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Bermudagrass Control in Sugarcane in Brazil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LCC, LRB, JEBB, VSB were responsible for the experimental conduction, author AADPMA for the laboratory analysis and its interpretation, author ALC for the translation and writing of the article and author CAMA for the operational and financial coordination of the team and writing of the article. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Evaluation of the chemicals in controlling bermudagrass weed and effects on sugarcane selectivity.
Study Design: Chamber growth studies: completely randomized design with nine treatments with five replicates. Field studies: Randomized block design with nine treatments with five replicates
Place and Duration of Study: Instituto Agronômico, Centro de Cana, São Paulo State, Brazil, between February/2018 and December/2019.
Methodology: Bermudagrass chemical control was studied in growth chamber in pots. In the first stage, imazapyr, clomazone, indaziflam, sulfentrazone and the control treatment were studied. In the second stage, imazapyr, clomazone, indaziflam were applied and a treatment with no herbicides

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was maintained. After 75 days of imazapyr application and 38 days of clomazone and indaziflam, clomazone + indaziflam and clomazone + sulfentrazone were applied, in addition to the control treatment. Sugar cane selectivity study was carried out in the field. Before sugarcane planting, imazapyr, clomazone, indaziflam were applied. After planting, clomazone + indaziflam and clomazone + sulfentrazone were applied, in addition to the control treatment.
Results: Clomazone at 1050.0 g ha^{-1} applied as pre plant at 38 days before planting followed by clomazone at 1050.0 g ha^{-1} plus sulfentrazone at 650.0 g ha^{-1} applied 2 days after sugar cane planting was the best treatment for bermudagrass control and yield of the crop**.** Other viable options for control involved clomazone plus sulfentrazone used after imazapyr or indaziflam.

Keywords: Saccharum spp.; weed, herbicides; α-esterase.

1. INTRODUCTION

Sugarcane in Brazil is important for sugar production and ethanol, in addition to generating electricity and weed control is very important for the crop.

Bermudagrass (*Cynodon dactylon* L. Pers.) is a dominant and very important weed species in this crop. Factors such as the spreading of rhizomes and stolons by equipment, sprouting under straw [1], rapid development of rhizomes (40 tons ha^{-1}) and stolons (5 m in 80 days) and different emergence flows [2], as well as herbicide tolerance, make this species abundant in fields.

The interference of bermudagrass on crop results in yield losses of up to 32% and reduces the ratoons longevity [3]. This interference also reduces the quality of stalks and increases mineral and vegetal impurities in industrial products [4].

Post-emergence chemical control is difficult as showed to be associated with the presence of the Casparian strips in plant tissues blocking herbicide translocation [5]. In contrast, other authors [6] observed that young plants have not yet developed anatomorphological structures that work as barriers to translocation.

In this context, herbicides applied during the pre-emergence period of young bermudagrass plants can move easily through the tissues, which constitutes the foundation for developing control strategies. In further study, herbicides applied in pre-emergence after plowing are absorbed as the sectioned propagules began to sprout [7]. In addition, it was also showed that the herbicides translocated until they reached the site of action [8].

Available herbicides for pre-emergence application and that effectively control bermudagrass include imazapyr, clomazone, and sulfentrazone [9]. As these chemicals have a sorption coefficient below 100 mL g^{-1} [10], they are gradually desorbed from soil colloids to the soil solution and are free to move [11], where plants can absorb them.

Sugarcane takes up to 120 days after planting to grow and cover the soil [12] and bermudagrass can develop during this period. Therefore, it is necessary to provide herbicide weed control during this phase.

Due to herbicides visible symptoms of injury on the leaves, decreased height and stand can occur [13]. It was also observed reactive forms of oxygen as well as the production of the α-esterase enzyme [14], which catalyzes such compounds before they interfere with the processes of oxidation, reduction or cellular hydrolysis [15].

To reduce the adverse effects of herbicides on sugarcane development and achieve better control effectiveness, it was hypothesized that herbicides applied at sugarcane pre-emergence and on sectioned vegetative plant parts of bermudagrass result in effective control and are selective to the sugarcane. To test this hypothesis, this research aimed to assess the chemical control of bermudagrass and an assessment of their effects on sugarcane selectivity, using herbicides sequential applications.

2. MATERIAL AND METHODS

This research was conducted in growth chamber and field. Growth chamber data provided information on bermudagrass control and field studies information on effects of the herbicides on sugarcane.

