

# HERBICIDE EFFICACY ON *Borreria densiflora* CONTROL IN PRE- AND POST-EMERGENCE CONDITIONS<sup>1</sup>

*Eficácia de Herbicidas no Controle de Borreria densiflora em Condições de Pré e Pós-Emergência*

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**ABSTRACT** - The weed *Borreria densiflora* is a management issue in soybean and sugarcane crops from North and Northeastern Brazil. Knowledge upon chemical control of *B. densiflora* contributes to the integrated management of this weed species, especially when active ingredient options become reduced due to the selection of herbicide resistant or tolerant weed species. Experiments in pre- and post-emergence of *B. densiflora* were conducted in greenhouse, in a randomized block design and four replications. In pre-emergence, the dose-response curve methodology was used and 7 herbicides were tested. In post-emergence, 9 herbicides at the recommended rate and 4 herbicide mixtures were tested. For pre and post-emergence conditions, evaluations were conducted at 60 and 21 days after treatment (DAT), respectively, and the variables analyzed were weed control and dry weight (%). The results showed options of pre-emergent herbicides that can be used for controlling *B. densiflora*, especially in sugarcane, where chemical weed control is mainly based on pre-emergent applications. In the current glyphosate resistance scenario, one should consider the use of pre-emergent herbicides within an integrated management of *B. densiflora*. For satisfactory post-emergence control, *B. densiflora* plants should be sprayed at the phenological stage of up to three pairs of leaves. Herbicide mixtures have been and will continue to be an important tool in chemical weed management, broadening the spectrum of weed control, while diversifying herbicide mechanisms of action, which helps to prevent or delay the appearance of herbicide resistance.

**Keywords:** vassourinha-de-botão, chemical control, dose-response, herbicide mixtures, integrated weed management.

**RESUMO** - A planta daninha *Borreria densiflora* é uma espécie de difícil manejo em soja e cana-de-açúcar no Norte e Nordeste do Brasil. O conhecimento do manejo químico de *B. densiflora* contribui para o manejo integrado dessa espécie, sobretudo quando opções de ingredientes ativos se tornam reduzidas devido à seleção de plantas daninhas resistentes e tolerantes. Experimentos em pré e pós-emergência de *B. densiflora* foram instalados em casa de vegetação em blocos casualizados com quatro repetições. Em pré-emergência, a metodologia de curvas de dose-resposta foi utilizada e sete herbicidas foram testados. Em pós-emergência, nove herbicidas e quatro misturas destes foram testados. Em pré e pós-emergência, as avaliações foram feitas aos 60 e 21 dias após aplicação (DAA), respectivamente, e as variáveis avaliadas foram controle e massa seca (%) de *B. densiflora*. Os resultados indicaram opções de herbicidas pré-emergentes que podem ser usados para controle de *B. densiflora*, especialmente em cana-de-açúcar, em que o manejo químico de plantas daninhas baseia-se em aplicações pré-emergentes. Diante do fenômeno da resistência de plantas daninhas ao glyphosate, devem-se considerar os herbicidas pré-emergentes como uma ferramenta importante dentro de um programa de manejo integrado de *B. densiflora*. Para controle pós-emergente satisfatório, plantas dessa espécie devem ser tratadas no estágio fenológico de até três pares de folhas. As misturas de herbicidas têm sido e continuarão a ser uma importante ferramenta no controle químico de plantas daninhas, aumentando o espectro de controle de plantas daninhas e diversificando os mecanismos de ação, o que ajuda a prevenir e retardar o aparecimento da resistência à herbicidas.

**Palavras-chave:** vassourinha-de-botão, controle químico, dose-resposta, mistura de herbicidas, manejo integrado de plantas daninhas.

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## INTRODUCTION

Weed management tactics are converging to the most sustainable manner possible, where cultural, mechanical and chemical control methods have been integrated within the production systems. Chemical control is the most predominant method in agricultural production systems worldwide (Zimdahl, 2013). Even before crop planting, mechanical soil operations have been widely replaced by herbicide applications in several cropping systems. The herbicides used in this pre-planting chemical method are known as burndown herbicides, within no-tillage systems.

