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Erigeron bonariensis L.: Caracterización de accesiones resistentes a glifosato en Colombia

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Resumen

El manejo efectivo de malezas es esencial en la agricultura moderna. Actualmente, glifosato es el herbicida más utilizado en el mundo, ofreciendo control efectivo de malezas, no-selectivo en post-emergencia al inhibir la enzima EPSP sintasa en los cloroplastos. El recurrente uso de un mismo modo de acción herbicida puede seleccionar biotipos resistentes al herbicida y resultar en la pérdida de eficacia. *Erigeron bonariensis* L. comúnmente llamada venadillo es una planta nativa de Sudamerica que ha invadido muchos ecosistemas en el mundo y que ha sido reportada como maleza resistente a glifosato en Colombia. *E. bonariensis* está adaptada a muchos nichos ecológicos, incluyendo agroecosistemas de cultivos esenciales. Se ha tenido sospecha de resistencia a glifosato en esta especie desde los años 90 y se confirmó resistencia desde 2006. El objetivo del presente estudio consistió en detectar la resistencia a glifosato en poblaciones de *E. bonariensis* en Colombia, estimar los niveles de resistencia y proponer medidas de control con herbicidas que fueran eficaces. En ensayos en invernadero, se confirmó que todas las poblaciones provenientes de agroecosistemas donde su había utilizado glifosato son resistentes a este herbicida, presentan porcentaje de supervivencia >80% a la dosis recomendada (1080 g ea ha^{-1}). Además el 90% de las poblaciones sobrevivió un 80% de las plantas al usar el doble de esta dosis. En dos poblaciones caracterizadas los factores de resistencia fueron de 3,15 y 22,3 veces la dosis necesaria para controlar la población más sensible. Ésta población presentó un ED_{50} en base a biomasa de 109 g ea ha^{-1}. Cinco herbicidas con diferente modo de acción fueron evaluados resultando pyraflufen-etyl y mesotrione los más efectivos y sugiriendo posibles casos resistencia múltiple con paraquat y a 2-4,D.

Palabras clave: dosis-respuesta, venadillo, rama negra, hormesis, log-logistic, buva, *Conyza* herbicida

Widespread occurrence of glyphosate-resistant hairy fleabane (*Erigeron bonariensis* L.) in Colombia and weed control alternatives

Abstract

Effective weed management is essential in modern agriculture. Currently, glyphosate is the most used herbicide globally, offering non-selective and post-emergence weed control by inhibiting the EPSP synthase in chloroplasts. Ubiquitous and recurrent use of the same herbicidal mode of action may concurrently select herbicide-resistant biotypes and thus result in loss of efficacy. Hairy fleabane (*Erigeron bonariensis* L.) is a native South American species that has invaded many agroecosystems worldwide, commonly reported as a glyphosate-resistant weed. In Colombia, *E. bonariensis* is adapted to many ecological niches, including essential crop systems. Putative hairy fleabane resistance to glyphosate was purported since the late '90s but eventually confirmed in Colombia's coffee plantations in 2006. Consequently, anecdotal accounts by farmers suggest a prevalence of glyphosate-resistant fleabane in several crop systems in Colombia and consistent with the dispersion of glyphosate-resistance hairy fleabane reported for this species in other countries. Objective in this investigation was to detect the resistance to glyphosate, also to estimate the levels of that resistance and to propose effective chemical options to control *E. bonariensis* in Colombia. We conducted a resistance profile test under a greenhouse to evaluate ten hairy fleabane populations collected from different agricultural systems in Colombia. We confirmed that all populations were glyphosate-resistant, with at least 80% survival to the recommended field rate of 1080 g ae ha^{-1}. Importantly, in 90% of populations, at least 80% of individuals survived to the double glyphosate field rate, suggesting high levels of glyphosate resistance in *E. bonariensis* from Colombia. As a reference, five pristine *E. bonariensis* populations collected from areas devoid of exposure to glyphosate were effectively controlled at the recommended rate, confirming that susceptibility still exists in non-sprayed areas. Characterization based on relative biomass through glasshouse dose-response studies identified one population with a low resistance factor (P_{10} with 3.15-fold) and a second, with a high resistance factor (P_{15} with 22.3-fold) when compared with the most sensitive population (P_7), which had an ED_{50} of 109 g ae ha^{-1}. Interestingly, both populations displayed hormesis at recommended glyphosate doses during this assessment. Finally, five herbicides with different modes of action were tested, identifying pyraflufen-ethyl as the most effective, followed by mesotrione; paraquat and glufosinate were the least effective. Our findings confirmed the prevalence of high glyphosate-resistant *E. bonariensis* in key crops throughout Colombia (i.e., plantain, banana, cassava, passionfruit, papaya, and red beans). Effective weed management strategies need to be implemented by Colombian farmers to mitigate the evolution of glyphosate resistance, combining mechanical and cultural control. Chemical alternatives include PPO and HPPD herbicides as part of the integrated weed management program.

***Keywords**:* dose-response, venadillo, rama negra, hormesis, log-logistic, buva, *Conyza,* herbicide

Introduction

Chemical weed control revolutionized agriculture worldwide in the 20th century. Advancements in cost-effective agricultural production systems, assisted by agrochemicals,

have sustained in part the demand for food of an increasing human population in the last decades (Heap, 2014; Kaushansky et al., 2018). Glyphosate is the world's most successful herbicide since its introduction in 1974; the molecule offers non-selective, systemic and effective post-emergence action (Duke, 2018; Heap & Duke, 2017). In Colombia, glyphosate is also the most sold and commonly used agrochemical (Valbuena et al., 2021). Thus, growers frequently spray glyphosate at pre-sowing of the crop as a broadcast application, directed spray in several annual and perennial crops, over-the-top applications in glyphosate-resistant crops and in non-agricultural areas.

Glyphosate acts by disrupting the shikimate pathway, ultimately resulting in the arrest of aromatic amino acid biosynthesis via inhibition of the enzyme 3-phosphoshikimate 1-carboxyvinyltransferase (EPSP synthase; EC 2.5.1.19) in the chloroplast (Heap & Duke, 2017; Morell et al., 1967). The shikimate pathway is responsible for *circa* 20% of the carbon fixed by plants, synthesizing essential precursors and metabolic compounds as vitamins, lignins, alkaloids and flavonoids. Thus, glyphosate's inhibition of EPSP synthase impacts several fundamental processes in the metabolism of the plant, mainly photosynthesis (Cobb & Reade, 2010; Heap & Duke, 2017). Inhibition of photosynthesis is triggered by a decreased concentration and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO; EC 4.1.1.39), ultimately instigating the generation of reactive oxygen species (ROS) as H_2O_2 (Ahsan et al., 2008). Thus, observed symptoms are growth inhibition, chlorosis is evident only several days after glyphosate and is followed by on necrosis, decay and plant death. These symptoms are consequences of aromatic amino acids scarceness, lack of essential metabolic compounds, photosynthesis reduction and oxidative stress (Cobb & Reade, 2010; Sammons & Gaines, 2014).

Hairy fleabane (*Erigeron bonariensis* L.) (synonym: *Conyza bonariensis* [L.] Cronquist) (Asteraceae), is a cosmopolitan plant native from South America (Fuentes et al., 2011). It was first described in Argentina and naturalized in warm areas worldwide (Wu et al., 2010). Together with *Erigeron* (=*Conyza*) *canadensis*, these species possess a weedy and aggressive invasive behaviour, characteristic noxiousness as well as resilience to environmental stress (Bajwa et al., 2016). Competition by *Erigeron* in soybean may result in >50% yield loss and is considered currently, the most problematic glyphosate-resistant broadleaf weed in South America (Peterson et al., 2018). Under tropical weather conditions as Colombia, hairy fleabane develops in a wide range of temperatures and altitudes, ranging from sea-level to above 3,900 m (Bajwa et al., 2016; Fuentes et al., 2011). Moreover, in Colombia, hairy fleabane has been reported as an important weed species in annual and perennial crops like banana, plantain, cassava, vegetables, coffee, rice, corn, and diverse fruits; commonly found on roadsides and waste areas (Menza-Franco & Salazar-Gutierrez, 2006; Montealegre, 2011; Quintero-Pértuz & Carbonó-DelaHoz, 2016).

Resistance to pesticides represents an imperil that may compromise sustainability of current crop production systems and consequently, food production, and quality (Kaushansky et al., 2018). Herbicide resistance is defined as the heritable ability of a weed biotype to survive the application of a recommended herbicide rate, that was effective prior to this event (Heap, 2005). Resistance to glyphosate on weeds was first confirmed in *Lolium rigidum* in Australia (Pratley et al., 1999). Nowadays, more than 55 weed species have been confirmed resistant to glyphosate. Approximately half of these reported cases have evolved resistance

to this herbicide under glyphosate-resistant cropping systems (Heap, 2021; Heap & Duke, 2017). The increased evolution of weeds resistant-to-glyphosate under glyphosate-resistant cropping systems is explained by higher selection pressure on such systems. There, growers rely on multiple glyphosate applications within the same growing season, without concern of crop injury (Owen et al., 2015; Owen & Zelaya, 2005). Similarly, perennial crops are routinely sprayed several times a year with glyphosate, favouring the evolution of resistance (Heap & Duke, 2017).

Hairy fleabane was firstly reported glyphosate-resistant in grape orchards in South Africa (Heap, 2021; Heap & Duke, 2017). In Colombia, first report was in 2006 in coffee bean groves (Heap, 2021; Menza-Franco & Salazar-Gutierrez, 2006). Recently, research characterized resistance to glyphosate in hairy fleabane populations from banana plantations in Magdalena province (Quintero-Pertuz et al., 2021).

Therefore, the objective of this research was to confirm glyphosate-resistance in hairy-fleabane. We also pursued to estimate the frequency and the level of resistance in populations from perennial and annual crops in Colombia (i.e. banana, plantain, papaya, passionfruit, cassava and red beans) and to assess alternative herbicide to control glyphosate-resistant hairy fleabane.

Materials and Methods

Plant material

A total of 10 locations were surveyed for presence of *E. bonariensis* in annual, and perennial crops and a roadside in Colombia (Table 1, Figure 1). Locations were selected based on the anecdotal reports of the weed by technicians and growers. Dry achenes (seeds) were acquired by removing florets from capitula from 10 to 15 plants per site, then samples were combined. As a reference of resistance, two previously-confirmed glyphosate resistant populations (Amaro-Blanco et al., 2018) collected in railway sides in Cordoba (Spain) were used; these accessions were kindly conveyed by Dr. Rafael de Prado. In addition, a third glyphosate-resistant and one glyphosate susceptible population collected in banana plantations was characterized by Dr. Irma Quintero were used in the study (Quintero-Pertuz et al., 2021). Another three putative sensitive populations were collected from understory in rainforests and one farm where glyphosate had not been sprayed over last ten years. At each location, coordinates and altitude were recorded using Garmin® 12 (Garmin International, Olathe, United States) to ascertain the specific location where collections were performed.

Capitula were placed in paper bags in the field and then transferred to the laboratory for drying, cleaning and storage. Achenes were sown in plastic pots (10 x 10 x 10 cm) filled with peat Projar® PS Seed PRO 8020 (Projar, Spain), then immediately watered and covered with plastic film to favour germination. Seedlings were placed in a germination chamber at 28-32 ºC, 16 hours photoperiod and fertilized bi-weekly with 0,1 g per pot of the water soluble fertilizer Soluplant® Inicio (Agafert, Italy). After 45 days of planting, 2 cm height seedlings were transplanted by placing one plant per pot that were filled with peat and organic soil mixture in a 1:1 proportion. Plants were grown under glasshouse conditions in Universidad Nacional de Colombia (4º38’10.8” N; 74º5’19.4” W, altitude 2,610 m) with day/night

temperature 36/28 ºC, 47% HR and 12 hours photoperiod. Plants were fertilized bi-weekly using the same fertilizer solution as in germination chamber. Plants were sprayed at rosette stage (BBCH 19), having 9 to 12 cm diameter. Herbicide solution was sprayed at 200 L ha^{-1} using de-ionized water in spray chamber equipped with flat-fan nozzle Tee-Jet® 80-02 (Spray Systems Co., United States) at 275 KPa.

