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Monitoring the occurrence of glyphosate-resistant sourgrass biotypes in the south region of Minas Gerais, Brazil[1]

Monitoramento da ocorrência de biótipos de capim-amargoso resistentes ao herbicida glyphosate na região do Sul de Minas Gerais, Brasil

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Abstract - This work was developed with the objective of monitoring the occurrence of glyphosate-resistant biotypes of sourgrass (*D. insularis*) in Machado, Alpinópolis, Serrania and Divisa Nova, in the south region of Minas Gerais, Brazil. In each area, seeds of at least 20 plants were collected in full physiological maturity stage. For operational reasons, the analysis of different biotypes was divided into two timings, the first held in the second half of 2013 and the second in the first half of 2014. Plants were treated in the 4-5 leaves stage / tillering (First timing) and pre-flowering (Second timing); with the following treatments (D = 720 g ha^{-1} a.e.): 4D, D, 1/4D, 1/16D, 1/64D and herbicide absence. The percentage of control was evaluated at 14 and 28 days after application (DAA) as well as the residual dry mass at 28 DAA. Twelve sourgrass biotypes were considered susceptible to glyphosate; glyphosate differential susceptibility was detected between sourgrass biotypes; different management measures must be adopted to reduce the pressure of selection and the worsening of the situation.
Keywords: *Digitaria insularis*; dose response; management; prevention; resistance

Resumo - Este trabalho foi desenvolvido com o objetivo de monitorar a ocorrência de biótipos de capim-amargoso (*D. insularis*) resistentes ao herbicida glyphosate em Machado, Alpinópolis, Serrania e Divisa Nova, na região de Sul de Minas Gerais, Brasil. Em cada área, foram coletadas sementes de, no mínimo, 20 plantas por biótipos, em estádio de plena maturidade fisiológica. Por questões operacionais, a análise dos diferentes biótipos foi dividida em duas fases, a primeira realizada no segundo semestre de 2013 e a segunda realizada no primeiro semestre de 2014. Foram realizadas pulverizações sobre plantas em estádio de 4-5 folhas / perfilhamento (Primeira Fase) e em pré-florescimento (Segunda Fase), com os seguintes tratamentos (D = 720 g ha^{-1} e.a.): 4D, D, 1/4D, 1/16D, 1/64D e ausência de herbicidas. Foi avaliado o controle percentual aos 14 e 28 dias após aplicação (DAA), bem como a massa seca residual aos 28 DAA. Os doze biótipos de capim-amargoso testados foram considerados suscetíveis ao herbicida glyphosate. Detectou-se suscetibilidade diferencial entre os biótipos. Assim sendo, medidas de manejo diferentes devem ser adotadas para evitar o agravamento da situação.
Palavras-chaves: *Digitaria insularis*; dose-resposta; manejo; prevenção; resistência

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Introduction

Weeds are among biotic factors that may directly or indirectly interfere on crops' growth and yield, competing by essential resources, releasing allelophatic substances in the environment or hosting pests (Leite Júnior & Mohan, 1990). Currently, the intensive use of herbicides for weed management is the most adopted practice in agriculture (Yang et al., 2007). Nowadays, glyphosate is the most important herbicide in the world, and it has been used for many years to control annual or perennial weeds, in many cropping systems (Faircloth et al., 2001; Blackshaw & Harker, 2002).

Currently, one of the most important discussion about weed management in Brazilian or Global agricultural crops is the selection of herbicide-resistant weed biotypes. Herbicide weed resistance may be defined as the inherent and inheritable capacity of certain biotypes, among weed population, to survive and reproduce after being exposed to a herbicide dose which would be lethal to susceptible individuals of the same species (Christoffoleti & López-Ovejero, 2008).

Resistance is a natural phenomenon that spontaneously occurs in weed populations; then, the herbicide does not cause resistance, it just works selecting resistant individuals that may be found naturally in the population, although with small initial frequency (López-Ovejero et al., 2006). This selection is related to the great genetic variability that is common on weed populations. This variability allows weed to adapt and survive in the most diverse environmental conditions (Christoffoleti & López-Ovejero, 2003).

