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Confirmation of synthetic auxin herbicide resistance in a green pigweed (*Amaranthus powellii*) population from Ontario, Canada

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Research Article

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Abstract

Following the application of MCPA/MCPB at 1.7 kg ae ha^{-1} at a field site near Dresden, ON, Canada, poor control (<50% visible control) of green pigweed (*Amaranthus powellii* S. Watson) was observed. *Amaranthus powellii* is a common weed in Ontario crop production, and its evolution of resistance to synthetic auxin herbicides (SAHs) could pose a risk to crop yields. The suspected resistant *A. powellii* population (R) was used in dose–response and field experiments to determine resistance to SAHs. The objective of these studies was to determine whether this population of *A. powellii* is resistant to MCPA and cross-resistant to other SAHs. The GR_{50} (herbicide dose that causes a 50% reduction in plant aboveground biomass) values were determined by fitting plant dry weight data, obtained following application with seven SAHs, to a four-parameter log-logistic equation and were compared between the suspected-resistant (R) population and a known susceptible (S) population of *A. powellii*. The field trial was conducted in 2017, 2018, 2019, and 2021 in corn (*Zea mays* L.) and consisted of 11 postemergence SAH treatments. The GR_{50} values differed between the R and S populations following application with MCPA, aminocyclopyrachlor, dichlorprop-p, and mecoprop, resulting in resistance factors of 4.4, 3.0, 2.5, and 2.4, respectively. In the field study, dicamba and MCPA ester controlled *A. powellii* 84% and 30%, respectively, at 8 wk after treatment application (WAA). The control of *Amaranthus powellii* with all SAHs applied POST in corn was poor (<90% visible control) at 8 WAA. Both studies confirmed resistance to SAHs in this population of *A. powellii*, which will create limitations for farmers aiming to control this weed.

Introduction

Synthetic auxin herbicides (SAHs) such as 2,4-D and MCPA have been on the market for more than seven decades since their introduction after World War II (Oerke 2006; Sterling and Hall 1997). These herbicides are primarily used for the control of dicot weeds in monocot crops; they act by mimicking endogenous auxins, phytohormones that are signaling molecules for vital plant processes (Busi et al. 2018; Sauer et al. 2013; Sterling and Hall 1997).

SAHs are primarily applied postemergence, although some have preemergence activity, are systemic, and are translocated primarily in the phloem, which makes them efficacious against many dicot weeds, including perennials. In addition, their relatively low cost makes this class of herbicides an excellent weed control option for farmers, and they have been widely adopted (Grossmann 2009; Jugulam et al. 2011). As a result, 366 million ha globally were treated with SAHs in 2014 (Busi et al. 2018). Because of their favorable characteristics, these herbicides have been and continue to be widely used, which has increased selection pressure for resistance.

An increasing number of synthetic auxin–resistant (SAH-R) weed biotypes have evolved in recent years. There are more than 40 weed species globally with confirmed resistance to SAHs (Heap 2024). Relative to time on the market, resistance to SAHs has evolved more slowly than to other herbicide modes of action (Jugulam et al. 2011). This has been attributed to the lack of soil residual activity of SAHs, the potential for functional redundancy between protein receptors at the target site, and the potential for fitness penalties to develop in resistant individuals, preventing resistance traits from being passed on to subsequent generations (Gressel 2009; Walsh et al. 2006).

Green pigweed (*Amaranthus powellii* S. Watson) is a small-seeded annual monoecious weed species that is highly competitive and is widespread in eastern North America (Uva et al. 1997; Weaver and McWilliams 1980). Coupled with small seed size and high seed viability, monoecious *Amaranthus* species have high fecundity and can produce up to 250,000 seeds per plant (Sellers et al. 2003). The competitiveness of *A. powellii* has contributed to significant crop

Table 1. Herbicide active ingredients, trade names, and manufacturers for treatments in dose–response and field trial studies[a]

Herbicide name	Trade name	Manufacturer
MCPA (amine formulation)	MCPA Amine 600	Nufarm Agriculture Inc., 5101, 333 96th Avenue NE, Calgary, AB T3K 0S3, Canada, https://nufarm.com/ca
MCPA (ester formulation)	MCPA Ester 600	
2,4-D	2,4-D Ester 700	
Mecoprop	Compitox	
Dichlorprop-p/2,4-D	Estaprop® XT	
2,4-DB	Embutox®	
Fluroxypyr	Fluroxypyr	
Dichlorprop-p	Duplosan™	Nufarm Americas Inc., 11901 S. Austin Avenue, Alsip, IL 60803, USA, https://nufarm.com/us
Halauxifen-methyl[b]	Elevore®	Corteva Agriscience Canada Company, 215 2nd Street SW, Suite 2450, Calgary, AB T2P 1M4, Canada, https://www.corteva.ca
Clopyralid	Lontrel™ XC	
Fluroxypyr/halauxifen-methyl	Pixxaro®	
Aminocyclopyrachlor[b]	Method®	Bayer Crop Science Inc., 160 Quarry Park Boulevard, Calgary, AB T2C 3G3, Canada, https://www.cropscience.bayer.ca/en
Dicamba	Engenia®	BASF Canada Inc., 28 Quarry Park Boulevard SE, Calgary, AB T2C 4P5, Canada, https://www.basf.com/ca/en.html
Dicamba	Banvel® II	
Dicamba/diflufenzopyr[c]	Distinct®	

[a] Herbicide rates for field trials are listed in Table 4. All treatments in dose–response and field study were applied postemergence.

