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ORIGINAL ARTICLE

Dose-response and growth rate variation among glyphosate resistant and susceptible *Conyza albida* and *Conyza bonariensis* populations

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Abstract

Plant responses to glyphosate applied at different doses were examined for one glyphosate resistant (R) and one glyphosate susceptible (S) population of *Conyza albida* and *C. bonarienis*. Growth rates and development stages of five R *C. albida* and three R *C. bonarienis* populations were also compared with those of their respective S counterparts to investigate the possible impact of the glyphosate resistance trait on their fitness. The GR_{50} values for *C. albida* R (3.94–5.22 kg a.i. · ha^{-1}) and S (0.24–0.31 kg a.i. · ha^{-1}) populations were higher than those of *C. bonariensis* R (0.60–1.51 kg a.i. · ha^{-1}) and S (0.10–0.13 kg a.i. · ha^{-1}). The growth rate (slope *b*) of one R *C. albida* population was lower than the respective S and other R populations, while growth rates of most R and S *C. bonariensis* populations were similar. Some R populations showed inconsistent differences in some development stages when compared to those of the S ones, which cannot be attributed to the glyphosate resistance trait.

Key words: *Conyza* resistance, development stage, fitness cost, glyphosate, growth rate

Introduction

Conyza species (members of the Asteraceae family) are the most important weeds in perennial cropping systems like orchards, olive groves, and vineyards in Greece (Mylonas *et al.* 2014). The genus *Conyza* includes weedy species such as *Conyza albida* Willd. ex Sprengel (Syn: *Conyza sumatrensis* (Retz.) E. H. Walker) (Fleabane or Sumatran fleabane), *Conyza bonariensis* (L.) Cronq. (Hairy fleabane), and *Conyza canadensis* (L.) Cronq. (Horseweed) and are the most common ones found worldwide and in Greece (Mylonas *et al.* 2014; Heap 2018).

Glyphosate [N-(phosphonomethyl)-glycine] is one of the most commonly used herbicides for the control of annual and perennial weeds in various cropping systems (Duke and Powles 2008; Kleinman and Rubin 2017). However, its intensive use has resulted in the evolution of 43 glyphosate R weed species (Heap 2018). The *Conyza* species are self-pollinating, produce massive quantities of wind-distributed seeds per plant, germinate under a wide range of conditions and produce non-dormant highly viable seeds, which has helped these annuals to invade and acclimate in many regions across the globe (Weaver 2001; Powles 2008; Kleinman *et al.* 2016). There have been 13 and 8 reported cases for glyphosate resistance in *C. bonariensis* and *C. albida*, respectively which is less than the 42 reported cases in *C. canadensis* (Heap 2018).

Conyza species exhibit high tolerance to glyphosate applied at high temperatures, in low humidities or in advanced weed growth stages (Walker *et al.* 2011; Kleinman and Rubin 2017). In addition, good control for R *C. canadensis* was achieved with glyphosate application under favorable weather conditions (average temperatures <16°C for 3 days prior to spraying, on the day of spraying and for 6 days after spraying) (Ge *et al.* 2011). Dennis *et al.* (2016) also reported that glyphosate provided good control of glyphosate S, R, and glyphosate and paraquat multiple resistant *C. bonariensis* populations at the five to eight leaf stages in the fall, but the control was poor in spring. Moretti *et al.* (2013) also found that control of *C. bonariensis* with glyphosate varied substantially due to environmental conditions prior to and after application time, whereas similar information was not found for *C. albida.*

Differences in growth and the amount of seed production between herbicide resistant and susceptible weed populations can affect the persistence and frequency of the herbicide resistant populations in a weed species (Davis *et al.* 2009). If relative growth and reproductive ability of the resistant populations are reduced, the resistance frequency is expected to decline over time once the selection pressure is ceased (Maxwell *et al.* 1990; Green 2007; Davis *et al.* 2009). Therefore, crop management decisions can be used to exploit the fitness cost to reduce the frequency of resistant populations (Williams *et al.* 1995; Zhang *et al.* 1999; Davis *et al.* 2009).

The possibility of a fitness cost associated with glyphosate resistance in *Conyza* species has been examined to some extent with *C. canadensis* and *C. bonariensis* but not with *C. albida*. Comparison of R and S *C. canadensis* populations in the greenhouse by Zelaya *et al.* (2004) showed no fitness difference. Similarly, Davis *et al.* (2009) reported that untreated plants from R *C. canadensis* populations, resistant to ALS-inhibitors or to glyphosate + ALS-inhibitors, produced similar amounts of biomass and seed compared to populations that were susceptible to either herbicides or the combination of herbicides. However, an R *C. canadensis* population from San Joaquin Valley (California) showed higher growth rate (Grantz *et al.* 2008a, b) and higher competitive ability than the S population when grown together in pots at high densities and under moisture-deficit stress (Shrestha *et al.* 2010). Furthermore, the above-mentioned R *C. canadensis* population reached reproductive maturity approximately 3 to 4 weeks earlier than the S population when grown (alone, in pots) in full sunlight (Shrestha *et al.* 2010) and approximately a week earlier when grown under a grapevine canopy in a vineyard (Alcorta *et al.* 2011). Regarding *C. bonariensis* from Greece, Travlos and Chachalis (2013) reported similar growth and seed production of one R and one S population originating from different geographical locations. Shrestha *et al.* (2014) also reported that five R *C. bonariensis* and five S populations had similar phenological development rates and biomass.

