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Article

RESISTANCE TO GLYPHOSATE IN POPULATIONS OF *Digitaria insularis*

Resistência ao Glyphosate em Populações de ***Digitaria insularis***

COSTA, N.V.[1]*
MORATELLI, G.[1]
FERREIRA, S.D.[1]
SALVALAGGIO, A.C.[1]
RODRIGUES-COSTA, A.C.P.[2]

ABSTRACT - The intensive use of glyphosate can select resistant populations. This study aimed at evaluating the occurrence of glyphosate resistance in populations of *D. insularis* in agricultural areas. Three experiments were conducted in randomized block experimental design with four replications. The Resistance Degree (RD) was determined by a dose-response curve in the evaluated populations: Experiment 2012, São Camilo (R01 and R02) and Palotina (R03, R04 and S05-susceptible); Experiment 2013 Palotina (R06 and R12), Katueté/Paraguay (R07), Toledo (R08 and R10), Cascavel (R09), São Miguel do Iguaçu (R11), Dom Eliseu/Pará (S13-susceptible); Experiment 2015 Nova Aurora (R14), Iracema do Oeste (R15 and R16), Jesuitas (R17 and R18), Tupãssi (R19), Jotaesse (R20), Espigão Azul (R21) and Palmitopolis (S22- susceptible). In 2012, it was possible to confirm a moderate resistance (RD = 1.1 to 5.0) only in R03; and populations R01, R02 and R04 were highly resistant (RD>5.0). In 2013, populations R09, R10, R11 and R12 showed resistance, while R06, R07 and R08 were highly resistant. In 2015, the R14, R16, R18, R19 and R20 populations showed moderateresistance, and R15 and R18 were highly resistant. Only R21 was susceptible. It was found that 83% of producers use the no-tillage system, 100% use the succession soybean/corn with 90% transgenic varieties, 45% of producers cannot explain the procedure regulation/sprayer calibration. Around 62% use practices to prevent the selection of resistant biotypes. Resistance was confirmed in 89.5% of the populations. The adopted agronomic practices may explain these results.

Keywords: sourgrass, agricultural practices, weed, herbicide.

RESUMO - O uso intensivo de glyphosate pode selecionar populações resistentes. Objetivou-se neste estudo avaliar a ocorrência de resistência em populações de ***D. insularis*** *ao glyphosate em áreas agrícolas. Foram realizados três experimentos no delineamento experimental de blocos ao acaso com quatro repetições. O Grau de Resistência (GR) foi determinado por meio de curva de dose-resposta nas populações avaliadas: Experimento 2012: São Camilo (R01 e R02) e Palotina (R03, R04 e S05-suscetível); Experimento 2013: Palotina (R06 e R12), Katuete/ Paraguai (R07), Toledo (R08 e R10), Cascavel (R09), São Miguel do Iguaçu (R11) e Dom Eliseu/Pará (S13-suscetível); Experimento 2015: Nova Aurora (R14), Iracema do Oeste (R15 e R16), Jesuítas (R17 e R18), Tupãssi (R19), Jotaesse (R20), Espigão Azul (R21) e Palmitópolis (S22-suscetítel). Em 2012 foi confirmada resistência moderada (GR=1,1 a 5,0) apenas na R03, sendo que as populações R01, R02 e R04 foram altamente resistentes (GR>5,0). Em 2013, as populações R09, R10, R11 e R12 apresentaram resistência moderada, enquanto R06, R07 e R08 mostraram-se altamente resistentes. Em 2015, as populações R14, R16, R18, R19 e R20 apresentaram-se moderadamente resistentes, e R15 e R18 foram altamente resistentes. Apenas a R21 foi suscetível. Verificou-se que 83% dos produtores*

*** Corresponding author:**
<neumarcio.costa@unioeste.br>

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[1] Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon-PR, Brasil; [2] Universidade Estadual de Maringá, Umuarama- PR, Brasil.

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utilizam plantio direto, 100% utilizam a sucessão soja/milho com 90% de variedades transgênicas e 45% dos produtores não sabem explicar o procedimento regulagem/calibração do pulverizador. Cerca de 62% utilizam práticas para evitar a seleção de biótipos resistentes. Foram confirmadas resistências em 89,5% das populações. As práticas agronômicas adotadas podem justificar esses resultados.

