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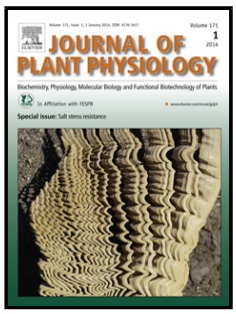

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Low temperatures enhance the absorption and translocation of ^{14}C-glyphosate in glyphosate-resistant *Conyza sumatrensis*

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ABSTRACT

Influence of low temperatures on the glyphosate efficacy was studied in glyphosate-resistant (R) and -susceptible (S) *Conyza sumatrensis* biotypes. For this purpose, the physiological and enzymatic aspects involved were characterized under two growing temperature regimes [high (30/20 ºC) and low 15/5ºC temperatures day/night]. The R biotype was 5.5 times more resistant than the S biotype at high temperatures; however, this R-to-S ratio

decreased to 1.6 at low temperatures. At 96 h after treatment (HAT), the shikimic acid accumulation was higher in the S biotype in both temperature regimes (4.6 and 1.9 more shikimic acid at high and low temperatures, respectively), but the accumulation of the R biotype increased 2.6 times at low temperatures compared to high ones. From 24 to 96 HAT, the ^{14}C-glyphosate absorption ranged from 28 to 65% (percentage reached from 48 HAT) at low temperatures and from 20 to 50% at high temperatures (gradual increase), but there were no differences between *C. sumatrensis* biotypes within each temperature regime. At high temperatures, the ^{14}C-glyphosate translocation was different between biotypes, where the R one retained at least 10% more herbicide in the treated leaves than the S biotype at 96 HAT. So, the S biotype translocated 40% of ^{14}C-glyphosate absorbed to roots, and the R biotype translocated only 28% of herbicide at the same period. At low temperatures, there were no differences between biotypes, and at 96 HAT, the ^{14}C-glyphosate found in treated leaves was ~47% and up to ~42% reached the roots, i.e., the resistance mechanism was suppressed. The basal and enzymatic activities of the 5-enolpyruvyishikimate 3-phosphate synthase were different between temperature regimes, but there was no differences between biotypes within each temperature regime, showing that target-site resistance mechanisms did not contribute in the glyphosate resistance of the R biotype. Low temperatures enhanced the absorption and translocation of glyphosate by suppressing the resistance mechanisms improving its efficacy on resistant plants. This is the first characterization about the role of temperatures in the glyphosate efficacy on *C. sumatrensis.*

Keywords: 5-enolpyruvyishikimate 3-phosphate synthase; glyphosate translocation, Sumatran fleabane, vacuolar sequestration

1. INTRODUCTION

The use of herbicides is increasing in worldwide crop production each year due, among other factors, to the reduction of workers for hand weeding (Gianessi 2013). Indiscriminate use of herbicides, together with a lack of an integrated weed management lead the appearance of biotypes resistant to herbicides in different cropping systems. A weed biotype resistant to a given herbicide is able to survive, complete its life cycle and reproduce by seed after application of the herbicide at a dose normally lethal for the wild biotype of the same species (Beffa et al. 2019). Herbicide resistance is one of the major concerns in the modern agriculture (Burgos et al 2013), and worldwide, there are 255 species (148 dicots and 107 monocots) resistant to herbicides (Heap 2019).

Glyphosate is a full spectrum herbicide that acts by inhibiting the enzyme 5-enolpyruvyishikimate 3-phosphate synthase (EPSPS) an important enzyme in the shikimate pathway (Steinruecken and Amrhein, 1980). This herbicide has been used to control weeds in different crop situations such as citrus orchards, olive groves, and vineyards in southern Spain. However, biotypes of *Conyza* species (*C. bonariensis, C. canadensis* and *C. sumatrensis*) has been reported to be glyphosate resistant in this country (Urbano et al, 2007; González-Torralva et al. 2012, 2014; Amaro- Blanco et al. 2018). *Conyza* spp. has evolved resistance to various herbicidal mechanisms of action (Kleinman and Rubin, 2017) such as ALS-inhibitors, paraquat, atrazine, and among other herbicides, with some populations evolving resistance to more than one herbicide mode of action (Heap 2019).

Conyza sumatrensis (Retz.) E. Walker is an annual, biennial or perennial herbaceous plant native to South America (Buhler and Owen, 1997). It can be found in subtropical and temperate climates, is a very invasive weed because produces high amounts of achenes which are easily dispersed by wind

(Hao et al. 2009). *Conyza* spp. have susceptibility differential to glyphosate with *C. sumatrensis* being the most susceptible (González-Torralva et al. 2010). Glyphosate resistant populations of *Conyza* spp. from several parts of the world has been described carrying target-site (TSR) and non-target-site (NTSR) resistance mechanisms, both isolated or associated (González-Torralva et al. 2012, 2014; Kleinman and Rubin, 2017; Page et al. 2018; Mei et al. 2018). *Conyza* spp. can exhibit high tolerance to glyphosate when treated in high temperature conditions or when plants are treated at advanced phenological stages (Shrestha et al. 2007; González-Torralva et al. 2010). However, there is evidence that environmental conditions, mainly the low temperatures (Vila-Aiub et al. 2013; Ghanizadeh et al. 2015a), may reduce the glyphosate resistance levels by suppressing the mechanism involved (Ge et al. 2011; Tani et al. 2016).

