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Article

MONITORING OF RESISTANCE OF SOURGRASS TO GLYPHOSATE HERBICIDE IN URBAN AREAS OF THE STATE OF SÃO PAULO, BRAZIL

Monitoramento da Resistência de Capim-Amargoso ao Herbicida Glyphosate em Áreas Urbanas do Estado de São Paulo

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ABSTRACT - The objective of this work was to monitor the resistance of sourgrass (*Digitaria insularis*) to glyphosate in urban areas of the State of São Paulo to understand the spread of resistant biotypes. Three experiments were conducted under greenhouse conditions in a completely randomized design, with four replications. In the first experiment, seven sourgrass biotypes were used, and the control of plants was evaluated at 7, 14, 21, 28, and 35 days after application (DAA) of glyphosate. In the second experiment, the shikimic acid accumulation was quantified at 72 hours after the glyphosate application, and the same evaluations of weed control were performed. In the third experiment, rate-response curves were developed, with glyphosate at rates of 0, 90, 180, 360, 720, 1,440, 2,880, and 5,760 g a.e. ha^{-1} applied on three progenies obtained from self-fertilized seeds; the percentage of control and shoot dry weight of the plants were evaluated at 28 DAA to determine their resistance factor. The results confirmed the occurrence of biotypes of sourgrass resistant to glyphosate in urban areas of Ipaussu and Santa Cruz do Rio Pardo and transmission of this resistance to the progenies obtained from self-fertilized seeds. These results indicate the possibility of resistant sourgrass seeds to be transported and disseminated to other Brazilian regions, thus, contributing to increase cases of resistance of sourgrass to this herbicide. However, it should be confirmed by more detailed studies involving DNA and family trees to determine the genetic proximity between resistant biotypes from different regions, since independent selection may also occur.

Keywords: shikimic acid, *Digitaria insularis*, EPSPS, resistance.

RESUMO - O objetivo deste estudo foi monitorar a resistência de ***Digitaria insularis*** *ao glyphosate em áreas urbanas do Estado de São Paulo, visando a compreensão da disseminação dos biótipos resistentes. Para isso, foram realizados três experimentos em casa de vegetação no delineamento inteiramente casualizado com quatro repetições. No primeiro experimento utilizaram-se sete biótipos de capim-amargoso e foi avaliado o controle das plantas aos 7, 14, 21, 28 e 35 dias após a aplicação (DAA) de glyphosate. No segundo experimento repetiu-se a avaliação de controle e, 72 horas após a aplicação do herbicida, quantificou-se o acúmulo de ácido chiquímico. No terceiro experimento foram conduzidas curvas de dose-resposta com glyphosate nas doses de 0, 90, 180, 360, 720, 1.440, 2.880 e 5.760 g e.a.* ha^{-1} *em três progênies obtidas por sementes autofecundadas, em que se avaliou a porcentagem de controle e massa seca aos 28 DAA, para obtenção do fator de resistência. Confirmou-se que em áreas urbanas de Ipaussu e Santa Cruz do Rio Pardo existem biótipos de capim-amargoso resistentes ao glyphosate e que*

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essa resistência é transmitida para as progênies obtidas com sementes autofecundadas. Esses resultados inferem que é possível que sementes de capim-amargoso resistentes sejam transportadas e disseminadas para outras localidades do Brasil e, assim, contribuir com o aumento dos casos de resistência a esse herbicida. No entanto, a confirmação exige estudos mais detalhados, que envolvam DNA e árvores genealógicas, para que seja determinada a proximidade genética entre biótipos resistentes que estejam localizados em regiões distintas, já que a seleção independente também pode ocorrer.

Palavras chave: ácido chiquímico, *Digitaria insularis*, EPSPS, resistência.

INTRODUCTION

Sourgrass (*Digitaria insularis* (L.) Mez ex Ekman) is indigenous to tropical and subtropical regions of America (Lorenzi, 2008). In Brazil, it has often been found in no-till crops, perennial crops, roadsides, and urban wastelands. Reproduction and dispersion of this species occur by seeds and rhizomes. (Machado et al., 2008). Its seeds are hairy, which allows their spread over long distances, are produced in large quantities, and have high germination percentage (Mendonça et al., 2014). In addition, when the plants develop rhizomes, they form clumps that make them perennial, and their capacity to produce and disseminate seeds starts to occur all year round (Kissmann and Groth, 1997; Lorenzi, 2008).

In addition, sourgrass is highly competitive, with potential to reduce maize and soybean yields by more than 32% and 44%, respectively (Gazziero et al., 2012; Gemelli et al., 2013). The use glyphosate-tolerant genetically modified crops have made this herbicide the most widely used in crop production systems. Therefore, control of sourgrass using glyphosate herbicide has becoming less efficient. There are several reports of resistance of *D. insularis* to glyphosate in Brazil, as reported by Correia et al. (2010), Carvalho et al. (2011, 2012), Reinert et al. (2013), Barroso et al. (2015), López-Ovejero et al. (2017), and Takano et al. (2018).

Considering these characteristics of this plant and its great difficulty of control, sourgrass is currently one of the main weeds in Brazil. However, it is not well known how its dispersion has been occurring throughout the country. The current hypothesis is that resistance transmission occurs through the spread of propagules by people and machine traffic (López-Ovejero et al., 2017) and/or by independent transmission, which can result in a rapid spread of resistance (Takano et al. 2018).

