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Sensitivity and resistance level of sourgrass population subjected to glyphosate application

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Abstract

The intensive use of glyphosate in agricultural areas has increased the frequency of weeds that are resistant to herbicides. Thus, this study was aimed to assess the sensitivity and resistance level of *Digitaria insularis* (L.) Fedde (sourgrass) populations to glyphosate. Sixty-two sourgrass populations were collected from the states of Paraná and São Paulo, Brazil, and subjected to glyphosate application at 1,080 and 2,160 g of acid equivalent (a.e.) · ha^{-1} in screening assays. Five sourgrass populations were selected, three of which are resistant and two of which are susceptible to glyphosate, to determine the resistance factors (RFs) through dose-response studies at two phenological stages of plant growth: the 2–4-leaf stages and the 2–4-tiller stage. The trials were conducted in a greenhouse in accordance with a completely randomized design. In both trials, the control was evaluated based on the score of the visual control symptoms (VC) and the percentage of dry matter (DM) in relation to those of the control (without application). In the screening test, the data obtained for the response variables were adjusted for frequency curves by following the regression model proposed by Gompertz. The results indicated low sensitivity of *D. insularis* to glyphosate in 100% of the samples from areas in which soybeans are tolerant to this herbicide. Populations with susceptible plants were found in fallow areas, pasture areas and sugar cane fields. Based on the values of VC_{50} and DM_{50}, the maximum RF obtained among the populations was 15. More advanced stages of development make sourgrass control difficult, requiring doses that are 3.5 times greater than those at the initial stage.

Keywords: chemical control, *Digitaria insularis*, Gompertz model, resistance factor

Introduction

Digitaria insularis (L.) Fedde (sourgrass), which is commonly known as sourgrass, is a perennial, rhizomatous weed with tillered growth. This grass naturally grows in a wide variety of environments, including fields with annual or perennial crop cultivations, pastures, vegetable gardens and ruderal areas such as roads and vacant lots (Silveira *et al.* 2018). Its development is interspersed between initial slow growth and subsequent exponential gains in biomass, which makes it highly competitive in capturing environmental resources (Gemelli *et al.* 2012).

The introduction of transgenic crops such as glyphosate-resistant soybean to Brazil in 1998 (CTNbio 2019) and the intensification of the use of this herbicide have caused significant changes in the composition of weed species, with a substantial increase in *D. insularis* infestation throughout the country. In the 2017/2018 cropping season, 92% of soybean crops were transgenic, and 65% of these were sown with herbicide tolerant cultivars (USDA 2018), which accounted for approximately 23 million hectares in total (CONAB 2018). This fact has contributed to the development of herbicide resistance, especially for glyphosate, which is the most widely used herbicide in the country.

The first case of *D. insularis* resistance in the world was confirmed in 2005 in Caaguazu, Alto Paraná

Departament, Paraguay (HEAP 2020). In 2008, the first confirmed case of glyphosate resistance in Brazil was found in the far west of Paraná state (HEAP 2020) near the first reported case in Paraguay. In addition, other studies reported the occurrence of glyphosate-resistant *D. insularis* in other regions and crops cultivated in the country (Carvalho *et al.* 2011; Silveira *et al.* 2018).

Considering the development and reproduction of *D. insularis*, several characteristics contribute to the exposure and evolution of herbicide resistance, such as high prolificacy, adaptability, capacity to grow year-round in most Brazilian regions (Gemelli *et al.* 2012), ease of wind dispersion, low dormancy percentage and high germination rate (Mondo *et al.* 2010; Mendonça *et al.* 2014). Furthermore, recent studies have shown that the migratory flow of agricultural machinery and the selection of resistant populations of independent origins are important factors that may contribute to the dispersion of resistance and occurrence in various locations (Takano *et al.* 2018).

In this scenario, the identification of unprecedented cases of resistance and knowing the actual situation of the resistance evolution in the field are important. Therefore, it is essential to develop studies that focus on the sensitivity of weed populations to herbicides to alert farmers and other interested parties about the dispersal of the species across different agricultural areas and to understand the migratory characteristics of the species from the results obtained in areas with no record of herbicide application.

In resistance monitoring studies, the amplitude of the weed populations in response to herbicides and the variation over time can be investigated in different ways, one of which is through the frequency of occurrence in the field. In this particular case, statistical methods that can facilitate data interpretation can contribute to a better understanding of the results and the evolution of herbicide resistance.

On the basis of the hypothesis that variation in the susceptibility of *D. insularis* populations to glyphosate herbicide, as well as the stage of development of this weed, can influence its management, this work proposed to evaluate the sensitivity of populations of *D. insularis* to glyphosate herbicide and to determine the resistance levels of sourgrass populations to the herbicide during two growth stages of this weed.