The response of one R and one S population of *C. albida* and *C. bonariensis,* respectively, to glyphosate season of application was studied. In addition, the growth rate and development stages of five R *C. albida* and three R *C. bonariensis* populations were also investigated under noncompetitive conditions and compared to that of their respective S counterparts.

Materials and Methods

Effect of glyphosate on resistant and susceptible *C. albida* and *C. bonariensis* populations

Seeds of one R population of *C. albida* (R1), one R *C. bonariensis* (R6) population and, one S population of *C. albida* (S1) and one S *C. bonariensis* (S2) population were used in this study, which were obtained from previous experiments conducted by Mylonas *et al.* (2014), where their resistance or susceptibility was confirmed. The seeds were sown in plastic seedling trays of 24 individual cells (dimensions 6 × 5.5 × 5 cm) filled with a 1 : 2 (v/v) mixture of white peat and potting soil. Each cell was seeded with approximately 20 seeds and the trays were placed in the greenhouse of the Benaki Phytopathological Institute for germination. After emergence, seedlings were thinned in order to have uniform seedlings in each population. Seedlings were grown, selected to be uniform in size (2–4 leaf stage) and transplanted to plastic pots (1.5 l) (two plants per pot) filled with the substrate of 1 : 2 (v/v) mixture of white peat and potting soil. The pots were placed in the greenhouse and watered as needed. One experiment was conducted in spring – summer 2012 with temperatures ranging from 16 to 42°C, and repeated in spring – summer 2013 with temperatures ranging from 17 to 41°C. Only natural light was used, and supplemental fertilizer was not added during the experiments. Plants were treated with glyphosate when they reached the six to eight leaf growth stage by using a custom-built, compressed-air, low-pressure flat-fan nozzle experimental sprayer calibrated to deliver 300 $l \cdot ha^{-1}$ of water at 280 kPa pressure. An isopropylamine salt formulation of glyphosate (Roundup 36 SL, 360 g a.i. $\cdot l^{-1}$, SL, Monsanto Hellas) was used. For the R populations, glyphosate was applied at 0.315, 0.630, 1.260, 2.520, 5.040, and 10.080 kg a.i. $\cdot$ ha^{-1}, which represent ½, 1, 2, 4, 8, and 16 times, respectively, the recommended dose. For the S populations, the glyphosate doses were 0.630, 0.315, 0.1575, 0.07875, 0.039375, and 0.0196875 kg a.i. · ha^{-1}, corresponding to 1, ½, ¼, 1/8, 1/16, and 1/32 times the recommended dose. Also, an untreated control for each population was included. At 6 weeks after treatment (WAT), control of *C. albida* and *C. bonariensis* populations was assessed by determining the fresh weight of all survived plants in each pot. Data were expressed as percentage % of the respective untreated control mean value of each population. At herbicide application, the temperatures were 27–31°C. The two identical experiments (one in 2012 and one in 2013) for the two R or for the two S populations were conducted in a 2 × 7 (2 R populations × 7 glyphosate doses) and a 2 × 7 (2 S populations × 7 glyphosate doses) split-plot arrangement, where populations were the main and the glyphosate doses the sub-plots. A randomized complete block design (RCBD) was used with four replications-pots per each combined treatment (population × glyphosate dose).

Growth rate and development stages of R and S *C. albida* and *C. bonariensis* populations

Seeds of six (5 R and 1 S) *C. albida* and four (3 R and 1 S) *C. bonariensis* populations, including the four populations used in the glyphosate efficacy experiments, were used in this study. They were obtained from previous experiments conducted by Mylonas *et al.* (2014), where their resistance or susceptibility was confirmed. Seeds and seedlings were treated using the same protocol as described in the previous experiments. However, the plastic pots used for transplanting had a capacity 8.5 l instead of 1.5 l. After transplanting in early May, the pots were placed outdoors so that the plants grew under natural environmental conditions. One experiment was conducted in spring – autumn 2012 with mean temperatures ranging from 20°C to 29°C, and repeated in spring-autumn 2014 with mean temperatures ranging from 17°C to 27°C. The daylight time was the natural one. For a uniform watering of the plants, pots were connected to a drip irrigation system using one self-compensating dripper (nominal flow rate 4 l · hr^{-1}) per pot. No supplemental fertilizer was provided during the experiments. Experiments with 6 × 5 (5 R and 1 S *C. albida* populations by 5 growth stages) and 4 × 5 (3 R and 1 S *C. bonariensis* populations by 5 growth stages) were conducted using split-plot arrangements, based on the RCBD. In each species experiment, populations were the main plots and growth stages the sub-plots. For each experiment conducted in 2012 and 2014, there were four replications-pots per each combined treatment (population × growth stage).

To assess population differences in growth rate, eight plants (two plants by four replicate-pots) per population were harvested at each of the five growth stages studied, namely at the rosette stage, bolting stage, full flowering, 50–80% of seed production, and 100% of seed production, and their fresh (data not shown) and dry weights (after two days in an oven at 60°C) were recorded.

Furthermore, the plants of the last evaluation (plants at 100% of seed production used for the growth rate) were also recorded daily before harvest for beginning of bolting (extension of the main stem), first appearance of a floral bud, first appearance of an open flower, and first appearance of a flower with seeds. The initiation of these development stages for each plant was recorded and expressed as days after plant establishment (DAE) outdoors.

Statistical analysis

A combined ANOVA over the two identically conducted experiments (one in 2012 and one in 2013) was used for the fresh weight data obtained from the glyphosate efficacy study against the R or the S populations of both species, using the experimental design described previously. The differences between treatment means of the R or S populations were tested at the 5% significance level using the LSD criterion.