Palavras-chave: capim-amargoso, práticas agrícolas, planta daninha, herbicida.

INTRODUCTION

Weed resistance to herbicides is an adaptive response to agricultural practices (Monquero and Christoffoleti, 2001). Thus, resistant weed biotypes were selected in response to the environmental disturbance caused by the selection pressure of herbicides provided by their intensive use over the last decades (Christoffoleti and López-Ovejero, 2003; Machado et al., 2006).

The concept of resistance consists in the natural and inheritable ability of certain biotypes within a population to survive and reproduce after the exposure to doses of herbicides that would be lethal to normal (susceptible) individuals from the same species (Christoffoleti and López-Ovejero, 2003). Thus, the countries with the highest numbers of glyphosate-resistant plants are the United States, Argentina and Brazil, since they are large consumers of glyphosate and intensively use high doses, which can more frequently select resistant populations (Heap, 2016).

Among the species that present resistant biotypes in Brazil, it is possible to highlight *Digitaria insularis*, whose resistance to glyphosate has already been confirmed in the states of São Paulo, Minas Gerais and Paraná (Lacerda and Victória Filho, 2004).

It is worth mentioning that agricultural producers from the western region of Paraná empirically reported the occurrence of resistance to glyphosate in populations of *D. insularis*; in most cases, suspicions of resistance in these weed populations have not been scientifically proven.

The observation of initial resistance is usually based on the unsatisfactory control of weeds after herbicide application, but this also occurs when there are usage defects in the application techniques. Thus, it is important to make careful observations on the field and in the laboratory, in the sense that any reduction in herbicide efficiency can be detected (Christoffoleti and López-Ovejero, 2003).

Therefore, the hypotheses of this work are based on the fact that the management used to control weeds in agricultural areas is selecting glyphosate-resistant biotypes in populations of *D. insularis*. The objective of this study was to evaluate the occurrence and quantification of glyphosate resistance levels in populations of *D. insularis*, characterizing the agronomic practices adopted in the agricultural areas.

MATERIAL AND METHODS

Three experiments were conducted in plastic pots maintained under field conditions. The first one was developed in the agricultural year of 2012, the second one in 2013 and the third one in 2015. A randomized block design with four replications was used. For each experiment, a susceptible population was used as a control treatment.

Table 1 describes the collection sites, as well as the year of the experiment, geographic coordinates and sowing system frequently used in the area. Specifically in the experimental year 2013, two populations did not originate in regions of the State of Paraná: Dom Eliseu, from the State of Pará (S13), and Katuete, from Paraguay (R07). All seeds were collected in areas of agricultural cultivation when seed dispersal by the wind was observed. At that moment, *D. insularis* panicles were wrapped in recyclable paper bags to collect as many mature seeds as possible.

In order to better understand the process of selecting populations of *D. insularis* that are resistant to glyphosate, at the time of seed collection from each population, a questionnaire was applied to producers, whose nine questions are described in Table 2.

