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Cross-resistance to diquat in glyphosate/paraquat-resistant hairy fleabane (*Conyza bonariensis*) and horseweed (*Conyza canadensis*) and confirmation of 2,4-D resistance in *Conyza bonariensis*

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Research Article

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Abstract

Hairy fleabane and horseweed are pervasive weed species in agriculture. Glyphosate-resistant (GR) and glyphosate/paraquat–resistant (GPR) biotypes challenge current management strategies. These GR and GPR biotypes have non–target site resistance, which can confer resistance to herbicides with different sites of action (SOAs). This study's objective was to characterize the response of GR, GPR, and glyphosate/paraquat–susceptible (GPS) biotypes of both weed species to herbicides with a different SOA. Whole-plant dose–response bioassays indicated a similar response among tested biotypes of both weed species to rimsulfuron, dicamba, hexazinone, glufosinate, flumioxazin, saflufenacil, or mesotrione. The hairy fleabane GR and GPR biotypes were 2.7- and 2.9-fold resistant to 2,4-D relative to the GPS biotype (GR_{50} 766.7 g ai ha^{-1}), confirming 2,4-D resistance in hairy fleabane for the first time in California. The GR and GPR biotypes were not cross-resistant to dicamba. No differences in response to 2,4-D were observed among horseweed biotypes with a GR_{50} ranging from 150.2 to 277.4 g ai ha^{-1}. The GPR biotypes of both species were cross-resistant to diquat, with a 44.0-fold resistance in hairy fleabane (GR_{50} 863.7 g ai ha^{-1}) and 15.6-fold resistance in horseweed (GR_{50} 563.1 g ai ha^{-1}). The confirmation of multiple resistances to glyphosate, paraquat, and 2,4-D in hairy fleabane curtails herbicide SOA alternatives and jeopardizes resistance management strategies based on herbicide rotation and tank mixtures, underscoring the critical need for nonchemical weed control alternatives.

Introduction

Hairy fleabane and horseweed are annual broadleaf weed species present in diverse cropping systems including row crops, perennial crops, and noncropped areas. Their prolific seed production, long-distance seed dispersal, plasticity in seed germination requirements, and tolerance to harsh environmental conditions (Bajwa et al. 2016) contribute to the invasive nature of these weeds. *Conyza* spp. are pervasive in production fields, and their management becomes more difficult with the evolution of herbicide-resistant biotypes. Reported cases of herbicide-resistant *Conyza* spp. are increasing, and they are present in multiple countries (Bajwa et al. 2016). As of 2020, there are 20 unique cases of herbicide-resistant hairy fleabane to four different herbicide sites of action (SOAs), and 65 unique cases of herbicide-resistant horseweed to five SOAs (Heap 2020). According to the International Herbicide-Resistant Weed Database, the herbicides with greatest reported resistance cases in these two *Conyza* spp. are glyphosate, an inhibitor of 5-enolpyurvylshikimate-3-phosphate (EPSP) synthase, and paraquat, a photosystem I (PSI) electron diverter. There are instances of multiple resistance to glyphosate and paraquat in both species (Heap 2020).

The current understanding of the mechanism of the glyphosate resistance and glyphosate/paraquat resistance in *Conyza* spp. in the western United States is limited. Reduced glyphosate translocation is often reported in GR *Conyza* spp., and although the molecular aspects of the non–target site resistance mechanism remain elusive, it is believed to be related to herbicide sequestration within the vacuole (Gaines et al. 2019). Similarly, paraquat resistance is often associated with reduced translocation and vacuole sequestration (Hawkes 2014). A previous study indicated reduced translocation in both glyphosate and paraquat resistance in both species in orchard production systems of California (Moretti and Hanson 2017). Additional mechanisms of resistance in these biotypes are possible. Non–target site resistance mechanisms may confer cross-resistance to herbicides with distinct SOAs (Han et al. 2020; Iwakami et al. 2019), and in some cases, to reactive oxygen species (ROS) generators (Ye and Gressel 2000).