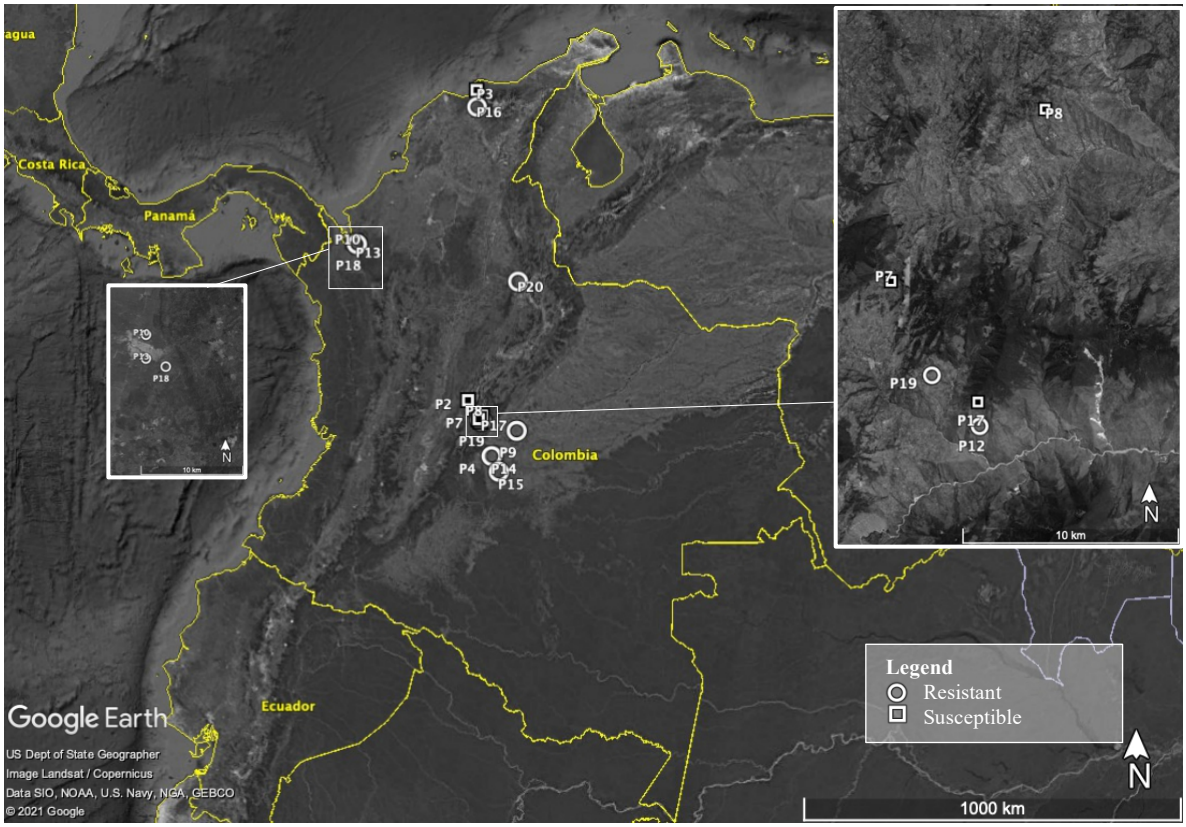

Figure 1. Map of Colombia describing the location where *E. bonariensis* accessions were collected.

Resistance profile test

The experimental design was a completely randomized, factorial arrangement with three replicates. The first factor was population (accession) with 18 levels, second factor was glyphosate rate with 3 levels: 0X, 1X and 2X, X being equivalent to field rate of 1,080 g ae ha^{-1} (Puricelli et al., 2015; Tahmasebi et al., 2018). The commercial glyphosate product used in the study was Round Up® Activo SL (Monsanto, United States) containing 363 g ae L^{-1} and formulated as glyphosate monopotasic salt.

After glyphosate application, plants were assessed for visual damage and survival at 21 Days after treatment (DAT). Plant survival assessment was based on visual appearance, colour and turgidity of the meristem; indicators that would suggest recover or not of plants and whether these would reach maturity and produce seed. Visual percent control was based on a scale from 0 to 100%, zero being equivalent to non-observable damage, whilst 100% was equivalent to a dry or necrotic, brown, non-phototactically active plant. Aerial parts of plants

were harvested at 21 DAT to determine fresh biomass (FW), while dry weight (DW) was determined by placing these tissues on individual paper bags at 40 ºC for 48 hours.

Table 1. *Erigeron bonariensis* populations tested.

ID	Crop	Department	Glyphosate use pattern Dose/sprays per year/time	Regional context	Coordinates Lat/Long	m a s l[1]
P2	Asparagus[s]	Cundinamarca	No use	H/CA	4º40'28.3"/74º13'1.2" W	2,516
P3	Rainforest[s]	Magdalena[a]	No use	U	11º6'24.9"/ 75º4'13" W	2,019
P4	Cassava	Meta	1,424 g ae ha-1/ 4 sprays/8 yr	CA	3º30'28.1"/73º43'21.8" W	371
P5, P6	Railway[c]	Cordoba, Spain	1,440 g ae ha-1/16 years	Non crop land	37º57'10,29"/ 4º53'20,09" E	ND
P7	Rainforest[s]	Cundinamarca	No use	U/near to CA	4º17'20.7"/ 73º59'42" W	2,855
P8	Rainforest[s]	Cundinamarca	No use	U/near to CA	4º21'38.5"/ 73º59'6,14" W	2,778
P9	Plantain	Meta	1,424 g ae ha-1/ 8 sprays/10 yr	CA	4º1'26.0"/73º13'51.5" W	221
P10	Plantain	Antioquia	1,068 g ae ha-1/ 4 sprays/>10 yr	CA	7º53'40,6/76º37'17,5 W	34
P12	Red Beans	Cundinamarca	1,068 g ae ha-1/ 1-2 sprays/18 yr	CA	4º13'42.6"/73º57'29.3" W	2,056
P13	Urban area	Antioquia	ND	Non crop land	7º51'59.6"/76º36'14.4" W	51
P14	Plantain	Meta	1,424 g ae ha-1/ 4 sprays/4 yr	CA	3º30'23.9"/73º44'49.8 W	321
P15	Papaya	Meta	1,424 g ae ha-1/ 10 sprays/5 yr	CA	3º11'16.2"/73º35'54.3" W	342
P16	Banana	Magdalena[b]	1,068 g ae ha-1/ 6 sprays/>20 yr	CA	10º45'3.6"74º7'10.5" W	53
P17	Shrubland[s]	Cundinamarca	No use	Sh	4º13'38.4"/ 74º57'13.3" W	2,026
P18	Plantain	Antioquia	1,068 g ae ha-1/ 6 sprays/>20 yr	CA	7º51'59.6"/76º36'14.4" W	56
P19	Red Beans	Cundinamarca	1,068 g ae ha-1/ 1-2 sprays/18 yr	CA	4º14'58.6"/73º58'41.2" W	2,316
P20	Passionfruit	Santander	1,424 g ae ha-1/ 6 sprays/8 yr	CA	7º4'35/73º12'46.7" W	1256

[1]meters above sea level
[s] Susceptible (*S*) populations
[a] Collected and characterized by (Quintero-Pertuz et al., 2021)
[c] Reference-resistant populations collected and characterized by (Amaro-Blanco et al., 2018)
ND = Not defined; no data available; H= Horticulture; CA=Conventional agriculture; Sh=Shrubland;U=Understory

Dose-response tests

Based on results from the resistant profile test, two confirmed resistant (*R*) populations with different levels of resistance were chosen for further characterization via dose-response tests. In addition, one *R* population from Spain (P_5) and two glyphosate-sensitive (*S*) populations identified from the discriminatory test, were included in the study as reference for resistance and susceptibility. Application timing for glyphosate was BBCH 19 (9-13 cm diameter). The experiment was conducted in a completely randomized design with factorial structure and four biological replicates (plants). The first factor was population (accession) with five levels and a second factor was rate with seven levels: 0, 102, 276, 750, 2,039, 5,542 and 15,064 g ae ha^{-1}. Visual control and plant survival were assessed at 21 DAT based on the aforementioned criteria. Aerial dry biomass was assessed by cutting the plant material at the soil surface at 21 DAT, drying the material for 48 hours at 40 ºC and letting samples cool, before determination. The dose-response tests were repeated in time to better understand a potential hormesis response, hence allowing comparison of results in an additional replication on a trial using same method described.

Alternative chemical control options for glyphosate-resistant populations

The efficacy of five herbicides with different modes of action were assessed on three *R* populations: paraquat (HRAC: 22), 2,4-D (HRAC: 4), mesotrione (HRAC: 27), glufosinate

(HRAC: 10) and pyraflufen-ethyl (HRAC: 14) (Heap, 2021). Commercial formulations of these herbicides and adjuvants were sprayed at the rates recommended by the label. *R* populations were selected based on the levels of glyphosate resistance observed in the resistance profile test and their geographical location in Colombia. Two selected *R* populations were those characterized via dose-response tests (papaya P_{15} and plantain P_{10}) and a third-one, from a passionfruit crop in Santander (P_{20}), demonstrating a high resistance level in the resistance profile test. The application timing and spray volumes were identical to those utilized in the dose-response and resistance profile tests (BBCH 19 and 200 L ha^{-1}).

The experiment consisted of a Complete Randomized Design in a factorial arrangement, the first factor being population (three levels; P_{10}; P_{15} and P_{20}) and the second factor herbicide rate (six levels). Each trial contained four biological replicates (plants). The commercial formulations tested were: Gramoxone® (Syngenta, Switzerland) containing paraquat dichloride at 200 g ai L^{-1}, SL formulation sprayed at a dose of 600 g ai ha^{-1}; Amina® (Invesa, Colombia), containing 2,4-D as dimethylamine salt at 720 g ae L^{-1}, SL formulation sprayed at a dose of 720 g ai ha^{-1}; Callisto® (Syngenta, Switzerland), containing mesotrione at 480 g ai L^{-1}, SC formulation sprayed at a dose of 100 g ai ha^{-1}; Finale® (BASF, Germany), containing glufosinate ammonium at 150 g ai L^{-1}, SC formulation sprayed at a dose of 225 g ai ha^{-1} and Et-Herb® (Nihon Nohyaku, Japan), containing pyraflufen-ethyl at a concentration of 26.5 g ai L^{-1}, EC formulation sprayed at a rate of 8 g ai ha^{-1}. Assessment variables were biomass DW at 28 DAT and visual control using the aforementioned criteria at 14 and 28 DAT.

Statistical analyses

Statistical analyses were performed using R-Studio version 1.1.463 (R-Studio, Inc., United States). For the resistance profile test (three-rate evaluation), data were analysed through the Effect Model with Restricted Maximum Likelihood (REML) using the *nmle* package (Pinheiro & Bates, 2020; R Core Team, 2018). This tool permitted to select a model evaluating the log-likelihood ratio based on the lowest Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Both AIC and BIC are complementary criterions that assisted in selecting the curve with best fit to data thus selecting the more parsimonious model (Aho et al., 2014; Zabala et al., 2019). Thus, the comparison of the variates to the fixed effects were conducted using the function *ghlt* package in R (Hothorn et al., 2008). Further, the response to glyphosate was scored independently for each variate, segregating in susceptible and resistant populations using the most repeated value or *mode* (Table 2) (Panozzo et al., 2015; Zabala et al., 2019). Finally, descriptive statistics were used to compare the responses in control, survival and the relatives fresh and dry biomass among the categories outputted.

Table 2. Separation of susceptibility and resistance level in the discriminatory test.

Category	Sub-category	Survival[a] (%)	Visual Control[c] (%)	Relative fresh biomass (%)	Relative dry biomass (%)
Susceptible	S	<80% at 1X[b]	>80% at 1X	Less than 20% at 1X	Less than 50% at 1X
Resistant	R_1	>80% at 1X	<80% at 1X	>20% at 1X but <20% at 2X	>50% at 1X but <50% at 2X
	R_2	>80% at 2X	<80% at 2X	>20% at 2X	>50% at 2X

[a]Adapted from (Beres et al., 2018);[b] *x*=1080 g ae ha^{-1};[c] Adapted from (Panozzo et al., 2015; Zabala et al., 2019)

For the dose-response determination, analyses were performed using the package *drc* in R (Knezevic et al., 2007; R Core Team, 2018; C. Ritz et al., 2015). The variates dry weight, relative weight, survival and visual control were fitted to the four-parameter log-logistic model (Burgos et al., 2013; Seefeldt et al., 1995). The best-fit model was selected using the function *mselect*, which considers the higher log-likelihood and lack-of-fit, yet the lower AIC and residuals variance (Knezevic et al., 2007; C. Ritz et al., 2015). The four-parameter log-logistic regression model is a curve symmetric at the inflection point "e" or ED_{50}, in the formula (1):

$$y = \mathrm{c} + \frac{\mathrm{d} - \mathrm{c}}{1 + e^{b \times (\log x - \log \mathrm{e})}} \quad \{1\}$$

Where: "*y"* is the response (survival, dry biomass, etc); "c" and "d" are lower and upper limits respectively; "*e"* is Euler`s number; "*b"* the slope at the parameter "e"; "*x"* the herbicide rate and "e" or ED_{50} which is also called GR_{50} and LD_{50} in the case of mortality/survival (Knezevic et al., 2007; C. Ritz et al., 2015; Christian Ritz & Strebig, 2016). Resistance factors are useful to estimate the magnitude of resistance to an herbicide within a population (Burgos et al., 2013; Heap, 2005). Resistance factors (RF) were calculated by the R/S ratios considering each GR_{50} estimated for resistant and sensitive populations respectively.

To the variable visual control percent, the parameter "d" was assigned to 100% while "c" was 0%, the function estimates the two remaining parameters. Idem, for survival rate where "d" was assigned to 1 and "c" was 0.

Dry biomass of two putative resistant populations collected in Colombia fitted best to the hormesis Brain-Cousens model with the formula (2):

$$y = \mathrm{c} + \frac{\mathrm{d} - \mathrm{c} + \mathrm{f}x}{1 + e^{b \times (\log x - \log \mathrm{e})}} \quad \{2\}$$

Where: "c" and "d" are the lower and upper asymptotes; "b" and "e" do not have interpretation and "f" is the size of the hormesis effect which must be different to zero for the model to have sense (Brain & Cousens, 1989; Knezevic et al., 2007; Ritz et al., 2015; Ritz & Strebig, 2016).

Statistical analyses for the alternative chemical control options were performed through variance analyses and Tukey test, using the function HSD test in the package *agricolae* (de Mendiburu, 2017; R Core Team, 2018; Tahmasebi et al., 2018).

