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Article

GOOSEGRASS RESISTANT TO GLYPHOSATE IN BRAZIL

Capim-Pé-de-Galinha Resistente ao Glyphosate no Brasil

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ABSTRACT - Several cases of herbicide resistance in goosegrass have been confirmed worldwide. Reports of control failures after glyphosate application have been observed, especially in the Midwest region of Paraná State. The objective of this study was to evaluate the existence of goosegrass populations resistant to glyphosate. For this, 25 populations collected in two consecutive seasons (2013/2014 and 2014/2015) were sown and grown in greenhouse. Glyphosate dose-response curve experiments were performed using doses of 0, 60, 120, 240, 480, 960, 1,920, 3,840, 7,680 and 15,360 g a.e. ha^{-1}. The application stages were from two to three tillers (E1) for the populations of 2013/2014 and E1 and five to six tillers (E2) for the populations of 2014/2015. Furthermore, three of the populations supposedly considered resistant in these experiments (populations 7, 19 and 25) have had their F1 submitted to the herbicide dose-response test in order to verify whether the resistance was inheritable. With the results obtained in this study, it was concluded that the populations 19 and 25, from Campo Mourão and Luziânia (Midwest of Paraná) are the first confirmed cases of goosegrass resistant to glyphosate in Brazil (RF = 3.99 to 6.81), following all the criteria for confirmation of new weed resistance cases.

Keywords: *Eleusine indica*, dose-response, application timming, resistance factor.

RESUMO - Diversos casos de resistência de capim-pé-de-galinha têm sido reportados mundialmente. Relatos de falhas de controle pela aplicação de glyphosate têm sido cada vez mais frequentes, especialmente na região Centro-Oeste do Paraná. O objetivo deste trabalho foi avaliar a existência de populações de capim-pé-de-galinha resistentes ao glyphosate. Para isso, 25 populações coletadas em duas safras consecutivas (2013/2014 e 2014/2015) foram semeadas e cultivadas em casa de vegetação. Experimentos de curva de dose-resposta de glyphosate foram realizados utilizando-se as doses de 0, 60, 120, 240, 480, 960, 1.920, 3.840, 7.680 e 15.360 g e.a. ha^{-1}. O estádio de aplicação foi de dois a três perfilhos (E1) para as populações de 2013/2014 e E1 e cinco a seis perfilhos (E2) para as populações de 2014/2015. Além disso, três das populações consideradas supostamente resistentes nesses experimentos (populações 7, 19 e 25) tiveram suas gerações F1 submetidas ao ensaio de dose-resposta do herbicida, visando verificar se a resistência era herdável. A partir dos resultados obtidos neste trabalho, conclui-se que as populações 19 e 25, oriundas de Campo Mourão e Luziânia (Centro-Oeste do Estado do Paraná), constituem o primeiro relato de capim pé-de-galinha resistente ao glyphosate no Brasil (RF = 3,99-6,81), atendendo a todos os critérios estabelecidos para confirmação de novos casos de resistência.

Palavras-chave: *Eleusine indica*, dose-resposta, estádio de aplicação, fator de resistência.

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INTRODUCTION

The weed resistance to herbicides is one of the main challenges in agriculture worldwide, especially in recent years with the number of cases of species resistant to herbicides increasing substantially. There are currently 41 cases of weed resistant to herbicide in Brazil, of which seven species are resistant to glyphosate, as follows, *Lolium perenne* ssp. *multiflorum*, *Conyza bonariensis*, *C. canadensis*, *C. sumatrensis*, *Digitaria insularis*, *Chloris elata* and *Amaranthus palmeri* (Carvalho et al., 2011; Heap, 2016).

For a population of a certain weed to be considered as a new case of resistance, it must meet the following criteria: 1. Survive and reproduce at a dose of herbicide normally lethal to the wild population; 2. Confirmation with results obtained through science-based protocols; 3. The resistance should be inheritable to subsequent generations; 4. The problem must have a practical impact on the field; 5. Botanical identification of the species of the weed in question (Heap, 2005; Cristoffoleti and Lopez-Ovejero, 2008).

The goosegrass is considered one of the most troublesome weeds, and is found in many regions of the world. It presents a fast growth, autogamy, C4 cycle and high seed production (over 120,000 plant^{-1}), which are spread by the wind (Kissmann and Groth, 1997; Mueller et al., 2011; Takano et al., 2016.).