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In California, herbicide resistance, mainly glyphosate resistance, affects production practices in multiple cropping systems (Hanson et al. 2014). Glyphosate is the most used pesticide in California, whereas paraquat is the third most used herbicide in the state (CDFA 2017). GR *Conyza* spp. are widespread across the state and present in nearly all tested populations from tree nut crops; GPR cases are not known to be widespread but appear to be increasing (Moretti et al. 2016). Worldwide, herbicide mixtures and rotations are often the first strategies to manage resistant biotypes (Peterson et al. 2018). The success of herbicide tank mixtures and rotations are reported by several studies evaluating GR *Conyza* spp. (Eubank et al. 2008; Tahmasebi et al. 2018; Urbano et al. 2007; Werth et al. 2010), a strategy also successful for GPR *Conyza* spp. biotypes (Eubank et al. 2012; Moretti et al. 2015). The pillars of this strategy are based on the diversity of SOAs with efficacy on the targeted resistant biotypes. This premise can be jeopardized by herbicide cross or multiple resistance. POST control strategies for GR *Conyza* spp. in California orchards crops often depend on glufosinate, various inhibitors of protoporphyrinogen oxidase (PPO), and to a lesser extent 2,4-D (Hanson 2020); however, previous field research has suggested poor or inconsistent control of multiple-resistant hairy fleabane with 2,4-D alone or in combination with glyphosate (Moretti et al. 2015). There have been no reports on the response of GR and GPR *Conyza* spp. to different SOAs or characterization of cross-resistance patterns. This study has examined the presence of resistance to other herbicide SOAs in GR, GPR, and susceptible biotypes of hairy fleabane and horseweed.

Materials and Methods

Plant Material

The experiments were conducted at the University of California–Davis greenhouse facilities in Davis, CA (38.54 N, 121.76 W). The hairy fleabane and horseweed biotypes used in this study were the descendants of single seeds and were self-pollinated for five or more generations to ensure a uniform phenotypic response. The biotypes were characterized as glyphosate/paraquat–susceptible (GPS), glyphosate-resistant (GR), and glyphosate/paraquat–resistant (GPR) (Moretti et al. 2016).

Plant Growth and Herbicide Application

Seeds of each biotype were sown in flats filled with a commercial potting medium (Sungro Horticulture, Agawam, MA, USA) and grown under natural-light conditions. Seedlings at the first-leaf stage were transplanted as one seedling per 7.5- by 7.5- by 10-cm pot filled with the same potting medium. Plants were maintained in the greenhouses at 30/15 C day/night temperatures under natural-light conditions. Irrigation and fertilization were provided as needed to promote vigorous growth. The experiments were initiated when plants were at the 5- to 6-leaf stage for hairy fleabane and the 8- to the 10-leaf stage for horseweed. All biotypes of both species were tested simultaneously during each herbicide study.

Whole-plant dose–response assays were conducted from April through October 2015. Nine herbicides representing seven distinct SOAs were selected for the study (Table 1). The active ingredient and respective WSSA SOA groups were rimsulfuron in Group 2 (acetolactate synthase inhibitors); 2,4-D and dicamba (Group 4–synthetic auxins); hexazinone (Group 5–an inhibitor of photosystem at PSII site A); glufosinate (Group 10–an inhibitor of glutamine synthetase); flumioxazin and saflufenacil (Group 14–inhibitors of PPO); diquat (Group 22–PSI electron diverter); and mesotrione (Group 27–an inhibitor of 4-hydroxyphenylpyruvate dioxygenase; 4-HPPD). Each herbicide was tested at eight rates plus a nontreated control. Adjuvants were selected based on manufacturer label recommendations (Table 1). Treatments were applied to plant foliage using a spray chamber (Technical Machinery Inc., Sacramento, CA) calibrated to deliver 187 L ha^{-1} at 207 kPa. The chamber was equipped with an even flat-fan nozzle 8002E (TeeJet Technologies, Wheaton, IL) placed 45 cm above the canopy. Plants were evaluated 28 d after application as alive or dead, and aboveground biomass was collected, dried, and recorded.