Table 1 **-** Characterization of collection points in each experimental year

Year	Population	Location	Coordinates	Sowing system
2012	R01	São Camilo(1)	24º14’35.89” S / 53º53’35.73” W	No-tillage
	R02	São Camilo(1)	24º12’01.78” S / 53º56’43.84 W	Conventional
	R03	Palotina	24º15’18.98” S / 53º53’42.25W	No-tillage
	R04	Palotina	24º16’18,64” S / 53º53’15.58 W	No-tillage
	S05	Palotina	24º14’03.54” S / 53º52’03.04 W	No-tillage
2013	R06	Palotina	24˚14’03.54” S / 53˚52’03.04 W	No-tillage
	R07	Katuete – PY(2)	25˚40’21.31” S / 54˚44’11.60 W	No-tillage
	R08	Toledo	24º71’48.97” S / 53˚68’63.28 W	No-tillage
	R09	Cascavel	24˚47’39.47” S / 53˚41’39.51 W	No-tillage
	R10	Toledo	24˚42’32.24” S / 53˚49’05.18 W	No-tillage
	R-11	São Miguel do Iguaçu	25˚25’21.24” S / 54˚13’02.99 W	No-tillage
	R12	Palotina	24º17’37.0 S / 53º49’41.9 W	Conventional
	S13	Dom Eliseu – PA(3)	04˚15’18” S / 47˚35’29 W	Conventional
2015	R14	Nova Aurora	24º27’51.90” S / 53º17’58.20 W	No-tillage
	R15	Iracema do Oeste	24º27’17” S / 53º20”19.10 W	No-tillage
	R16	Iracema do Oeste	24º34’28.30 S / 53º25’03.80 W	No-tillage
	R17	Jesuítas	24º30’06.00 S / 53º23’50” W	No-tillage
	R18	Jesuítas	24º27’17” S / 53º20’19,1 W	No-tillage
	R19	Tupãssi	24º38’29.9 S / 53º28’34.0 W	No-tillage
	R20	Jotaesse(4)	24º34’39.5 S / 53º30’24.4 W	Conventional
	R21	Espigão Azul(5)	24º49’18.2 S / 53º27’39.2 W	No-tillage
	S22	Palmitopolis(6)	24º34’57.4S / 53º22’36.5 W	No-tillage

R- resistant population. S- susceptible population (control treatment). (1) District of Palotina-Paraná, (2) Paraguay-PY, (3) State of Pará-Brazil, (4) Village of Tupãssi-Paraná, (5) District of Cascavel-Paraná, (6) District of Nova Aurora-Paraná.

Table 2 **-** Questionnaire submitted to producers at the time of collection of *Digitaria insularis* populations

Question	Option
1. Main cultivation system used in the property.	Conventional - No-tillage - Organic
2. Do you use crop rotation?	See producer response
3. What are the main herbicides used? 4. Do you use transgenic varieties?	See producer response Yes – No
5. Do you use the recommended dose of herbicides?	Yes – No
6. Do you understand the concept of resistance? 7. Do you use management practices to avoid resistance? 8. Do you adjust/calibrate the sprayer before the applications? 9. Can the producer explain how to perform the sprayer adjustment/calibration procedure?	Yes – No Yes – No Yes – No Yes – No

The resistance degree (RD) was determined using a dose-response curve with the following glyphosate doses for the experimental years 2012 and 2013: 0, 43, 90, 360, 720, 1,440, 2,880, 7,200 g ha^{-1}. For the experimental year 2015, doses were 0, 28, 56, 112, 225, 450, 900, 1,800, 3,600 and 7,200 g ha^{-1}. A commercial formulation containing 360 g L^{-1} of acid equivalent and without the addition of adjuvant was used. Applications were made when the plants had two to three tillers.

Populations were individually seeded in polystyrene trays for germination and subsequent transplanting of the seedlings in plastic pots filled with soil. The experimental units were represented by a plastic pot with a capacity of 3.0 dm^3, filled with soil with the following chemical composition: 13.95 mg dm^{-3} of P; 0.36 cmol dm^{-3} of K +; 4.34 cmol dm^{-3} Ca; and 1.23 cmol dm^{-3} of Mg.

Transplanting was performed 20 days after sowing, when *D. insularis* plants had a leaf and a well developed root system. The standardization of four plants per experimental unit (plastic pot) was carried out.

In the application of the herbicide treatments, a CO_2 pressurized backpack sprayer was used, working at a constant pressure of 2.0 kgf to obtain a spray volume corresponding to 200 L ha^{-1}. The bar was composed of four fan-type XR 110.02 nozzles spaced 0.5 m apart and positioned at 0.5 m from the target. Climatic conditions during the applications ranged from 26.2 to 30.1 °C, relative humidity from 54 to 60% and wind speed from 3.2 to 4.6 km h^{-1}.

The visual control evaluation was carried out 30 days after the application (DAA), using a 0 to 100% scale, in which 0% corresponds to non-control and 100% to the plant death, proposed by the Brazilian Society of Weed Science (Sociedade Brasileira da Ciência das Plantas Daninhas - SBCPD, 1995). After completing the visual evaluations, the analysis of the dry matter (g) of plants was carried out, and plants were collected and stored in paper bags and placed in a forced air circulation oven at 65 °C for 72 hours, until reaching constant weight.

