

Response of *Conyza bonariensis*, *Conyza canadensis* and *Conyza sumatrensis* to glufosinate

Fidel González-Torralva^{1*}, Julia Ríos-Gómez², Hugo Enrique Cruz-Hipolito³ and Rafael De Prado⁴

¹Post-graduate student at University of Cordoba, Spain, z82gotof@uco.es. ²Graduate student at University of Cordoba, Spain, a42rigoj@uco.es. ³Technical representative at Bayer, Mexico, cruzhipolito@yahoo.com.mx. ⁴Professor at University of Cordoba, Spain, qe1pramr@uco.es.

*Author for correspondence

Conyza bonariensis, *C. canadensis* and *C. sumatrensis* are problematic weeds in citrus orchards and olive trees in southern Spain. The aim of this work was to determine the efficacy of glufosinate in these species, and also to establish a suitable growing stage for application in *C. bonariensis*. For this purpose, dose-response and spray retention assays were carried out in susceptible biotypes of *C. bonariensis*, *C. canadensis* and *C. sumatrensis* at the rosette stage (BBCH 14-15). Additionally, the ED₅₀ and spray retention at two later growth stages were determined in *C. bonariensis*. Results at rosette stage (BBCH 14-15) showed an ED₅₀ of 0.216 in the case of *C. bonariensis*; 0.058 for *C. canadensis* and 0.090 L ha⁻¹ for *C. sumatrensis*. The spray retention values did not show any significant differences between the three species at rosette stage. In *C. bonariensis*, at the second stage of its growth (10-15 cm in height), the ED₅₀ obtained was 0.517 and 1.297 L ha⁻¹ for the third stage (with formed capitula). Also, the spray retention in the second and third stage was of 0.44 and 0.38 mL of glufosinate g⁻¹ of dry weight, respectively. These species treated in an early developmental stage are more susceptible to glufosinate herbicide.

Keywords: *Conyza* spp., dose-response, spray retention, DL-phosphinothricin.

Introduction

The genus *Conyza* Less (Asteraceae) is a native of North and South America. Different species of this genus were introduced in Europe, and, currently, due to its rapid adaptation and prolific seed production they are considered to be noxious weeds in more than 40 crops in 70 countries (Thébaud and Abbot 1995, Holm et al. 1997). In Spain, six species in different Spanish crops have been described, but only three of them are the most important, mainly affecting permanent crops such as citrus orchards and olive groves: hairy fleabane [*Conyza bonariensis* (L.) Cronq.], horseweed [*Conyza canadensis* (L.) Cronq.], and tall fleabane [*Conyza sumatrensis* (Retz.) E. Walker = *Conyza albida* Willd. ex Spreng.] (Saavedra and Pastor 2002, Carretero 2004, Bastida et al. 2005). For all three species, seedling emergence is preferably in winter (Recasens and Conesa 2009) with flowering and seed production in summer-autumn (Carretero 2004).

The common way to control these problematic weeds is with the use of different non selective herbicides such as glufosinate. Glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] belongs to the glutamine synthetase inhibitors. It is used as a non-selective post-emergence herbicide and it controls a broad spectrum of annual and perennial grass and broadleaf weeds (Senseman 2007). Glufosinate works by inhibiting the glutamine synthetase (GS, EC 6.3.1.2) enzyme, which is very important in the nitrogen metabolism (Bayer et al. 1972, Lea et al. 1984, Wendler et al. 1990, Devine et al. 1993). After the herbicide application, a deficiency of glutamine occurs (Tachibana et al. 1986a) and an inhibition of photosynthesis, so, therefore, a rapid accumulation of ammonia is produced by the plant. The final result is the death of the plant cells (Bayer et al. 1972,

Tachibana et al. 1986a and 1986b, Coetzer and Al-Khatib 2001, Eubank et al. 2008).