The fresh weight data, resulting from each of the experiments (2012, 2013) × population × glyphosate doses × species combination, were used separately to determine the amount of glyphosate required for 50% growth reduction (GR_{50} value) of each R or S *C. albida* and *C. bonariensis* population. In particular, the fresh weight data were subjected to nonlinear regression analysis using the log-logistic equation proposed by Seefeldt *et al.* (1995):

$$y = C + \frac{D - C}{1 + \exp\left\{b\left[\log(x) - \log(GR_{50})\right]\right\}},$$

where: C = the lower limit of fresh weight, D = the upper limit of fresh weight, b = the slope at the GR_{50}, and GR_{50} = the estimated herbicide dose required for 50% growth reduction. In this nonlinear regression equation, the glyphosate dose (kg a.i. · ha^{-1}) was the independent variable (x) and the fresh weight (percentage % of the untreated control mean value for each population) was the dependent one (y).

Goodness of fit of the log-logistic models was assessed according to Motulsky and Christopoulos (2003), by a) visual inspection of the overall fit on the corresponding graphs with the superimposed regression curves, b) computing and evaluating the R^2 coefficient of determination of the corresponding models, c) computing and evaluating the standard deviations of the corresponding models' residuals (s_e), d) computing and evaluating the range of the estimated GR_{50}

confidence intervals (*CI*), and e) evaluating the scientific plausibility of the estimated parameters.

The R^2 coefficient was computed according to the formula:

$$R^2 = 1 - \frac{RSS}{CTSS},$$

where: RSS = the Residual Sum of Squares and $CTSS$ = the Corrected Total Sum of Squares computed from the nonlinear regression.

For the growth rate, a combined ANOVA over the two identically conducted experiments (2012, 2014) was performed for each species, according to the corresponding experimental designs described previously. The dry weight data, before the application of the ANOVA, were log transformed. As the ANOVA results indicated a significant ($p < 0.05$) three-way interaction [two experiments by *C. albida* (5R and 1S) or *C. bonariensis* (3R and 1S) populations by growth stages], the experiments × population × growth stage means were used to determine the growth rate for each population of either species. Therefore, mean dry weight raw data were fitted to a quadratic model, and the growth rate (slope b) along with the coefficients of determination (R^2) were estimated from the regression model. In the quadratic regression equation, the dry weight was the dependent variable (y) and the time (days after plant establishment outdoors) was the independent one (x). Estimated days for maximum dry weight were determined after differentiation (finding the first and the second derivative) of the quadratic model using the estimated least squares coefficients a, b, and c.

For each development stage assessment, a combined ANOVA over the two identically conducted experiments was made on data (days after plant establishment outdoors) obtained from the six (5 R and 1 S *C. albida*) populations or the four (3 R and 1 S *C. bonariensis*) populations. Differences among treatment means were tested at the 5% significance level using the LSD criterion.

All statistical analyses were performed using the SPSS v15.0 statistical software.

Results

Effect of glyphosate on resistant and susceptible *C. albida* and *C. bonariensis* populations

The combined ANOVAs over the two identically conducted experiments (one in 2012 and repeated in 2013) for the fresh weight data obtained from the R (*C. albida* and *C. bonariensis*) or from the S (*C. albida* and *C. bonariensis*) populations showed significant interaction ($p < 0.05$) due to experiments (2012, 2013) on R or S population species (*C. albida* and *C. bonariensis*) with different glyphosate doses. Therefore, the estimated GR_{50} values from the fitted log-logistic equation (Fig. 1) are presented separately for each species population by experimental year. These data revealed that the R1 and S1 *C. albida* populations showed higher GR_{50} values than those of the R6 and S2 *C. bonariensis* populations in both 2012 and 2013 experiments (Table 1).

Growth rate and development stages of R and S *C. albida* and *C. bonariensis* populations

The estimated slope b (growth rate), for *C. albida* populations in 2012, indicated that the R2 population had the highest slope b (6.6) and the R4 population the lowest (0.4), whereas, in 2014, the highest slope b was estimated for the R5 population (1.9) and the lowest for the R4 population (0.4) (Table 2). According to slope b, the decreasing order for *C. albida* populations in 2012 was R2 > R3 > S1 > R1 > R5 > R4, whereas the respective order in 2014 was R5 > S1 > R3 > R2 > R1 > R4. Regarding *C. bonariensis* populations, in 2012, the highest slope b was estimated for the R8 population (1.4) and the lowest for the S2 population (0.7), whereas the highest slope b in 2014 was estimated for the S2 population (1.0) and the lowest for the R6 population (0.7) (Table 3). Taking into consideration slope b, the decreasing order for *C. bonariensis* populations in 2012 was R8 > R7 > R6 > S2, whereas the respective order in 2014 was S2 > R7 > R8 > R6.

Regarding *C. albida* development stages, in 2012, the beginning of bolting, first appearance of a floral bud, first appearance of an open flower, and first appearance of a flower with seeds of the R4 population were observed 5–13 days later than the respective parameters for the S1 population (Fig. 2). In addition, in 2014, the above-mentioned development stages for the R4 population were observed 8–9 days later than for the S1 population. However, most of the development stages for the R1, R2, R3, and R5 populations were similar to those of the S1 population.

Concerning *C. bonariensis* development stages, in 2012, beginning of bolting, first appearance of a floral bud, first appearance of an open flower, and first appearance of a flower with seeds of the R7 population were observed 3–8 days earlier than the respective days of the S2 population, whereas, in 2014, the above-mentioned development stages for R7 population were observed 4–5 days later than for the S2 population (Fig. 3).

