Sci* 102:459-464
- Geddes CM, Owen ML, Ostendorf TE, Leeson JY, Sharpe SM, Shirriff SW, Beckie HJ (2022c) Herbicide diagnostics reveal multiple patterns of synthetic auxin resistance in kochia (*Bassia scoparia*). *Weed Technol* 36:28-37
- Geddes CM, Pittman MM, Hall LM, Topinka AK, Sharpe SM, Leeson JY, Beckie HJ (2023) Increasing frequency of multiple herbicide-resistant kochia (*Bassia scoparia*) in Alberta. *Can J Plant Sci* 103:233-237
- Geddes CM, Pittman MM, Sharpe SM, Leeson JY (2024) Distribution, frequency and impact of herbicide-resistant weeds in Saskatchewan. *Can J Plant Sci* [In press]. doi:10.1139/cjps-2024-0017

- Hall LM, Beckie HJ, Low R, Shirriff SW, Blackshaw RE, Kimmel N, Neeser C (2014) Survey of glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Alberta. *Can J Plant Sci* 94:127-130
- Heap I (2024) The International Herbicide-Resistant Weed Database. www.weedscience.org. Accessed: September 17, 2024
- Herbicide Resistance Action Committee (HRAC) (2024a) Criteria for confirmation of herbicide-resistant weeds. Global Herbicide Resistance Action Committee. <https://hracglobal.com/herbicide-resistance/confirming-resistance>. Accessed: June 04, 2024
- Herbicide Resistance Action Committee (HRAC) (2024b) HRAC mode of action classification 2024. Global Herbicide Resistance Action Committee. <https://hracglobal.com/tools/2024-hrac-global-herbicide-moa-classification>. Accessed: June 12, 2024
- Ikley J, Christoffers M, Dalley C, Endres G, Gramig G, Howatt K, Jenks B, Law Q, Lim C, Ostlie M, Peters T, Robinson A, Thostenson A, Valenti HH (2024) North Dakota Weed Control Guide. North Dakota State University, Fargo, ND. 135 p.
- Kumar V, Jha P (2015) Effective preemergence and postemergence herbicide programs for kochia control. *Weed Technol* 29:24-34
- Kumar V, Jha P, Dille JA, Stahlman PW (2018) Emergence dynamics of kochia (*Kochia scoparia*) populations from the U.S. Great Plains: A multi-site-year study. *Weed Sci* 66:25-35
- Kumar V, Jha P, Jugulam M, Yadav R, Stahlman PW (2019) Herbicide-resistant kochia (*Bassia scoparia*) in North America: A review. *Weed Sci* 67:4-15
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Shabenberger O, eds (2006) SAS[®] for Mixed Models. 2nd ed. Cary, NC: SAS Institute Inc. 634 p
- Martin SL, Benedict L, Sauder CA, Beckie HJ, Hall LM (2020) High gene flow maintains genetic diversity following selection for high EPSPS copy number in the weed kochia (Amaranthaceae). *Sci Rep* 10:18864
- Matringe M, Camadro J-M, Labbe P, Scalla R (1989a) Protoporphyrinogen oxidase inhibition by three peroxidizing herbicides: oxadiazon, LS 82-556 and M&B 39279. *FEBS Lett* 245:35-38
- Matringe M, Camadro J-M, Labbe O, Scalla R (1989b) Protoporphyrinogen oxidase as a molecular target for diphenyl ether herbicides. *Biochem J* 260:231-235

- Mosqueda EG, Lim CA, Sbatella GM, Jha P, Lawrence NC, Kniss AR (2020) Effect of crop canopy and herbicide application on kochia (*Bassia scoparia*) density and seed production. *Weed Sci* 68:278-284
- Nair J, Lima PMT, Abdalla AL, Molnar LJ, Wang Y, McAllister TA, Geddes CM (2021) Kochia (*Bassia scoparia*) harvest date impacts nutrient composition, *in vitro* degradability, and feed value more than pre-harvest herbicide treatment or herbicide resistance traits. *Anim Feed Sci Technol* 280:115079
- Obour AK, Holman JD, Simon LM, Schlegel AJ (2021) Strategic tillage effects on crop yields, soil properties, and weeds in dryland no-tillage systems. *Agronomy* 11:662
- R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- Ritz C, Baty F, Streibig FC, Gerhard D (2015) Dose response analysis using R. *PLoS ONE* 10:e0146021
- Schwinghamer TD, Van Acker RC (2008) Emergence timing and persistence of kochia (*Kochia scoparia*). *Weed Sci* 56:37-41
- Sharpe SM, Novek T (2024) Sublethal dosing exposure risk of kochia [*Bassia scoparia* (L.) A.J. Scott] to carfentrazone-ethyl. *Can J Plant Sci* [In press]. doi: [10.1139/cjps-2023-0168](https://doi.org/10.1139/cjps-2023-0168)
- Sharpe SM, Leeson JY, Geddes CM, Willenborg CJ, Beckie HJ (2023) Survey of glyphosate- and dicamba-resistant kochia (*Bassia scoparia*) in Saskatchewan. *Can J Plant Sci* 103:472-480
- Sheldrake T-Jr, Boodley JW (1966) Plant growing in light-weight artificial mixes. *Acta Horti* 4:155-157
- Shoup DE, Al-Khatib K, Peterson DE (2003) Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. *Weed Sci* 51:145-150
- Torbiak AT, Blackshaw RE, Brandt RN, Hall LM, Hamman B, Geddes CM (2021a) Herbicide mixtures control glyphosate-resistant kochia (*Bassia scoparia*) in chemical fallow, but their longevity warrants careful stewardship. *Can J Plant Sci* 101:188-198
- Torbiak AT, Blackshaw RE, Brandt RN, Hamman B, Geddes CM (2021b) Herbicide strategies for managing glyphosate-resistant and -susceptible kochia (*Bassia scoparia*) in spring wheat. *Can J Plant Sci* 101:607-620

- Torbiak AT, Blackshaw RE, Brandt RN, Hamman B, Geddes CM (2022) Glyphosate- and acetolactate synthase inhibitor-resistant kochia (*Bassia scoparia*) control in field pea. *Can J Plant Sci* 102:553-565
- Torbiak AT, Blackshaw RE, Brandt RN, Hamman B, Geddes CM (2024) Multiple herbicide-resistant kochia (*Bassia scoparia*) control in glufosinate-resistant canola. *Can J Plant Sci* 104:298-310
- Varanasi VK, Godar AS, Currie RS, Dille AJ, Thompson CR, Stahlman PW, Jugulam M (2015) Field-evolved resistance to four modes of action of herbicides in a single kochia (*Kochia scoparia* L. Schrad.) population. *Pest Manag Sci* 71:1207-1212
- Westra EP, Nissen SJ, Getts TJ, Westra P, Gaines TA (2019) Survey reveals frequency of multiple resistance to glyphosate and dicamba in kochia (*Bassia scoparia*). *Weed Technol* 33:664-672
- Yadav R, Kumar V, Jha P (2020) Herbicide programs to manage glyphosate/dicamba-resistant kochia (*Bassia scoparia*) in glyphosate/dicamba-resistant soybean. *Weed Technol* 34:568-574

