PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Chemical Control and Morphoanatomical Analysis of Leaves of Different Populations of Sourgrass

Controle Químico e Análise Morfoanatômica das Folhas de Diferentes Populações de Capim-Amargoso

GOMES, L.J.P.[1]*
SANTOS, J.I.[1]
GASPARINO, E.C.[1]
CORREIA, N.M.[2]

ABSTRACT - *Digitaria insularis* has been selected by frequent glyphosate applications both in no-till areas and in fruit orchards. The objective of this paper was to evaluate control efficacy of *D. insularis* populations by glyphosate, alone and mixed with quizalofop-p-tefuryl, as well as classify them for herbicide sensitivity as susceptible, moderately susceptible and tolerant. This study also aimed to evaluate the relations between herbicide sensitivity and morphoanatomic features of leaf tissues. For chemical control, the experiment was conducted in a completely randomized design, with a 12 x 4 factorial arrangement with four replications. Twelve populations of *D. insularis* were treated with glyphosate alone at two concentrations (1.44 and 2.16 kg a.e. ha^{-1}) and with the mixture of glyphosate (2.16 kg a.e. ha^{-1}) and quizalofop-p-tefuryl (0.12 kg a.i. ha^{-1}). One treatment without herbicide application was maintained for each plant population. Evaluations about the morphoanatomic features and wax content of leaves from the twelve plant populations were made under laboratory conditions. Populations 3, 5, 6 and 8 were considered to be susceptible; 9, 10 and 12 were considered as moderately susceptible; 1, 2, 4, 7 and 11 were considered tolerant to glyphosate. However, populations within each group (susceptible, moderately susceptible and tolerant) did not have similar characteristics that justify their response to glyphosate. Differences relative to herbicide translocation and/or plant physiology could account for the chemical control results for these populations.

Keywords: *Digitaria insularis*, glyphosate, tolerance, genetic variability.

*RESUMO - A espécie **Digitaria insularis** tem sido selecionada por aplicações frequentes de glyphosate, tanto em áreas de plantio direto como em pomares de frutíferas. Assim, objetivou-se neste trabalho o controle de diferentes populações de **D. insularis** pelo herbicida glyphosate, isolado e em mistura com quizalofop-p-tefuryl, além de classificar as populações quanto à sensibilidade (suscetível, medianamente suscetível ou tolerante) ao glyphosate e analisar características morfoanatômicas das folhas das populações. Para o experimento de controle químico, o delineamento experimental foi o inteiramente casualizado com quatro repetições, em esquema fatorial 12 x 4. Doze populações de **D. insularis** foram pulverizadas com glyphosate isolado (1,44 e 2,16 kg e.a. ha^{-1}) e em mistura (2,16 kg e.a. ha^{-1}) com quizalofop-p-tefuryl (0,12 kg i.a. ha^{-1}). Ademais, uma testemunha sem aplicação foi mantida para cada população. Em laboratório, foram realizadas análises morfoanatômicas e de quantificação de cerosidade das folhas. As populações 3, 5, 6 e 8 foram consideradas suscetíveis ao glyphosate; 9, 10 e 12, medianamente suscetíveis; e 1, 2, 4, 7 e 11, tolerantes. Todas as populações apresentaram as mesmas características morfológicas, mas diferiram nas*

*** Corresponding autor:**
<leo_pgomes@hotmail.com>

Received: January 11, 2016
Approved: March 29, 2016

Planta Daninha 2017; v35:e017158021

[1] Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal-SP, Brasil; [2] Embrapa Hortaliças, Brasília-DF, Brasil.

Doi: 10.1590/S0100-83582017350100008

anatômicas e na quantidade de cera. Contudo, as populações dentro de cada grupo (suscetíveis, medianamente suscetíveis ou tolerantes) não tiveram características semelhantes que justificassem a resposta diferencial ao herbicida glyphosate. Diferenças relacionadas com a translocação dos herbicidas e/ou fisiologia das plantas poderiam explicar os resultados do controle químico dessas populações.

Palavras-chave: *Digitaria insularis*, glyphosate, tolerância, variabilidade genética.

INTRODUCTION

Sourgrass (*Digitaria insularis*) is a plant native to the American continent, in tropical and subtropical regions. This is a perennial herbaceous species, upright and of great potential as an invasive species, as its seeds are covered in long hairs, are carried long distances by the wind and have good germination capacity. It reproduces through rhizomes and/or seeds and may reach 50 to 150 cm in height (Kissmann, 1997).

In agricultural areas, where there is no establishment of cover crops in the off season, there has been an increase in infestation of that species (Correia et al., 2010). What hinders the management of *D. insularis* is that, once established, the plant becomes very rustic because of the formation of numerous rhizomes, which leads to the formation of large shrubs. Thus, after the process of perennation has occurred, sourgrass can flourish and scatter its seeds with low levels of dormancy during the whole year (Gemelli et al., 2012).

This species has been selected by frequent glyphosate applications, both in no-till fields and in fruit orchards (Timossi, 2009). Changes relative to absorption, translocation, site of action, metabolism or even compartmentalization of glyphosate by *D. insularis* plants may explain why the specimens respond differently to the action of the herbicide (Correia et al., 2010). There are two reports of resistance of *D. insularis* to glyphosate in the world: the first one in corn, soybean, sunflower and cotton crops in 2005 in Paraguay; the second report, in soybean and corn crops in 2008 in Brazil (Weed Science, 2016).

When studying the control of 12 *D. insularis* populations, with average height of 50 cm, by the herbicide glyphosate alone (1.44 and 2.16 kg a.i. ha^{-1}) and in mixture (1.44 and 2.16 kg a.i. ha^{-1}) with quizalofop-p-tefuryl (0.12 kg a.i. ha^{-1}), Correia et al. (2015) found that population 11 (grain production area in Itumbiara-GO) was sensitive to glyphosate alone; populations 5 (citrus orchard in Colômbia-SP) and 9 (area with sugarcane crops in Pradópolis-SP) were moderately susceptible; and populations 1, 2, 3 and 4 (grain production area in Jaboticabal-SP), 6, 7 and 8 (citrus orchard in Bebedouro, SP, Ibitinga-SP and Balbinos-SP), 10 (rubber tree area in Bebedouro, SP) and 12 (urban area in Jaboticabal-SP) were tolerant, in the light of biotype sensitivity to increased rates of glyphosate alone.