Materials and Methods

Seed sampling and locations of populations

For the trial installations, 62 populations of *D. insularis* were collected from agricultural areas of the western, midwestern and northern regions of Paraná state (PR) and the southwest and central south regions of São Paulo state (SP), where a high frequency of *D. insularis* occurrence is found in the field. Fifty-three of the populations came from glyphosate-resistant transgenic soybean crops that received herbicide application but did not present satisfactory control, which characterized areas with suspected resistance. Nine populations were sampled from fields with no record of herbicide application, including fallow areas, pastures and sugarcane fields, to collect the susceptible populations required in the comparative evaluation of herbicide resistance.

The seeds were collected from several locations and distributed at 17 locations: Boa Esperança (PR), Goioerê (PR), Mariluz (PR), Quarto Centenário (PR), Janiópolis (PR), Campo Mourão (PR), Bandeirantes do Oeste (PR), Moreira Sales (PR), Peabiru (PR), Ivailândia (PR), Palotina (PR), Marechal Cândido Rondon (PR), Maripá (PR), Formosa do Oeste (PR), Avaré (SP), Botucatu (SP) and Itaí (SP). At the time of collection, the geographical coordinates of the sampling points, altitude, state, city and conditions of occupation of the area were recorded.

Each population of sourgrass was represented by a sample of seeds collected from 20–40 plants according to the methodology proposed by Burgos *et al.* (2013). The seeds were removed from the panicles of maturing plants, which were stored in properly identified paper bags. After sampling, the seeds were cleaned of impurities and treated with phosphine pellets to avoid the incidence of insects and pathogens during storage and trial conduction. The seeds were stored in a cold chamber at 10°C and 37% relative humidity (RH).

Screening assay

A screening assay was carried out at São Paulo State University (UNESP), School of Agriculture, Botucatu, SP (22°50’41.20”S; 48°26’6.65”W), to determine the sensitivity and amplitude of the response of sourgrass populations to glyphosate by applying doses of 1,080 and 2,160 g a.e. · ha^{-1}, which corresponded to 1 and 2 times the commercial dose of glyphosate (Roundup WG 72% a.e., Monsanto of Brazil, São Paulo, SP) registered to control *D. insularis* (Rodrigues and Almeida 2018). The assay was conducted in a completely randomized design with five replicates. The experimental units were represented by 0.5-l capacity polyethylene pots, which were filled with commercial substrate (Carolina Soil®). Thirty seeds were sown in each pot with subsequent thinning after seedling emergence so that three plants were evenly distributed.

When the plants had produced 2–4 fully expanded leaves, the herbicide was applied in the laboratory using a mobile spraying system with velocity and pressure control. The system was equipped with a 3.0-m-long boom spray, which contained AXI 11003 flat fan nozzles (Jacto®) spaced 0.5 m apart. The equipment was set to operate at a pressure of 200 kPa and displacement velocity of 5.0 km · h^{-1} to provide a spray volume of 200 l · h^{-1}.

Dose-response assay

A dose-response assay was carried out at UNESP, School of Agriculture, Botucatu, SP (22°50'41.20"S; 48°26'6.65"W). The populations in the dose-response assay were selected based on data obtained from the screening assay, which used the results of the visual control symptoms (VC) at 21 days after application (DAA) as the reference, and reduction in dry matter (DM). Thus, the three populations that showed the lowest glyphosate control efficiency and the two populations that showed the highest sensitivity to the herbicide were selected.

Two glyphosate dose-response assays were carried out at different sourgrass growth stages. Spraying was conducted when the plants reached 2–4 leaves and 2–4 tillers (0.20 and 0.40 m). The trials were conducted in a completely randomized design with nine treatments and seven replicates. The location and application conditions were the same as those used for the screening assay.

To prepare the dose-response curves, in the first assay, the doses were 135, 270, 540, 1,080, 2,160, 4,320, 8,640 and 17,280 g a.e. · ha^{-1}, which corresponded to 1/8, 1/4, 1/2, 1, 2, 4, 8 and 16 times the commercial dose of glyphosate (Roundup WG®), respectively; 1 is the recommended dose for the *D. insularis* control (1,080 g · a.e. ha^{-1}). These treatments were compared to the control treatment (without application). In the second assay, using plants with 2–4 tillers, the lowest dose was 270 g a.e. · ha^{-1}, and the highest was 34,560 g a.e. · ha^{-1} (which corresponded to 32 times the commercial dose) to obtain total control of resistant populations.

