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Inheritance of Glyphosate Resistance in Hairy Fleabane (*Conyza bonariensis*) from California

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Inheritance of glyphosate resistance was investigated in hairy fleabane populations from California as part of providing the information needed to predict and manage resistance and to gain insight into resistance mechanism (or mechanisms) present in the populations. Three glyphosate-resistant individuals grown from seed collected from distinct sites near Fresno, CA, were crossed to individuals from the same susceptible population to create reciprocal F_1 populations. A single individual from each of the F_1 populations was used to create a backcross population with a susceptible maternal parent, and an F_2 population. Based on dose response analyses, reciprocal F_1 populations were not statistically different from each other, more similar to the resistant parent, and statistically different from the susceptible parent, consistent with nuclear control of the trait and dominance to incomplete dominance of resistance over susceptibility in all three crosses. Glyphosate resistance in two of the three crosses segregated in the backcross and the F_2 populations as a single-locus trait. In the remaining cross, the resistant parent had approximately half the resistance level as the other two resistant parents, and the segregation of glyphosate resistance in backcross and F_2 populations conformed to a two-locus model with resistance alleles acting additively and at least two copies of the allele required for expression of resistance. This two-locus model of the segregation of glyphosate resistance has not been reported previously. Variation in the pattern of inheritance and the level of resistance indicate that multiple resistance mechanisms may be present in hairy fleabane populations in California.

Nomenclature: Glyphosate; hairy fleabane, *Conyza bonariensis* (L.) Cronq.
Key words: Glyphosate resistance, inheritance.

Treatment with herbicides is the primary method of controlling weed populations in modern agriculture, and strong selection by herbicides has resulted in widespread evolution of resistance to herbicides in weed populations (Délye et al. 2013; Powles and Yu 2010). Glyphosate is the most widely used herbicide because it has many favorable attributes, including effectiveness on a wide range of species, low toxicity to animals, and rapid inactivation in the soil, and is relatively inexpensive (Baylis 2000; Duke and Powles 2008; Woodburn 2000). In recent years, glyphosate has been widely used in reduced-tillage systems that have many environmental and economic benefits but rely heavily on herbicides for weed control, and often include transgenic glyphosate-resistant crops (Owen 2008; Powles 2008; Shaner 2000). A recent significant decrease in the cost of glyphosate due to the availability of generic equivalents resulted in further increase in the use of the herbicide. The overreliance on glyphosate has led to evolution of glyphosate resistance in populations of 24 weed species in 20 countries to date and an increasing risk due to the sustained use of the herbicide (Duke and Powles 2008; Heap 2013).

In the Central Valley of California, glyphosate has been the primary herbicide used for weed control in low-tillage systems—including orchards, vineyards, field edges, roadsides, and irrigation ditches—for decades (CADPR 2009). In the low-tillage systems in the valley, hairy fleabane was confirmed resistant to glyphosate in 2007 (Shrestha et al. 2008). Two years prior to hairy fleabane, the closely related horseweed [*Conyza canadensis* (L.) Cronq.] was confirmed resistant to the herbicide (Shrestha et al. 2007). A main factor hypothesized to underlie the evolution of glyphosate resistance in horseweed is the increased use of glyphosate following the recent implementation of state regulations restricting certain pesticides vulnerable to leaching and runoff into groundwater (CADPR 2004; Okada et al. 2013; Shrestha et al. 2007). Hairy fleabane is a major weed in crop and noncrop habitats similar to those occupied by horseweed (Shrestha et al. 2008). Based on a survey conducted in 2010, glyphosate resistance is more widespread in

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hairy fleabane than in horseweed in the Central Valley (Okada, Hanson, Hembree, Shrestha, Wright, and Jasieniuk, unpublished data). The reason for more widespread glyphosate resistance in hairy fleabane is unknown. However, with an increasing number of glyphosate-resistant weed species in California (Heap 2013), information on genetic and ecological factors underlying the evolution of the resistance trait in each species may be becoming more important to understand and manage the evolution of glyphosate resistance in multiple weed species in the region (reviewed in Norsworthy et al. 2012).