Genetic variability of weed populations is affected by many factors, such as evolution of production system, crop-weed interaction (gene flow through pollen and seed dispersal), geographical distribution and natural selection (Huangfu et al., 2009). Knowledge is required of whether genetic diversity of weeds is the same across different populations, especially comparing those subject to selection caused by repetitive use of herbicides and those without a history of application (Huangfu et al., 2009).

According to Procópio et al. (2003), leaf morphology of plants affects the amount of herbicide intercepted and retained, while leaf anatomy determines the process whereby herbicides will be absorbed. These authors also mentioned that high trichome density, great cuticle thickness of the adaxial surface and low stomatal density in the adaxial surface were the main potential barriers detected for herbicide retention and absorption in *Conyza bonariensis*; for *Crotalaria incana*, the main obstacle in leaves was high epicuticular wax content.

Thus, based on the hypothesis that *D. insularis* populations have different levels of sensitivity to glyphosate and that there are morphological and anatomical changes among specimens of these plants, the present research aimed to study the control of different of *D. insularis* populations by the herbicide glyphosate, isolated and mixed with quizalofop-p-tefuryl. Another objective was to group the populations for sensitivity (susceptible, moderately susceptible or tolerant) to glyphosate and analyze morpho-anatomical characteristics of the leaves in these populations.

MATERIAL AND METHODS

Between the months of March and November 2014, seeds from 12 populations of *D. insularis* were collected in the states of GO, MS and SP. All areas showed a history of successive glyphosate applications: two for grain production in Pedrinhas Paulista (SP), one for grain production in Jaboticabal (SP), one for citrus production in Taquaral (SP), one for grain production in Chapadão de Céu (GO), two for grain production in Rio Verde (GO), two for grain production in Cristalina (GO), one for grain/vegetable production in Morrinhos (GO), one for grain production in Chapadão do Sul (MS) and one for grain production in Itaquiraí (MS).

In the first half of December 2014, the seeds of the populations were sown in cells of styrofoam trays filled with composite plant, for seedling formation. When the plants had between two and three leaves, at 15 days after sowing (DAS), three cells were transplanted to pots, with subsequent thinning, thus maintaining three plants per pot.

Each experimental unit was consisted of a plastic pot with 3.5 liter capacity, filled with a mixture of soil, sand and composite plant, in a 3:1:1 ratio, respectively. Irrigation was performed with the purpose of keeping substrate moisture. The substrate was fertilized fortnightly with a nutrient solution containing 18 mg of nitrogen, 45 mg of phosphorus and potassium per kg of soil. The pots were kept at ambient temperature from December 2014 to April 2015.

For chemical control, the experimental design was completely randomized with four replications, in a 12 x 4 factorial arrangement. Twelve populations of *D. insularis* were sprayed with glyphosate alone (1.44 and 2.16 kg a.i. ha^{-1}) and mixed (2.16 kg a.i. ha^{-1}) with quizalofop-p-tefuryl (0.12 kg a.i. ha^{-1}) when plants measured an average of 42.9 cm and had 6.0 tillers per plant. In addition, a control without application was kept for each population. In addition, a control without application was kept for each population. Mineral oil at 0.5% v/v was added to the spray with quizalofop-p-tefuryl.

The commercial products used were Roundup Transorb® (glyphosate), Panther® (quizalofop-p-tefuryl) and Assist® (mineral oil).

The herbicides were applied with the help of backup sprayer, under constant pressure (maintained by compressed CO_2) of 2.2 kgf cm^2, fitted with bars with two XR 110.015 flat fan nozzles spaced at 0.5 m, with spray volume equivalent to 150 L ha^{-1}.

In the course of the experiment, a qualitative scale (high, medium or low) was used to visually determine pilosity in the sheath and in the leaves and plant growth habit (droopy or upright). At 15 and 60 days after application (DAA) of herbicides, visual control evaluations were made by using a scale from 0 to 100%, in which zero represents the absence of visual injuries and 100, the death of all the plants in the pot. At 60 DAA, all the green shoots of the plants were collected. The material was dried in an oven at 50 °C, until constant mass, when it was evaluated.

Based on data on dry matter, the populations were categorized as follows: susceptible, when controlled with the two glyphosate rates; moderately susceptible, when it responded satisfactorily to an increase in the glyphosate rate; and tolerant, when the population was not controlled by glyphosate, even at the highest rate.

The results were submitted to analysis of variance (F). The effects of the treatments (populations and herbicides/control), in addition to the interaction between them, when significant, were compared by Tukey's test at 5% probability. The statistical software program SISVAR (Ferreira, 2011) was used.

In the laboratory, morpho-anatomical analyses and wax content quantification were performed fpr the leaves. At this stage of the research, only the control leaves (without herbicide application) of the 12 *D. insularis* populations, collected at 125 days after the seedlings were transplanted to the pots, were used.

Wax content quantification was based on the change of color caused by reaction of wax with potassium dichromate acid, as described by Ebercon et al. (1977). The second fully expanded leaf from the apex of the culm was collected, totaling six leaves (of six plants), corresponding to 48 cm² of leaves per population. The leaves were immersed in 15 mL of chloroform for 30 seconds. The extract obtained was filtered and evaporated in a water bath, until the smell of chloroform could no longer be smelled. Then, 50 mL of the potassium dichromate acid solution was added by heating it in a water bath for 30 minutes. After cooling, 2 mL of deionized water was added. After the change of color and full cooling, optical density was measured with 590 mn.

For leaf anatomical analysis, again, the second fully expanded leaf from each population (three leaves of three plants) was collected and 1 cm² of the leaf blade was sampled in the central region. Then, the material was fixed in FAA 50 (formaldehyde + acetic acid + 50% alcohol) for conservation, in accordance with the methodology of Johansen (1940).

