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ORIGINAL PAPER

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Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L.) Cronq.

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Abstract *N*-(phosphonomethyl)glycine (glyphosate) resistance was previously reported in a horseweed [*Conyza* (= *Erigeron*) *canadensis* (L.) Cronq.] population from Houston, DE (P_0^R). Recurrent selection was performed on P_0^R, since the population was composed of susceptible (5%) and resistant (95%) phenotypes. After two cycles of selection at 2.0 kg ae glyphosate ha^{-1}, similar glyphosate rates that reduced plant growth by 50%, glyphosate rates that inflicted 50% mortality in the population, and accumulations of half of the maximum detectable shikimic acid concentration were observed between the parental P_0^R and the first (RS_1) and second (RS_2) recurrent generations. In addition, RS_1 and RS_2 did not segregate for resistance to glyphosate. This suggested that the RS_2 population comprised a near-homozygous, glyphosate-resistant line. Whole-plant rate responses estimated a fourfold resistance increase to glyphosate between RS_2 and either a pristine Ames, IA (P_0^P) or a susceptible *C. canadensis* population from Georgetown, DE (P_0^S). The genetics of glyphosate resistance in *C. canadensis* was investigated by performing reciprocal crosses between RS_2 and either the P_0^P or P_0^S populations. Evaluations of the first (F_1) and second (F_2) filial generations suggested that glyphosate resistance was governed by an incompletely dominant, single-locus gene (*R* allele) located in the nuclear genome. The proposed genetic model was confirmed by back-crosses of the F_1 to plants that arose from achenes of the original RS_2, P_0^P, or P_0^S parents. The autogamous nature of *C. canadensis*, the simple inheritance model of glyphosate resistance, and the fact that heterozygous genotypes (F_1) survived glyphosate rates well above those recommended by the manufacturer, predicted a rapid increase in frequency of the *R* allele under continuous glyphosate selection. The impact of genetics on *C. canadensis* resistance management is discussed.

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Introduction

Since its commercial introduction in 1974, *N*-(phosphonomethyl)glycine (glyphosate) has become the most important herbicide worldwide, primarily for its favorable characteristics: low mammalian toxicity, rapid degradation in the environment and resultant minimal ground water contamination, and effective systemic activity on a diverse flora (Baylis 2000). Glyphosate inhibits 3-phosphoshikimate 1-carboxyvinyltransferase (EPSPS; EC 2.5.1.19), blocking the synthesis of important compounds derived from the shikimic acid pathway, instigating ultrastructural atrophy, and arresting protein synthesis (Mollenhauer et al. 1987; Muñoz-Rueda et al. 1986; Steinrücken and Amrhein 1980). Glyphosate resistance has been engineered through transformation with the metabolizing genes glyphosate oxidoreductase (*GOX*) and glyphosate *N*-acetyltransferase (*GAT*) (Barry et al. 1992; Castle et al. 2004), expression of an insensitive *EPSPS* (Padgette et al. 1991), *EPSPS* amplification (Shah et al. 1986), and enhanced *EPSPS* transcription (Klee et al. 1987). However, few resistance cases have evolved despite the prolonged glyphosate use worldwide. The short half-life ($t_{1/2}$) in the environment, unique biochemical characteristics, and complex molecular modifications required to engineer glyphosate-resistant crops were purported reasons for the low frequency of glyphosate resistance in weeds (Bradshaw et al. 1997).

The first confirmed glyphosate-resistant weed was *Lolium rigidum* Gaudin, where a seven- to 11-fold

resistance evolved after 15 years of continuous glyphosate application (Powles et al. 1998). Differences in glyphosate uptake, translocation, or metabolism were disregarded as potential resistance mechanisms in *L. rigidum*, suggesting that resistance may be conferred by *EPSPS* overexpression, an insensitive EPSPS, or improper targeting of glyphosate to the loci of action (Feng et al. 1999; Lorraine-Colwill et al. 1999). More recently, the mechanism of resistance in *L. rigidum* was credited to differences in cellular translocation of glyphosate (Lorraine-Colwill et al. 2003). Since reports of the *L. rigidum* biotype, glyphosate resistance was confirmed in *Eleusine indica* (L.) Gaertner (Lee and Ngim 2000), *L. multiflorum* Lam. (Pérez and Kogan 2003), *Conyza bonariensis* (L.) Cronq., and *Plantago lanceolata* L. (Heap 2004). Resistance in *L. multiflorum* is apparently not associated with differences in glyphosate absorption or translocation (Pérez et al. 2004). The resistance mechanism(s) in *C. bonariensis* and *P. lanceolata* is/are unknown to date; however, glyphosate resistance in *E. indica* was ascribed to a polymorphic, resistant *EPSPS* (Baerson et al. 2002b). At least one more glyphosate resistance mechanism, in addition to target site modification, apparently exists in another *E. indica* population from Malaysia (Ng et al. 2004a). Plausible glyphosate resistance mechanisms include sequestration (Foley 1987), cellular compartmentation (Hetherington et al. 1998), differential translocation (Tucker et al. 1994), enhanced metabolism (Komoßa et al. 1992), increased transcription, or extended $t_{1/2}$ of the peptide encoded by *EPSPS* (Holländer-Czytko et al. 1992).

Conyza (= *Erigeron*) *canadensis* (L.) Cronq. (Asteraceae) is a winter or summer annual North American native weed of importance in no-tillage crop production systems (Buhler and Owen 1997). *C. canadensis* is considered one of the ten most important herbicide-resistant weeds, evolving resistance to triazine, amide, bipyridilium, imidazolinone, and sulfonylurea herbicides in more than ten countries worldwide (Heap 2004). Northeast US farmers rely on glyphosate in combination with residual herbicides for full-season *C. canadensis* management in glyphosate-resistant crops (VanGessel et al. 2001). Increased selection pressure resulted in inconsistent *C. canadensis* control with two split applications of 1.6-kg acid equivalents (ae) ha^{-1} of glyphosate in glyphosate-resistant soybean [*Glycine max* (L.) Merr.] fields near Houston, DE. Whole-plant rate responses confirmed that the Houston biotype had an eight- to 13-fold resistance increase compared to a susceptible Georgetown, DE, biotype, requiring rates of 0.84 kg ha^{-1} and 8.8 kg ha^{-1} glyphosate to achieve control of the susceptible and resistant *C. canadensis* biotypes, respectively (VanGessel 2001). Noteworthy is the confirmation of least ten additional independent glyphosate-resistant *C. canadensis* populations throughout the United States (Heap 2004).

Despite the global importance of glyphosate, limited information exists regarding the identity, frequency, and cellular location of genes associated with glyphosate resistance in plants. Herein we report on the inheritance of glyphosate resistance in the *C. canadensis* population from Houston, propose a model for the resistance gene (*R* allele), and assess the level of allogamy between *Conyza* populations.

Materials and methods

Source of plant materials

The pristine *C. canadensis* population (P_0^P) was obtained from the Weed Science seed collection at Iowa State University (ISU), Ames, IA. ISU records indicated that P_0^P evolved without the selection pressure of glyphosate, in a wild, undisturbed area in the vicinity of Gateway Park in Ames. The glyphosate-resistant *C. canadensis* population (P_0^R) was collected in a soybean field near Houston, where plants survived 1.6 kg glyphosate ha^{-1}, a rate that effectively controlled the population in years prior. Evolution of the resistant population occurred in a no-tillage production system where glyphosate applied preplant and in glyphosate-resistant soybeans was the sole control method in 1998–2000. The glyphosate-susceptible *C. canadensis* population (P_0^S) was collected at the University of Delaware's Research and Education Center (UD–REC) near Georgetown, in a field untreated with glyphosate for at least 5 years (VanGessel 2001). The P_0^P, P_0^S, and P_0^R populations possessed stems with coarsely spreading hirsute and lacked purple tips on bracts; therefore, the populations were classified as *C. canadensis* var. *canadensis* (Gleason and Cronquist 1991).