In Brazil, herbicides that are registered to be used in pre-planting burndown operations are: 2,4-D, diquat, glyphosate, MSMA, paraquat, glufosinate-ammonium (Melhorança, 2002). Herbicide tank mixes, including glyphosate + 2,4-D and carfentrazone-ethyl + glyphosate, are also used by growers for burndown operations. Among the burndown herbicides, paraquat and glyphosate are the most used, and therefore, subjected to several efficacy evaluation studies (Voll, 1987). The tank mixture of 2,4-D and glyphosate results in greater broadleaf activity, without losing the graminicide activity of glyphosate (Koch et al., 1989; Oliveira Jr., 2001). Herbicide efficacy depends on several factors, including physico-chemical characteristics and dose of the product, the weed species to be controlled and the phenological stage of weed and crop (Christoffoleti & López-Ovejero, 2004).

The weed *B. densiflora* is reported to be a difficult-to-control weed species, especially with burndown herbicide applications in no-tillage fields, where it has been reported as occurring in large infestations in soybean and sugarcane production areas in North and Northeastern Brazil. This weed is a simple perennial species within the Rubiaceae family and belongs to the genus *Borreria* G. Mey. Thermal amplitudes, temperatures above 25 °C, presence of light and capacity of regrowth after cutting influence the population dynamics of *B. densiflora* (Martins et al., 2009, 2010).

The present study had the objective to evaluate the efficacy of some herbicides and herbicide mixtures recommended for pre and

post-emergence management, as well as in burndown applications, on the control of *B. densiflora*.

## MATERIAL AND METHODS

**Pre-emergence** - Seeds were collected in 2007 from soybean production fields infested with *B. densiflora*, in North Tocantins State (08°58'03"S 48°10'29"N). The experimental design used was the randomized block design, with four replications, under an 8 x 7 factorial arrangement. The herbicides tested were chosen based on their representativeness in pre-emergent applications in soybean and sugarcane (Table 1). Eight doses of each herbicide tested were used (1/16D, 1/8D, 1/4D, 1/2D, 1D, 2D, 4D and 0D). D is the recommended dose in the label of each herbicide (Table 1), and the experiment comprised 224 experimental units.

A 3L plastic pot represented each experimental unit, which contained clay soil (46,5% of clay, 14,5% of silt e 39% of sand) and 40 seeds of *B. densiflora*. Proportional fertilization of 37,5 kg ha<sup>-1</sup> of nitrogen, 37,5 kg ha<sup>-1</sup> of phosphorus and 50 kg ha<sup>-1</sup> of potassium was provided to all pots containing the seeds. The chemical properties of the soil used in the pre-emergence experiment are presented in Table 2.

Herbicide spraying was done using a XR80.02, even, flat-fan nozzle and an overhead, compressed-air sprayer, calibrated to deliver 216 L ha<sup>-1</sup>, working at a pressure of 2.5 bar. Following herbicide spraying, all pots received a water lamina proportional to a 10 mm precipitation. Irrigation was provided as necessary.

**Post-emergence** - Seeds of *B. densiflora* were germinated in germination chamber under 20/30 °C day/night temperatures with a 12-hour photoperiod (Martins et al., 2010). The seedlings were transplanted to 1.5 L plastic pots. Ten days after transplanting, the seedlings were thinned to one plant per pot. The pots were filled with commercial potting mix (Plantmax<sup>®</sup>) and fertilized with 4 g of the granulated commercial formula 10-10-10 and 0.1 g of Ouro Verde<sup>®</sup> commercial liquid fertilizer diluted in 50 mL of water.

At the 3 pairs of leaves stage, treatments were applied at the recommended rate of each herbicide (Table 3). Herbicide spraying was done using a XR110.02, even, flat-fan nozzle and an overhead, compressed-air sprayer, calibrated to deliver 195 L ha<sup>-1</sup>, working at a pressure of 2 bar.