Statistical Analysis

The experiments were organized in a two-factor randomized complete block design. The three biotypes (GPS, GR, and GPR) were the first factor, and the nine herbicide rates were the second factor. There were four replicates per biotype by treatment level, and the experiment was conducted twice. Each species and herbicide was analyzed independently. Data were analyzed in R version 4.0.3 (R Core Team 2020). Plant mortality was analyzed using logistic regression with a mixed-effect model using the glm function from package stats version 3.6.2. Experimental blocks and experimental runs were treated as random factors. Herbicide rate, biotype, and interaction were tested using a Wald test ($P < 0.05$) (Table 2). Mortality data were analyzed using nonlinear logistic regression (drc package version 3.01) with two parameters (Ritz et al. 2015). The parameters were the herbicide dose required to kill 50% of the plants (LD_{50}) and the slope of the regression near the LD_{50} parameter. The ratio of LD_{50} for each biotype was used to compare biotypes by a z-test ($P < 0.05$) using the compParm procedure in the drc package. The resistance index (RI) was calculated as the ratio of LD_{50} values relative to the GPS biotype of each species. When the regression models were fitted for each biotype (full model), the model was compared to a simplified model using a common regression for all biotypes. Models were compared using a chi-square test with the ANOVA command in the drc package. The reduced model was used when no statistical difference in LD_{50} was reported or when the chi-test result was not significant. Plant biomass data were analyzed using a mixed model with the lmer function in the lme4 package version 1.123. The herbicide rate, biotype, and their interactions were tested as fixed factors (Table 2). Data were submitted to a nonlinear regression using the drc package. Multiple models were tested and compared using a Akaike information criterion to decide the best fit. Diagnostic plots were used to check normality and heteroskedasticity assumptions. The three-parameter log-logistic model was appropriate in all cases (Equation 1).

$$Y = \frac{d}{1 + \left(\frac{x}{\mathrm{ED}_{50}}\right)^{\mathrm{b}}}$$

Where Y was the response measured, d references the upper limit, x refers to the herbicide rate, ED_{50} denotes the herbicide dose causing 50% reduction in the response measured, and b is the relative slope of the curve around the ED_{50}. The three-parameter log-logistic model was with slope of the regression, upper as the maximum value of the regression, and GR_{50} as the herbicide rate required to reduce biomass accumulation by 50%. Biotype effects were also tested using compParm function and fitting a regression to each biotype (full model) compared to a common regression (reduced model). The reduced model was used when the models were not different.

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Table 1. POST herbicides used in the experiments. Site of action, active ingredient, trade name, rates, reference rate, and manufacturers.

WSSA SOA[a]	Common name	Trade name	Rate[b]								Reference rate	Manufacturer
			g ai or ae ha^{-1}									
2	Rimsulfuron	Matrix SG	1	3	8	23	70	210	630	1,900	70	Corteva AgriScience
4	2,4-D	Saber	11	33	99	296	880	2,660	8,000	24,000	880	Loveland Products
4	Dicamba	Clarity	85	170	340	680	1,360	2,730	5,450	10,800	1,120	BASF Corp.
5	Hexazinone	Velpar	5	15	48	146	440	1,320	3,950	11,800	1,320	Bayer CropScience
10	Glufosinate	Rely 280	6	18	56	166	500	1,500	4,500	13,500	980	BASF Corp.
14	Flumioxazin	Chateau	53	107	214	430	860	1,710	3,400	6,900	430	Valent USA
14	Saflufenacil	Treevix	2	5	9	18	35	70	140	280	49	BASF Corp.
22	Diquat	Reglone	2	10	40	158	630	2,530	10,120	40,500	560	Syngenta Crop Protection
27	Mesotrione	Broadworks	13	26	53	105	210	420	840	1,680	210	Syngenta Crop Protection

[a]Weed Science Society of America site of action (SOA) group number (https://wssa.net/wssa/weed/herbicides): 2, Acetolactate synthase inhibitor; 4, synthetic auxin; 5, inhibitor of photosynthesis at photosystem II; 10, glutamine synthase inhibitor; 14, protophorphyrinogen oxidase inhibitor; 22, photosystem I electron diversion; 27, inhibition of the hydroxyphenylpyruvate dioxygenase.
[b]All treatments included ammonium sulfate at equivalent of 2% wt/vol. Nonionic surfactant (Rainier; Wilbur Ellis, Aurora, CO) 0.25% vol/vol was included in rimsulfuron, hexazinone, 2,4-D, dicamba, diquat, and flumioxazin treatments. Methylated seed oil (Mor-act Crop Oil; Wilbur Ellis, Aurora, CO) was included at 1% vol/vol in saflufenacil and mesotrione treatments.