The mode of inheritance of the resistance trait is one of the factors needed to predict and manage the evolution and spread of herbicide resistance (Jasieniuk et al. 1996; Maxwell et al. 1990). In addition, variation in the mode of inheritance, if detected, provides insights into the diversity of mechanisms underlying glyphosate resistance in weeds. The mechanism of glyphosate resistance in hairy fleabane in California has not been identified, and whether multiple mechanisms are present is unknown. Glyphosate resistance segregates as a single-locus trait in rigid ryegrass (*Lolium rigidum* Gaudin) from Australia (Lorraine-Colwill et al. 2001) and horseweed from Delaware (Zelaya et al. 2007), both with altered translocation as the mechanism of resistance (Feng et al. 2004; Lorraine-Colwill et al. 2001), and in goosegrass [*Eleusine indica* (L.) Gaertn.] from Malaysia with a target-site mutation in the gene encoding the herbicide's target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; Baerson et al. 2002; Ng et al. 2004). In rigid ryegrass in California, glyphosate resistance segregated at two loci (Simarmata et al. 2005) in a population with a target-site mutation in the EPSPS gene that conferred glyphosate resistance (Simarmata and Penner 2008), suggesting one of the segregating loci may be the target-site mutation (Preston et al. 2009). Glyphosate resistance in several rigid ryegrass populations segregates as a single-locus trait in most individuals in populations with altered translocation as the mechanism of resistance (Preston et al. 2009; Wakelin and Preston 2006; Wakelin et al. 2004). The objective of this study was to investigate the mode of inheritance of glyphosate resistance in individuals from different populations of hairy fleabane from California.

Materials and Methods

Parental Plants. Glyphosate-susceptible (S) parental plants, S-19, S-2, and S-14, used in controlled crosses were grown from seed from the susceptible population previously characterized (Shrestha et al. 2008). Glyphosate-resistant (R) parental plants were obtained from seed collected from three sites near Fresno, CA. Eighteen to 36 plants were grown from seed collected from each site and treated with 0.84 kg ae ha^{-1} of glyphosate as described below. One surviving plant per collection site was chosen 21 d after treatment to be used in controlled crosses as the R parent. The R parent plants, R-38, R-8, and R-4, were from populations HF21 (36.5938° N, 119.5117°W), BH51 (Moretti et al. 2013), and BH55 (36.6021°N, 119.5112°W), respectively. Because hairy fleabane is a predominantly self-pollinating species (Okada and Jasieniuk, unpublished data), there was a high probability that parental plants would be homozygous for resistance and susceptibility. We tested to confirm parental homozygosity by screening the progeny of self-pollinated plants for segregation of resistance and susceptibility. The parental plants were self-pollinated by placing glassine bags over capitula before anthesis and leaving the bags in place until seed set.

Controlled Crosses. Capitula of hairy fleabane consist of pistillate ray florets and perfect disk florets (Keil and Nesom 2012). Capitula of plants serving as female parents were emasculated by removing the disk florets before anthesis. Emasculated capitula were covered with glassine bags with nonemasculated capitula removed from the capitulescence. Capitula of plants serving as male parents were covered with glassine bags before anthesis. Controlled pollination was made 2 to 3 d after emasculation by touching the capitula of the female parent and the male parent to transfer pollen. Glassine bags were placed back on the female capitula until seed set, and the male capitula were discarded. Reciprocal F_1 generations were produced in controlled crosses consisting of three resistant-and-susceptible parental pairs, R-38 × S-19, R-8 × S-2, and R-4 × S-14. A single F_1 plant with the susceptible plant as the female parent from each of the three crosses was used to produce both the backcross (BC) and F_2 generations. The BC populations were produced in controlled crosses with the F_1 plant as the female back to its maternal parent as the male. The F_2 populations were produced by self-pollinating the same F_1 plants used to produce the BC populations.

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Table 1. Number and proportion of hairy fleabane progeny from self-pollination of resistant and susceptible parents that survived treatment with glyphosate at varying rates. The resistant and susceptible parents were crossed to produce the F_1 populations for this study. Bolded numbers indicate the glyphosate treatment under which the proportion of progeny of the self-pollinated R parent tested homozygous for glyphosate resistance.