Temperature influences the glyphosate efficacy (Ghanizadeh et al. 2015a) and low temperatures can reduce the resistance levels by suppressing the NTSR mechanism involved (Ge et al. 2011). The aims of this research were a) to characterize the glyphosate efficacy through dose-response assays in a resistant (R) *C. sumatrensis* biotype in comparison to one susceptible (S); and b) to determine the physiological and enzymatic aspects (shikimic acid accumulation, absorption and translocation, and EPSPS activity assays), influencing the glyphosate under two temperatures regimes.

2. MATERIAL AND METHODS

2.1 *Plant material and temperature conditions*

Two biotypes of *C. sumatrensis* (S and R) were used in all the experiments described below. Seeds of R biotype were collected from plants that survived glyphosate field doses (1080 g ae ha^{-1}) used in a citrus area

located in the Southern Spain (37º13'35.8"N 7º17'02.8"W). S seeds were harvested from a nearby area never treated with herbicide (37º14'15.2"N 7º16'38.0"W).

Seeds of both biotypes were sown in trays containing moistened peat and covered with transparent film until the emergence. Trays were placed under controlled conditions (30/20 °C day/night, 6-h photoperiod, 300-µmol m^{-2} s^{-1} photosynthetic photonflux density, and 80% relative humidity). Seedlings were transplanted individually into 250-mL pots containing peat and sand (1:1, v/v). Two set of plants of each *C. sumatrensis* biotype were placed in growth chambers under the same growing conditions described above, except for the temperature which were 20/30 ºC day/night (high) for a growing chamber and 15/5 ºC day/night (low) for the other. Both R and S plants, from the two growing temperatures tested, at the rosette stage (6-8 true leaves) were used in all experiments.

2.2 *Whole plant dose-response assays*

Plants R and S, grown at high and low temperatures, were sprayed with glyphosate (Roundup Energy 450 g ae L^{-1}, Monsanto Agricultura, España). Treatments were performed in an automatic track sprayer equipped with flat fan nozzles (Tee Jet 8002 Even Flat Spray Tip) at a height of 50 cm above the plants and calibrated to spray 200 L ha^{-1} at 200 kPa. The glyphosate doses applied were: 0, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4000 g ae ha^{-1}. Treatments were repeated three times in a completely randomized design (each replication with four plants). Plants at both temperatures were maintained outside the growth chamber until the sprayed solution was dried at room temperature, and immediately were placed in their corresponding

growing conditions. Twenty-one days after herbicide treatment, plants were cut at ground level and, the fresh weight was registered.

2.3 *Shikimic acid accumulation*

Glyphosate was sprayed on R and S *C. sumatrensis* plants (grown at high and low temperatures) as described above at the rate of 360 g ae ha^{-1} in a volume of 200 L. Shikimic acid was extracted at different time intervals (24, 48, 72, and 96 h after treatment). Fresh tissues (50 mg) were harvested in each time interval, placed in an Eppendorf containing 0.25N HCl, and immediately freeze in liquid nitrogen. Shikimic acid accumulation was performed following the procedure described by Cromartie and Polge (2002). Different known concentrations of shikimic acid added to 0.25N HCl were used to establish a standard curve, and to calculate the shikimic acid present in each sample at an optical density of 382 nm wavelength. Absorbance lectures were obtained by using a Beckman DU-640 spectrophotometer. Experiment was arranged in a completely randomized design with three replications per biotype including an untreated control plants.

2.4 *^{14}C-glyphosate absorption and translocation*

Absorption and translocation of ^{14}C-glyphosate was carried out according to Palma-Bautista (2019a). A commercial glyphosate solution of 360 g ae ha^{-1} was mixed with radiolabel ^{14}C-glyphosate in order to prepare a solution with an activity of 0.834 kBq μL^{-1}. Plants R and S from both growing temperatures were treated with the radiolabeled solution by applying one droplet of 1.0 µL in the third leaf in each plant. Plants were kept outside growth chamber as long as the droplet had dried on the application site, then immediately a returned accordingly. At different time intervals of droplet application (24, 48, 72 and 96 h), the treated leaf was washed in batches with

3 mL of a water:acetone (1:1 v/v) solution in order to remove and quantify the unabsorbed ^{14}C-glyphosate. Then, 7 mL of scintillation liquid was added to each rinsate, and the radioactivity measured by liquid scintillation spectrometry (LSS) using a Scintillation Counter LS 6500 (Beckman Coulter) instrument. Finally, plants were sectioned into treated leaf, rest of shoot and roots. Plant sections were placed into cellulose cones, dried at 60 °C during 72 h and combusted in a biological sample oxidizer (307, PerkinElmer). The 14C02 released by combustion was trapped and mixed with 18 mL of a 9:9 v/v mixture of Carbo-Sorb® E and Permaf1uor® (PerkinElmer), and quantified by LSS as stated before. Experiment was arranged in a completely randomized design using five replicates per biotype.

2.5 *Visualization of ^{14}C-glyphosate translocation*

Plants were treated and leaves washed at the same time intervals as described in the previous section. However, the whole plants were removed from the pot and roots washed with distilled water. Whole plants were fixed in filter papers (12.5 x 25 cm) and dried at room temperature. Then, plants were placed on a phosphor storage film during 8 h in the dark. Films were scanned for ^{14}C dispersion in a storage phosphor system (Cyclone, Perkin-Elmer). The experiment was performed with three samples per biotype and harvest time.