Thus, there is a need for information on how glyphosate-resistant sourgrass transmission occurs in urban areas, especially in municipalities near agricultural production areas. This information may allow a better understanding of how the dispersal of resistant populations has been occurring throughout Brazil, and can be useful to adopt other control management methods for an effective resistance management program. In this context, the objective of this work was to monitor the resistance of *D. insularis* to glyphosate herbicide in urban areas of the state of São Paulo, Brazil, to understand the spread of resistant biotypes throughout the country.

MATERIAL AND METHODS

Three greenhouse experiments were conducted in a completely randomized design, with four replications. Experiments 1 and 2 consisted of seven treatments (biotypes), and experiment 3 consisted of eight treatments (glyphosate rates).

The herbicide was applied using a stationary sprayer that contained a metal structure supporting a 2 meters spray bar, which ran 6 meters with the aid of an electric motor and had a frequency modulator that controls the working speed. The bar contained four XR 11002 VS tips spaced 0.5 meter apart and positioned at 0.5 meter height in relation to the plants. The working pressure used was 196.13 kPa and the speed was 3.6 km h^{-1}, generating a solution flow of 200 L ha^{-1}. The commercial product used in all experiments was the Original Roundup® (360 g a.e. L^{-1}) at a rate of 4 L ha^{-1}.

Plant material collection

Seven sourgrass biotypes were collected in urban areas of the state of São Paulo between January and February 2016 (Table 1). Clumps were collected to preserve the plant materials, which were manually fragmented and their propagules were transplanted into 25 liters pots filled with a clayey soil to preserve their plant material. The soil used presented the following physical and chemical characteristics: 552, 199, and 245 g dm^{-3} of clay, silt, and sand, respectively; pH ($CaCl_2$) = 4.9; organic matter = 22.5 g dm^{-3}; P (resin) = 15.5 mg dm^{-3}; Al^{3+} = 1.8 $mmol_c$ dm^{-3}; H+Al = 47.5 $mmol_c$ dm^{-3}; K^+ = 1.4 $mmol_c$ dm^{-3}; Ca^{2+} = 22.5 $mmol_c$ dm^{-3}; Mg^{2+} = 10.5 $mmol_c$ dm^{-3}; sum of bases = 35 $mmol_c$ dm^{-3}; CEC = 82.5 $mmol_c$ dm^{-3}; S = 55 mg dm^{-3}; and base saturation = 40.5%.

Table 1 - Location of the collection of the *Digitaria insularis* biotypes used in the experiments

Biotype	Latitude	Longitude	Altitude	Municipality
IPA	23º 03' 24"	49º 37' 20"	568	Ipaussu, SP
BOT 1	22º 51' 28"	48º 26' 5"	804	Botucatu, SP
BOT 2	22º 51' 20"	48º 26' 20"	805	Botucatu, SP
BOT 3	22º 50' 39"	48º 25' 41"	730	Botucatu, SP
STA 1	22º 53' 24"	49º 36' 39"	526	Santa Cruz do Rio Pardo, SP
STA 2	22º 52' 42"	49º 36' 37"	555	Santa Cruz do Rio Pardo, SP
MAR	22º 15' 38"	49º 95' 12"	593	Marília, SP

Experiments 1 and 2 – Weed control and shikimic acid quantification tests

The clumps were transplanted for tests of weed control on February 11, 2016, for the first experiment; and on October 5, 2016, for the second experiment. The preserved plant materials were manually fragmented, keeping one propagule (a defragmented rhizome + root system + tillers above the first internode) per pot. The pots had capacity of 2 liters and were filled with a commercial substrate (Carolina II®), which was composed of sphagnum peat, expanded vermiculite, roasted rice husk, dolomitic limestone, agricultural gypsum, and NPK traces, and presented electrical conductivity of 0.7±0.3 mS cm^{-1}, pH of 5.5, density of 155 kg m^{-3}, and 55% water retention capacity.

Glyphosate was applied after transplantation, when the plants were between 40 and 60 cm tall, at the vegetative stage. The average temperatures and relative humidity at the time of application were, respectively, 32 ºC and 51% (Experiment 1), and 28 ºC and 59% (Experiment 2).

In the first experiment, visual control evaluations were performed at 7, 14, 21, 28, and 35 days after application (DAA), according to a percentage scale, in which 0% means no control and 100% means death of the plants, according to Sociedade Brasileira da Ciência das Plantas Daninhas (SBCPD, 1995).

In the second experiment, shikimic acid quantification in the plants and visual control evaluation were performed. All leaves of three random tillers were collected from each plot at 72 hours after herbicide application for shikimic acid quantification, which was performed using high-performance liquid chromatography and mass spectrometry. The remaining plants were kept alive for visual control evaluation, using the same methodology of the first experiment.