Achene storage and plant growth conditions

P_0^P achenes were collected in 1994 by removing the inflorescence of mature *C. canadensis* plants in the field. The capitula were then allowed to dry at room temperature and achenes stored at 5°C until 2002. Similarly, P_0^R and P_0^S achenes were harvested from mature plants grown in the greenhouse, allowed to dry at room temperature, and stored at 5°C. For all three populations, achenes were planted in flats containing a peat:perlite:loam (1:2:1) soil-mix media, and 1 week after seedling emergence, individual plants were transplanted to 12-cm diameter pots. Plants were grown in a greenhouse set at 28–35°C and 50–80% relative humidity (RH) day and 20–25°C and 50% RH night conditions, and natural light was supplemented to a 16-h photoperiod with artificial illumination at 600–1,000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Plants were irrigated as needed and fertilized (Miracle Gro Excel, Scott–Sierra Horticultural Products, Marysville, OH, USA) 1 month after transplanting. Prior to anthesis, plants used in crosses were transferred to a growth cabinet set at a 16-h photoperiod, 35°C day, 25°C night, 70–90% RH, and 600 μmol m^{-2} s^{-1} PPFD conditions.

Parental *Conyza* populations response to glyphosate

Classification of glyphosate-resistant, intermediate-resistant, and susceptible phenotypes

The manufacturer's recommended glyphosate rate is 0.85 kg ae ha^{-1} of the isopropylamine salt of glyphosate (Roundup UltraMAX, Monsanto, St. Louis, MO, USA) sprayed on 10-cm diameter *C. canadensis* rosettes (Anonymous 2004). Typical glyphosate toxicity symptoms on *C. canadensis* included meristematic and leaf margin necrosis, leaf chlorosis especially in the area between veins, and arrested plant growth. Treatment of P_0^P or P_0^S rosettes at the 10-cm diameter stage with 2.0 kg glyphosate ha^{-1} caused visual herbicide injury levels ≥70% and uniform mortality 20 days after treatment (DAT). These parameters were adopted to define the glyphosate-susceptible (S) phenotype. Conversely, treatment of P_0^R rosettes at the same stage and glyphosate rate resulted in marginal visual herbicide injuries (≤ 30%), thus prompting classification of the glyphosate-resistant (R) phenotype. A third phenotype was identified in the progenies of crosses (explained below): the intermediate-resistant (IR) classification comprised plants that developed 31–69% visual injuries when treated as described for the other two classifications. Both R and IR phenotypes reached reproductive stage; however, IR phenotypes demonstrated slower growth rates than R phenotypes. No visual difference in growth rates was observed between R phenotypes and untreated *C. canadensis* plants. Phenotypic proportions within populations were estimated following treatment of plants with 2.0 kg glyphosate ha^{-1} as described above.

Glyphosate rate responses

The performance of *C. canadensis* populations to glyphosate was evaluated by testing the response of 10-cm diameter rosettes to deionized water ($_dH_2O$, control), 0.42, 0.85, 1.69, 3.38, 6.77, or 13.54 kg glyphosate ha^{-1}. Glyphosate treatments were applied 30 cm from the plant canopy with an even, flat-fan nozzle (80015-E, TeeJet Spraying Systems, IL, USA) in a CO_2-powered spray chamber (SB5–66, DeVries Manufacturing, MN, USA) delivering 187 l ha^{-1} at 2.8 kg cm^{-2}. Each treatment had four replicates, and the experiment was repeated ($n = 8$). The herbicide was applied in the morning and plants returned to the greenhouse. Glyphosate efficacy was evaluated 20 DAT by calculating the percentage of visual injury of treated plants compared to the $_dH_2O$-treated control. Biomass measurements were determined by cutting rosettes at the soil surface, drying at 80°C for 48 h in paper bags, and estimating the weight of individual plant samples. Glyphosate efficacy was also assessed by monitoring the accumulation of endogenous shikimic acid (3*R*, 4*S*, 5*R* trihydroxy-1-cyclohexene-1-carboxylic acid) in a dry sub-sample of each treated *C. canadensis* plant (explained below).

Endogenous shikimic acid extraction and determination

A 0.5 g of biomass sub-sample was assayed in duplicate to estimate endogenous shikimic acid levels, using a spectrophotometric protocol modified from Cromartie and Polge (2002). The dry *C. canadensis* tissue was ground with 2.5-mm glass beads for 10 min in a Bead-Beater (BioSpec Products, Bartlesville, OK, USA) and shikimic acid extracted in a 1:10 tissue:0.25 N HCl for 48 h at 5°C. The samples were then centrifuged at 15,000 *g* for 15 min to precipitate cell debris and a 5–10 µl aliquot sample was oxidized with 22 mM periodate plus sodium *meta*-periodate for 45 min at 45°C. The shikimic acid chromophore was generated by adding 1 M NaOH and immediately stabilized with 56 mM Na_2SO_3. Finally, absorbance was detected at 382 nm (A_{382}), and a previously prepared standard curve at 1–60 µmol ml^{-1} shikimic acid (Sigma-Aldrich, Saint Louis, MO, USA) was used to convert A_{382} data to micromole shikimic ae per gram dry weight.

Genetic analysis of glyphosate resistance

Recurrent selection of the resistant C. canadensis material

Greenhouse evaluations indicated that the P_0^P and P_0^S populations were uniformly susceptible to glyphosate, while approximately 95% of the individuals in the P_0^R population were resistant to glyphosate. Therefore, a stable, homogenous, resistant population was isolated through two cycles of recurrent selection. P_0^R rosettes were treated with 2.0 kg glyphosate ha^{-1} at the 10-cm diameter stage, evaluated for efficacy 20 DAT, and ten plants with a resistant phenotype allowed to grow and self-pollinate in the greenhouse. The resulting population comprised the first recurrent generation (RS_1). Accordingly, the second recurrent generation (RS_2) was isolated by undergoing another cycle of selection on RS_1 material as indicated in this section. Intraspecific and back-crosses were conducted with P_0^P, P_0^S, and RS_2 plants with a confirmed phenotype (explained below).

Phenotypic confirmation of parents utilized in crosses

Both P_0^P and P_0^S populations were not selected, since rate responses confirmed that these populations were susceptible for glyphosate. Treatment of 10-cm diameter P_0^P or P_0^S rosettes with 0.4 kg glyphosate ha^{-1} resulted in ≤ 60% visual injuries and a reduction in rosette growth rate compared to untreated *C. canadensis* rosettes. However, P_0^P or P_0^S rosettes treated with the 0.4-kg ha^{-1} rate recovered from injuries within 2–4 weeks and reached reproductive stage. Concomitantly, the 0.4-kg ha^{-1} sub-lethal glyphosate rate permitted non-destructive confirmation of the susceptible parents utilized in the interspecific and back-crosses (explained below). The resistant parents were confirmed by treating

10-cm diameter RS_2 rosettes with 2.0 kg glyphosate ha^{-1} and evaluating efficacy to the herbicide 20 DAT; rosettes with $\leq 30\%$ injury were used in the crosses.