Results and discussion

Resistance profile test

Consistently, survival, percent control, FW and DW data fitted better to the mixed model using doses as fixed effects and population as a random effect (Table 3). The interaction between populations and replicates was not significant (data not shown) and the mixed model had higher explanatory power than using a linear model. The treatment (dose) effect was significant for all variates tested and the model was effective in removing the variation caused

by populations' factor. For further and detailed description of these analyses refer to the supplementary table 1. Finally, the mixed model enabled proper assessment of the response to glyphosate based on the response of each population in terms of efficacy. Thereby, the discriminatory test allowed to effectively separate the response to glyphosate into: susceptible populations (*S*) and the two resistant population levels as described in table 2.

Table 3. Explanatory power and random effects' importance in the mixed model for survival, percent control, fresh (FW) and dry (DW) biomass in the 18 *E. bonariensis* populations.

Variable	Fix effect	Pop.	Pop. x Rep	Log-Lik[c]	AIC[d]	BIC[e]	Lik.[c] ratio	df[f]	p-value
Survival	Dose	x	-	-38.80	85.61	97.91	30,90	4	<0,0001
Control	Dose	x	-	-74. 85	1495.70	1507.99	68.43	4	<0,0001
FW[a]	Dose	x	-	-338.85	685.71	698.02	42.71	4	<0,0001
DW[b]	Dose	x	-	-100.73	209.45	221.75	52.82	4	<0,0001

[a]FW = Fresh biomass; [b]DW=Dry biomass; [c]Lik=Likelihood; [d]AIC = Akaike information criterion
[e]BIC = Bayesian information criterion; [f]df= Degrees of freedom; See supplementary Table 1 for detailed analyses.

All reference glyphosate resistant (*R*) and glyphosate susceptible (*S*) populations were classified as expected (Table 4). Where, putative sensitive (P_3) from the understory resulted glyphosate-sensitive as reported (Quintero-Pertuz et al., 2021). Conversely, the three *R* accessions (P_5-P_6-P_{16}) were rated as resistant (Amaro-Blanco et al., 2018; Quintero-Pertuz et al., 2021). Meanwhile, the putative sensitives were classified as sensitive, these populations were collected from: rainforests (P_7, P_8), a shrubland (P_{17}) and a non-glyphosate-sprayed farm (P_2).

Table 4. Resistance profile test output. Hairy fleabane response to glyphosate in reference and susceptible populations.

	Crop/ Landscape	Code	Survival	Control	Rel-FW[a]	Rel-DW[b]	Overal (mode)
Reference Resistant	**Railway (Spain)**	**P_5**	R_2	R_2	R_2	R_2	**R_2**
		P_6	R_2	R_2	R_1	R_2	**R_2**
	Banana	**P_{16}**	R_2	R_2	R_2	R_2	**R_2**
Reference Sensitive	**Rainforest**	**P_3**	S	S	S	S	**S**
Putative Sensitive	**Asparagus**	**P_2**	S	S	S	S	**S**
	Understory	**P_7**	S	S	S	S	**S**
		P_8	R_1	S	S	S	**S**
	Shrubland	**P_{17}**	S	S	S	S	**S**

[a]Rel-FW= relative fresh weight 21 DAT, [b]Rel-DW= relative dry weight 21 DAT; Mode= Most repeated value; S=Susceptible; R_1= Low resistance level; R_2=High resistance level, according to table 2

Sensitive populations (*n*=5) were successfully controlled with the 1X dose (1,080 g ae ha^{-1}), where visual control averaged 89,2% (SE ±5,92) (Fig. 3a). With 2X rate, visual control average was 96% (SE ±2.45). Conversely, the populations with lower resistance level R_1 averaged 67.5% (SE ±5.81) visual control at the 1X dose and only 80% (SE ±3.87) when plants were sprayed with the 2X field rate. Exceedingly, poor visual control 16,82% (SE ±6.96) at the 1X rate was observed with the R_2 group and only 43.03% (SE ±9.54) visual control with the 2X rate (Figure 2). Tahmasebi, et al (2017) found that *S* populations were

effectively controlled with the 1X dose with average controls above 90%, whilst one *R* population had only 40% visual control at the 1X and 70% at 2X rate, these values were similar to the averages reported on the R_1 populations described on this study.

All nine populations sampled from agricultural glyphosate-sprayed systems were resistant to glyphosate (Figure 2). In addition, the population collected on a street (non-agriculture) near to a banana plantation were also classified as R_2. In Iowa and Ohio (United States), researchers found that 45% of the non-agricultural sampled places were *R* and may account for high survival (>80%) even at 40 times the recommended field rate (*i.e*. 33,600 g ae ha^{-1}) (Beres et al., 2018). Other results confirmed that non-agricultural areas are also serve as refugee for *R* genes (Amaro-Blanco et al., 2019).

Surprisingly, eight out of ten populations (80%) originating from agroecosystems with history of glyphosate use in Colombia resulted in high *R* level "R_2" (See supplementary table 2). Two populations sampled from plantain areas, reported a lower level for resistance to glyphosate "R_1", explained by a lower selection pressure with fewer sprays per year (Table 1). Surveys performed in Brazil detected *R* on 71,2% of *Erigeron* sp. populations out of 1.184 samples collected between 2014 and 2018 (Mendes et al., 2021). Previously in the United States, 100% of the *E. canadensis* from agricultural areas had high levels of resistance in Ohio, while in Iowa more than 90% were *R* sites. These results describe the seriousness of the problem and suggest that resistance to glyphosate will evolve in *E. bonariensis* within agricultural landscapes as those sampled in Colombia.

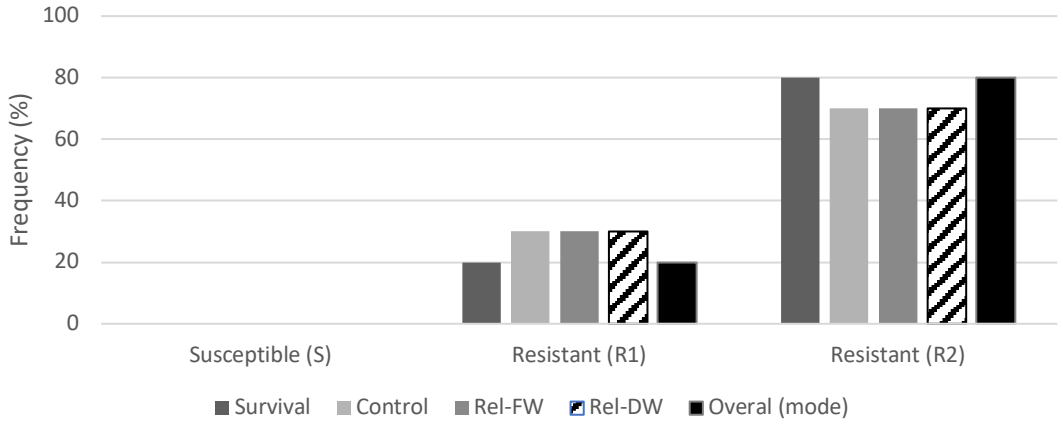

Figure 2. Response to glyphosate in *E. bonariensis* collected from different agroecosystems. Rel-FW= Relative fresh weight 21 DAT; Rel-DW= Relative dry weight 21 DAT. R1= Low resistance level; R2=High resistance level, according to table 2

The calculated relative fresh biomass (Rel-FW) and dry weight (Rel-DW) averages demonstrated significant differences between 1X and 2X rates (Fig. 3c-3d). Furthermore, in "S" populations, the Rel-FW was on average only 10,49% (±3.51) in contrast with the untreated control at dose the 1X rated and was reduced to only 4.68% (±1.62) at 2X. The Rel-FW average for R_1 spraying the recommended rate was affected much less than with the S populations and reached 60.63% (±1.62). When using 2X, that relative biomass was also

higher in R_1 than in S 17.36% (±5.04). Considering the R_2 populations, Rel-FW averaged biomass increased by 109.16% (±13.99) at 1X rate, suggesting a hormesis response at the recommended glyphosate dose versus the untreated. Interestingly, several populations classified as R_2 plants had height greater than the untreated and earlier flowering (Figure 4e-4f) at the 1X dose. Hormesis occurs when a dose-response relationship has a characteristic opposite effect at low dose compared to high doses (Kendig et al., 2010) in other words, this effect represents an stimulatory response at a subtoxic level of toxin (R. G. Belz & Duke, 2014). Other authors have described hormesis as a non-monotonic or biphasic effect (Zhu et al., 2013). Several researchers have reported a hormesis response to glyphosate applications in *Erigeron* (Brito et al., 2018).

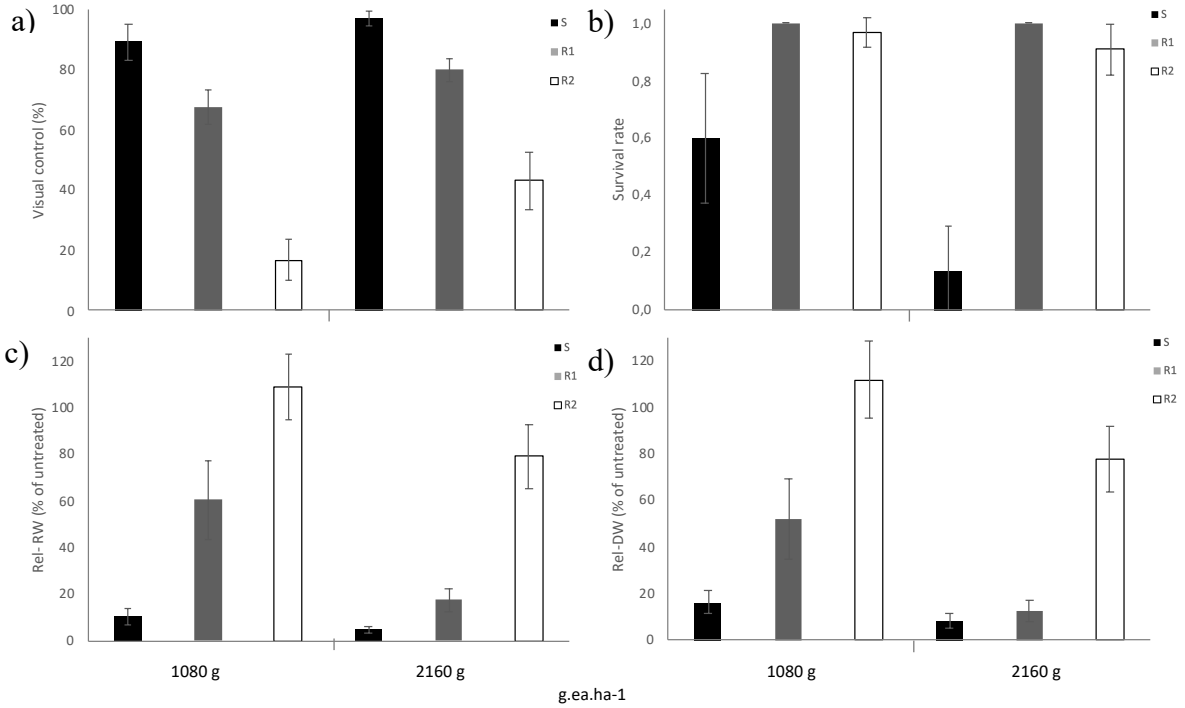

Figure 3. a) Visual control, b) survival rate, c) relative fresh weight (Rel-FW) and d) relative dry weight (Rel-DW) in *E. bonariensis* 21 days after treatment with glyphosate. Bar size= Average per class, S (*n*=5), R_1 (*n*=2) and R_2 (*n*=11), error bars = ± Standard Error (SE).

In addition to *Erigeron* species, at least other 20 cultivated plants and weeds have expressed a hormesis response to glyphosate applied from 1.9 to 730 g ae ha^{-1} (Brito et al., 2018). In South Africa hairy fleabane accessions classified as *R* showed hormesis when the trials were performed under 27 ºC conditions but not at 15 ºC (Okumu et al., 2019). In *E. canadensis* field *R* populations from Indiana (United States), five out of nine accessions revealed hormesis when measured by fresh weight in glyphosate dose-response trials (Davis et al., 2010).

A research in Brazil found that a glyphosate dose of 90 to 360 g ae ha^{-1} in *E. sumatrensis* increased plant height and had earlier flowering while there was no effect in number of capitula per plant after treatment (Gomes, 2014). Our results found a hormesis response at the recommended rate, indicating that some Colombian biotypes may have a larger tolerance to glyphosate applications than on the aforementioned studies in South Africa, Brazil or United States.

In particular, 6 out of 11 populations (55.55%) collected in Colombia had hormetic responses at dose the 1X, and surprisingly two (22.2%) had hormesis at the dose 2X, having values of biomass higher compared to the untreated control (UTC). These results contrast with other researchers where hormesis was observed with somehow lower rates. Nevertheless, hormesis has been found to occur at field rates in the past (R. G. Belz & Duke, 2014; Petersen et al., 2008). In practice, it means that when growers spray the recommended field rate of glyphosate to control *E. bonariensis* populations, the result would be observed as an increase in plant growth and vigour, instead of a fitness penalty.