The results obtained in the experiments were submitted to the F test in the analysis of variance. Data were adjusted to the logic-biological non-linear regression, represented in Equation 1:

$$y = \frac{a}{1 + e^{-\left(\frac{x - C_{50}}{b}\right)}}$$

where y is the control percentage; "x" represents the herbicide dose (g ha^{-1}); and "b" and "a" are curve parameters, so that "b" corresponds to the slope of the curve, "a" represents the upper limit of the curve and "C_{50}" corresponds to the dose providing 50% of the variable response - the lower limit of the curve was considered zero.

After the statistical analyses, it was possible to the resistance degree (RD) related to the use of glyphosate doses and required to provide 50% control on plants, adapted from Seefeldt et al. (1995). According to the results, populations were classified as susceptible with RD≤1.0, moderately resistant with an RD between 1.1 and 5.0 and highly resistant with RD>5.0.

RESULTS AND DISCUSSION

Table 3 shows the resistance levels of the populations, as well as the parameters evaluated for dose-response curves.

During the experimental year 2012, populations R01 and R02 were classified as highly resistant (RD>5.0), population R03 presented moderate resistance (RD = 1.1 to 5.0), while population R04 was classified as susceptible (RD≤1.0).

In the experimental year 2013, the populations from Palotina (R06 and R12), Toledo (R09 and R08) and Cascavel (R10) presented high resistance; 62% of the populations were evaluated. The population of Paraguay (R07) presented high resistance (RD = 7.9). Some researchers point out that resistant biotypes found in Paraná come from this region of the neighboring country, since the first occurrence of resistance in *D. insularis* was found in Paraguay in 2006 (Heap, 2016). However, this information needs to be confirmed with genomic studies between Brazilian and Paraguayan biotypes.

In the experiment conducted in 2015, populations R15 (Iracema do Oeste) and R17 (Jesuítas - Paraná state) were the ones with the highest resistance degrees, with an RD of 7.9 and 12.5, respectively. These locations are about 50 km away from the region of Toledo and São Camilo, where populations showed a high degree of resistance (RD>5.0). Probably, seed dispersal may have occurred among these locations, since they are easily carried by the wind due to the caryopsis included in their chaff (Kissmann and Groth, 1997). Another important point to be observed is the chemical management used to control *D. insularis*, which is probably selecting resistant biotypes of this species.

It is possible to observe that the great majority of the evaluated populations ranged from moderately resistant to highly resistant. Licorini et al. (2015), in studies with different populations

Table 3 - Resistance degree (RD) of populations of *D. insularis* collected in agricultural areas, based on control percentage, 30 days after application

Year	Population	$y = \dfrac{a}{1 + e^{-\left(\frac{x - C_{50}}{b}\right)}}$					
		a	*b*	C_{50}	R^2	*P*	RD
2012	R01	93.7468	513.7712	1193.1870	0.97	<0.0001	21.2
	R02	84.4114	438.9998	741.4787	0.83	0.0111	13.1
	R03	92.6560	151.1182	246.5553	0.93	0.0011	4.4
	R04	100.8447	19.1496	51.9011	0.99	<0.0001	1.0
	S05	99.9158	15.1750	56.4083	0.99	<0.0001	1.0
2013	R06	100.0537	208.3990	1436.3904	0.99	<0.0001	6.0
	R07	100.9667	473.7762	1889.2987	0.99	<0.0001	7.9
	R08	99.9221	514.1456	2438.0736	0.99	<0.0001	10.2
	R09	93.7708	172.6850	1201.4570	0.99	<0.0001	5.0
	R10	90.5208	310.0051	1096.9060	0.91	0.0024	4.6
	R11	91.0890	235.4546	1144.3032	0.95	0.0030	4.8
	R12	94.1316	170.5039	1207.2096	0.99	<0.0001	5.0
	S13	98.7030	58.8093	237.4046	0.99	<0.0001	1.0
2015	R14	100.4339	100.1183	231.1867	0.98	<0.0001	2.9
	R15	95.6211	760.4443	629.3600	0.82	0.0023	7.9
	R16	85.6515	218.0721	251.2081	0.90	0.0003	3.1
	R17	98.5216	951.3884	993.6637	0.81	0.0026	12.4
	R18	88.1046	234.2751	256.1433	0.86	0.0009	3.2
	R19	100.7695	55.5004	89.8850	0.94	<0.0001	1.1
	R21	100.1140	33.8552	81.0097	0.99	<0.0001	1.0
	R20	100.3045	51.5945	111.9962	0.99	<0.0001	1.4
	S22	99.9948	11.9522	80.1216	0.99	<0.0001	1.0

R- populations with suspected resistance to glyphosate. S- populations susceptible to glyphosate.