2.1 Growth Chamber Studies – Effects on *Cynodon dactylon*

A sample of sandy soil was collected (629, 335 and 36 g kg^{-1} sand, clay and silt, respectively), sieved and treated with dolomitic limestone (1.5 t ha^{-1}), placed into 18-L plastic pots, kept in an open environment (17.3°C +/- 5.9), with irrigation (150 mm $month^{-1}$). After incubation period with liming (15 days), vegetative parts of bermudagrass, i.e., vigorous stems were planted in pots, sectioned and replanted after 30 days making suitable for herbicide application simulating field environment, with developed rhizomes, stolon and new shoots. Then, study was planned to conducted on twenty five pots to assess the treatments with no mixtures of herbicides (Table 1).

Other pots were used to study sequential applications of herbicides in mixture according to the treatments described in Table 2.

Both experiments used a CO_2 pressurized backpack sprayer equipped with a boom with six nozzles (TT11002) and regulated to provide a spray volume of 200 L ha^{-1}. Before each application, the vegetative plant parts were cut and incorporated into the soil; herbicide applied and superficially tilled the soil to simulate pre-incorporated application (PPI) transfering the pots to a growth chamber regulated to simulate spring and summer conditions.

After that, pots from both the experiments were arranged inside the plant growth chamber (photoperiod of 12/12 hours (light/dark), temperature of 27°C (+/-2) and 55-65% relative air humidity) according to a completely randomized design, replicated five times. It was provided daily irrigation with 3.32 mm/day/pot (100 mm per month), which is similar to the amount of rainfall under summer conditions in the Awa climate (Köppen classification).

We assessed shoots of bermudagrass for injury symptoms (%) visually and obtained the plant dry mass at 60 days after the last application (DALA) by a visual scale, with zero corresponding to the absence of injury and 100 corresponding to total plant death [16]. Dry weight was obtained by weighing the material after harvesting and drying in a forced-air circulation oven at 70°C to constant weight. Data was analyzed by ANOVA, and the means were compared using student's t-test ($p<0.05$).

Table 1. Single herbicide treatments used in the screening experiment in pre-emergence in growth chamber with *Cynodon dactylon*

Treatments (Herbicides)	Rate (g i.a ha^{-1})
Check	0.0
Imazapyr	375.0
Clomazone	1050.0
Indaziflam	75.0
Sulfentrazone	650.0

Table 2. Treatments and timing of herbicide application in pre-emergence in growth chamber on *Cynodon dactylon*

Treatments		
0 DAP	**38 DAP**	**75 DAP**
control	**control**	**control**
0.0	0.0	clomazone +sulfentrazone
0.0	0.0	clomazone + indaziflam
imazapyr	0.0	clomazone + sulfentrazone
imazapyr	0.0	clomazone + indaziflam
0.0	clomazone	clomazone + indaziflam
0.0	clomazone	clomazone + sulfentrazone
0.0	indaziflam	clomazone + sulfentrazone
0.0	indaziflam	clomazone + indaziflam

DAP (days after planting), imazapyr (375.0 g ha^{-1}), clomazone (1050.0 g ha^{-1}), indaziflam (75.0 g ha^{-1}), sulfentrazone (650.0 g ha^{-1})

Table 3. Treatments and timing of herbicide application to evaluate sugarcane selectivity in the field

treatments		
Pre planting		**Post planting**
75 DBP	38 DBP	2 DAP
control	control	control
0.0	0.0	clomazone +sulfentrazone
0.0	0.0	clomazone + indaziflam
imazapyr	0.0	clomazone + sulfentrazone
imazapyr	0.0	clomazone + indaziflam
0.0	clomazone	clomazone + indaziflam
0.0	clomazone	clomazone + sulfentrazone
0.0	indaziflam	clomazone + sulfentrazone
0.0	indaziflam	clomazone + indaziflam

DBP (days before planting), DAP (days after planting), imazapyr (375.0 g ha-1), clomazone (1050.0 g ha-1), indaziflam (75.0 g ha-1), sulfentrazone (650.0 g ha-1)

2.2 Field Studies - Effects on Sugarcane

The area was prepared by eradicating the sugarcane ratoons using glyphosate (3600 g ha^{-1}); followed up by dolomitic limestone (1.5 t ha^{-1}), subsoiling and harrowing of the sandy soil (623, 326 and 51 g kg^{-1} sand, clay and silt, respectively) using a randomized block design with nine chemical treatments with five replicates in plots with rows spaced 1.50 m apart and 25 m long (Table 3).