The first glyphosate resistance report on goosegrass occurred in orchards in Malaysia in 1997 (Lee and Ngim, 2000). Thereafter, resistant populations were found in different continents around the world (Yuan et al., 2005; Kaundun et al, 2008; Mueller et al, 2011; Molin et al., 2013; Heap, 2016). In addition to the resistance to glyphosate, some populations of goosegrass may have resistance up to four mechanisms of action simultaneously (EPSPS, ACCase, Photosystem I and GS-GOGAT) (Jalaludin et al., 2014).

Although there are no goosegrass population resistant to glyphosate in Brazil, populations with low levels of resistance have been detected in Rio Grande do Sul (Vargas et al., 2013). Even though it is important for the scientific community, this kind of resistance has low practical impact on the field, since the recommendation dose is normally lethal to these populations. However, several technicians and producers in the Midwest region of Paraná state have seen goosegrass plants surviving the application of glyphosate in their crops.

Considering this context, the objective of this study was to evaluate the existence of goosegrass populations resistant to glyphosate in Brazil.

MATERIAL AND METHODS

Goosegrass seeds were collected in harvests in 2013/2014 and 2014/2015 in areas with a history of glyphosate applications and grown in succession system soybean/corn, on which the soybean cultivation, in all cases, were cultivars resistant to glyphosate. In these areas, the collection was done in at least 20 random plants not controlled by the application of glyphosate in the soybean's post emergence, forming a *bulk.*

In the season 2013/2014, the collections were held in 13 locations in the states of Parana, Santa Catarina and Goiás. In the same period, a well known susceptible population was collected and used as a standard. Depending on the results obtained in the first year, in the season 2014/2015, the collection was carried out only in the state of Paraná, in 12 locations. Each location was considered a distinct population of goosegrass and the collection period was during the months of January and February in 2014 and 2015 (Table 1). Plants in reproductive stage and representative of each population were properly identified in the Herbarium of the State University of Maringá (HUEM).

To confirm the resistance in science-based protocol, three experiments were conducted. The experiment 1 was made for 14 populations from 2013/2014, the experiment 2 for 13 populations from 2014/2015, and the experiment 3, for the F1 generation of the populations 7, 19 and 25. All experiments were conducted in a completely randomized outlining. Experiments 1 and 3 were arranged in double factorial scheme, with the first factor composed of different populations and the second factor for 10 doses of glyphosate (0, 60, 120, 240, 480, 960, 1,920,

Table 1 **-** Geographical coordinates of the different collection sites of *Eleusine indica populations in* seasons 2013/2014 and 2014/2015

Population	Season	Coordinates	City	State
Susceptible	2014	S 23º 19'35" W 52º 00'05"	Mandaguaçu	PR
1	2014	S 24º 27'24" W 54º 16'16"	Rondon	PR
2	2014	S 24º 20'42" W 53º 24'57"	Jesuítas	PR
3	2014	S 23º 51'10" W 52º 11'29"	Quinta do Sol	PR
4	2014	S 24º 13'25" W 52º 27'56"	Goioerê	PR
5	2014	S 24º 20'34" W 52º 33'25"	Mamborê	PR
6	2014	S 23º 19'11" W 51º 18'54"	Rolândia	PR
7	2014	S 24º 46'32" W 51º 45'42"	Pitanga	PR
8	2014	S 18º 46'37" W 52º 37'02"	Chapadao do Céu	GO
9	2014	S 26º 23'22" W 52º 48'45"	São Lourenço do Oeste	SC
10	2014	S 24º 12'59" W 52º 57'46"	Campo Mourão	PR
11	2014	S 24º 18'39" W 52º 43'16"	Juranda	PR
12	2014	S 24º 12'46" W 52º 48'32"	Boa Esperança	PR
13	2014	S 24º 06'27" W 52º 51'25"	Campo Mourão	PR
14	2015	S 24º 04'11" W 52º 52'51"	Moreira Sales	PR
15	2015	S 24º 13'44" W 52º 50'53"	Goioerê	PR
16	2015	S 24º 14'06" W 52º 52'22"	Boa Esperança	PR
17	2015	S 24º 01'52" W 52º 49'27"	Janiópolis	PR
18	2015	S 24º 02'27" W 52º 52'33"	Janiópolis	PR
19	2015	S 24º 09'29" W 52º 29'04"	Campo Mourão	PR
20	2015	S 24º 07'12" W 53º 13'37"	Mariluz	PR
21	2015	S 24º 01'52" W 52º 49'07"	Janiópolis	PR
22	2015	S 24º 02'42" W 52º 27'55"	Campo Mourão	PR
23	2015	S 24º 08'19" W 52º 28'50"	Campo Mourão	PR
24	2015	S 24º 13'51" W 52º 52'31"	Rancho Alegre	PR
25	2015	S 24º 13'09" W 52º 18'05"	Luziânia	PR