Assay evaluation

Both assays were evaluated based on the visual symptoms of control (Association Latin American Weed – ALAM 1974) and assigned scores from 0% (no symptoms) to 100% (plant death) at 21 DAA. Then, the plants were cut near the soil and stored in properly identified paper bags. Subsequently, the samples were taken to dry in a Fanem model 320/5-MP forced-air circulation oven (220 V and 5200 W) at a temperature of 65 ± 5°C until they reached constant weight. Afterwards, the plants were weighed with a Marte model AY220 precision analytical scale (0.001 g), and the data were used to calculate the percentage of DM in relation to the control (without application).

Data analysis

Screening assays

The populations sampled from areas with no record of application were grouped and analyzed separately from the populations from fields with a record of application. This procedure was necessary to determine particular responses to the groups that were considered resistant and susceptible.

Data obtained for populations with suspected resistance were subjected to analysis of variance and *F* test. When significance was found, the data were adjusted to the sigmoidal regression model proposed by Gompertz (1825) to determine the cumulative frequencies, as shown in equation 1 (E1):

$$y = e^{\left[a - e^{(-b-c.x)}\right]}. \qquad \text{(E1)}$$

In this equation, e^a corresponds to the asymptote of the model. Thus, the value 4.605170 was assigned to parameter a so that $e^a = 100$; x represents the estimated values of the response variables, b is the displacement of the curve along the x-axis, and c is the slope of the curve in relation to the cumulative frequency (Souza *et al.* 2007). Parameters b and c were estimated by the model.

The arithmetic means of the response variables were calculated based on the original data and position measurements (mode and median) obtained according to equations 2 (E2) and 3 (E3), respectively:

$$y = -\frac{b}{c}, \qquad \text{(E2)}$$

$$y = -\frac{\ln(a-3.912)+b}{c}. \qquad \text{(E3)}$$

A graphical visualization of the dispersion measurements and data amplitude was obtained by the non-cumulative frequency, which is given by the first derivative of the Gompertz model, using equation 4 (E4) according to Souza *et al.* (2007):

$$y' = c * e^{(a-b-c*x-e^{(-b-c*x)})}. \qquad \text{(E4)}$$

Dose-response assays

The data obtained from the dose-response assays were subjected to analysis of variance and *F* test. When significant, the data were adjusted to the log-logistic nonlinear regression models proposed by Seefeldt *et al.* (1995) to analyze the visual control and DM reduction according to equations 5 (E5) and 6 (E6), respectively:

$$y = \frac{a}{\left[1+\left(\frac{x}{b}\right)^{c}\right]}, \quad \text{(E5)}$$

$$y = P_{\min} + \frac{a}{\left[1+\left(\frac{x}{b}\right)^{c}\right]}, \quad \text{(E6)}$$

where: y – the percentage of control or remaining DM; x – the herbicide dose in g a.e. · ha^{-1}; a, b and c – the parameters of the models; P_{min} – the minimum value obtained for residual DM; a – the difference between the maximum and minimum point; b – provides 50% of the asymptote; c – the slope of the curve at x.

Since the 100% control value is not always obtained in dose-response assays, the value of parameter b, estimated by the model, was disregarded, and the dose required for 50% control (VC_{50} or DM_{50}) of the population was calculated based on the inverse equations of the models, according to Carvalho *et al.* (2005). Thus, the y value of the inverse equation is replaced by 50 to obtain VC_{50} or DM_{50}. Similarly, replacing y with 80 gives the dose necessary to achieve satisfactory population control (≥80% – VC_{80} or DM_{80}), according to the percentage control scale established by ALAM (1974).

The relationship between C_{50} or GR_{50} values obtained by inverse equations and the log-logistic models of suspected resistance and susceptible populations provided the resistance factor (RF), which expresses the number of times the dose required to control 50% of the resistant population is greater than the dose controlling 50% of the susceptible population (Hall *et al.* 1998; Christoffoleti 2002).

The analysis of nonlinear, sigmoidal and logistic regression models was performed with the aid of SAS statistical software (Statistical Analysis System, SAS Institute, version 9.3., Cary, North Carolina, USA) and the graphs were obtained through software SigmaPlot (Systat Software, version 12.5, San Jose 2013).

Results and Discussion

Screening assay

Regarding the populations sampled from the fields with no record of glyphosate use, the application of the registered herbicide dose (1,080 g a.e. · ha^{-1}) was sufficient to obtain satisfactory control (>80% control) of 67% of the population evaluated, achieving the highest sensitivity to this herbicide (Table 1). Additionally, populations 55 and 57 were efficiently controlled when subjected to two times the registered dose (2,160 g a.e. · ha^{-1}), whereas population 56 from Palotina (PR) showed no satisfactory control (<80% control), even with twice the recommended dose. The overall result obtained for this group of plants shows that there are morphological characteristics favorable to the seed dispersal of this species, such as the presence of hair-like appendages, which are useful for seed adhesion and dispersion (Kissmann and Groth 1997) and the low weight of seedlings. Hence, the migratory flow of seeds manifested in the field is not expressive and is evidenced by the high percentage of efficiently controlled populations.