2.6 *EPSPS enzyme activity assays*

Five grams of young leaves were harvested from R and S plants (grown at high and low temperatures) for the extraction of EPSPS following the methodology described by Sammons et al (2007). The total soluble protein (TPS) in the extract (EPSPS basal activity in absence of glyphosate) was measured using a kit for protein determination (Sigma-Aldrich, Madrid, Spain) according to Bradford (1976). EPSPS activity was determined using an

EnzChek phosphate assay kit (Invitrogen, Carlsbad, CA, USA). The glyphosate concentrations assayed to inhibit the EPSPS inhibition by 50% (I_{50}) were 0, 0.1, 1, 10 100 µM. The release of phosphate at the lower level was measured for 10 min at 360 nm wavelength in a spectrophotometer (DU-640, Beckman Coulter Inc., Fullerton, CA, USA). The basal EPSPS activity was expressed as the amount (µmol) of phosphate released per µg of TSP min^{-1}. Three replications of each biotype per glyphosate concentration were analyzed. EPSPS inhibition was expressed as a percentage relative to the control.

2.7 *Data processing*

Fresh weight and EPSPS enzyme activity data were transformed to percentage respect to control, and fitted to the nonlinear log-logistic regression model: $Y = c + \{(d-c)/[1 + (x/g)^b]\}$, where: Y represents the fresh weight; letters c and d represent the lower and upper asymptotes; letter b is the slope of the line; g is the herbicide rate at the point of inflection halfway between the upper and lower asymptotes, i.e., the GR_{50} or I_{50} (herbicide rate producing the fresh weight reduction or enzymatic inhibition by 50%); and x represents the herbicide rate. Regression analysis were performed in SigmaPlot 10.0 software (Systat Software Inc.). The resistance index (RI) was obtained by dividing the GR_{50} or I_{50} value of the R biotype by the GR_{50} or I_{50} values of the S biotype, respectively.

Data obtained in the shikimic acid accumulation, and absorption-translocation of ^{14}C-glyphosate were submitted to ANOVA, and the effects of biotype, time (HAT), and temperature, and their interaction were tested. Biotype was treated as a fixed factor whereas time and growing temperatures were considered as a random factor. When necessary, the Tukey HSD test at

the 5% probability was used to separate means. Shapiro-Wilk test was performed to assume a normal distribution of data, and if required data were transformed (arcsine) prior to analysis. Statistical analysis was conducted using the Statistix (version 8.0) (Analytical Software, United States of America) software.

3. RESULTS

3. 1 *Whole plant dose-response assays*

The biotype R, grown at high temperatures (30/20 ºC), showed the highest GR_{50} value (281.2 g ae ha^{-1}), meanwhile this value was almost halved (GR_{50} = 152.1 g ae ha^{-1}) at 15/5ºC (low temperatures). The susceptibility of the biotype S because showed a GR_{50} values of 50.9 g ae ha^{-1} at high temperatures and increased to 94.3 g ae ha^{-1} at low temperatures. Thus, the proportion of resistance indexes (RI= R/S) decreased from 5.5 (high temperatures) to 1.6 (low temperatures) (**Table 1, Fig. 1**).

3.2 *Shikimic acid accumulation*

The presence of shikimic acid was higher in the S biotype than the R biotype in both growth temperature regimes from the initial monitoring time. At 24 HAT, S plants grown at low temperatures accumulated more shikimate than those grown at 30/20 ºC (2.5 and 1.7 mg shikimic acid g^{-1} fresh weight, respectively). However, from 48 HAT there was no differences for this biotype (~5.7 mg g^{-1} fresh weight at 96 HAT), regardless of the growth temperature regime. On the other hand, the accumulation of shikimate was higher (2.6 times at 96 HAT) in R plants that grew at low temperatures than those grown at high temperatures at any time of evaluation. Finally, S plants

4.6 and 1.9 times more shikimic acid at 30/20 ºC and 15/5 ºC, respectively, than the R plants at 96 HAT (**Fig. 2**).

3.3 *Absorption and translocation of ^{14}C-glyphosate*

The R and S *C. sumatrensis* biotypes did not show differences in ^{14}C-glyphosate absorption within each temperature regime; however, the absorption was higher and faster in plants maintained at low temperatures. At high temperatures, the ^{14}C-glyphosate absorption increased gradually from 20 to 50% between 24 and 96 HAT. In contrast, at low temperatures, the ^{14}C-glyphosate absorption presented an initial average of ~28% (24 HAT) that exceeded 65% from 48 HAT until the end of the experiment (**Fig. 3**).

The translocation speed of ^{14}C-glyphosate differed between R and S plants according to the temperature regime. In plant grown at high temperatures, the ^{14}C-glyphosate translocation rates were different between the R and S biotypes. The ^{14}C-herbicide found in the treated leaf of R plants diminished from 73 to 53% from 24 to 96 HAT, as for the plants S that decrease was from 66 to 41%. The ^{14}C-glyphosate found in the rest of plant showed no marked differences between biotypes because the herbicide was translocated to the roots. Thus, the S biotype translocated 40% of ^{14}C-glyphosate absorbed from the treated leaf to roots, while the R biotype only 28% at 96 HAT. By other one hand, there were no differences between biotypes at low temperatures. At 24 HAT, both R and S plants retained higher amounts of herbicide in treated leaves (~83%) translocating less than 12% of ^{14}C-glyphosate absorbed to the roots at this time. At 96 HAT, the ^{14}C-herbicide found in treated leaves was ~47% and up to ~42% of ^{14}C-glyphosate reached the roots (**Fig. 4A**). These results can be visualized in the images

obtained by phosphor imager where the red color shown high concentration of radiolabeled herbicide (**Fig. 4B**).