Estimates of allogamy through assisted intraspecific crosses

Ten RS_2 and $P_0{}^S$ rosettes confirmed phenotypically, as indicated in the previous section, were grown in the greenhouse and transferred to the growth cabinet approximately 2 weeks prior to anthesis. Assisted crosses were performed between the glyphosate-resistant and -susceptible phenotypes to assess the levels of cross-pollination (allogamy) between *C. canadensis* plants. Ten RS_2 and $P_0{}^S$ plant-pairs (families) were allowed to grow in isolation. At anthesis, the inflorescences of RS_2 and $P_0{}^S$ plant pairs were permitted to interact physically inside a PQ218 DelNet bag (DelStar Technologies, Middletown, DE, USA), thus restricting pollen release within the bag and limiting contamination from external pollen sources. Percentage allogamy was estimated by determining the frequency of IR phenotypes within full-sibling populations.

Intraspecific artificial crosses and back-crosses

Given that *C. canadensis* has white pistillate ray and yellow, perfect disk florets and that some self-fertilization can occur prior to anthesis (Weaver 2001), capitula emasculation was performed in artificial crosses to ensure the origin of pollen used to fertilize the ovum in pollen-receptor plants. Disk florets from unopened capitula were removed with forceps under a magnifying lens so that only ray florets remained (emasculation); an estimated 50 capitula per plant were emasculated. The remaining non-emasculated capitula were removed from plants to limit self-fertilization. Approximately 5 days post-emasculation, the remaining pistillate florets became receptive, upon which stigmas were fertilized by gently rubbing the intact capitula of pollen-donor plants. Emasculated capitula were fertilized daily for 1 week, achenes allowed to mature on the mother plant, and removed when the pappus became visible. Finally, the mature achenes were germinated in soil-mix media, and the resulting seedlings grown in the greenhouse. If emasculation were completely effective at eliminating self-fertilization in *C. canadensis*, emasculated capitula that matured in the absence of pollen would produce non-viable achenes. Therefore, the efficiency of emasculation was tested by assessing the non-germination of 50 emasculated capitula in each of ten *C. canadensis* plants that developed inside a DelNet bag.

Twenty RS_2 and ten $P_0{}^P$ and $P_0{}^S$ plants previously confirmed phenotypically were crossed in reciprocal (R×S, S×R), totaling ten families per parent pair combination. The progeny of these crosses, representing the first filial generation (F_1), were treated with 2.0 kg glyphosate ha^{-1} and individual F_1 plants classified phenotypically. One F_1 plant per family was allowed to self-pollinate in isolation, and the efficacy to glyphosate in the second filial generation (F_2) was assessed through whole-plant rate responses and phenotypically at the single 2.0-kg ha^{-1} rate. To test the genetic model, one F_1 plant per family was back-crossed to plants that derived from achenes of the original parents; these populations were labeled BC_R (RS_2), BC_P ($P_0{}^P$), or BC_S ($P_0{}^S$), depending on the parent used in the back-cross. Three plants from each of the 20 generated families were randomly selected ($n = 60$) to assess the rate response of F_1 and F_2 rosettes to glyphosate.

Statistical analysis

All statistical analyses were conducted with the Statistical Analysis System (SAS 2000). Replications in time were tested for patterns of covariance matrices that satisfied the Huynh–Feldt condition (option PRINTE) (Huynh and Feldt 1970). When the sphericity test confirmed that the covariances were type H, *F*-statistics tested the univariate analyses for within time effects and related interactions. Whole-plant rate responses were evaluated by analysis of variance (ANOVA) as a randomized complete block design with four replications and repeated once in time (PROC GLM). When ANOVA identified significant population effects, mean separation was conducted with Fisher's least significant difference test at the $\alpha \leq 0.05$ level. Visual injury data were converted to a dichotomous distribution, following the classification for R ($< 69\%$) and S ($\geq 70\%$) phenotypes. The transformed injury data were then analyzed with a modified Newton–Raphson algorithm (PROC PROBIT) to estimate the glyphosate rate that inflicted 50% mortality in the population (LD_{50}) (Collett 2002). In addition, biomass and shikimic acid data were subjected to log-logistic analysis (Gauss–Newton method) and the glyphosate rate that reduced plant growth by 50% (GR_{50}) or instigated accumulation of half of the maximum detectable shikimic acid concentration (I_{50}) was calculated (PROC NLIN) (Seefeldt et al. 1995). The non-linear model fit to the data was assessed graphically by the distribution of residuals and statistically by lack-of-fit (LOF) tests and pseudo-coefficients of determination [$R^2_{(pseudo)}$] (Schabenberger et al. 1999; Seefeldt et al. 1995). Biomass data model to the reparameterized Brain–Cousens equation (Marquardt–Levenberg method) allowed for estimation of the probability (P) for the absolute difference between two calculated GR_{50} values ($|\lambda_{50}|$) (Schabenberger et al. 1999). The relationship strength between the estimated whole-plant rate response parameters and endogenous shikimic acid levels was determined by Spearman's linear correlation analysis.

The phenotypic F_1, F_2, BC_R, BC_P, and BC_S data were analyzed according to Cochran–Mantel–Haenszel statistics. The proposed genetic model was tested by comparing the observed R, IR, and S segregation ratios in full-siblings (families) against the expected Mendelian

proportions for the model with a chi-square (χ^2) goodness-of-fit (GOF) test. Homogeneity χ^2 analysis was performed to ascertain whether combination of the segregation data within families was suitable. The null hypothesis (H_0) of monofactorial inheritance was tested by comparing the observed F_2 mortality to that expected as suggested by Tabashnik (1991): $Y_\chi = W_R\ (0.25) + W_{IR}\ (0.50) + W_S\ (0.25)$.

Results

C. canadensis populations responded distinctively to glyphosate

Recurrent selection increased the frequency of resistant phenotypes

Greenhouse experimentation established that all 59 P_0^P and 73 P_0^S rosettes evaluated were uniformly susceptible to glyphosate at the 2.0-kg ha^{-1} rate; in contrast, only 78 of 82 P_0^R rosettes treated at this same rate demonstrated a resistant phenotype (Tables 3, 4). This suggested that the P_0^R population was composed of homozygous, susceptible (5%) and resistant (95%) genotypes. Thus, recurrent selection was imposed on P_0^R plants to isolate a stable, homogenous, resistant population. Evaluations of RS_1 and RS_2 plants confirmed that all 79 and 84 rosettes evaluated, respectively, had a resistant phenotype at the 2.0-kg glyphosate ha^{-1} rate (Table 3). Recurrent selection results therefore suggested that the RS_2 population comprised a homogenously resistant line. Resistant RS_2 phenotypes demonstrated only limited injury to glyphosate at the 2.0-kg ha^{-1} rate, had growth rates analogous to the untreated P_0^P or P_0^S rosettes, and were able to complete the reproductive cycle. To investigate the rate response of the parental and selected populations to glyphosate, the sums of squares and cross-products matrix of experiments conducted in time were first estimated to assess the suitability for a combined data analysis. These estimates provided a statistically significant partial correlation estimate for biomass ($r^2 = 0.33$, $P < 0.001$) and visual injury ($r^2 = 0.54$, $P < 0.001$), suggesting a strong relationship strength between the measurements acquired in time. Concurrently, multivariate ANOVA test for the H_0 of no-time effect resulted in non-significant estimates for biomass (Wilks' $\lambda = 0.99$, $P = 0.66$) and visual injury (Wilks' $\lambda = 0.99$; $P = 0.62$); therefore, a negligible effect of replication in time was inferred, and data were combined.