Table 1. Estimated GR_{50} values from the log-logistic model fitted for the *Conyza albida* R1, S1 and *C. bonariensis* R6, S2 populations examined in the two experiments (spring – summer 2012, spring – summer 2013)

Populations	GR_{50} (95% CI) [kg a.i. · ha^{-1}]	R^2	s_e
Spring – summer 2012 (application at 27–31°C)			
C. albida			
R1	3.94 (3.10–4.57)	0.863	12.6
S1	0.31 (0.23–0.39)	0.840	14.6
C. bonariensis			
R6	1.51 (1.15–1.93)	0.908	14.1
S2	0.13 (0.10–0.16)	0.940	11.3
Spring – summer 2013 (application at 27–31°C)			
C. albida			
R1	5.22 (2.67–8.41)	0.624	15.5
S1	0.24 (0.19–0.29)	0.932	10.0
C. bonariensis			
R6	0.60 (0.47–0.75)	0.811	15.2
S2	0.10 (0.07–0.12)	0.947	11.1

GR_{50} – glyphosate dose for 50% reduction of *C. albida* or *C. bonariensis* fresh weight
CI – Confidence Interval
R^2 – log-logisitc model's coefficient of determination
s_e – standard deviation of the log-logistic model's residuals

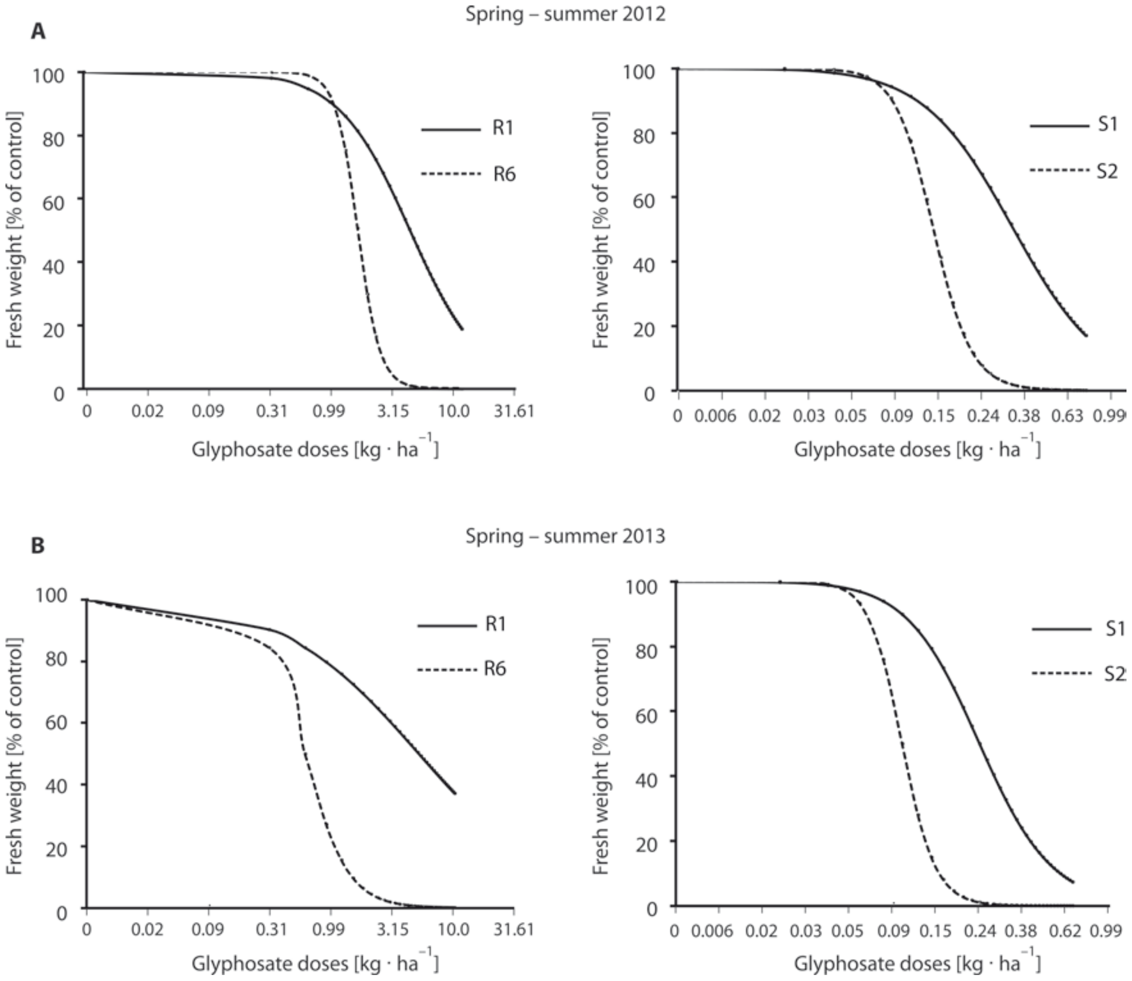

Fig. 1. Glyphosate dose response of the *Conyza albida* R1, S1 and *C. bonariensis* R6, S2 populations evaluated in the two experiments: 1 – spring – summer 2012 (A), 2 – spring – summer 2013 (B). Each data point is a mean of four replicates. Curve fitting was initially made using glyphosate doses in $\log_{10}(X + 1)$ values; the glyphosate doses presented in the four graphs are back transformed to X values

