Weed Technology 2014 28:587–591

Different Glyphosate Susceptibility in *Chloris polydactyla* Accessions

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Glyphosate is now the most widely used herbicide; after years of frequent utilization, resistant weeds were selected, mainly due to widespread adoption of glyphosate-resistant crops and no-tillage sowing system. Increasing difficulty in controlling *Chloris polydactyla* with glyphosate has been noticed in agricultural areas. Here, the susceptibility level of various *C. polydactyla* accessions from Brazil is examined. Two whole-plant studies were conducted to confirm the presence and extent of glyphosate susceptibility among accessions, which involved the application of seven glyphosate doses on four accessions. The four accessions showed different glyphosate susceptibilities. The "Matão" accession presented major tolerance for glyphosate compared to "Palotina" accessions. "Jaboticabal" plants showed an intermediary susceptibility. The resistance factor (RF) was 3.76 between the "Matão" and "Palotina" accessions. All biotypes died at 2,880 g ae ha^{-1} glyphosate.
Nomenclature: Glyphosate; *Chloris polydactyla* (L.) Sw.
Key words: Control, herbicide resistance.

Glyphosate es actualmente el herbicida más ampliamente usado. Después de años de uso frecuente, malezas resistentes fueron seleccionadas, principalmente debido a la amplia adopción de cultivos resistentes a glyphosate y de sistemas de siembra con labranza cero. En áreas agrícolas, se ha notado el incremento en la dificultad para controlar *Chloris polydactyla* con glyphosate. Aquí, se examinó el nivel de susceptibilidad de varias accesiones de *C. polydactyla* de Brasil. Dos estudios de plantas enteras fueron realizados para confirmar la presencia y la magnitud de la susceptibilidad a glyphosate entre accesiones, los cuales involucraron la aplicación de siete dosis de glyphosate sobre cuatro accesiones. Las cuatro accesiones mostraron diferentes susceptibilidades a glyphosate. La accesión "Matão" presentó una tolerancia mayor al compararse con la accesión "Palotina". Las plantas de "Jaboticabal" mostraron una susceptibilidad intermedia. El factor de resistencia (RF) fue 3.76 entre las accesiones "Matão" y "Palotina". Todos los biotipos murieron al ser expuestos a 2,880 g ae ha^{-1} de glyphosate.

Agriculture is considered a high-technology process; however, average crop yields remain suboptimal. One of the causes of production loss is weed competition (Teixeira et al. 2000). The use of herbicides is the most common weed control method. There are currently at least 200 herbicides registered with different mechanisms of action around the world (WSSA 2010). Of these, the most commonly used herbicide is glyphosate, which was commercially introduced in the early 1970s (Woodburn 2000).

The primary mechanism of glyphosate herbicidal activity is the inhibition of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Steinrucken and Amrhein 1980), which is required for the biosynthesis of essential amino acids (Herrmann and Weaver 1999). This results in a decline of protein, vitamin cofactors, secondary metabolites, and photosynthesis, resulting in plant death (Duke et al. 2003; Duke and Powles 2008). Low cost, broad-spectrum weed control, and high efficacy makes glyphosate a useful tool for farmers (Preston and Wakelin 2008). However, its exclusive and extensive utilization in Brazil has resulted in the selection of resistant plants such as ryegrass (*Lolium* spp.), *Conyza* spp., and sourgrass [*Digitaria insularis* (L.) Mez ex Ekman] (Heap 2014).

Weed resistance was first observed for organic compounds (Switzer 1957) and can be defined as

DOI: 10.1614/WT-D-14-00039.1

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https://doi.org/10.1614/WT-D-14-00039.1 Published online by Cambridge University Press

the natural ability of a biotype to survive and reproduce after exposure to doses of herbicide that are considered lethal to normal individuals (susceptible) of the same species (WSSA 1998). Among the causes of resistance selection are the continued use of the same reagent (Gressel and Segel 1990) and the use of doses below the recommended levels (Powles et al. 2011).

No glyphosate-resistant weeds were observed for the first 20 yr after glyphosate was introduced in the market (Dyer 1994; Powles et al. 1998). Genetically modified (GM) crops, especially glyphosate-resistant cultivars, have been a technical, economic, and agronomic success for the companies that developed them and the farmers who use them. With the high adoption of glyphosate, new cases of glyphosate-resistant plants are routinely being discovered.