Parental populations represent near-homozygous lineages

Adequacy of the log-logistic model for describing the population response to increasing glyphosate rates was calculated by LOF and coefficient-of-determination estimates. Satisfactory overall quality model fit was confirmed by the resulting $R^2_{(pseudo)}$ values for biomass (0.73) and shikimic acid (0.72) measurements, and the LOF test (biomass: $F = 0.58$, $P = 0.97$; shikimic acid: $F = 0.47$, $P = 0.99$). Therefore, it was inferred that parameters estimated by the log-logistic model described the response of *C. canadensis* populations to glyphosate. Approximately 0.5 kg glyphosate ha^{-1} was the effective rate reducing plant growth by 50% in either P_0^P or P_0^S populations (Table 1), and the absolute difference between the estimated GR_{50} values was not statistically different ($|\lambda_{50}| = 0.03$; $F_{obs} = 0.82$; $P = 0.96$). The glyphosate rate required to inflict 50% mortality on either P_0^P or P_0^S populations was also similar (Table 1). Hence, the performance to glyphosate of both the pristine P_0^P and susceptible P_0^S populations was considered equivalent.

At least fourfold and sevenfold resistance increases to glyphosate, respectively, were estimated in the P_0^R

Table 1 Summary of whole-plant rate responses for the evaluated *Conyza canadensis* populations. Numbers in *parenthesis* designate the 95% confidence intervals [plant growth reduced by 50% (GR_{50}), half of the maximum detectable shikimic acid concentration (I_{50})] or 95% fiducial limits [*N*-(phosphonomethyl)glycine (glyphosate) rate that inflicted 50% mortality in the population (LD_{50})] for the preceding estimated parameter. Spearman's correlation (r^2) estimated the association strength between shikimic acid levels and biomass or visual injury

Population[a]	Source	GR_{50}[b]	LD_{50}[c]	I_{50}[d]	Biomass (r^2)[e]	Injury (r^2)[e]
P_0^P	Ames, IA	0.53 (0.43–0.63)	0.91 (0.61–1.34)	1.88 (1.20–2.57)	−0.64	0.75
P_0^S	Georgetown, DE	0.50 (0.41–0.59)	1.16 (0.77–1.76)	2.06 (1.44–2.67)	−0.60	0.74
P_0^R	Houston, DE	2.11 (1.43–2.79)	8.80 (6.22–12.63)	3.87 (0.95–6.80)	−0.73	0.82
RS_1	Houston, DE	2.00 (1.37–2.63)	9.69 (6.72–16.30)	4.38 (0.18–8.59)	−0.68	0.84
RS_2	Houston, DE	2.03 (1.36–2.70)	10.49 (7.40–16.72)	3.10 (1.27–4.94)	−0.67	0.76
F_1[f]	Artificial cross	1.21 (0.85–1.57)	2.80 (1.83–4.40)	2.33 (1.48–3.19)	−0.79	0.85
F_2[f]	Artificial cross	1.62 (1.11–2.14)	3.62 (2.32–6.14)	3.38 (1.17–5.59)	−0.69	0.70

[a] P_0^P pristine population; P_0^S glyphosate-susceptible population; P_0^R glyphosate-resistant population; RS_1, P_0^R selected at 2.0 kg glyphosate ha^{-1}; RS_2, RS_1 selected at 2.0 kg glyphosate ha^{-1}; F_1 first filial generation; F_2 second filial generation

[b] Glyphosate rate in kg ha^{-1} that reduced biomass accumulation by 50%

[c] Glyphosate rate in kg ha^{-1} that inflicted 50% mortality in the population

[d] Glyphosate rate in kg ha^{-1} that resulted in accumulation of half of the total extractable shikimic acid in the tissue of treated plants

[e] The probability of $|r|$ was greater than 0.001 for all estimates; therefore, the null hypothesis (H_0) that $r = 0$ was rejected

[f] Three randomly selected rosettes per each of the 20 generated families ($n = 60$) were used to test the rate response to glyphosate

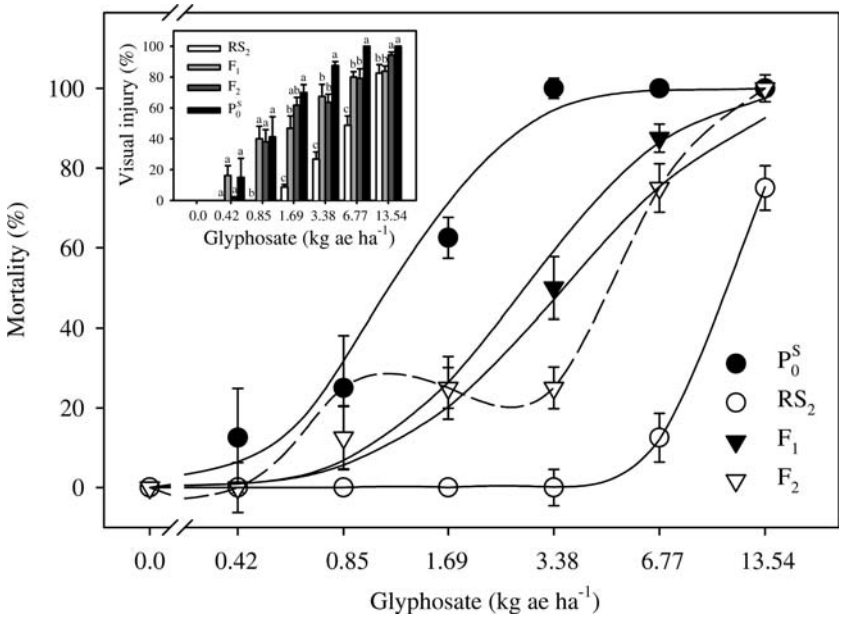

Fig. 1 *Main plot* Observed mortality at 20 days after treatment (DAT) of *Conyza canadensis* from Georgetown, DE [(P_0^S) *filled circle*, *black bar*], the Houston, DE, selected twice at 2.0 kg glyphosate ha^{-1} [(RS_2) *open circle*, *white bar*], the first filial [(F_1) *filled inverted triangle*, *gray bar*], and the second filial [(F_2) *inverted triangle*, *dark gray bar*] populations to *N*-(phosphonomethyl)glycine (glyphosate). *Solid lines* represent the percentage mortality estimated by PROBIT, whereas the *broken line* represents the expected F_2 mortality calculated by assuming monogenic inheritance. *Inset* Visual herbicide injury of treated *C. canadensis* plants. *Letters above the bars* designate the minimum statistical difference ($LSD_{\alpha 0.05}$) between populations for a single rate. Each *data point* or *bar* represents the mean of four replications and two experiments conducted at different times ($n = 8$). Three randomly selected plants per each of the 20 generated families ($n = 60$) were used to estimate the response of the F_1 and F_2. *Extensions on symbols or bars* designate the standard error associated with individual means (σ_M)

population compared to either P_0^P or P_0^S based on biomass (GR_{50}) or mortality (LD_{50}) responses (Table 1). More visual injury was also recorded in susceptible, compared to resistant phenotypes, above the 0.85-kg ha^{-1} glyphosate rate (Fig. 1). It was of interest to ascertain whether the calculated GR_{50} value for the resistant and susceptible populations differed statistically. Therefore, $|\lambda_{50}|$ values were calculated for contrasts between P_0^R and P_0^P or P_0^S, resulting in values of 1.61 kg ha^{-1} ($F_{obs} = 1.33$, $P = 0.006$) and 1.58 kg ha^{-1} ($F_{obs} = 1.27$, $P = 0.016$), respectively. The statistical significance of these contrasts confirmed that the *C. canadensis* P_0^R population differed in response to glyphosate from the P_0^S and P_0^P populations. Other confirmed cases of glyphosate resistance ascribed GR_{50} values of 1.2 kg ha^{-1} in *L. multiflorum*, 4.9 kg ha^{-1} in *E. indica*, and 4.6–5.1 kg ha^{-1} in *L. rigidum* (Lee and Ngim 2000; Lorraine-Colwill et al. 2001; Pérez and Kogan 2003).