Weed management is also essential in coffee (*Coffea* spp.) plantations, since this crop is extremely sensible to weed competition by nutrients (Ronchi & Silva, 2006), light and water, causing damages to flowering, fruiting and hence its yield (Alcântara & Ferreira, 2000). In the south region of Minas Gerais State, Brazil, there are no confirmed cases of glyphosate-resistant weed species; however, frequently, weed management in coffee plantations is strongly based on several annual applications of this herbicide. This environment is extremely favorable for selecting herbicide-resistant weed biotypes, which must be monitored carefully.

Sourgrass (*Digitaria insularis*) is a weed frequently found in Brazilian pastures, coffee plantations, orchards, roadsides and vacant lands. This weed used to be less common in cultivated soils, however currently it has been identified as one of the most important weed in no-tillage areas of the cerrado and in southern Brazil. It grows vigorously, forming clumps that bloom during almost all the summer (Lorenzi, 2008). The first world case of a glyphosate-resistant sourgrass biotype was reported in Paraguay, in 2005 (Heap, 2015). In Brazil, the first cases were reported in 2008 and 2011 (Melo, 2011; Carvalho et al., 2011; Heap, 2015), in soybean and maize fields and in citrus orchards.

Considering sourgrass is easily found in coffee plantations frequently managed with glyphosate, the objective of this work was monitoring the occurrence of glyphosate-resistant biotypes of sourgrass (*D. insularis*) in Machado, Alpinópolis, Serrania and Divisa Nova, in the south region of Minas Gerais, Brazil.

Material and Methods

This work was carried out in greenhouse condition at the Federal Institute of Education, Science and Technology of the South of Minas Gerais (MG), Machado campus, Brazil (21º 40' S; 45º 44' W; 850 m of altitude).

Twelve independent trials were developed to evaluate glyphosate control of sourgrass (*Digitaria insularis*) biotypes. These biotypes were sampled on different growth environments (coffee plantations, glyphosate applying areas, urban area and fallow land), in the cities of Machado, Alpinópolis, Serrania and Divisa Nova (MG). In each area, seeds were collected in bulk from at least 20 plants, in representative infestations, in the stage of complete physiological maturity. At the harvest moment, geographical coordinates were noted for each sampling point (Table 1).

Table 1. Sampling points for biotypes of sourgrass (*Digitaria insularis*), cities, geographic coordinates and altitude.

Biotypes	Municipality	Soil Use	Geographic Coordinate		Altitude (m)
			Latitude	Longitude	
A	Machado	Coffee	21° 39' 55"	45° 51' 05"	876
B	Machado	Coffee	21° 43' 05"	45° 53' 32"	909
C	Machado	Corn / Fallow	21° 40' 15"	45° 55' 03"	849
D	Machado	Coffee	21° 36' 56"	45° 57' 15"	970
E	Machado	Cucumber	21° 43' 01"	45° 57' 07"	853
F	Machado	Urban Area	21° 40' 53"	45° 55' 49"	859
G	Alpinópolis	Soybean	20° 47' 58"	46° 21' 39"	790
H	Serrania	Coffee	21° 34' 31"	46° 08' 50"	955
I	Divisa Nova	Sunflower	21° 33' 20"	46° 10' 54"	825
J	Serrania	Coffee	21° 29' 28"	46° 03' 03"	870
K	Serrania	Coffee	21º 29' 36"	46º 02' 59"	882

Seeds of each biotype were homogenized and stored in paper bags, in a dry place at room temperature, until the beginning of the trials. For growing the seedlings, seeds were distributed on 4 L commercial plastic pots, filled with a mix of commercial substrate (*Pinus* bark, turf and vermiculite) and vermiculite (3:1 v/v). At two unfolded leaves stage, seedlings were transplanted to experimental plots, where they remained up to the end of the trials. Experimental plots consisted of 1L plastic pots, filled with the same mixture of substrate and vermiculite, properly fertilized. Plots had the mean of ten plants, without nutritional or water stress.

For operational reasons, the analysis of several biotypes was divided into two phases. First, half of the biotypes were evaluated in the second half of 2013. After that, the other half of the biotypes were studied in the first half of 2014. Biotypes susceptibility to glyphosate was quantified through the method of dose-response curves. Each biotype was considered as an independent trial installed on a randomized blocks experimental design, with six treatments (glyphosate doses) and five replicates, totaling 30 plots. In this case, small variability in size or number of plants per pot was considered the factor for blocking the trials. On each biotype, it was applied the same six glyphosate (Roundup Original®) doses, as follows: 4D, D, 1/4D, 1/16D, 1/64D and herbicide absence. The D is the herbicide recommended dose, proportional to 720 g ha^{-1} of glyphosate acid equivalent (a.e.). Doses were chosen considering susceptible biotypes, since using higher doses could not allow the comparisons, if all the doses might promote 100% of control.