The permanent blades were prepared in accordance with the methods of ethanol dehydration series (70, 85, 95 and 100%) and paraffin-embedding. Transversal histological sections were performed in a Leica RM 2065 rotary microtome at a thickness of 10 μm (Johansen, 1940). After 24 hours at ambient temperature, the material was stained with 0.05% toluidine blue for about two minutes (Sakai, 1973). After that, the blades were prepared with synthetic resin (Entellan®).

The anatomical structures were observed in an optical microscope (Bel photonics, 40 X objective lens), and measured with the aid of a lens containing a micrometric ruler. Three slides were prepared for each population; in each one of them, the leaves were different and had four cuts. Each variable was measured 75 times for each population, i.e., 25 times per slide. The images were captured with the aid of a light chamber fitted to a Bel photonics microscope.

The following leaf anatomical characters were quantitatively evaluated: thickness of adaxial and abaxial epidermis, total blade outside the vein, total blade on the vein, length from center of blade, length and width of sustaining tissue, length and width of main vein and length and width of the secondary veins.

The results were submitted to the F-test for analysis of variance; when significant, they were compared by Tukey's test at 5% probability. The statistical software program SISVAR (Ferreira, 2011) was used.

For morphological evaluation by means of scanning electronic microscopy (SEM), the second fully expanded leaf was also collected (one per population). After collection, 0.25 cm² of the leaf was sampled; then, they were fixed in 25% glutaraldehyde solution and 0.1 M potassium phosphate buffer, and dehydrated in an increasing ethanol series (30, 50, 70, 85 and 95%), once each for 20 minutes, and in 100% ethyl alcohol three times for 20 minutes.

After that, the material was dried up to the critical point, with the objective of removing the ethyl alcohol from the leaf samples, replacing it by liquid CO, and later changing it into gaseous state. After drying, the samples were placed on aluminum stubs with the aid of double-sided carbon adhesive tape, and metallized with gold (20 nm of gold), in a SCD005 Baltec metallizer.

The images were captured in a Phillips Scanning Electron Microscope (SEM). They were scanned and edited on Adobe Photoshop CS 5.1, for composition of the figures.

In addition, principal component analysis was performed on the basis of the evaluated morpho-anatomical characteristics, with the purpose of identifying possible differentiation among populations (susceptible, moderately susceptible and tolerant).

RESULTS AND DISCUSSION

For the chemical control of different *D. insularis* populations, there was a significant effect ($p<0.01$) of isolated factors and the interaction (population vs. herbicide) in all assessed variables (Table 1).

At 15 and 60 DAA, glyphosate alone (at the two rates) resulted in greater percentage of control for populations 3, 5, 6 and 8 (96% to 100%) and population 12 (85%), at the highest rate, at 60 DAA, which differed from the other populations (Tables 2 and 3). Initially, population 4 was the most tolerant to isolated glyphosate, with control of less than 24%, regardless of spray rate. The same result was found in the subsequent evaluation, but only at the highest rate of the herbicide. On the other hand, in the two periods of evaluation, the mixture glyphosate + quizalofop was efficient in the control (87% to 100%) of all the populations, with the exception of population 11, at 15 DAA.

Table 1 - Results of the F-test for analysis of variance for percentage of plant control of 12 populations of *Digitaria insularis* at 15 and 60 days after herbicide application (DAA), and plant dry matter at 60 DAA. Jaboticabal, SP. 2014/2015

Sources of variation	Control - DAA		Dry matter
	15	60	Plant
Population	108.0**	3.5**	10.1**
Herbicide	366.2**	190.9**	63.9**
Pop. vs. Herb.	21.8**	11.1**	4.3**
VC (%)	8.4	9.4	83.7

** significant at 1% probability by the F test for analysis of variance.

When comparing the treatments of herbicides in each population, at 15 and 60 DAA, for populations 1, 2, 4, 7, 9, 10 and 11, the association of glyphosate with quizalofop resulted in greater control percentage of *D. insularis*, with an increase of 18% to 76%, different from glyphosate alone, at the two tested rates. For populations 7, 9, 10, 11 and 12, the highest glyphosate rate differed from the lowest rate and resulted in an increase of up to 68% of control. By contrast, for populations 3, 5, 6 and 8, there was no significant difference between the treatments of herbicides, with control of 96% to 100%.

In some agricultural areas of Central Southern Brazil, adult populations of this species have required very high rates of glyphosate, with unsuccessful control (Correia and Durigan, 2009). As an alternative for management, ACCase inhibitors are currently used (chemical group aryloxyphenoxypropionate and cyclohexadienes), mixed with glyphosate for control of resistant *D. insularis* populations. There are studies that have proven the synergy of these mixtures, as reported by Melo et al. (2012), in which the herbicides sethoxydim (0.23 kg ha^{-1}), haloxyfop-methyl (0.06 kg ha^{-1}), fenoxaprop + clethodim (0.05 + 0.05 kg ha^{-1}), fluazifop-p-butyl (0.12 kg ha^{-1}) or tepraloxydim (0.10 kg ha^{-1}), associated with glyphosate (1.44 kg ha^{-1}), were effective in the control of glyphosate-resistant biotopes of *D. insularis*, when they had three to five tillers and measured 30 to 40 cm in height.