Many investigations have shown the differential susceptibility/response among weed species, in the same genus, or even in crop cultivars when they are treated with a same or different herbicide(s). Those differences are very important when the chemical control is carried out because it could determine the application timing and the herbicide rate to be used (Carvalho et al. 2006, Ruiz-Santaella et al. 2006, Campos et al. 2009, Ferreira et al. 2010, Bond and Walker, 2011). We hypothesized that these species naturally exhibit different responses to glufosinate.

The objectives of this research were a) to determine under greenhouse conditions the response of *C. bonariensis*, *C. canadensis* and *C. sumatrensis* to glufosinate; b) to evaluate the efficacy of glufosinate under greenhouse conditions at two additional growth stages on *C. bonariensis*; c) to characterize spray retention as a physical factor that could explain differential susceptibility to glufosinate in *C. bonariensis*, *C. canadensis* and *C. sumatrensis*.

Material and methods

Plant material and growing conditions. Glufosinate-susceptible biotypes of *C. bonariensis*, *C. canadensis* and *C. sumatrensis* were used in the experiments described below. Seeds were collected in 2006 from orchards in southern Spain and kept in paper bags at room temperature until their use. *C. bonariensis* and *C. canadensis* were collected from olive orchards in Córdoba, while *C. sumatrensis* was collected from citrus orchards in Huelva. In none of the cases had glufosinate ever been applied to control these weeds. The seeds were sown in 663 cm³ pots filled with moistened peat and were covered with transparent film

until the seeds germinated. Seedlings were planted in pots (one plant per pot) containing a 1:1 (v/v) peat:sand mix and placed in a growth chamber at 28/18 °C (day/night), 16 h photoperiod, 850 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density, 80% relative humidity, and watered as required (González-Torralva et al. 2010).

Dose-response assays. Glufosinate was applied to plants at the BBCH 14-15 scale (Hess et al. 1997) using a laboratory spray chamber at a height of 50 cm above the plants. Herbicide solution doses were applied with flat fan nozzles (Tee Jet 8002 EVS) at 200 kPa and an output volume equivalent to 300 L ha^{-1} . Doses ranged from 0 to 0.4 L (formulated product) ha^{-1} . Additionally, in *C. bonariensis* a species with a rapid growth compared with the other two species, glufosinate was applied at two later growth stages: 10-15 cm in height and prior to flowering (with capitula formed), with doses ranging from 0 to 3 L ha^{-1} . To evaluate herbicide efficacy, plants were cut at ground level 21 days after treatment, and their fresh weight recorded and expressed as a percentage with respect to untreated control plants. Treatments were replicated 4 times in a completely randomized design (each replication with three plants). The same procedure was performed in the additional two growth stages of *C. bonariensis*. The experiments were repeated at least twice.

Dose response curves were determined for each population according to González-Torralva et al. (2010). Data were pooled and fitted to a nonlinear, log-logistic regression model:

$$Y = c + \{(d - c) / [1 + (x / g)^b]\}$$

where Y is the fresh above-ground weight expressed as a percentage of the untreated control, c and d are coefficients corresponding to the lower and upper asymptotes, b is the slope of the line, g is the herbicide rate at the point of inflection halfway between the upper and lower asymptotes, and x (independent variable) is the herbicide dose. Regression analysis was conducted using SigmaPlot for Windows Version 10.0.

Spray retention assays. Plants at BBCH 14-15 scale (Hess et al. 1997) were sprayed with a colored glufosinate solution using the spray chamber and conditions as described in the dose-response assays. Solution contained glufosinate at a rate of 1 L (formulated product) ha^{-1} plus 100 mg L^{-1} Na-fluorescein (Grangeot et al. 2006, Michitte et al. 2007) in an application volume of 300 L ha^{-1} . After the spray had dried on the foliage (15 min.), plants were cut off at ground level and immersed for 30 s in 50 mL of 5 mM NaOH, next the washed solution was filtered and kept in vials. Plants were then placed at 60 °C for 72 h and the dry matter weighed. The amount of Na-fluorescein in each sample was measured by its absorbance with a Hitachi F-2500 Fluorescence Spectrophotometer at 490_{exc}/510_{em} nm. This procedure was carried out with *C. bonariensis* at the different growth stages described. Experiments were arranged in a completely randomized design with four replications (3 plants each) for each population. Results were expressed as mL of spray retention per g^{-1} dry weight. The experiments were repeated at least twice.