For both pre-emergence and post-emergence experiments, control was

visually recorded, at 60 and 28 DAT, respectively, ranging from 0% (lack of control) to 100% (absolute control), according to the scale developed by the Latin America Weed Association (ALAM, 1974). Shoot biomass was collected, stored in paper bags and kept in air flow chamber at 70 °C for 48 hours. Dry biomass data are expressed as percentages of the untreated control.

**Table 1** - Pre-emergent herbicide treatments at the recommended rate (1D)

Treatment		Rate	
Commercial product (c.p.)	Active ingredient (a.i.)	(mL or g p.c. ha <sup>-1</sup> )	(g a.i. ha <sup>-1</sup> )
Untreated Control		--	--
Gamit	clomazone	2,500	1,250
Spider 840 WG	diclosulan	41.7	35.02
Dual Gold	S-metolachlor	2,000	1,920
Sencor 480	metribuzin	1,000	480
Herbadox 500 CE	pendimethalin	3,000	1,500
Scepter	imazaquin	1,100	165
Boral 500 SC	sulfentrazone	1,200	600

**Table 2** - Chemical properties of the soil used in the pre-emergence experiment

M.O.	P resin	K	Ca	Mg	H+Al	Al	SB	CTC	V	m
(g dm <sup>-3</sup> )	(mg dm <sup>-3</sup> )	(mmol <sub>c</sub> dm <sup>-3</sup> )						(%)		
8	1	0,3	36	11	15	0	47	62	76	0

**Table 3** - Post-emergent herbicide treatments at the recommended rate

Commercial product (c.p.)	Active Ingredient (a.i.)	Rate	
		(mL or g c.p. ha <sup>-1</sup> )	(g a.i. ha <sup>-1</sup> )
Untreated Control		--	--
Cobra	lactofen	500	120
Flex	fomesafen	600	150
Pivot	imazethapyr	700	74.2
Classic	chlorimuron-ethyl	50	12.5
Pivot + Cobra	imazethapyr + lactofen	600 + 300	60 + 72
Pivot + Classic	imazethapyr + chlorimuron-ethyl	600 + 0.04	60 + 10
Pivot + Classic + Cobra	imazethapyr + chlorimuron + lactofen	600 + 0.04 + 300	60 + 10 + 72
MSMA San. 720 SL	MSMA	4,000	2,880
DMA 806	2,4-D	1,000	670
Roundup WG	glyphosate	1,500	1,080
Roundup WG + DMA 806	glyphosate + 2,4-D	1,500 + 1,000	1,080 + 670
Aurora + Roundup WG	carfentrazone-ethyl + glyphosate	32 + 1,500	12.8 + 1,080
Gramoxone	paraquat	2,000	400



**Statistical Analysis** - For the dose-response experiment in pre-emergence, herbicide was considered as a factor, and the doses represented the levels of the factor. Control (%) data were adjusted to a hyperbolic model of 2-parameters,  $a$  and  $b$ , as follows:

$$y = \frac{(a * b)}{(b + x)}$$

where  $y$  is *B. densiflora* control (%),  $a$  and  $b$  are the slope and asymptote of the hyperbolic curve, respectively, and  $x$  is the herbicide rate. Significance of factorial interactions was analyzed (herbicide\*dose) using the least square means test with Tukey adjustment for multiple comparisons via the PROC GLM statement in SAS (version 9.3, SAS Institute, Cary, NC). For the post-emergence experiment with a single dose for each herbicide tested, data were subjected to ANOVA to test significant differences among herbicides for control and dry biomass (%) using ANOVA with the PROC GLM statement in SAS (version 9.3, SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

For the experiment in pre-emergence, a significant interaction between herbicide and dose was detected ( $p < 0.0001$ ), and control data were fit to a hyperbolic regression (Figure 1, Tables 4 and 5). Studies have been reported using hyperbolic regressions for building dose-response curves to other weed species (Bósnic & Swanton, 1997; Monquero, 1999; Knezevic et al., 2009).