[b] Halauxifen-methyl applied in the field trial and aminocyclopyrachlor in the dose–response experiment were tank mixed with 1.00 % v/v of the adjuvant MSO Concentrate (Loveland Products Inc., 3005 Rocky Mountain Avenue, Loveland, CO 80538, USA).

[c] Dicamba/diflufenzopyr was tank mixed with 0.25% v/v of the adjuvant Agral® 90 (Syngenta Canada Inc., 140 Research Lane, Guelph, ON N1G 4Z3, Canada). Dicamba/diflufenzopyr was tank mixed with 1.25% v/v of urea ammonium nitrate (UAN-28-0-0).

yield reductions, and *A. powellii* has been found to reduce overall crop quality (Aicklen et al. 2022a, 2022b; Costea et al. 2004). While the negative impact of *A. powellii* can be alleviated with the application of many broadleaf herbicides, including SAHs, the evolution of herbicide resistance further impacts producers' ability to manage this species.

A farmer near Dresden, ON, Canada reported poor control of *A. powellii* with a mixture of MCPA/MCPB in a field of processing peas (*Pisum sativum* L.) Field observations identified a high density of surviving *A. powellii* plants to the exclusion of other weeds, indicating possible resistance to MCPA and other SAHs in this population. These observations prompted further research to confirm suspected synthetic auxin resistance in this *A. powellii* population. The objective of this research was to confirm MCPA resistance and to determine cross-resistance to other SAHs in this *A. powellii* population through dose–response experiments and a field trial study.

Materials and Methods

Confirmation of Resistance

Preparation of Plant Material

Samples from the suspected SAH-R *A. powellii* population (accession AMAPO 501; hereinafter referred to as R) from Dresden, ON, Canada (42.582811°N, 82.113953°W) were collected following survival of a field application of MCPA ester at 600 g ae ha^{-1}. The field site had minimal historical exposure to SAHs, with these herbicides being used once or twice over a 6-yr crop rotation. Seed heads from the suspected resistant plants were collected from the field, dried at room temperature, and threshed. To optimize germination, cleaned seed was scarified by soaking in 97% H_2SO_4 for 30 s, followed by neutralization using a 0.1 M solution of sodium bicarbonate, and a rinse with water. The seeds were then air-dried and stored at 5 C until required (Ferguson et al. 2001).

Seeds were collected directly from surviving plants following application of MCPA in the field, which would negate the presence of susceptible individuals in the resulting population. To select an appropriate susceptible population, several populations of *A. powellii* were screened for susceptibility to MCPA. A known synthetic auxin–susceptible population (AMAPO 511; hereinafter referred to as S) from the Elora Research Station (43.645472°N, 80.400444°W) was selected to be used in the dose–response study. Although other populations demonstrated susceptibility to MCPA in preliminary tests, there was limited seed supply or their germinability was low, and they were therefore not included in the dose–response study.

Seeds of R and S were germinated in petri dishes containing a 0.6% standard agar–water solution and placed in sealed plastic bags. To promote germination, the seeds were then placed in a growth chamber for 22 h at 40 C in the dark, followed by 2 h at 15 C in the light (adapted from Ferguson et al. 2001). Following heat treatment, seed was removed from the growth chamber and placed in a growth room to promote further germination. Conditions in the growth room were programmed for a 16-h photophase at 25 C and an 8-h scotophase at 20 C. The main light source in the growth room was from LED bulbs and tubes with a photosynthetic photon flux density of 450 $\mu mol\ m^{-2}\ s^{-1}$. Once seedlings had reached the cotyledon stage, two plants per pot were transplanted into 14-cm-diameter pots containing an artificial potting mix (PRO-MIX PGX, Premier Tech Home and Garden, 1 Avenue Premier, Rivière-du-Loup, QC G5R 6C1, Canada) for use in the dose–response study. The plants were watered as required with deionized water and fertilized at a 1:100 ratio using 20:20:20 (N: P_2O_5: K_2O) fertilizer (Plant Prod 20-20-20 Classic, Plant Products, 50 Hazelton Street, Leamington, ON N8H 3W1, Canada).

Dose–Response Study

Amaranthus powellii plants at the 4- to 6-leaf stage (between 5 and 8 cm in height) were treated with various rates of MCPA amine, 2,4-D ester, dicamba, halauxifen-methyl, mecoprop, dichlorprop-p, or aminocyclopyrachlor with the appropriate adjuvants (Table 1). The most uniform plants were selected to ensure homogeneity across

Table 2. Trial year, soil characteristics, corn planting, emergence, and harvest dates, and treatment application dates for trial site near Dresden, ON, Canada, in 2017, 2018, 2019, and 2021