Since P_0^R had some susceptible phenotypes (5%), and if resistance to glyphosate in *C. canadensis* were inherited as a dominant trait, recurrent selection would increase the frequency of resistant individuals and therefore, the overall population response to glyphosate. To examine this possibility, rate responses were conducted on the RS_1 and RS_2 populations, and response parameters were compared to those of the original P_0^R population. The estimated GR_{50} and LD_{50} values overlapped at the 95% confidence and fiducial intervals, and $|\lambda_{50}|$ comparisons were non-significant, thus suggesting similar population responses to glyphosate (Table 1). These results reaffirmed the notion of parallel performances of P_0^R and RS_1 ($F_{obs} = 0.82$, $P = 0.96$) and P_0^R and RS_2 ($F_{obs} = 0.89$, $P = 0.85$) to glyphosate. Hence, the RS_2 population was considered near-homozygous resistant, given that the parental and selected populations performed similarly to glyphosate, and RS_1 and RS_2 did not segregate for glyphosate resistance (Tables 3, 4).

Less shikimic acid accumulates in resistant plants

In plants, glyphosate causes cytoplasmic accumulation of the substrate and unphosphorylated substrate of EPSPS at a 1:20 proportion of 3-phosphoshikimate (3PS):shikimic acid (Gout et al. 1992). Ultimately, putative phosphorylases hydrolyzed the phosphoryl group in 3PS, and the aromatic compound is accumulated as shikimic acid in cell vacuoles (Holländer-Czytko and Amrhein 1983). Whole-plant response to glyphosate can therefore be confirmed by monitoring endogenous shikimic acid concentrations (Harring et al. 1998). In addition, shikimic acid levels may serve as an indirect indicator of the level of EPSPS inhibition by glyphosate.

In the absence of glyphosate, *C. canadensis* rosettes contained extractable shikimic acid concentrations of 18–25 μmol g^{-1} of dry tissue across all populations. These basal levels increased sigmoidally with increasing glyphosate rates to an approximate maximum of

113–133 μmol shikimic acid g^{-1} of dry tissue at 20 DAT (Fig. 2). The glyphosate rate required to inhibit half of EPSPS in the P_0^P or P_0^S populations was close to 2.0 kg ha^{-1}, in contrast to 3.1–4.4 kg ha^{-1} required for the resistant or recurrent selected populations (Table 1). Marginal differences in shikimic acid levels were observed 20 DAT at the 0.42-kg glyphosate ha^{-1} or 0.85-kg glyphosate ha^{-1} rates, while maximum differences occurred at 3.38 kg glyphosate ha^{-1} (Fig. 2). This confirmed that EPSPS in RS_2 was less inhibited at glyphosate rates > 0.85 kg ha^{-1} compared to P_0^P or P_0^S. Patterns of shikimic acid accumulation also correlated negatively with biomass and positively with visual injury assessments (Table 1). Mueller et al. (2003) reported that in a glyphosate-resistant *C. canadensis* biotype from Tennessee, shikimic acid levels decreased significantly 4 DAT compared to 2 DAT at the 0.84-kg ha^{-1} glyphosate rate. *C. canadensis* possesses three EPSPS isoforms (Montgomery et al. 2003), each with apparently different kinetic constants, thus potentially explaining the differential EPSPS inhibition reported in resistant plants.

C. canadensis is essentially autogamous

Estimates of emasculation efficiency suggested that some (< 1%) self-fertilization (autogamy) may occur prior to capitula opening (Table 2). An alternative explanation for these results was that some pollen was released during removal of the disk florets. Of the total florets produced by *C. canadensis*, approximately 45% self-fertilized and develop into viable achenes (current study). Thus, the emasculation method was approximately 98% effective at preventing autogamy in *C. canadensis*. Estimates of assisted cross pollination (allogamy) across families ranged from 0% to 14% in the RS_2 to P_0^P or P_0^S cross and 0% to 10% in the reciprocal P_0^P or P_0^S to RS_2 cross (Table 2). Weaver (2001) reported an average 4% allogamy, ranging from 1.2% to 14.5%, in a paraquat-resistant *C. canadensis* biotype. Assisted crosses estimated allogamy under ideal conditions; in nature, inflorescent proximity, abiotic factors such as wind, and biotic agents such as insects may modulate allogamy dynamics between *C. canadensis* plants.

The *R* allele is nuclear encoded

Artificial crosses provided an estimate of the intraspecific compatibility within *C. canadensis* and ascertained whether glyphosate resistance was maternally inherited. Across all families and artificial reciprocal crosses, > 92% of treated rosettes demonstrated an IR phenotype (Table 2). This confirmed that *C. canadensis* plants were overall genetically compatible. The unexpected levels of susceptible and resistant phenotypes in the F_1 were attributed to the inefficiency (2%) associated with emasculation or autogamy prior to anthesis. Artificial reciprocal crosses also established that the *R* allele was pollen-borne, since the vast majority of F_1 rosettes displayed an IR phenotype. In the event of cytoplasmic inheritance of glyphosate resistance, susceptible phenotypes would have predominated the RS_2 to P_0^P or P_0^S artificial cross. With the exception of some instances in resistance to triazine herbicides, the predominant cases of herbicide resistance are conferred by nuclear gene(s) (Gasquez 1997).

Glyphosate resistance in *C. canadensis* follows the 1:2:1 model

Segregation ratios were monitored in F_2 full-siblings to ascertain the number of genes and based on phenotypic