The management programs consisted of a weed-free treatment and combinations of herbicides applied before and after planting the sugarcane, using the same herbicide combination from bermudagrass control (Table 3).

After the herbicide fallow period applied before planting, sugarcane cultivar CTC4 was mechanically planted and applied mineral fertilizer (500 kg ha^{-1} of 4-14-08) and fipronil insecticide (270 g ha^{-1}) to the furrows.

A tractor sprayed the herbicide at a rate of 200 L ha^{-1} of spray volume controlled by an onboard computer coupled to a self-propelled sprayer equipped with a spraying boom with ADD08-type nozzles spaced 0.50 m apart. Height; stand and yield at 315 days after planting (DAP). For height, the distance from the ground to the dewlap of ten stalks chosen at random in each plot was used. The stand was determined by counting the stalks in three central rows of each plot and later expressed in stalks m^{-1}. The yield was estimated by the stalk diameter, height and stand according to the traditional methodology [17].

The variables were submitted to ANOVA by the F-test according to the proposed experimental design, and means were compared by Student´s t-test ($p<0,5$).

At 315 DAP, the profile of the α-esterase isoenzyme was characterized as an indirect measure to assess the oxidative stress caused by the herbicides to the crop, selecting the middle third of three leaves+1, which together composed the sample for each treatment. The samples were put in cassettes and stored on ice when they were still in the field and at -80°C when in the laboratory. The profile was characterized for α-esterase isoenzyme via polyacrylamide gel electrophoresis, followed by the staining and drying processes of the gels according to literature [18]. Then, the gels were scanned and analyzed by ImageJ software to obtain the total area occupied by the bands of each isoenzyme profile of each treatment [19]. The variables were submitted to ANOVA by the F-test according to the proposed experimental design and compared the means by Student's t-test ($p < 0.5$).

3. RESULTS AND DISCUSSION

3.1 Growth chamber studies – *Cynodon dactylon* control

Bermudagrass area covered 61% of the control pots and produced 6.03 g dry mass,

corresponding to 952.86 kg ha^{-1} of the grass, indicating the adaptation of the species to the tropical climate, with rapid development of rhizomes and stolons [2]. All herbicides affected its growth, except indaziflam (Table 4).

Clomazone and sulfentrazone most effectively suppressed growth with a control of 74.41 and 59.44% respectively. Both herbicides also reduced the dry weight and caused more injury to the grass (Table 4). This effect was also visible on injury and dry mass reduction. Clomazone caused white spots on the leaves of bermudagrass, while sulfentrazone brown spots that evolved to necrosis. The albinism in plants under the effect of clomazone is due to inhibition in the biosynthesis of carotenoids and the dark spots caused by sulfentrazone to the inhibition of protoporphyrinogen oxidase (Protox) enzyme [20].

Imazapyr also had limited effect in control (43.19%) and caused injuries to the branches and leaves (Table 4). This herbicide is registered for sugarcane and is effective in suppressing emergency flows of bermudagrass [9]. However, by itself it failed to control the development of the species. Other authors [1, 21] also found similar results with this molecule and proposed sequential applications to obtain less infestation.

Indaziflam, the least effective (Table 4), caused fragility by breaking down the bermudagrass' stocks and roots with less development, certainly because the herbicide inhibited cellulose biosynthesis affecting cell wall formation leading to less vigorous plants [22]. The properties of indaziflam, with low solubility (2.8 ppm) and strong colloid retention (1000 mL g) blocks its movement in the soil [10] affecting the contact of the herbicide resulting in slight injuries (8%) and virtually no control.

This prior screening showed the need for chemical management programs using sequential applications of different herbicides to contain the development of bermudagrass. In this high-population density scenario, it was found that the combined treatments of herbicides were more efficient [1].

When used in combination the best treatments involved clomazone with other herbicides. Clomazone plus sulfentrazone after clomazone or indaziflam caused high injury, decrease on dry weight and control of bermudagrass (Table 5).

Single application of clomazone plus sulfentrazone or clomazone plus indaziflam without previous application of another herbicide showed inadequate control efficacy. They caused 68.00% and 60.00% of injury to bermudagrass with accumulation of 3.08 g and 3.12 g of dry mass respectively, and around 50% of control (Table 5), insufficient for control. Other studies obtained similar results in which non-sequential managements resulted in greater re-infestation of bermudagrass [1, 21].