In the first phase, glyphosate was applied on plants at the 4-5 leaves / full tillering stage (biotypes A to G); in the second phase, plants were applied on pre-flowering stage (biotypes H to K + F) at the application timing. Herbicide treatments were applied using a CO_2-backpack sprayer, coupled to a two nozzles bar (flat fan - TeeJet XR 110.02), positioned at 0.50 m above the targets and with consumption of spray solution proportional to 200 L ha^{-1}. After herbicide application, pots were placed in the greenhouse and irrigated on the following day to secure adequate foliar absorption of the molecules. Meteorological data were locally collected at the time of applications (Table 2).

Sourgrass control was evaluated at 14 and 28 days after application (DAA), as well as the residual dry mass was measured at 28 DAA. For control evaluations, it was considered a percentage scale variable from zero up to 100%, in which zero means the absence of symptoms and 100 means plant death. Dry mass values were obtained from the harvest of all remaining plant material in the plots, with subsequent drying in an oven at 70 °C for 72 h. These values were corrected to percentage by comparing the residual mass of each herbicide treatment with the mass of control plots (herbicide absence), considered as 100%.

Table 2. Description of days and meteorological conditions of herbicide application on sourgrass (*Digitaria insularis*) biotypes. Machado (MG), 2013/14.

Application	Application		
	10/09/2013	10/24/2013	03/24/2014
Biotype	A, B, C, D, E, F	G	H, I, J, K, F
Start time	15:55 h	09:01 h	15:35 h
End time	16:02 h	09:13 h	15:50 h
Mean Temperature (°C)	25.6	25.3	29.8
Relative Humidity (%)	54.1	73.7	50.3
Wind (m/s)	0.1	0.0	0.2
Atmosphere condition	Partly covered (80%)	Clean	Covered

Initially, data analysis was performed by applying the F test on analysis of variance. Considering that the maximum dose has reached 100% of control, dose-response curves were fitted according to non-linear log-logistic model with two parameters, adapted by Carvalho et al. (2010):

$$y = \frac{100}{\left[1+\left(\frac{x}{LD_{50}}\right)^{\alpha}\right]}$$

In which: y is the variable (control or percentage of dry mass), x is the herbicide dose (g ha^{-1} a.e.), LD_{50} is the herbicide lethal dose that reduces 50% of variable response (50% of control or reduction of mass) and α is the slope of the curve around LD_{50}.

The log-logistic model presents advantages once one of the equation parameter is an estimative of LD_{50}. LD_{50} (lethal dose to 50%) is the herbicide dose (g ha^{-1} a.e.) that promotes 50% of control or weeds' weight reduction (Carvalho et al., 2009). Considering agronomic efficacy, it was also calculated LD_{80}, i.e., the herbicide dose necessary to control the biotype up to 80% or to reduce 80% of dry mass.

Results and Discussion

Log-logistic models were fit to the data, with coefficients of determination always above 0.90 (Tables 3 and 4). Considering control evaluation at 14 DAA, LD_{50} values were below 720 g ha^{-1} a.e. for all biotypes, what is equivalent to 2 L ha^{-1} of commercial product (Roundup Original®; 360 g L^{-1} a.e.). In this evaluation, it was also possible to observe that plants on pre-flowering stage (second phase) were more difficult to control with glyphosate than plants on tillering stage (first phase), mainly if LD_{80} is considered. In both phases, biotype F was the most difficult to control, demanding doses up to 2,650 g ha^{-1} a.e. for LD_{80}, that is highly above commercial recommendation (Rodrigues & Almeida, 2011) (Table 3).

Curiously, biotype F was sampled in Machado urban area. Once herbicide application in urban area is legally forbidden, and considering the possibilities of sourgrass dispersal, it is possible that this biotype may be exposed to glyphosate elsewhere and then transported to urban areas.

Table 3. Parameters[1] of the equation for sourgrass (*Digitaria insularis*) control after the application of six glyphosate doses, evaluated at 14 and 28 DAA. Machado (MG), 2013/14.