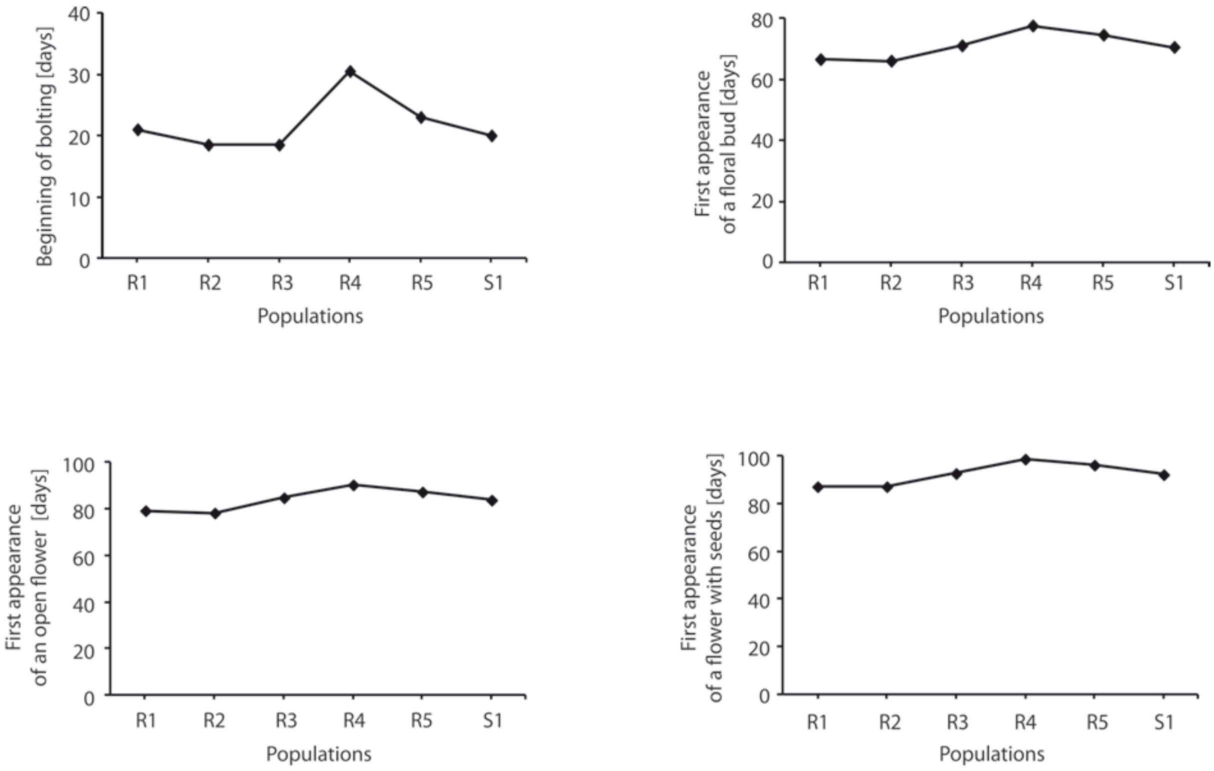

Fig. 2. Average time (days after plant establishment outdoors) required by the R1–R5 and S1 *Conyza albida* populations to reach four development stages in the two experiments (2012, 2014)