3.4 *EPSPS enzyme activity assays*

The EPSPS basal activity was different among temperature regimes, but no between biotypes within each temperature regime. Thus, the basal EPSPS activities of *C. sumatrensis* plants were in average ~0.21 and ~0.33 μmol μg TSP^{-1} min^{-1} at low and high temperatures, respectively. By having less EPSPS, R and S plants grown at low temperatures needed less glyphosate to inhibit the enzyme by 50% (I_{50}= 0.42 μM) in relation to plants grown at high temperatures (I_{50}= 0.42 μM) (**Fig. 5, Table 2**).

4. DISCUSSION

Results of this work demonstrated the selected resistance to glyphosate in the R biotype of *C. sumatrensis* in relation to the S biotype. Glyphosate efficacy is dependent on the temperature (Ghanizadeh et al. 2015a), as demonstrated in this study when the response to glyphosate of the two S and R biotypes assessed was different depending to the growth temperature regime. By other hand, several studies highlight that the effectiveness of glyphosate is higher at high temperatures than low ones (Adkins et al. 1998, Ghanizadeh et al. 2015a). This information corroborates the results observed in the S biotype of *C. sumatrensis* since its sensitivity to glyphosate decreased at low temperatures; however, it is apparently contradictory with the glyphosate response of the R biotype, which was more susceptible in this same temperature regime (≠ the half of GR_{50} value estimated at high temperatures). The lower susceptibility observed in the R biotype at low temperatures reinforces that the glyphosate resistance depends on the temperature (Tani et

al. 2016). Our results are in agreement with those observed in *Sorghum halepense*, *Lolium rigidum* (Vila-Aiub et al. 2013) and *Echinoclhoa colona* (Nguyen et al. 2016), where the low temperatures decreased the plant survival and biomass only in the R biotypes and the R-to-S ratios were also lower than at high temperatures.

Quantifying the accumulation of shikimic acid after glyphosate treatment is often a parameter to determine susceptibility or resistance to this herbicide (Shaner et al. 2005), because glyphosate is a potent inhibitor of the EPSPS (Steinruecken and Amrhein, 1980). However, differences in the accumulation of shikimic acid can reveal unclear results due to the growth conditions, the dose of glyphosate applied and the phenological stage of plants, as observed in glyphosate-susceptible and -resistant *C. bonariensis* biotypes treated at 360 g ae ha^{-1} that presented similar shikimate accumulation levels at 72 HAT (Dinelli et al. 2008). Here, the differences in the accumulation of shikimic acid between R and S *C. sumatrensis* biotypes were more contrasting when plants of both biotypes grew in high temperatures, highlighting the important role of temperature in the effectiveness of glyphosate. Optimal temperatures have a positive effect on the shikimate pathway, thus shikimate accumulation is more evident in plants maintained under high conditions after glyphosate treatment (Shaner et al. 2005; Tani et al. 2016).

Exploring the biochemical basis of resistance, differences in absorption rates were found between the temperature regimes, but not between *C. sumatrensis* biotypes within each temperature regime. Reduced absorption has rarely been reported as resistance mechanism to glyphosate (Ghanizadeh et al. 2015b), and when there were differences, they were due to inherent characteristics of a specific population/biotype and not of the species. For

example, a R *S. halepense* population absorbed less glyphosate compared to three other R populations that absorbed similar amounts of herbicide to the control S (Vila-Aiub et al., 2012). Glyphosate R and S populations of *Conyza* species have generally shown no differences in the absorption of herbicide (González-Torralva et al. 2012; Moretti and Hanson, 2017). On the other hand, temperature seem to play a relevant role in the glyphosate absorption. For example, *Kochia scoparia* showed the lowest glyphosate absorption rate at 32.5/22.5°C (day/night), that increased as temperature decreased (25/15 and 17.5/7.5°C) (Ou et al. 2018). In the same way, *E. colona* plants absorbed two-fold less glyphosate at 30/28 ºC that at 20/18 ºC (Nguyen et al. 2016). Therefore, it can be inferred that at high temperatures the absorption rate of the herbicide is low and vice versa, as was corroborated in the R and S *C. sumatrensis* biotypes of this study.

The reduced translocation of ^{14}C-glyphosate in the R *C. sumatrensis* biotype was observed under optimal growth temperatures (high). This non-target site resistance mechanism has been observed conferring resistance to glyphosate in several weeds (González-Torralva et al. 2012; Vila-Aiub et al. 2012; Ghanizadeh et al. 2015b; Kleinman and Rubin, 2017; Amaro- Blanco et al. 2018; Palma-Bautista et al. 2019b), but was not completely unraveled. Glyphosate follows the movement of sucrose in the phloem (Shaner et al. 2012; Yanniccari et al. 2012), and enters plant cells through a phosphate pump (Preston, 2008). Within cells of the R biotype, the glyphosate was possibly linked to active tonoplast transporters (Ge et al. 2014), especially ABC transporters (Nol et al. 2012; Tani et al. 2015), which carried it quickly into the vacuole. The sequestration and immobilization of glyphosate in the vacuole has been reported in *C. canadensis* from USA (Ge et al. 2010) and *Lolium* spp. from Australia, South America, and Europe (Ge et al. 2012).