As regards shoot dry mass, (Table 4), as there was a mortality of all plants of the populations 3, 5, 6 and 8, the values were null for all treatments with glyphosate and in the comparison of

Table 2 - Percentage of palnt control of 12 populations[1] of *Digitaria insularis* at 15 days after the application of isolated glyphosate and mixed with quizalofop-p-tefuryl, in addition to the control without herbicide. Jaboticabal, SP. 2014/2015

Population	Herbicide/control			
	Glyphosate		Glyphosate+quizalofop	Control[2]
	1.44 kg ha^{-1}	2.16 kg ha^{-1}	2.16+0.12 kg ha^{-1}	
	Control (%) - 15 DAA			
1	42.5 bc B[3]	48.8 d B	86.9 ab A	0.0
2	51.2 b C	71.2 bc B	95.0 a A	0.0
3	95.6 a A	98.8 a A	98.8 a A	0.0
4	22.5 d B	23.8 e B	97.5 a A	0.0
5	98.1 a A	99.4 a A	97.5 a A	0.0
6	100.0 a A	100.0 a A	100.0 a A	0.0
7	38.8 bc C	60.0 bcd B	90.0 ab A	0.0
8	96.9 a A	98.8 a A	100.0 a A	0.0
9	40.0 bc C	63.8 bc B	88.1 ab A	0.0
10	33.8 bc C	72.5 b B	90.0 ab A	0.0
11	37.5 bc C	57.5 cd B	80.0 b A	0.0
12	47.5 bc C	71.2 bc B	92.5 ab A	0.0
LSD (in the row)	10.5			
LSD (in the column)	14.8			

[1] 1 and 11 - area of Pedrinhas Paulista (SP); 2 - area of Jaboticabal (SP); 3 - area of Chapadão do Céu (GO); 4 - area of Itaquiraí (MS); 5 and 7 - area of Rio Verde (GO); 6 and 8 - area of Cristalina (GO); 9 - area of Morrinhos (GO); 10 - area of Chapadão do Sul (MS); 12 - area of Taquaral (SP). [2] Data on the control without herbicide application were not included in the statistical analysis. [3] Based on Tukey's test at 5% probability. Means followed by lowercase letter, in the columns, compare the populations within each herbicide treatment, and capital letters in the rows, compare herbicide treatments within each population.

Table 3 - Percentage of plant control of 12 populations[(1)] of *Digitaria insularis* at 60 days after the application of isolated glyphosate and mixed with quizalofop-p-tefuryl, in addition to the control without herbicide. Jaboticabal, SP. 2014/2015

Population	Herbicide/control			
	Glyphosate		Glyphosate+quizalofop	Control[(2)]
	1.44 kg ha^{-1}	2.16 kg ha^{-1}	2.16+0.12 kg ha^{-1}	
	Control (%) - 60 DAA			
1	16.2 b B[(3)]	30.0 de B	100.0 a A	0.0
2	26.2 b B	41.2 cde B	100.0 a A	0.0
3	100.0 a A	100.0 a A	100.0 a A	0.0
4	33.8 b B	23.8 e B	100.0 a A	0.0
5	100.0 a A	100.0 a A	100.0 a A	0.0
6	100.0 a A	100.0 a A	100.0 a A	0.0
7	21.2 b C	52.5 cde B	100.0 a A	0.0
8	100.0 a A	100.0 a A	100.0 a A	0.0
9	20.0 b C	67.5 bc B	100.0 a A	0.0
10	5.0 b C	56.2 bcd B	100.0 a A	0.0
11	8.8 b C	40.0 cde B	100.0 a A	0.0
12	17.5 b B	85.0 ab A	100.0 a A	0.0
LSD (in the row)	23.1			
LSD (in the column)	32.5			

[(1)] 1 and 11 - area of Pedrinhas Paulista (SP); 2 - area of Jaboticabal (SP); 3 - area of Chapadão do Céu (GO); 4 - area of Itaquiraí (MS); 5 and 7 - area of Rio Verde (GO); 6 and 8 - area of Cristalina (GO); 9 - area of Morrinhos (GO); 10 - area of Chapadão do Sul (MS); 12 - area of Taquaral (SP). [(2)] Data on the control without herbicide application were not included in the statistical analysis. [(3)] Based on Tukey's test at 5% probability. Means followed by lowercase letter, in the columns, compare the populations within each herbicide treatment, and capital letters in the rows, compare herbicide treatments within each population.

Table 4 - Plant dry matter of 12 populations[(1)] of *Digitaria insularis* collected at 60 days after application of isolated glyphosate and mixed with quizalofop-p-tefuryl, in addition to the control without herbicide. Jaboticabal, SP. 2014/2015

Population	Herbicide/control			
	Glyphosate		Glyphosate+quizalofop	Control
	1.44 kg ha^{-1}	2.16 kg ha^{-1}	2.16+0.12 kg ha^{-1}	
	Dry matter (g vase-1) - 60 DAA			
1	19.7 bc B[(2)]	17.7 c B	0.0 a A	31.7 a C
2	17.0 bc B	12.4 abc B	0.0 a A	36.4 ab C
3	0.0 a A	0.0 a A	0.0 a A	37.2 ab B
4	12.3 ab B	16.7 c B	0.0 a A	36.7 ab C
5	0.0 a A	0.0 a A	0.0 a A	42.6 ab B
6	0.0 a A	0.0 a A	0.0 a A	35.5 ab B
7	20.8 bc B	11.4 abc B	0.0 a A	32.0 a C
8	0.0 a A	0.0 a A	0.0 a A	46.0 b B
9	23.6 bc B	8.0 abc A	0.0 a A	41.8 ab C
10	29.0 c B	8.3 abc A	0.0 a A	37.7 ab B
11	24.9 bc BC	15.9 bc B	0.0 a A	33.5 ab C
12	19.8 bc B	2.5 ab A	0.0 a A	34.5 ab C
LSD (in the row)	10.9			
LSD (in the column)	13.9			

[(1)] 1 and 11 - area of Pedrinhas Paulista (SP); 2 - area of Jaboticabal (SP); 3 - area of Chapadão do Céu (GO); 4 - area of Itaquiraí (MS); 5 and 7 - area of Rio Verde (GO); 6 and 8 - area of Cristalina (GO); 9 - area of Morrinhos (GO); 10 - area of Chapadão do Sul (MS); 12 - area of Taquaral (SP). [(2)] Based on Tukey's test at 5% probability. Means followed by lowercase letter, in the columns, compare the populations within each herbicide treatment, and capital letters in the rows, compare herbicide treatments within each population.

the herbicide treatments within each population. To compare the herbicide treatments for populations 9, 10 and 12, the highest rate of glyphosate alone did not differ from the mixture with quizalofop, contrary to populations 1, 2, 4, 7 and 11. For these populations, the two glyphosate rates did not differ among themselves, with a reduction of up to 66% in mass accumulation by plants, but they differed for glyphosate + quizalofop.