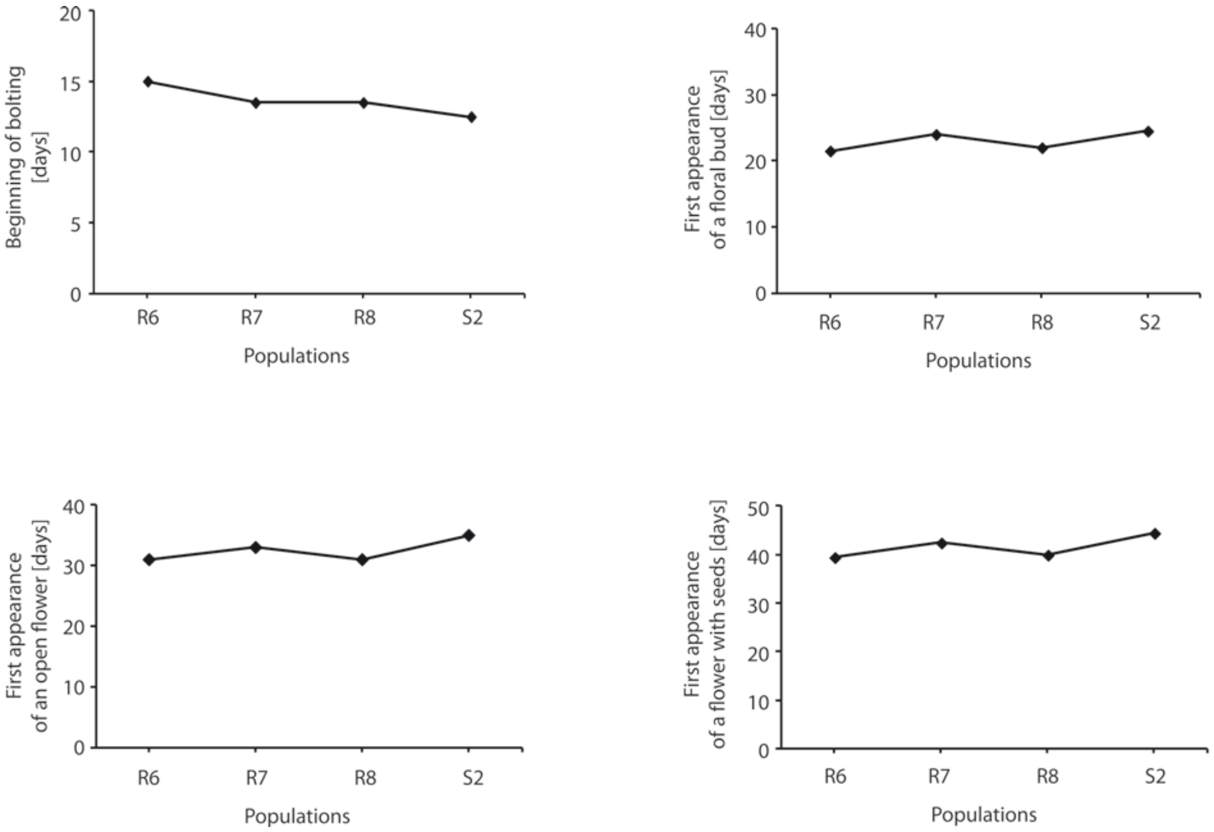

Fig. 3. Average time (days after plant establishment outdoors) required by the R6–R8 and S2 *Conyza bonariensis* populations to reach four development stages in the two experiments (2012, 2014)

Table 2. Estimated growth rate (slope $b \pm$ SE) and coefficient of determination (R^2) from the quadratic model fitted for the R1–R5 and S1 *Conyza albida* populations examined in the two experiments (2012, 2014)

Populations	Slope ($b \pm$ SE)	Estimated days for maximum dry weight	R^2
First experiment (2012)			
R1	3.6 ± 1.5	>118	0.963
R2	6.6 ± 2.3	80	0.922
R3	5.4 ± 2.4	90	0.924
R4	0.4 ± 1.2	>118	0.985
R5	1.9 ± 1.8	>118	0.960
S1	3.7 ± 1.9	98	0.926
Second experiment (2014)			
R1	0.6 ± 0.3	>133	0.965
R2	1.1 ± 0.5	114	0.949
R3	1.2 ± 0.5	120	0.944
R4	0.4 ± 0.1	>133	0.994
R5	1.9 ± 0.5	95	0.958
S1	1.2 ± 0.8	120	0.897

SE – standard error of the estimate

Table 3. Estimated growth rate (slope $b \pm$ SE) and coefficient of determination (R^2) from the quadratic model fitted for the R6–R8 and S2 *Conyza bonariensis* populations examined in the two experiments (2012, 2014)

Populations	Slope ($b \pm$ SE)	Estimated days for maximum dry weight	R^2
First experiment (2012)			
R6	0.8 ± 0.2	>97	0.998
R7	1.2 ± 0.2	>97	0.993
R8	1.4 ± 0.3	>97	0.990
S2	0.7 ± 0.1	>97	1.000
Second experiment (2014)			
R6	0.7 ± 0.1	>92	0.989
R7	0.8 ± 0.2	>92	0.980
R8	0.8 ± 0.1	>92	0.984
S2	1.0 ± 0.1	>92	0.996

SE – standard error of the estimate

Discussion

The GR_{50} values for all populations were higher (R *C. albida* 3.94–5.22 kg a.i. · ha^{-1}, R *C. bonariensis* 0.60–1.51 kg a.i. · ha^{-1}, S *C. albida* 0.24-0.31 kg a.i. · ha^{-1}, S *C. bonariensis* 0.10-0.13 kg a.i. · ha^{-1}) after glyphosate application in spring – summer than with the respective GR_{50} values obtained (R *C. albida* 2.45–2.61 kg a.i. · ha^{-1}, R *C. bonariensis* 0.28–0.39 kg a.i. · ha^{-1}, S *C. albida* 0.07–0.08 kg a.i. · ha^{-1}, S *C. bonariensis* 0.04–0.05 kg a.i. · ha^{-1}) in the same area of Greece after glyphosate application in autumn 2011 – winter 2012 (Mylonas *et al.* 2014). A comparison of these findings suggests that the R and S populations of both *Conyza* species in Greece were more susceptible to glyphosate applied in autumn – winter than in spring-summer. This strongly supports that management of *Conyza* species with glyphosate is dependent on seasonal effect (this is reported for the first time for *C. albida* in Greece and worldwide). Similar results were reported by Kleinman *et al.* (2016) for R and S *C. bonariensis* populations. They found higher population tolerance to glyphosate as temperatures rose. Dennis *et al.* (2016) also found that the efficacy of glyphosate against R *C. bonariensis* population was reduced when applied in spring than under lower temperature regimes. In addition, Moretti *et al.* (2013) reported that, during summer, a higher glyphosate dose is required to reduce growth by 50% (GR_{50} value = = 0.94 kg a.i. · ha^{-1}) of an R *C. bonariensis* population as compared with the respective GR_{50} value (0.22 kg a.i. · ha^{-1}) for the winter glyphosate application. Furthermore, Ge *et al.* (2011) found that the control of a R *C. canadensis* population varied inversely with temperature (less control at higher temperature), and Vila-Aiub *et al.* (2013) also reported that glyphosate efficacy against R *Sorghum halepense* and *Lolium rigidum* populations increased with decreasing temperatures.

The methodological approach proposed by Vila-Aiub *et al.* (2011) to control genetic background was not used because the resistance mechanism to glyphosate of the populations studied was not molecularly elucidated. In addition, competition studies between R and S populations were not conducted because any significant fitness differences may not correspond to resistance traits but possibly to pleiotropic effects of resistance gene(s) or to different geographical locations of origin of the populations. Therefore, a comparison of differences between the growth rates of the R and S populations was conducted under noncompetitive conditions, since it gives the opportunity for plants to express their genotype in a more efficient way than with those grown under competitive conditions. In addition, although this approach does not record differences in the genetic background of R and S lines, it helps to determine the influence of different genetic backgrounds on the fitness of resistance genes in natural populations (Vila-Aiub *et al.* 2011).