Although we did not study this phenomenon, we can infer that the vacuolar sequestration was responsible for the reduced translocation at high temperatures in the R biotype, since key ABC-transporter genes regulate this mechanism (Nol et al. 2012; Tani et al. 2015). These key genes are affected by environmental conditions and their expression is reduced at low temperatures (Tani et al. 2016), i.e., there are not enough active transporters carrying glyphosate to the vacuole, therefore, this mechanism is suppressed (Ge et al. 2011), allowing the translocation patterns of glyphosate to normalize and reach its target-site. This explaining because at low temperatures there were no differences in the translocation of ^{14}C-glyphosate between the R and S biotypes of *C. sumatrensis*.

The expression key ABC-transporter genes involves the synchronization of the *EPSPS* gene expression (Tani et al. 2015), i.e., the reduced expression of key ABC-transporter genes at low temperatures also causes a reduced expression of the *EPSPS* gene (Tani et al. 2016). Thus, once there was less amount of EPSPS (basal activity) in plants of both R and S *C. sumatrensis* biotypes that grew at low temperatures, the glyphosate concentration required to inhibit EPSPS (enzyme activity) by 50% was lower compared to EPSPS from plants grown at high temperatures. The no differences in basal and enzymatic activity of the EPSPS between plants R and S shows that TSR mechanisms do not contribute to glyphosate resistance (Bracamonte et al. 2018, Garcia et al. 2019). Therefore, there were no TSR mechanisms involved in the glyphosate resistance of the R biotype, since differences in EPSPS activities occurred within the temperature regimes and not between the biotypes of *C. sumatrensis*.

5. CONCLUSION

Control of some herbicide resistant weeds could be realized more efficiently if the herbicide is applied when low temperatures occurs, but previously knowledge on the physiology and biochemical basis of resistance are needed. Summarizing, as was demonstrated, the low temperatures enhanced the absorption and translocation of ^{14}C-glyphosate in the glyphosate-resistant and-susceptible plants of *C. sumatrensis* showing a better efficacy of herbicide at low temperatures.

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FIGURE LEGENDS

Fig. 1. Dose-response curves of glyphosate-resistant (solid lines) and -susceptible (dotted lines) *Conyza sumatrensis* biotypes grown at low (15/5 ºC day/night; blue lines) and high (30/20 ºC day/night; red lines) temperatures. Vertical bars of each point correspond to standard deviations of the mean (*n*= 12).

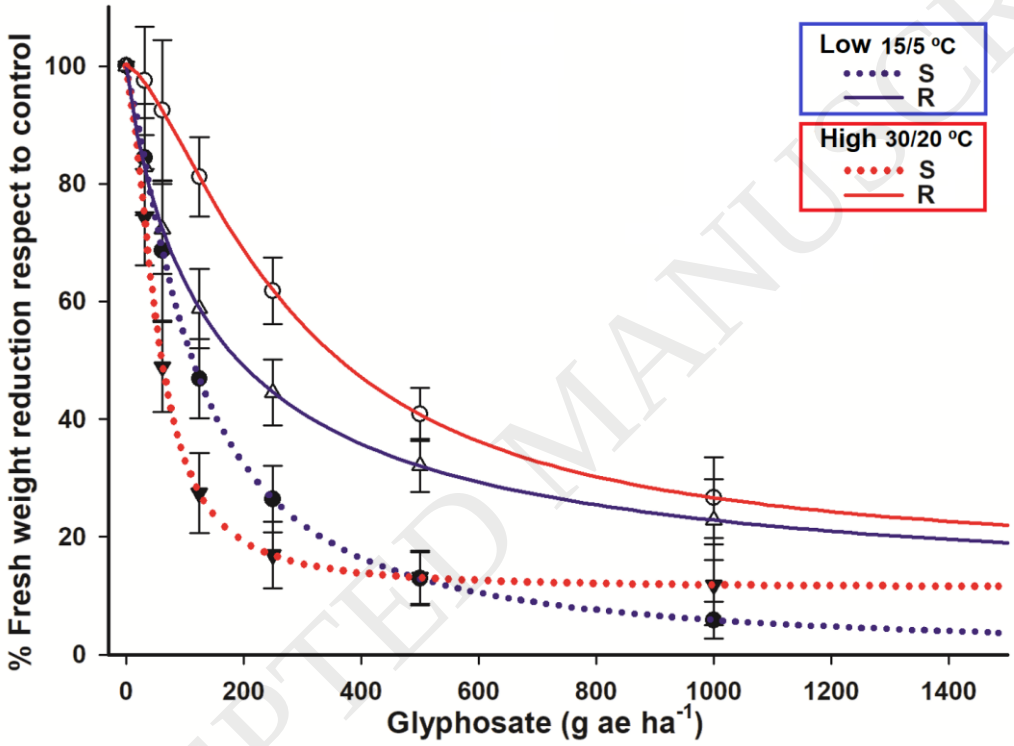

Fig. 2. Shikimic acid accumulation in glyphosate-resistant (solid lines) and -susceptible (dotted lines) *Conyza sumatrensis* plants grown at low (15/5 ºC day/night; blue lines) and high (30/20 ºC day/night; red lines) temperatures. Vertical bars of each point correspond to standard deviations of the mean (*n*= 3).