		Glyphosate treatment (kg ae ha^{-1})							
		0.21		0.42		0.84		1.68	
Parent	Treatment date	No. tested	Proportion alive	No. tested	Proportion alive	No. tested	Proportion alive	No. tested	Proportion alive
R-38	October 21, 2011	132	1.00	132	1.00	132	**0.99**	132	0.68
S-19	October 21, 2011	12	0.58	12	0.42	12	**0.00**	12	0.00
R-8	October 17, 2011	128	1.00	128	0.99	132	**1.00**	131	0.99
S-2	October 17, 2011	161	0.69	16	0.19	12	**0.00**	13	0.00
R-4	October 21, 2011	98	**0.96**	99	0.88	99	0.48	99	0.05
S-14	October 21, 2011	10	**0.00**	9	0.11	9	0.00	9	0.00

Plant Response to Glyphosate. Seeds were germinated on the surface of moist soil in 20 by 20–cm flats. Young seedlings were transplanted into 5 by 5–cm square pots with one seedling per pot. All plants were grown using modified University of California soil mix (sand : compost : peatmoss in 1 : 1 : 1 ratio and dolomite at 1.78 g L^{-1}) and watered daily with fertilizer water (nitrogen : phosphorus : potassium in 2 : 1 : 2 ratio) in a greenhouse with no supplementary lighting in Davis, CA. The glyphosate treatments were applied at the five- to eight-leaf stages using a track sprayer (Technical Machinery Inc., Sacramento, CA) in a spray volume of 140 L ha^{-1} with deionized water as the carrier at 207 kPa. To test if the resistant parents were homozygous and not segregating for glyphosate resistance, 99 to 132 plants of the progeny of the self-pollinated resistant parents were treated with 0.21, 0.42, 0.84, or 1.68 kg ae ha^{-1} of glyphosate (Weathermax, Monsanto Company, St. Louis, MO) with 9 to 16 plants from the self-pollinated progeny of susceptible parents (Table 1). To test for nuclear or cytoplasmic control and dominance of glyphosate resistance, the reciprocal F_1 populations and the progeny of the self-pollination of resistant and susceptible parents were treated at 0, 0.21, 0.42, 0.84, 1.68, 3.36, 6.72, and 13.44 kg ae ha^{-1} of glyphosate with two replications over time (Table 2). Glyphosate was not applied to plants for the 0 rate. To assess the number of loci segregating in the BC and F_2 populations, 70 to 90 BC plants and 120 F_2 plants per glyphosate rate and per population were treated at 0.21, 0.42, 0.84, or 1.68 kg ha^{-1} glyphosate (Table 3). Eight to 12 progeny plants produced from self-pollination of the resistant and susceptible parents were used as controls with the exception of the S8 × R2 BC, for which only resistant control plants were used. Plants were assessed visually as alive or dead 35 d after treatment.

Statistical Analyses. Dose response data on the proportion of survivors in reciprocal F_1 populations and progeny of self-pollination of respective resistant and susceptible parents were analyzed simultaneously with log-logistic equations using the R package drc (R Development Core Team 2012; Ritz and Streibig 2005). The four-parameter log-logistic model (LL.4) and the two-parameter log-logistic model (LL.2) with the lower and upper limits fixed at 0 and 1, respectively, were used. Since comparisons of the four-parameter and two-parameter models using an *F*-test were nonsignificant ($\alpha = 0.05$), the simpler two-parameter model was used to estimate glyphosate dose required to reduce the proportion of survivors to 0.5 (LD_{50}, i.e., 50% survival) and for comparisons of LD_{50} among the populations using the compParm function. The function compParm implements approximate *t* tests for parameter ratios deviating from 1 (Ritz and Streibig 2013). The P values were adjusted for multiple tests per experiment using Bonferroni corrections ($\alpha = 0.05$).