Tables

Table 1. Recent herbicide use history in the Kindersley, Saskatchewan field where protoporphyrinogen oxidase (PPO) inhibitor-resistant *Bassia scoparia* was confirmed in 2021

Application timing					Herbicide information	
Year	Month	Day	Window ^a	Crop ^b	Common name ^c	Rate
						--- g ai/ae ha ⁻¹ ---
2019			PRE	Flax	Glyphosate + <u>Sulfentrazone</u>	890 + 113
2019			POST	Flax	Bromoxynil + MCPA	278 + 278
2020			PRE	Chickpea	Glyphosate + <u>Sulfentrazone</u>	890 + 113
2021	May	06	PRE	Brown mustard	Glyphosate + <u>Sulfentrazone</u> + <u>Carfentrazone</u>	890 + 75 + 18
2021	May	16	PRE	Brown mustard	Glyphosate + <u>Carfentrazone</u> + Bromoxynil	890 + 9 + 70

^aAbbreviations: POST, postemergence; PRE, preemergence

^bScientific names: Brown mustard, *Brassica juncea* (L.) Czern.; Chickpea, *Cicer arietinum* L.; Flax, *Linum usitatissimum* L.

^cPPO-inhibiting active ingredients are underlined

Table 2. Recent protoporphyrinogen oxidase (PPO)-inhibiting herbicide use in the Mandan and Minot, North Dakota fields where PPO inhibitor-resistant *Bassia scoparia* was confirmed in 2022

Year	Mandan		Minot
	Crop ^a	Herbicide common name	Herbicide common name ^b
2016	Wheat		
2017	Corn		Carfentrazone; Flumioxazin; Saflufenacil; Sulfentrazone
2018	Sunflower	Carfentrazone + Sulfentrazone	
2019	Wheat		Saflufenacil; Sulfentrazone
2020	Field pea	Carfentrazone + Sulfentrazone	Sulfentrazone
2021	Wheat		Carfentrazone; Flumioxazin; Sulfentrazone
2022	Sunflower	Carfentrazone + Sulfentrazone	Saflufenacil; Sulfentrazone

^aScientific names: Corn, *Zea mays* L.; Field pea, *Pisum sativum* L.; Sunflower *Helianthus annuus* L.; Wheat, *Triticum aestivum* L

^bThese herbicides were applied in research plots in various parts of the field, and research plots were moved around the field each year. The rest of the field was typically seeded to wheat where no PPO-inhibiting herbicides were used

Table 3. Saflufenacil and carfentrazone resistance indices for one putative protoporphyrinogen oxidase (PPO) inhibitor-resistant *Bassia scoparia* accession collected from Saskatchewan in 2021 and two putative PPO inhibitor-resistant *Bassia scoparia* accessions collected from North Dakota in 2022 compared with two locally-relevant susceptible control accessions based on visible control at 7 and 21/28 days after treatment (DAT) and biomass fresh weight (FW) and dry weight (DW) 21/28 DAT

Province/State	Herbicide	Accession	Response variable	R/S1 ^{a,b}	R/S2 ^{a,c}
Saskatchewan		KindersleyR	Visible control 7 DAT	47.4***	46.9***
		KindersleyR	Visible control 21 DAT	56.5	101.1***
		KindersleyR	Biomass FW 21 DAT	63.4***	82.2***
		KindersleyR	Biomass DW 21 DAT	57.0***	87.2***
		KindersleyR	Visible control 7 DAT	45.5***	48.1***
		KindersleyR	Visible control 21 DAT	38.0***	24.3***
		KindersleyR	Biomass FW 21 DAT	70.6**	72.8*
		KindersleyR	Biomass DW 21 DAT	120.9**	97.0*
North Dakota	Saflufenacil	MandanR	Visible control 7 DAT	292.8	294.8
		MandanR	Visible control 28 DAT	460.6	419.8
		MandanR	Biomass FW 28 DAT	158.9**	164.2*
		MandanR	Biomass DW 28 DAT	204.0*	320.5*
		MinotR	Visible control 7 DAT	355.6	358.1
		MinotR	Visible control 28 DAT	504.1	459.5
		MinotR	Biomass FW 28 DAT	38.3*	39.6*
		MinotR	Biomass DW 28 DAT	45.4*	71.3*
	Carfentrazone	MandanR	Visible control 7 DAT	292.1	180.4
		MandanR	Visible control 21 DAT	1008.1*	515.7*
		MandanR	Biomass FW 21 DAT	506.8	149.8
		MandanR	Biomass DW 21 DAT	330.1	110.5
		MinotR	Visible control 7 DAT	166.4***	102.8***

MinotR	Visible control 21 DAT	210.9***	107.9**
MinotR	Biomass FW 21 DAT	336.4	99.5
MinotR	Biomass DW 21 DAT	264.1	88.4

^a*, **, and *** indicate significant difference of the resistance index from unity at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

^bR/S1 indicates the resistance index relative to the first susceptible control accession; EastendS for Saskatchewan and FargoS for North Dakota

^cR/S2 indicates the resistance index relative to the second susceptible control accession; RosetownS for Saskatchewan and MinotS for North Dakota

Table 4. Regression parameter estimates for the three-parameter log-logistic model fit to describe the response of three Saskatchewan *Bassia scoparia* accessions to a rate titration of saflufenacil or carfentrazone based on visible control at 7 and 21 days after treatment (DAT) and shoot biomass fresh weight (FW) and dry weight (DW) 21 DAT^a

