Australian Journal of Crop Science AJCS

AJCS 10(10):1466-1473 (2016) ISSN:1835-2707
DOI: 10.21475/ajcs.2016.10.10.p7761

Plant growth and genetic polymorphism in glyphosate-resistant sourgrass (*Digitaria insularis* L. Fedde)

Juliano Francisco Martins, Arthur Arrobas Martins Barroso*, Leonardo Bianco de Carvalho, Anne Elise Cesarin, Cárita Liberato do Amaral, Mariluce Pascoina Nepomuceno, Janete Aparecida Desidério, Pedro Luis da Costa Aguiar Alves

São Paulo State University-UNESP, Department of Applied Biology, 14884-200, Jaboticabal, São Paulo, Brazil

***Corresponding author: arthuragro07@hotmail.com**

Abstract

Weed herbicide resistance has been a challenge in agriculture. The objective of this study was to detect sourgrass (*Digitaria insularis* (L.) Fedde) glyphosate-resistant plants and to evaluate the growth and polymorphism rate between the resistant and susceptible biotypes, as a subsidy for the integrated management of species. An experiment was conducted primarily for the detection of weed resistance using increasing doses of glyphosate to generate a dose-response curve. Then, an experiment consisting of eight treatments (destructives analysis of growth) and five replications was conducted to characterize and compare the phenological stages of each biotype. In addition to visual assessments, the dry weight and leaf area, absolute and relative growth rates, net assimilation rate and leaf area ratio of plants were determined. Finally, polymorphism among biotypes was determined using inter simple sequence repeat molecular markers. A resistant factor of 3.12 was found among the biotypes. The resistant biotype grew faster and was more robust than the susceptible biotype, arriving first to flowering. The resistant biotype was better adapted to light interception. The 25 inter simple sequence repeat molecular markers analysis showed a polymorphism rate of 56.6% among the analyzed biotypes.

Keywords: *Digitaria insularis*, genetic variance, herbicide resistance, phenology, roundup.
Abbreviations: AGR_absolute growth rate; ALS_acetolactate synthase; AMPA_ aminomethylphosphonic acid; DAS_days after sowing; FS II_photosystem II; GR_{50}_growth reduction by 50%; ISSR_inter simple sequence repeat; LAR_leaf area ratio; NAR_net assimilation rate; RDM_root dry matter accumulation; RF_resistance factor; RGR_relative growth rate; SDM_shoot dry matter accumulation; TDM_total dry matter accumulation.

Introduction

Weed herbicide resistance has been a challenge in agriculture. Glyphosate-resistant weed populations are occurred in all agricultural areas, becoming a major problem all over the world. In fact, there were six glyphosate-resistant species in Brazil out of the 32 listed worldwide (Heap, 2015). In Brazil, the sourgrass (*Digitaria insularis* (L.) Feed) occurs in the majority of crops (Mondo et al., 2010). This species is an annual herbaceous plant with growth bunches and rhizomes, making herbicide control difficult. Main mechanisms of glyphosate-resistance are reported for sourgrass, as being a slower herbicide absorption in the resistant biotype and an indication of herbicide metabolization (glyphosate to aminomethylphosphonic acid [AMPA], glycosylate and sarcosine). The differential translocation between biotypes was also observed and seems to be the major cause of glyphosate insensibility. The resistant plants translocated less herbicide than susceptible plants after the first hours of application (Carvalho et al., 2012).

A consistent Integrated Weed Management System requires information of the basic biology of weeds that is essential for the development of viable control from economic and environmental viewpoints (Carvalho, 2005). Gomes and Christoffoleti (2008) reported the biology of plants as a fundamental tool for this management development.

The evolution of weeds occurs in spite of the introduction of genes from wild biotypes. After sexual reproduction, the beneficial characteristics are propagated through descendants, who have herbicide resistance. With the development of molecular biology, it is possible to estimate the genetic variability within a species using molecular markers (Faleiro, 2007). Assessment of dispersity and genetic variation of *Solanum nigrum* plants that are resistant and sensitive to herbicide inhibitors of FS II was done in 25 sites in France, Poland and the United Kingdom (Stankiewicz et al., 2001). A high genetic similarity among the various populations was found. The results of that research suggested that the dispersion of *Solanum nigrum* seeds occurred through migratory birds among the sampled countries. However, among three populations the resistance occurred independently due to the low similarity (Stankiewicz et al., 2001). In addition, studies evaluating the genetic similarity between biotypes of *Euphorbia heterophylla* showed that resistance to ALS inhibitors occurred independently in the evaluated sites (Winkler et al., 2002). Thus, studies on the genetic variability of species are relevant, and allow biology to help develop effective management strategies for weeds that are resistant to herbicides. This study aimed to detect the polymorphism rate and phenological differences between two biotypes of *Digitaria insularis*: one susceptible and one resistant to glyphosate.