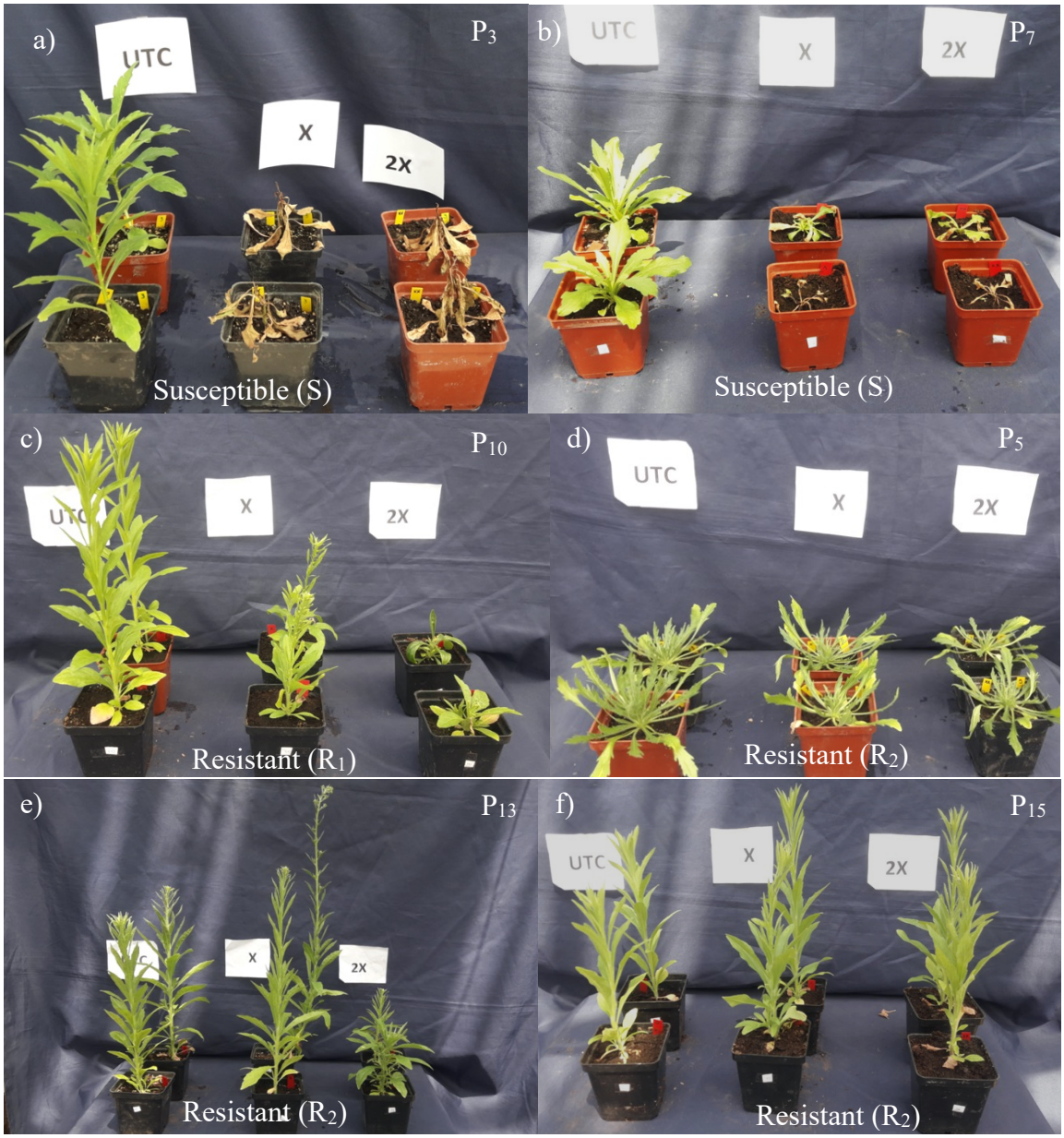

Figure 4. Effect of two glyphosate rates on susceptible and resistant *E. bonariensis* populations. UTC= Untreated check; X=1080 g ae ha^{-1}; 2X= 2160 g ae ha^{-1}

Dose-response tests

Models fitted

For parameters plant survival rate and visual control all five populations fitted to the log-logistic with four parameter model. Two populations tested were *S* populations collected from understory in Colombia (P_3-P_7) and three were *R*. One population collected in plantain P_{10} had the R_1 sub-category on discriminatory test, another P_{15} from a papaya orchard (R_2 level) and P_5, collected from railway in Spain and used as GR reference (R_2 level). Biomass assessed as DW and Rel-DW in the *R* population reference P_5 as well as the S populations had best fit to the log-logistic model with four parameters (Table 5). In contrast, for the P_{10} and P_{15} populations, the DW and Rel-DW fitted best to the Brain-Counsens model with 5 parameters and hormesis response, together with the *S* from understory P_7 (Brain & Cousens, 1989; Knezevic et al., 2007; Ritz et al., 2015; Christian Ritz & Strebig, 2016).

Table 5. Models fitted and parameters in dose-response test on *E. bonariensis* populations.

Model fitted	Lack-of fit p-value[a]	Pop.	b	c	d	e g ae ha^{-1}	f (p-value)[c]
Survival							
LL4	0.9959664	P_7	1.9	0	1.0	326.6	-
		P_3	7.8	0	1.0	651.3	-
		P_5	0.8	0	1.0	45,454.9	-
		P_{10}	0.9	0	1.0	6,857.9	-
		P_{15}	1.8	0	1.0	6,638	-
Visual control							
LL4	0.506233	P_7	-3.5	0	100	129.5	-
		P_3	-2.2	0	100	152	-
		P_5	-1	0	100	1,278.1	-
		P_{10}	-9.9	0	100	1,581.6	-
		P_{15}	-6.3	0	100	4,552.3	-
Dry biomass (DW)							
LL4	0.961805	P_7	3.3	0.2	1.8	109,0	-
		P_3	2.5	0.2	2.1	165,7	-
		P_5	1.0	0.0	1.8	1,935.5	-
BC 5 param.	0.081436	P_7	3.2	0.2	1.8	92.1	0.0052 (0.9411)
		P_{10}	13.0	0.3	0.9	763.1	0.0050 (7.51e-7)
		P_{15}	7,0	0.4	1.3	4490.4	0.0007 (5.05e-8)
Relative biomass (Rel-DW)							
LL4	0.992759	P_7	3.3	9.4	100	109.0	-
		P_3	2.5	8.5	100	166.0	-
		P_5	1.0	-0.8	100	1.950.3	-
BC 4 param.	0.143583	P_7	3.4	9,2	100	67.6	1.5803 (0,9364)
		P_{10}	13.0	36,0	100	753.0	0.7549 (2.2e-16)
		P_{15}	8.7	33.7	100	4,652.5	0.0641 (2.2e-10)

FW= Fresh biomass; DW=Dry biomass; Rel-DW= Relative dry weight;
LL4=log-logistic model 4 parameters; BC=Brain-Cousens.

[a] p-value for lack-of-fit test should be >0.05 for the model to describe properly the data

[b] For log-logistic models parameters e is the ED_{50} in g ae ha^{-1}

[c] Parameter f must be ≠0 for the hormetic significative effect; so, p-value for t-test must be <0.05 (Knezevic et al., 2007; Ritz et al., 2015; Ritz & Strebig, 2016)

As aforementioned, the lower and higher asymptotes assigned were 0 and 1.0 for survival rate and 0% and 100% for visual control; therefore, the algorithm estimated the remaining two parameters on the log-logistic model. Biomass data (DW and Rel-DW) fitted well to the

hormesis model using the Brain-Cousens formula in both populations from papaya (P_{15}) and plantain (P_{10}). In both cases, the "f" parameter was significatively different to zero (t-test p-value <0.05), indicating a significative hormesis effect. In contrast, in the P_7 (*S*) population the "f" parameter was not different to zero, concluding that the response did not describe a hormesis-like response (Knezevic et al., 2007; Ritz et al., 2015). All selected models had a non-significative p-value (>0.05) in the lack-of-fit test, therefore indicating that the models described the data adequately (Knezevic et al., 2007; Ritz et al., 2015; Ritz & Strebig, 2016). For further reference, please see detailed description of analyses in supplementary table 3.

The dose that delivers 50% reduction (ED_{50}) in plant biomass represents a reliable estimate of the weed's resistance level (Burgos et al., 2013; Knezevic et al., 2007). The ED_{50} dry biomass values reported in literature for *E. bonariensis* in the *S* biotypes may vary from 34.8 to 335 g ae ha^{-1}. On the other hand, for *R* populations the same values may vary from 1,129.9 to 6,264.1 g ae ha^{-1} (Gomes, 2014; González-Torralva et al., 2010, 2012; M. Moretti et al., 2016; Okumu et al., 2019; Puricelli et al., 2015; Quintero-Pertuz et al., 2021; Tahmasebi et al., 2018). Please refer to supplementary table 4.

Glyphosate-sensitive populations

Sensitivity to glyphosate was higher in P_7 than in P_3 for all the variables evaluated (Table 6). Thus, ED_{50} values in P_3 and P_7 were intermediate in comparison to 34.8 to 335 g ae ha^{-1} reported by previous studies (Gomes, 2014; González-Torralva et al., 2010; M. Moretti et al., 2016; M. L. Moretti & Hanson, 2017; Okumu et al., 2019; Puricelli et al., 2015; Quintero-Pertuz et al., 2021; Tahmasebi et al., 2018). These intermediate ED_{50} values were useful for appropriate resistance factors (RF) determinations (Burgos et al., 2013).

Table 6. Comparative estimates LD_{50}, ED_{50} and RFs in *E. bonariensis* populations.

Crop	Survival[a]			Control			DW			Rel-DW		
Pop	LD_{50} g ae ha^{-1} ±SE	RF_7	RF_3	ED_{50} g ae ha^{-1} ±SE	RF_7	RF_3	ED_{50} g ae ha^{-1} ±SE	RF_7	RF_3	ED_{50} g ae ha^{-1} ±SE	RF_7	RF_3
Und. P_7	326.6 ±79.5	-	-	129.5 ±8.5	-	-	109.0 ±15.0	-	-	109.0 ±12.7	-	-
Und. P_3	651.3 ±1346.4	-	-	152.0 ±11.3	-	-	165.7 ±27.01	-	-	166.0 ±25.0	-	-
Railway P_5	45,454.9 ±58,863	139.2	69.8	1,278.1 ±138.0)	9.9	8.4	1,935.5 ±1,071.6	17.8	11.7	1,950.3 ±1,053.8	17.9	11.7
Plantain P_{10}	6,857.9 ±1,682.4	20.3	10.2	1,581.6 ±1,883.0	12.2	10.4	1,222.5	8.7[a]	-	1,283.0	3.15[a]	-
Papaya P_{15}	6,638.0 ±2,549.6	21.0	10.5	4,552.3 ±964.9)	35.1	29.9	10,330.8	73.7[a]	-	9,102.3	22.3[a]	-

Und=Understory; LD_{50}=Lethal Dose 50; DW=Dry weight 21 DAT; Rel-DW=Relative dry weight 21 DAT.
[a]ED_{50} and RF calculated for DW and Rel-DW calculated through the Brain-Cousens formula (2) vs P_7 fitted to the hormesis model; RF_7= Resistance factor vs P_7; RF_3= Resistance factor vs P_3;

Glyphosate-resistant populations

The model for plant survival in *R* populations, predicted much higher lethal dose (LD_{50}) in the population from railway Spain (P_5) compared to the populations collected in Colombia on plantain (P_{10}) or papaya (P_{15}) (Table 7). In the *R* reference P_5 (from Railway), the model

estimated a high LD_{50} (45,454.9 g ae ha^{-1}). Likewise, LD_{50} value based on survival from railway (P_5) was similar to one *R* biotype described by Moretti, et al. (2016), but higher than the ED_{50} reported by Tahmasebi et al. (2018). When comparing the estimated ED_{50} for visual control, dry biomass and relative biomass, values were higher for the populations collected in Colombia (i.e. P_{15}>P_{10}>P_5).

Accordingly, the calculated biomass RFs determined as the ED_{50} ratio between *R* and *S*; were highest in P_{15}. Glyphosate treated plants from railway (P_5) had high survival, but also had evident damage, reduced growth (Figures 5c, 6a) in comparison to untreated control plants. In contrast, considering the hormetic populations from papaya and plantain (P_{10} and P_{15}), the control resulted in low values with the lower doses tested while plant size and DW increased (Figure 6b, Table 6).

Figure 5. Dose response *E. bonariensis* test at 21 days after treatment. a) P_3= Sensitive (*S*) reference population; b) P_7= Sensitive (*S*) population collected from understory; (c) P_5=Resistant (*R*) reference population from railway in Spain; d) P_{10}: Low resistance (*R*) level collected in plantain; e) P_{15}= High resistance (*R*) level population from papaya.

The resistant-reference population from railway (P_5) showed ED_{50} 1,950.3 g ae ha^{-1} for Rel-DW, being very similar to previous results that yielded an ED_{50} of 1920 g ea ha^{-1} for Rel-DW with the same population from Spain (Amaro-Blanco et al., 2019). RFs for this populations from railway are >10-fold based on survival, DW and Rel-DW, thus resistance levels for P_5 on this study can be accepted to be high (Heap, 2005) (Table 6).