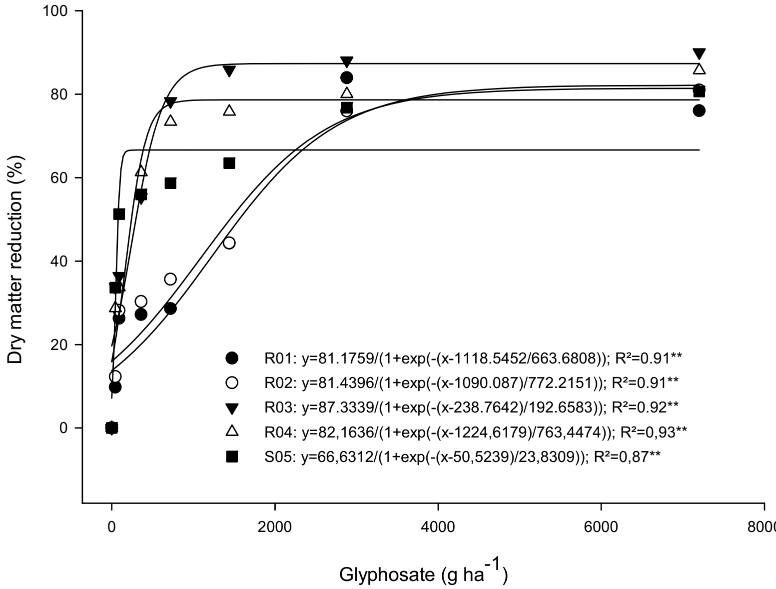
of glyphosate-resistant *D. insularis*, confirmed the occurrence of this weed's resistance in the cities of Cascavel (RD = 16.88), Maringá (RD = 9.12) and Palotina (RD = 6.32), in Paraná.

Figures 1, 2 and 3 show data about the dry matter reduction (%) at 30 DAA for the experimental years 2012, 2013 and 2015, respectively.

In the 2012 experiment, like what was verified in visual control data, it is possible to observe that populations R01 and R02 were considered highly resistant (RD = 22.14 and 21.56, respectively), while the R03 population was considered moderately resistant (RD = 4.73). Based on the dry matter the population R04 was considered highly resistant (GR = 24.24). This can be explained by the variation in plant size, where the behavior of the populations with higher dry matter accumulation at the beginning of the evaluations adjusted to models that determined the need to use high doses for biomass reduction, even though they were considered susceptible by visual control data (Table 3), and vice versa. However, for the R04 population the dry matter reduction data confirm the high resistance in disregard of visual control data at 30 DAA.

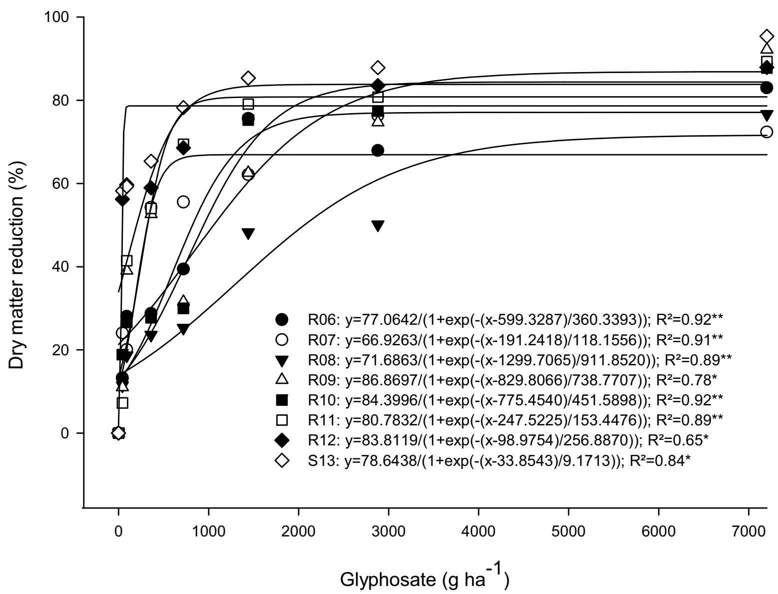
To reduce by 50% the dry matter of the shoot of the R04 population, a dose of 1,224.61 g ha^{-1} was necessary, while in the susceptible population S05 the same reduction was provided by a dose of 50.52 g ha^{-1}. Similarly, Reinert et al. (2013) found that a glyphosate dose of 108.70 g ha^{-1} reduced by 50% the shoot dry matter of susceptible *D. insularis* populations, requiring the use of 1,811,20 g ha^{-1} to obtain the same effect in the resistant population; these values are similar to those obtained in this study.