Herbicide	Response variable	Accession	<i>b</i> (\pm SE)	<i>d</i> (\pm SE)	<i>e</i> (\pm SE)	ED ₈₀ (\pm SE)
					----- g ai ha ⁻¹ -----	
Saflufenacil	Visible control 7 DAT <i>RSE</i> = 12.2	EastendS	-1.95 (0.21)		0.8 (0.1)	1.5 (0.2)
		RosetownS	-1.98 (0.21)		0.7 (0.1)	1.5 (0.2)
		KindersleyR	-0.73 (0.05)		35.2 (3.6)	234.3 (33.9)
	Visible control 21 DAT <i>RSE</i> = 17.6	EastendS	-2.19 (0.47)		1.0 (0.2)	2.0 (0.3)
		RosetownS	-6.66 (79.14)		1.9 (6.6)	2.3 (2.4)
		KindersleyR	-0.60 (0.05)		104.9 (16.3)	1049.0 (239.7)
	Biomass FW 21 DAT <i>RSE</i> = 1.2	EastendS	1.78 (0.58)	8.0 (0.3)	0.3 (0.4)	0.7 (0.3)
		RosetownS	2.03 (0.72)	6.4 (0.3)	0.4 (0.1)	0.9 (0.4)
		KindersleyR	0.83 (0.09)	7.0 (0.2)	27.5 (4.5)	147.6 (29.1)
	Biomass DW 21 DAT <i>RSE</i> = 0.2	EastendS	1.39 (0.33)	0.7 (0.1)	0.3 (0.1)	0.8 (0.2)
		RosetownS	1.74 (0.53)	1.0 (0.1)	0.5 (0.1)	1.0 (0.4)
		KindersleyR	0.88 (0.10)	1.2 (0.1)	26.2 (4.2)	126.9 (25.0)
Carfentrazone	Visible control 7 DAT <i>RSE</i> = 15.4	EastendS	-1.12 (0.17)		1.1 (0.1)	3.9 (0.9)
		RosetownS	-1.95 (0.73)		1.2 (0.2)	2.4 (0.4)
		KindersleyR	-0.93 (0.09)		55.0 (5.9)	241.0 (37.3)
	Visible control 21 DAT <i>RSE</i> = 16.3	EastendS	-1.05 (0.11)		4.3 (0.6)	16.3 (3.1)
		RosetownS	-1.11 (0.13)		2.8 (0.4)	9.7 (2.1)
		KindersleyR	-1.11 (0.12)		105.3 (10.2)	365.8 (55.5)
	Biomass FW 21 DAT	EastendS	0.93 (0.24)	6.7 (0.5)	0.7 (0.3)	3.3 (1.5)

<i>RSE</i> = 2.2	RosetownS	1.28 (0.32)	8.3 (0.5)	0.8 (0.2)	2.2 (0.6)
	KindersleyR	1.33 (0.42)	6.0 (0.3)	53.6 (12.8)	151.7 (48.8)
Biomass DW 21 DAT <i>RSE</i> = 0.2	EastendS	1.01 (0.31)	0.6 (0.1)	0.75 (0.3)	3.0 (1.4)
	RosetownS	1.16 (0.27)	0.8 (0.1)	0.6 (0.2)	2.0 (0.7)
	KindersleyR	1.80 (0.56)	0.6 (0.1)	73.2 (14.5)	157.9 (48.1)

^aAbbreviations: *b*, slope of the response curve at inflection point; *d* upper asymptote; DAT, days after treatment; DW, dry weight; FW, fresh weight; *e*, response curve inflection point considered ED₅₀ for visible control or GR₅₀ for biomass; ED₈₀, effective dose of herbicide resulting 80% visible control (ED₈₀) or biomass reduction (GR₈₀); RSE, residual standard error; SE, standard error

Table 5. Regression parameter estimates for the three-parameter log-logistic model fit to describe the response of four North Dakota *Bassia scoparia* accessions to a rate titration of saflufenacil or carfentrazone based on visible control at 7 and 21/28 days after treatment (DAT) and shoot biomass fresh weight (FW) and dry weight (DW) 21/28 DAT^a

Herbicide	Response variable	Accession	<i>b</i> (±SE)	<i>d</i> (±SE)	<i>e</i> (±SE)	ED ₈₀ (±SE)
					----- g ai ha ⁻¹ -----	
Saflufenacil	Visible control 7 DAT <i>RSE</i> = 15.3	FargoS	-2.11 (0.32)	91.0 (1.5)	0.8 (0.1)	2.0 (0.5)
		MinotS	-2.29 (0.39)	90.5 (1.5)	0.8 (0.2)	1.9 (0.5)
		MandanR	-0.52 (0.11)	83.4 (11.8)	115.8 (79.3)	50969.0 (94450.0)
		MinotR	-0.64 (0.13)	79.3 (9.6)	131.9 (66.5)	N/A
	Visible control 28 DAT <i>RSE</i> = 22.1	FargoS	-3.53 (7.81)		1.3 (1.9)	2.0 (1.1)
		MinotS	-2.94 (3.93)		1.5 (1.1)	2.3 (0.3)
		MandanR	-0.48 (0.06)		613.9 (137.4)	10719.0 (5407.1)
		MinotR	-0.66 (0.09)		671.9 (117.9)	5423.6 (2018.1)
	Biomass FW 28 DAT <i>RSE</i> = 0.2	FargoS	1.97 (0.42)	0.61 (0.03)	0.7 (0.2)	1.3 (0.4)
		MinotS	2.40 (0.81)	0.61 (0.03)	0.6 (0.2)	1.1 (0.5)
		MandanR	0.93 (0.28)	0.42 (0.02)	105.2 (27.8)	468.9 (211.1)
		MinotR	0.51 (0.06)	0.72 (0.03)	25.4 (7.6)	391.5 (124.5)
	Biomass DW 28 DAT <i>RSE</i> = 0.1	FargoS	2.19 (0.94)	0.18 (0.01)	0.5 (0.2)	0.9 (0.5)
		MinotS	7.74 (9.75)	0.18 (0.01)	0.3 (0.1)	0.4 (0.2)
		MandanR	0.89 (0.24)	0.12 (0.01)	102.1 (28.7)	485.6 (221.9)
		MinotR	0.49 (0.05)	0.22 (0.01)	22.7 (7.1)	387.8 (126.2)
Carfentrazone	Visible control 7 DAT <i>RSE</i> = 17.6	FargoS	-1.19 (0.19)	99.55 (1.92)	1.12 (0.2)	3.7 (0.8)
		MinotS	-1.46 (0.41)	99.12 (1.98)	1.81 (0.2)	4.8 (1.5)
		MandanR	-0.63 (0.20)	84.10 (17.47)	180.0 (144.5)	20094.0 (44566.0)
		MinotR	-1.09 (0.20)	92.59 (7.15)	162.4 (36.1)	889.0 (431.5)
	Visible control 21 DAT	FargoS	-1.50 (0.52)		1.9 (0.3)	4.9 (1.9)

<i>RSE</i> = 23.8	MinotS	-2.27 (0.80)		3.8 (1.1)	7.0 (3.4)
	MandanR	-0.46 (0.09)		1955.6 (788.1)	40404.6 (37105.2)
	MinotR	-1.05 (0.15)		409.2 (57.5)	1532.0 (393.7)
Biomass FW 21 DAT <i>RSE</i> = 0.3	FargoS	0.75 (0.29)	0.66 (0.06)	0.2 (0.1)	1.3 (0.9)
	MinotS	1.12 (0.45)	0.56 (0.06)	0.7 (0.3)	2.4 (1.4)
	MandanR	0.51 (0.12)	0.89 (0.05)	106.1 (45.1)	1582.8 (795.7)
	MinotR	0.80 (0.15)	0.86 (0.04)	70.4 (20.0)	401.2 (130.2)
Biomass DW 21 DAT <i>RSE</i> = 0.1	FargoS	0.73 (0.29)	0.18 (0.02)	0.2 (0.1)	1.6 (1.2)
	MinotS	1.11 (0.46)	0.16 (0.02)	0.7 (0.3)	2.5 (1.4)
	MandanR	0.54 (0.11)	0.25 (0.01)	78.6 (31.6)	1040.3 (458.7)
	MinotR	0.76 (0.13)	0.24 (0.01)	62.9 (17.0)	390.0 (124.8)