Table 2. Fixed factors for logistic and ANOVA analysis for mortality and biomass of *Conyza* spp.

	Hairy fleabane						Horseweed					
Wald test	Mortality			Biomass			Mortality			Biomass		
Factor	Rate	Biotype	R × B	Rate	Biotype	R × B	Rate	Biotype	R × B	Rate	Biotype	R × B
Rimsulfuron	*	NS	NS	*	NS	NS	*	NS	NS	*	NS	NS
2,4-D	*	NS	*	*	*	*	*	NS	NS	*	NS	NS
Dicamba	*	NS	NS	*	NS	NS	*	NS	NS	*	NS	NS
Hexazinone	*	*	NS	*	NS	NS	*	NS	NS	*	NS	NS
Glufosinate	*	NS	NS	*	NS	NS	*	NS	NS	*	NS	NS
Flumioxazin	*	NS	*	*	NS	NS	*	*	NS	*	NS	NS
Saflufenacil	*	NS	NS	*	NS	NS	*	*	NS	*	*	NS
Diquat	*	*	NS	*	*	*	*	*	NS	*	*	NS
Mesotrione	*	NS	NS	*	NS	NS	*	*	NS	*	NS	NS

Abbreviations: R, rate; B, biomass; significance levels based on Wald Test for mortality and ANOVA for biomass: NS, not significant (>0.05); *, $P < 0.05$.

Results and Discussion

All tested herbicides affected *Conyza* spp. mortality and biomass, but biotype response depended on the herbicide tested, and it differed between species (Table 2). The hairy fleabane mortality response to hexazinone and diquat depended on biotype tested, whereas the interaction of rate and biotype was significant for 2,4-D and flumioxazin. Biomass analysis also indicated a biotype and rate-by-biotype effect for 2,4-D and diquat, but not for the other herbicides. The mortality of horseweed differed among biotypes for flumioxazin, saflufenacil, diquat, and mesotrione. When considering biomass, the biotype effect was only significant for saflufenacil and diquat. A common regression model for the three biotypes was fitted for rimsulfuron and dicamba for both species. For the herbicides in which biotype or biotype-by-rate interactions were significant, a regression model was fitted for each biotype (full model). The comparison of LD_{50} or GR_{50} ratio indicated no differences among biotypes for hexazinone, glufosinate, flumioxazin, saflufenacil, and mesotrione. The full model did not differ from the reduced model ($P < 0.05$), so a single regression model for all three biotypes was fitted.

All the *Conyza* spp. biotypes tested were sensitive to the acetolactate synthase inhibitor (ALS) herbicide rimsulfuron. The rimsulfuron rates that caused 50% mortality (LD_{50}) or reduction of biomass (GR_{50}) of hairy fleabane were 78 and 1.1 g ai ha^{-1}, respectively (Table 3). Similar values were observed for horseweed, and in all cases, the values were close to the reference 1× rate of 70 g ai ha^{-1}. These results are comparable to previously reported results regarding the response of susceptible horseweed to chlorimuron, a sulfonylurea herbicide, with a GR_{50} of 0.1 g ai ha^{-1} (Zheng et al. 2011). The response to the auxinic herbicide dicamba was similar among tested biotypes of both *Conyza* spp. The hairy fleabane LD_{50} was 879.9 g ae ha^{-1}, and the GR_{50} was 72.6 g ae ha^{-1} of dicamba. Horseweed was more sensitive to dicamba than hairy fleabane, with an LD_{50} 2.5-fold lower and a GR_{50} 7.8 times lower (Table 3). The dicamba GR_{50} of both *Conyza* species is comparable to the findings of Zheng et al. (2011). Horseweed populations from Indiana were reported to have a GR_{50} ranging from 31 to 127 g ae ha^{-1} depending on the developmental stage of the plants (Kruger et al. 2010; McCauley and Young 2019).