Quantification of shikimic acid

Shikimic acid was quantified using the methodology proposed by Gomes et al. (2015) with adaptations. The leaves were placed in paper bags for drying in a forced-air circulation oven at 40 ºC for five days. The samples were, then, macerated in a mortar containing liquid nitrogen, and an aliquot of 0.1 g of the material was placed in a 15 mL centrifuge tube. Then, 10 mL of acid water (pH 2.5) was added to the tube, using an automatic pipette (Gilson). The tubes were gently shaken and subjected to an ultrasound bath (Elma – Elmasonic P 180 H) at 37 Khz and temperature of 55 ºC for 30 minutes. After extraction, all samples were centrifuged at 2,755 g for 10 minutes

at 20 °C (Centrifuge Routine 38R). Subsequently, the supernatant was collected, filtered through a Millex-HV (Millipore) 0.45 µm filter with a 13 mm Durapore membrane, and placed in a 9 mm amber vial (Flow Supply) of 2 mL capacity for quantitation by high-efficiency liquid chromatography and mass spectrometry (HPLC-MS/MS).

The HPLC-MS/MS quantification was performed using a system consisted of a Shimadzu Proeminence UFLC model High Performance Liquid Chromatograph (HPLC), which has two LC-20AD pumps, a SIL-20AC autoinjector, a DGU-20A5 degasser, a CBM-20A controller system (makes operation automated), a CTO-20AC oven (controls column temperature), and a triple quadrupole hybrid TRIPLE QUAD 4500 (AB SCIEX) mass spectrometer, wherein Q1 and Q3 are used as mass filter, and Q2 is a collision cell where intact molecules and Q1 fragments are broken into smaller mass fragments.

The chromatographic conditions used to quantify the compounds in positive and negative ionization modes were: Gemini 5 µ C18 110 Å (150 mm × 4.6 mm) analytical column, with two mobile phases, namely: phase A (FA) = 5 mM ammonium acetate in water; and phase B (FB) = 5 mM ammonium acetate in methanol. The gradient used was 0-1 minute = 10% FB and 90% FA; 1-4 minutes = 95% FB and 5% AF; 4-8 minutes = 95% FB and 5% FA; 8-10 minutes = 10% FB and 90% FA; and 10-12 minutes = 10% FB and 90% FA. The flow rate was 0.600 mL min^{-1}.

Detection and separation of the compounds were performed on the run, with ionization in positive and negative modes, under a total time of 12 minutes, simultaneously. Retention time in the chromatographic column for glyphosate was 2.89 minutes, and 2.95 minutes for shikimic acid. The ion used for quantification was always the first fragment generated from each molecule, being: Glyphosate – molecular mass 169.08 and fragments – 163.1; 150.0; 78.9; and Shikimic acid – molecular mass 174.15 and fragments – 93.0; 111.0; 73.0.

The analytical curves for the compounds were developed in concentration ranges, as follows: Glyphosate – line equation y = 2.79e + 003x + 1.6e + 004; r^2 0.9931; and linear range 2.34-600; and Shikimic Acid – line equation y = 226x + 1.15e + 004; r 0.9907; and linear range 18.93-4800.

Experiment 3 – Rate-response curves with glyphosate herbicide

Self-fertilized seeds of three sourgrass progenies were obtained from the preserved material (IPA, BOT 3, and STA 2). The seeds were sowed on January 5, 2017 in 350 mL pots filled with a commercial substrate (Carolina II®). Thinning was performed after emergence, keeping four plants per pot.

The treatments were applied on February 6, 2017, when the plants were at vegetative stage and presented heights of 40 to 60 cm and four to six tillers. Glyphosate was applied at rates of 0, 90, 180, 360, 720, 1,440, 2,880, and 5,760 g a.e. ha^{-1}. The average relative humidity and temperature at the time of application were 70% and 27 °C, respectively.

The plants were subjected to visual control and shoot dry weight evaluations at 28 DAA. For shoot dry weight determination, the shoot was cut and dried in a forced-air circulation oven at 70 °C until constant weight; Subsequently, it was weighed in a precision balance of 0.0001 g^{-1}.

Statistical analysis of data

The results of visual control analysis and shikimic acid concentrations (µg g^{-1} shoot dry weight) for experiments 1 and 2 were subjected to analysis of variance by the F test and the means were compared by the Tukey's test ($p \leq 0.05$), using the AgroEstat 1.1.0.712 program.

The results of control percentage and shoot dry weight from experiment 3 were subjected to analysis of variance by the F test. When significant, rate-response curves were developed and the data were fitted to the log-logistic nonlinear regression model proposed by Seefeldt et al. (1995):

$$y = a + \frac{b}{\left[1 + \left(\frac{x}{c}\right)d\right]}$$

where: y = control percentage or shoot dry weight; x = herbicide rate; a = lower limit of the curve; b = difference between the maximum and minimum points of the curve; c = rate providing 50% response of the variable; and d = slope of the curve.

The herbicide rates that control 50% of the population (CE_{50}) or promote a 50% reduction in shoot dry weight (GR_{50}) were used to obtain the resistance factor (RF = R/S), using the R statistical program (Development Core Team, 2008). The graphs were plotted using the SigmaPlot 12.5 program.

RESULTS AND DISCUSSION

The glyphosate herbicide showed inefficient control of *D. insularis* biotypes in experiments 1 and 2 (Figure 1). The most sensitive plants (BOT 1, BOT 2, BOT 3, and MAR) to the herbicide had control percentages close to 100% at 14 days after application (DAA); for the less sensitive ones (IPA STA 1 and STA 2), the maximum control percentage did not exceed, on average, 40%. No regrowth was found in susceptible plants at 35 DAA, because the control remained at 100% (Figure 1). This shows that glyphosate is still an important control management tool for some populations of *D. insularis*, even for mature and perennial plants.