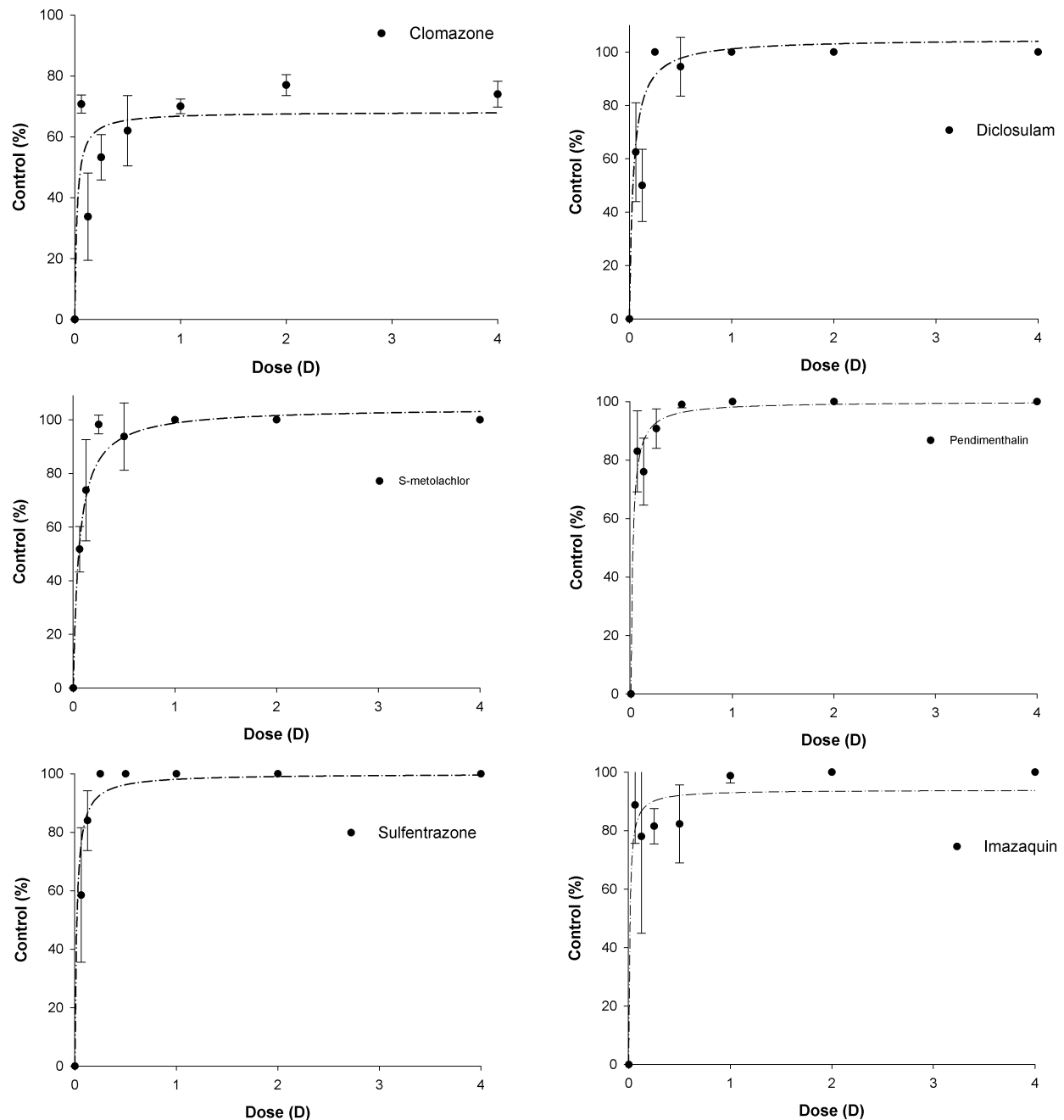
All herbicides, except clomazone, controlled *B. densiflora* since the lowest rate, indicating the high susceptibility of this species to these herbicides (Figure 1). There was no difference in control or dry biomass (%) among the herbicides tested, except clomazone. We decided to test clomazone because despite of being recommended to control grass species, clomazone has activity on some broadleaf weeds. *Borreria densiflora* control with clomazone at the field rate was not satisfactory and was lower compared to the other herbicides tested (Table 5).