Table 1. Visual weed control – VC (%) and residual dry matter – DM (%) of *Digitaria insularis* populations from fields with no record of glyphosate application at 21 days after application (DAA)

Population	Location	Field use	Herbicide dose			
			1,080 [g a.e. · ha^{-1}]		2,160 [g a.e. · ha^{-1}]	
			VC	DM	VC	DM
2	24°11’38”S; 053°1’13’W	fallow area	82.50	63.12	100.00	17.59
3	24°11’19”S; 053°1’57”W	fallow area	100.00	16.21	100.00	12.12
55	24°18’24”S; 053⁰50’18”W 053°50’18,18”	fallow area	58.75	49.80	100.00	17.66
56	24°18’22”S; 053°50’10”W	fallow area	20.00	140.36	60.00	32.07
57	22°50’02”S; 048°25’31”W	pasture	57.00	36.99	95.00	17.89
58	22°49’54”S; 048°25’29”W	pasture	100.00	23.76	100.00	12.35
59	22°52’10”S; 048°45’74”W	pasture	95.50	20.02	98.50	18.34
60	23°50’81”S; 048°82’43”W	fallow area	100.00	27.79	100.00	17.60
61	2331’55”S; 049°04’46”W	sugarcane	100.00	25.31	100.00	11.35
Mean			79.31	44.82	94.83	17.44
Median			57.00	36.99	95.00	17.89
CV%			35.73	86.76	13.88	35.49

CV – coefficient of variation in percentage

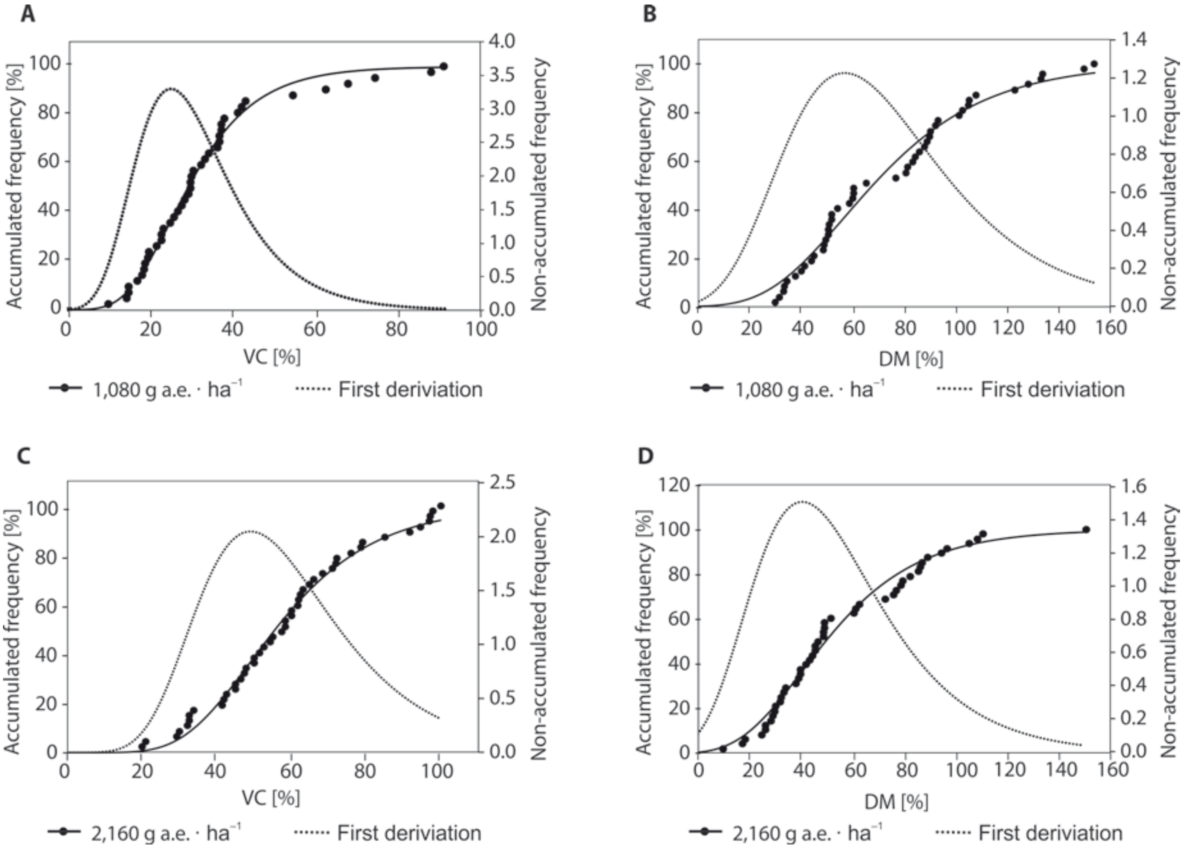

Fig. 1. Cumulative and noncumulative frequency curves obtained for the first derivative of the Gompertz model for visual weed control – VC (A, C) and residual dry matter – DM percentage (B, D) and those obtained for *Digitaria insularis* populations collected from areas suspected of glyphosate resistance and treated with doses of 1,080 and 2,160 g a.e. · ha^{-1}