However, the number of cases of resistance to glyphosate is increasing, and many sourgrass populations are no longer effectively controlled with this herbicide. This has hindered the management of this species, which is currently widespread through practically all producing regions of the country, as reported by López-Ovejero et al. (2017). In addition, resistant sourgrass populations to some ACCase inhibitor herbicides, such as haloxyfop and fenoxyprop (Heap, 2018), have already been found. This restricts chemical control options and makes the control more difficult.

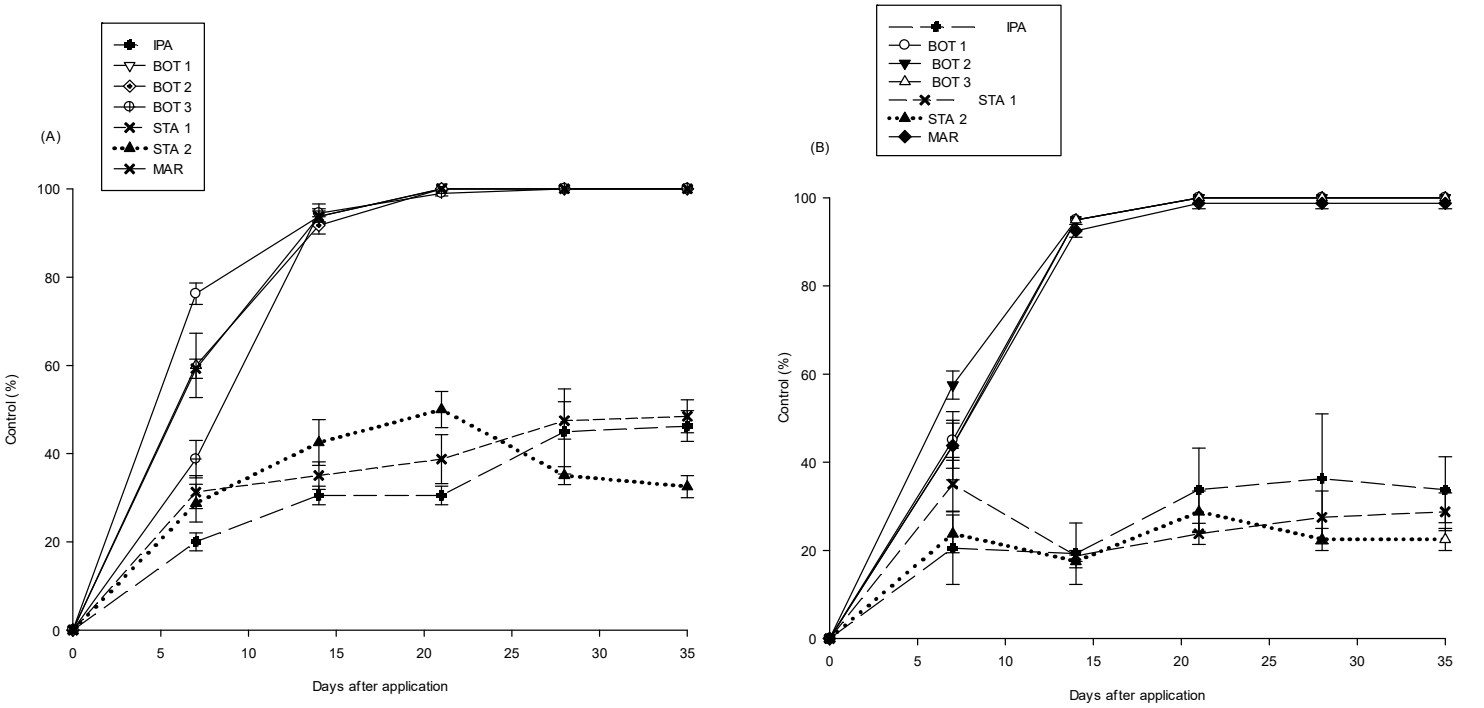
The shikimic acid accumulation in the plants after application of glyphosate was quantified to confirm the resistance of the evaluated sourgrass biotypes. Shikimic acid is an important marker of glyphosate-resistant plants, as described by Singh and Shaner (1998), Zablotowicz and Reddy (2004), De Maria et al. (2006), Nandula et al. (2007), Matallo et al. (2009), Reddy et al. (2010), and Carvalho et al. (2012). Figure 2 shows that the IPA, STA 1, and STA 2 biotypes, which presented control percentages below 40% (Figure 1), had lower shikimic acid accumulation.

This result is because glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), which catalyzes the condensation of shikimic acid and pyruvate phosphate, which are essential for plant growth and development (Zablotowicz and Reddy, 2004). When applying this herbicide to susceptible plants, shikimate accumulation occurs due to deregulation of its route, indicating that EPSPS is being inhibited (Powles and Preston, 2006). When glyphosate is applied to resistant plants, the non-inhibition of all EPSPS prevents the accumulation of shikimic acid and, consequently, allows the plant to survive.

Thus, the sourgrass biotypes collected in the urban areas of Ipaussu and Santa Cruz do Rio Pardo are resistant to glyphosate herbicide. These municipalities are in southwestern São Paulo, an important grain producer region of the state. Considering the marketing and purchase of inputs and the short distance between rural properties in this region, the traffic of agricultural machinery and implements is common and may be contributing to the dispersion of resistant sourgrass, since weed control herbicides is not usually used in urban areas, increasing the presence of weeds in them.