% of control – 14 DAA				
Biotype	Statistical Parameter			
	LD_{50}	α	LD_{80}	R^2
	Plants with 4 to 5 leaves/Tillering			
A	250.462	-1.801	540.797	0.997
B	263.281	-1.922	541.584	0.997
C	245.943	-1.799	531.494	0.996
D	246.125	-1.561	598.197	0.996
E	215.829	-1.628	505.738	0.998
F	332.578	-1.204	1051.825	0.998
G	356.928	-1.524	886.407	0.997
	Plants on pre-flowering			
H	283.286	-1.059	1048.921	0.998
I	379.500	-1.043	1433.674	0.994
J	354.417	-1.136	1200.873	0.998
K	317.108	-1.109	1106.860	0.998
F	516.059	-0.847	2651.636	0.991
% of control – 28 DAA				
Biotype	Statistical Parameter			
	LD_{50}	α	LD_{80}	R^2
	Plants with 4 to 5 leaves/Tillering			
A	227.460	-3.340	344.479	0.996
B	224.589	-2.851	365.228	0.994
C	196.039	-1.970	396.239	0.993
D	236.279	-4.644	318.469	0.997
E	207.706	-3.363	313.671	0.999
F	207.007	-3.120	322.814	0.999
G	239.123	-3.311	363.461	0.999
	Plants on pre-flowering			
H	158.563	-1.423	420.043	0.994
I	236.914	-1.239	725.295	0.997
J	198.804	-1.703	448.698	0.998
K	161.079	-1.547	394.655	0.997
F	241.089	-1.482	614.364	0.999

[1]Mathematical model: $y=100/(1+(x/R_{50})^{\alpha})$; LD_{50} = dose of glyphosate that controls 50% of weed; α = slope of the curve; R^2 = coefficient of determination; LD_{80} = dose of glyphosate that controls 80% of weed.

At 28 DAA, 360 g ha^{-1} a.e. of glyphosate were enough to ensure LD_{50} of all biotypes. Considering LD_{80}, biotype *I* was the most difficult to control, demanding 725 g ha^{-1} a.e. of glyphosate (Table 3). In this second evaluation, LD_{50} and LD_{80} values were lower than at 14 DAA, that may be explained by the time necessary for glyphosate killing the weeds, about 14 to 21 days. Considering dry mass evaluation at 28 DAA, LD_{80} could be reached with the glyphosate commercial dose, up to 1,440 g ha^{-1} a.e., what indicates these biotypes are not resistant to the product, however they have differential levels of susceptibility (Table 4).

Table 4. Parameters[1] of the equation for sourgrass (*Digitaria insularis*) dry mass after the application of six glyphosate doses, evaluated at 28 DAA. Machado - MG, 2013/14

Biotype	Statistical Parameter			
	LD_{50}	α	LD_{80}	R²
	Plants with 4 to 5 leaves/Tillering			
A	301.503	2.453	530.554	0.986
B	124.585	1.140	420.328	0.991
C	117.878	1.032	451.673	0.998
D	233.008	1.320	666.004	0.993
E	173.739	1.883	362.770	0.991
F	183.838	1.782	400.213	0.929
G	193.501	3.002	307.069	0.996
	Plants on pre-flowering			
H	140.581	1.034	537.266	0.983
I	151.965	0.787	884.606	0.988
J	242.544	0.923	1089.122	0.983
K	130.798	0.819	710.750	0.984
F	239.942	0.816	1311.973	0.995

[1]Mathematical model: $y=100/(1+(x/R_{50})^{\alpha})$; LD_{50} = dose of glyphosate that controls 50% of weed; α = slope of the curve; R² = coefficient of determination; LD_{80} = dose of glyphosate that controls 80% of weed.

Frequently, control of sourgrass adult plants demands the application of glyphosate doses superior than those recommended to control other species of the Poaceae family. Timossi et al. (2006) observed that 1,440 g ha^{-1} a.e. of glyphosate were necessary to promote satisfactory control of the infesting population, but it did not prevent its regrowth. In field condition, in areas with glyphosate continuous use, young plants originated from seeds have been adequately controlled by the herbicide; however, when they develop and create rhizomes, its control is inefficient (Machado et al., 2006).

Machado et al. (2008) commented that the higher difficult to control sourgrass plants emerged from rhizomes may be related to the increased thickness of adaxial and abaxial epidermis as well as to the increased thickness of the leaf blade, when compared to plants grown from seeds. They also observed great amount of starch in the rhizomes, that may difficult glyphosate translocation and allow fast shoot regrowth. Therefore, not always a hard-to-kill biotype is a resistant biotype. Low susceptibility may also be related to advanced phenological stage, plant physiology and morphology.