Results and Discussion

Detection of resistance and biotypes development

The reduction of dry matter for the two biotypes was similar when increasing glyphosate doses were applied, reaching approximately a 90% dry weight reduction (compared to the development of non-applied plants) at the end of evaluated period. Thus, the dose required to reduce the resistant sourgrass dry weight (GR_{50}) by 50% was 301.7 g e.a. ha^{-1}, while for the susceptible biotype, this dose was 96.7 g e.a. ha^{-1} (Table 2). Calculating the RF, a value of 3.12 was found (Fig 1). The analysis shows a significant difference controlling the two biotypes ($p \leq 0.01$), confirming the greater glyphosate resistance in biotype R, in agreement with other studies (Correia et al., 2010; Carvalho et al., 2011).

Considering the evolution of the phenological stages (Table 3) between the two biotypes, after the first true leaf emission, the R biotype developed more slowly than S biotype (from five to 34 days after sowing - DAS). In the beginning of tillering until flowering, the R plants began to develop more quickly. The time to reach flowering stage was 74 DAS for the R biotype and 78 DAS for the S biotype, corroborating other studies, which observed this period 63 and 70 DAS (Machado et al., 2006). The slow initial development of resistant plants could be explained by the fact that these individuals take a longer time to develop morphological and physiological structures, preparing for a perpetuation strategy with vigorous vegetative growth, ensuring an early fruiting, being more aggressive.

Considering the plant development, the shoot and root dry matter accumulation (SDM and RDM) were higher in resistant plants from 30 to 70 DAS. Consequently, the total dry matter accumulation (TDM) was higher for the R biotype during this period (Fig 2). The susceptible biotype showed a TDM of 5.7 g at 30 DAS and practically doubled its mass at 21 days, with a TDM of 11.5 g at 51 DAS. The resistant biotype, which had a TDM of 4.7 g at 30 DAS, practically doubled its mass in seven days, totaling 8.9 g at 37 DAS. This result indicates that the plants had a slow initial growth (until 30 DAS) when start to tiller and then rapidly grew until flowering, displaying a resistant biotype with major growth compared to the susceptible biotype during this period. At 79 DAS, the R biotype had a TDM of 23.9 g and the S biotype 27.5 g, representing a slowing in the development of the resistant biotype after flowering (Fig 2c). The higher herbicide-resistant growth of biotypes was also observed for *Ambrosia trifida*, which accumulates more root dry matter than susceptible biotype (Alcorta et al., 2011).

The dry matter accumulation in sourgrass plants was also described by Machado et al. (2006), where one plant accumulated 30.66 g at 98 days after the emergency. These values are higher than those have been observed for other species, such as *Urochloa plantaginea* (syn. *Brachiaria plantaginea*), which accumulated 23.87 g per plant only at 143 DAS, and lower than others, such as *Urochloa brizantha* (syn. *Brachiaria brizantha*), which accumulated 53.6 g (Silva et al., 2005, Carvalho et al., 2007). The slowing in resistant biotype development is related to foliar area stabilization. The foliar area of R plants stopped growth at approximately 65 DAS, close to flowering initiation (Fig. 2d). The rapid growth of resistant plants can play an important role in glyphosate non-efficacy. A plant in rapid development displays a major development of secondary meristematic tissues, which could result in a lower translocation of the herbicide, as observed for *D. insularis* (Timossi et al., 2006). Another important point is that the R plants will reach their stage of application faster, whereas S plants will be more sensible (less developed), contributing to a faster resistance spread in the area due to accelerated individual selection (Rodrigues and Almeida, 2011). One possible reason for this faster development should be attributed to a more efficient *EPSPs* activity in the R biotypes, which contain a double mutation in their sequences or by the wyld-type EPSPs enzyme that can provide advantage in both the presence and absence of herbicide, as observed by other studies (Cross, et al., 2015).

The net assimilation rate (NAR) of the two biotypes exhibited a tendency to decrease over time (Fig 3a; Table 5 and 5.1), indicating the reduction of the photosynthetic capacity of plants. This tendency was also observed in other studies (Machado et al., 2006). Comparing the NAR of R and S plants, higher values for resistant plants were observed until 65 DAS, whereas the mean NAR was 0.01085 g cm^{-2} day^{-1} for S and 0.01706 g cm^{-2} day^{-1} for R plants. This allocation could be explained by the need for more photosynthetic structures in R plants. The higher NAR that was observed in resistant plants indicates that the R biotype is more adapted to higher irradiance levels during the initial stages of growth because of its more efficient photosynthetic machinery (Shipley 2002, Shipley 2006, Seker et al., 2015).