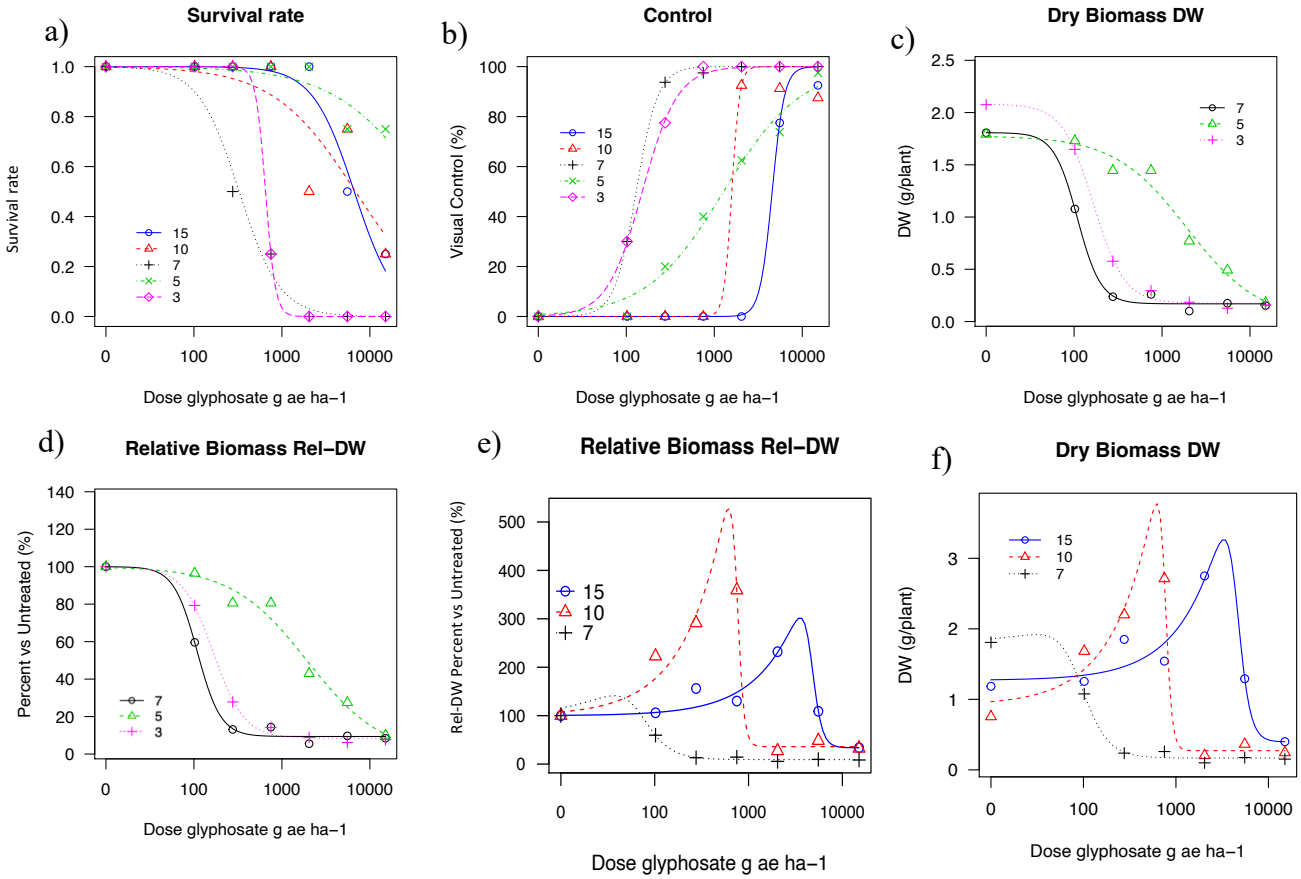

Figure 6. Dose-response curve for glyphosate response in five *E. bonariensis* populations a) Survival rate; b) Visual control (%); c) Dry biomass (g plant^{-1}) P_3, P_7 and P_5; d) Relative dry weight (% over untreated) P_3, P_7 and P_5; e) Relative dry weight (% over untreated), P_7, P_{10} and P_{15}; f) Dry biomass (g plant^{-1}) P_7, P_{10} and P_{15}.

In the case of the population from plantain (P_{10}), ED_{50} for Rel-DW was 1,283 g ae ha^{-1}, being lower than previous findings on banana in Colombia, where the ED_{50} was 6,264 g ae ha^{-1} (Quintero-Pertuz et al., 2021). The respective ED_{50} values for visual control were somehow high in comparison with other several of the previous studies for *E. bonariensis* (supplementary table 4); anyhow, it was similar to the levels found by Okumu et al. (2019). On this biotype DW and Rel-DW had ED_{50} values were similar to those found in Spain by Tahmasebi et al. (2018) and Moretti et al. (2016). Furthermore, the RF is scored as high (RF >10) for survival and control but low for DW and Rel-DW, therefore the resistance level in P_{10} could be interpreted as intermediate (Heap, 2005). The hormesis response found in P_{10} vs P_{15} have a similar non-monotonic response. But it is evident that the peak in P_{10} occurs at a lower dose than in P_{15} ~750 vs ~2,039 g ae ha^{-1} (Figure 6e-6f).

The population collected from a papaya grove (P_{15}) has a RF above 10-fold for all the variates assessed and thus had the highest resistance level in this study. The ED_{50} levels estimated for P_{15} were also high and similar to the large values found in United States by Moretti et al. (2016) and Quintero-Pertuz, et al. (2021) for populations from banana in Colombia. Further, P_{15} has a marked hormesis response as aforementioned in the discriminatory test where the

sprayed plants with 1X and 2X rates had height greater than the untreated and premature flowering. In addition, a second dose-response test demonstrated that the hormesis response is consistent across different trials in population P_{15} from the papaya crop (Figure 7).

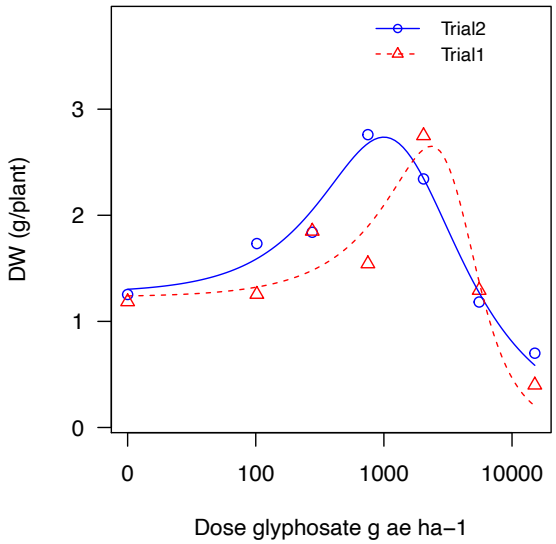

Figure 7. Dose-response for glyphosate in one *E. bonariensis R* population (P_{15}) collected form papaya orchard across two trials showing hormesis effect.

In summary, the susceptibility levels in this study were reliable for RF estimations on both sensitive populations. The resistance levels were high in the reference-resistant population from railway (P_5) and the one from papaya crop (P_{15}). But, the population from plantain grove (P_{10}) had an intermediate resistance level. Dose-response results are consistent with the discriminatory tests and previous researchers results on *E. bonariensis*.

Several glyphosate resistance mechanisms have been elucidated for the genus *Erigeron* (synonym: *Conyza*). In the particular case of *E. bonariensis*, a concomitant effect of target site mutation at *EPSPS2* plus reduced glyphosate absorption-translocation (González-Torralva et al., 2014). In contrast, transcriptome analysis of *E. bonariensis*, discarded target site mutation on the *EPSPS* gene as a potential mechanism, but identified other 834 candidate genes for non-target site resistance in Australian *R* populations (Hereward et al., 2018). In resistant biotypes from California, two mechanisms for reduced glyphosate translocation have been associated with resistance to glyphosate in *E. bonariensis*; interestingly, one of these impaired translocation mechanisms also effectively limited paraquat translocation to chloroplast (Moretti & Hanson, 2017).

Overall, data thus far suggest that the active ingredient exclusion mechanisms is the most common in *R E. canadensis* resistant to glyphosate (Sammons & Gaines, 2014). Nuclear magnetic resonance (NMR) studies utilizing phosphorus isotope ^{31}P, demonstrated that *R* plants can sequestrate glyphosate into vacuole. Thus, plant have the ability to limit the amount of compound available for translocation and this resulted in decreased herbicide content in the phloem (Ge et al., 2010).

Moreover, synchronized overexpression of *EPSPS* and transporters *ATP-binding cassette* (ABC) genes *M7*, *M10*, *M11* and *P3* conferred resistance to *E. canadensis* (Tani et al., 2015). In summary, *R* mechanisms described in *E. canadensis* and *E. bonariensis* seem to be common but complex in terms of genes involved in both species, this is explained at least partially by the phylogenetic close distance and the hybridization among both species (Zelaya et al., 2007). If we compare both resistance mechanisms, current knowledge suggests that the glyphosate sequestration and translocation mechanism of is more important in terms of level of resistance conferred, in comparison to target site mutations (Amaro-Blanco et al., 2018; Kleinman & Rubin, 2017; Mora et al., 2019). This is also linked with the observed hormesis in this study for the tested populations in papaya and plantain (P_{15} and P_{10}), where very low amounts of glyphosate are released into chloroplast enhancing growth as reported by other authors in *R Erigeron.* Plants which had lower levels of shikimic-acid and indol-acetic acid but higher amounts of salicylic acid at 21 DAT with glyphosate in comparison to the *S* populations (Gomes, 2014).
Based on the low resistance levels (RF=8.7 in DW) observed for P_{10} from plantain, we hypothesize that the resistance mechanism might be linked with vacuolar sequestration (Ge et al., 2011). The populations from papaya (P_{15}) and from railways (P_5) had higher RFs for DW and Rel-DW. On both populations, another mechanism maybe involved, possibly i.e. overexpression of *EPSPS* transcription or the *ABC-transporters* that effectively pump glyphosate into the vacuole (Tani et al., 2015). In the population from railways in Spain P_5, there is less probability that target site mutation is a factor since the DW is impacted with increases of glyphosate dose. For hormesis, the mechanism may be associated with the sequestration mechanism into the vacuole, where lower amounts of glyphosate would target the chloroplast and consequently having less chance to inhibit EPSPS.

Glyphosate mode of action in plants is reported to trigger oxidative stress in cells (Ahsan et al., 2008). Low amounts of reactive oxygen species (ROS) generated after glyphosate exposure in *R*, may cascade an stimulatory effect at higher doses than in *S* biotypes (R. G. Belz & Duke, 2014; Brito et al., 2018) and may trigger the overcompensation processes by ROS scavengers. The observed hormesis effect with longer plants, branching and earlier flowering can be explained by a decrease on auxin oxidation and the diminution of phenols caused by glyphosate (Cobb & Reade, 2010; Gomes, 2014). Fitness increase linked to hormetic populations in Colombia has also been demonstrated in research conducted in Brazil in *Erigeron* resistant to glyphosate with earlier flowering and growth increase at glyphosate at 360 g ae ha^{-1}; however, this study detected hormesis at field doses *circa* 1,080 g ae ha^{-1}. Other cases reporting a hormesis effect using the recommended doses of the herbicide have been found in ACCase-target-site-resistant (*Alopercus myosuroides* Huds.) biotypes; this resulted in a 147% increase in shoot fresh weight after exposure to rates similar to those recommended for the field. Such increase in biomass results in higher ability for weed competition with the crop and an enhancement of the reproductive potential for resistance biotypes (R. Belz & Duke, 2014; Petersen et al., 2008).

The hormetic response was not significative in the reference population from railway in Spain (P_5) (data not shown). We hypothesize that this differential response might be explained by a response to the environment on which each biotype has evolved. Similarly to abiotic-stress, herbicides may trigger similar stress' signalling, gene expression and physiological responses (Dyer, 2018).

Recent research has demonstrated that drought-stress or heat-stress traits are also linked to herbicide-resistance. In (*Echinochloa colona*), sublethal herbicide doses instigated upregulation of metabolic and protection genes (Benedetti et al., 2020). Heat shock proteins are subjected to protein post-translational modifications as response to heat stress and are implicated in hormesis responses (Dyer, 2018). Under temperate conditions the plants are exposed to seasonal changes in temperature and photoperiod, allows the progressive acclimation of plants. Opposite, tropical hot weather conditions where P_{10} and P_{15} are adapted in Colombia require plants to keep high higher adaptation to stress (T^{o}_{min} 23.2/21.4 ºC, T^{o}_{max} 31.2/30.7 ºC average 26.6/25.6ºC, annual average RH 87%/80%, rainfall 2,658/4,329 mm.yr^{-1} with 209/207 days with rain per year) (Ideam, 2020). Probably, population from railway in Spain have not evolved same stress-resistant traits and overcompensation systems as biotypes from Colombia. Further research may be performed to confirm this.

Alternative herbicides evaluation

The study was performed using P_{10} (low resistance level), P_{15} and P_{20} both with high resistance level to glyphosate. The factorial ANOVA analyses for Rel-DW at 28 DAT demonstrated that population, herbicide and their interaction were highly significant (p-value <0.001). Therefore, performance and response of each herbicide differs depending on the targeted population evaluated. Population P_{20} from passionfruit location had the highest dry biomass values. In particular, in P_{20} the herbicides 2,4-D, glufosinate and paraquat delivered the lowest biomass reductions (Figure 8c). On the other hand, in P_{15} from papaya 2,4-D, glufosinate, mesotrione and pyraflufen-ethyl reduced effectively biomass and are efficacious alternatives to manage this biotype (Figure 8b). Biomass (Rel-DW) assessment in population from plantain (P_{10}) indicated that the best alternative for effective control were attained with 2,4-D or pyraflufen-ethyl (Figure 8a); the second alternatives were mesotrione and glufosinate and lastly paraquat.