The growth rate (slope b) and development stage differences between *C. albida* and *C. bonariensis* populations grown in the two years indicated that fitness of *C. albida* and *C. bonariensis* is not affected by the glyphosate resistance trait. Other non-resistant fitness traits selected by different environmental conditions in the geographical origins of the populations could account for this (Vila-Aiub *et al.* 2011; Papapanagiotou *et al.* 2015). The greater growth rate and development stage differentiation of *C. albida* and *C. bonariensis* populations due to warmer and drier weather conditions in 2012 than in 2014 agree with results reported by Alcorta *et al.* (2011).

The fact that beginning of bolting, first appearance of a floral bud, first appearance of an open flower, and first appearance of a flower with seeds in *C. albida* R4 population were observed 5–13 days later than the respective parameters for a S1 population is in contrast with results reported by Alcorta *et al.* (2011) for a R *C. canadensis* population. They found budded, flowered, and set seeds 6, 10, and 7 days, respectively, earlier than in the S population. Sherstha *et al.* (2010) also found that a R *C. canadensis* population budded, flowered, and set seeds 3 to 4 weeks earlier than the S population. The similar development stages of the *C. albida* R1, R2, R3, and R5 populations as compared with those of S1 are also in contrast with the above-mentioned findings.

The similar growth rates and development stages of the *C. bonariensis* R6, R7, and R8 populations as compared with those of S2 agree with the results reported by Travlos and Chachalis (2013), who also found in Greece that growth and seed production of one R and one S *C. bonariensis* population were similar when they were grown either under noncompetitive or competitive conditions. Shrestha *et al.* (2014) also reported that five R *C. bonariensis* and five S populations had similar development stage rates and biomass. Additionally, Pedersen *et al.* (2007) found no significant differences in vegetative growth or competitiveness between the R and S *Lolium rigidum* populations. Furthermore, Westhoven *et al.* (2008) did not find any apparent fitness penalty between R and S *Chenopodium album* populations.

Conclusions

Data from this study, combined with those of Mylonas *et al.* (2014), revealed that glyphosate efficacy against R and S *C. albida* and *C. bonariensis* populations was lower after its application in spring – summer than in autumn – winter. In addition, the *C. bonariensis* R and S populations were more susceptible to glyphosate than the *C. albida* ones. Furthermore, the growth rate and development stages, defined as fitness cost, did not show any significant differences for the R *C. albida* and *C. bonariensis* populations as compared with the respective S populations.

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References

Alcorta M., Fidelibus M.W., Steenwerth K.L., Shrestha A. 2011. Effect of vineyard row orientation on growth and phenology of glyphosate-resistant and glyphosate-susceptible horseweed (*Conyza canadensis*). Weed Science 59 (1): 55–60. DOI: https://doi.org/10.1614/WS-D-10-00097.1

Davis V.M, Kruger G.R., Stachler J.M., Loux M.M., Johnson W.G. 2009. Growth and seed production of horseweed (*Conyza canadensis*) populations resistant to glyphosate, ALS-inhibiting, and multiple (glyphosate + ALS-inhibiting) herbicides. Weed Science 57 (5): 494–504. DOI: https://doi.org/10.1614/WS-09-024.1

Dennis M., Hembree K.J., Bushoven J.T., Shrestha A. 2016. Growth stage, temperature, and time of year affects the control of glyphosate-resistant and glyphosate-paraquat resistant *Conyza bonariensis* with saflufenacil. Crop Protection 81: 129–137. DOI: https://doi.org/10.1016/j.cropro.2015.12.019

Duke S.O., Powles S.B. 2008. Glyphosate: a once-in-a-century herbicide. Pest Management Science 64 (4): 319–325. DOI: https://doi.org/10.1002/ps.1518

Ge X., d'Avignon D.A., Ackerman J.J., Duncan B., Spaur M.B., Sammons R.D. 2011. Glyphosate-resistant horseweed made sensitive to glyphosate: low-temperature suppression of glyphosate vacuolar sequestration revealed by ^{31}P NMR. Pest Management Science 67 (10): 1215–1221. DOI: https://doi.org/10.1002/ps.2169

Green J.M. 2007. Review of glyphosate and ALS-inhibiting herbicide crop resistance and resistant weed management. Weed Technology 21 (2): 547–558. DOI: https://doi.org/10.1614/WT-06-004.1

Grantz D.A., Shrestha A., Vu H-B. 2008a. Early vigor and ozone response in horseweed (*Conyza canadensis*) biotypes differing in glyphosate resistance. Weed Science 56 (2): 224–230. DOI: https://doi.org/10.1614/WS-07-130.1

Grantz D.A, Shrestha A., Vu H-B. 2008b. Ozone enhances adaptive benefit of glyphosate resistance in horseweed (*Conyza canadensis*). Weed Science 56 (4): 549–554. DOI: https://doi.org/10.1614/WS-07-202.1

Heap I. 2018. The international survey of herbicide resistant weeds. Available on: http://www.weedscience.org. [Accessed: December 20, 2018]

Kleinman Z., Ben-Ami G., Rubin B. 2016. From sensitivity to resistance – factors affecting the response of *Conyza* spp. to glyphosate. Pest Management Science 72 (9): 1681–1688. DOI: https://doi.org/10.1002/ps.4187