Complementing the NAR, the leaf area ratio (LAR) decreased for both biotypes during the analyzed period (Fig. 3b). The results show that for the two biotypes, a reduction occurred in the useful photosynthetic area. These reductions were 38.9 cm^2 g^{-1} for S and 35.1 cm^2 g^{-1} for R. In addition, when comparing the biotypes, lower values of LAR were observed for R plants up to 65 DAS. Interestingly, from 72 DAS, an inversion of this pattern was observed (with the LAR being higher for R). This trend is a result of the possible allocation of photoassimilates to reproductive structures in R plants at the beginning of plant development. The higher values of LAR in R plants at the end of the analyzed period indicated an adaptation for light interception compared to the S. The higher specific leaf area maximizes the light interception in R biotypes in the late stages of plant growth, when weeds should be competing with crops for light in the canopy closing (Bell 2005, Seker et al., 2015). Similar to the dry matter accumulation by plants, the NAR and LAR could be related to the competitive ability of weeds. For LAR, the higher is the value, the smaller the competitiveness of the species, with an opposite trend for NAR. It is important to note that under different development conditions or under natural ones, other attributes of plants and their interactions with ecological factors (biotic or abiotic) could influence the competitiveness of species (Roush and Radosevich, 1985). Thus, the major competitiveness that is displayed by the resistant biotype can be reported. However, new studies are needed for confirmation. Obeying the theory of species evolution, the R biotype (a mutated plant that is mainly found in low frequencies in the field in absence of herbicide), should display a fitness penalty in its development. However, it was not observed in this study. The fitness penalty can be present in other plant processes that were not analyzed, such as plant defense to biotic or abiotic stresses or in reproduction factors, as seed production or fecundity, as observed in *Ambrosia trifida* (Brabham, 2011).

Attesting the comparison of the development of the R and S plants, the absolute and relative growth rates (Fig 3c and Fig 3d) provide estimates of the growth rate in plants during the

Table 1. Nucleotides sequences (primers) and temperatures that were used for amplification.

Primers (UBC)	Sequence	TºC	Primers (UBC)	Sequence	TºC
807	AGA GAG AGA GT	55	842	AGA GAG AGA AYG	55
808	AGA GAG AGA GC	54	843	CTC TCT CTC TRA	55
809	AGA GAG AGA GG	54	855	ACA CAC ACA CYT	55
811	GAG AGA GAG AC	54	856	ACA CAC ACA CYA	54
816	CAC ACA CAC AT	55	857	ACA CAC ACA CYG	55
818	CAC ACA CAC AG	54	861	ACC ACC ACC ACC	55
823	TCT CTC TCT CC	55	864	ATG ATG ATG ATG	54
825	ACA CAC ACA CT	55	884	HBH AGA GAG AG	55
826	ACA CAC ACA CC	55	885	BHB GAG AGA GA	55
827	ACA CAC ACA CG	55	886	VDV CTC TCT CT	55
836	AGA GAG AGA GYA	55	887	DVD TCT CTC TC	55
840	GAG AGA GAG AYT	54	888	BDB CAC ACA CA	55
841	AGA GAG AGA AYC	55			

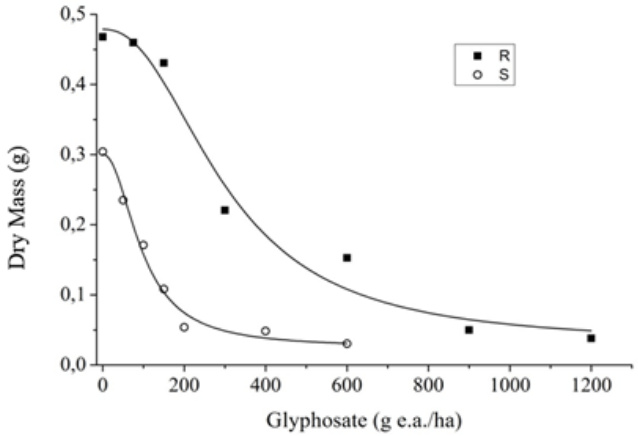

Fig 1. Response (dry mass values) of susceptible (S) and resistant (R) biotypes of sourgrass plants 21 days after crescent glyphosate herbicide application.

Table 2. Logistics equations parameters that were used to calculate the glyphosate dose that was required for 50% dry matter reduction in sourgrass plants.

Biotypes[a]	a	b/GR_{50}[b]	c	R^{2c}	RF[d]
R	0.03028	301.7	2.27103	0.959**	1.00
S	0.02489	96.7	2.08467	0.975**	3.12

[a]S and R indicate susceptible and resistant biotype curves values, respectively.
[b]GR_{50} is the concentration or rate of glyphosate that inhibits 50% of the dry weight accumulation.
[c]Curve determination coefficient, and** indicates the significance of the regression adjustment model by a 1% probability F test.
[d]RF indicates the resistance factor as expressed by the $GR_{50}(R)/GR_{50}(S)$ ratio.

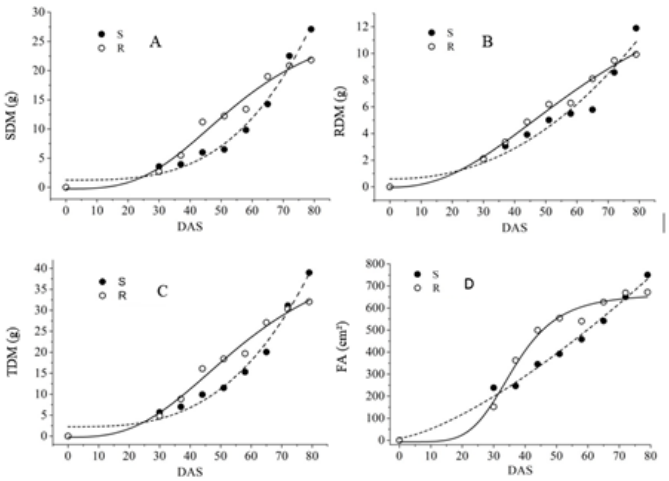

Fig 2. Mean values of growth parameters of resistant (R) and susceptible (S) sourgrass biotypes during time. a- Shoot Dry Matter (SDM), b-Root Dry Matter (RDM), c-Total Dry Matter (TDM) and d-Plant Foliar Area (FA).