Only 3% of the populations collected from the fields with a record of glyphosate application showed satisfactory control (>80% control) when subjected to the registered dose (Fig. 1A); at twice the recommended dose, the rate of control was 22%. Lopez Ovejero *et al.* (2017) studied the resistance monitoring of populations from the Paraná (PR) and Mato Grosso do Sul (MS) states in 2014. The occurrence of over 80% of the *D. insularis* population exhibiting some level of resistance indicates that the lack of adequate management programs and quarantine implementation are the main factors responsible for the increasing resistance of *D. insularis* in the locations studied.

The alteration in the concavity of the noncumulative frequency curve obtained for the dose of 1,080 g a.e. · ha^{-1} compared to that for the dose of 2,160 g a.e. · ha^{-1} (Fig. 1A–C) demonstrated that with increasing dose, there was greater sensitivity of the populations to the herbicide and greater variability in control responses. In general, visual symptoms increased from 34% at the recommended dose to 59% at twice the dose (Table 2). Nonetheless, the overall results indicated the low sensitivity of *D. insularis* populations collected from soybean fields to glyphosate.

The frequency curve parameters (Table 2) show the evolution and presence of alarming indices of glyphosate-resistant *D. insularis*. Twice the recommended herbicide dose was not sufficient to satisfactorily control most of the evaluated populations, which shows the difficulties in re-establishing herbicide susceptibility in the field.

These results suggest that the resistance of *D. insularis* to glyphosate was more intimately associated with the continuous application of the herbicide in the field, especially in fields with transgenic crops, where the herbicide use is more intensively conducted instead of the dissemination of propagules to the surrounding areas. The increase in herbicide dose did not appear to be a viable measure of the progress of this weed control.

Dose-response assay of the glyphosate–2-4-leaf plant developmental stage

Significant differences were found between the populations sampled in areas without application records (3 and 60) and the supposedly resistant populations (19, 49 and 53) from areas with records of herbicide use (Quarto Centenário [PR], Palotina [PR] and Maripá [PR], respectively) when subjected to glyphosate, which confirms the resistance of the last three populations (Fig. 2A–B).

Table 2. Gompertz model equation parameters adjusted for visual weed control – VC (%) and residual dry matter – DM (%) data of *Digitaria insularis* populations suspected of glyphosate resistance

Dose of 1,080 [g a.e. · ha^{-1}]						
Variable	*b*	*c*	Mean	Median	Mode	r^2
VC	–2.2632	0.0899	34.22	29.25	25.17	0.9980
DM	–1.8806	0.0332	74.51	67.68	56.64	0.9951
Dose of 2,160 [g a.e. · ha^{-1}]						
Variable	*b*	*c*	Mean	Median	Mode	r^2
VC	–2.7211	0.0554	58.60	55.73	49.12	0.9980
DM	–1.6528	0.0408	56.31	49.49	40.51	0.9957

b – displacement of the curve along the x-axis; *c* – slope of the curve in relation to the cumulative frequency; r^2 – determination coefficient