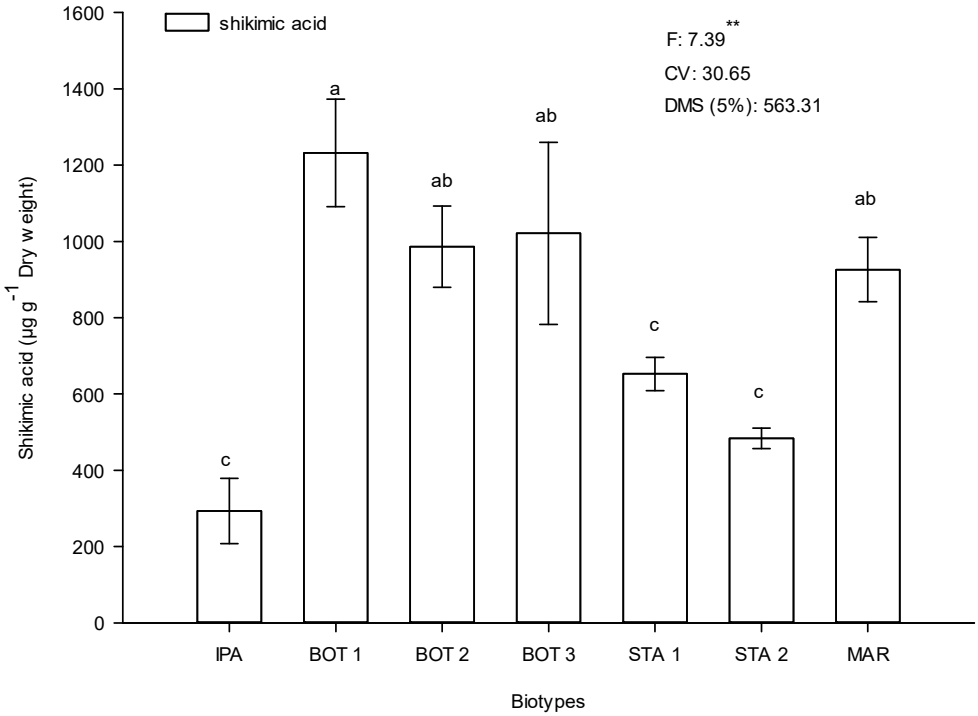
This is worrying because most farmers in this region also own farms in other states and often transport their machinery and implements to these other locations, favoring the spreading of resistant plants to other states.

In the municipalities of Botucatu and Marília, SP, which are in regions with no significant grain productions, glyphosate-resistant sourgrass is not found. This reinforces the idea that the increase in resistance cases may be associated with dissemination of propagules by the traffic of machinery and implements from grain producing regions of Brazil. This may be one of the reasons for the presence of resistant sourgrass in other grain producing regions of the country, as in the states of Mato Grosso, Mato Grosso do Sul, Goiás, and Bahia; the first glyphosate-



The error bars represent the mean standard error (n=4). Values of (A): F = 24.87**; 97.96**; 111.32**; 34.76**; 133.60**, respectively; CV (%) = 18.31; 7.66; 6.93; 9.44; 5.1, respectively. Values of (B): F = 4.90**; 210.92**; 85.25**; 38.93**; 125.45**, respectively; CV (%) = 30.52; 9.03; 11.88; 17.64; 9.89, respectively. **Significant at p<0.01.

Figure 1 - Glyphosate phytotoxicity in the tests of control 1 (A) and 2 (B), at 7, 14, 21, 28, and 35 days after application on seven *Digitaria insularis* biotypes propagated vegetatively.



The error bars represent the mean standard error (n=4), and bars with the same letters do not differ by the Tukey's test at p≤0.05. ** Significant at p<0.01.

Figure 2 - Shikimic acid accumulation in seven *Digitaria insularis* biotypes at 72 hours after glyphosate application.

resistant sourgrass in Brazil was reported in 2008 for the municipality of Guaíra, PR (Heap, 2018), which is very distant from these other regions.

This hypothesis of dissemination of glyphosate-resistant sourgrass is supported by the fact that the evolution of resistance is affected by several factors, classified as genetic, bioecological, and agronomic, which determine the time required for resistant biotypes to become predominant in an area (Christoffoleti et al., 2016). In addition, genetic variability among weeds of the same species allows them to evolve and adapt to new locations. Sourgrass plants have high variability. Martins et al. (2016) evaluated random primers and found that the DNA of glyphosate-resistant and susceptible sourgrass plants presented a variation of 56.6%. This is a highly competitive plant with great infestation potential and fast and aggressive growth, which develops well in poor and acidic soils and has high seed germination percentages (above 90%) (Mondo et al., 2010). Thus, *D. insularis* is fully capable of adapting to other regions, as has been occurring. López-Ovejero et al. (2017) reported the presence of sourgrass in practically all agricultural regions of Brazil.