Table 3. Comparison and characterization of the phenological stages of growth of resistant (R) and susceptible (S) sourgrass biotypes.

DAS R[2]	DAS S[3]	Phenological stages of growth
0	0	sowing
5	6	1st true leaf
10	9	2 true leaves
15	14	3 true leaves
22	21	4 true leaves
31	29	5 true leaves
34	34	5 true leaves and tillering
38	39	5 true leaves (PS[1]) and 2 tillers
44	48	5 true leaves (PS[1]) and 3 tillers
58	60	6 true leaves (PS[1]) and 2 tillers
65	66	7 true leaves (PS[1]) and 3 tillers
70	74	8 true leaves (PS[1]), 3 tillers and pre-flowering
74	78	8 true leaves (PS[1]), 3 tillers and flowering
79	>80	8 true leaves (PS[1]), 3 tillers and full flowering

[1]PS = principal stem, [2]DAS R = Days after sowing resistant biotype.[3]DAS S = Days after sowing susceptible biotype.

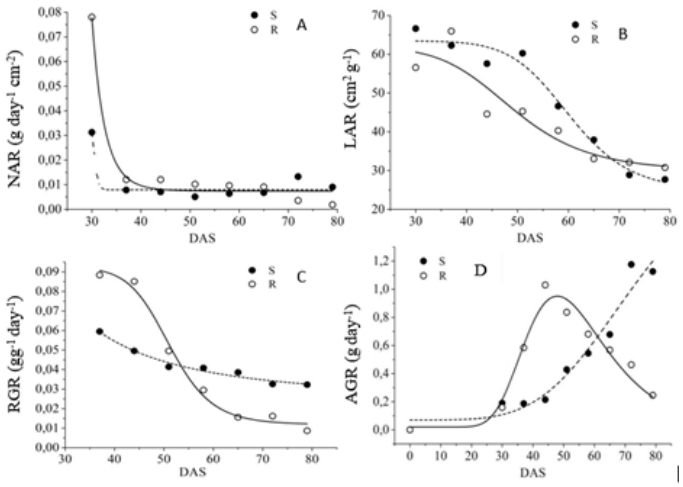

Fig 3. There should be a geral legend. Then a, b c etch be explained separately. a-Net Assimilation Rate (NAR), b-Leaf Area Ratio (LAR), c-Relative Growth Rate (RGR) e d-Absolute Growth Rate (AGR) per plant during the cycle of resistant (R) and susceptible (S) glyphosate sourgrass plants.

Table 4. Parameters of the log-logistic model for Shoot Dry Matter (SDM), Root Dry Matter (RDM), Total Dry Matter (TDM) and Foliar Area (FA) for resistant (R) and susceptible (S) glyphosate sourgrass biotypes.

Parameters	SDM		RDM		TDM		FA	
	R[a]	S[a]	R	S	R	S	R	S
a	29.70	22583.1	17.58	83356.7	46.89	142575	664.1	825915
b	56.27	605.706	69.52	4695.15	59.57	1187.39	36.42	671170
c	-0.22	1.265	-0.03	0.59583	-0.24	2.2377	-6.97	10.22
R[2b]	0.967**	0.975**	0.984**	0.924**	0.976**	0.975**	0.975**	0.987**

[a]S shows glyphosate susceptible biotype and R, the resistant. [b]Determination coefficient of the regression, and ** indicates the significance adjusting regression models at a 1% probability F test.

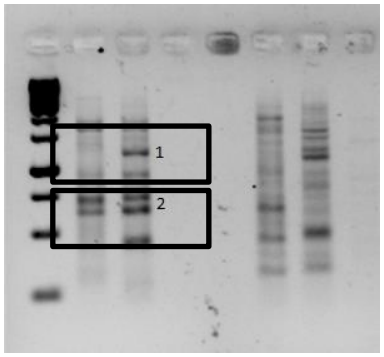

Fig 4. Polymorphic (1) and monomorphic bands (2) electrophoretic profiles in glyphosate-resistant (R) and -susceptible (S) sourgrass.

Table 5. Parameters of the log-logistic model for Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Relative Growth Rate (RGR) and Absolute Growth Rate (AGR) for resistant (R) and susceptible (S) glyphosate sourgrass biotypes.