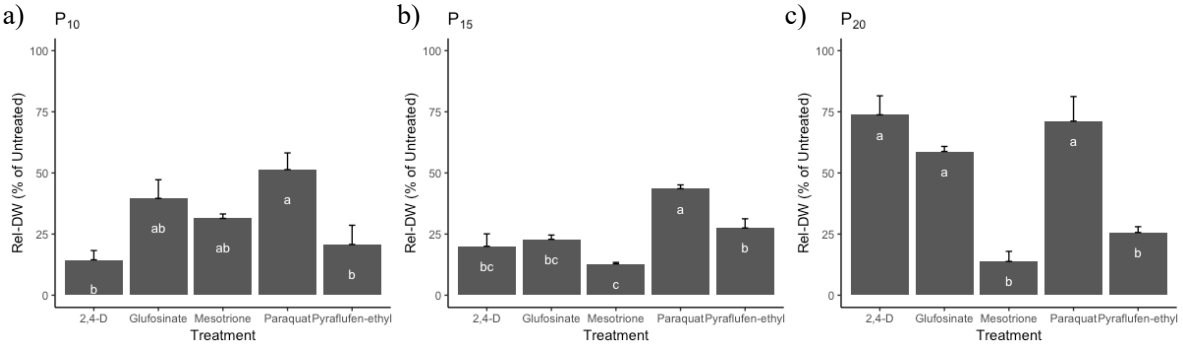

Figure 8. Effect of five alternative herbicides on relative biomass (Rel-DW) 28 DAT in three *R* populations of *E. bonariensis* from plantain (P_{10}), papaya (P_{15}) and passionfruit (P_{20}). Doses: 2,4-D (700 g ai ha^{-1}), glufosinate (225 g ai ha^{-1}), mesotrione (100 g ai ha^{-1}), paraquat (600 g ai ha^{-1}), and pyraflufen-ethyl (8 g ai ha^{-1}). Letters= Tukey test. Error bars= +SE.

ANOVA estimated significant effects for all factors (herbicide, population and time) on visual control assessment, as well as the interaction at the evaluation time of 14 and 28 DAT. The effect on evaluation time, explained on the speed and mode of action for each herbicide (Table 7).

The pyridinium paraquat had the highest biomass and the lowest visual control percentages and was the least effective herbicide tested on all three populations. This is in contrast with results reported with diquat (pyridinium) that demonstrated 80% control on *R* populations from Spain (Tahmasebi et al., 2018). Our results suggest that a multiple resistance mechanism, including vacuolar sequestration by ABC-transporters, may have conferred resistance either to paraquat and glyphosate in these populations as reported in *E. bonariensis* populations from United States (M. L. Moretti & Hanson, 2017). Further research is needed to confirm multiple resistance on these populations from Colombia. Nevertheless, paraquat can be discarded as alternative and effective control method for the cases reported in Colombia.

Table 7. Visual control on glyphosate-resistant *E. bonariensis* populations with different herbicides 14 and 28 days after treatment (DAT).

Herbicide[a]	**Dose**	**P_{10}**				**P_{15}**				**P_{20}**			
	g ai-ea ha^{-1}	14 DAT[b]		28 DAT		14 DAT		28 DAT		14 DAT		28 DAT	
2,4-D	720	85.0	a	88.75	a	83.75	a	85.0	a	82.5	a	25.0	b
Glufosinate	150	78.75	ab	47.5	b	81.25	a	56.25	b	56.65	b	25.0	b
Mesotrione	100	66.25	ab	40.0	b	80.0	a	92.5	a	83.75	a	86.25	a
Paraquat	600	52.5	a	20.0	b	60.0	a	50.0	b	20.0	c	30.0	b
Pyraflufen-ethyl	8	80.0	ab	82.5	a	72.5	a	77.5	ab	80.0	a	90.0	a
Pr(>F)		0.0176		7.27E-05		0.0712		0.00113		1.77E-07		1.01E-08	

[a] 2,4-D (HRAC: 4); glufosinate (HRAC: 10); mesotrione (HRAC: 27); paraquat (HRAC: 22); pyraflufen-ethyl (HRAC: 14)
[b] Letters= Tukey groups (HSD); See detailed analyses in Supplementary table 5.

Pyraflufen-ethyl had the best performance across all populations for both assessment times, followed by mesotrione and 2,4-D. Mesotrione effectively controlled P_{15} and P_{20}, while 2,4-D controlled well P_{10} and P_{15} populations. Glufosinate offered acceptable control levels (>80%) at 14 DAT only in one population, but the control declined over the time and at 28 DAA, plants recovered from the application. This is in part explained by the pseudo-contact action of the mode of action associated with glufosinate (Cobb & Reade, 2010). In the screening performed by Tahmasebi, et al, (2017) efficacy to pyraflufen-ethyl had similar values than in this investigation (80 to 90% control). On that study, the 2,4-D visual control was lower than in our results for P_{10} and P_{15}. While glufosinate had much higher levels of control (>95%) than those found in our paper.

Interestingly, in the case of the auxin herbicide 2,4-D, the population from passionfruit field (P_{20}) had a satisfactory control (82.5%) in the 14 DAT assessment; however, the control reduced significantly to only 25% at 28 DAT. This indicates that possibly, this population might have evolved resistance to 2,4-D as it has been recently discovered (Moretti et al., 2021; Palma-Bautista et al., 2021). It is also possible that requires a higher dose to prevent plant recover. 2,4-D can also be tank-mixed with glyphosate to improve the synergistic effect on either resistant or sensitive populations (Tahmasebi et al., 2018). In addition, 2,4-D can be sprayed in preplant-burndown or in post-emergence to crop on those crops where selectivity can be achieved and when crop growth and competition may complete effective control of weed (Håkansson, 2003). It was also demonstrated that very high control levels can be reached by tank-mixing glyphosate with pyraflufen-ethyl, glufosinate or mesotrione

which also widens spectrum (Tahmasebi et al., 2018). However, tank-mixes would increase production cost, which is a consequence of resistance.

Conclusions

Results of glyphosate performance over *S* and *R* reference populations were as expected, allowing confirmation of susceptibility and resistance in the *E. bonariensis* accessions collected in Colombia. In addition, the discriminatory test and dose-response trials were consistent to segregate between susceptible and resistance levels to glyphosate. In all glyphosate-treated agroecosystems, *R* in hairy fleabane was present at medium and high levels. This means that glyphosate-resistant *E. bonariensis* is found over the five provinces sampled: Antioquia, Magdalena, Santander, Cundinamarca and Meta. In all these cases, conventional farming is a practice which includes the use of agrochemicals where glyphosate plays an important role in weed management.

The high prevalence and high resistance levels to glyphosate found in *E. bonariensis* have an impact on weed management in traditional and export crops of Colombia. In particular, high levels of resistance to glyphosate (RF: 22.3-fold) can silently be spread throughout several agroecosystems. In Colombia, non-agricultural landscapes are also resistance reservoirs as reported in Spain or United States. Crop practices that prevent the spread of resistance are necessary and are seldom implemented in Colombia. However, these practices may increase management cost across several crops and threatening growers' net incomes. This is a clear consequence of resistance which relates to the fact that proper management should be implemented. Moreover, populations with limited exposure to the glyphosate are sensitive to the herbicide, demonstrating that resistance has evolved in the agroecosystems sampled and is expected to increase under those scenarios with higher selection pressure (dose, frequency and time). The lower resistance level was found in plantain crop (RF: 3.1-fold), where the growers spray less frequently glyphosate and at lower rates. In addition, two resistant biotypes revealed hormesis in the dose-response trials at recommended rates, therefore plants are able to increase growth, branching and premature flowering under grower practice increasing fitness and resistance spread. Our results in the discriminatory test suggest that other six populations may also have hormesis at field rates.

Finally, our research demonstrated that two modes of action evaluated (HRAC: 14 and 27) are effective alternatives to control of *E. bonariensis*. In particular, herbicides pyraflufen-ethyl and mesotrione are the most promising alternatives to control this weed as confirmed by our results. These herbicides may be applied solo or in mixture with glyphosate to delay herbicide resistance evolution in *E. bonariensis*, either on perennial or annual crops as well as to achieve successful weed control. In special, in the population from passionfruit (P_{20}) further research is also needed to confirm putative resistance to 2,4-D (HRAC: 4). Importantly, control with glufosinate (HRAC:10) and paraquat (HRAC: 22) was un-satisfactory; research is necessary to confirm whether probable multiple resistance may be present (glyphosate, glufosinate and paraquat) as reported in United States. A proper guidance should be delivered to technicians and growers in order to improve weed management practices and mitigate higher glyphosate resistance dispersion.

Recommendations

Our results confirmed that discriminatory tests are useful to evaluate populations and determine the frequency and level of resistance. This type of tests save time and resources,

and inform about the relative resistance levels in populations with previous studies (Panozzo et al., 2015; Zabala et al., 2019). Further research is necessary to find the resistance to glyphosate and hormesis mechanisms found on the populations from Colombia.

Integrated weed management is necessary and should include cultivation, ground coverage, mechanical control, hand pull-up, herbicide-tank mixes and crop rotation in order to prevent and/or to manage resistance (Bajwa et al., 2016).

In most of the sampled crop conditions *E. bonariensis* mesotrione and pyraflufen-ethyl were effective post-emergence herbicides to control weed. Further research is required to confirm possible multiple resistance cases including paraquat, glufosinate and 2,4-D in glyphosate-resistant hairy fleabane from Colombia.

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Supplementary table 1. Statistical analyses Resistance profile test

Explanatory power and importance of the aleatory effects on the non-linear mixed model for the variates fresh biomass (FW), dry biomass DW, control and survival with glyphosate at two doses (X and 2X; X=1080 g ea ha^{-1}) compared with the untreated check (UTC).

Fresh biomass:

Var.	Fixed effect	Random effect		Model	Log-Lik	AIC	BIC	Test	L.ratio	df	p-value
		P	P*R								
FW				1 lme.0	-360.2150	726.4300	735.6555			3	
	Dose			**2 lme.1**	**-338.8589**	**685.7179**	**698.0186**	**1 vs 2**	**42.7121**	**4**	**<.0001**
	Dose	X		3 lme.1ML	-368.2990	742.5981	751.8609			3	
	Dose		X	4 lme.1ML	-337.8763	683.7526	696.1030	3 vs 4	60.84552	4	<.0001

Multiple Comparisons of Means: Tukey Contrasts

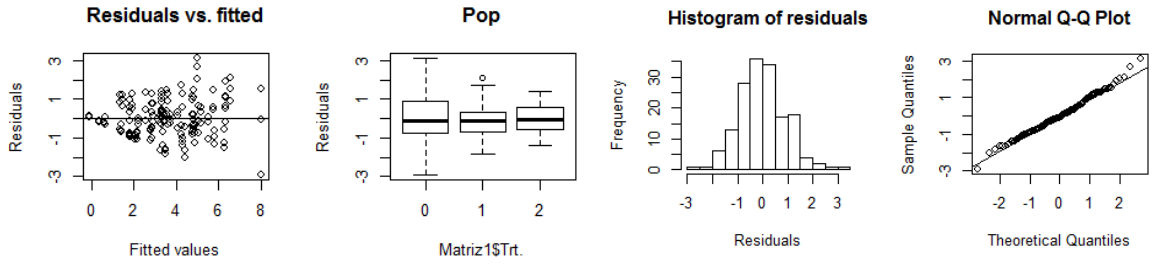
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(>|z|)	
1 - 0 == 0	-1.6809	0.3402	-4.940	< 1e-04	***
2 - 0 == 0	-2.9452	0.3402	-8.656	< 1e-04	***
2 - 1 == 0	-1.2643	0.3402	-3.716	0.000577	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)

Normality and homoscedacity tests

Shapiro-Wilk normality test
data: residuals(lme.1)
W = 0.99201, p-value = 0.5063



Supplementary table 1. Statistical analyses Resistance profile test (continuation)

Dry biomass

Var.	Fixed effect	Random effect		Model	Log-Lik	AIC	BIC	Test	L.ratio	df	p-value
		P	P*R								
DW				1 lme.0	-127.1350	260.2700	269.4955			3	
	Dose			**2 lme.1**	**-100.7269**	**209.4538**	**221.7545**	**1 vs 2**	**52.81618**	**4**	**<.0001**
	Dose	X		3 lme.1ML	-122.07611	250.1522	259.4150			3	
	Dose		X	4 lme.1ML	-96.83748	201.6750	214.0254	3 vs 4	50.47725	4	<.0001

Multiple Comparisons of Means: Tukey Contrasts

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(>|z|)	
1 - 0 == 0	-0.27833	0.07621	-3.652	0.000767	***
2 - 0 == 0	-0.58852	0.07621	-7.723	< 1e-04	***
2 - 1 == 0	-0.31019	0.07621	-4.070	0.000155	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Adjusted p values reported -- single-step method)

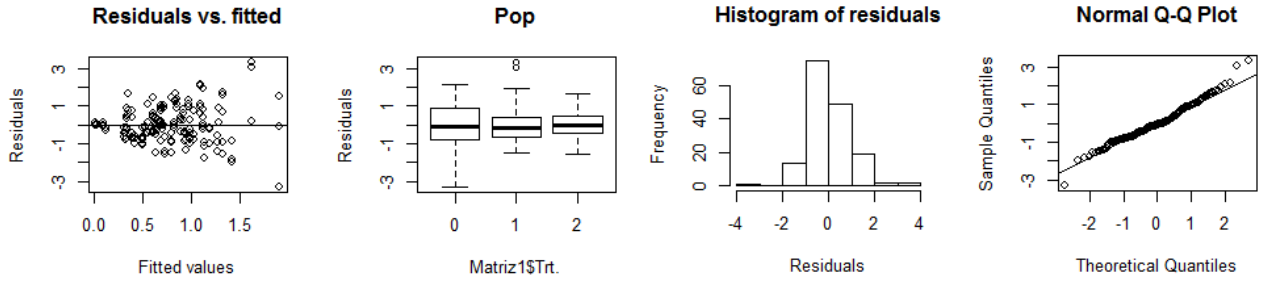
Normality and homoscedacity test

Pearson.test(residuals(lme.1))