Another hypothesis is the independent selection of new resistant biotypes in each region, since resistant plants will always be present at a low frequency in an area (Christoffoleti et al., 2016). According to Christoffoleti et al. (2016), when an herbicide is applied, it acts as an agent of selection pressure; susceptible weeds die and resistant ones survive and multiply without the presence of the susceptible ones. When a resistant plant completes its cycle, it produces hundreds or thousands of seeds, multiplying the plants for the subsequent harvests. The higher the frequency of a resistance-promoting gene, the shorter the time required for resistant individuals to increase their proportion under successive applications of the same herbicide (Vidal, 2002). This selection also depends on the herbicide that is used; the selection of resistant plants by glyphosate takes around 20 years to occur (Preston, 2018).

Sourgrass possibly present independent selection. Takano et al. (2018) evaluated the resistance of *D. insularis* to glyphosate in Brazil (states of Paraná and São Paulo) and in Paraguay and found that the selection of resistant populations in São Paulo possibly occurred independently of other sites, since the resistance evolved in highly divergent populations based on ISSR sequences. According to these authors, the level of genetic divergence in *D. insularis* populations was considered high (GST = 0.63) due to the high level of polymorphism found in the ISSR sequences. This indicates that resistant sourgrass populations were independently selected between the state of Paraná and São Paulo, since they were genetically structured populations (GST = 0.66) and with low gene flow rate (Nm = 0.25). However, further studies on divergences between resistance mechanisms in different populations are needed to a better understanding of how this independent selection occurs.

The rate-response curve experiment showed that the resistant progenies (IPA and STA 2) obtained from self-fertilized seeds presented satisfactory control and reduction of shoot dry weight above 80% only at rates above 2,000 g a.e. ha^{-1} of glyphosate, whereas susceptible plants (BOT 3) presented 100% control and approximately 80% reduction in shoot dry weight with only 360 g a.e. ha^{-1} of glyphosate (Figure 3). The recommended rates described in the glyphosate label for the control of *D. insularis* are 720 to 1,920 g a.e. ha^{-1}; the lowest one is recommended for younger plants, therefore, much lower than the control rate needed for the IPA and STA 2 biotypes in this experiment.

The herbicide rates that control 50% of the population (CE_{50}) or promote a 50% reduction in shoot dry weight (GR_{50}) were determined to obtain the resistance factor (RF = R/S) for confirmation of resistance. This factor corresponds to the number of times that the CE_{50} and GR_{50} of the resistant population is higher than the CE_{50} and GR_{50} of the susceptible population (Christoffoleti et al., 2016). The resistance was confirmed when the R/S factor was greater than 1.0 (Saari et al., 1994). The resistance factors of the sourgrass to glyphosate were 7.75 (IPA) and 9.77 (STA 2) for the percentage of control, i.e., approximately seven to nine times more glyphosate was needed for the resistant biotypes to promote the same symptoms observed in the susceptible biotype (BOT 3) (Table 2). The resistance factors were 9.44 (IPA) and 9.97 (STA 2) for the shoot dry weight reduction (Table 3).

These data corroborate those found in different studies that reported cases of resistance of *D. insularis* to glyphosate throughout Brazil, as described by Reinert et al. (2013), who found the

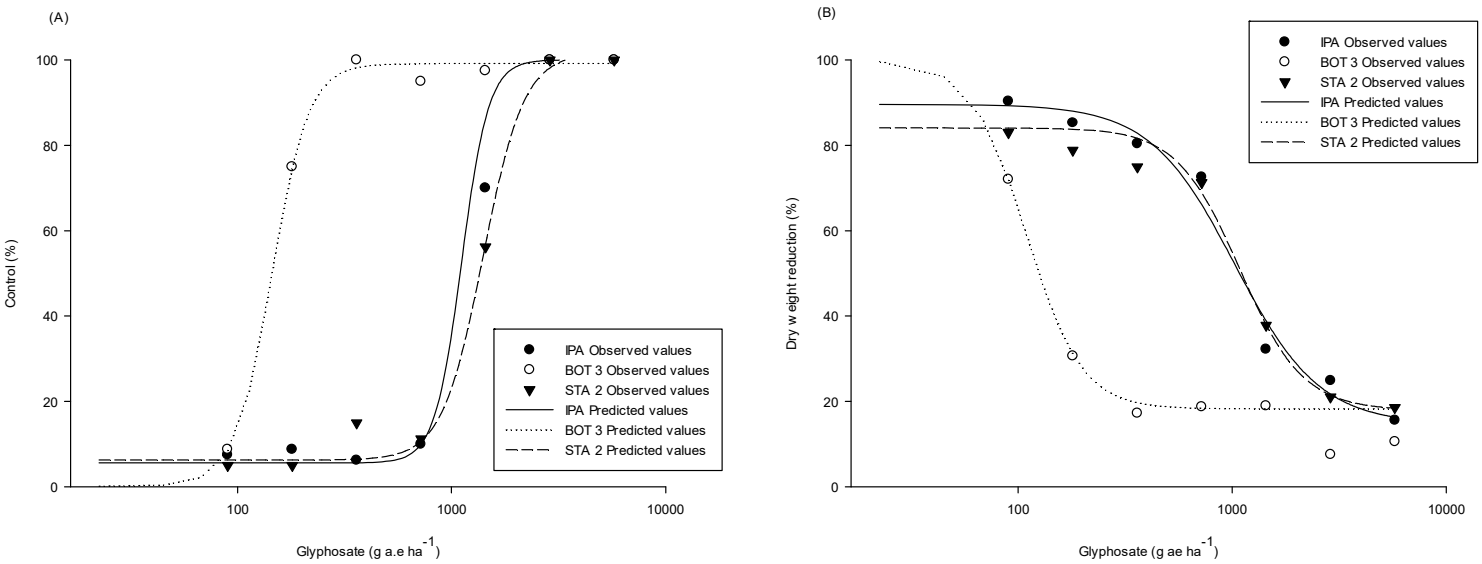


Figure 3 - Percentage of control (A) and reduction of shoot dry weight in relation to the control treatment (B) for the IPA, BOT 3, and STA 2 biotypes subjected to different rates of glyphosate herbicide, at 28 days after application.