	Soil characteristics[a]						Agronomic information			
Year	Texture	Sand	Silt	Clay	OM[b]	pH	Planting date	Emergence date	Harvest date	Application date
		%								
2017	N/A[c]	N/A	N/A	N/A	N/A	N/A	June 12	June 18	N/A[d]	2017A: July 6 2017B: July 14
2018	N/A	N/A	N/A	N/A	N/A	N/A	May 10	May 21	N/A	2018A: June 6 2018B: July 3 2018C: June 28
2019	Sandy loam	62	23	15	2.9	7.0	June 8	June 15	November 23	July 7
2021	Loam	41	33	26	3.1	6.8	May 13	May 21	November 11	June 3

[a] Soil data were provided by A&L Canada Laboratories Inc. and was recorded for samples taken at a 15-cm depth below the soil surface (2136 Jetstream Road, London, ON N5V 3P5, Canada).
[b] OM, organic matter.
[c] No soil characteristic data were collected in 2017 or 2018.
[d] No harvest data were collected in 2017 or 2018.

experimental units. The experiment was set up according to a randomized complete block design (RCBD) with four blocks. Fourteen doses of MCPA and 12 doses of each other herbicide were applied to both *A. powellii* populations, including an untreated control. Each experiment was repeated twice for each herbicide. The plants were sprayed using an indoor track sprayer pressurized to 276 kPa delivering 210 L ha^{-1} at a speed of 4 km h^{-1} through an even fan spray tip (TeeJet® TP8002E-SS, Spraying Systems, 200 West North Avenue, Glendale Heights, IL 60139, USA) at a height of 46 cm above target. Following spraying, the plants were returned to the growth room, where they were maintained as described earlier. Fourteen days after treatment, all aboveground plant material was harvested by cutting off the aboveground portion of the plant and placing it in paper envelopes. The plants were then oven-dried at 70 C for a minimum of 72 h before aboveground biomass was recorded.

Table 3. Average corn height and growth stage and *Amaranthus powellii* height, number of leaves, and density at time of treatment application for seven trials conducted near Dresden, ON, Canada, in 2017, 2018, 2019, and 2021

	Corn		*Amaranthus powellii*[a]		
Year	Size	Development stage[b]	Height	Stage	Density
	cm		cm	no. of leaves	plants m^{-2}
2017A	30	V5	10	8	1,272
2017B	48	V5	28	10	961
2018A	39	V4	10	14	28
2018B	62	V6	24	12	193
2018C	38	V4	21	10	471
2019	38	V5	8	14	202
2021	22	V3	5	6	14

[a] Average height, staging, and density was recorded for two 0.25-m^{2} quadrats in the nontreated control plots.
[b] Development stage determined using McWilliams et al. (1999) corn staging guide.

Field Evaluation of the Efficacy of SAHs

Field experiments were conducted in 2017, 2018, 2019, and 2021 at a site near Dresden, ON, Canada (42.582811°N, 82.113953°W), the same site where the seed for the dose–response experiments was collected. Two experiments were completed in 2017, three in 2018, and one in 2019 and 2021 for a total of 7 site-years. Table 2 contains information pertaining to soil characteristics; corn (*Zea mays* L.) planting, emergence, and harvest dates; and herbicide application dates. Furthermore, data on average corn height and growth stage and *A. powellii* height, leaf number, and density are presented in Table 3. The experiments were arranged as an RCBD with four blocks. The study consisted of 11 SAH treatments, including a weedy control and a weed-free control. Information on herbicide active ingredients, trade names, and manufacturers are displayed in Table 1. A complete herbicide treatment list and rates are presented in Table 4.

Before planting, the trial plots were conventionally tilled. Each plot was 3-m wide (4 corn rows spaced 75 cm apart) and 10-m long; the center 2 m were sprayed. Corn was planted between mid-May to early June using the 'BSS8040' sweet corn hybrid (Green Giant Canada, B&G Foods, 5935 Airport Road, Mississauga, ON L4V 1W5, Canada) in 2017 and 2018 at a rate of 40,000 plants ha^{-1}. In 2019, 'DKC45-65' (Bayer Crop Science, 160 Quarry Park Boulevard, Calgary, AB T2C 3G3, Canada) corn was planted, and in 2021, 'B79N56PWE' (Corteva Agriscience Canada, 215 2nd Street SW, Suite 2450, Calgary, AB T2P 1M4, Canada) corn hybrid was planted at a rate of 83,000 seeds ha^{-1}. The corn was planted to a depth of approximately 4 cm. Weed-free plots were maintained with *S*-metolachlor/atrazine/mesotrione/bicyclopyrone (Acuron®, 2,022 g ai ha^{-1}, Syngenta Canada, 140 Research Lane, Guelph, ON N1G 4Z3, Canada) applied preemergence followed by glyphosate applied postemergence (Roundup WeatherMax®, 900 g ae ha^{-1}, Bayer Crop Science Inc., 160 Quarry Park Boulevard, Calgary, AB T2C 3G3, Canada) in 2019 and 2021. In 2019 and 2021, the trial site was fertilized with 448 kg ha^{-1} of urea, and grass control was provided by a cover spray of quizalofop-p-ethyl (AMVAC Assure® II, plus Sure-Mix™, 0.5% v/v, Belchim Crop Protection Canada, 104 Copper Drive, Unit 3, Guelph, ON N1C 0A4, Canada) at 36 g ai ha^{-1}.