^aAbbreviations: *b*, slope of the response curve at inflection point; *d* upper asymptote; DAT, days after treatment; DW, dry weight; FW, fresh weight; *e*, response curve inflection point considered ED₅₀ for visible control or GR₅₀ for biomass; ED₈₀, effective dose of herbicide resulting 80% visible control (ED₈₀) or biomass reduction (GR₈₀); RSE, residual standard error; SE, standard error

Table 6. Herbicide options registered for *Bassia scoparia* control or suppression in western Canada assuming blanket resistance to all active ingredients within Groups 2, 4, 9, and 14^a

Use window and herbicide	MOA	Wheat ^b	Barley	Oat	Corn	Canola	Mustard	Flax	Soybean	Field pea	Lentil
Soil-applied preemergence											
Ethalfluralin ^c	3					C	C ^d		C	C	C
Triallate/Trifluralin ^c	15/3	S	S			S	S	S			
Trifluralin + Metribuzin ^c	3 + 5									C	
Pyroxasulfone	15	S			S				S	S	S
Foliar-applied preplant											
Bromoxynil ^c	6	C	C	C	C	C					
Bromoxynil + Topramezone	6 + 27					C					
Bromoxynil + Pyrasulfotole ^c	6 + 27	C	C								
Pyridate	6				C	C				C	
Foliar-applied postemergence											
Bromoxynil	6	C	C	C	C			C			
Bromoxynil + Tolpyralate	6 + 27	C	C								
Bromoxynil + Pyrasulfotole	6 + 27	C	C								
Glufosinate ^f	10					C					
Pyridate	6				C						
Pyroxasulfone	15				S				S		
Topramezone	27				C ^g						
Tembotrione	27				C						

^aAdapted from Anonymous (2024a, 2024b); C indicates control ($\geq 80\%$ control), S indicates suppression (60-79% control)

^bScientific names: wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; oat, *Avena sativa* L.; corn, *Zea mays* L.; canola, *Brassica napus* L.; mustard, *Brassica juncea* (L.) Czern. or *Sinapis alba* L.; flax, *Linum usitatissimum* L.; soybean, *Glycine max* (L.) Merr.; field pea, *Pisum sativum* L.; lentil, *Lens culinaris* Medik.

^cPreplant incorporated or late fall-applied

^dYellow mustard only

^eMixed with glyphosate

^fGlufosinate-resistant varieties

^gMust be applied with tank mix partner

Table 7. Herbicide options registered for *Bassia scoparia* control or suppression in the United States assuming blanket resistance to all active ingredients within Groups 2, 4, 9, and 14^a

Use window and herbicide	MOA	Wheat ^b	Barley	Oat	Corn	Canola	Mustard	Flax	Soybean	Field pea	Lentil
Soil-applied preemergence											
Atrazine	5				S/C						
Ethalfluralin ^c	3					S/C	S/C	S/C	S/C	S/C	S/C
Isoxaflutole	27				C						
Metribuzin	5								C	C	C
Pendimethalin	3				S				S	S	S
Pyroxasulfone	15	S			S				S	S	S
Trifluralin ^c	3	S	S		S	S	S	S	S	S	S
Foliar-applied preplant											
Glufosinate	10				C	C			C		
Paraquat	22	C	C	C	C	C	C	C	C	C	C
Pyridate	6				C						S
Foliar-applied postemergence											
Bentazon	6				S				S	S	
Bromoxynil	6	C	C	C	C			C			
Bromoxynil + Bicyclopyrone	6 + 27	C	C								
Bromoxynil + Pyrasulfotole	6 + 27	C	C								
Bromoxynil + Tolpyralate	6 + 27	C	C								
Glufosinate ^d	10				C	C			C		

Mesotrione + Atrazine	27 + 5	C	
Pyridate	6	C	S
Tembotrione + Atrazine	27 + 5	C	
Tolpyralate + Atrazine	27 + 5	C	
Topramezone + Atrazine	27 + 5	C	

^aAdapted from Ikley et al. (2024); C indicates control ($\geq 80\%$ control), S indicates suppression (60-79% control)

^bScientific names: wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; oat, *Avena sativa* L.; corn, *Zea mays* L.; canola, *Brassica napus* L.; mustard, *Brassica juncea* (L.) Czern. or *Sinapis alba* L.; flax, *Linum usitatissimum* L.; soybean, *Glycine max* (L) Merr.; dry pea, *Pisum sativum* L.; lentil, *Lens culinaris* Medik.