To date, resistance to glyphosate has resulted in the evolution of 29 resistant species; five of these are found in Brazil, corresponding to almost 20% of the total number reported (Heap 2014). In agricultural areas where soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), and orchards are raised in Brazil, farmers have recently reported that *Chloris polydactyla* control with glyphosate is less effective compared to previous years, which also coincides with increased infestations of the species in Paraná (Lorenzi 2006). This weed species can produce more than 96,000 seeds (Brighenti et al. 2007). In addition, 4 plants m^{-2} can reduce soybean growth by 50% (AAM et al. unpublished data).

C. polydactyla is a C_4 grass native to the Americas, and propagates through seeds and/or rhizomes (Carvalho et al. 2005; Kissmann and Groth, 2007). This weed species exhibits tolerance to several acetyl CoA carboxylase herbicides once in tillering stage (Brighenti et al. 2007).

Based on the widespread evolution of herbicide resistance in agricultural weeds (Jasieniuk et al. 2008), variation in susceptibility to glyphosate among *C. polydactyla* accessions collected in different states of Brazil was investigated.

Materials and Methods

Plant Material. From 2012 to 2013, *C. polydactyla* seeds were collected from three accessions from São Paulo (21.61S°S; 48.44°W, Matão), (21.26°S; 48.30°W, Jaboticabal), (22.74°″S; 50.19°W, Palmital), and one accession from Paraná (24.31°S; 53.83°″W, Palotina). In Matão, plants were previously reported surviving several applications of glyphosate in orchards (glyphosate-putative resistance). Seeds from Jaboticabal were collected from an urban area; this accession had not received any glyphosate applications at the last 4 yr (subjected only to mechanical control). Seeds from Palotina and Palmital were collected in areas with no history of limited *C. polydactyla* control and were thus regarded as susceptible accessions. All four collection sites were located within 10 km from each other. In all cases, seeds were pooled from 20 to 40 randomly selected plants.

Whole-Plant Dose-Response Assay. Seeds were germinated in polystyrene trays with commercial potting mix (Hortimix®; Agristar, Rio de Janeiro, Brazil), and following emergence, individual plants at the two-leaf stage were transplanted to 2-L plastic pots containing a sandy loam soil maintaining one plant per pot in a greenhouse under conditions of 35/25 C day/night temperature and 14-h photoperiod. Pots were watered daily to the field capacity.

The first experiment included only the Matão and Palotina accessions. The second experiment included Palmital, Jaboticabal, Matão, and Palotina accessions. Herbicide application was done at the four-leaf growth stage using an automated spray chamber equipped with a flat-fan nozzle (8002; Jacto, São Paulo, Brazil) and delivering a spray volume of 200 L ha^{-1} at 200 kPa.

Glyphosate (Roundup Original, 360 g ae L^{-1}; Monsanto, São José dos Campos, Brazil) was applied at 0, 90, 180, 360, 720, 1,440, and 2,880 g ae ha^{-1}. After treatment, plants were returned to the greenhouse. Twenty-eight d after treatment (DAT), visual estimates of control from 0 to 100%, with 0 being no control and 100 being plant death, were assessed. Additionally, aboveground fresh weight per plant was determined and expressed as the percentage of the nontreated control. The experiments were arranged in a completely randomized design and had four and three replications in the first and second experiment, respectively.

Statistical Analysis. For each biotype, the control estimates and fresh weight reduction at 28 d after treatment were fitted to a nonlinear, log-logistic regression model after the means of control proved to be significantly different between biotypes by ANOVA (Equation 1):

https://doi.org/10.1614/WT-D-14-00039.1 Published online by Cambridge University Press

Table 1. Parameters of the log-logistic equation[a] used to calculate the herbicide concentration (g per equivalent acid) required for 50% control (I_{50}) or 80% control (I_{80}) of susceptible (S1) and putative-resistant (PR1) biotypes by whole-plant assay in the first study.[b]

Accession	R^2	c	d	b	I_{50} (g ea^{-1} ha^{-1})	RF	I_{80} (g ea^{-1} ha^{-1})	RF
S1	0,96	0.0 ± 2.9	101.1 ± 1.7	−2.0 ± 0.31	81.5 ± 5.6	3.9	161.6 ± 15.5	2.8
PR1	0,99	1.2 ± 2.1	99.7 ± 1.9	−3.4 ± 0.58	318.5 ± 11.6		452.7 ± 25.2	

[a] Equation $Y = c + \{(d - c)[1 + (x/g)^b]\}$ where Y is the control or the aboveground fresh weight expressed as a percentage of the nontreated control; c and d are the coefficients corresponding to the lower and upper asymptotes; b is the slope of the line; g is the herbicide rate at the point of inflection halfway between the upper and lower asymptotes; and x is the herbicide rate used. Control data for second study did not meet the criteria for regressions. Data are expressed as the mean ± standard error of four replicates.