Statistical analyses. Statistical analyses among the *Conyza* spp. populations were performed using Statistix version 8.0 Analytical Software. Data obtained in spray retention assays were subjected to analysis of variance and means were compared using Tukey's honestly significant difference (HSD) test at the 5% probability level.

Results and discussion

Dose-response assays. Different degrees of sensitivity and control have been found in different species when they are treated with glufosinate (Mersey et al. 1990, Everman et al. 2009). Dose-response assays showed a high susceptibility among the different *Conyza* spp. with differences between species. At 4 days after treatment all the plants showed phytotoxicity symptoms due to the herbicide application.

The dose inhibiting the above-ground biomass by 50% (ED_{50}) decreased in the order: *C. bonariensis* > *C. sumatrensis* > *C. canadensis*. The ED_{50} values found were 0.216; 0.09 and 0.058 L ha^{-1} , respectively (Figure 1). *C. bonariensis* was 3.7 and 2.4 times less susceptible than *C. canadensis* and *C. sumatrensis*, respectively. These results are similar to those reported by Fernandez-Cerejido et al. (2009) whose work with several adjuvants on the effectiveness of *Conyza* spp. reported that *C. albida* showed a higher susceptibility to glufosinate than *C. bonariensis* regardless of the adjuvant.

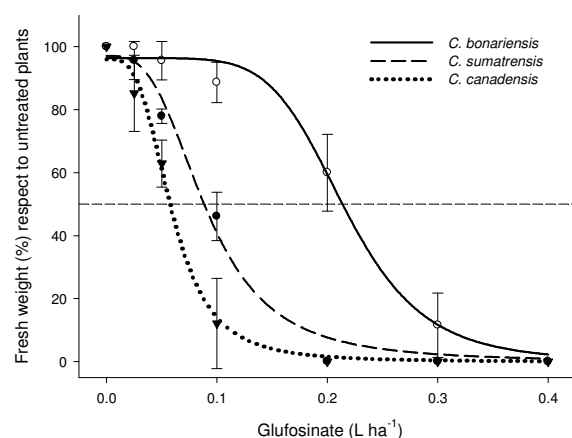


Figure 1. Dose response curves of the three species of *Conyza* spp. Fresh weight was determined 21 days after treatment, data were expressed as percentage with respect to untreated plants. Vertical bars represent \pm standard errors of the mean. Córdoba, Spain, 2011.

At later developmental growth stages in *C. bonariensis*, the dose inhibiting the above ground biomass by 50% increased considerably. ED_{50} showed values of 0.517 and 1.297 L ha^{-1} for the second and third growth stage, respectively (Table 1). Other studies carried out on *A. artemisiifolia* L. showed that the most effective control in this species with glufosinate or glyphosate is at bud appearance (BBCH 53-55) growth stage (Gauvrit and Chauvel 2010). Assays made on different species such as *Amaranthus rudis* Sauer; *Ambrosia trifida* L.; *Ipomoea hederacea* (L.) Jacq.; *Eriochloa contracta* Hitchc.; *Abutilon theophrasti* medicus and *Cyperus esculentus* L.

treated with glufosinate at three different growth stages showed different responses among species, locations and growth stage application. *Ambrosia trifida* L., *Ipomoea hederacea* (L.), and *Eriochloa contracta* Hitchc. showed a major percentage damage when treated at an early growth stage compared to later developmental stages. However, when glufosinate was applied on *A. trifida* L. in another location the visual damage was different, and major injury was obtained when plants were treated at 15 cm in height (Hoss et al. 2003). The differences in sensitivity found in this work could be due to differences in absorption/translocation or metabolism as has been demonstrated in other studies. The study of those techniques requires further investigations.