Glyphosate resistance was analyzed as a trait with two discrete phenotypic classes of R or S at specific rates of glyphosate treatment because some level of survival of an S genotype is expected at treatments below those rates and some level of mortality is expected for an R genotype above those rates. In addition, response of hairy fleabane to glyphosate is affected by seasonality and the associated environmental conditions (Moretti et al. 2013; Shrestha et al. 2008). Thus, the pattern of inheritance was analyzed at the rate of glyphosate treatment determined to be the lowest rate at which all R plants survived and all S plants died in each of the experiments conducted over time. The observed ratio of R and S was obtained by counting alive and dead plants in the segregating populations as R and S, respectively, at the determined rate in each

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Table 2. Glyphosate dose required to reduce the percentage of survivors to 50% (LD_{50}) ± SE for the self-pollinated progeny of the susceptible and resistant parents and the reciprocal F_1 populations of hairy fleabane. LD_{50} and standard error were calculated using the two-parameter log-logistic analysis (Ritz and Steibig 2005). LD_{50} values were compared within experiments using approximate *t* tests (Ritz and Steibig 2013) with Bonferroni corrections for multiple tests ($\alpha = 0.05$).

	S-19/R-38				S-2/R-8				S-14/R-4			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2	
	June 30, 2011		July 28, 2011		June 30, 2011		July 28, 2011		July 19, 2011		August 30, 2011	
	n	LD_{50}[a]	*n*	LD_{50}	*n*	LD_{50}	*n*	LD_{50}	*n*	LD_{50}	*n*	LD_{50}
S parent self	8–12	0.233 ± 0.008 a	8	0.091 ± 0.007 a	12	0.219 ± 0.036 a	12	0.201 ± 0.011 a	12	0.095 ± 0.004 a	8	ns[b]
R parent self	4–5	nm[b]	8–12	0.862 ± 0.022 b	12	0.829 ± 0.058 b	12	0.608 ± 0.024 b	4	0.451 ± 0.152 b	12	0.383 ± 0.033 a
F1(S × R)	8–13	1.300 ± 0.134 b	8–12	0.703 ± 0.020 c	8	0.685 ± 0.085 b	8	0.561 ± 0.035 bc	8	0.366 ± 0.015 bc	4	Ns
F1(R × S)	8–12	1.313 ±0.381 b	8	0.753 ± 0.879 bc	4	0.781 ± 0.150 b	4	0.452 ± 0.023 cd	12	0.293 ± 0.011 bd	12	0.181 ± 0.031 b

[a] LD_{50} in $\log_{10}$ glyphosate dose (kg ae ha^{-1}).
[b] Abbreviations: nm, no mortality; ns, no survivors.

experiment. The segregation data in the BC and F_2 populations were tested for goodness of fit to expected segregation ratios using the chi-square test ($\alpha = 0.05$) in R (R Development Core Team 2012). Expected segregation ratios were calculated for disomic rather than polysomic inheritance models because hairy fleabane is an allohexaploid with disomic inheritance (Thébaud and Abbott 1995).

Glyphosate resistance is controlled by a single locus in most cases within the three weed species in which inheritance of the trait has been investigated, including rigid ryegrass (Lorraine-Colwill et al. 2001; Wakelin and Preston 2006), goosegrass (Ng et al. 2004), and horseweed (Zelaya et al. 2007). However, two loci were reported to underlie glyphosate resistance in rigid ryegrass from California (Simarmata et al. 2005). Thus, chi-square tests were conducted for the one-locus model initially. If the observed ratio deviated significantly from the one-locus model, two-locus models were tested. Four two-locus models were tested, including additive and epistatic models that were consistent with expression of glyphosate resistance in the F_1 populations. In the additive models, the effects of the R alleles at the two independently segregating loci are equal and additive (Lande 1981). The additive models tested included those in which one or two copies of the alleles are required for expression of resistance. Two epistatic two-locus models were tested. In one model, two copies of the R alleles, at least one from each locus, are required for expression of resistance (Petit et al. 2010). In the other model, the R allele is dominant at one locus and recessive at the other locus.