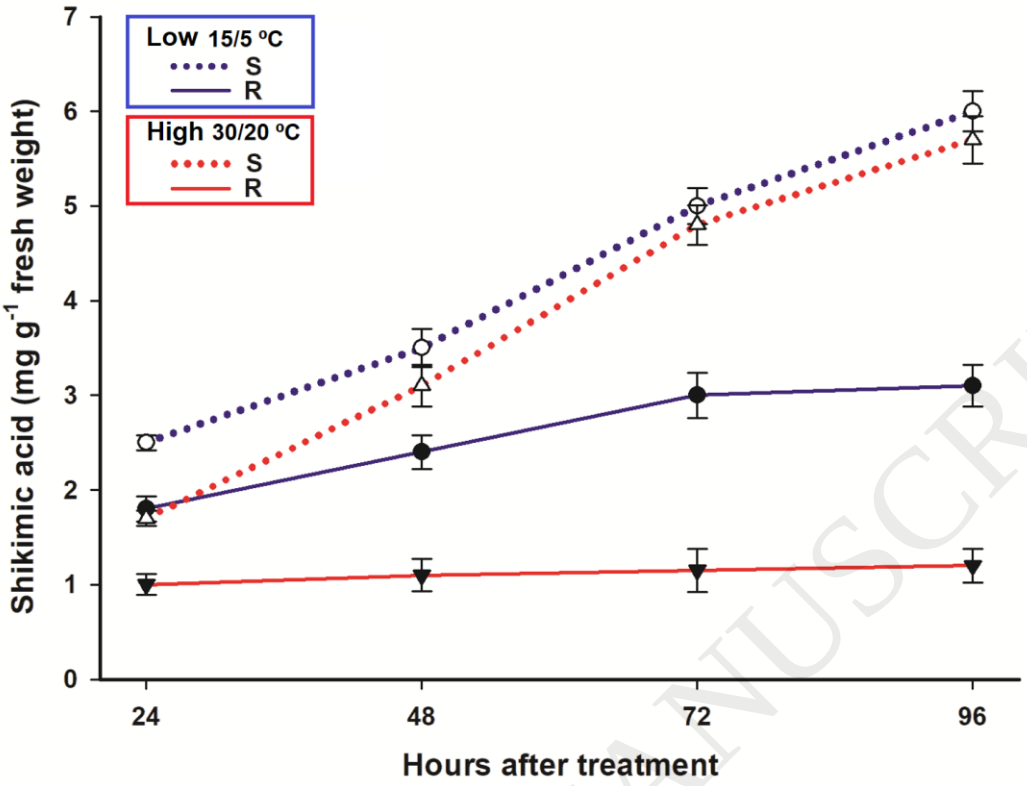

Fig. 3. Absorption of ^{14}C-glyphosate in glyphosate-resistant (solid lines) and -susceptible (dotted lines) *Conyza sumatrensis* plants grown at low (15/5 ºC day/night; blue lines) and high (30/20 ºC day/night; red lines) temperatures. Vertical bars of each point correspond to standard deviations of the mean (*n*= 5).