A CO$_2$-pressurized backpack sprayer equipped with a handheld boom at an operating pressure of 207 kPa and water volume of 200 L ha^{-1} was used to apply the herbicide treatments. The boom was fitted with four ULD-120-02 (Pentair Canada, 490 Pinebush Road, Cambridge, ON N1T 0A5, Canada) nozzles spaced 50 cm apart, producing a spray width of 2 m. Treatments were applied when the *A. powellii* was approximately 10 cm in height (Table 3).

Visible *A. powellii* control was assessed at 1, 2, 4, and 8 wk after treatment application (WAA). Visible *A. powellii* control was estimated as aboveground biomass reduction in comparison to the weedy control on a 0% to 100% scale, with 0% indicating no visible

Table 4. Visible weed control ratings (1 WAA, 2 WAA, 4 WAA, and 8 WAA), density, and aboveground biomass (8 WAA) for *Amaranthus powellii* as impacted by postemergence treatments with synthetic auxin herbicides from field trials conducted in 2017, 2018, 2019, and 2021 near Dresden, ON, Canada[a]

		Visible *Amaranthus powellii* control											
Treatment[b]	Rate	1 WAA		2 WAA		4 WAA		8 WAA		Density		Biomass	
	g ae ha^{-1}	%								plants m^{-2}		g m^{-2}	
Weedy control	—	0		0		0		0		111	a	552	a
Weed-free control	—	100		100		100		100		0		0	
Clopyralid	200	3	c	1	f	1	f	1	f	99	a	385	b
Fluroxypyr	108	12	bc	11	ef	7	ef	7	f	90	a	303	bc
Halauxifen-methyl	5	18	b	22	de	20	de	16	ef	82	ab	302	bc
MCPA (ester formulation)	850	23	b	27	cd	24	c-e	30	de	69	ab	199	cd
Fluroxypyr/halauxifen-methyl + MCPA	77/5 + 372	26	b	33	cd	29	cd	36	cd	65	ab	194	c-e
Fluroxypyr + MCPA	108 + 600	27	b	40	bc	40	c	46	cd	49	a-c	125	d-f
2,4-DB	1,500	25	b	36	cd	42	bc	51	bc	50	a-c	113	d-f
Dichlorprop-p/2,4-D	346/671	44	a	55	ab	59	ab	67	ab	35	bc	72	d-f
Dicamba/diflufenzopyr	142/58	54	a	65	a	70	a	74	a	25	c	69	d-f
2,4-D	850	43	a	60	a	63	a	75	a	26	c	46	ef
Dicamba	600	53	a	69	a	76	a	84	a	19	c	28	f

[a] Same letter following treatment means within each column are not statistically different based on Tukey's honestly significant difference (HSD) of $P < 0.05$. WAA, weeks after application.

[b] Treatments that are preformulated mixtures are distinguished using a slash (/), whereas separate products in a tank mix are distinguished using a plus sign (+).

reduction in biomass and 100% indicating complete reduction in biomass. At 8 WAA, *A. powellii* density and aboveground biomass were measured and recorded using a square quadrat measuring 0.25 m^2. To measure *A. powellii* density, the number of plants in the quadrat was recorded and repeated in two separate areas of each plot. After weed density was recorded, the aboveground portion of the plants in each quadrat was cut at the soil surface, placed in brown paper bags, and placed in a kiln at 45 C for approximately 14 d. After this period, the aboveground biomass was recorded.

Statistical Analysis

Dose–Response Study

The statistical analysis for the dose–response study was conducted using SAS (v. 9.4, SAS Institute, 100 SAS Campus Drive, Cary, NC 27513, USA). When the data were subjected to an ANOVA, no significant differences between experiments was observed based on $P = 0.05$, which allowed for the data to be pooled. A nonlinear regression analysis using PROC NLMIXED was conducted to generate dose–response curves for each herbicide. The herbicide dose that reduced aboveground biomass by 50% (GR_{50}) was determined using a log-logistic equation by Seefeldt et al. (1995), which associates plant biomass y to herbicide dose x:

$$y = C + D - C/1 + (x/GR_{50})^b \qquad [1]$$

where D refers to the upper limit, C refers to the lower limit, b refers to the slope of the curve at the inflection point, and GR_{50} refers to the dose of the herbicide causing a 50% reduction in aboveground biomass. The GR_{50} for the R population was divided by the GR_{50} for the S population to calculate the resistance factor (RF). Average aboveground biomass as a percentage of the untreated control for each population was used to construct the dose–response curve. A significance level of $P = 0.05$ was used to determine differences between the GR_{50} values for the two populations for each herbicide dose response.

Field Evaluation of the Efficacy of SAHs

The statistical analysis for the field study was conducted in SAS v. 9.4 (SAS Institute) using PROC GLIMMIX. The fixed effect for this study was herbicide, and the random effects were environment (year) and block. Statistical analysis revealed no significant interactions between treatment and environment ($P = 0.05$), which allowed the data from all site-years to be pooled. The five assumptions of normality were met by assessing the residuals against predicted, treatment, year, and replicate. PROC UNIVARIATE was used to generate the Shapiro-Wilk test statistic to ensure the data fit a normal distribution. The data were fit to a normal distribution for all variables, except weed density, which was fit to a lognormal distribution and back-transformed for presentation. Treatments were separated at a significance level of $P = 0.05$ using Tukey's honestly significant difference (HSD).