Other options of control done with imazapyr applied at 75 DBLP followed by clomazone plus sulfentrazone or indaziflam caused symptoms, reduction in dry weight and control between 91% to 96%. Similar results were found with clomazone or indaziflam used 38 days before last application (DBLP) followed by clomazone plus indaziflam (Table 5). Clomazone was involved in most of the options.

Table 4. Effect of herbicide on *Cynodon dactylon* in pre-emergence in growth chamber at 60 days after planting (DAP)

Treatments (Herbicides)	Injury (%)	Dry mass (g)	(%) Control
control	0.00 a	6.03 a	0.00 c
imazapyr	44.00 c	3.30 b	43.19 b
clomazone	50.00 bc	1.46 c	74.41 a
indaziflam	8.00 d	7.68 a	0.00 c
sulfentrazone	62.00 b	2.25 bc	59.44 ab

Means followed by the same letter in the same column do not differ statistically among themselves by Student's t-test (P <.05), imazapyr (375.0 g ha^{-1}), clomazone (1050.0 g ha^{-1}), indaziflam (75.0 g ha^{-1}), sulfentrazone (650.0 g ha^{-1}). Means followed by the same letter in the same column do not differ statistically among themselves by Student's t-test (P < .05)

Table 5. Effect of herbicide treatments and mixtures on *Cynodon dactylon* in growth chamber at 60 days after last application

75 DBLA	38 DBLA	0 DBLA	Injury (%)	Dry Weight (g)	Percent Control
0.0	0.0	0,0	0.00 c	6.64 a	0.00 d
0.0	0.0	clomazone+sulfentrazone	68.00 b	3.08 b	56.73 c
0.0	0.0	clomazone+ indaziflam	60.00 b	3.12 b	52.30 c
imazapyr	0.0	clomazone+sulfentrazone	84.00 ab	0.54 cde	91.02 ab
imazapyr	0.0	clomazone+ indaziflam	78.00 ab	0.24 de	96.48 ab
0.0	clomazone	clomazone+ indaziflam	74.00 ab	0.88 cd	85.78 b
0.0	clomazone	clomazone+sulfentrazone	100.00 a	0.00 e	99.68 a
0.0	indaziflam	clomazone+sulfentrazone	80.00 ab	0.04 e	99.37 a
0.0	indaziflam	clomazone+ indaziflam	74.00 ab	1.03 c	84.71 b

DBLA (days before last application), imazapyr (375.0 g ha^{-1}), clomazone (1050.0 g ha^{-1}), indaziflam (75.0 g ha^{-1}), sulfentrazone (650.0 g ha^{-1}). Means followed by the same letter in the same column do not differ statistically among themselves by Student's t-test (P < .05)

Table 6. Effect of herbicides treatments on sugarcane in the field during the total life cycle of 315 days after sugarcane planting

Pre plant		Post plant	Total bands of α-esterase	Height	Stand	Yield
75 DBP	38 DBP	2 DAP	(pixel cm^2)[a/]	(cm)	Stalk (m^{-1})	Tons (ha)
0.0	0.0	0.0	100	276.53 a	13.20 a	122.90 ab
0.0	0.0	clomazone+sulfentrazone	103	272.13 ab	13.20 a	114.09 ab
0.0	0.0	clomazone+indaziflam	109	268.60 bc	13.20 a	112.64 abc
imazapyr	0.0	clomazone+sulfentrazone	124	269.13 bc	12.80 ab	114.07 ab
imazapyr	0.0	clomazone+indaziflam	135	261.53 de	11.40 c	99.53 c
0.0	clomazone	clomazone+indaziflam	102	265.10 cd	11.80 c	109.78 bc
0.0	clomazone	clomazone+sulfentrazone	101	271.70 ab	13.00 a	124.95 a
0.0	indaziflam	clomazone+sulfentrazone	116	264.07 cd	12.00 bc	108.58 bc
0.0	indaziflam	clomazone + indaziflam	91	258.40 e	11.80 c	113.36 abc

DBP (days before planting), DAP (days after planting), imazapyr (375.0 g ha-1), clomazone (1050.0 g ha-1), indaziflam (75.0 g ha-1), sulfentrazone (650.0 g ha-1), a/obtained from a composite sample and not subjected to statistical analysis. Means followed by the same letter in the same column do not differ statistically among themselves by Student's t-test (P < .05)

Various symptoms were observed on the leaves of the bermudagrass due to the herbicides: small yellow spots due to the use of imazapyr, white spots due to clomazone and weak less vigorous leaves due to indaziflam. Indaziflam inhibits cellulose biosynthesis, which in turn hinders the formation of cell walls promoting formation of branches, leaves and roots that are less vigorous [22]. We also observed thin, fasciculate branches in bermudagrass, in addition to the easy plucking of the soil.