The paraquat resistance mechanism of hairy fleabane was previously attributed to enhanced enzymatic detoxification of ROS conferring cross-resistance to oxidant stress (Shaaltiel et al. 1988; Shaaltiel and Gressel 1986). Several herbicides work by generating ROS upon light exposure, including PSII inhibitors, glutamine synthetase inhibitors, PPO inhibitors, PSI electron diverters, and inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HRAC 2020). Hexazinone, a PSII inhibitor, is not used in tree nuts but is commonly used in small fruit crops like blueberry. Other chemicals with the same SOA most widely used in tree nuts are diuron and simazine. Hexazinone was effective in all biotypes of *Conyza* spp. The LD_{50} ranged from 27 to 62 g ai ha^{-1}, whereas the reference rate is 1,320 g ai ha^{-1}. There was no difference among tested biotypes for glufosinate, a glutamine synthetase inhibitor and ROS producer (Takano et al. 2019). The LD_{50} for glufosinate was 574

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Table 3. Regression parameter estimates and standard errors for plant mortality and biomass of hairy fleabane and *Conyza* spp. 28 d after foliar treatment with different herbicides. A common regression logistic regression was fitted across biotypes within each species and herbicide tested.[a]

	Mortality				Biomass					
Herbicide	LD_{50} (±SE)[b]		Slope (±SE)		Slope (±SE)		Upper (±SE)		GR_{50} (±SE)	
	g ai ha^{-1}								g ai or ae ha^{-1}	
Rimsulfuron										
Hairy fleabane	78.1	(17.8)	−0.9	(0.1)	0.3	(0.0)	0.4	(0.0)	1.1	(0.5)
Horseweed	55.8	(12.3)	−0.9	(0.4)	0.4	(0.0)	0.6	(0.0)	2.0	(0.5)
Dicamba										
Hairy fleabane	879.9	(125.6)	−1.4	(0.1)	0.5	(0.0)	0.5	(0.0)	72.6	(12.3)
Horseweed	348.2	(76.6)	−0.8	(0.1)	0.3	(0.0)	0.4	(0.0)	9.2	(4.5)
Hexazinone										
Hairy fleabane	62.5	(15.2)	−1.4	(0.3)	2.4	(1.1)	0.5	(0.0)	85.8	(17.5)
Horseweed	27.8	(8.5)	−1.1	(0.2)	2.3	(0.8)	0.5	(0.0)	77.7	(13.1)
Glufosinate										
Hairy fleabane	397.1	(68.5)	−1.5	(0.2)	0.6	(0.1)	0.4	(0.0)	106.4	(28.2)
Horseweed	574.5	(105.3)	−1.3	(0.6)	0.6	(0.1)	0.5	(0.0)	135.2	(32.8)
Flumioxazin										
Hairy fleabane	1,473.7	(198.9)	−1.6	(0.2)	0.7	(0.1)	0.4	(0.0)	153.2	(14.7)
Horseweed	1,326.9	(243.8)	−1.0	(0.7)	0.7	(0.1)	0.4	(0.0)	72.7	(11.1)
Saflufenacil										
Hairy fleabane	2.2	(0.3)	−2.2	(0.4)	0.6	(0.1)	0.7	(0.0)	0.3	(0.1)
Horseweed	2.1	(0.4)	−1.4	(0.5)	0.6	(0.1)	0.7	(0.0)	0.3	(0.1)
Mesotrione										
Hairy fleabane	117.6	(14.7)	−1.8	(0.2)	0.2	(0.1)	0.6	(0.0)	80.8	(67.2)
Horseweed	388.5	(62.7)	−1.2	(0.2)	0.2	(0.1)	0.7	(0.0)	36.4	(21.4)

[a]Abbreviations: LD_{50}, effective dose killing 50% of the population; slope, relative slope around the LD_{50} or GR_{50}; upper, upper limit of the response; GR_{50}, effective doses reducing shoot dry biomass by 50%.
[b]Means are pooled across two experiments and three biotypes ($n = 24$) for hairy fleabane and horseweed. Standard errors are in parentheses.