3,840, 7,680 and 15,360 g e.a. ha^{-1}). The experiment 2, on the other hand, was arranged in triple factorial scheme had it's the first factor composed of different populations, the second factor by the same 10 doses of glyphosate and the third factor of two stages of development.

In all experiments, the experimental units were composed by jars of 1 dm^3, which were filled with soil up to the half presenting pH values in water of 5,9; 11,89 g dm^{-3} of C; 720 g kg^{-1} of sand; 20 g kg^{-1} of silt and 260 g kg^{-1} of clay. Above this layer of soil, the experimental units were filled with coconut fiber substrate, aiming to prevent the emergence of possible seeds of weeds present in the soil used.

Thirty seeds of each population were sown per pot and after the emergence a thinning was performed, keeping three seedlings per experimental unit. For the 2013/2014 populations, the stage of the plants at the time of the application was from two to three tillers per plant, with two to three leaves per tiller and 5-8 cm (E1). As for the 2014/2015 populations, in addition to E1, plants were also evaluated in 5-6 tiller stage and 15 cm. Surviving plants from populations 7, 19 and 25 were grown separately and their seeds were collected to form their F1 generations.

The applications were made by spraying pressurized CO_2, equipped with a 1.5 m long bar containing three spray tips AI 110.02 type (0.5 m between tips), with pressure of 2.5 kgf cm^{-2}, providing an application volume equivalent to 200 L ha^{-1}. Applications were always held in the morning in suitable weather conditions.

The recommended registration dose of glyphosate to control goosegrass varies between 720 and 1,200 g e.a. ha^{-1} in more than 80% of this herbicide's trademarks (Brazil, 2015), which is the range of doses normally used by most producers in the region. In addition, several literature studies report that the application of 840 g e.a. ha^{-1} of glyphosate provides goosegrass control above 96% (Corbett et al., 2004; Thomas et al., 2006; Clewis et al., 2006). Therefore, the average value recommendation was considered as a reference dose for setting the dose response curve (960 g ha^{-1}).

Was evaluated the control percentage (visual scale from 0 to 100%) at 28 DAA, where 0% represents no injury and 100% represents the plant's death. At the end of this period, we also determined the dry mass of the aerial part, by collecting the plant's aerial part which survived the application and subsequent drying in a greenhouse at 65 °C for 72 hours.

Data were submitted to analysis of variance and regression, adjusted to logistic nonlinear regression model proposed by Streibig (1988):

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

where: *y* is the percentage control or the dry mass of the aerial part; *x* is the dose of the herbicide (g e.a. ha^{-1}); and *a*, *b* and *c* are the equation's estimated parameters, in such a way that: *a* is the amplitude existent between the maximum and the minimum point of the variable; *b* is the dose which promotes a 50% response of the variable and *c* is the declivity of the curve around *b*.

One of the terms integrating the equation of the logistic model *(b)* is an estimate of C_{50} or $GR_{50,}$ which represent the herbicide dose required for control or reduction of the plant's dry mass by 50% (Christoffoleti 2002). Although one of the logistic model parameters is an estimate of the value of $C_{50,}$ it was also decided to perform its mathematical calculation by inverse equation, according to the discussion proposed by Carvalho et al. (2005).

$$x = b\left(\left|\frac{a}{y} - 1\right|\right)^{\frac{1}{c}}$$

In addition to the C_{50} or GR_{50} values, were also obtained the values of C_{80} and GR_{80}, using the inverse equation. The estimate of these values is important from a practical point of view, since it is the dose required to achieve minimum levels of control considered acceptable in the field. Based on the values of C_{50} and GR_{50}, we calculated the resistance factor (RF = C_{50} or GR_{50} from the supposedly resistant population/C_{50} or GR_{50} from the susceptible population) to the resistant populations. The resistance factor expresses the number of times on which the necessary dose to control 50% of the resistant population is superior to the dose that controls 50% of the susceptible population (Hall et al., 1998).