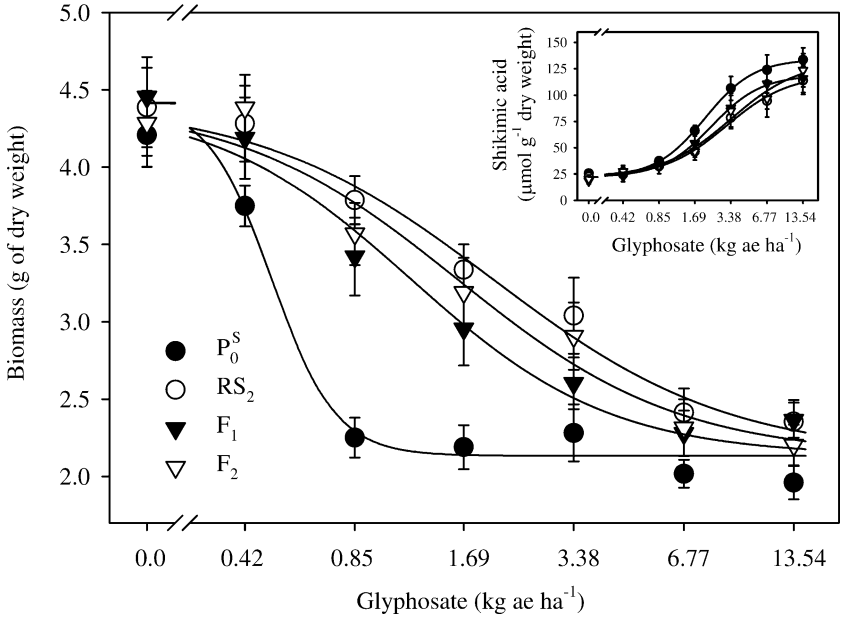

Fig. 2 *Main plot* Rate response at 20 DAT of *C. canadensis* populations P_0^S (*filled circle*), RS_2 (*open circle*), F_1 (*filled inverted triangle*), and F_2 (*inverted triangle*) to glyphosate. *Inset* Endogenous shikimic acid levels of treated *C. canadensis* plants. Each *data point* represents the mean of four replications and two experiments conducted at different times ($n = 8$). F_1 and F_2 rate responses were conducted on a population composed of three randomly selected plants per each of the 20 generated families ($n = 60$). *Extensions on symbols* designate the standard error associated with individual means (σ_M)

Table 2 Estimates of emasculation efficiency (*EE*) and cross pollination (allogamy) between *C. canadensis* populations from Ames, IA (P_0^P), Georgetown, DE (P_0^S), and Houston, DE selected twice at 2.0 kg glyphosate ha^{-1} (RS_2). EE represents the percentage of germinated achenes (*G*) from the total estimated achenes emasculated (*AE*). Percentage allogamy (*PA*) and percentage compatibility (*PC*) were estimated from the frequency of glyphosate-resistant (*R*), intermediate-resistant (*IR*), and susceptible (*S*) first filial descendants within each family

Family	Emasculated[a]			Assisted cross[b]						Artificial cross[c]					
	RS_2			RS_2 to P_0^P			P_0^P to RS_2			RS_2 to P_0^P			P_0^P to RS_2		
	AE	G	EE[d]	S[e]	IR	PA[f]	IR	R	PA	S	IR	PC[f]	IR	R	PC
1	280	2	99.3	24	4	14.3	3	25	10.7	1	31	96.9	28	1	96.6
2	350	4	98.9	24	2	7.7	0	25	0.0	0	25	100.0	22	0	100.0
3	245	0	100.0	25	0	0.0	2	24	7.7	0	29	100.0	26	0	100.0
4	245	2	99.2	33	4	10.8	1	31	3.1	2	30	93.8	27	0	100.0
5	315	0	100.0	32	4	11.1	2	34	5.6	1	24	96.0	32	0	100.0
	RS_2			RS_2 to P_0^S			P_0^S to RS_2			RS_2 to P_0^S			P_0^S to RS_2		
1	280	1	99.6	27	0	0.0	0	25	0.0	0	28	100.0	25	1	96.2
2	350	4	98.9	31	4	11.4	3	26	10.3	0	25	100.0	31	1	96.9
3	350	3	99.2	24	3	11.1	0	28	0.0	2	32	94.1	25	1	96.2
4	315	0	100.0	25	2	7.4	2	27	6.9	1	27	96.4	24	0	100.0
5	350	3	99.2	29	5	14.7	3	26	10.3	2	26	92.9	29	0	100.0
Total	3080	19	99.4	274	28	9.3	16	271	5.6	9	277	96.9	269	4	98.5

[a]Yellow, perfect florets were manually excised from the capitula pre-anthesis and the white pistillate florets allowed to mature inside a DelNet bag

[b]At anthesis, intact RS_2 and P_0^S inflorescences were covered with a DelNet bag and florets permitted to cross-pollinate

[c]The receptor capitula were emasculated pre-anthesis, and the remaining pistillate florets were fertilized with intact capitula from the pollen donor plant

[d]EE represents the percentage non-germinated achenes from the total estimated achenes evaluated

[e]S comprised 10-cm diameter rosettes killed at 2.0 kg glyphosate ha^{-1}. Per contra, R and IR represents rosettes that reached reproductive stage and demonstrated ≤ 30 and 31–69% visual injuries at the same glyphosate rate and phenological stage, respectively

[f]PA or PC represents the proportion IR phenotypes within the total rosettes treated

frequencies, a model was constructed to explain the inheritance of glyphosate resistance in *C. canadensis*. The purported genetic model was tested by back-crosses of F_1 plants to a progenitor from the original RS_2, P_0^P or P_0^S parent. Moreover, glyphosate rate responses were conducted to confirm intermediacy of the putative heterozygous F_1 and the H_0 of monogenic inheritance tested, based on the expected F_2 mortality (Tabashnik 1991). Efficacy trials of F_2 full-siblings at 2.0 kg glyphosate ha^{-1} identified R, IR, and S phenotypes, as defined earlier, within each family. Visual assessments suggested that glyphosate resistance in *C. canadensis* segregated following partially dominant Mendelian genetics, consistent with a single-gene effect. F_2 families generated from the RS_2 to P_0^P cross had observed phenotypic ratios that converged to the expected 1:2:1 proportion predicted by Mendelian genetics ($\chi^2 < 2.79$, $P > 0.25$, Table 3). Concomitantly, GOF analysis for the reciprocal P_0^P to RS_2 families, and the combined homogenous data set for all F_2 families ($\chi^2 = 0.44$, $P = 0.80$) provided non-significant χ^2 values, reaffirming appropriateness of the incompletely dominant monogenic model. Results from the χ^2 homogeneity test permitted combined analysis of the back-cross data; GOF results were consisted with the expected 1:1 ratio of the proposed genetic model (Table 3). To further investigate the genetics of glyphosate resistance, ten additional reciprocal families were created from the $RS_2 \times P_0^S$ crosses. Family 1 in the P_0^S to RS_2 cross displayed an above-expected number of resistant individuals, which resulted in a non-Mendelian phenotypic ratio ($\chi^2 = 6.73$, $P = 0.03$, Table 4). Regardless, the combined GOF analysis for the $RS_2 \times P_0^S$ cross converged to the expected 1:2:1 (F_2) and 1:1 (back-cross) ratios for the proposed genetic model (Table 4). Further evidence for the proposed partially dominant model was substantiated graphically, where the distribution of observed F_2 mortality had three distinct segments that resembled a 1:2:1 segregation pattern (Fig. 1). The putative homozygous, susceptible genotype was killed at glyphosate rates of 0.85–3.38 kg ha^{-1} (12.5–25% mortality). Per contra, the putative heterozygous and homozygous, resistant genotypes were controlled at the 6.77-kg ha^{-1} (75% mortality) and 13.54-kg ha^{-1} (100% mortality) glyphosate rates, respectively (Fig. 1).