Results and Discussion

Dose–Response Study

The dose–response experiment revealed that more MCPA was necessary to reduce biomass of the putative R population compared with the S population (Figure 1A), which suggests resistance to MCPA has evolved in this biotype. Calculated GR_{50} values revealed a 4.4-fold RF to MCPA in R compared with S (Table 5).

The results from the dose–response study found varying levels of cross-resistance to three of the six remaining SAHs. Cross-resistance to the structurally similar herbicides mecoprop and dichlorprop-p was confirmed with RFs of 2.4 and 2.5 (Figure 1B and 1D; Table 5). Additionally, there was 3.0-fold cross-resistance to aminocyclopyrachlor, an active ingredient that is structurally unrelated to the phenoxy carboxylate MCPA (Figure 2A; Table 5). Finally, the R population exhibited no cross-resistance to 2,4-D, dicamba, and halauxifen-methyl (Figures 1C, 2B and 2C; Table 5) as the dose–response curves and the calculated GR_{50} values did not differ from those of the S population.

Cross-resistance to mecoprop can be expected, as this molecule has a structure very similar to that of MCPA; they share the same phenoxy ring, with the difference being an acetic acid and a propionic acid side chain for MCPA and mecoprop, respectively (Loos 1975). Based on this reasoning, the lack of cross-resistance to 2,4-D is unexpected, as it is structurally very similar to MCPA, differing only in the phenoxy ring; 2,4-D has a chlorine substituent

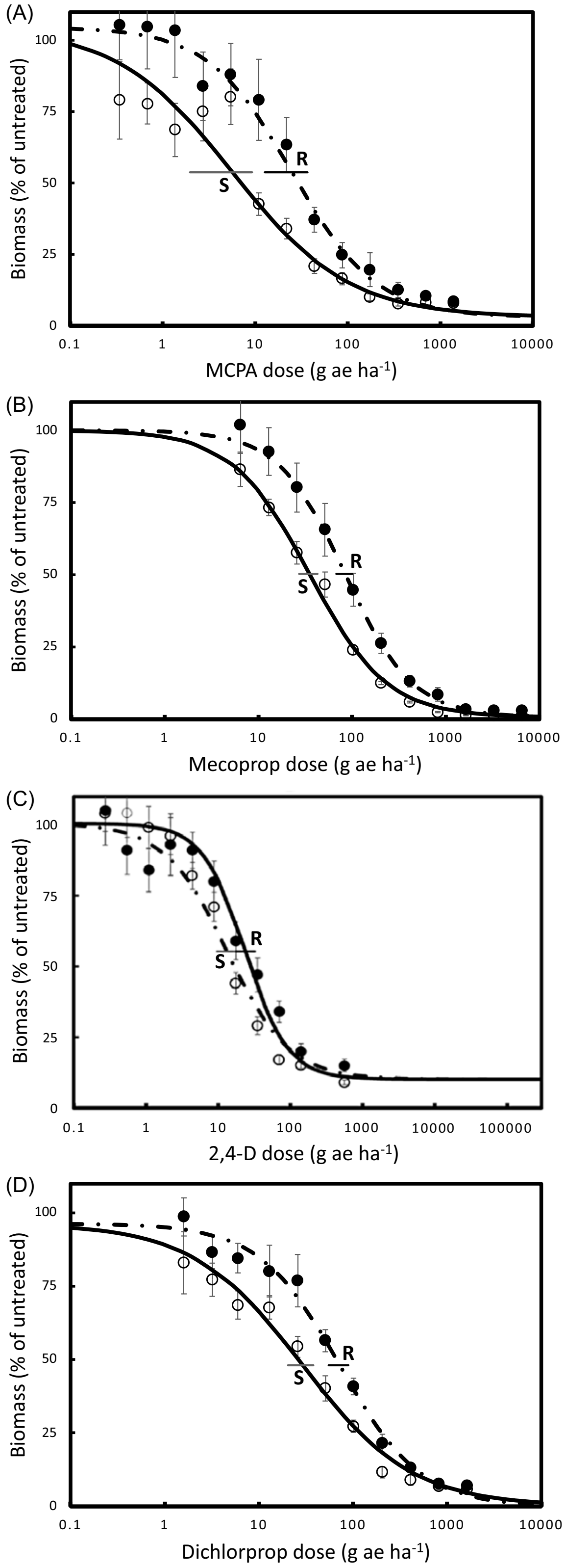

Figure 1. Nonlinear dose–response curves for *Amaranthus powellii* R and S populations following treatment with phenoxy carboxylic acids, MCPA (A), mecoprop (B), 2,4-D (C), and dichlorprop-p (D) as determined using a four-parameter log-logistic equation: $y = C + D - C/1 + (x/\mathrm{GR}_{50})^b$. Each point represents the average percent reduction in aboveground biomass relative to the untreated control across two experimental runs with four replicates per treatment. Error bars represent the standard error. The GR_{50} indicates the herbicide dose causing a 50% reduction in aboveground biomass as represented by 95% confidence intervals.

at position 2, while it is a methyl group for MCPA. In addition, dichlorprop-p is the propionic acid equivalent to 2,4-D, and it could have been expected that the R population would have responded similarly to these two molecules; however, the R population was resistant to dichlorprop-p. Aminocyclopyrachlor is a relatively new SAH and is used mostly for vegetation management in non-crop areas. It is from the pyrimidine-carboxylate class of SAHs, of which it is the only active ingredient, and was introduced in the late 2000s. No cases of resistance to this herbicide have been reported, although multiple species are resistant to a range of SAHs.