The organic matter content in the soil is the first factor that influences the sorption of

many herbicides, including metribuzin (Harper, 1994). Control and dry biomass (%) at 60 DAT for metribuzin were 100% and 0%, respectively, since the lowest rate. Thus, plots for metribuzin were not included in Figure 1. Organic matter increases microbial activity, increasing the speed with which herbicides are degraded in the soil, resulting in reduced persistence (Prata & Lavorenti, 2000). The low organic matter content in the soil used in this experiment might have contributed to the availability and persistence of the pre-emergent herbicides tested, even in low rates, except clomazone, until 60 DAT.

Diclosulam controlled *B. densiflora* since  $\frac{1}{4}$  of its recommended rate. This herbicide possesses great efficacy in broadleaf weed species such as *Spermacoce latifolia*, in the Rubiaceae family, with which *B. densiflora* share similar characteristics. Imazaquin provided satisfactory control of *B. densiflora* with the recommended rate (Table 5). This herbicide was widely used in soybean as an alternative to metribuzin tolerant weed species. Despite that, in the mid 1990's, imazaquin resistant weed species were selected after several years of repetitive use of this herbicide. These species include *Euphorbia heterophylla* and *Bidens pilosa* and tolerant species were also selected, such as the weed *Cardiospermum halicacabum* (Vargas & Gazziero, 2014).

Pre-emergent herbicides, including metribuzin and imazaquin, and selective post-emergent, herbicides were gradually replaced by glyphosate after the glyphosate resistant soybean was launched. The repetitive use of glyphosate lead to the selection of resistant and/or tolerant weed biotypes to this herbicide. As examples, *B. latifolia* is known to be tolerant to glyphosate (Gazziero & Prette, 2005; Maciel et al., 2009), and woody borerria (*B. verticillata* syn. *Hedyotis verticillata*) was found to be resistant to both paraquat and glyphosate in oil palm plantations from Malaysia (Chuah et al., 2005). Therefore, the pre-emergent herbicides tested in this study represent options to be included in the integrated management of not only species within the *Borreria* genus, but also other weed species in glyphosate resistant soybean, which was estimated to be 90% of the planted



**Figure 1** - Control (%) of *B. densiflora* at 60 DAT as a function of pre-emergent herbicide dose. Dose 1 represents the herbicide recommended rate (1D).

soybean area in Brazil in the 2012/2013 season (Gomes & Borém, 2013). Moreover, several weed species, including those from the *Borreria* genus, possess ecological plasticity throughout Brazil and are on the list of species likely to develop resistance to herbicides used in cultivation, being the soybean crop genetically modified or not (Vivian et al., 2013).

It is necessary to avoid the continuous use of herbicides with the same mechanism of action in the same area, favoring the rotation of mechanisms of action in order to collaborate with the reduction of selection pressure upon the agroecosystem (Christoffoleti et al., 1994). Understanding herbicide efficacy on weed species over time is important because



**Table 4** - Estimates of parameters a and b and determination coefficients ( $r^2$ ) and parameter probabilities (p) of the hyperbolic model for the response variable control (%), at 60 DAT<sup>1/</sup>

Herbicide	Parameter		$r^2$	p (model)
	a	b		
Clomazone	68.1610	0.0207	0.71	< 0.0001
p	< 0.001	0.05		
Diclosulan	104.5696	0.0588	0.90	< 0.0001
p	< 0.0001	< 0.0001		
S-metolachlor	104.7782	0.0518	0.93	< 0.0001
p	< 0.0001	< 0.0001		
Pendimethalin	100.0205	0.0195	0.95	< 0.0001
p	< 0.0001	< 0.0001		
Sulfentrazone	105.0040	0.0378	0.93	< 0.0001
p	< 0.0001	< 0.0001		
Imazaquin	93.9178	0.0105	0.82	< 0.0001
p	< 0.0001	0.05		

<sup>1/</sup> Model:  $y = a \cdot x / (b + x)$ .