Table 2 - Estimates of parameters of the log-logistic model *a, b, c,* and *d*, coefficient of determination (R^2), and coefficient of variation (CV%) for young plants of *Digitaria insularis* biotypes regarding the percentage of control at 28 days after application of glyphosate

Variable	Biotype	CV (%)	a	b	c (CE_{50})	d	R^2	RF*
Control (%)	IPA	9.32	5.61	94.45	1115.21	-6.9	0.99	7.75
	BOT 3	16.97	0.00	99.00	144.00	-5.07	0.96	-
	STA 2	32.25	6.00	95.43	1405	-4.53	0.96	9.77

CE_{50} expressed in g a.e. ha^{-1} of glyphosate; Equation of the model: y=a+(b/((1+(x/c)d))); * Resistance factor = CE_{50} R/CE_{50} S.

Table 3 - Estimates of parameters of the log-logistic model *a, b, c,* and *d*, coefficient of determination (R^2), and coefficient of variation (CV%) for young plants of *Digitaria insularis* biotypes regarding the percentage of shoot dry weight in relation to the control treatment at 28 days after application of glyphosate

Variable	Biotype	CV (%)	a	b	c (GR_{50})	d	R^2	RF*
shoot dry weight reduction (%)	IPA	22.96	14.67	74.95	1028.18	2.19	0.90	9.44
	BOT 3	24.01	18.00	82.00	109	3.36	0.91	-
	STA 2	15.50	18.00	66.17	1086	2.95	0.92	9.97

GR_{50} expressed in g a.e. ha^{-1} of glyphosate; Equation of the model: y=a+(b/((1+(x/c)d))); * Resistance factor = GR_{50} R/GR_{50} S.

need for 1,732.26 g a.e. ha^{-1} to obtain GR_{50}; Melo et al. (2015), who found rates between 456.33 and 1,030.89 g a.e. ha^{-1} for GR_{50}; and Licorini et al. (2015), who found the need for glyphosate rates above 1,437.68 g a.e. ha^{-1} for GR_{50}.

Therefore, the occurrence of glyphosate-resistant sourgrass biotypes in urban areas of the State of São Paulo was confirmed. The resistance trait of sourgrass is transmitted to progenies obtained from self-fertilized seeds. According to these results combined and the literature reports described, it is possible that resistant sourgrass seeds are being transported and disseminated to other regions of Brazil, contributing to increase cases of resistance of this species to glyphosate. However, this confirmation requires more detailed studies involving DNA and family trees to determine the genetic proximity between resistant biotypes of different regions, since independent selection of resistance can also occur.

In addition, this shows the need for resistance management measures, such as the properly cleaning of agricultural machinery and implements used in areas with presence of this species

and caution while transporting such equipment between agricultural regions, to reduce the spread of glyphosate-resistant sourgrass biotypes and to prevent the aggravation of problems of control of this weed species.

REFERENCES

Barroso AAM, Galeano E, Albrecht A, Reis F, Victoria Filho R. Does sourgrass leaf anatomy influence glyphosate resistance? Comun Sci. 2015;6(4):445-53.

Carvalho LB, Cruz-Hipolito H, González-Torralva F, Alves PLCostaA, Christoffoleti PJ, Prado R. Detection of sourgrass (*Digitaria insularis*) biotypes resistant to glyphosate in Brazil. Weed Sci. 2011;59(2)171-6.

Carvalho LB, Alves PLCA, González-Torralva F, Cruz-Hipolito HE, Rojano-Delgado AM, Prado R, et al. Pool of resistance mechanisms to glyphosate *Digitaria insularis*. J Agric Food Chem. 2012;60(2):615-22.

Christoffoleti PJ, cordenador. Aspectos da resistência de plantas daninhas a herbicidas. 4ª.ed. Piracicaba: Hrac – BR; 2016.

Correia NM, Leite GJ, Garcia LD. Resposta de diferentes populações de *Digitaria insularis* ao Herbicida Glyphosate. Planta Daninha. 2010;28(4):769-76.

De Maria N, Becerril JM, García-Plazaola JI, Hernandez A, De Felipe MR, Fernandez-Pascual M. New insights on glyphosate mode of action in nodular metabolism: role of shikimate accumulation. J Agric Food Chem. 2006;54(7):2621-8

Gazziero DLP, Voll E, Fornarolli D, Vargas L, Adegas FS. Efeitos da convivência do capim-amargoso na produtividade da soja. In: Anais do 28º Congresso Brasileiro da Ciência das Plantas Daninhas. Campo Grande: SBCPD; 2012. p.345-50.

Gemelli A, Oliveira Jr RS, Constantin J, Braga G, Braz P, Jumes TMC, et al. Estratégias para o controle de capim-amargoso (*Digitaria insularis*) resistente ao glyphosate na cultura milho safrinha. Rev Bras Herb. 2013;12(2):162-70.