It should be noted that, for population 1,4% of the plants sprayed with glyphosate alone (at the two rates) died, unlike the other plants (96%), which survived. This equates to one out of 24 evaluated plants. This result indicated that this population is composed of susceptible individuals in a smaller percentage, and tolerant ones in more expressive amounts. The emergent flora is a reflection of the soil seed bank; thus, it can be inferred that the seed bank of the assessed location has susceptible and tolerant specimens. The same result was found for populations 7, in 8% of the plants; 9, 13%; 11, 4%; and 12, 17.8%. This characteristic has influenced on the scores for control and shoot dry matter, because the values were obtained per pot (experimental unit) rather than per plant (specimen) in the pot.

In short, the results indicated that the lowest rate of glyphosate was enough to control populations 3, 5, 6 and 8; and with the increased rate, results were satisfactory for populations 9, 10 and 12. However, none of the rates of isolated glyphosate was efficient for populations 1, 2, 4, 7 and 11. In this sense, the populations were classified based on their response to treatments with isolated glyphosate (at two rates), particularly for dry matter accumulation by the plants. Populations 3, 5, 6 and 8 were considered to be susceptible to glyphosate; 9, 10 and 12, moderately susceptible; and 1, 2, 4, 7 and 11, tolerant.

Population 6 showed high pilosity, without consequences for the efficacy of isolated glyphosate (at two rates), because the plants were susceptible to it (Table 5). Populations 4 and 7 had mean pilosity, while the plants of other populations showed low pilosity. The presence of hairs on the leaves can assist in retention, reflecting in greater herbicide absorption on the leaf blade (Silva and Silva, 2007); however, very pillous leaves may retain and absorb a lesser amount of the product because the density and the type of trichomes can intercept the drops, thus preventing them from reaching the epidermis.

The plants of population 4 showed a drooping growth pattern, unlike others, which were upright. A drooping growth habit may favor herbicide retention by the leaf and, consequently, greater absorption by the leaf blade. However, although population 4 has this characteristic, this has not benefited the action of glyphosate, because this population was tolerant to the product.

Populations 8 and 3 were the ones that showed the highest and lowest leaf wax contents, respectively, both of them being susceptible to glyphosate. This fact evidenced that, even with a variation in wax content between leaves of populations, this did not affect plant control by glyphosate, especially in populations classified as sensitive. The process of herbicide absorption through the epicuticular layer may be influenced by environmental factors (light, temperature and relative humidity), cuticle thickness, wax content and pilosity of leaves. However, absorption is not necessarily linked to cuticular weight or thickness but rather to lipid peroxidation and degree of impediment of the passage of solutes (Silva and Silva, 2007).

Table 5 **-** Classification of populations of *Digitaria insularis* according to sensitivity to glyphosate, quantification of wax content, visual assessment of pilosity (high, medium or low) and plant growth habit (drooping or upright). Jaboticabal, SP. 2014/2015

Population	Classification	Wax content (absorbance cm²)	Pilosity	Growth
1(1)	Tolerant	0.0060	Low	Upright
2	Tolerant	0.0028	Low	Upright
3	Susceptible	0.0021	Low	Upright
4	Tolerant	0.0037	Mean	Drooping
5	Susceptible	0.0042	Low	Upright
6	Susceptible	0.0047	High	Upright
7	Tolerant	0.0055	Mean	Upright
8	Susceptible	0.0072	Low	Upright
9	Mean for susceptible populations	0.0056	Low	Upright
10	Mean for susceptible populations	0.0048	Low	Upright
11	Tolerant	0.0036	Low	Upright
12	Mean for susceptible populations	0.0043	Low	Upright

(1) 1 and 11 - area of Pedrinhas Paulista (SP); 2 - area of Jaboticabal (SP); 3 - area of Chapadão do Céu (GO); 4 - area of Itaquiraí (MS); 5 and 7 - area of Rio Verde (GO); 6 and 8 - area of Cristalina (GO); 9 - area of Morrinhos (GO); 10 - area of Chapadão do Sul (MS); 12 - area of Taquaral (SP).

As regards leaf anatomical analysis, there was a significant effect ($p<0.01$) of the populations on all assessed variables (Tables 6 and 7). The leaves of population 7 showed higher total blade on the vein, higher main vein length and width and higher secondary vein length and width, in addition to longer center of the blade. Population 10 had the largest mean values of leaf blade length and the length of the adaxial and abaxial epidermis. Populations 1 and 4 showed the highest values for length and width of sustaining tissue, respectively.

In general, the histological analysis of the leaves of the 12 populations of *D. insularis* indicated that the adaxial epidermis showed thicker bulliform cells, when compared with the abaxial epidermis, which may interfere in and hinder herbicide absorption in the leaves. The secondary veins were non-uniform on the leaf surface: some were smaller while others were larger. In the periphery of the main veins, and there was schlerenchimain some secondary veins. It is a woody sustaining tissue that, depending on width and length, may also influence herbicide absorption (Figure 1).

However, the comparison across the populations within each group (susceptible, moderately susceptible and tolerant) showed that they have not had similar characteristics which would justify leaf anatomy as the main factor for tolerance of the populations to glyphosate. In this respect, Barroso (2013), when evaluating leaf anatomy characteristics of two biotypes (one susceptible and one resistant) of *D. insularis*, reported that there was no difference between them for any evaluated characteristic.

Scanning electron microscope (SEM) (Figure 2) has not identified morphological differences in the leaf surface of the 12 populations of *D. insularis*. The leaf epidermis showed elongated cells with four sides and straight anticlinal walls, clarly arranged longitudinally. Leaf epidermis showed elongated cells with four sides and straight anticlinal walls, clearly arranged longitudinally. The stomata guard cells were dumbbell-shaped and surrounded by two subsidiary cells. They had extended ends with thin walls, while the median region, facing the ostiole, was narrower and had thickened walls. There were unbranched unicellular or multicellular tector trichomes. There were longitudinal veins on the leaves, whose cells were small and often suberized or had a silica body.