Results and Discussion

Response to Glyphosate in Progeny of Self-Pollinated Parents. Based on the mortality of progeny from self-pollinated R and S parents, the threshold rate of glyphosate treatment at which to assess the homozygosity of parental plants was determined to be 0.84 kg ha^{-1} for R-38 and R-8 and 0.21 kg ha^{-1} for R-4 (Table 1). The proportion of progeny of self-pollination of the R parents that survived the threshold rate of glyphosate was 0.99, 1.00, and 0.96 for R-38, R-8, and R-4, respectively (Table 1), indicating that the three R parental parents were homozygous for resistance, based on evaluation of 132, 132, and 98 progeny, respectively. All S plants tested died at these three rates. Although treatment dates were the same or

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Table 3. Segregation of glyphosate resistance in backcross (BC) and F_2 populations in hairy fleabane and the chi-square tests for goodness of fit to the expected ratio under the one-locus model. The chi-square test was conducted on segregation data in the lowest glyphosate treatment rate at which all resistant controls survived and all susceptible controls died, shown in bold.[a]

Cross	Treatment date	Glyphosate treatment	S[b] control		R control		Observed		One-locus model	
			Alive	Dead	Alive	Dead	Alive	Dead	χ^2	P
		kg ae ha^{-1}								
S-19 × R-38										
BC	September 20, 2011	0.21	2	7	9	0	49	26	–	–
		0.42	**0**	**9**	**9**	**0**	**38**	**37**	0.01	0.91
		0.84	0	8	8	0	47	30	–	–
		1.68	0	9	9	0	26	49	–	–
F_2	October 21, 2011	0.21	8	4	12	0	113	7	–	–
		0.42	3	9	12	0	107	13	–	–
		0.84	**0**	**12**	**12**	**0**	**89**	**31**	0.04	0.83
		1.68	1	11	6	6	55	65	–	–
S-2 × R-8										
BC	September 23, 2011	**0.21**	**nd**	**nd**	**9**	**0**	**48**	**22**	9.66	1.9×10^{-3}
		0.42	**nd**	**nd**	**9**	**0**	**34**	**36**	0.06	0.81
		0.84	nd	nd	6	3	21	49	–	–
		1.68	nd	nd	1	8	8	62	–	–
F_2	October 17, 2011	0.21	7	5	12	0	112	8	–	–
		0.42	**0**	**12**	**12**	**0**	**95**	**25**	1.11	0.29
		0.84	0	12	10	2	77	43	–	–
		1.68	0	12	9	3	13	107	–	–
S-14 × R-4										
BC	January 6, 2012	0.21	9	0	9	0	90	0	–	–
		0.42	8	1	9	0	82	8	–	–
		0.84	6	3	9	0	67	23	–	–
		1.68	**0**	**9**	**9**	**0**	**19**	**71**	30.0	4.2×10^{-8}
F_2	January 6, 2012	0.21	12	0	12	0	120	0	–	–
		0.42	11	1	12	0	116	4	–	–
		0.84	4	8	12	0	111	9	–	–
		1.68	**0**	**12**	**12**	**0**	**80**	**40**	4.44	0.04

[a] Expected segregation ratio under the one-locus model for BC is 1 : 1 (R : S), and for F_2 is 3 : 1 (R : S).
[b] Abbreviations: S, glyphosate-susceptible; R, glyphosate-resistant; nd, not done.

similar for all the parental plants screened, suggesting similar environmental conditions, the parental pair R-4 and S-14 showed survival proportions of 0.96/0.00 (R/S) at a lower rate of 0.21 kg ha^{-1} glyphosate.

F_1 Dose Response Analysis. Log-logistic analysis of the dose response of the reciprocal F_1 populations and the progeny of self-pollinated S and R parents was conducted with the exception of the parent R-38 in experiment 1 and the parent S-14 and F_1 (S-14 × R-4) in experiment 2 (Figure 1, Table 2). Selfed progeny of the parent R-38 in experiment 1 survived all eight glyphosate doses: 0.21, 0.42, 0.84, 1.68, 3.36, 6.72, and 13.44 kg ha^{-1}. The parent S-14 and F_1 (S-14 × R-4) in experiment 2 had no survivors over the eight doses of glyphosate. No mortality observed for parent R-38 at all treatment rates and no survivors for F_1 (S-14 × R-4) under the lowest glyphosate doses may be mainly due to stochasticity associated with the small sample size, although particular environmental conditions may have influenced the results. The time of application of glyphosate, thus the environmental conditions of the experiment, appeared to affect the parental pair S-19 and R-38 and the parental pair S-14 and R-4 more than the parental pair S-2 and R-8. Consistent with the screening of the R parents for segregation (Table 1), the LD_{50} of the parents S-14 and R-4 tended to be lower than for the other parental pairs.