Parameters	NAR		LAR		RGR		AGR
	R[a]	S[a]	R	S	R	S	S
a	26.5628	0.03887	61.7164	63.4483	0.09222	3.9792	0.07028
b	18.316	30.4453	49.2291	60.3453	51.2230	2.88955	74.4384
c	0.00742	0.00794	29.7491	24.5102	0.01183	0.02372	2.071
R^{2b}	0.969**	0.897**	0.870**	0.956**	0.981**	0.949**	0.927**

[a]S shows glyphosate susceptible biotype and R, the resistant.
[b]Determination coefficient of the regression, and ** indicates the significance adjusting regression models at a 1% probability F test.

Table 5.1. Model parameters log-normal carried out for the Absolute Growth Rate (AGR) for the resistant sourgrass biotype.

	AGR
Parameters	R[a]
x	47.9
R^{2b}	0.918**

[R]Indicates sourgrass glyphosate-resistant biotype. [b]Determination coefficient of the regression, and ** indicates the significance adjusting regression models at a 1% probability F test

Table 6. Genetic variation detected between two biotypes (resistant and susceptible to glyphosate) of sourgrass by 25 ISSR molecular markers.

Primers (UBC)	Band number	Polymorphic band number	Polymorphism (%)
807	13	9	69.2
808	14	11	78.6
809	7	4	57.1
811	7	5	71.4
816	8	4	50.0
818	4	2	50.0
823	4	3	75.0
825	9	5	55.6
826	7	2	28.5
827	6	2	33.3
836	15	10	66.7
840	8	2	25.0
841	11	9	81.8
842	7	3	42.9
843	8	2	25.0
855	10	5	50.0
856	8	5	62.5
857	9	7	77.8
861	7	6	85.3
864	12	6	50.0
884	5	2	40.0
885	6	2	33.3
886	5	2	40.0
887	6	3	50.0
888	7	4	57.1
Total	203	115	56.6

cycle of the experiment. Analyzing the relative growth rate (RGR) for the biotypes, a decrease in the matter accumulation according to the advanced period was observed (Fig 3c). Once the plants accumulate more mass, the need for photoassimilates for structural maintenance increases; therefore, the photoassimilates that are available for growth tend to be lower. Consequently, the RGR assumes this model. Comparing the RGR between R and S plants, higher values for R plants were noted up to 51 DAS, confirming the aggressive growth characteristic. This result may be an evolutionary trait that was developed for this biotype to dominate the environment and perpetuate the species. Furthermore, weeds with rapid early growth require that management of this species should be undertaken in young plants, as later control is not the best option for sourgrass (Campos et al., 2012). Looking at the absolute growth rate (AGR), there is a rapid growth of the resistant biotype from 30 DAS, peaking at 47 DAS in association with the formation of tillers and leaves, followed by a decrease in growth (Fig 3d). For the susceptible biotype, the accumulation of dry matter was slower, peaking at 74 DAS, when it began its reproductive stage of growth. Analyzing all of the data together, the parameter "b" from the curves shows that the resistant biotype has a faster life cycle compared to that of the susceptible biotype. In addition, to 50% of dry matter accumulation (shoots and roots), foliar area and maximum period of absolute growth was reached in R biotype (Tables 4, 5 and 5.1). The lack of a fitness penalty in the absence of glyphosate would complicate the long-term management of glyphosate-resistant sourgrass because periods of alternate methods of management would not be expected to reduce the resistant trait frequency (Preston et al., 2009). Thus a long-

term integrated weed management plan that does not involve glyphosate should be adopted, using pre-emergency herbicides, such as Trifluralin and S-metolachlor, or post-emergency herbicides, such as ACCase inhibitors, controlling all the biotypes (Norsworthy et al., 2012).

Genetic polymorphism analysis

The analysis of the genetic distance between biotypes of sourgrass showed high levels of genetic dissimilarity. The ISSR technique identified 203 bands in 25 oligonucleotides, reaching a mean of 8.12 bands per primer. Still, 115 bands of the total showed to be polymorphic (56.65%) of the total (Table 6).

Of these, the UBC 841 and 861 nucleotides showed higher percentage of polymorphic bands, above 80%, against the UBC 826 and 840 nucleotides that showed the lowest percentages (less than 30%). The electrophoretic profile of the UBC 887 and UBC 857 primers showed one polymorphic and one monomorphic band when comparing the biotypes (Fig 4).

The general rate of polymorphism was 56.6%, featuring a high genetic dissimilarity between biotypes, as explained by the fact that sourgrass is a species of outcrossing fecundity, offering varied genetic load in reproduction. Studying the ALS-resistant biotypes of *Bidens pilosa*, an average genetic dissimilarity from 27% to 37% was found, confirming the obtained data (Lamego et al., 2006, Vidal et al., 2006).

Furthermore, Souza et al. (2005) found a 32.7% polymorphism rate in an oat population and observed that some oligonucleotides were missing only in organic-compound-resistant populations. The relationship between herbicide resistance and polymorphic profile is not a rule in weeds, similar to *Digitaria nuda* resistant to atrazine (Vieira et al., 2010). Thus, it can be suggested that there is a higher genetic variability between the biotypes, but it cannot be inferred that this variability is linked to the susceptibility of the species to glyphosate. However, once the species has a high dissimilarity, it has been suggested that there are more genes for outcrossing between plants, which could generate a different response to herbicide stress. However, after demonstrating the resistance in the Matão biotype and observing its faster development, the relationship between variability in bands was observed. It should be inferred that a high variability in some primers could be related to a trade-off in the fitness of plants.