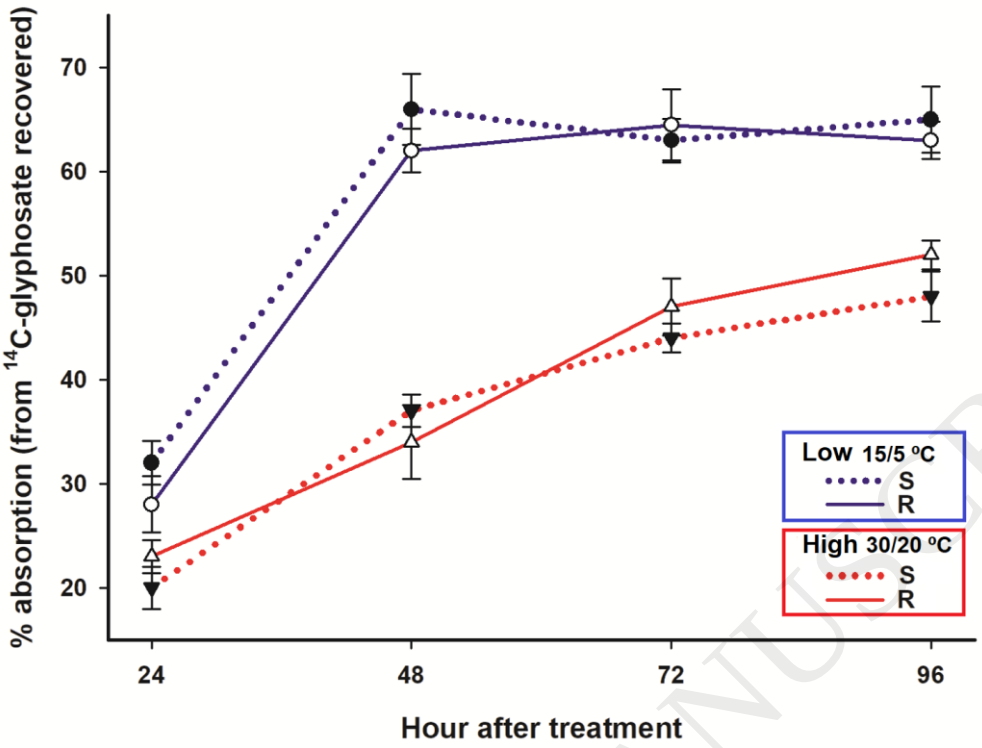

Fig. 4. A) Percentage of ^{14}C-glyphosate translocation in glyphosate-resistant (solid lines) and -susceptible (dotted lines) *Conyza sumatrensis* plants grown at low (15/5 ºC day/night; top panels) and high (30/20 ºC day/night; lower panels) temperatures from 24 to 96 h after treatment. Vertical bars of each point correspond to standard deviations of the mean (*n*= 5). B) Digital (left plants) and autoradiograph (right plants) images that show the distribution of ^{14}C-glyphosate within *C. sumatrensis* plants at 96 h after treatment. Red color intensity indicates the highest glyphosate concentrations. Arrows shows the initial droplet site application.

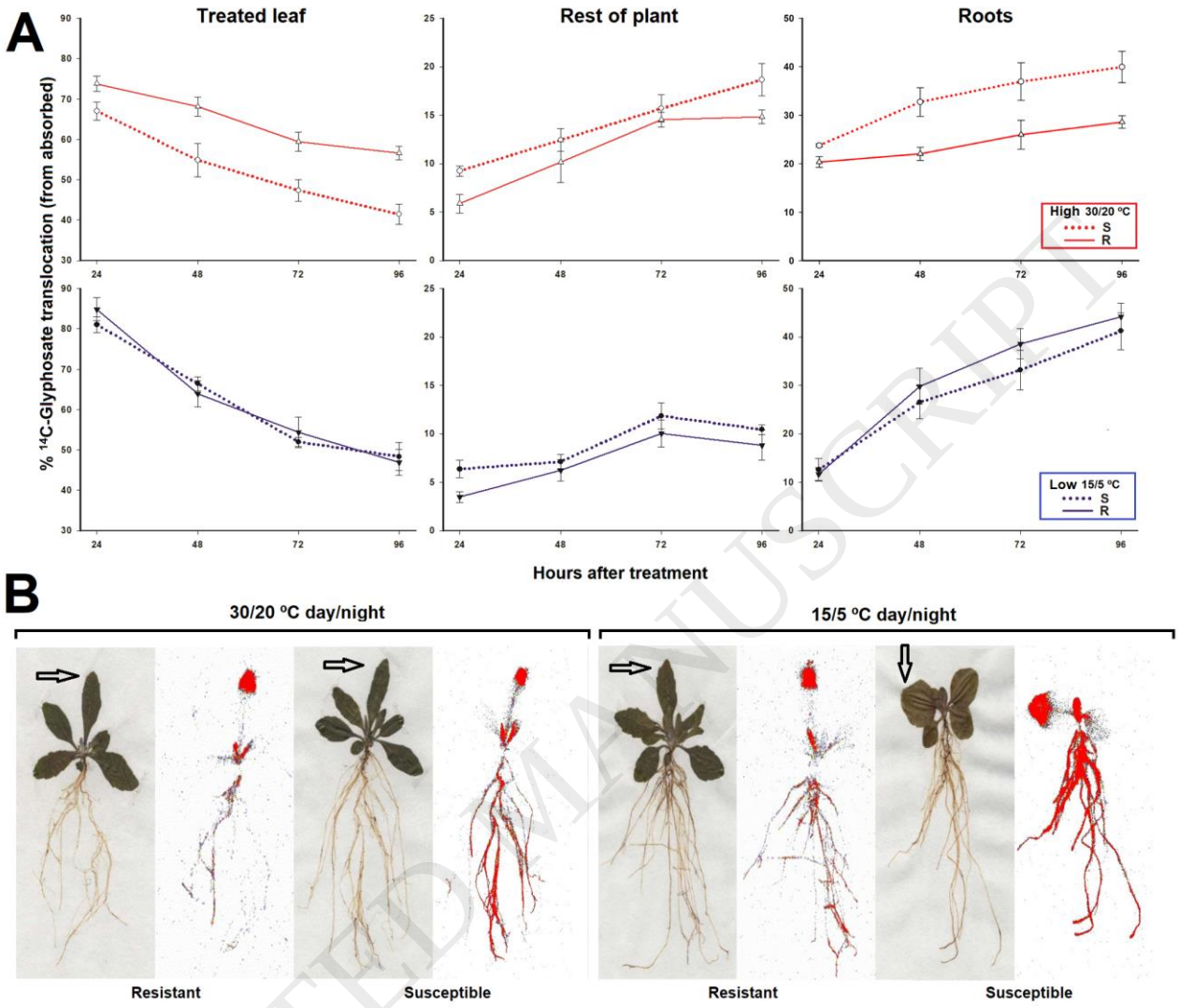

Fig. 5. Activity of the total soluble protein (TPS) 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in glyphosate-resistant (solid lines) and -susceptible (dotted lines) *Conyza sumatrensis* plants grown at low (15/5 ºC day/night; blue lines) and high (30/20 ºC day/night; red lines) temperatures. Basal activity (absence of glyphosate) and dose-response curves of the EPSPS enzyme, expressed as percentage of the untreated control, exposed to different glyphosate concentrations (µM). Vertical bars of histograms and each point represent the standard error of the mean (n=3).