Pearson chi-square normality test

data: residuals(lme.1)

P = 18.543, p-value = 0.138



Supplementary table 1. Statistical analyses Resistance profile test (continuation)

Control

Var.	Fixed effect	Random effect		Model	Log-Lik	AIC	BIC	Test	L.ratio	df	p-value
		P	P*R								
Control				1 lme.0	-778.0623	1562.125	1571.350			3	
	Dose			**2 lme.1**	**-743.8491**	**1495.698**	**1507.999**	**1 vs 2**	**68.42636**	**4**	**<.0001**
	Dose	X		3 lme.1ML	-815.4205	1636.841	1646.104			3	
	Dose		X	4 lme.1ML	-748.0930	1504.186	1516.536	3 vs 4	134.655	4	<.0001

Multiple Comparisons of Means: Tukey Contrasts

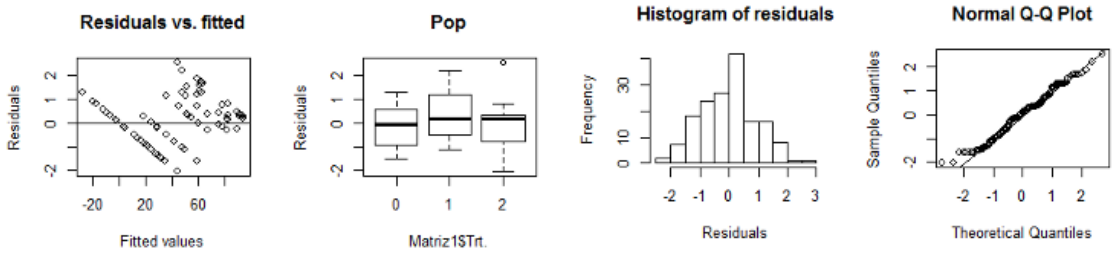
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(>|z|)	
1 - 0 == 0	42.556	4.039	10.536	< 1e-06	***
2 - 0 == 0	62.111	4.039	15.378	< 1e-06	***
2 - 1 == 0	19.556	4.039	4.842	3.51e-06	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Normality and homoscedacity test

Shapiro-Wilk normality test
data: residuals(lme.1)
W = 0.9846, p-value = 0.06967



Supplementary table 1. Statistical analyses Resistance profile test (continuation)

Survival

Var.	Fixed effect	Random effect		Model	Log-Lik	AIC	BIC	Test	L.ratio	df	p-value
		P	P*R								
Survi-val				1 lme.0	-54.25396	114.50792	123.73344			3	
	Dose			**2 lme.1**	**-38.80372**	**85.60745**	**97.90814**	**1 vs 2**	**30.90048**	**4**	**<.0001**
	Dose	X		3 lme.1ML	-48.33282	102.66564	111.92843			3	
	Dose		X	4 lme.1ML	-33.97487	75.94974	88.30012	3 vs 4	28.7159	4	<.0001

Multiple Comparisons of Means: Tukey Contrasts

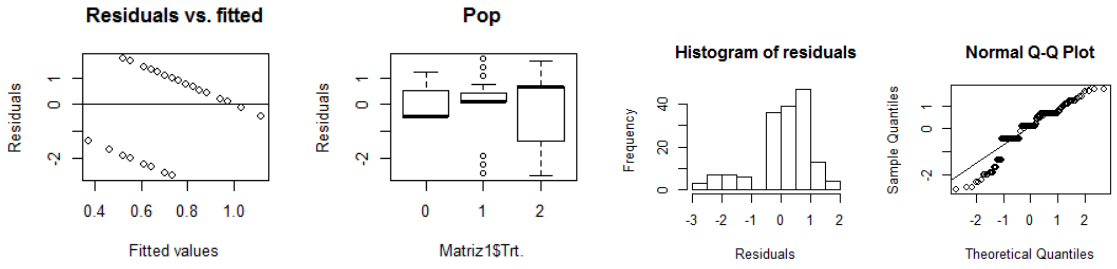
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(>|z|)	
1 - 0 == 0	-0.1296	0.0529	-2.451	0.03782	*
2 - 0 == 0	-0.2963	0.0529	-5.601	< 0.001	***
2 - 1 == 0	-0.1667	0.0529	-3.151	0.00465	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Normality and homoscedacity test

Pearson chi-square normality test
data: residuals(lme.1)
P = 256.57, p-value < 2.2e-16



Supplementary table 2. *E. bonariensis* response to glyphosate from different agroecosystems.

Crop/ Landscape	Plantain			Red beans		Papaya	Banana	Passion-fruit	Cassava	Urban area
Code	**P_9**	**P_{10}**	**P_{14}**	**P_{12}**	**P_{19}**	**P_{15}**	**P_{18}**	**P_{20}**	**P_4**	**P_{13}**
Survival[a]	R_2	R_2	R_2	R_2	R_1	R_2	R_1	R_2	R_2	R_2
Control[b]	R_2	R_1	R_1	R_2	R_2	R_2	R_1	R_2	R_2	R_2
Rel-FW[b]	R_2	R_1	R_1	R_2	R_2	R_2	R_2	R_2	R_1	R_2
Rel-DW[b]	R_2	R_1	R_1	R_1	R_2	R_2	R_1	R_2	R_2	R_2
Overal (mode)	**R_2**	**R_1**	**R_1**	**R_2**	**R_2**	**R_2**	**R_2**	**R_2**	**R_2**	**R_2**

[a]Adapted from (Beres et al., 2018)
[b]Adapted from (Panozzo et al., 2015; Zabala et al., 2019); Rel-FW= relative fresh weight, Rel-DW= relative dry weight

Supplementary table 3. Statistical analyses Dose-response test

Variate: Survival rate
Model selection:

Model	**logLik**	**AIC**	**Lack.of.fit**	**Res.var**
LL.2	**-6.618261217**	**35.23652243**	**0.99596638**	**0.069306089**
LL.2.1	-6.618261217	35.23652243	0.99596638	0.069306089
LL2.2	-6.618511745	35.23702349	0.99596508	0.069306338
LL2.3	-6.409354185	44.81870837	0.97352653	0.071863544
LL.3u	-6.619354273	45.23870855	0.96768999	0.072079459
LL2.3u	-6.620356212	45.24071242	0.96766015	0.07208049
bcl3	-5.556161221	53.11232244	0.91878477	0.073950994

Model parameters: Survival rate

	coefficients.lph.LL2.	Std. Error	t-value	p-value	
b:15	1.835409446	0.7108	2.58232	0.010921	*
b:10	0.93010475	0.3044	3.05564	0.002726	**
b:7	1.881152422	0.6927	2.71560	0.007515	**
b:5	0.833306816	0.5914	1.40902	0.161218	
b:3	7.794235361	114.0000	0.06837	0.945594	
e:15	6638.014633	1682.4000	3.94556	0.000130	***
e:10	6857.933205	2549.6000	2.68981	0.008087	**
e:7	326.6373824	79.5520	4.10596	0.070720	***
e:5	45454.88929	58863.0000	0.77221	0.441392	
e:3	651.2751405	1346.4000	0.48372	0.629412	

Res. Std.err: 0.2632605 (130 degrees of freedom)

Lack-of-fit test: Survival rate

Model	**Df**	**RSS**	**Df**	**F.value**	**p.value**
ANOVA	105	8.25			
DRC Model	130	9.00979163	25	0.38680301	0.99596638

Supplementary table 3. Statistical analyses Dose-response test (continuation)

ED Estimations: Survival rate

	Estimate	Std. Error
e:15:50	8.773.5	0.259
e:15:80	9.553.2	0.432
e:15:90	10.009.3	0.586
e:10:50	8.748.2	0.457
e:10:80	10.349.5	0.694
e:10:90	11.286.2	0.994
e:7:50	573.0	0.275
e:7:80	649.6	0.391
e:7:90	694.4	0.520
e:5:50	1.077.2	1.378
e:5:80	12.646.5	2.819
e:5:90	13.742.8	3.719
e:3:50	606.1	9.135
e:3:80	615.4	10.768
e:3:90	620.8	11.763

Variate: Visual control

Model selection

Model	**logLik**	**AIC**	**Lack.of.fit**	**Res.var**
LL.4	-486.60090	1015.20200	0.88333	71.35836
L3m	**-495.62560**	**1013.25100**	**0.50620**	**68.95520**
BC.5	-482.38710	1016.77400	0.99519	70.11084
bcl4	-482.38710	1016.77400	0.99519	70.11084
LL.5	-484.69470	1021.38900	0.83766	72.46064

Lack-of-fit test: Visual control

L3m

Model	**ModelDf**	**RSS**	**Df**	**F.value**	**p.value**
ANOVA	105	7906.25			
DRC Model	130	9741.31743	25	0.97483424	0.506233

Supplementary table 3. Statistical analyses Dose-response test (continuation)

Parameter estimates: Visual control

	Estimate	Std. Error	t-value	p-value	
b:15	-7.847149	14.06143	-0.5581	0.5778	
b:10	-20.143684	167.274198	-0.1204	0.90434	
b:7	-3.637575	0.877433	-4.1457	6.20E-05	***
b:5	-0.94251	0.155807	-6.0492	1.565E-08	***
b:3	-2.138665	0.303581	-7.0448	1.102E-10	***
d:15	92.495553	4.156758	22.2519	2.20E-16	***
d:10	90.416561	2.397196	37.7176	2.20E-16	***
d:7	99.440929	2.111516	47.0946	2.20E-16	***
d:5	103.282865	9.755448	10.5872	2.20E-16	***
d:3	100.767928	2.265515	44.479	2.20E-16	***
e:15	8.410814	0.37562	22.3918	2.20E-16	***
e:10	7.000015	3.529983	1.983	0.04956	
e:7	4.855522	0.071406	67.9992	2.20E-16	***
e:5	7.248242	0.300889	24.0894	2.20E-16	***
e:3	5.034367	0.07776	64.742	2.20E-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 8.303917 (125 degrees of freedom)

ED Estimates: Visual control

	Estimate	Std. Error
e:15:50	8,410.8	0.3756
e:15:80	8,587	0.0777
e:15:90	8,691	0.1412
e:10:50	7,000	3.5300
e:10:80	7,069	3.8380
e:10:90	7,109	4.0448
e:7:50	486	0.0714
e:7:80	524	0.1486
e:7:90	546	0.1996
e:5:50	7,248	0.3009
e:5:80	8,719	0.5185
e:5:90	9,579	0.6541
e:3:50	503	0.0778
e:3:80	568	0.1264
e:3:90	606	0.1722

Supplementary table 3. Statistical analyses Dose-response test (continuation)

Variate: Dry weight
Populations: P_3. P_7 and P_5
Model selection

	logLik	IC	Lack of fit	Res. Var
LL.4	**-11.19895**	**48.3979**	**0.96181**	**0.08918123**
LL.4	-11.19895	48.3979	0.96181	0.08918123
LL2.3	-15.93628	51.8726	0.54792	0.09583636
BC.4	-13.92664	53.8533	0.59821	0.09516533
BC.5	-11.01851	54.0370	0.84369	0.09265973

	Estimate	Std. Error	t-value	p-value	
b:7	3.27E+04	2.52E+04	12.963	0.199004	
b:5	1.05E+04	5.29E+03	19.838	0.051096	.
b:3	2.47E+04	7.34E+03	33.590	0.001254	**
c:7	1.69E+03	7.73E+02	21.915	0.031649	*
c:5	6.84E+01	4.00E+03	0.0171	0.986415	
c:3	1.76E+03	8.07E+02	21.781	0.032672	*
d:7	1.81E+04	1.49E+03	121.077	<2.2E-16	***
d:5	1.78E+04	1.39E+03	127.997	<2.2E-16	***
d:3	2.08E+04	1.49E+03	139.988	<2.2E-16	***
e:7	1.09E+06	1.50E+05	72.808	3.34E-10	***
e:5	1.94E+07	1.07E+07	18.062	0.075066	.
e:3	1.66E+06	2.70E+05	61.351	4.16E-05	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error:

0.2986323 (72 degrees of freedom)

ED Estimates: Dry weight

	Estimate	Std. Error
e:7:50	108.99	14.97
e:7:80	166.61	65.16
e:7:90	213.56	122.83
e:5:50	1,935.50	1,071.59
e:5:80	7,249.79	8,058.27
e:5:90	15.70	23,228.26
e:3:50	165.73	27.01
e:3:80	290.81	66.33
e:3:90	404.08	123.16

Supplementary table 3. Statistical analyses Dose-response test (continuation)

Lack-of-fit test: Dry weight

Model	**Df**	**RSS**	**Df**	**F.value**	**p.value**
ANOVA	63	6.1319			
DRC Model	72	6.421	9	0.3301	0.9618

Variate: Dry weight
Populations: P_7, P_{10} and P_{15}

Model selection:

	logLik	**IC**	**Lack.of.fit**	**Res.var**
BC.5	-25.579545	83.1590907	0.08143591	0.13105608
BC.4	-31.425957	88.851914	0.01398097	0.14435362
LL.3	-66.323131	152.646261	1.4764E-11	0.31808831
LL.4	-63.923739	153.847479	6.687E-12	0.31294351
LL.2	-113.56494	241.129877	2.8669E-25	0.94191336