The incompletely dominant model predicted that the heterozygous genotype would display an intermediate phenotype compared to both parents. This was confirmed by the prevalence of IR phenotypes in the heterozygous F_1 population that arose from crosses between the near-homozygous RS_2 and P_0^P or P_0^S parents (Table 2). Furthermore, the F_1 population demonstrated an intermediate GR_{50}, mortality, visual injury, and shikimic acid levels when contrasted to both resistant and susceptible parents (Table 1; Figs. 1, 2). Glyphosate resistance in another *C. canadensis*

Table 3 Resistance gene (*R* allele) segregation in 10 second filial (F_2) families generated by artificial crosses between the pristine *C. canadensis* populations P_0^P and RS_2. An F_2 family originated from a single first filial (F_1) *C. canadensis* plant allowed to self-pollinate in isolation. For the back-crosses, the F_1 served as the pollen donor to a previously emasculated RS_2 (BC_r) or P_0^P (BC_p) pollen-receptor plant that arose from an achene of the original RS_2 or P_0^P parent

Origin of F_1 Parents[a]		F_2 family no.	Observed phenotype[b]				Expected[c]	χ^2	$P > \chi^2$
Donor	Receptor		R	IR	S	Total	R:IR:S		
RS_2	P_0^P	1	5	13	11	29	7.25:14.5:7.25	2.79	0.25
		2	10	15	6	31	7.75:15.5:7.75	1.06	0.59
		3	6	16	11	33	8.25:16.5:8.25	1.54	0.46
		4	5	16	5	26	6.5:13:6.5	1.38	0.50
		5	6	11	8	25	6.25:12.5:6.25	0.68	0.71
		Total	32	71	41	144	36:72:36	1.15	0.56
P_0^P	RS_2	1	7	17	6	30	7.5:15:7.5	0.60	0.74
		2	9	15	7	31	7.75:15.5:7.75	0.29	0.86
		3	4	17	7	28	7:14:7	1.93	0.38
		4	12	12	5	29	7.25:14.5:7.25	4.24	0.12
		5	5	14	11	30	7.5:15:7.5	2.53	0.28
		Total	37	75	36	148	37:74:37	0.04	0.98
Combined F_2 families[d]			69	146	77	292	73:146:73	0.44	0.80
Combined BC_r families[d]			62	55	–	117	58.5:58.5:0	0.42	0.52
Combined BC_p families[d]			–	41	47	88	0:44:44	0.41	0.52
Performance of parents									
P_0^P			0	0	59	59	0:0:59	–	–
P_0^R			78	0	4	82	82:0:0	–	–
RS_1			79	0	0	79	79:0:0	–	–
RS_2			84	0	0	84	84:0:0	–	–

[a] F_1 plants were produced by reciprocal intraspecific artificial crosses between the RS_2 and P_0^P parents. *Donor* represents the pollen donor *C. canadensis* parent with intact capitula. *Receptor* was the *C. canadensis* parent with pistillate florets (emasculated) that accepted the pollen

[b] Observed R, IR, and S phenotypes in the progeny of a single F_1 per family allowed to self-pollinate (F_2). Twenty days after treatment (DAT) of 2.0 kg glyphosate ha^{-1}, R, IR, and S phenotypes comprised rosettes with ≤ 30, 31–69, and ≥70% visual herbicide injury, respectively. Only S individuals failed to reach reproductive stage. All plants were treated at the 10-cm diameter rosette stage

[c] Expected Mendelian R, IR, and S segregation ratios for the incompletely-dominant, single-gene model (1:2:1)

[d] The homogeneity χ^2 test among families was non-significant; therefore, data were combined for the χ^2 goodness-of-fit (GOF) test. Combined F_2 families, $\chi^2 = 1.77$, $P = 0.99$; combined BC_r families, $\chi^2 = 4.79$, $P = 0.85$; combined BC_s families, $\chi^2 = 7.68$, $P = 0.57$

population was conferred by a single, dominant nuclear gene, and the mechanism was apparently reduced glyphosate translocation within the plant (Montgomery et al. 2003; Feng et al. 2004). Our results clearly demonstrate an intermediate response to glyphosate of the heterozygous F_1 and thus confirm suitability of the incompletely dominant model for the P_0^R *C. canadensis* populations. Since dominant and incompletely dominant models have been proposed for the inheritance of glyphosate resistance in *C. canadensis*, two distinct mechanisms of resistance may exist. Other investigations focus on elucidating the mechanism(s) of glyphosate resistance in *C. canadensis* and would certainly provide evidence as to the identity of the gene responsible for the resistant trait.

Discussion

Genetics of evolved glyphosate resistance in plants

Approximately 300 herbicide-resistant weed biotypes have been confirmed to date; however, only in less than 10% of the confirmed cases have the resistance mechanism and genetics of resistance been conclusively elucidated (Heap 2004). Herbicide resistance in the majority of characterized cases is conferred by a single, nuclear-encoded allele inherited as a dominant or incompletely dominant trait (Gasquez 1997). Examples of recessive inheritance include resistance of several grasses to dinitroaniline herbicides (Wang et al. 1996; Zeng and Baird 1999). Only in triazine resistance has maternal inheritance been shown (Jasieniuk et al. 1996). Examples of more complex genetics include reports in *Avena fatua* L. of dominant diclofop resistance at low rates and reversal, dominant susceptibility, at high rates of the herbicide (Seefeldt et al. 1998). In another *A. fatua* example, triallate resistance was governed by two unlinked recessive alleles, and inheritance was apparently maternal only at high triallate rates (Kern et al. 2002). Examples of polygenic resistance comprise the description of two independent nuclear alleles conferring fenoxaprop-P-ethyl resistance in *Alopecurus myosuroides* Huds.; identity of the resistance genes was ascribed to a mutant acetyl-CoA carboxylase (EC 6.4.1.2) and a cytochrome P-450 mono-oxygenase (Letouzé and Gasquez 2001). More complex scenarios include additive gene effects in cross-resistant weeds, where a single allele modulates the overall level of resistance (Preston 2003).

Table 4 *R* allele segregation in F_2 families generated by artificial crosses between the susceptible *C. canadensis* populations P_0^S and RS_2. An F_2 family originated from a single F_1 *C. canadensis* plant allowed to self-pollinate in isolation. For the back-crosses, the F_1 served as the pollen donor to a BC_r or BC_s pollen-receptor plant that arose from an achene of the original RS_2 or P_0^S parent

Origin of F_1 Parents[a]		F_2 family no.	Observed phenotype[b]				Expected[c]	χ^2	$P > \chi^2$
Donor	Receptor		R	IR	S	Total	R:IR:S		
RS_2	P_0^S	1	6	22	8	36	9:18:9	2.00	0.37
		2	12	13	6	31	7.75:15.5:7.75	3.13	0.21
		3	9	13	12	34	8.5:17:8.5	2.41	0.30
		4	5	19	5	29	7.25:14.5:7.25	2.79	0.25
		5	6	15	14	35	8.75:17.5:8.75	4.37	0.11
		Total	38	82	45	165	41.25:82.5:41.25	0.60	0.74
P_0^S	RS_2	1	16	15	6	37	9.25:18.5:9.25	6.73	0.03
		2	11	14	5	30	7.5:15:7.5	2.53	0.28
		3	7	10	9	26	6.5:13:6.5	1.69	0.43
		4	9	14	8	31	7.75:15.5:7.75	0.36	0.84
		5	5	20	5	30	7.5:15:7.5	3.33	0.19
		Total	48	73	33	154	38.5:77:38.5	3.34	0.19
Combined F_2 families[d]			86	155	78	319	79.75:159.5:79.75	0.65	0.72
Combined BC_r families[d]			57	54	–	111	55.5:55.5:0	0.08	0.78
Combined BC_s families[d]			–	46	53	99	0:49.5:49.5	0.49	0.48
Performance of parents									
P_0^S			0	0	73	73	0:0:73	–	–
RS_2			84	0	0	84	84:0:0	–	–