The LD_{50} of the reciprocal F_1 populations, when both were available, were not significantly different from each other in all crosses based on the approximate *t* test (Table 2), indicating nuclear control of glyphosate resistance. Consistent with dominant or incompletely dominant expression of resistance over susceptibility, the LD_{50} of the S parent was significantly lower than the R parent and

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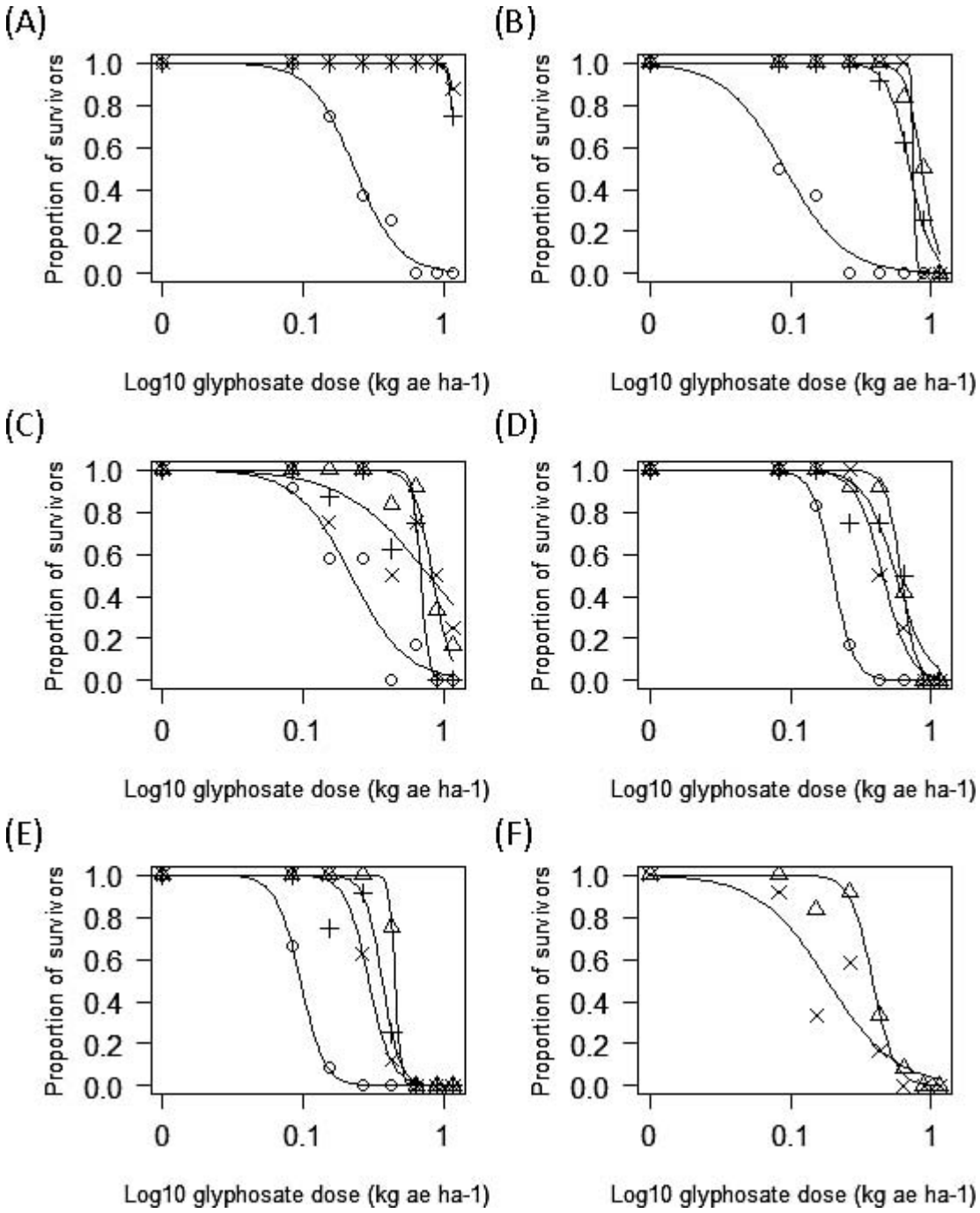