There are at least 43 other species with reported resistance to SAHs in the International Survey of Herbicide-Resistant Weeds Database (Heap 2024), and they represent 86 cases. In 25 of those species, resistance to 2,4-D has been reported, while resistance to MCPA is found in 12 species. There are eight reported cases with 2,4-D and MCPA resistance occurring jointly in one population. Unfortunately, it is not possible to determine whether the lack of a mention of resistance to a herbicide indicates that the population is susceptible to it, or whether the herbicide molecule was not tested. These results, however, probably reflect the wider use of 2,4-D over MCPA (Busi et al. 2018).

Resistance to MCPA has been confirmed in 12 other weed species globally (Heap 2024), although the level of resistance has only been fully characterized in a few of those cases. In most cases, resistance to MCPA is low level and similar to the level of MCPA resistance in *A. powellii* in the current study. Resistance to MCPA in a brittlestem hempnettle (*Galeopsis tetrahit* L.) population from Alberta, Canada, was confirmed through dose–response experiments with a RF of 3.3 (Weinberg et al. 2006), similar to the RF determined for MCPA in the present study. Resistance to MCPA varied among populations of tall buttercup (*Ranunculus acris* L.) in New Zealand, with RFs ranging between 2.2- and 4.2-fold compared with the most susceptible population based on a survival dose–response curve analysis (Bourdôt et al. 1990). Based on LD_{50} analysis, a clopyralid-selected SAH-R population of field burrweed (*Soliva sessilis* Ruiz & Pav.) had 2.0- to 2.9-fold resistance to MCPA and up to 13-fold resistance to dicamba; however, this population was susceptible to mecoprop (Ghanizadeh et al. 2021). Genetic variation among populations of scentless false mayweed [*Tripleurospermum inodorum* (L.) Sch. Bip.] from roadsides in England resulted in up to 2.5-fold resistance between the most resistant and the most susceptible based on biomass reduction, and 2.1-fold based on mortality measurements (Ellis and Kay 1975). A Palmer amaranth (*Amaranthus palmeri* S. Watson) population from a long-term conservation tillage experimental field in Kansas that had evolved a high level of resistance to 2,4-D (11-fold) (Shyam et al. 2021, 2022) also had low-level cross-resistance to MCPA (2.8- to 3.0-fold) (Singh et al. 2023).

Populations in other species have evolved higher levels of resistance to MCPA. For example, wild radish (*Raphanus raphanistrum* L.) from Western Australia has 10-fold resistance to MCPA following multiple exposures to SAHs during a 17-yr period in a wheat (*Triticum aestivum* L.)–lupin (*Lupinus angustifolius* L.) rotation (Jugulam et al. 2013). In a wild mustard (*Sinapis arvensis* L.) population resistant to picloram, dicamba, and MCPA, the resistance level to MCPA based on a seedling growth inhibition test was 10-fold; that population exhibited no resistance to mecoprop (Webb and Hall 1995). A population of Oriental mustard (*Sisymbrium orientale* L.) from a wheat field in South Australia was 20-fold more resistant to MCPA than a known susceptible population based on survival and had a similar level of

Table 5. Parameters and resistance factors for whole-plant dose response for *Amaranthus powellii* populations R and S following postemergence applications of MCPA, 2,4-D, halauxifen-methyl, dicamba, dichlorprop-p, mecoprop, and aminocyclopyrachlor as determined using a four-parameter log-logistic equation[a]

Herbicide	Population	*D*	*C*	*b*	GR_{50}	RF[b]
		——————% db[c]	——————	% db/g ae ha^{-1}	g ae ha^{-1}	
MCPA	R	104.7 (90.6–118.9)	3.0 (−7.0–13.0)	1.0 (0.5–1.5)	24.8 (12.8–36.9)	4.4*
	S	104.7 (90.6–118.9)	3.0 (−7.0–13.0)	0.7(0.4–1.0)	5.6 (2.0–9.3)	
Mecoprop	R	100.0 (0.0)	0.9 (0.9–4.3)	1.3 (1.0–1.5)	87.1 (72.0–102.2)	2.4*
	S	100.0 (0.0)	0.9 (0.9–4.3)	1.1 (0.9–1.3)	36.2 (29.5–43.0)	
2,4-D	R	96.1 (90.6–101.6)	9.7 (1.3–18.0)	1.0 (0.6–1.5)	17.9 (10.9–25.0)	1.4
	S	96.1 (90.6–101.6)	9.7 (1.3–18.0)	1.5 (0.8–2.2)	12.6 (8.8–16.4)	
Dichlorprop-p	R	96.2 (91.2–101.2)	0.0 (0.0)	1.0 (0.8–1.2)	72.5 (55.2–89.8)	2.5*
	S	96.2 (91.2–101.2)	0.0 (0.0)	0.8 (0.6–0.9)	29.1 (20.5–37.7)	
Aminocyclopyrachlor	R	97.0 (90.2–103.9)	0.0 (0.0)	1.3 (0.8–1.7)	119.9 (85.1–154.7)	3.0*
	S	97.0 (90.2–103.9)	0.0 (0.0)	0.8 (0.6–0.9)	40.2 (24.7–55.7)	
Dicamba	R	84.3 (76.3–92.4)	7.7 (7.7–19.8)	1.0 (0.3–1.7)	5.4 (3.4–7.3)	1.0
	S	84.3 (76.3–92.4)	7.7 (7.7–19.8)	0.9 (0.4–1.4)	5.1 (3.1–7.1)	
Halauxifen-methyl	R	93.5 (87.5–99.4)	7.6 (−4.1–19.4)	0.8 (0.4–1.2)	0.6 (0.2–1.1)	1.3
	S	93.5 (87.5–99.4)	7.6 (−4.1–19.4)	1.2 (0.6–1.9)	0.5 (0.2–0.7)	