Principal component analysis was performed with the purpose of identifying possible differentiation among populations (susceptible, moderately susceptible and tolerant) based on

Table 6 - Mean values for total blade size on the nerve, length and width of main nerve and length and width of sustaining tissue of leaves of 12 populations of *Digitaria insularis*. Jaboticabal, SP. 2015

Population	Total blade	Main nerve		Sustaining tissue	
		Length	Width	Length	Width
	(µm)				
1[(1)]	185.0 b[(2)]	98.5 bc	92.1 de	26.4 d	53.6 bc
2	192.6 bc	97.0 ab	84.7 b	20.0 bc	53.5 bc
3	189.4 bc	102.3 bcd	87.2 bcd	19.9 bc	57.9 d
4	186.1 b	102.5 bcd	87.4 bcde	19.5 b	64.0 e
5	173.5 a	100.7 bcd	84.9 bc	19.8 bc	54.0 bc
6	167.8 a	90.0 a	78.1 a	17.7 a	46.5 a
7	222.2 d	115.0 f	106.0 g	20.2 bc	58.7 d
8	198.5 c	106.8 de	92.7 e	21.4 c	54.0 bc
9	186.4 b	104.1 bcde	92.6 de	19.7 bc	54.1 bc
10	219.1 d	110.3 ef	99.2 f	16.0 a	51.1 b
11	188.6 bc	105.3 cde	91.8 de	19.6 b	55.9 cd
12	194.4 bc	106.8 de	90.2 cde	17.0 a	52.0 b
F	50.4 **	16.1 **	37.1 **	45.4 **	27.6 **
VC (%)	10.1	13.6	11.3	16.8	13.0
LSD	10.3	7.5	5.5	1.8	3.8

(1) 1 and 11 - area of Pedrinhas Paulista (SP); 2 - area of Jaboticabal (SP); 3 - area of Chapadão do Céu (GO); 4 - area of Itaquiraí (MS); 5 and 7 - area of Rio Verde (GO); 6 and 8 - area of Cristalina (GO); 9 - area of Morrinhos (GO); 10 - area of Chapadão do Sul (MS); 12 - area of Taquaral (SP). (2) Based on the Tukey's test at 5% probability. Means followed the same letter, in the columns, compare the populations (control without herbicide application), within each analyzed variable.

Table 7 **-** Mean values for leaf blade, adaxial and abaxial epidermis, length and width of secondary vein and length from center of leaf blade of 12 populations of *Digitaria insularis*. Jaboticabal, SP. 2015

Population	Leaf blade	Epidermis		Secondary vein		Length of center of blade
		Abaxial	Adaxial	Length	Width	
	(µm)					
1[1]	134.2 a[2]	12.6 a	41.9 a	46.7 abcd	45.5 de	341.9 b
2	163.4 bcd	16.3 bcde	48.7 abc	44.7 a	41.9 abc	277.3 a
3	169.4 de	18.6 e	55.8 def	45.3 a	41.8 ab	449.8 f
4	165.2 cde	15.4 abcd	53.1 cde	46.2 abc	40.6 a	443.6 f
5	154.4 b	13.7 ab	48.1 abc	49.2 bcde	44.4 bcd	346.4 b
6	140.1 a	13.7 abc	42.1 ab	46.1 ab	42.5 abc	285.9 a
7	174.6 ef	16.5 cde	56.4 ef	55.6 f	49.6 f	478.2 g
8	160.4 bcd	17.5 de	54.3 cdef	49.4 cde	42.5 abc	413.0 e
9	156.5 bc	15.5 bcd	49.1 bcd	49.7 de	44.8 cde	391.2 d
10	183.3 f	22.1 f	60.9 f	52.2 e	47.4 ef	373.5 c
11	164.4 bcd	17.8 de	57.5 ef	46.8 abcd	43.1 abcd	448.1 f
12	166.6 cde	16.8 de	53.1 cde	49.1 bcde	43.1 abcd	408.0 e
F	39.4 **	17.3 **	15.4 **	18.9 **	16.8 **	1060.3**
VC (%)	11.7	32.31	25.2	12.9	12.3	1.80
LSD	10.1	2.8	7.0	3.3	2.9	9.4

[1] 1 and 11 - area of Pedrinhas Paulista (SP); 2 - area of Jaboticabal (SP); 3 - area of Chapadão do Céu (GO); 4 - area of Itaquiraí (MS); 5 and 7 - area of Rio Verde (GO); 6 and 8 - area of Cristalina (GO); 9 - area of Morrinhos (GO); 10 - area of Chapadão do Sul (MS); 12 - area of Taquaral (SP). [2] Based on the Tukey's test at 5% probability. Means followed the same letter, in the columns, compare the populations (control without herbicide application), within each analyzed variable.