Figure 1. Fit of the two-parameter log-logistic model for proportion of survivors in response to glyphosate treatments in the progeny of self-pollinated susceptible (circle) and resistant (triangle) parents and the reciprocal F_1 populations, F_1 (S × R) (+) and F_1 (R × S) (×) in hairy fleabane. Plots for parents S19 and R28 in the (A) first experiment and (B) second experiment, parents S2 and R8 in the (C) first experiment and (D) second experiment, and parents S14 and R4 in the (E) first experiment and (F) second experiment.

the reciprocal F_1 populations in all crosses, based on available estimates. The LD_{50} value of the R parent was greater than those of the F_1 populations but was not significantly different from either or only one of the reciprocal F_1 populations based on the approximate *t* test, also consistent with dominance to incomplete dominance of resistance. Based on the dose response analysis of the F_1 populations, glyphosate resistance that is segregating in the three crosses is under nuclear control and is dominant to incompletely dominant over susceptibility consistent with all previous studies of inheritance of glyphosate resistance, including, rigid ryegrass (Lorraine-Colwill et al. 2001; Simarmata et al. 2005; Wakelin and Preston 2006), goosegrass (Ng et al. 2004), and horseweed (Zelaya et al. 2007).

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Table 4. Segregation of glyphosate resistance (R) in backcross (BC) and F_2 populations derived from S-14 and R-4 as parents in hairy fleabane and expected ratios under five two-locus models and the P values from chi-square tests for goodness of fit.

		Observed		Additive		Epistatic	
	Glyphosate treatment	Alive	Dead	One copy	Two copies	Two copies, at least one in each locus	Dominant R at one locus, recessive R at the other
	kg ae ha^{-1}						
BC	1.68	19	71	3 : 1 2.2×10^{-16}	1 : 3 0.39	1 : 3 0.39	1 : 1 4.2×10^{-8}
F_2	1.68	80	40	15 : 1 2.2×10^{-16}	11 : 5 0.62	7 : 9 4.2×10^{-7}	13 : 3 4.3×10^{-5}

Segregation in BC and F_2 Populations. Using the chi-square test, the segregation ratios observed for the BC and F_2 populations for all crosses were initially tested for goodness of fit to one-locus phenotypic ratios expected for a nuclear, dominant, or incompletely dominant trait at the lowest glyphosate rates at which all S controls died and all R controls survived (Table 3). Because there were no data available for the S control for the BC population of the S-2 and R-8 parents, the segregation data under both 0.21 and 0.42 kg ha^{-1}, at which all R controls survived, were tested. The levels of resistance of the F_1 populations were not significantly different from the R parents (Table 2). Thus, based on the dose response analysis, heterozygous plants of the BC and F_2 populations, like the F_1 populations, should survive at the lowest rate at which all R control plants survived and all S control plants died.

The segregation ratios observed in the BC and F_2 populations for the parents S-19 and R-38 did not deviate significantly from the expected ratio of 1 : 1 in BC and 3 : 1 in F_2 populations for a dominant trait controlled by a single nuclear locus (Table 3). Similarly, for the parents S-2 and R-8, the observed segregation ratios in the BC and F_2 generations conformed to the expected ratios under the single-locus model at 0.42 kg ha^{-1} of glyphosate treatment. The observed ratio for the BC population of the same parents under 0.21 kg ha^{-1} of glyphosate treatment deviated significantly from the expected ratio, but a substantial number of S individuals likely would have survived the treatment rate, based on the screening of the R parents at four different glyphosate rates (Table 1) and the dose response analysis (Table 2). Unlike the parents S-19 and R-38 and parents S-2 and R-8, the observed ratios in the BC and F_2 populations for the parents S-14 and R-4 deviated significantly from the expected ratios under the single-locus model, indicating that glyphosate resistance is segregating at more than one locus in this cross. The observed ratios deviated significantly from expected ratios under one of the two additive two-locus models and both epistatic two-locus models (Table 4). However, the observed BC and F_2 ratios conformed to the expected ratios of a two-locus additive model in which at least two doses of the resistance allele are needed for the expression of resistance based on the chi-square test of goodness of fit (Table 4). Under this two-locus, additive-two-dose model, the R-4 parent would have been homozygous at the two loci at 0.21 kg ha^{-1} of glyphosate treatment (Table 1, $P > 0.05$).