[a] Four-parameter log-logistic equation: $y = C + D - C/1 + (x/GR_{50})^{b}$, where *D* refers to the upper limit of curve of best fit, *C* refers to the lower limit of curve of best fit, *b* refers to the slope of the curve best fit, and GR_{50} refers to the herbicide dose in g ae ha^{-1} causing a 50% reduction in aboveground dry weight.

[b] RF, resistance factor as determined by dividing the GR_{50} for population R by the GR_{50} for population S. An asterisk (*) indicates GR_{50} values are significantly different based on non-overlapping 95% confidence intervals (values in parentheses).

[c] db, dry biomass.

resistance to 2,4-D (Preston et al. 2013). Patterns of cross-resistance among SAHs as well as the amplitude of resistance among species suggest various mechanisms of resistance are involved.

Field Evaluation of the Efficacy of SAHs

All of the SAHs evaluated controlled *A. powellii* <85% (Table 4). Clopyralid, fluroxypyr, and halauxifen-methyl controlled SAH-R *A. powellii* <20% at 8 WAA. At 8 WAA, MCPA ester provided 30% control of SAH-R *A. powellii*, surpassing clopyralid and fluroxypyr but falling short of 2,4-DB, dichlorprop-p/2,4-D, dicamba/diflufenzopyr, 2,4-D, and dicamba. *Amaranthus powellii* control with MCPA was similar to control with halauxifen-methyl, fluroxypyr/halauxifen-methyl + MCPA, and fluroxypyr + MCPA. Dichlorprop-p/2,4-D, dicamba/diflufenzopyr, 2,4-D, and dicamba controlled SAH-R *A. powellii* similarly at 1, 2, 4, and 8 WAA. Numerically, dicamba provided the greatest control (84%) of SAH-R *A. powellii* at 8 WAA. The findings from the dose–response study and the field study support that the SAH-R *A. powellii* population is resistant to MCPA.

The level of visible SAH-R *A. powellii* control can be linked to reductions in density and biomass. Clopyralid, fluroxypyr, halauxifen-methyl, MCPA, fluroxypyr/halauxifen-methyl + MCPA, fluroxypyr + MCPA, and 2,4-DB did not reduce SAH-R *A. powellii* density relative to the non-treated control. Fluroxypyr + MCPA, 2,4-DB, dichlorprop-p/2,4-D, dicamba/diflufenzopyr, 2,4-D, and dicamba reduced *A. powellii* density 56%, 55%, 68%, 77%, 77%, and 83%, respectively; these values were statistically similar. All of the SAHs reduced SAH-R *A. powellii* biomass relative to the nontreated control. Clopyralid, fluroxypyr, and halauxifen-methyl reduced SAH-R *A. powellii* biomass similarly at 30% to 45%. Fluroxypyr + MCPA, 2,4-DB, dichlorprop-p/2,4-D, dicamba/diflufenzopyr, 2,4-D, and dicamba reduced *A. powellii* biomass 77%, 80%, 87%, 88%, 92%, and 95%, respectively; the biomass reduction was similar with the six aforementioned herbicides.

Clopyralid, fluroxypyr, and halauxifen-methyl provided the lowest control (<20% visible control) of SAH-R *A. powellii* at 8 WAA, consistent with *Amaranthus* species control ratings (multiple species) in the Ontario Guide to Weed Control, Field Crops 2021; these active ingredients do not provide effective control of *Amaranthus* species (OMAFRA 2021). Therefore, low control with these herbicides cannot be attributed to the occurrence of SAH resistance in this population.

In the absence of resistance, MCPA ester should control *Amaranthus* species 70% in corn and 90% to 100% in cereal crops in Ontario based on label recommendations (OMAFRA 2021). However, the development of resistance to MCPA in this population of *A. powellii* explains the low-level control (30%) observed at 8 WAA.

Although none of the herbicides evaluated controlled SAH-R *A. powellii* >90%, dichlorprop-p/2,4-D, dicamba/diflufenzopyr, 2,4-D, and dicamba controlled *A. powellii* 67%, 74%, 75%, and 84%, respectively, at 8 WAA. Benoit et al. (2019) found that 2,4-D, dicamba/diflufenzopyr, and dicamba applied postemergence in corn at 560, 200, and 600 g ai ha^{-1} controlled *A. tuberculatus* 85%, 74%, and 82%, respectively, at 8 WAA. These control values are comparable to the control obtained with the same herbicides in the present study. Although these herbicides applied postemergence were the most efficacious for SAH-R *A. powellii* control in corn, the control was <85%.