RESULTS AND DISCUSSION

The goosegrass plants had their species identified as *Eleusine indica* (Linn.) Gaertn, Poaceae family (protocol number: HUEM 30031).

The analysis of data variance showed there were significance for the variation sources, as well as the interaction between populations and doses. There was a proper adjustment of the model to the control data and dry mass of the aerial part, setting *a*, *b* and *c* parameters of the log-logistic equation. The R^2 values were close to 1, indicating adequate model adjustment to the observed data. From the adjusted models, it was possible to calculate, to the control data and the dry mass of aerial part, the doses necessary to control 50% and 80% of each population tested.

For the variable control in the season 2013/2014, with the exception of population 8, all populations showed RF>1.0 (Table 2). However, in most of them, the glyphosate dosage necessary for the control or reduction of dry mass was 80% lower than the dose recommended for the control of the species. On the other hand, populations 5 and 7 presented C_{80} of 1,217.73 and 1,443.01 g e.a. ha^{-1} and GR_{80} 1,102.74 and 1,248.20, respectively, which is greater than the average dose recommended for the species. In these cases where the recommended dose did not provide satisfactory control, the RF varied between 4.16 and 7.99. For the susceptible population, the dry mass of the aerial part was reduced by 80% with 172.18 g e.a. ha^{-1} of glyphosate.

Table 2 **-** Estimated glyphosate doses for control and reduction of dry mass in the aerial part in 505 and 80% and their resistance factors for different populations of *Eleusine indica* on stage of two to three tillers (E1) (season 2013/2014)

Population	C_{50}	C_{80}	RF_{50}	GR_{50}	GR_{80}	RF_{50}
	(g e.a. ha^{-1})					
Susceptible	100.45	150.78	-	100.97	172.18	-
1	194.94	284.24	1.94	152.39	353.92	1.51
2	278.15	389.06	2.77	110.99	235.05	1.10
3	154.12	212.92	1.53	99.74	216.45	0.99
4	162.26	249.62	1.62	133.23	322.51	1.32
5	604.72	1217.73	6.02	420.34	1102.74	4.16
6	117.08	164.61	1.17	61.05	118.89	0.60
7	802.43	1443.01	7.99	726.13	1248.20	7.19
8	88.32	140.71	0.88	59.62	122.28	0.59
9	248.35	393.99	2.47	225.16	348.26	2.23
10	276.01	402.30	2.75	81.31	260.33	0.81
11	105.11	142.72	1.05	61.59	114.68	0.61
12	313.98	479.52	3.12	167.25	375.21	1.66
13	179.71	258.01	1.79	103.38	168.22	1.02

C_{50} or C_{80}: Necessary dose to control the population in 50% and 80%; GR_{50} or GR_{80}: Necessary dose to reduce the dry mass of the aerial part of the population in 50% and 80%; FR_{50}: resistance factor (C_{50} or GR_{50} of the resistant population/C_{50} or GR_{50} of the susceptible population.

In the season 2014/2015, for the first stage (E1), based on the results of C_{50} and $GR_{50,}$ all populations showed RF>1.0 (Table 3).The values of C_{80} and GR_{80} were higher than the recommended dose only for the populations 19 and 25. In the case of population 19, the value of GR_{80} (1,653.49 g e.a. $ha^{-1)}$ was higher than the highest recommended dose ever recorded for goosegrass control (1,620 g e.a. ha^{-1}).

The results obtained for the 2015 population which received application in E2 stage showed lower susceptibility to glyphosate in all populations compared to the E1 stage (Table 4). Except for the populations 23 and 24, C_{80} and GR_{80} values of all populations were greater than 960 g e.a. ha^{-1}. This shows the importance of the stage of the plants at the time of the application, which may be collaborating with application failures observed in the field.