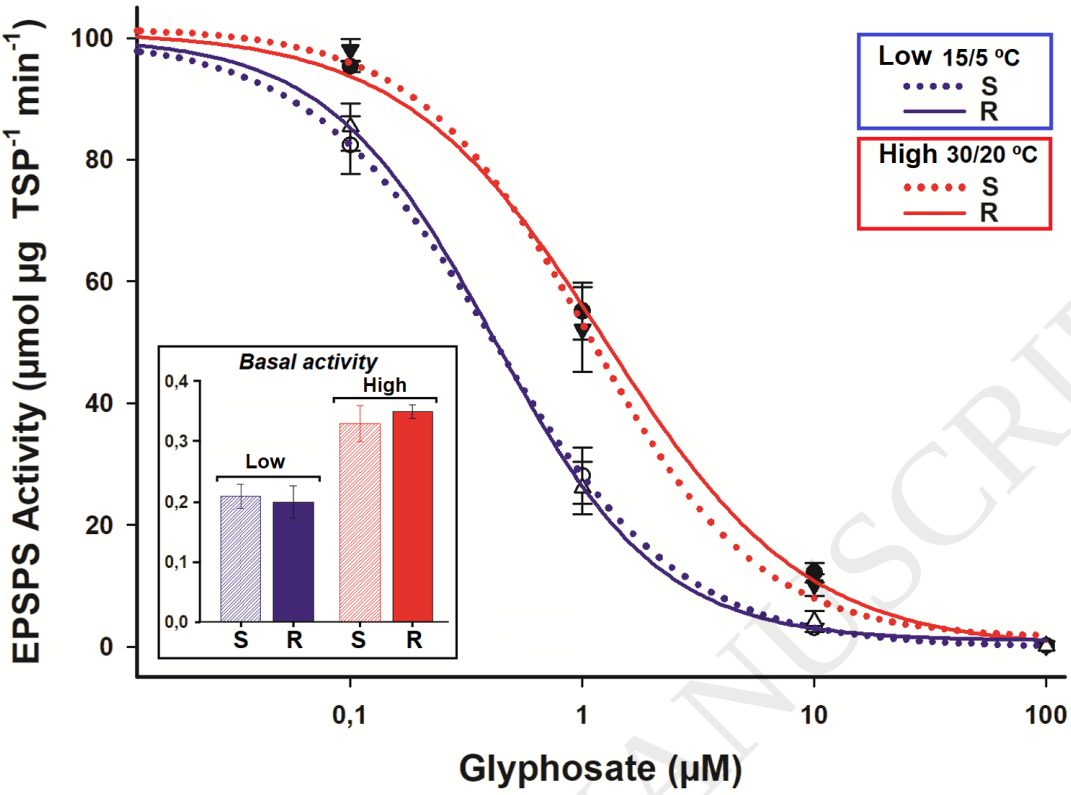

Table 1. Parameters of the equation[a] used to calculate the glyphosate rate (g ae ha^{-1}) that reduced the fresh weight by 50% (GR50) in glyphosate-resistant and -susceptible *Conyza sumatrensis* plants grown at low (15/5 ºC day/night) and high (30/20 ºC day/night) temperatures (Tº regime)

Tº regime	Biotype	*c*	*d*	*b*	pseudo r^2	p[b]	GR_{50}	RI[c]
High	S	11.3	101.8	1.7	0.94	<0.0001	50.9±3.2	5.5
	R	15.7	100.5	1.5	0.96	<0.0001	281.2±33.2	
Low	S	0.4	99.3	1.2	0.99	<0.0001	94.3±10.6	1.6
	R	8.6	100.8	0.9	0.98	<0.0001	152.1±24.0	

[a] $Y= c +\{(d–c)/[1+(x/g)^b]\}$, where *Y* is the is the fresh weight reduction; *c* and *d* are the coefficient corresponding to the upper and lower asymptote, respectively; x the herbicide dose; *b* is the slope of the line; and *g* is the dose at inflection point, hence the GR_{50}. [b] p value= probability level of significance of the non-linear model. [c] Resistance index ($RI=GR_{50}$ R/GR_{50} S). ± Standard error of the mean (n= 12).

Table 2. Parameters of the equation[a] used to estimate the glyphosate concentration (µM) that inhibited the 5- enolypyruvylshikimate- 3-phosphate synthase by 50% (I_{50}) in glyphosate-resistant and -susceptible *Conyza sumatrensis* plants grown at low (15/5 ºC day/night) and high (30/20 ºC day/night) temperatures (Tº regime)

Tº regime	Biotype	*c*	*d*	*b*	pseudo r^2	p[b]	I_{50} (µM)	RI[c]
High	S	0.21	100.1	1.00	0.99	0.0853	1.24±0.16	0.87
	R	1.36	100.6	1.18	0.99	0.1063	1.05±0.17	
Low	S	0.11	100.0	1.07	1.00	0.0020	0.42±0.01	0.97
	R	0.97	100.1	1.21	0.99	0.0477	0.41±0.03	

[a] $Y= c +\{(d–c)/[1+(x/g)^b]\}$, where *Y* is the inhibition by 50% of the enzyme with respect to the control; *c* and *d* are the coefficient corresponding to the upper and lower asymptote, respectively; x the glyphosate concentration; *b* is the slope of the line; and *g* is the glyphosate concentration at inflection point (I_{50}). [b] p value= probability level of significance of the non-linear model. [c] RI=I□$_{50}$ R/I□$_{50}$ S). ± Standard error of the mean (*n*= 3).