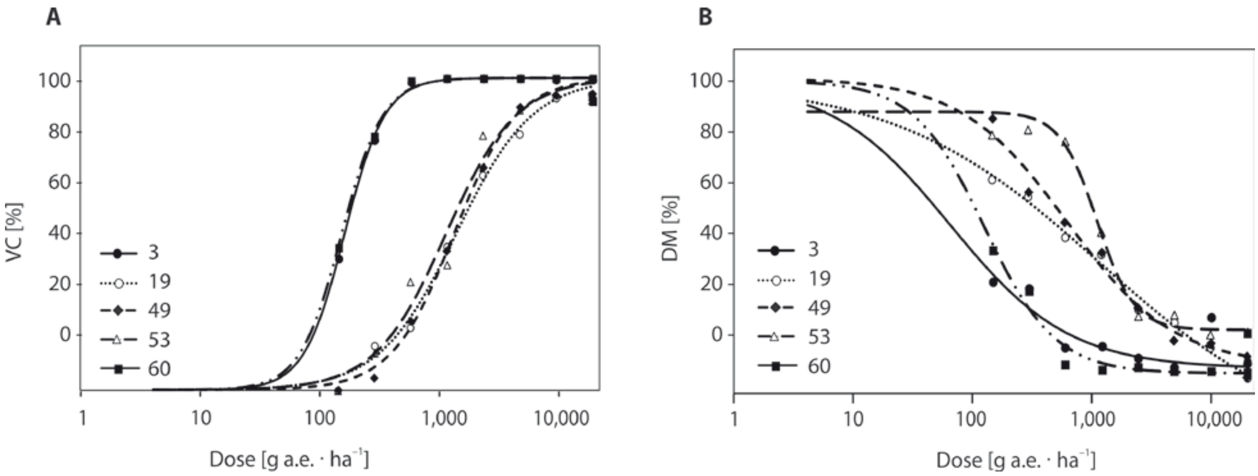

Fig. 2. Log-logistic curves of the visual weed control – VC (A) and residual dry matter – DM (B) variables of *Digitaria insularis* populations that were resistant and susceptible to glyphosate (3 and 60 susceptible; 19, 49 and 53 resistant) at the 2–4-true leaf-stage and at 21 days after treatment (DAA) of the herbicide

For the susceptible populations, the estimated VC_{50} (dose required for visual control of 50% of the population) was 150 g a.e. · ha^{-1} (Table 3); for the resistant populations, the average index was 1,200 g a.e. · ha^{-1}, with no significant differences from the resistant ones. The ratio of VC_{50} of the most resistant population with the most sensitive index resulted in an RF of 8.84.

The susceptible populations had a DM_{50} (dose required for 50% reduction in DM) of 73.3–126.8 g a.e. · ha^{-1}, while the maximum value of DM_{50} among the resistant populations was 1,104 g a.e. · ha^{-1}, which was obtained for population 53, with an RF of 15.06.

Previous studies have reported the resistance of sourgrass to glyphosate, with different RF values of 2.1–26.7 (Carvalho *et al.* 2011; Silveira *et al.* 2018). Nevertheless, RFs are not considered the most appropriate parameter for comparing between studies, since this index may vary due to the higher or lower sensitivity of the susceptible population examined in the studies. For example, Carvalho *et al.* (2011) and Reinert *et al.* (2013) obtained VC_{50} smaller than 100 g a.e. · ha^{-1} for the control of a susceptible population, while Silveira *et al.* (2018) required 431 g a.e. · ha^{-1}. Thus, it is assumed that such a comparison should be made based on the doses required to achieve the considered inhibition level while always observing the stage of plant development.

Considering the inhibition level of 80% obtained for the control variable (Table 3), up to approximately 3.3 times the commercial glyphosate dose was necessary to achieve satisfactory control (>80%) of population 19, which was collected from Quarto Centenário, PR. Regarding the DM variable, the inhibition level of 80% would be reached if a dose of 379–513 g a.e. · ha^{-1} was applied to susceptible populations and a dose of 4,147–11,078 g a.e. · ha^{-1} was applied to resistant populations, which makes evident the differences in the control between resistant and susceptible populations of the present work and the ineffective chemical control with glyphosate herbicide in the three resistant populations.

Although producers recognize the limitations of glyphosate on *D. insularis* control, the herbicide has been commonly used to control other weeds in the same areas, which causes continued exposure of *D. insularis* populations in the field.

Table 3. Parameters a, b, c of the log-logistic model in response to glyphosate application. Doses (g a.e. · ha^{-1}) required to inhibit 50% (VC_{50} or DM_{50}) and 80% (VC_{80} or DM_{80}) of *Digitaria insularis* populations at 2–4 leaf stage at 21 DAA

VC [%]								
Population		*a*	*b*	*c*	VC_{50}	VC_{80}	r^2	RF
3		100.00	153.30	–2.60	153.30	261.49	0.9999	1.06
19		100.00	1,282.80	–1.37	1,282.80	3,522.75	0.9981	8.84
49		100.00	1,233.50	–1.68	1,233.50	2,818.52	0.9985	8.50
53		100.00	1,057.40	–1.49	1,057.40	2,685.93	0.9919	7.29
60		100.00	145.10	–2.53	145.10	251.16	0.9999	1.00
DM [%]								
Population	P_{min}	*a*	*b*	*c*	DM_{50}	DM_{80}	r^2	RF
3	7.9	92.07	60.43	0.88	73.33	513.94	0.9895	1.00
19	–16.6	116.60	1,050.80	0.51	600.10	4,836.08	0.9944	8.18
49	8.7	92.18	561.90	0.98	694.67	4,147.77	0.9881	9.47
53	20.1	69.98	995.20	2.82	1104.65	11,078.27	0.9782	15.06
60	6.2	93.67	116.30	1.48	126.80	379.72	0.9903	1.73

VC – visual weed control; DM – residual dry matter; DAA – days after treatment
a – difference between the maximum and minimum point; *b* – provides 50% of the asymptote; *c* – slope of the curve at *x*; r^2 – coefficient of determination; RF – resistance factor

Dose-response assay of the glyphosate--2-4-leaf plant developmental stage

Similar to the observation made in the first stage of *D. insularis* development of 2–4 tillers, the populations with suspected resistance (19, 49, and 53) significantly differed from the susceptible populations (3 and 60) when subjected to the application of different doses of glyphosate (Fig. 3A–B).