Glyphosate applications in goosegrass in early development stage (E1) usually imply more effective, because it is the moment of greatest susceptibility of these plants to herbicides (Ulguim et al., 2013). One of the goosegrass characteristics is its rapid tillering capacity, as the stadium of 2-3 tillers and 5-6 tillers can be reached at nine and twenty-four days after its emergence (DAE) respectively (Takano et al., 2016). The rapid growth of this species suggests that, for operational reasons, applications in the field cannot always be carried out in suitable stadium for its control.

Nevertheless, all populations had higher C_{50} or GR_{50} than the susceptible population. Populations 17, 19 and 25 showed values of C_{80} and GR_{80} higher than the maximum recommended dose of glyphosate to control goosegrass, while the susceptible population was controlled at 80% with 478.18 g e.a. ha^{-1} of glyphosate. The resistance factors in these three populations varied from 3.69 to 5.37.

The results obtained for the F1 generation of populations 7, 19, and 25 showed that the resistance was inherited only in populations from season 2014/2015 (19 and 25) (Table 5). In the case of the population 7 (season 2013/2014), the RF>1.0, however the values of C_{80} and GR_{80} were below the recommended dose (653.27 and 647.27 g e.a. $ha^{-1,}$ respectively) not attending, to this population, the heritability criteria of the resistance. On the other hand, the values of these parameters were above 1,200 g e.a. ha^{-1} for populations 19 and 25 and the resistance factors varied from 3.99 to 6.81, meeting these populations heritability criteria.

The population of goosegrass in Rio Grande do Sul characterized as low-level resistant to glyphosate showed a resistance factor of 1.52 (Vargas et al., 2013). As for the resistance factors obtained for the populations 19 and 25 in this study (RF = 3.99 to 6.81) are more similar to reports of goosegrass resistant to glyphosate in Tennessee, USA (RF = 3,1 to 7,4) (Mueller et al., 2011) and in Malaysia (RF = 8 to 12) (Lee and Ngim, 2000).

The results obtained in this study show that populations 19 and 25 meet all the criteria set for the confirmation of a case of resistance by Heap (2005).Criterion 1: the plants from these populations have survived and reproduced after their exposure to a herbicide dose that was lethal to the susceptible population; Criterion 2: the resistance factors were higher than one

Table 3 **-** Estimated doses of glyphosate for control and reduction of dry mass from the aerial part in 50% and 80% and their resistance factors for different populations of *Eleusine indica* for applications performed in stage of two to three tillers (E1) (season 2014/2015)

Population	C_{50}	C_{80}	RF_{50}	GR_{50}	GR_{80}	RF_{50}
	(g e.a. ha^{-1})					
Susceptible	113.26	204.39	-	101.14	240.22	-
14	299.43	504.80	2.64	178.45	387.66	1.76
15	273.31	419.75	2.41	155.58	341.76	1.54
16	397.20	797.43	3.51	298.28	609.01	2.95
17	367.13	629.17	3.24	444.80	688.38	4.40
18	363.66	650.49	3.21	305.72	519.79	3.02
19	503.49	1571.42	4.45	503.41	1653.49	4.98
20	320.95	620.41	2.83	277.55	873.90	2.74
21	239.14	444.47	2.11	137.88	310.16	1.36
22	267.72	428.39	2.56	249.82	495.75	2.47
23	289.94	554.91	2.36	313.94	708.92	3.10
24	320.63	594.91	2.83	107.54	222.77	1.06
25	536.54	1471.71	4.74	552.02	1524.49	5.46

C_{50} or C_{80}: Necessary dose to control the population in 50% and 80%; GR_{50} or GR_{80}: Necessary dose to reduce the dry mass of the aerial part of the population in 50% and 80%; RF_{50}: resistance factor (C_{50} or GR_{50} of the resistant population/C_{50} or GR_{50} of the susceptible population.