Melo et al. (2012), while evaluating the control of glyphosate-resistant and -susceptible *D. insularis*, observed that the glyphosate doses required for a satisfactory control were 310.9 and 632.6 g ha^{-1} for resistant plants, and 175.5 and 474.1 g ha^{-1} for susceptible plants.



**significant at 5% probability by F test.

***Figure 1* -** Dry matter reduction (%) of resistant and susceptible *D. insularis* populations, 30 days after the application of glyphosate in the experimental year of 2012.

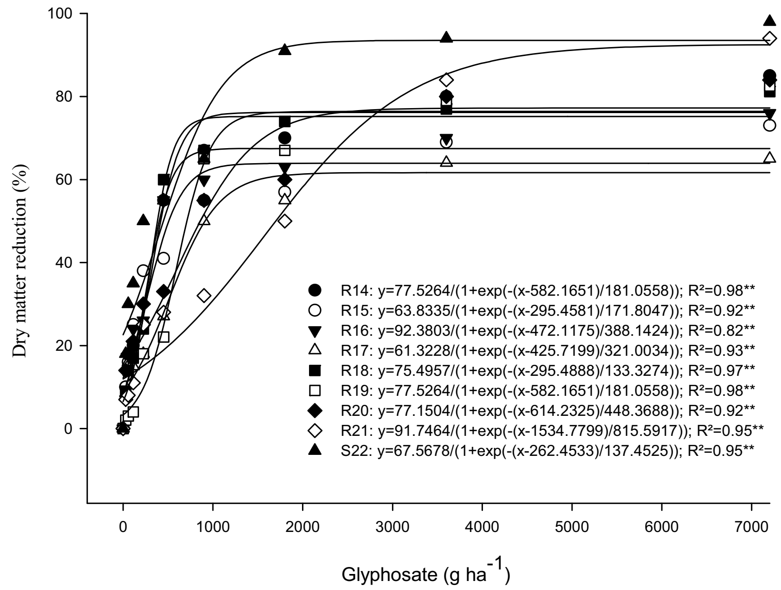


* a **significant at 1 and 5% probability by F test, respectively.

***Figure 2* -** Dry matter reduction (%) of resistant and susceptible *D. insularis* populations, 30 days after the application of glyphosate in the experimental year of 2013.

In the experimental year 2013, only the R07 and R08 populations showed the lowest dry matter reductions with the highest glyphosate dose, in relation to the others (Figure 2). For the 2015 experiment, it was observed that the R21 population was considered highly resistant (RD = 5.84), but it is possible to observe that at the highest evaluated dose, the reduction was equivalent to that of the susceptible population S22 (Figure 3). The other populations were considered moderately resistant.

Generally speaking, these results confirm the occurrence of resistance to glyphosate in populations of *D. insularis* in agricultural areas of western Paraná.



** significant at 5% probability by F test.

Figure 3 **-** Dry matter reduction (%) of resistant and susceptible *D. insularis* populations, 30 days after the application of glyphosate in the experimental year 2015.

After submitting the questionnaire to producers from the collection areas of the period from 2012 to 2015, data were transformed into percentage and presented graphically (Figure 4).

Approximately 83% of the 22 interviewed producers practiced the no-tillage system, and 100% used the soybean/maize succession system. Producers justify the use of this system as the best economic alternative for their property.