Table 1. Parameters found in the model^a used to calculate the glufosinate dose required for 50% plant injury (ED₅₀) in *C. bonariensis* plants treated at three different growth stages. Córdoba, Spain, 2011.

Growth stage	c	d	b	ED ₅₀ (L ha ⁻¹)	pseudo r ^{2b}	P ^c
Rosette (BBCH 14-15)	0.89	100.00	5.68	0.216	0.97	<0.001
10-15 cm in height	43.54	97.58	3.99	0.517	0.87	<0.001
Capitula formed (prior to flowering)	60.25	95.98	4.28	1.297	0.93	<0.001

^a $Y = c + \{(d - c) / [1 + (x / g)^b]\}$, where Y is the percent plant injury; x (independent variable) is the herbicide rate; c and d are the lower and upper, asymptote, respectively; b is the slope of the curve; and ED₅₀ is the effective herbicide rate required for 50% plant injury. Data were pooled and fitted to a non-linear regression model. ^b Approximate coefficient of determination of non-linear models with a defined intercept calculated as pseudo r² = 1 - (sums of squares of the regression/corrected total sums of squares). ^c Probability level of significance of the non-linear model.

Spray retention assays. *Conyza canadensis* retained more glufosinate than *C. sumatrensis* and *C. bonariensis*, but there were no significant differences between populations at BBCH 14-15 stage (Table 2). In the second and third growth stage in *C. bonariensis* the values in spray retention decreased, with the values ranging from 0.35 to 0.45 mL spray retention g⁻¹ dry weight. There were no significant differences between those two growth stages, although when compared with the amount retained in the BBCH 14-15 stage there were significant differences.

Species like *Ambrosia artemisiifolia*, *Triticum aestivum* L., *Pisum sativum* L., *Chamomilla recutita* L., *Solanum nigrum* L., *Lycopersicon esculentum* Mill. have shown differences in the amount of spray retention with values ranging from 0.3 to 0.4 mL g⁻¹ dry weight (De Ruiter et al. 1990). In the BBCH 14-15 stage, the data in spray retention are not in accordance with the ED₅₀ values found in the different *Conyza* spp., *C. bonariensis* retained almost the same amount of herbicide solution (with no significant differences), but the ED₅₀ value was higher compared with the other two species. In the other two growth stages of *C. bonariensis* the amount of herbicide retained was similar without significant differences. However, as in the BBCH 14-15 stage, the ED₅₀ values are not in accordance; this could be due to the amount

of herbicide absorption by the plant in the different growth stages, and different factors such as temperature, humidity, spray volume and the plant physiology (Grangeot et al. 2006).

Table 2. Spray retention values obtained with treated plants of *Conyza* spp. in the BBCH 14-15 stage and two additional growth stages in *C. bonariensis*. Córdoba, Spain, 2011.

Species	Stage	mL spray retention g ⁻¹ dry weight ^a
<i>C. canadensis</i>	BBCH 14-15	1.16 ± 0.29 A
<i>C. sumatrensis</i>	BBCH 14-15	0.99 ± 0.17 A
	BBCH 14-15	0.98 ± 0.20 A
<i>C. bonariensis</i>	10-15 cm in height	0.44 ± 0.05 B
	Capitula formed (Prior to flowering)	0.38 ± 0.05 B

^a Mean values within a column followed by the same letter are not significantly different at the 0.05 level as determined by the Tukey HSD test. Coefficient of variation (CV) = 21.64. Mean values ± standard errors of the mean.

Conclusions

Results obtained show the differential response of the three *Conyza* in southern Spain to glufosinate. As demonstrated by the dose response curves, the optimal application timing is in the rosette stage. Investigations are under way to elucidate the physiological factors that could explain this differential response.

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Received on January 4, 2012.

Accepted on February 20, 2012.

Online published on February 28, 2012.

Quality of English writing is responsibility of authors.