Materials and methods

Plant material and growth conditions

From 2011 to 2012, sourgrass seeds were collected from two accessions in Brazil in at least 40 plants: a natural field (17º49’23’’S and 50º35’18’’W) in Goiás State (GO) and a cultivated perennial field (21º36’12’’S and 48º21’57’’W) from São Paulo State (SP). The biotype from the natural field did not receive any glyphosate application (mechanical control), hereafter designated as the glyphosate-susceptible biotype (S), while that from the cultivated field (a citrus orchard) was exposed to glyphosate over the past 20 years, hereafter designated as the glyphosate-resistant biotype (R). This R biotype was used in previous research studies for glyphosate-resistance (Carvalho et al., 2012). In every case, the seeds were pooled from 20 to 40 randomly selected plants and were germinated in polystyrene trays with commercial potting mix. 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 32/25°C day/night temperature and a 14-h-photoperiod. The pots were watered daily to field capacity.

Herbicide resistance confirmation

Herbicide application was performed at the four-leaf growth stage using a backpack sprayer (Herbicat, Catanduva, Brazil) with constant compression (CO_2), equipped with a bar and two flat fan nozzles (XR 110,015; Jacto, São Paulo, Brazil), delivering a spray volume of 150 L ha^{-1} at 274 kPa. Glyphosate (Round up Original, 360 g ae L^{-1}; Monsanto, São José dos Campos, Brazil) was applied at 0, 50, 100, 150, 200, 400 and 600 g ae ha^{-1} for the S biotype and at 0, 75, 150, 300, 600, 900 and 1,200 g ae ha^{-1} for the R biotype.

Thirty days after treatment (DAT), the aboveground dry weight per plant was determined and expressed as a percentage of the non-treated control. The experiment was arranged in a completely randomized design, in a factorial scheme (2 biotypes × 7 doses) with four replications. In addition, for further studies, seeds were gathered from the remaining plants (of the R biotypes that were exposed to glyphosate) that grew and reproduced while isolated from other plants to guarantee a specific resistant biotype and not just a putative resistant population. For each biotype, the dry weight reduction was fitted to a nonlinear, log-logistic (Table 4) regression model after the means proved to be significantly different between biotypes by ANOVA (Equation 1):

$Y = a/[1 + (\frac{x}{b})^c]$; [Eq. 1]

Where, *Y* is the aboveground dry weight; *x* is the number of accumulated days; and *a*, *b* and *c* are estimated parameters of the equation (“*a*” is the amplitude between the maximum and minimum points of the variable, “*b*” corresponds to the number of days that are required for the occurrence of 50% of the response variable (EC_{50}), and “*c*” is the slope of the curve around *b*). The herbicide rates needed to cause a 50% reduction in weight, growth reduction (GR_{50}), compared to the non-treated control as determined using the first equation. The resistance factor (RF), was also computed by the division of GR_{50} of the resistant and susceptible biotype. The regression analysis was computed using the OriginPro® program software.

Plant growth analysis

In this study, the growth of the R and S biotypes was analyzed. We used 3-L plastic pots containing a sandy loam soil with the previous application of 300 kg ha^{-1} N-P-K in a 4:14:8 proportion. The pots were maintained under field conditions and were watered daily to the field capacity. Each pot contained two plants. The treatments consisted of different periods of harvest that were spaced seven days apart after 30 days of sowing until 79 days after sowing (DAS), totally eight treatments. The experiment was arranged in a completely randomized design, in a factorial scheme (2 biotypes × 8 periods) with five replications.

The plants were observed and characterized by their phonological stage, considering the stage when 50% + 1 individuals reached the characteristic (Bleiholder *et al*., 1991). The leaf area was also measured using a leaf area integrator I 3100 (LI-COR, Lincoln, Nebraska, USA). Then, the dry weight of different plant tissues was obtained by drying these tissues in an oven with forced ventilation at 70°C to a constant weight.

Based on the results of the leaf area and dry weight, the following were determined: Relative Growth Rate (RGW),

Absolute Growth Rate (AGR), Net Assimilation Rate (NAR) and Leaf Area Ratio (LAR) by Benincasa formulas (Benincasa, 2003). Quantitative variables that were related to plant growth were analyzed statistically by the F test for the variance analysis, followed by non-linear logistic regression using a logistic model (Streibig, 1998). The AGR curve of the R biotype was better adjusted in a non-linear lognormal regression: y = a+b*exp{-0,5*[LN(x/c)/d]*[LN(x/c)/d]}, where, *y* is the interest variable; *x* is the number of accumulated days; and *a*, *b*, *c* and *d* are the estimated parameters of the equation.