The VC_{50} estimates reveal that the doses required to control susceptible *D. insularis* (S) populations at this stage of development were close to 418 g a.e. · ha^{-1}, while VC_{50} of the resistant populations (R) was 4,125–5,659 g a.e. · ha^{-1}, which represents a significant increase over the first evaluation period. The relationship between the doses needed to control the R/S populations resulted in RFs of up to 13.54 (Table 4).

These results are similar to those reported by Silveira (2018), who identified high levels of resistance of *D. insularis*, in contrast to those of Carvalho *et al.* (2011). In the latter study, the authors obtained data of low resistance levels, where the highest VC_{50} dose evaluated to control the R population was 249 g a.e. · ha^{-1}, which presented an RF of 5.6. These results suggest that sourgrass populations have increased their resistance over time, most likely due to continued exposure to glyphosate herbicide in the field. As discussed

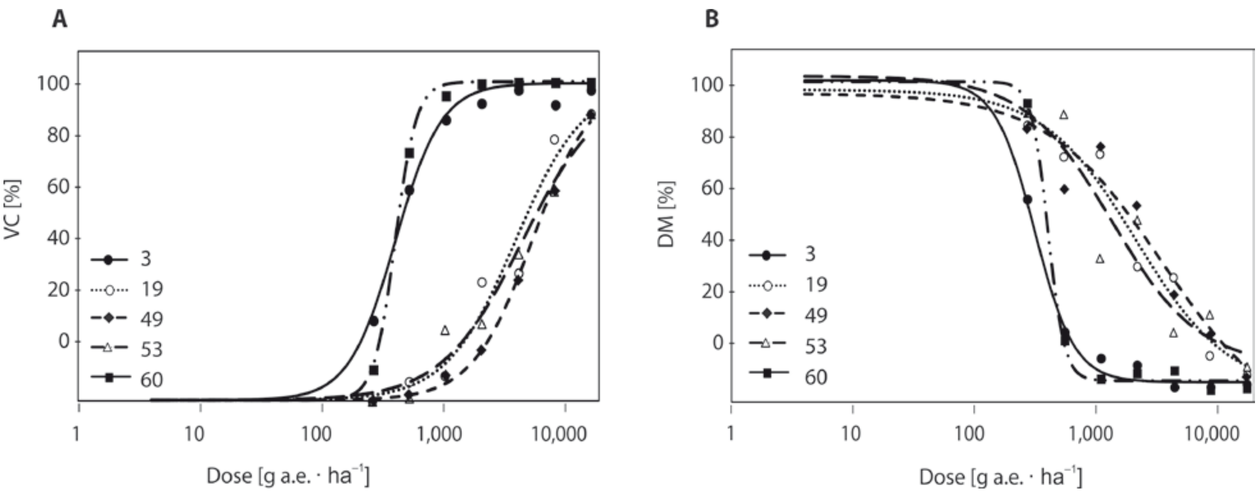

Fig. 3. Dose-response curves of the visual weed control – VC (A) and residual dry matter – DM (B) variables of *Digitaria insularis* populations that were resistant and susceptible to glyphosate (3 and 60 susceptible; 19, 49 and 53 resistant) at the 2–4 tiller stage and at 21 days after treatment (DAA) of the herbicide

Table 4. Parameters *a*, *b* and *c* of the log-logistic model in response to glyphosate application at the 2–4 tiller stage. Doses (g ae · ha^{-1}) required to control 50% (VC_{50} or DM_{50}) and 80% (VC_{80} or DM_{80}) of *Digitaria insularis* populations at 21 DAA

VC [%]								
Population		*a*	*b*	*c*	VC_{50}	VC_{80}	r^2	RF
3		100.00	418.10	–2.26	418.10	772.57	1.00	1.00
19		100.00	4,125.90	–1.52	4,125.90	10,253.13	0.99	9.88
49		100.00	5,658.50	–1.75	5,658.50	12,470.74	1.00	13.54
53		100.00	4,690.80	–1.30	4,690.80	13,611.54	0.99	11.23
60		100.00	417.80	–4.96	417.80	552.47	1.00	1.00
DM [%]								
Population	P_{min}	*a*	*b*	*c*	DM_{50}	DM_{80}	r^2	RF
3	6.36	93.85	314.30	2.66	331.37	612.49	0.9924	1.00
19	3.15	94.37	2,155.50	1.13	2,182.26	8,285.63	0.9727	6.59
49	–8.54	104.70	3,473.00	0.92	2,683.00	10,088.15	0.9596	8.10
53	11.24	90.55	1,354.70	1.21	1,723.15	8,650.76	0.9283	5.20
60	6.80	93.22	403.80	6.38	413.18	535.56	0.9971	1.25