g ai ha^{-1} or less, and the GR_{50} was 135 g ai ha^{-1}; both parameters were below the reference rate of 980 g ai ha^{-1}. Glufosinate is commonly used in tree nut crops, and resistance has been reported in Italian ryegrass [*Lolium perenne* L ssp. *multiflorum* (Lam.) Husnot] (Brunharo et al. 2019). *Conyza* biotypes responded similarly to flumioxazin and saflufenacil, the PPO herbicides tested. Flumioxazin LD_{50} was 1,473 and 1,326 g ai ha^{-1} for hairy fleabane and horseweed, respectively, values over three times greater than the reference rates for tree nut crops. Flumioxazin is used primarily for its PRE activity in *Conyza* spp. Flumioxazin in POST reduces *Conyza* biomass, as indicated by the GR_{50} lower than 153 g ai ha^{-1}, but it often does not control the plants. Similar to these findings, flumioxazin applied POST did not control *Conyza* spp. populations from Europe (Tahmasebi et al. 2018). In contrast, saflufenacil has an excellent POST activity on *Conyza* spp. with an LD_{50} of 2.2 g ai ha^{-1}, and the GR_{50} was lower than 0.7 g ai ha^{-1}. Saflufenacil is often used to manage GR *Conyza* spp. in tree nuts, and it has been shown to control GR horseweed in other cropping systems (Budd et al. 2016; Mellendorf et al. 2013). Response to mesotrione did not differ among the tested biotypes of *Conyza* spp. (Table 3). The mesotrione doses to cause 50% mortality were 117.6 and 388.5 g ai ha^{-1} for hairy fleabane and horseweed, respectively. In general, more *Conyza* spp. survived mesotrione treatment relative to the other products evaluated in this research. This herbicide is often recommended as a tank-mixture partner to provide adequate control of horseweed (Armel et al. 2009).

The *Conyza* spp. response to 2,4-D was species- and biotype-dependent (Table 2). The 2,4-D doses required to kill 50% of the hairy fleabane biotypes GR and GPR were 3.9 and 6.8 times greater than the GPS biotype, with an LD_{50} of 1,338.1 g ai ha^{-1} (Table 4 and Supplementary Figure S1). The biomass data also indicate that hairy fleabane GR and GPR are resistant to 2,4-D, with an RI of 2.7 and 2.9 (Table 4). The LD_{50} for horseweed GR and GPR biotypes showed an RI of 3.0 and 2.9, but biomass data indicate that the biotypes responded similarly, with a RI of 1.3 and 1.8, respectively (Table 4). The estimated 2,4-D GR_{50} of horseweed from California is comparable to levels reported elsewhere, such as in Indiana, where reported GR_{50} values ranged from 131.6 to 314.1 g ai ha^{-1} of 2,4-D (Kruger et al. 2010; McCauley and Young 2019). These data confirm the first case of 2,4-D–resistant hairy fleabane in California. Although no attempt was made to study the mechanism of resistance to 2,4-D, none of the plants exhibited the rapid-necrosis phenotype reported in 2,4-D–resistant Sumatran fleabane [*C. sumatrensis* (Retz.) E. Walter] from Brazil (de Queiroz et al. 2020). It is important to note that the GR_{50} level of the GPS population was 766.7 g ai ha^{-1}, which approaches the reference rate. For comparisons, hairy fleabane populations from Australia did not survive 2,4-D treatment of 875 g ae ha^{-1}(Aves et al. 2020). Previous studies have documented the low efficacy of 2,4-D in hairy fleabane from California (Moretti et al. 2015). Hairy fleabane biotypes resistant to 2,4-D curtail the benefits of 2,4-D use in the tree nut crops, including the potential use of low-volatility formulations like the 2,4-D choline salt (Peterson et al. 2016). However, the absence of cross-resistance to dicamba suggests that other auxinic herbicides, such as halauxifen-methyl, could be effective against this biotype. Halauxifen-methyl has been shown to be effective against GR horseweed (McCauley et al. 2018).