Genetic polymorphism analysis

Samples were collected (0.1 g of fresh leaves from young plants; four to five expanded leaves) from the two biotypes, ground to a fine powder in liquid nitrogen using a sterilized mortar and pestle for DNA Extraction. Them, 1 ml of extraction buffer (20 nM EDTA; 100 nM Tris-HCL (pH 8.0); 1.4 M NaCl and 2% (v/v) of CTAB and 0.2% of β-mercaptoethanol) was added to the mixture, which was stirred and transferred to a microcentrifuge tube of 2 ml. Then, 10 mg of PVP (p polyvinylpyrrolidone - Sigma) was added to the mixture, and the tube was gently inverted several times for optimal mixture and incubated at 60 °C for 25 minutes and cooled at room temperature. In the next step, 1 ml of chloroform/isoamyl alcohol (24:1) was added with stirring and then centrifuging at 10.621 × g for 15 minutes at room temperature.

The supernatant (20 mL) was transferred to a new tube, to which 0.5 volumes of 5 M NaCl and 1.0 volumes of 95% ethanol (-20°C) was added. The solution was stored at -80°C for 20 minutes for total DNA precipitation and then centrifuged at 4.460 × g for five minutes and at 10.621 × g for 5 minutes at 4°C for "pellet" precipitation. The supernatant was discarded, and the "pellet" was cleaned with 75% ethanol (4°C), reacting for approximately 1 minute and then centrifuged at 10.621 × g for 5 minutes at 4°C. After discarding the ethanol, the DNA was dried and resuspended in 50 µl of 10:1 TE buffer (Tris 10 nM, 1 mM, pH 8.0), treated with 10 µL of RNase (10 mg mL^{-1}) and incubated at 37°C for 30 minutes. The DNA samples were stored at -20°C.

A DNA extraction quality analysis was performed by agarose gel electrophoresis (0.8%) using a TBE 1× buffer (Tris 89 mM, H_3BO_3 89 mM, 2.5 mM EDTA, pH 8.2) and ethidium bromide (0.5 g/ml). The genomic DNA fragments were visualized under UV lights and documented in a Gel Doc 2000 (Bio-Rad) photodocumentation system. For band size comparison, a standard DNA of known molecular size was used (1 kb DNA Ladder).

Plasmid DNA was quantified in a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, USA) measuring the absorbance at 260 and 280 nm wavelengths, calculating the 260/280 nm ratio according to Sambrook and Russel (2001), determining the DNA quality and calculating the DNA concentration. To estimate the concentration of total DNA, a standard of 50 µg/ml DNA was used at a 260-nm wavelength. The final concentration that was standardized for analysis was 10 µg g L^{-1}. The genetic distance between biotypes was determined using an ISSR molecular marker (Inter Simple Sequence Repeats). For this purpose, 25 primers were selected (Table 1). The sequences were aligned with the CP ATLAS® program and submitted to genetic distance analysis (PAUP 4.0b10®).

Conclusion

Glyphosate-resistant (R) and susceptible (S) sourgrass biotypes showed different growth patterns. The resistant biotype grew faster than the susceptible and is more adapted to light interception. The different growth patterns between both biotypes are probably due to the high degree of genetic polymorphism of the species.

Acknowledgments

The authors thank the FAPESP and CNPq for the scholarship granted to the authors.

References

Alcorta M, Fidelibus MW, Steenwerth KL, Shrestha A (2011) Competitive effects of glyphosate-resistant and glyphosate-susceptible horseweed (*Conyza canadensis*) on young grapevines (*Vitis vinifera*). Weed Sci. 59:489–494.

Bell LW (2005) Relative growth rate, resource allocation and root morphology in the perennial legumes, *Medicago sativa*, *Dorycnium rectum* and *D. Hirsutum* growth under controlled conditions. Plant Soil. 270:195-211.

Benicasa MMP (2003) Análise de crescimento de plantas (noções básicas). Funep, São Paulo, Brazil.

Bleiholder H, Kirfel H, Langeluddecke P, Stauss R (1991) Codificação unificada dos estádios fenológicos de culturas e ervas daninhas. Pesq Agropec Bras. 26:1423-1429.

Brabham CB, Gerber CK, Johnson WG (2011) Fate of glyphosate-resistant giant ragweed (*Ambrosia trifida*) in the presence and absence of glyphosate. Weed Sci. 59:506–511.

Campos LHF, Mello MSC, Carvalho SJP, Nicolai ME, Christoffoleti PJ (2012) Initial growth of *Merremia cissoides*, *Neonotonia wightii* e *Stizolobium aterrimum*. Planta Daninha. 30:497-504.

Carvalho LB, Alves, PL, González-Torralva F, Cruz-Hipolito HE, Rojano-Delgado AM, De Prado R, Gil-Humanes J, Barro F, de Casto MD (2012) Pool of resistance mechanisms to glyphosate *Digitaria insularis*. J Agr Food Chem. 60:615−622.

Carvalho LB, Cruz-Hipólito H, González-Torralva F, Alves PLCA, Christoffoleti PJ, De Prado R (2011) Detection of sourgrass (*Digitaria insularis*) biotypes resistant to glyphosate in Brazil. Weed Sci. 59:171-176.