The dose–response study revealed that the population is resistant to three out of four phenoxy carboxylic acid herbicides, specifically MCPA, mecoprop, and dichlorprop-p, but susceptible to 2,4-D. Although many weed species with resistance to MCPA are also cross-resistant to 2,4-D, this is not always the case (Heap 2024). For example, a *G. tetrahit* population studied by Weinberg et al. (2006) was resistant to MCPA but not cross-resistant to 2,4-D. Similarly, results from the present dose–response and field trial studies demonstrate that this SAH-R *A. powellii* population is still susceptible to 2,4-D; control was significantly improved compared with MCPA, as 2,4-D controlled the population 75% at 8 WAA. Control of *A. powellii* with dichlorprop-p/2,4-D was moderate in the field study. Because dichlorprop-p was not applied alone, it is difficult to ascertain whether resistance to dichlorprop-p affected control. It is likely that 2,4-D compensated for resistance to dichlorprop-p, as efficacy was similar with dichlorprop-p/2,4-D and 2,4-D.

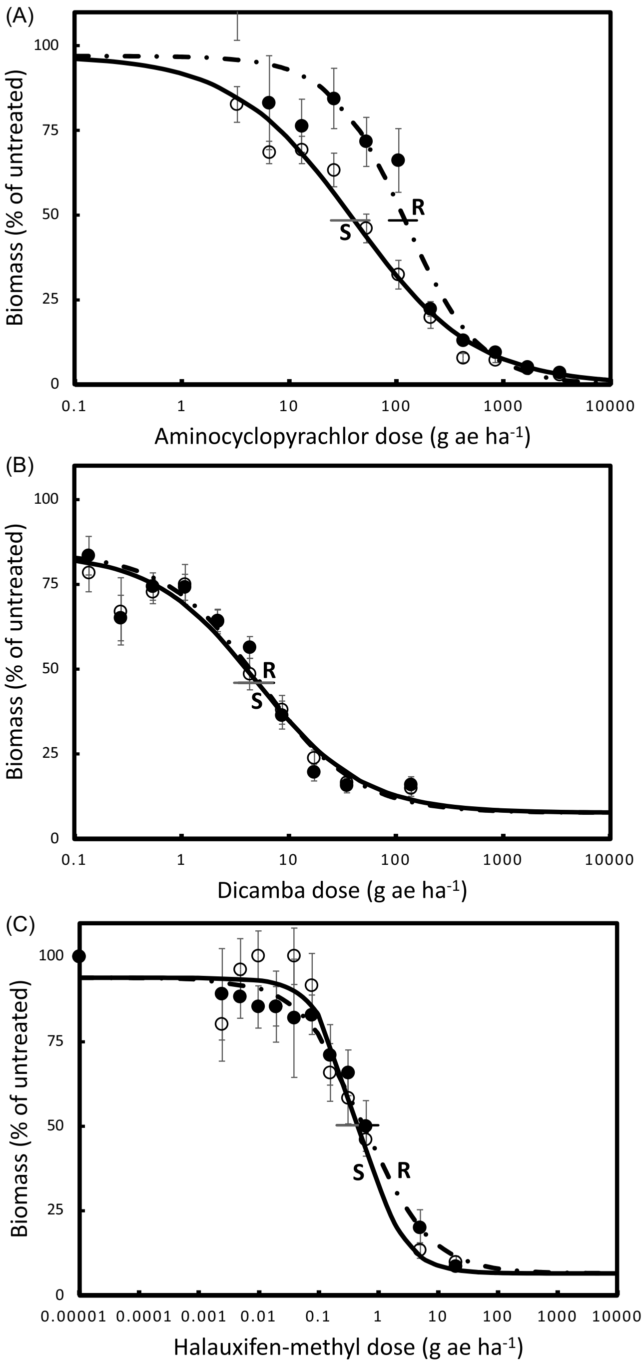

Figure 2. Nonlinear dose–response curves for *Amaranthus powellii* R and S populations following treatment with aminocyclopyrachlor (A), dicamba (B), and halauxifen-methyl (C) as determined using a four-parameter log-logistic equation: $y = C + D - C/1 + (x/\text{GR}_{50})^b$. Each point represents the average percent reduction in aboveground biomass relative to the untreated control across two experimental runs with four replicates per treatment. Error bars represent the standard error. The GR_{50} indicates the herbicide dose causing a 50% reduction in aboveground biomass as represented by the 95% confidence intervals.

These studies confirm that this SAH-R *A. powellii* population is resistant to MCPA and cross-resistant to mecoprop, dichlorprop-p, and aminocyclopyrachlor. Results of the dose–response study support the findings of significantly reduced SAH-R *A. powellii* control with MCPA in the field. Based on the results of the field study and the dose–response study, there are differences in the level of control of SAH-R *A. powellii* with different synthetic auxin subclasses. Given that all the SAHs tested in the field provided <85% control of the resistant population, alternative herbicide options must be identified to prevent unacceptable crop yield losses. The presence of SAH resistance would complicate management, as current practices would need to be adjusted to control this *A. powellii* population.

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