Kleinman Z., Rubin B. 2017. Non-target-site glyphosate resistance in *Conyza bonariensis* is based on modified subcellular distribution of the herbicide. Pest Management Science 73 (1): 246–253. DOI: https://doi.org/10.1002/ps.4293

Maxwell B.D., Roush M.L., Radosevich S.R. 1990. Predicting the evolution and dynamics of herbicide resistance in weed populations. Weed Technology 4 (1): 2–13. DOI: https://doi.org/10.1017/S0890037X0002488X

Moretti M.L., Hanson B.D., Hembree K.J., Shrestha A. 2013. Glyphosate resistance is more variable than paraquat resistance in a multiple-resistant hairy fleabane (*Conyza bonariensis*) population. Weed Science 61 (3): 396–402. DOI: https://doi.org/10.1614/WS-D-12-00201.1

Motulsky H.J., Christopoulos A. 2003. The first five questions to ask about nonlinear regression results. p. 29–31. In: "Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting". GraphPad Software Inc. San Diego, USA, 351 pp.

Mylonas P.N., Giannopolitis C.N., Efthimiadis P.G., Menexes G.C., Madesis P.B., Eleftherohorinos I.G. 2014. Glyphosate resistance of molecularly identified *Conyza albida* and *Conyza bonariensis* populations. Crop Protection 65: 207–215. DOI: https://doi.org/10.1016/j.cropro.2014.07.012

Papapanagiotou A.P., Paresidou M.I., Kaloumenos N.S., Eleftherohorinos I.G. 2015. ACCase mutations in *Avena sterilis* populations and their impact on plant fitness. Pesticide Biochemistry and Physiology 123: 40–48. DOI: https://doi.org/10.1016/j.pestbp.2015.01.017

Pedersen B.P., Neve P., Andreasen C., Powles S.B. 2007. Ecological fitness of a glyphosate-resistant *Lolium rigidum* population: growth and seed production along a competition gradient. Basic and Applied Ecology 8 (3): 258–268. DOI: https://doi.org/10.1016/j.baae.2006.01.002

Powles S.B. 2008. Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64 (4): 360–365. DOI: https://doi.org/10.1002/ps.1525

Seefeldt S.S., Jensen J.E., Fuerst E.P. 1995. Log-logistic analysis of herbicide dose-response relationships. Weed Technology 9 (2): 218–227. DOI: https://doi.org/10.1017/S0890037X00023253

Shrestha A., Hanson B.D., Fidelibus M.W., Alcorta M. 2010. Growth, phenology, and intraspecific competition between glyphosate-resistant and glyphosate-susceptible horseweeds (*Conyza canadensis*) in the San Joaquin Valley of California. Weed Science 58 (2): 147–153. DOI: https://doi.org/10.1614/WS-D-09-00022.1

Shrestha A., Steinhauer K.M., Moretti M.L., Hanson B.D., Jasieniuk M., Hembree K.J., Wright S.D. 2014. Distribution of glyphosate-resistant and glyphosate-susceptible hairy fleabane (*Conyza bonariensis*) in central California and their phenological development. Journal of Pest Science 87 (1): 201-209. DOI: https://doi.org/10.1007/s10340-013-0524-8

Travlos I.S., Chachalis D. 2013. Relative competitiveness of glyphosate-resistant and glyphosate-susceptible populations of hairy fleabane, *Conyza bonariensis*. Journal of Pest Science 86 (2): 345–351. DOI: https://doi.org/10.1007/s10340-012-0446-x

Vila-Aiub M.M., Neve P., Roux F. 2011. A unified approach to the estimation and interpretation of resistance costs in plants. Heredity 107 (5): 386–394. DOI: https://doi.org/10.1038/hdy.2011.29

Vila-Aiub M.M., Gundel P.E., Yu Q., Powles S.B. 2013. Glyphosate resistance in *Sorghum halepense* and *Lolium rigidum* is reduced at suboptimal growing temperatures. Pest Management Science 69 (2): 228–232. DOI: https://doi.org/10.1002/ps.3464

Walker S., Bell K., Robinson G., Widderick M. 2011. Flaxleaf fleabane (*Conyza bonariensis*) populations have developed glyphosate resistance in north-east Australian cropping fields. Crop Protection 30 (3): 311–317. DOI: https://doi.org/10.1016/j.cropro.2010.11.010

Weaver S.E. 2001. The biology of Canadian weeds. 115. *Conyza canadensis*. Canadian Journal of Plant Science 81 (4): 867–875. DOI: https://doi.org/10.4141/P00-196

Westhoven A.M., Kruger G.R., Gerber C.K., Stachler J.M., Loux M.M., Johnson W.G. 2008. Characterization of selected common lambsquarters (*Chenopodium album*) biotypes with tolerance to glyphosate. Weed Science 56 (5): 685–691. DOI: https://doi.org/10.1614/WS-08-018.1

Williams II M.M., Jordan N., Yerkes C. 1995. The fitness cost of triazine resistance in jimsonweed (*Datura stramonium* L.). The American Midland Naturalist 133: 131–137.

Zelaya I.A., Owen M.D.K., VanGessel M.J. 2004. Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L.) Cronq. Theoretical and Applied Genetics 110 (1): 58–70. DOI: https://doi.org/10.1007/s00122-004-1804-8

Zhang J., Salas M.L., Jordan N.R., Weller S.C. 1999. Biorational approaches to managing *Datura stramonium*. Weed Science 47 (6): 750–756. DOI: https://doi.org/10.1017/S0043174500091438