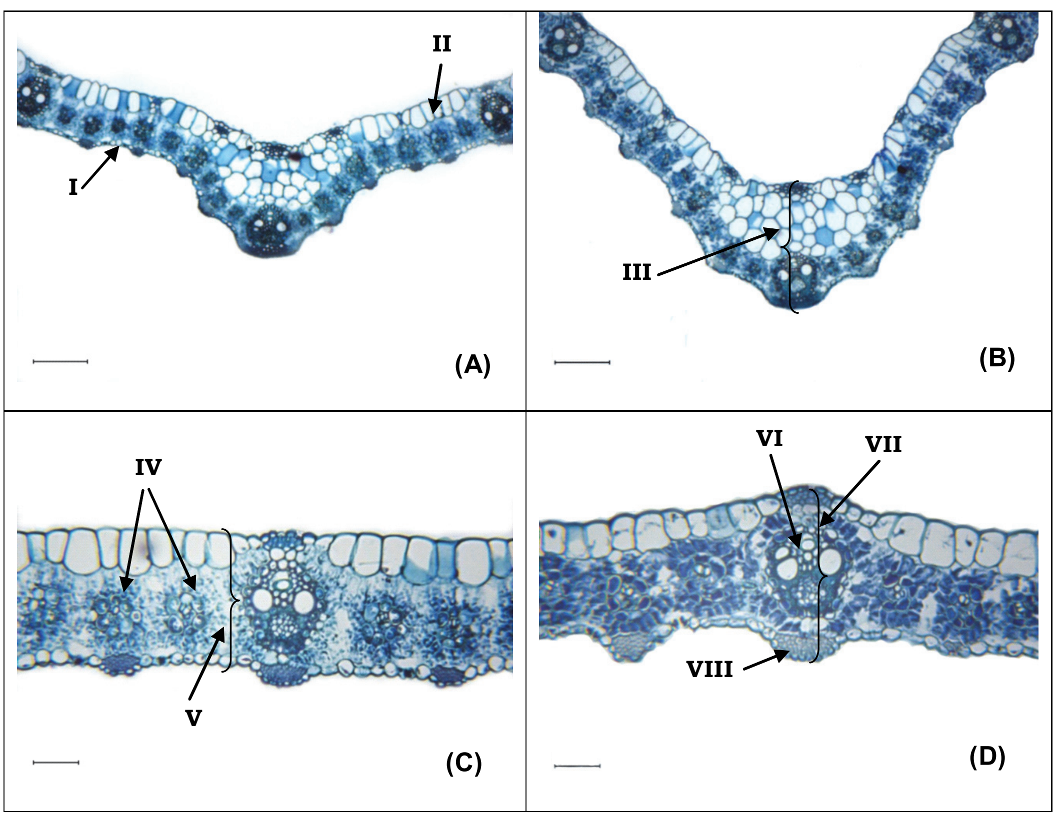
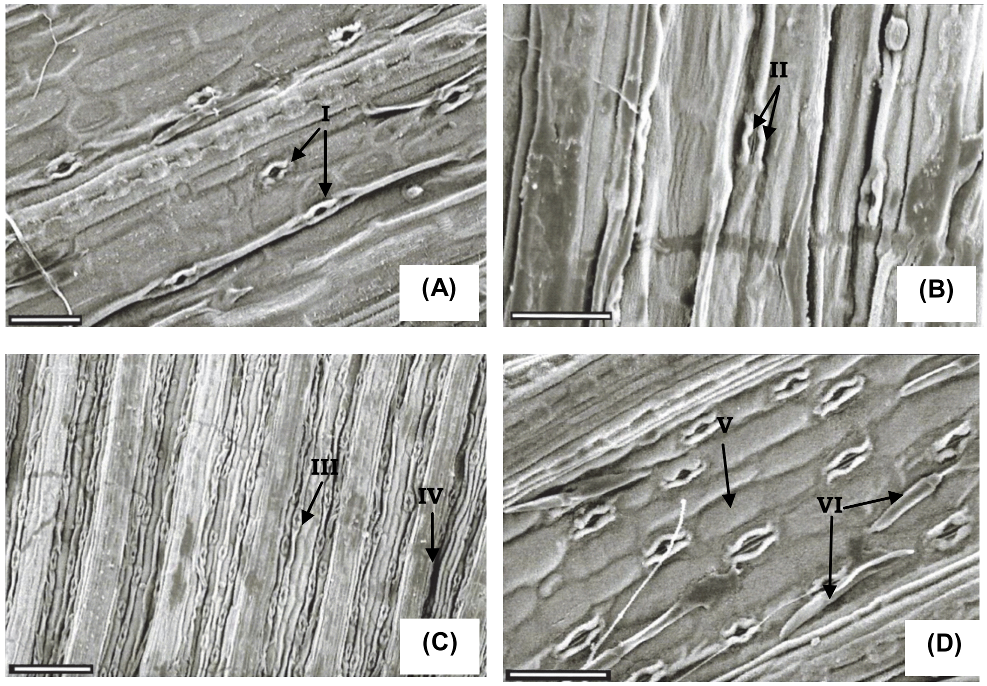


Figure 1 **-** Cross sections of the leaf surface of *Digitaria insularis* (A, B - 150 µm / C, D - 50 µm). (I) abaxial epidermis; (II) adaxial epidermis; (III) length from center of blade; (IV) secondary veins; (V) LEAF; (VI) main vein; (VII) total blade; (VIII) sustaining tissue.



A - (50 µm) - (I) stomata in evidence; B - (50 µm) - (II) subsidiary cells bypassing the guard cells; C - (100 µm) - (III) stomata in a row; (IV) main vein; D - (50 µm) - (V) elongated cells; (VI) trichomes in evidence.

Figure 2 - Scanning Electron Microscopy (SEM) of abaxial leaf epidermis (A, B) and adaxial leaf epidermis (C, D) of *Digitaria insularis*.