The GPR biotypes of both *Conyza* spp. showed cross-resistance to diquat, whereas the GR biotypes were as sensitive to diquat as the GPS. Compared to the GPS biotypes within species, the hairy fleabane GPR LD_{50} was 4,961.8 g ai ha^{-1}, with a RI of 90.2, and GR_{50} was 863.7 g ai ha^{-1}, with a RI of 44 (Table 4 and Supplementary Figure S2). In horseweed, the LD_{50} of the GPR biotype was 1,791.2 g ai ha^{-1} (RI 11.3) and the GR_{50} 563.1 g ai ha^{-1} (RI 15.6). Cross-resistance between PSI herbicides is commonly observed in paraquat-resistant weeds (Hawkes 2014). A previous study reported that paraquat-resistant hairy fleabane exhibited a cross-resistance to diquat 10-fold lower than to paraquat

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Table 4. Estimated nonlinear regression parameters for hairy fleabane and horseweed biotypes from California in response to 2,4-D and diquat. Mortality and dry-biomass whole-plant dose–response assay were estimated for glyphosate/paraquat-susceptible (GPS), glyphosate-resistant (GR), and glyphosate/paraquat-resistant (GPR) biotypes of each species.[a]

	Hairy fleabane						Horseweed					
	Mortality			Biomass			Mortality			Biomass		
	LD_{50} (±SE)[b]		RI	GR_{50} (±SE)		RI	LD_{50} (±SE)		RI	GR_{50} (±SE)		RI
	g ai or ae ha^{-1}			g ai or ae ha^{-1}			g ai or ae ha^{-1}			g ai or ae ha^{-1}		
2,4-D												
GPS	1,172.7	(386.9)	1.0	766.7	(150.8)	1.0	1,164.1	(324.2)	1.0	150.2	(48.5)	1.0
GR	4,613.0	(967.1)	3.9*	2,090.6	(241.5)	2.7*	2,705.4	(881.6)	3.0*	200.2	(57.3)	1.3
GPR	8,000.1	(515.3)	6.8*	2,231.3	(384.2)	2.9*	2,685.1	(824.4)	2.9	277.4	(115.3)	1.8
Diquat												
GPS	54.9	(22.1)	1.0	19.5	(5.0)	1	157.9	(12.5)	1.0	35.9	(8.9)	1.0
GR	77.4	–	1.3	20.1	(3.4)	1.1	46.7	(13.4)	0.3	20.9	(4.7)	0.6
GPR	4,961.8	–	90.2*	863.7	(210.4)	44.0*	1,791.2	(576.9)	11.3*	563.1	(106.7)	15.6*

[a]Abbreviations: LD_{50}, effective dose killing 50% of the population; slope,relative slope around the LD_{50} or GR_{50}; upper, upper limit of the response; GR_{50}, effective doses reducing shoot dry biomass by 50%; RI, resistance index relative to susceptible biotype.
[b]Means are pooled across two experiments and three biotypes ($n = 24$) for hairy fleabane and horseweed. Standard errors are in parentheses. * Significance level based on z-test at $P < 0.05$.

(Vaughn et al. 1989). In this study, both GPR biotypes were more tolerant to paraquat than diquat. The paraquat GR_{50} (RI) for these GPR biotypes were previously reported as 1,161 (RI 278) and 1,390 g ai ha^{-1} (RI 322) for the hairy fleabane and horseweed, respectively (Moretti et al. 2016). These RI values are 6- and 20-fold higher than the observed RI based on the diquat GR_{50} in this study. An absence of cross-resistance to various ROS-generating herbicides in GPR biotypes was observed in this study, suggesting that enhanced enzymatic detoxification of ROS is not associated with paraquat resistance in these biotypes. These findings agree with the previous report of a paraquat-resistant hairy fleabane biotype that showed no cross-resistance to ROS (Vaughn et al. 1989).

The confirmation of multiple resistance to glyphosate, paraquat, and 2,4-D in hairy fleabane indicates that herbicide-based strategies to manage herbicide resistance are only a short-term approach. Increasing cases of cross- and multiple-herbicide resistance will curtail the SOA availability, the pillar for its success, jeopardizing this resistance management strategy. This finding underscores the critical need for nonchemical weed control alternatives in perennial crops.

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