^cPreplant incorporated or late fall-applied

^dGlufosinate-resistant varieties

Figures

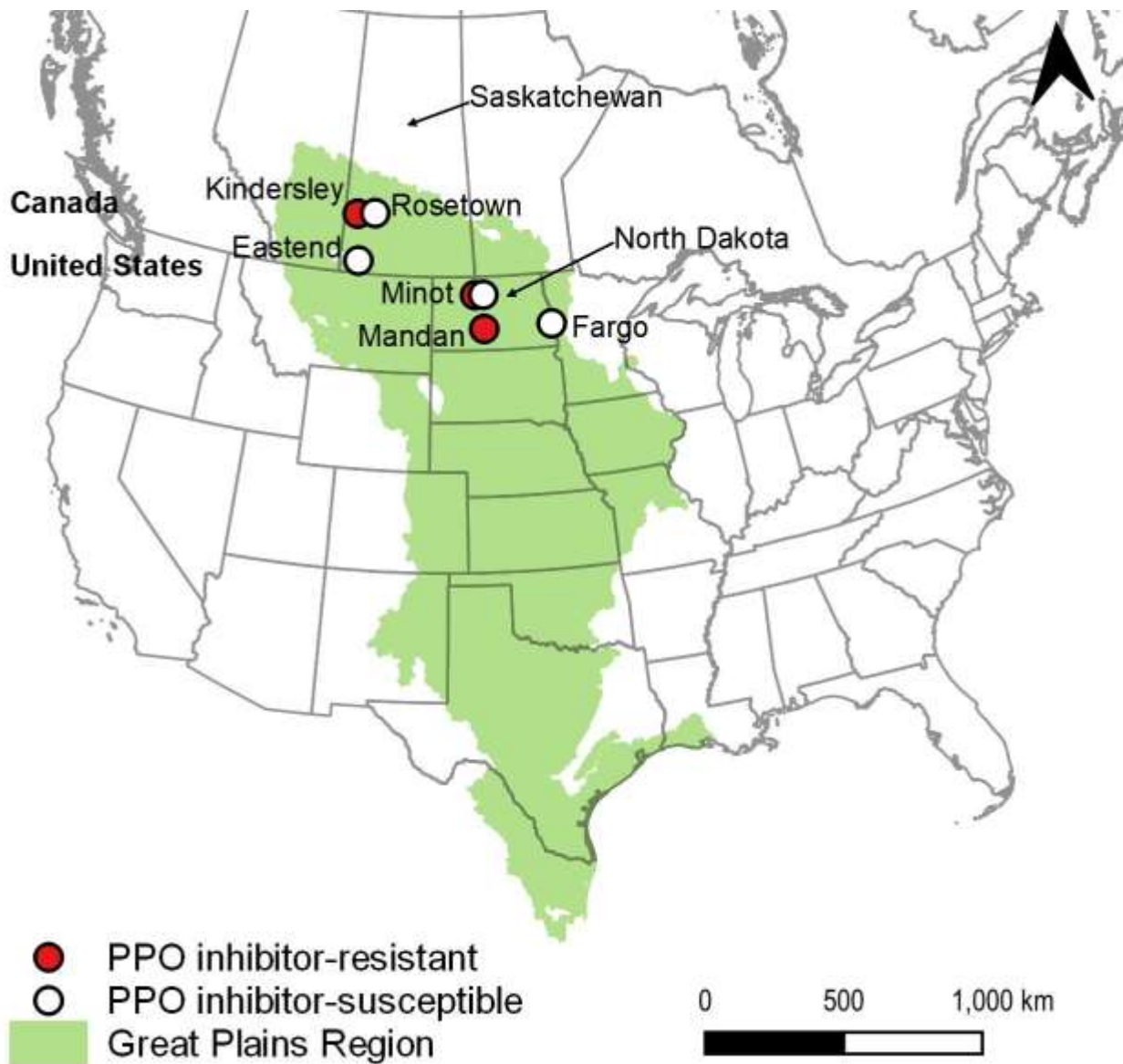


Figure 1. Map of Canada and the United States showing the collection locations of the protoporphyrinogen oxidase (PPO) inhibitor-resistant *Bassia scoparia* accessions and the susceptible control accessions used for the first confirmations of PPO inhibitor-resistant *Bassia scoparia* in 2021 and 2022. Collection locations are adjusted to the nearest city or town.

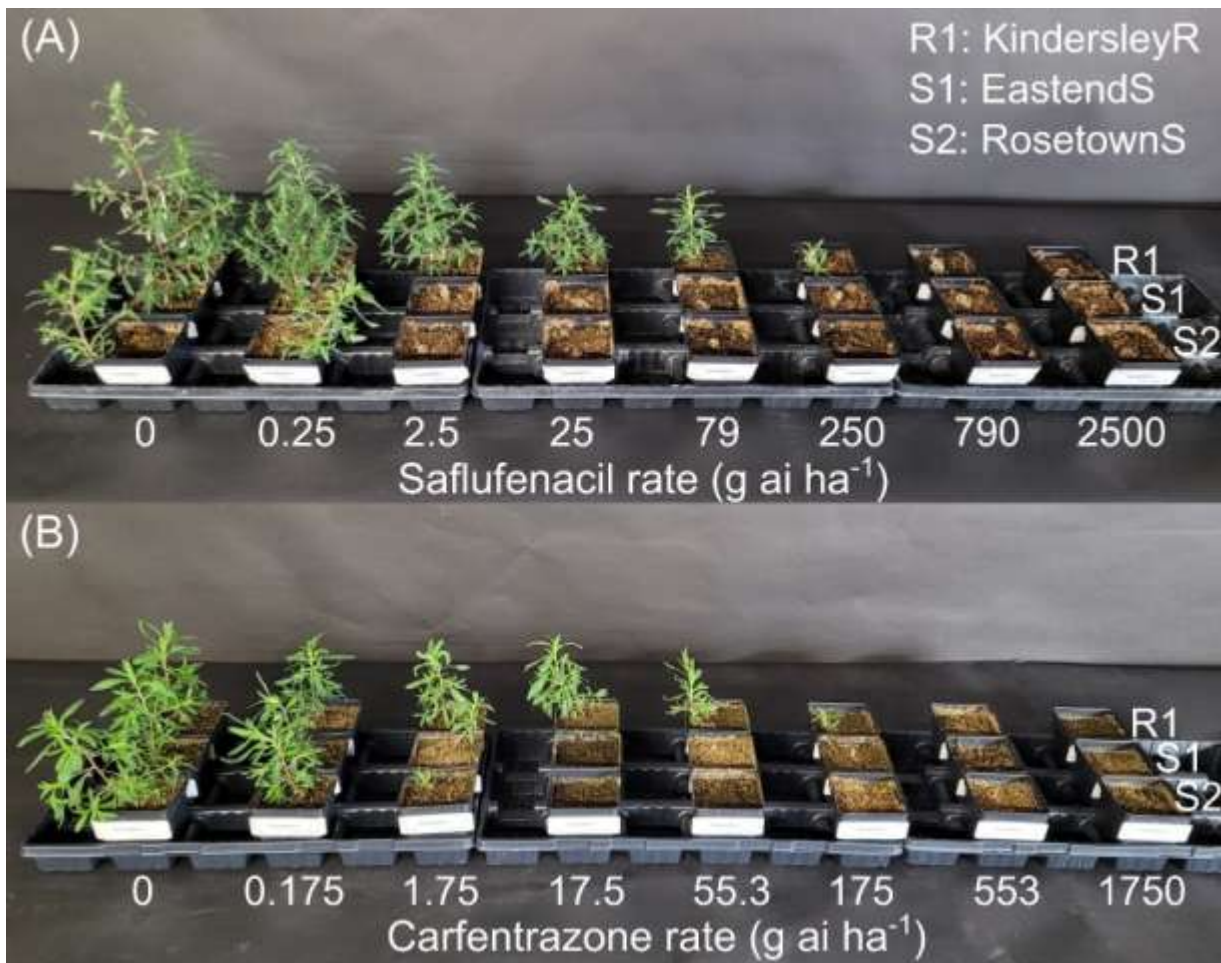


Figure 2. One replicate of the (A) saflufenacil and (B) carfentrazone dose-response experiments 21 days after treatment for one putative-resistant (KindersleyR) and two susceptible (RosetownS and EastendS) *Bassia scoparia* accessions from Saskatchewan