The main herbicides were glyphosate at the mean dose of 928.8 g ha^{-1} and 2,4-D at the mean dose of 443.3 g ha^{-1}, applied alone or in a tank-mix in the desiccation management of weeds for the no-tillage system; they were used by 90% of the producers.

It is also worth mentioning that 96% of the producers use transgenic varieties with glyphosate resistance technology. This contributes to the excessive use of glyphosate applications per year (≥3) and at higher doses than the recommended one (≥720 g ha^{-1}), since only 78% of the producers use the recommended dose. These results support those obtained by Vargas et al. (2013), which

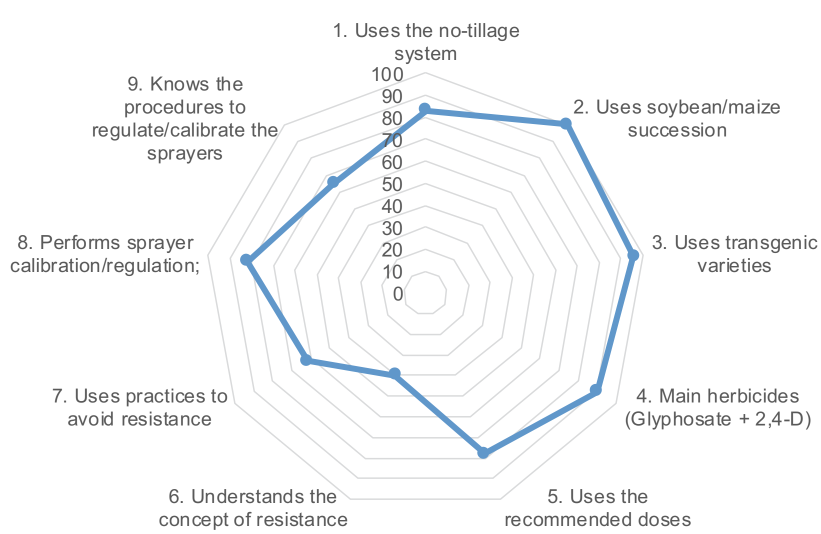


Figure 4 **-** Results of the questionnaires submitted to agricultural producers for the experiments conducted in 2012, 2013 and 2015.

found that approximately 80% of farmers in Rio Grande do Sul performed three or more applications of glyphosate per year, and 76% cultivated RR soybean for five years or more. These authors also emphasized that the intensive use of glyphosate can exert a high selection pressure on weed populations, and the advantages derived from the control of these weeds in transgenic crops can be lost in the short or medium run, making it impossible to use this technology.

It is worth highlighting that failures in the control of *D. insularis* populations with the use of glyphosate have occurred in areas where the number of applications of this herbicide has gradually increased. Thus, the intensified use of glyphosate increases the risk of selection of resistant biotypes (Beckie and Reboud, 2009; Melo et al., 2012).

Only 36% of the interviewed farmers understand the concept of resistance, although 60% affirm that they adopt practices to avoid weed resistance selection in their cultivated areas.

Approximately 82% of the producers stated that they perform the correct regulation of the spraying equipment. However, when asked if they could explain the adjustment and calibration procedure of sprayers, only 65% could explain them clearly and correctly. The lack of attention by producers on the regulation and calibration of sprayers before the applications can indicate the use of doses above or below the recommendation, besides increasing the risk of contamination of the applicator and of the environment.

Bauer et al. (2009) verified that, after diagnosing conditions, time of use and maintenance in sprayers in the State of Mato Grosso do Sul, 51.1% of them had nozzles that should have been replaced, since they had a flow variation higher than the established limits and the equipment was being used outside the legal specifications.

In general, these results may contribute to a better understanding of the selection process of herbicide-resistant weed biotypes in agricultural production areas, since it was verified that most producers use only a system of culture succession and doses of glyphosate, successively, throughout the year and for several years.

Based on the results, glyphosate resistance was confirmed in 89.5% of the evaluated *D. insularis* populations, and it is possible to infer that the agronomic practices adopted in the agricultural areas contribute to the selection process of glyphosate-resistant *D. insularis*.

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