VC – visual weed control; DM – residual dry matter; DAA – days after treatment
a – difference between the maximum and minimum point; *b* – provides 50% of the asymptote; *c* – slope of the curve at *x*; r^2 – coefficient of determination; RF – resistance factor

by Sammons and Gaines (2014), the justification for continuously increasing doses required to control the R populations may be a function of the accumulation of different mutation events in the same plant, which may or may not be expressed by different mechanisms of resistance.

Indeed, information in the literature differs as to the likely mechanisms of the resistance expression of *D. insularis*. Some authors point out that the combination of lower glyphosate absorption and translocation in the plant, higher herbicide metabolization to nontoxic compounds and mutation of the enzyme EPSPS are mechanisms responsible for affording the resistance of this species (Carvalho *et al.* 2012). However, recently, Melo *et al.* (2019) did not verify the expression of the previously mentioned resistance mechanisms, and the resistance attributes may differ depending on the plant origin considering the selection of resistant populations in the field with independent origins (Takano *et al.* 2018).

The maximum dose needed to obtain satisfactory control (>80%) was up to 12 times the commercial dose of glyphosate, which suggests that the application of this compound is considered an unsustainable agricultural practice to control resistant *D. insularis* populations in the studied regions. It is essential to adopt other measures to obtain satisfactory control of this species.

Relationship between *Digitaria insularis* growth stages and doses necessary to achieve control

Considering the ratio between doses required to control 50 and 80% of the populations, for the variable VC and DM responses, 3.5 times larger doses of glyphosate were required at the 2–4 tiller stage to obtain the same level of inhibition for both resistant and susceptible populations (Table 5).

Table 5. Relationship between values obtained for the control parameters VC_{50} and VC_{80} and DM_{50} and DM_{80} from the second (2–4 tillers) and first (2–4 leaves) stages of development of *Digitaria insularis* populations. All data are related to 21 DAA of glyphosate

Population	VC		DM	
	VC_{50}	VC_{80}	DM_{50}	DM_{80}
3	2.73	2.95	4.52	1.19
19	3.22	2.91	3.64	1.71
49	4.59	4.42	3.86	2.43
53	4.44	5.07	1.56	0.78
60	2.88	2.20	3.26	1.41
Mean	3.57	3.51	3.37	1.51

VC – visual weed control; DM – residual dry matter; DAA – days after treatment

However, the ratio between the doses required to reduce DM by 50 and 80% was 3.35 and 1.51 times higher at the most advanced developmental stage, respectively. These indices suggest that for both resistant and susceptible populations, higher doses are required to control *D. insularis*. This result may be related to the greater amount of leaf tissue formed and, consequently, the greater amount of EPSPS enzyme in plant tissues. Another possibility is attributed to the development of the leaf cuticle, which becomes thicker at more advanced developmental stages and represents a more expressive barrier to herbicide absorption.

In this study, in 97% of the cases, the *D. insularis* populations collected from the states of Paraná and São Paulo in areas with suspected glyphosate resistance showed herbicide insensitivity. The study of the cumulative frequency of herbicide responses performed using the Gompertz model was adequate to evaluate the effect of herbicide application on *D. insularis* populations and showed variability of population responses and the possibility of using different species resistance monitoring programs to better understand the evolution of this characteristic in the field.

The maximum RF of *D. insularis* populations was 15 for both developmental stages under study. In general, *D. insularis* is significantly less sensitive to glyphosate control at more advanced stages of development, which requires higher doses for satisfactory species control.

The results presented in this research show the current state of sourgrass resistance to glyphosate in the southern and southeastern regions of Brazil. The evolution of sourgrass resistance is rapid in response to the simplification of chemical weed control methods used in production systems based on the succession between soybeans (first crop) and corn (second crop) crops, both of which experience transgenic events in response to this herbicide.

In this scenario, it is essential to adopt alternative methods for the management of weeds and the prevention of resistance, such as the use of crop rotation, forage crops, and intercropping systems, which promote the maintenance of vegetation cover and reduce the need for frequent use of chemical control. An increase in chemical alternatives for weed control is necessary. In addition, mechanical control through mowing and soil tillage and the use of herbicides applied in the preemergence of weeds, such as herbicides from the chemical groups of dinitroanilines and chloroacetamides, stand out among the main measures taken to reduce the development of weed resistance in the field.

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