Considering control at 14 and 28 DAA and dry mass, it was evident that plants phenological stage at the application moment is a very important detail to be observed for reaching complete control with glyphosate. Always, applying glyphosate on pre-flowering plants demanded higher doses than applying glyphosate on tillering plants (Tables 3 and 4). For example, considering dry mass of biotype F, dose necessary to control plants on pre-flowering was three times higher than the necessary dose to control the same biotype on tillering stage (Table 4).

The influence of phenological stage on glyphosate efficacy has been greatly reported in literature. For example, 15.8 g ha^{-1} a.e. of glyphosate were enough to reach LD_{50} of *Commelina benghalensis* on cotyledon leaves stage. However, more than 2,880 g ha^{-1} a.e. of glyphosate were necessary to reach LD_{50} of the same population, when considering pre-flowering plants. In the same work, glyphosate dose necessary to control pre-flowering *Brachiaria plantaginea* was five times fold than application on cotyledon leaves plants (Dias et al., 2013).

Christoffoleti et al. (2005) and Ribeiro (2008) also reported the interference of plants phenological stage on glyphosate efficacy. These authors evaluated resistant biotypes of *Lolium multiflorum* and observed lower levels of control on the most developed plants. Lacerda & Victoria Filho (2004) also evaluated glyphosate control of *D. insularis*. These authors observed that only 128.5 g ha^{-1} a.e. of glyphosate was enough to reach R_{50} of young plants on the stage of four unfolded leaves.

Nicolai et al. (2010) carried out experiments with sourgrass biotypes collected in the region of Matão, São Paulo State, Brazil. These authors reported the necessity of glyphosate doses between 4,320 and 5,760 g ha^{-1} a.e. to control some biotypes, with resistance factor of 7,5 (R/S). Adegas et al. (2010) carried out another similar work, in the municipality of Guaíra, Paraná State, Brazil. These authors found out biotypes which 8,640 g ha^{-1} a.e. of glyphosate resulted on only 77% of control, with factor R/S of 6,4. The R/S factor corresponds to the division of LD_{50}, LD_{80} or LD_{95} of the unknown susceptibility biotype by the susceptible biotype (Christoffoleti & López-Ovejero, 2008).

Correia et al. (2010) also reported glyphosate differential susceptibility of sourgrass populations, what enunciated the selection of glyphosate-resistant biotypes. In this work, doses up to 3,988 g ha^{-1} a.e. were necessary to reach LD_{50}, at 28 DAA. Although it has been commercialized since 70s, the first world case of a glyphosate-resistant weed was only reported in 1996 (Pratley et al., 1996). This several years delay may be explained mainly due to biochemical characteristics of the molecule when it is in the plants or soil, such as: absence of soil residual activity, presence of multiple physiological paths related to the action mechanism, low ecological adaptability of surviving individuals, low initial frequency of resistant individuals, absence of other herbicides with the same action mechanism and limited metabolism in the plants (Bradshaw et al., 1997).

In addition, in the last few years, higher number of glyphosate applications have been observed in agricultural areas as consequence of soil management conservationist systems (no-tillage) as well as the possibility of glyphosate application on transgenic crops. The higher number of annual glyphosate applications has increased significantly the risk of new cases of glyphosate-resistant biotypes, due to the pressure of selection created by the herbicide (Neve et al., 2003). In this way, since 2008 and 2011, new cases of glyphosate-resistant biotypes of sourgrass have been published in Brazil (Melo, 2011; Carvalho et al., 2011; Heap, 2015).

Monitoring glyphosate susceptibility of sourgrass biotypes is very important, mainly in coffee plantations of the south of Minas Gerais State. In general, differential susceptibility of sourgrass biotypes was identified in samples collected in the cities of Machado, Alpinópolis, Serrania and Divisa Nova, Minas Gerais State. Level of control obtained up to this moment do not characterize cases of resistance, however new management programs may be structured considering these data, including different herbicides or management practices, in order to prevent or avoid cases of sourgrass glyphosate resistance in the areas.

Conclusions

All sourgrass biotypes were considered susceptible to glyphosate.

Glyphosate differential susceptibility was detected between sourgrass biotypes.

The different management measures must be adopted to reduce the pressure of selection and the worsening of the situation.

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