Gomes GLGC, Velini ED, Carbonari CA. Fosfito de potássio não protege plantas de milho contra os efeitos fitotóxicos do glyphosate. Pesq Agropec Trop. 2015;45(3):291-6.

Heap IA. The international survey of herbicide resistant weeds. [accessed on: 27 Apr. 2018. Available at: http://www.weedscience.org.

Kissmann KG, Groth D. Plantas infestantes e nocivas. Plantas inferiores e monocotiledôneas. 2ª.ed. ed. São Paulo: BASF; 1997.

Licorini LR, Gandolfo MA, Sorace MA, Osipe R, Cossa CA, Osipe JB. Identificação e controle de biótipos resistentes de *Digitaria insularis* (L.) Fedde ao glyphosate. Rev Bras Herb. 2015;14(3):141-7.

Lorenzi H. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. 4ª.ed. Nova Odessa: Instituto Plantarum; 2008.

López-Ovejero R, Takano HK, Nicolai M, Ferreira A, Melo MSC, Cavenaghi AL, et al. Frequency and dispersal of glyphosate-resistant sourgrass (*Digitaria insularis*) populations across brazilian agricultural production areas. Weed Sci. 2017;65(2):285-94.

Machado AFL, Meira RMS, Ferreira LR, Ferreira FA, Tuffi Santos LD, Fialho CMT, et al. Caracterização anatômica de folha, colmo e rizoma de *Digitaria insularis*. Planta Daninha. 2008;26(1):1-8.

Martins JF, Barroso AAM, Carvalho LB, Cesarin AE, Amaral LC, Nepomuceno MP, et al. Plant growth and genetic polymorphism in glyphosate-resistant sourgrass (*Digitaria insularis* L. Fedde). Aust J Crop Sci. 2016;10(10):1466-73.

Matallo MB, Almeida SDB, Cerdeira AL, Franco DA, Blanco FMG, Menezes PTC, et al. Microwave-assisted solvent extraction and analysis of shikimic acid from plant tissues. Planta Daninha. 2009;27:987-94.

Melo MSC, Silva DCP, Rosa LE, Nicolai M, Christoffoleti PJ. Herança genética da resistência de capim-amargoso ao glyphosate. Rev Bras Herb. 2015;14(4)296-305.

Mendonça GS, Martins CC, Martins D, Costa NV. Ecophysiology of seed germination in *Digitaria insularis* ((L.) Fedde). Revi Cienc Agron. 2014;45:823-32.

Mondo VHV, Carvalho SJP, Dias ACR, Marcos Filho J. Efeitos da luz e temperatura na germinação de sementes de quatro espécies de plantas daninhas do gênero Digitaria. Rev Bras Sementes. 2010;32(1):31-7.

Nandula VK, Reddy KN, Rimando AM, Duke SO, Poston DH. Glyphosate-resistant and susceptible soybean (*Glycine max*) and canola (*Brassica napus*) dose response and metabolism relationship with glyphosate. J Agric Food Chem. 2007;55(9):3540-5.

Powles SB, Preston C. Evolved glyphosate resistance in plants: biochemical and genetic basis of resistance. Weed Technol. 2006;20:282-9.

Preston C. Threat from herbicide resistant weeds. [accessed on: 01 May. 2018] Available at: http://www.grdc.com.au/growers/res_upd/hirain/02/1longford_spring2002.

R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2008. [accessed on: 14 June 2018]. Available at: http://www.r-project.org.

Reddy KN, Bellaloui N, Zablotowicz RM. Glyphosate effect on shikimate, nitrate reductase activity, yield, and seed composition in corn. J Agric Food Chem. 2010;58(6):3646-50.

Reinert SC, Prado ABCA, Christoffoleti PJ. Curvas de dose-resposta comparativas entre os biótipos resistente e suscetível de capim-amargoso ao herbicida glyphosate. Rev Bras Herb. 2013;12(3):260-7.

Saari LL, Cotterman JC, Thill DC. Resistance to acetolactate synthase inhibiting herbicides. In: Powles SB, Holtum JAM. Herbicide resistance in plants: biology and biochemistry. Boca Raton: 1994. p.83-139.

Seefeldt SS, Jensen JE, Fuerst EP. Loglogistic analysis of herbicide dose response relationship. Weed Technol. 1995;9(2):218-27.

Singh BK, Shaner DL. Rapid determination of glyphosate injury to plants and identification of glyphosate-resistant plants. Weed Technol. 1998; 12(3):527-30.

Sociedade Brasileira da Ciência das Plantas Daninhas – SBCPD. Procedimentos para instalação, avaliação e análise de experimentos com herbicidas. Londrina: 1995. 42p.

Takano HK, Oliveira Jr RS, Constantin J, Mangolim CA, Machado MFPS, Bevilaqua MRR. Spread of glyphosate-resistant sourgrass (*Digitaria insularis*): Independent selections or merely propagule dissemination? Weed Biol Manage. 2018;18(1):50-9.

Vidal RA. Ação dos herbicidas. Ribas Vidal; 2002.

Zablotowicz RM, Reddy KN. Impact of glyphosate and *Bradyrhizobium japonicum* symbiosis; with glyphosate-resistant transgenic soybean: a minireview. J Environ Qual. 2004;3:825-31.