Carvalho LB, Bianco S, Pitelli RA, Bianco MS (2007) Dry matter and macronutrient accumulation by maize and *Brachiaria plantaginea*. Planta Daninha. 25:293-301.

Carvalho SJP, Pereira Silva RF, López-Ovejero RF, Nicolai M, Christoffoleti PJ (2005) Growth, development and seed production of *Chloris polydactyla*. Planta Daninha. 23:603-609.

Correia NM, Leite GJ, Garcia LD (2010) Response of different *Digitaria insularis* (L.) populations to glyphosate. Planta Daninha. 28:769-776.

Cross RB, Mccarty LB, Mcelroy JC, Tharayil N, Bridges WCJ (2015) Comparison of enzyme and growth characteristics in ALS-inhibitor susceptible and resistant annual bluegss (*Poa annua*) biotypes. Weed Sci. 63:220-228.

Faleiro FG (2007) Marcadores genético-moleculares aplicados a programa de conservação e uso de recursos genéticos. Planaltina, DF: Embrapa Cerrados.

Gomes FG, Christoffoleti PJ (2008) Biologia e manejo de plantas daninhas em área de plantio direto. Planta Daninha. 26:789-798.

Heap I (2015) The International Survey of Herbicide Resistant Weeds. Available from URL: http://weedscience.com. Accessed 23 May 2015.

Lamego FP, Resende LV, Silva PR, Vidal RA, Nunes AL (2006) Distância genética e geográfica entre acessos de picão-preto suscetíveis e resistentes a herbicidas inibidores da acetolactato sintase. Pesq Agropec Bras. 41: 963-968.

Machado AFL, Ferreira LR, Ferreira FA, Fialho CMT, Tuffi Santos LD, Machado MS (2006) Growth analysis of *Digitaria insularis*. Planta Daninha. 24:641-647.

Mondo VHV, Carvalho SJP, Dias ACRD, Filho JM (2010) Light and temperature effects on the seed germination of four *Digitaria* species. Rev Bras Sementes. 32:131-137.

Norsworthy JK, Sarah MW, David RS, Rick SL, Robert LN, Theodore MW, Kevin WB, George F, Stephen BP, Nilda RB, William WW, Michael B (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci. 60:31–62.

Preston C, Wakelin AM, Dolman FC, Bostamam Y, Boutsalis P (2009) A decade of glyphosate-resistant *Lolium* around the world: mechanisms, genes, fitness, and agronomic management. Weed Sci. 57: 435–441

Sambrook J, Russel DW (2001) Molecular cloning. A laboratory manual. 3rd ed. Cold spring harbor laboratory press, New York.

Seker H, Yolui H, Acikgaz R (2015) Primary growth parameters of three alfafa cultivars adapted to highland climatic conditions. J Agron Crop Sci. 21:219-227.

Shipley B (2006) Net assimilation rate, specific leaf area and leaf mass ratio: Which is most closely correlated with relative growth rate? A meta-analysis. Funct Ecol. 20:565-574.

Shipley B (2002) Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. Funct Ecol. 16:682-689.

Silva AC, Ferreira LR, Silva AA, Ferreira FA (2005) Growth analysis of *Brachiaria brizantha* under reduced rates of fluazifop-p-butyl. Planta Daninha. 23:85-91.

Timossi PC, Leite GJ, Durigan JC (2006) Eficácia de glyphosate em plantas de cobertura. Planta Daninha. 24:475-480.

Rodrigues BN, Almeida FS (2011) Guia de Herbicidas. 6.ed. Londrina, Brazil.

Roush ML, Radosevich SR (1985) Relationships between growth and competitiveness of four annual weeds. J Appl Ecol. 22:895-905.

Souza VQ, Pereira AS, Kopp MM, Coimbra JLM, Carvalho FIF, Luz VKL, Oliveira AC (2005). Genetic dissimilarity in oat (*Avena sativa* L.) tolerant and sensitive mutants to organic acids. Bragantia. 64:569-575.

Stankiewicz M, Gadamski G, Gawronski (2001) Genetic variation and phylogenetic relationships of triazineresistant and triazine-susceptible biotypes of *Solanum nigrum* analysis using RAPD markers. Weed Res. 41:287-293.

Streibig JC (1998) Herbicide bioassay. Weed Res. 28:479-484.

Vidal RA, Hernandes GC, Winkler LM, Federizzi LC, Da Silva PR (2006) Relation between geographic distance and genetic variability within a population of *Bidens* spp. with resistance to ALS inhibitors. Planta Daninha. 24:149-155.

Vieira VC, Alves PLCA, Picchi SC, Lemos MVF. Sena JAD (2010) Molecular characterization of accessions of crabgrass (*Digitaria nuda*) and response to ametryn. Acta Sci Agron. 32:255-261.

Winkler LM, Vidal RA, Barbosa Neto JF (2002) Aspectos genéticos envolvidos na resistência de plantas daninhas aos herbicidas. Revista Plantio Direto. 70:21-24.