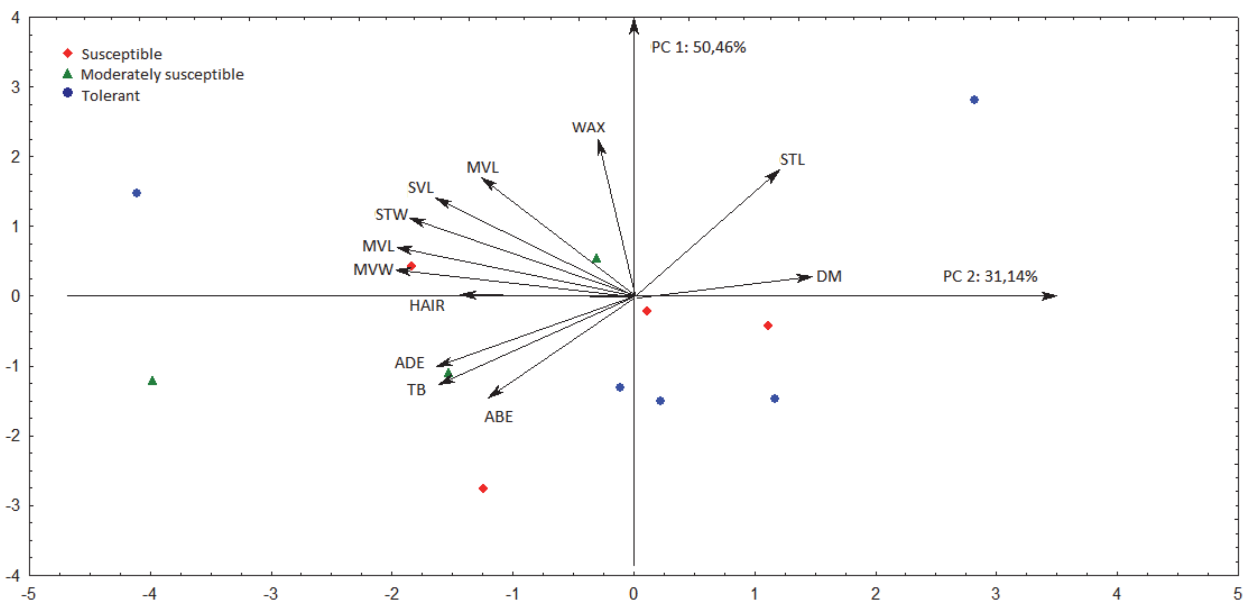


Figure 3 - Principal components analysis (PCA) of PC1 and PC2 with the variables: pilosity (hair), epicuticular wax (wax), plant dry matter with application of 2.16 kg a.i. ha^{-1} (DM), total blade (TB), main vein length (MVL), main vein width (MVW), sustaining tissue length (STL), sustaining tissue width (STW), total blade of vein (TBV), secondary vein length (SVL), secondary vein width (SVW), adaxial epidermis (ADE) and abaxial epidermis (ABE).

the evaluated morpho-anatomical characteristics. It showed that there was no difference across the groups (Figure 3). Principal components analysis showed the formation of a 2-dimensional plane generated by two main components, PC1 and PC2, which accounted for 81.6% of the original information (50.46% in PC1 and 31.14% in PC2). This result is in line with the criterion established by Sakai (1973), whereby the PC number used in the interpretation of data must be such that it accounts for at least 70% of the total variance of the data.

The lack of differentiation between the groups (susceptible, moderately susceptible and tolerant), by principal component analysis, can be justified by the fact that the evaluated characters are not decisive parameters for the distinction of populations. In addition to the morpho-anatomical characteristics relative to herbicide absorption the by plant, other factors may affect herbicide effectiveness, such as translocation, metabolization and even mutation in the target enzyme.

The most expressive attributes extracted by CP1 were total blade of vein (r = 0.93), main vein length (r = -0,95), main vein width (r = -0.89), total blade (r = -0.84), abaxial epidermis (r = 0.74), adaxial epidermis (r = 0.86), secondary vein length (r = -0.78) and secondary vein width (r = -0.63), which showed an inverse correlation, while the variables extracted by CP2 were wax content (r = 0.78), sustaining tissue length (r = 0.65) and secondary vein width (r = 0.60), with a positive correlation.

Populations 3, 5, 6 and 8 were considered susceptible to glyphosate; 9, 10 and 12, moderately susceptible; 1, 2, 4, 7 and 11, tolerant. All populations have the same morphological features, but they differ in their anatomical characteristics and wax content. However, the populations within each group (susceptible, moderately susceptible or tolerant) had similar characteristics that justify their response to glyphosate.

REFERENCES

Barroso A.A.M. **Caracterização genética e foliar de capim-amargoso resistente ao herbicida glyphosate e eficácia do seu controle com associação de herbicidas** [dissertação]. Piracicaba: Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 2013.

Correia N.M., Acra L.T., Balieiro G. Chemical control of different *Digitaria insularis* populations and management of a glyphosate-resistant population. **Planta Daninha**. 2015;33:93-101.

Correia N.M., Leite G.J., Garcia L.D. Resposta de diferentes populações de *Digitaria insularis* ao herbicida glyphosate. **Planta Daninha**. 2010;28:769-76.

Correia N.M., Durigan J.C. Manejo químico de plantas adultas de *Digitaria insularis* com glyphosate isolado e em mistura com chlorimuron-ethyl ou quizalofop-p-tefuryl em área de plantio direto. **Bragantia**. 2009;68:689-97.

Ebercon A., Blum A., Jordan W.R. A rapid colorimetric method for epicuticular wax content of sorghum leaves. **Crop Sci.** 1977;17:179-80.

Ferreira D.F. Sisvar: a computer statistical analysis system. **Ci Agrotecnol.** 2011;35:1039-42.

Gemelli A. et al. Aspectos da biologia de *Digitaria insularis* resistente ao glyphosate e implicações para o seu controle**. Rev Bras Herbic.** 2012;11:231-40.

Huangfu C.H., Song X.L., Qiang S. ISSR variation within and among wild *Brassica juncea* populations: implication for herbicide resistance evolution. **Genetic Res Crop Evol.** 2009;56:913-24.

Johansen D.A. **Plant microtechnique**. New York: McGrow-Hill Book, 1940. 523p.

Kissmann K.G. **Plantas infestantes e nocivas**. São Paulo: BASF Brasileira, 1997. p.675-8.

Melo M.S.C. et al. Alternativas para o controle químico de capim-amargoso (*Digitaria insularis*) resistente ao glyphosate. **Rev Bras Herbic.** 2012;11:195-203.

Procópio S.O. et al. Estudos anatômicos de folhas de espécies de plantas daninhas de grande ocorrência no Brasil. III - *Galinsoga parviflora*, *Crotalaria incana*, *Conyza bonariensis* e *Ipomoea cairica*. **Planta Daninha**. 2003;21:1-9.

Sakai W.S. Simple method for differential staining of paraffi n embedded plant material using toluidine blue O. **Stain Technology**, v.43, p.247-249, 1973.

Silva A.A., Silva J.F. **Tópicos em manejo de plantas daninhas**. Viçosa, MG: Universidade Federal de Viçosa, 2007. 367p.

Timossi P.C. Manejo de rebrotes de *Digitaria insularis* no plantio direto de milho. **Planta Daninha**. 2009;27:175-9.

Weed Science. **International survey of herbicide resistant weeds**. [accessed on: 27 mar. 2016]. Available at: http://weedscience.org/details/case.aspx?ResistID=5351.