Multiple Mechanisms of Glyphosate Resistance. Based on our results, glyphosate resistance is controlled by a single locus between parents S-19 and R-38 and parents S-2 and R-8. In goosegrass, glyphosate resistance due to the target-site mutation in the gene for the herbicide's target enzyme, EPSPS, segregates as a single locus (Ng et al. 2004) as expected, and resistance due to altered translocation also segregates as a single locus in rigid ryegrass (Lorraine-Colwill et al. 2001) and in horseweed (Zelaya et al. 2007), which is closely related to hairy fleabane. The mechanism of glyphosate resistance in hairy fleabane in California is unknown. However, a glyphosate-resistant biotype of hairy fleabane from Spain showed differential translocation of the herbicide and increased expression of the herbicide's target enzyme, EPSPS, but a target-site mutation in the EPSPS gene is unlikely to be a mechanism based on shikimate accumulation in R plants (Dinelli et al. 2008).

Interestingly, for parents S-14 and R-4, the observed segregation ratio in both BC and F_2 populations conformed to a two-locus model in which the R alleles across loci work additively, and at least two doses of the R allele are needed for the R phenotype. This model of glyphosate resistance differs from the only reported case of glyphosate resistance segregating at two loci, which was reported for rigid ryegrass in California (Simarmata

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et al. 2005). The additive two-locus model also has never been tested in any glyphosate-resistant weed populations investigated. The different pattern of inheritance is unlikely to be a result of the genetic background of the susceptible parent, S-14, since all S parents were obtained from a seed lot that showed no genetic variation, based on microsatellite marker analysis of a sample of 30 plants grown from the seed lot (Okada, Hanson, Hembree, Peng, Shrestha, Stewart, Wright, and Jasieniuk, unpublished data). Also, none of the S parents appears to be segregating for resistance (Table 1). Based on the distinct pattern of inheritance in the parents S-14 and R-4 (Table 4) and approximately half the resistance level of the R-4 parent in comparison to the other two R parents (Table 1), the mechanism (or mechanisms) of resistance is different from the one segregating in the parents S-19 and R-38 and the parents S-2 and R-8. Thus, at least two different mechanisms of glyphosate resistance are present in hairy fleabane populations in California.

The two loci controlling resistance may represent two different mechanisms within an R plant as observed in hairy fleabane from Spain (Dinelli et al. 2008) and rigid ryegrass from South Africa (Kaundun et al. 2011; Yu et al. 2007). However, the requirement of two doses for expression of resistance in the model of segregation is inconsistent with presence within a plant of two mechanisms of glyphosate resistance that are each under single-locus control with dominance or incomplete dominance such as the target-site mutation and altered pattern of translocation identified in populations of other weed species (Lorraine-Colwill et al. 2001; Ng et al. 2004; Wakelin and Preston 2006; Zelaya et al. 2007). The mechanism (or mechanisms) of resistance underlying the distinct pattern of inheritance in the cross between S-14 and R-4 may also be different from previously identified mechanisms of glyphosate resistance, such as has been observed in a South African rigid ryegrass population (Kaundun et al. 2011) or in a rigid ryegrass population after selection under sublethal doses (Busi and Powles 2009, 2011). Further investigations are required to determine the underlying cause of the plasticity in response to glyphosate and the effect of the plasticity on the evolution of glyphosate resistance in California populations of hairy fleabane.

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