Model Parameters: Dry weight

	coefficients.cv.bc5.	Std.Err	t-value	p-value	
b:15	6.97786361	8.61E+00	0.8106	0.4204003	
b:10	13.0010515	1.21E+02	0.107	0.9150904	
b:7	3.25263659	2.72E+00	11.979	0.2350539	
c:15	0.39611472	1.83E-01	21.637	0.0339523	*
c:10	0.27208908	1.05E-01	26.035	0.01129	*
c:7	0.16725836	9.68E-02	17.271	0.088623	.
d:15	1.27069492	1.06E-01	11.934	<2.2e-16	***
d:10	0.91690971	1.56E-01	5.885	1.30E-07	***
d:7	1.80739142	1.81E-01	9.986	4.91E-15	***
e:15	4490.38218	1.20E+03	3.746	0.0003686	***
e:10	763.093737	1.25E+02	6.123	4.96E-08	***
e:7	92.1230096	1.60E+02	0.5751	0.5670681	
f:15	0.00070665	1.16E-04	6.118	5.05E-08	***
f:10	0.00499193	9.17E-04	5.443	7.51E-07	***
f:7	0.00522215	7.05E-02	0.0741	0.9411455	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error:

0.3620167 (69 degrees of freedom)

Supplementary table 3. Statistical analyses Dose-response test (continuation)

Lack-of-fit test: Dry weight

Model	ModelDf	RSS	Df	F.value	p.value
ANOVA	63	7.6067			
DRC Model	69	9.0429	6	1.9824	0.0814

Calculated ED: Dry weight

	ED50	ED80	ED90
15	10330.81	16235.20	22661.99
10	1222.53	1495.91	1735.50
7	140.230	519.05	1543.00

Variate: Relative biomass (Rel-DW)
Populations: P_3, P_7 and P_5
Model selection

	LogLik	AIC	Lack of fit	Res var
LL4.m	**-345.229**	**710.4587**	**0.992759**	**243.5197**
LL.4	-345.221	716.4420	0.953892	253.6158
LL2.3	-349.719	719.4374	0.565437	270.9914
BC.4	-347.874	721.7473	0.591751	270.1505
BC.5	-345.079	722.1577	0.814939	263.7485
LL.5	-345.085	722.1706	0.813698	263.7890

Lack-of-fit test: Relative biomass (Rel-DW)

Model	ModelDf	RSS	Df	F.value	p.value
ANOVA	63	17390			
DRC Model	75	18264	12	0.2637	0.9928

Model Parameters: Relative biomass (Rel-DW)

	coefficients	Std. Error	t-value	p-value	
b:7	3.26571452	2.37418	13.755	0.173066	
b:5	1.010904628	0.35181	28.734	0.005279	**
b:3	2.470450952	0.75235	32.836	0.001558	**
c:7	9.374533251	4.03979	23.205	0.023033	*
c:5	-0.837888162	19.35525	-0.0433	0.965585	
c:3	8.470483202	4.2122	20.109	0.047926	*
e:7	108.9986323	12.72486	85.658	9.98E-10	***
e:5	1950.336522	1053.76349	18.508	0.068132	.
e:3	166.0215529	25.02117	66.352	4.45E-09	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual Standard Error 15.60512 (75 degrees of freedom)

Supplementary table 3. Statistical analyses Dose-response test (continuation)

ED Estimates: Relative biomass (Rel-DW)

	Estimate	Std..Error
e:7:50	108.9986323	12.7248649
e:7:80	166.6398488	61.12725758
e:7:90	213.609848	115.7442812
e:5:50	1950.336522	1053.763489
e:5:80	7685.55303	7412.821934
e:5:90	17141.88782	21098.31143
e:3:50	166.0215529	25.02117105
e:3:80	290.9839015	71.35694815
e:3:90	404.0424083	133.4583316

Variate: Relative biomass (Rel-DW)
Populations: P_7, P_{10} and P_{15}
Model selection

	logLik	IC	Lack.of.fit	Res.var
BC.5	**-415.60659**	**857.213186**	**0.14358344**	**1355.1517**
LL.5	-458.91203	949.824051	3.737E-15	3965.19387
LL.4	-470.82569	961.651379	2.1557E-16	4844.44075
LL.3	-505.24061	1024.48122	3.4465E-26	10569.9388

Lack-of-fit test: Relative biomass (Rel-DW)

Model	ModelDf	RSS	Df	F.value	p.value
ANOVA	63	79687			
DRC Model	72	97571	9	1.571	0.1436

Model Parameters: Relative biomass (Rel-DW)

	coefficients.	Std.Err	t-value	p-value	
b:15	8.71136612	35.483	0.2455	0.806763	
b:10	13.0233784	115.07	0.1132	0.910202	
b:7	3.37090327	5.3814	0.6264	0.533033	
c:15	33.7046305	18.469	1.8249	0.072165	.
c:10	35.9727509	10.627	3.3849	0.001156	**
c:7	9.2244268	10.057	0.9172	0.362101	
e:15	4652.45619	1.3973	13.973	0.166617	
e:10	752.996658	26.3369	263.369	<2.2E-16	***
e:7	67.5610202	205.4	0.3289	0.743163	
f:15	0.06409517	7.3763	73.763	2.22E-10	***
f:10	0.75487964	12.0667	120.667	<2.2E-16	***
f:7	1.58035271	19.751	0.08	0.936447	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 36.81239 (72 degrees of freedom)

Supplementary table 3. Statistical analyses Dose-response test (continuation)

ED Calculated: Relative biomass (Rel-DW)

	ED50 Calc	ED80 Calc	ED90 Calc
15	407.26	2603.50	11248.11
10	9102.33	12703.40	16232.94
7	1283.01	1567.13	1817.95

Supplementary table 4. Studies on resistance levels of *E. bonariensis* to glyphosate.

			ED_{50} g ae ha^{-1}				
Country	**Class**	**Stage**	**Control**	**Survival**	**DW**	**FW**	**Reference**
Colombia	*S*	rosette			729.4[a]		Quintero-Pertuz et al., 2021
	R	rosette			2,545.2[a]		
	R	rosette			6,264.1[a]		
Brazil	*S*	rosette	88.4	-	34.8		Gomes, 2014
Spain	*S*	rosette	15.7	-	-		González-Torralva et al., 2010
	S	bolting	86.6	-	-		
		flowering	117.5				
Spain	*S*	rosette				50.7[a]	González-Torralva et al., 2012
	R	rosette				311.2[a]	
Spain	*S*	rosette		327.2	75.2[a]		Tahmasebi et al., 2018
	R	rosette		4,985	1,129.9[a]		
South	*S*	rosette	88.5	-	-		Okumu et al., 2019
Africa	*S*	rosette	268.0	-	-		
	R	rosette	344.1				
	R	rosette	3,908.4				
Argentina	*S*	rosette	88	-	-		Puricelli et al., 2015
	S	bolting	182	-	-		
	R	rosette	308.8				
	R	bolting	861.2				
United	*S*	rosette	-	607	219		Moretti et al., 2016
States	*S*	rosette	-	1016	335		
	R	rosette	-	40,862	1,279		
	R	rosette	-	56,153	14,261		

[a] Evaluated as relative biomass

S= Glyphosate sensitive; *R*=Glyphosate resistant

Supplementary table 5. Statistical analyses alternative herbicides evaluation

Explanatory variable: Relative biomass (Rel-DW)

Anova Table

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Trat	4	9566	2391.4	21.463	5.85E-10	***
Pob	2	5823	2911.3	26.129	2.94E-08	***
Trat:Pob	8	7939	992.3	8.906	3.25E-07	***
Residuals	45	5014	111.4			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Population 20

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Trat	4	11932	2983.1	19.81	7.53E-06	***
Residuals	15	2258	150.6			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Tukey Print (TkRDW20)

MSerror	Df	Mean	CV	MSD
150.5535	15	48.64553	25.22333	26.79151

$parameters

test	name.t	ntr	Studentized Range	alpha
Tukey	Trat	5	4.366985	0.05

$means

	Rel.DW	std	r	Min	Max	Q25	Q50	Q75
T1	71.18156	20.106932	4	53.025937	99.13545	59.07781	66.28242	78.38617
T2	73.77522	15.551282	4	55.331412	93.37176	68.29971	73.19885	78.67435
T3	13.83285	8.205247	4	2.305476	20.74928	10.95101	16.13833	19.02017
T4	58.78963	4.102624	4	55.331412	64.55331	56.19597	57.63689	60.23055
T5	25.64841	4.741196	4	19.596542	31.12392	23.91931	25.9366	27.66571

$comparison
NULL
$groups

	Rel.DW	groups
T2	73.77522	a
T1	71.18156	a
T4	58.78963	a
T5	25.64841	b
T3	13.83285	b

Supplementary table 5. Statistical analyses alternative herbicides evaluation (cont.)

Population 10

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Trat	4	3462	865.4	5.887	0.0047	**
Residuals	15	2205	147			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Tukey print(TkRDW10)

MSerror	Df	Mean	CV	MSD
147.0134	15	31.53005	38.45509	26.47465

$parameters

test	name.t	ntr	Studentized Range	alpha
Tukey	Trat	5	4.366985	0.05

$means

	Rel.DW	std	r	Min	Max	Q25	Q50	Q75
T1	51.36612	15.222282	4	36.065574	72.13115	43.442623	48.63388	56.55738
T2	14.48087	13.661202	4	4.371585	33.87978	5.191257	9.836066	19.12568
T3	31.42077	7.591499	4	20.765027	38.25137	28.961749	33.333333	35.79235
T4	39.61749	3.61097	4	37.15847	44.80874	37.15847	38.251366	40.71038
T5	20.76503	15.686011	4	6.557377	42.62295	11.47541	16.939891	26.22951

$groups

	Rel.DW	groups
T1	51.36612	a
T4	39.61749	ab
T3	31.42077	ab
T5	20.76503	b
T2	14.48087	b

Population 15

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)	
Trat	4	2110.2	527.6	14.37	5.13E-05	***
Residuals	15	550.5	36.7			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

MSerror	Df	Mean	CV	MSD
36.70068	15	25.35714	23.89113	13.22783

$parameters

test	name.t	ntr	Studentized Range	alpha
Tukey	Trat	5	4.366985	0.05

Supplementary table 5. Statistical analyses alternative herbicides evaluation (continuation)

$means

	Rel.DW	std	r	Min	Max	Q25	Q50	Q75
T1	43.57143	7.514159	4	37.14286	54.28571	39.28571	41.42857	45.71429
T2	20	3.086067	4	17.14286	24.28571	18.21429	19.28571	21.07143
T3	12.85714	1.166424	4	11.42857	14.28571	12.5	12.85714	13.21429
T4	22.85714	3.499271	4	18.57143	27.14286	21.78571	22.85714	23.92857
T5	27.5	10.193702	4	17.14286	41.42857	22.5	25.71429	30.71429

$groups

	Rel.DW	groups
T1	43.57143	a
T5	27.5	b
T4	22.85714	bc
T2	20	bc
T3	12.85714	c

Explanatoty variable: Visual Control 14 DAA

ANOVA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Trat	4	15585	3896	8.88	4.34E-06	***
Pob	2	6928	3464	7.89	0.000695	***
Time	1	2253	2253	5.13	0.025857	*
Trat:Pob	8	7216	902	2.06	0.048603	*
Trat:Time	4	1424	356	0.81	0.521385	
Pob:Time	2	1858	929	2.12	0.126395	
Trat:Pob:Time	8	6703	838	1.91	0.068158	.
Residuals	90	39500	439			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Population P20

Control 14DAA

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	
Trat	4	11907	2977	35.42	1.77E-07	***
Residuals	15	1260	84			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

HSD Tukey test

groups	Control14	groups
T3	83.75	a
T2	82.5	a
T5	80	a
T4	56.65	b
T1	20	c

Supplementary table 5. Statistical analyses alternative herbicides evaluation (continuation)

Population P10

Control 14DAA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Trat	4	2762	690.6	4.207	0.0176	*
Residuals	15	2462	164.2			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

HSD Tukey test

$groups	**Control14**	**groups**
T2	85	a
T5	80	ab
T4	78.75	ab
T3	66.25	ab
T1	52.5	b

Population P15

Control 14DAT

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	
Trat	4	1482	370.6	2.695	0.0712	.
Residuals	15	2062	137.5			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

HSD Tukey test

$groups	Control14	groups
T2	83.75	a
T4	81.25	a
T3	80	a
T5	72.5	a
T1	60	a

Explanatoty variable: Visual Control 28 DAT

Population P20

Control 28DAA

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	
Trat	4	18225	4556	53.87	1.01E-08	***
Residuals	15	1269	85			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Supplementary table 5. Statistical analyses alternative herbicides evaluation (continuation)

HSD Tukey test

$groups	Control28	groups
T5	90.00	a
T3	86.25	a
T1	30.00	b
T2	25.00	b
T4	25.00	b

Population P10

Control 28DAA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Trat	4	13595	3399	13.53	7.27E-05	***
Residuals	15	3769	251			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

HSD Tukey test

$groups	Control28	groups
T2	88.75	a
T5	82.5	a
T4	47.5	b
T3	40	b
T1	20	b

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Population P15

Control 28DAA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Trat	4	5405	1351.3	8.047	0.00113	**
Residuals	15	2519	167.9			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

HSD Tukey test

$groups	**Control28**	**groups**
T3	92.50	a
T2	85.00	a
T5	77.50	ab
T4	56.25	b
T1	50.00	b