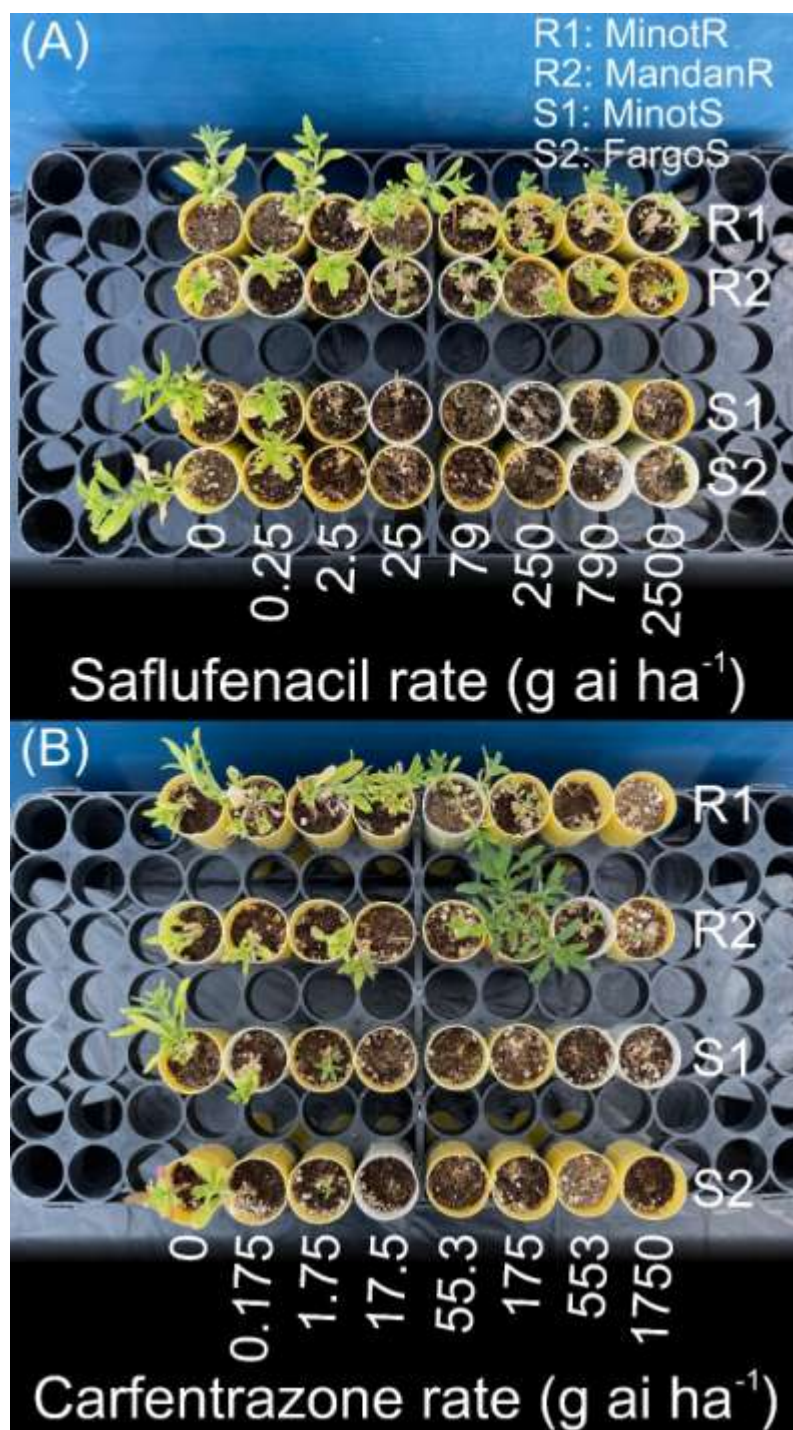


Figure 3. One replicate of the (A) saflufenacil 28 days after treatment (DAT) and (B) carfentrazone 21 DAT dose-response experiments for two putative-resistant (MandanR and MinotR) and two susceptible (MinotS and FargoS) *Bassia scoparia* accessions from North Dakota.

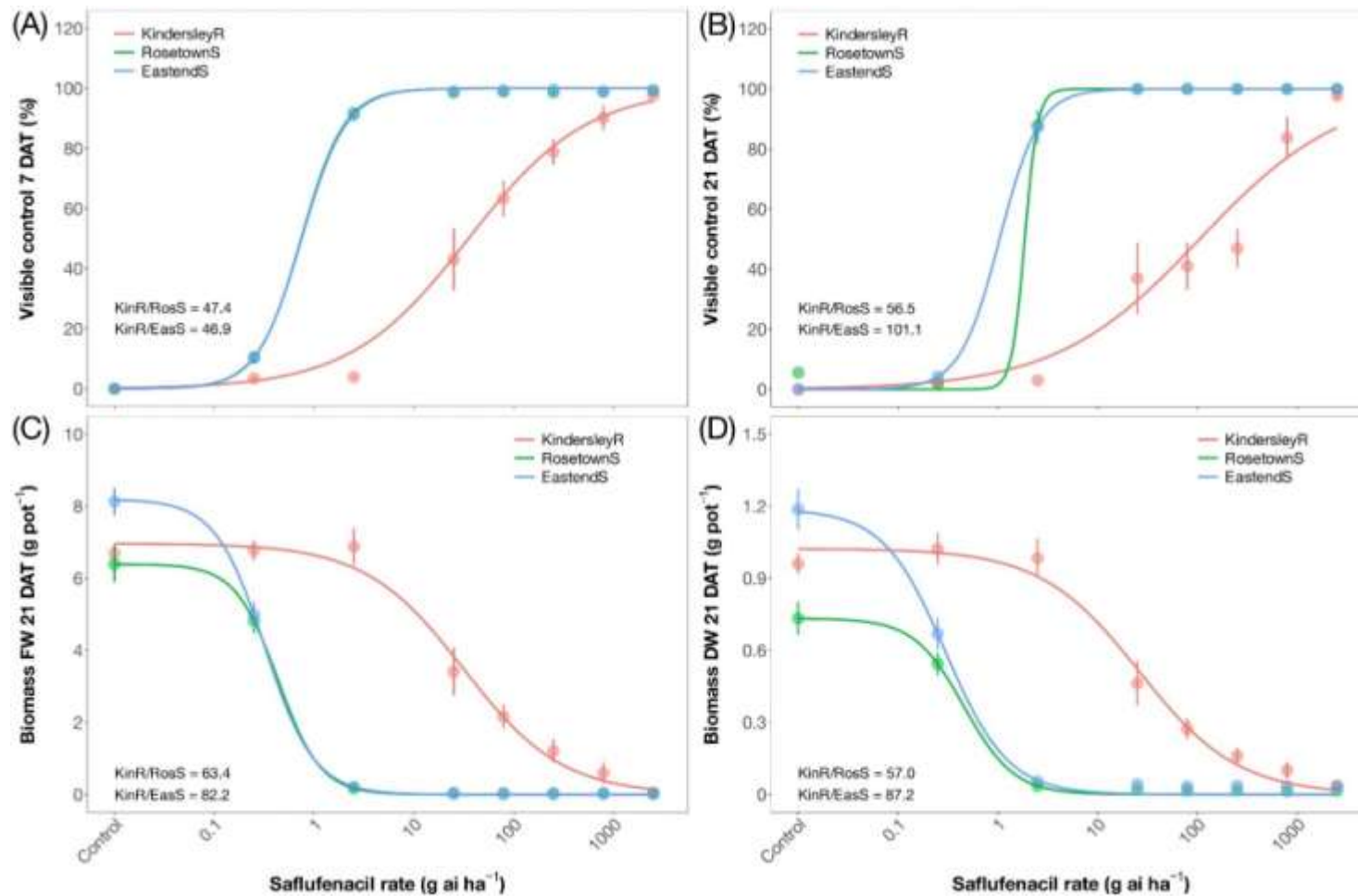


Figure 4. The response of one putative-resistant (KindersleyR) and two susceptible (RosetownS and EastendS) *Bassia scoparia* accessions from Saskatchewan to a range of foliar-applied saflufenacil rates based on visible control at [A] 7 and [B] 21 days after treatment (DAT) and shoot biomass [C] fresh weight (FW) and [D] dry weight (DW) 21 DAT. Dots indicate treatment means; bars represent standard error. Embedded text indicates the resistance index (R/S ratio) for the putative-resistant accession relative to each susceptible accession.