**Table 5** - Pre-emergent control (%) of *B. densiflora*, 60 DAT

Herbicide	Control (%)	
	Comercial dose	Half of commercial dose
Untreated control	0 c	0 c
Clomazone	80 b	70 b
Diclosulan	100 a	100 a
Pendimethalin	100 a	100 a
S-metolachlor	100 a	100 a
Metribuzin	100 a	100 a
Sulfentrazone	100 a	100 a
Imazaquin	100 a	100 a

Means followed by the same letters, in columns, do not differ at  $p = 0.005$ .

knowledge about this characteristic helps when choosing herbicide rates to obtain the desired residual effect, in case of chemical management in pre-emergence (Carvalho et al., 2005) - especially in sugarcane, given that it is the main weed management tactic used. In pre-planting operations, pre-emergent herbicides can also be mixed with post-emergence herbicides, aiming residual weed control. Thus, it is important to know the physico-chemical characteristics of each herbicide formulation to be mixed, as well as the cropping system, e.g., if tillage has been adopted or if the land is under no-tillage.

According to the germination dynamics of *B. densiflora* (Martins et al., 2010), the pre-emergent herbicides tested in this study, except clomazone, showed the necessary residual period for controlling this weed species.

For the experiment in post-emergence, the F value was significant for control and dry biomass, at 5% of probability ( $p < 0,001$ ). The herbicide MSMA had an inferior control of *B. densiflora* compared to the other herbicides tested (Table 6). At 21 DAT, *B. densiflora* control was 78.5% with MSMA. *Borreria densiflora* dry biomass (%) from all herbicide treatments was lower than that from MSMA (Table 7).

All the other herbicides tested controlled *B. densiflora* at the recommended rate, despite differences have been detected (Table 6). The control achieved with glyphosate was lower compared with all herbicide mixtures and herbicides lactofen and chlorimuron-ethyl alone. All herbicide mixtures tested provided control of *B. densiflora* above 97% (Table 6).

The mixture of glyphosate with other herbicides possessing different mechanisms of action is recommended for the prevention and control of glyphosate tolerant weed species in areas intensively treated with this herbicide, in which a reduced amount of each herbicide is used in the tank mixture (Kruse et al., 2000; Gazziero & Prette, 2005). The susceptible and resistant weed biotypes are controlled by the herbicide mixture, that is, the resistant biotype to one of the active ingredients is controlled by the other added in the mixture (Powles & Holtum, 1994).

In a study with *Spermacoce latifolia* and *Richardia braziliensis*, the mixture of glyphosate with other herbicides provided control above 95%, compared to 85% when glyphosate was applied alone (Ferreira et al., 2006). In the present study, a similar pattern was observed (Table 6).

The mixture of active ingredients has the objective to enhance weed control in comparison to the use of the same ingredients alone. When the control obtained with the mixture is greater than the expected control with the herbicides alone, such mixture is called synergistic; when the control is less than

**Table 6** - Post-emergence control (%) of *B. densiflora*, 21 DAT

Treatment	Control (%)
Untreated control	0.0 d
Glyphosate + 2,4-D	100.0 a
Paraquat	94.5 ab
Lactofen	100.0 a
Fomesafen	90.0 ab
Chlorimuron-ethyl	98.7 a
Imazethapyr	95.0 ab
Glyphosate	87.5 b
Imazethapyr + lactofen	100.0 a
Carfentrazone-ethyl + glyphosate	100.0 a
Imazethapyr + chlorimuron-ethyl	97.5 a
Imazethapyr + chlorimuron-ethyl + lactofen	100.0 a
MSMA	76.5 c
VC (5%) <sup>1/</sup> = 4.96	F <sub>(treatments)</sub> = 124.13 <sup>*</sup>

<sup>1/</sup> VC, variation coefficient at p = 0.005. Means followed by the same letters, in columns, do not differ at p = 0.005.

<sup>\*</sup> F significant at p = 0.05.