[a] F_1 plants were produced by reciprocal intraspecific artificial crosses between the RS_2 and P_0^S parents. *Donor* represented the pollen donor *C. canadensis* parent with intact capitula. *Receptor* was the *C. canadensis* parent with pistillate florets (emasculated) that accepted the pollen

[b] Observed R, IR, and S phenotypes in the progeny of a single F_1 per family allowed to self-pollinate (F_2). Twenty DAT of 2.0 kg glyphosate ha^{-1}, R, IR, and S phenotypes comprised rosettes with ≤ 30, 31–69, and ≥70% visual herbicide injury, respectively. Only S individuals failed to reach reproductive stage. All plants were treated at the 10-cm diameter rosette stage

[c] Expected Mendelian R, IR, and S segregation ratios for the incompletely-dominant, single-gene model (1:2:1)

[d] The homogeneity χ^2-test among families was non-significant; therefore, data were combined for the χ^2 GOF test. Combined F_2 families, $\chi^2 = 3.41$, $P = 0.95$; combined BC_r families, $\chi^2 = 3.68$, $P = 0.93$; combined BC_s families, $\chi^2 = 3.32$, $P = 0.95$

Evolved glyphosate resistance was first confirmed in two independent *L. rigidum* populations of Orange, New South Wales, and Echuca, Northern Victoria, Australia (Powles et al. 1998; Pratley et al. 1999). Genetic analysis of the Orange *L. rigidum* population revealed that glyphosate resistance was conferred by a single, incompletely dominant allele under nuclear control (Lorraine-Colwill et al. 2001); to date, however, the identity of the resistance gene remains elusive. Initial investigations found no indication that metabolism, uptake, or ztranslocation mechanisms were involved in glyphosate resistance, nor did differences in EPSPS and 3-deoxy-7-phosphoheptulonate (EC 2.5.1.54) synthase activities or *EPSPS* expression (Feng et al. 1999; Lorraine-Colwill et al. 1999). A more robust investigation found no evidence of *EPSPS* amplification or co-segregation of specific *EPSPS* isoforms with resistance; however, mRNA levels and EPSPS specific activity were higher in resistant plants (Baerson et al. 2002a). Enhanced *EPSPS* mRNA levels and endogenous activity of the enzyme, in addition to possible post-translational regulation of EPSPS, were also cited as resistant mechanisms in a glyphosate-resistant *Dicliptera chinensis* (L.) Juss. population (Yuan et al. 2002). More recently, evidence was put forward that glyphosate resistance in *L. rigidum* was mediated by differences in the cellular transport of the herbicide (Lorraine-Colwill et al. 2003). Glyphosate import into plant cells is apparently ATP driven by a phosphate transporter in the plasmalemma (Hetherington et al. 1998). Mutations in phosphate transporters significantly diminish movement of inorganic phosphate within plants and thus potentially the translocation of glyphosate (Versaw and Harrison 2002). Analogously, a mutant phosphate transporter in resistant plants could reduce glyphosate cellular transport and explain the proposed mechanism and genetic model for *L. rigidum*.

While evolved glyphosate resistance in *E. indica* was attributed to a $C^{875} \rightarrow T$ transition coding for an insensitive $prolyl^{101} \rightarrow seryl$ EPSPS isoform (Baerson et al. 2002b), no genetic analysis was conducted to validate the proposed single-mechanistic model. A transversion at this same site, $C^{875} \rightarrow A$, codes for a $threonyl^{101}$ EPSPS isoform that is apparently also insensitive to glyphosate (Ng et al. 2004a). In addition, a glyphosate-resistant population from Lenggeng, Malaysia, possessed an *EPSPS* sequence identical to the susceptible biotype, suggesting that at least another mechanism is capable of conferring glyphosate resistance in *E. indica* (Ng et al. 2004a). Glyphosate resistance in *E. indica* was purportedly governed by an incompletely dominant, single, nuclear gene (Ng et al. 2004b). Conversely, in *Ceratopteris richardii* (L.) Brongn., glyphosate resistance is governed by the independent nuclear *glt1* and *glt2* loci that are inherited as incompletely dominant or recessive traits, respectively (Chun and Hickok 1992). Results from these two species

entertain the possibility that two or more mechanisms may modulate survival to glyphosate in some cases of evolved resistance. This assertion is supported by studies in *Convolvulus arvensis* L., where several mechanisms at the cellular and metabolic levels modulate tolerance to glyphosate (Westwood and Weller 1997). Concurrently, it was demonstrated that inheritance of glyphosate tolerance within *C. arvensis* biotypes is the result of maternal effects and additive gene actions (Duncan and Weller 1987). Quantitative genetics of glyphosate tolerance was also cited in maize (*Zea mays* L.) somaclones (Racchi et al. 1997). In the event that inheritance of glyphosate resistance is polygenic, weak selection pressure from sublethal applications and recombination through several generations may be necessary to increase resistant allele frequencies and select for the highest level of resistance. Mitigation of evolved polygenic resistance was proposed by periodically alternating sublethal herbicide applications with high rates of the herbicide, in addition to alternative control strategies (Gardner et al. 1998).

Impact of genetics on *C. canadensis* resistance management

No fitness penalty was observed between the P_0^P, P_0^S, or RS_2 populations under greenhouse conditions, suggesting that in the absence of glyphosate, resistant and susceptible *C. canadensis* plants would be present at equal proportions in the environment. Even under fitness penalty against RS_2, the *R* allele would reside in the environment at lower frequencies (Gasquez 1997). In addition, expression of the *R* allele in the heterozygous genotype (F_1) estimated a GR_{50} of 1.21 kg glyphosate ha^{-1} (Table 1), which is well above the 0.85-kg ha^{-1} rate recommended by the manufacturer. Hence, under field conditions, both homozygous and heterozygous genotypes would behave as a dominant trait. Finally, data from the reciprocal crosses confirmed that *C. canadensis* is essentially autogamous and self-compatible (Table 2). These combined statements would predict a rapid increase of resistant individuals within *C. canadensis* populations under continuous glyphosate selection. Not surprisingly, resistance in the P_0^R populations evolved after 3 years of continuous glyphosate selection (VanGessel 2001). Considering that glyphosate resistance has evolved in at least ten independent *C. canadensis* populations (Heap 2004), we suggest that enough genetic variability exists in *Conyza* for resistance to evolve rapidly.

Glyphosate resistance in *C. canadensis* is pollen-borne (Tables 3; 4). Evidence of entomophilous interactions has been cited in *Conyza* (Weaver 2001), entertaining the possibility of resistance transfer to adjacent *C. canadensis* populations. Furthermore, the anemochory nature of *C. canadensis* allows for achene dispersal to a maximum of 30 m in 16-km h^{-1} wind (Dauer et al. 2003). This effective dispersal mechanism combined with *C. canadensis* potential to produce 240,000 achenes per growing season (Muenscher 1935) would certainly facilitate resistance spread to adjacent areas. Containment of evolved glyphosate resistance may require the use of an integrated management approach. For example, mechanical control and a combination of pre-emergence and residual herbicides provide effective *C. canadensis* management (Brown and Whitwell 1988; VanGessel et al. 2001). Farmers should not only contemplate the economics associated with weed management, but rather focus on adopting effective and long-term strategies that will preserve the sustainability of current production systems.

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