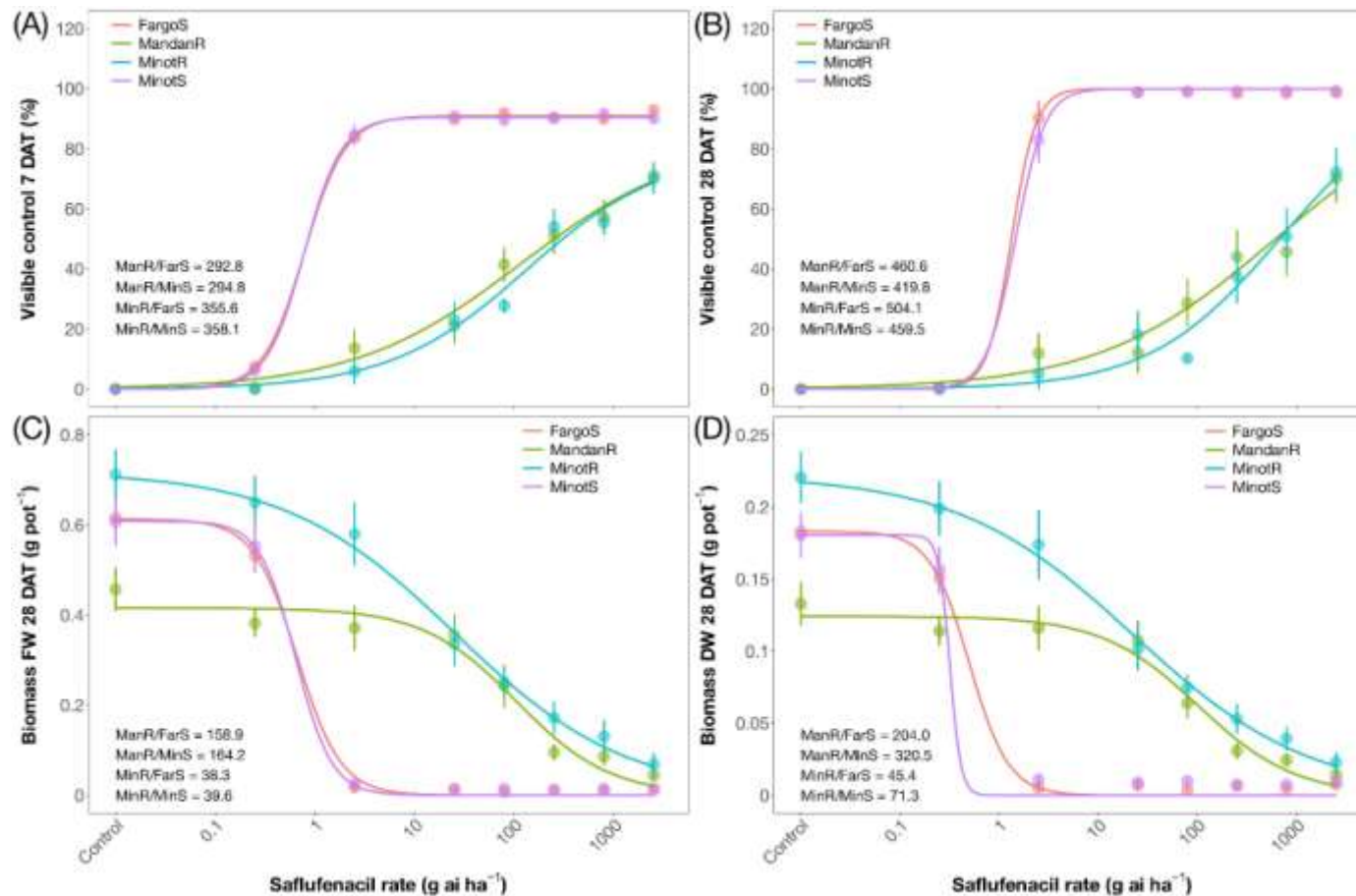


Figure 5. The response of two putative-resistant (MandanR and MinotR) and two susceptible (FargoS and MinotS) *Bassia scoparia* accessions from North Dakota to a range of foliar-applied saflufenacil rates based on visible control at [A] 7 and [B] 28 days after treatment (DAT) and shoot biomass [C] fresh weight (FW) and [D] dry weight (DW) 28 DAT. Dots indicate treatment means; bars represent standard error. Embedded text indicates the resistance index (R/S ratio) for the putative-resistant accession relative to each susceptible accession.