[b] Abbreviation: RF, resistant factor = I_{50}(PR1) / I_{50}(S1) or I_{80}(PR1) / I_{80}(S1).

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

where Y is the control or the aboveground fresh weight expressed as a percentage of the nontreated control; c and d are the coefficients corresponding to the lower and upper asymptotes; b is the slope of the line; g is the herbicide rate at the point of inflection halfway between the upper and lower asymptotes; and x is the herbicide rate used.

The herbicide rates needed to cause 50 and 80% control (I_{50}, I_{80}, respectively) and reduction in fresh weight (GR_{50}, GR_{80}, respectively) compared to nontreated control were determined using equation 1. The resistant factor (RF) was also computed as (Equation 2):

$$\begin{aligned} RF &= I_{50}(PR)/I_{50}(S) \text{ or } RF \\ &= GR_{50}(PR)/GR_{50}(S); [2] \end{aligned}$$

where PR represents accessions with putative resistance, and S represents accessions without previous glyphosate use and with the lowest I_{50} and GR_{50} in the present study. For fresh weight reduction in study 1, the lower limit was fixed at 0, with no prejudicial regression fitness (lack-of-fit test = 0.50). Regression analysis was computed using the R program software, using a drc package (Knezevic et al. 2007).

Results and Discussion

First Experiment. In the first study, all plants from the putative resistant accession (Matão), designated hereafter as PR1, survived (less than 80% control) glyphosate rates below 720 g ha^{-1}, which provided 100% control. Plant death was reached for the susceptible accession (Palotina), hereafter referred to as S1, with rates of 180 g ha^{-1} of glyphosate or higher.

Determination of I_{50} and GR_{50} indicated that the PR1 accession had a low level of glyphosate resistance. The I_{50} value of PR1 accession was 319 g ha^{-1}, which was 3.9-fold higher than the I_{50} value of the S1 accession 82 g ha^{-1} (Table 1). GR_{50} was 164 g ha^{-1} in PR1 and 64 g ha^{-1} in S1 plants for RF = 2.5 (Table 2).

In order to achieve I_{80} (control level required for herbicide registration in Brazil); 453 and 162 g ha^{-1} were required for PR1 and S1, respectively, representing a RF = 2.8 (Table 1). For GR_{80}, 388 and 215 g ha^{-1} were required for the R1 and S1 biotypes with RF = 1.8 (Table 2).

Second Experiment. GR_{50} for PR1 plants was 277 g ha^{-1} and 147 g ha^{-1} for S1. The RF calculated was 1.88, which was slightly smaller than in the first experiment. Differences in the amount of herbicide required to control the same accession in the two studies might have been due to an environmental factor as differences in day length or temperature during the studies occurred.

The two new accessions evaluated in this study (Jaboticabal and Palmital) showed different glyphosate susceptibilities. The Jaboticabal accessions (hereafter referred to as PR2), exhibited GR_{50} of 183 g ha^{-1}. Comparing this accession with S1, RF was 1.7. The Palmital accession (hereafter referred to as S2), only required 73 g ha^{-1} of glyphosate to achieve a 50% reduction in biomass. The RF value from PR1/S2 showed a value of 3.9 (Table 2).

To reduce 80% of the biomass of the R accessions, 366 g ha^{-1} were required for both PR1 and PR2 biotypes. To reduce 80% of the biomass of the S accessions, 389 and 114 g ha^{-1} were required for the S1 and S2 biotypes, respectively. RF ranged from 1 to 3.38 (Table 2). This close dose between

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Table 2. Parameters of the log-logistic equation[a] used to calculate the herbicide concentration (g per equivalent acid) required for 50% or 80% fresh weigh plants reduction (GR_{50} and GR_{80}) of susceptible (S1 and S2) and putative-resistant (PR1 and PR2) biotypes by whole-plant assay in first and second study.[b]