**Table 7** - Residual dry biomass (%) of *B. densiflora*, 21 DAT

Treatment	Residual dry biomass (%)
Untreated control	100.00 a
Glyphosate+2,4-D	3.52 bc
Paraquat	5.30 bc
Lactofen	1.21 c
Fomesafen	10.34 bc
Chlorimuron-ethyl	8.17 bc
Imazethapyr	12.86 b
Glyphosate	7.69 bc
Imazethapyr + lactofen	4.74 bc
Carfentrazone-ethyl + glyphosate	3.83 bc
Imazethapyr + chlorimuron-ethyl	4.52 bc
Imazethapyr + chlorimuron-ethyl + lactofen	4.88 bc
MSMA	27.65 ab
VC (5%) <sup>1/</sup> = 49.8	F <sub>(treatments)</sub> = 5.16 <sup>*</sup>

<sup>1/</sup> VC, variation coefficient at p = 0.005. Means followed by the same letters, in columns, do not differ at p = 0.005. <sup>\*</sup> F significant at p = 0.05.

the expected, the herbicide mixture is antagonistic and when control is the same between the mixture and the herbicides alone, it is called additive (Colby, 1967). Results of the present study suggest a synergistic effect of the herbicide mixtures on the control of *B. densiflora* (Table 6). However, further analyses are necessary to classify the conjoint effect of the active ingredients in the mixtures tested. Knowing both site and mode of action of the compounds in a mixture provides the biological basis for choosing the appropriate reference model to assess the effects of these compounds in the mixture (Streibig, 2003).

Due to the great weed species diversity in the region under vegetation of cerrado, where *B. densiflora* has been a management issue, the combination of glyphosate with other herbicides have been used, in order to maximize its control, while increasing the number of weed species controlled. It is important to recognize that glyphosate can be combined not only with some post-emergence herbicides used in conventional soybean, but also with some herbicides that have pre-emergence activity.

*Borreria densiflora* becomes increasingly tolerant to post-emergence herbicides during its development. In a study, when *B. densiflora* was at the phenological stage of 4-5 pairs of leaves, lack of control was observed by some herbicides at the recommended rate, including imazethapyr, chlorimuron-ethyl, fomesafen and glyphosate (data not shown). Thus, herbicide dose appears to be correlated with phenological stage for the control of *B. densiflora*. The presence of lateral buds along the main stem nodes makes the herbicide contact with the lower leaves difficult, which allows survivor of the plant even after herbicide spraying. Thus, *B. densiflora* control becomes challenging after the phenological stage of 3 pairs of leaves, even using systemic herbicides.

*Borreria densiflora* is a difficult-to-control weed species in soybean pre-planting burndown, causing growers to use high glyphosate rates to obtain satisfactory control. Therefore, within the chemical management of *B. densiflora* in post-emergence, herbicide application should be done until the 3 pairs of leaves phenological stage because once the plant produces more leaves and lateral buds, its control becomes poor. This information is important, given that glyphosate, a post-emergence herbicide, is one of the most used herbicides in soybean in Brazil (Meyer & Cederberg, 2010), both in pre-planting burndown and post-emergence applications in glyphosate resistant soybeans.

All pre-emergent herbicides tested, except clomazone, are options to control *B. densiflora*, especially in sugarcane production areas, to which chemical weed control is based predominantly on pre-emergent applications. In the current context of glyphosate resistant



weeds in the agricultural areas in Brazil and worldwide, it is important to recognize the use of pre-emergent herbicides as an important tool within integrated weed management programs. All post-emergent herbicides, except MSMA, provided satisfactory control of *B. densiflora*, when the herbicides were applied at the phenological stage of 3 pairs of leaves. It is recommended that post-emergence applications of *B. densiflora* be done until the growth stage of 3 pairs of leaves. The use of higher rates than the recommended in burndown operations to control *B. densiflora* should not be encouraged. It is important to follow the recommended rates in herbicide applications within each production system, as well as knowing the most appropriate time of spraying. Adoption of herbicide tank mixtures, although not allowed in official recommendations in Brazil, has been and still is an important operation to increase the spectrum of weed control within a chemical management, while saving costs of application, and slowing down the selection pressure of herbicide resistant and/or tolerant weeds.

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