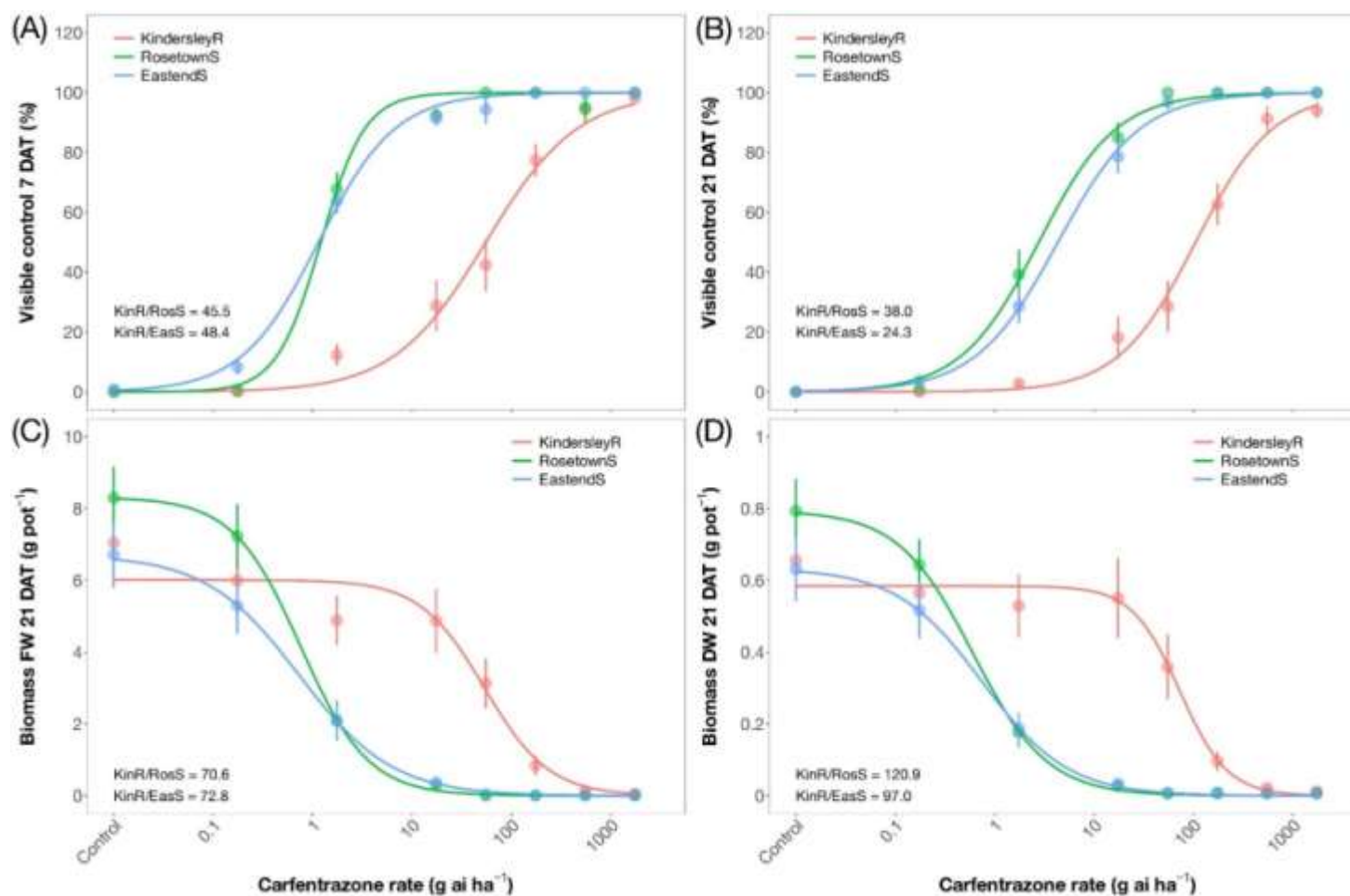


Figure 6. The response of one putative-resistant (KindersleyR) and two susceptible (RosetownS and EastendS) *Bassia scoparia* accessions from Saskatchewan to a range of foliar-applied carfentrazone rates based on visible control at [A] 7 and [B] 21 days after treatment (DAT) and shoot biomass [C] fresh weight (FW) and [D] dry weight (DW) 21 DAT. Dots indicate treatment means; bars represent standard error. Embedded text indicates the resistance index (R/S ratio) for each putative-resistant accession relative to each susceptible accession.

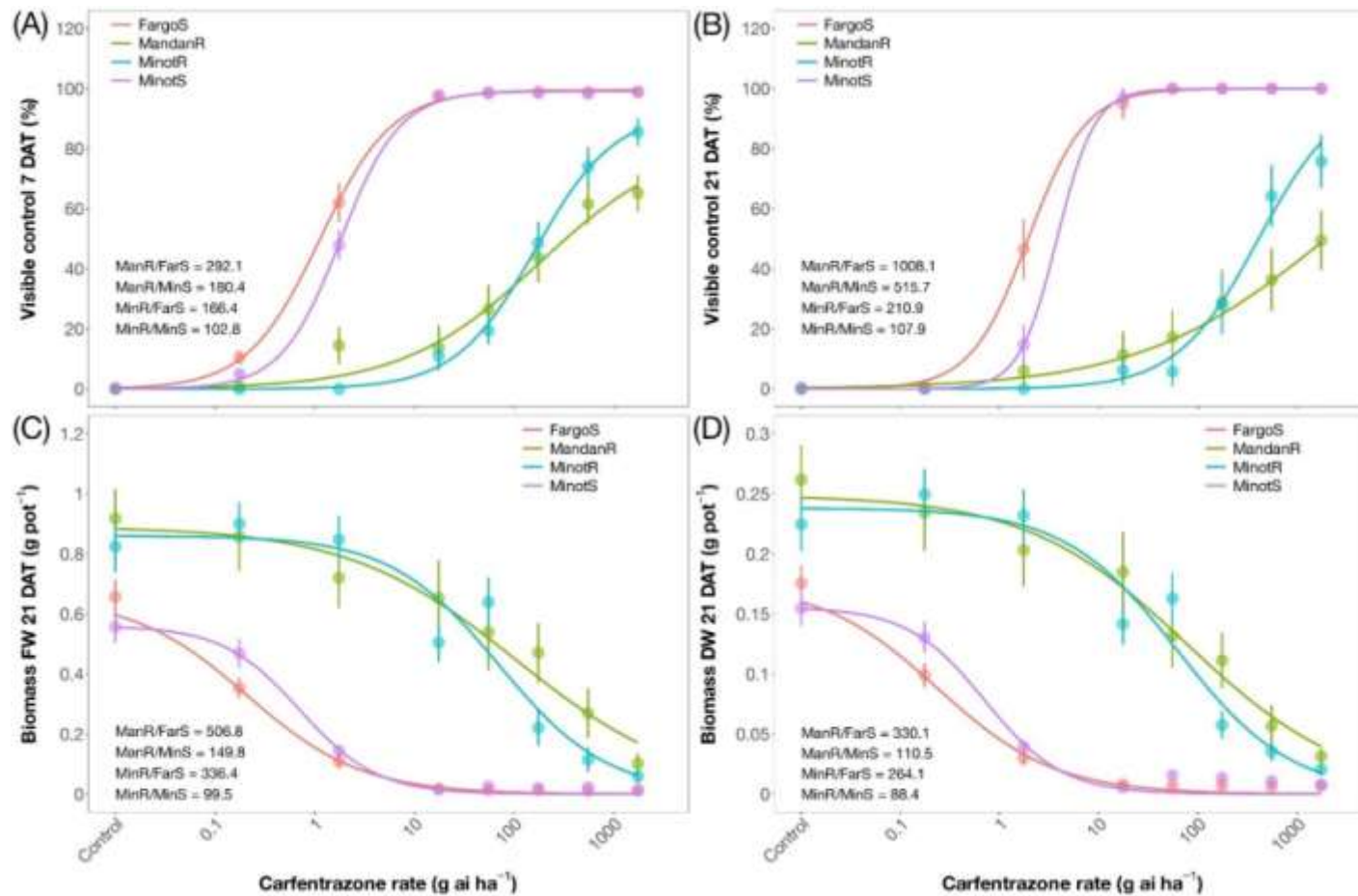


Figure 7. The response of two putative-resistant (MandanR and MinotR) and two susceptible (FargoS and MinotS) *Bassia scoparia* accessions from North Dakota to a range of foliar-applied carfentrazone rates based on visible control at [A] 7 and [B] 21 days after treatment (DAT) and shoot biomass [C] fresh weight (FW) and [D] dry weight (DW) 21 DAT. Dots indicate treatment means; bars represent standard error. Embedded text indicates the resistance index (R/S ratio) for the putative-resistant accession relative to each susceptible accession.