Experiment	Accessions	R^2	c	d	B	GR_{50} (g ea^{-1} ha^{-1})	RF	GR_{80} (g ea^{-1} ha^{-1})	RF
1	S1	0.84	0.0 ± 0.0	102.9 ± 3.3	−1.1 ± 0.2	63.9 ± 9.5	2.5	214.8 ± 48.1	1.8
1	PR1	0.97	0.0 ± 0.0	103.2 ± 2.8	−1.6 ± 0.1	163.8 ± 11.8		388.4 ± 53.3	
2	PR1	0.96	0.5 ± 2.6	86.4 ± 3.5	−4.0 ± 1.3	253.8 ± 24.5		366.6 ± 40.3	
2	PR2	0.97	0.0 ± 3.2	89.8 ± 4.4	−1.6 ± 0.3	146.2 ± 14.9	1.7	366.4 ± 83.0	1.0
2	S1	0.91	0.0 ± 3.7	86.8 ± 5.2	−1.1 ± 0.2	107.9 ± 15.1	2.3	389.3 ± 124.0	0.9
2	S2	0.86	0.0 ± 1.5	72.6 ± 4.2	−2.5 ± 1.5	63.8 ± 5.7	3.9	114.8 ± 43.5	3.1

[a] Equation $Y = c + \{(d - c)[1+(x/g)^b]\}$ where Y is the control or the aboveground fresh weight expressed as a percentage of the nontreated control; c and d are the coefficients corresponding to the lower and upper asymptotes; b is the slope of the line; g is the herbicide rate at the point of inflection halfway between the upper and lower asymptotes; and x is the herbicide rate used. Data are expressed as the mean ± standard error of four replicates.

[b] Abbreviation: RF, resistant factor = GR_{50}(PR1) / GR_{50}(x) or GR_{80}(PR1) / GR_{80}(x).

Jaboticabal and Matão accessions could be explained because of the geographical proximity of the cities (45 km) or by possible gene flow.

These results suggest that the PR1 and PR2 accessions possessed similar levels of glyphosate susceptibility, and that biotype S2 was more susceptible to the herbicide than S1. In addition, the S1 biotype has an intermediate susceptibility to the herbicide compared to the PR1 and S2 accessions.

The observed values of RF are consistent with the reported glyphosate resistance levels of other weeds. Steckel et al. (2008) reported that the resistance levels of the Palmer amaranth values ranged from 1.5 to 5.0. The RF values ranging from 1.5 to 5.0 were also reported in horseweed [*Conyza canadensis* (L.) Cronq.] accessions (Main et al. 2004). For grass weeds, these values range from 2.0 to 5.5 (Carvalho et al. 2012; Perez-Jones et al. 2005; Ribeiro et al. 2008).

The Jaboticabal accession, which was not treated with glyphosate during the previous 4 yr, also showed an intermediated susceptibility to glyphosate. Variations in the different level of resistance among biotypes have also been observed in another study (Carvalho et al. 2011). These variations could indicate that more than one glyphosate-resistance mechanism occurs in the plant. The herbicide resistance observed in *C. polydactyla*, together with previous reports, has not been well-accepted by the corresponding government authorities. In Brazil, there is no glyphosate label recommendation for this species using glyphosate.

Heap (2006) stated that resistance is an event that reflects the existence of differential susceptibility; however, the usual recommended rate (for other species) controls the occurrence of specific biotypes. These differences between biotypes should persist in more advanced stages of growth of plants. *C. polydactyla* plants from Matão (PR1), required 2,880 g ha^{-1} of glyphosate to provide 100% control at the tillerings stage (AAM Barroso, et al. unpublished data).

Once resistant plants are selected, their rapid dispersion will likely occur, as documented with other species such as hairy fleabane [*Conyza bonariensis* (L.) Cronq.] and sourgrass. In addition, the mixture of glyphosate with acetyl CoA carboxylase-inhibiting herbicides showed no high efficacy (AAM Barroso, et al. unpublished data) on tillerings plants. If control with glyphosate is lost, the infested areas will experience problems with weed interference.

The rotation of POST herbicides with different mechanisms of action and the use of PRE applications (Brighenti et al. 2007) of acetochlor or trifluralin are effective strategies to manage *C. polydactyla* (Brighenti et al. 2007). The possible existence of glyphosate resistance mechanisms in *C. polydactyla* should be further investigated.

Acknowledgments

The authors would like to thank the FAPESP (São Paulo State Research Foundation) and CNPq (National Counsel ofTechnological and Scientific Development) for the researcher's scholarships and PhD Maria do Carmo Pavani, Pedro Luis da Costa Aguiar Alves, and Antonio Sergio Ferraudo from UNESP (São Paulo State University) - Jaboticabal

https://doi.org/10.1614/WT-D-14-00039.1 Published online by Cambridge University Press

for biotype identifications, suggestions and statistical support.

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Received April 15, 2014, and approved July 30, 2014.

https://doi.org/10.1614/WT-D-14-00039.1 Published online by Cambridge University Press