Table 4 **-** Estimated doses of glyphosate for control and reduction of dry mass from the aerial part in 50% and 80% and their resistance factors from different populations of *Eleusine indica* for applications carried out in stage of five to six tillers (E2) (season 2014/2015)

Population	C_{50}	C_{80}	RF_{50}	GR_{50}	GR_{80}	RF_{50}
	(g e.a. ha^{-1})					
Susceptible	190.31	478.18	-	120.67	298.42	-
14	546.29	1412.49	2.87	397.07	969.40	3.29
15	782.83	1734.77	4.11	259.56	1059.55	2.15
16	624.54	1727.47	3.28	219.76	1029.19	1.82
17	843.13	2137.20	4.43	444.80	1878.16	3.69
18	630.48	1508.86	3.31	469.14	1352.47	3.88
19	978.04	2451.96	5.14	648.21	1943.56	5.37
20	713.05	1873.06	3.75	397.84	1519.33	3.30
21	712.11	1796.25	3.74	466.63	1421.12	3.87
22	832.80	1900.60	3.72	401.87	1008.55	3.33
23	507.87	767.01	2.67	383.29	729.57	3.18
24	430.59	793.04	2.26	281.63	582.66	2.33
25	784.95	2575.59	4.12	511.20	1856.07	4.24

C_{50} or C_{80}: Necessary dose to control the population in 50% and 80%; GR_{50} or GR_{80}: Necessary dose to reduce the dry mass of the aerial part of the population in 50% and 80%; RF_{50}: resistance factor (C_{50} or GR_{50} of the resistant population/C_{50} or GR_{50} of the susceptible population.

Table 5 **-** Estimated doses of glyphosate for control and reduction of dry mass from the aerial part in 50% and 80% and their resistance factors from different populations of *Eleusine indica* for applications carried out in stage of two to three tillers (E1)

Population	C_{50}	C_{80}	RF_{50}	GR_{50}	GR_{80}	RF_{50}
	E1: 2-3 tillers (g e.a. ha^{-1})					
Susceptible	113.26		-	100.14		-
7 - F1	419.68	653.27	3.71	397.78	647.27	3.97
19 - F1	573.76	1290.77	5.07	399.59	1407.04	3.99
25 - F1	554.65	1268.62	4.90	681.86	1554.95	6.81

C_{50} or C_{80}: necessary dose for control of the population in 50% e 80%; GR_{50} or GR_{80}: necessary dose for the reduction of the dry mass of the aerial part of the population in 50% e 80%; RF_{50}: resistance factor (C_{50} or GR_{50} of the resistant population/C_{50} or GR_{50} of the susceptible population. F1: is the seed or F1 generation of that population.

(RF = 3,99 to 6,81) and the recommended dose to the species did not provide satisfactory control (C_{80} e GR_{80} > 1,200 g e.a. ha^{-1}); Criterion 3: the plants from the F1 generation of these populations were also considered resistant; Criterion 4: control flaws complaints are being observed on the field; Criterion 5: random plants of these populations were properly classified as *Eleusine indica*.

In this sense, complementary measures handling with herbicides should be designed to reduce the selection pressure, such as the association and the use of herbicides with different mechanisms of action (Shaner, 2000; Johnson and Gibson, 2006).Research papers show that ACCase inhibitors are one of the alternatives for goosegrass control (Ulguim et al., 2013). However, it is important to remember that goosegrass resistance to this mechanism of action has been previously reported in Brazil (Vidal et al., 2006), which shows that the continuous application of graminicides may not be effective in all situations. Alternatively, other mechanisms of action may exhibit effectiveness in controlling these populations, such as GS-GOGAT inhibitors, carotenoid synthesis inhibitors (Ulguim et al., 2013), cell division inhibitors, and photosystem I inhibitors (Molin et al., 2013).

The resistance mechanism to glyphosate in other countries' goosegrass involves replacement of a proline by serine or threonine at position 106 in the EPSPS gene (P106S or P106T) (Baerson et al., 2002; Ng et al.2004; Yu et al.2007; Kaundun et al, 2008, 2011), or the EPSPS gene amplification in resistant plants (Chen et al., 2015). Such single mutation confers intermediate levels of glyphosate resistance (RF = 2 to 4). However, double mutation at positions 106 (T102I and P106S) of the mutant population can confer higher levels of resistance (RF>10) (Yu et al., 2015). Nevertheless, the mechanism that confers resistance in populations from Brazil is still unknown. Therefore, further studies to elucidate the mechanism that confers resistance, as well as the development of new alternative tools for the management of resistant populations are needed.

With the results obtained in this study, it is concluded that the populations 19 and 25, coming from Campo Mourão and Luziânia (Midwest of Paraná), are the first report of goosegrass resistant to glyphosate in Brazil (RF = 3.99 to 6.81), meeting all the criteria for confirmation of new cases of resistance.

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