Imazapyr inhibits acetolactate synthase (ALS) enzyme and interrupts the formation of amino acids, it interferes with the growth of the plant [11], resulting in yellow leaves. Clomazone interferes with carotene biosynthesis and leaf albinism is the main symptom [23].

3.2 Field studies – Effects on Sugarcane

Imazapyr followed by clomazone plus indaziflam controlled 96.48% of the weed in the green house experiment (Table 5). The same treatments affected the height, stand and yield of sugarcane in the field in addition to increase the level of isoenzymatic profile of α-esterase. We observed a similar effect on the enzyme with imazapyr followed by clomazone plus sulfentrazone, although with less injury to sugarcane (Table 6).

Imazapyr is effective in controlling the species but is not selective for sugarcane [24] and should be used before the establishment of the crop and weeds according to the Brazilian Department of Agriculture [9]. Imazapyr inhibits acetolactate

synthase (ALS) the enzyme responsible for reducing pyruvate to acetolactate, precursors of amino acids valine and leucine. The interruption in the biosynthesis of amino acids affects the formation of cell membranes and the subsequent growth of plants [11].

The interference with the height and stand of sugarcane in treatments with indaziflam (Table 6), may be related to its mechanism of action that inhibits cellulose biosynthesis and hinders cell wall formation [22], which impairs tissue formation and plant growth.

Chemical management consisting of clomazone plus sulfentrazone applied after planting or its association with clomazone 38 days before planting, or last application, were selective; it did not harm sugarcane stand, height and yield. Even when combined with imazapyr or indaziflam before planting it did not reduce height and productivity, with less alteration in the α-esterase profile (Table 6).

The best treatment for sugarcane selectivity was clomazone followed by clomazone plus sulfentrazone and the worst was imazypyr followed by clomazone plus indaziflam. All other treatments were intermediate in terms of sugarcane yield (Table 6).

The best management programs showed a profile of α-esterase within the range of normality margin of the control (100 +/- 5%). This observation reinforces that in these chemical managements, herbicides no longer produced harmful free radicals to cellular structures [14,15].

The management program consisting of clomazone 38 days before planting (DBP) followed by clomazone plus sulfentrazone 2 days after planting (DAP) did not affect the crop and did not alter the α-esterase profile (Table 6). This treatment also provided better bermudagrass control in green house and did not impair productivity in the field (Table 7). Such treatment, under field conditions, would allow the development of the crop and the control of bermudagrass.

At the end of the experiment, the residual effects of the herbicides in the soil remained. Because they are residual, non-volatile and non-photodegraded [10], the herbicides used resisted for a long time until rain and became more available to the soil solution [25].

The management program consisting of clomazone plus sulfentrazone applied after planting followed by clomazone applied at 38 DBP of the crop also provided effective control of bermudagrass promoting a better yield. (Table 7).

Table 7. Effect of herbicides treatments on sugarcane in the field 315 days after sugarcane planting comparing to bermudagrass control in growth chamber

Pre plant		Post plant	Bermudagrass	Yield
75 DBP	**38 DBP**	**2 DAP**	**Control (%)**	**(t ha)**
hoed	hoed	hoed	100.00 a	122.90 ab
0.0	0.0	clomazone+sulfentrazone	56.73 d	114.09 ab
0.0	0.0	clomazone+indaziflam	52.30 d	112.64 abc
imazapyr	0.0	clomazone+sulfentrazone	91.02 abc	114.07 ab
imazapyr	0.0	clomazone+indaziflam	96.48 ab	99.53 c
0.0	clomazone	clomazone+indaziflam	85.78 bc	109.78 bc
0.0	clomazone	clomazone+sulfentrazone	99.68 a	124.95 a
0.0	indaziflam	clomazone+sulfentrazone	99.37 a	108.58 bc
0.0	indaziflam	clomazone + indaziflam	84.71 c	113.36 abc

DBP (days before planting), DAP (days after planting). Means followed by the same letter in the same column do not differ statistically among themselves by Student's t-test (P < 0.05), imazapyr (375.0 g ha-1), clomazone (1050.0 g ha-1), indaziflam (75.0 g ha-1), sulfentrazone (650.0 g ha-1), Means followed by the same letter in the same column do not differ statistically among themselves by Student's t-test (P < .05)

4. CONCLUSION

Clomazone treatment followed by clomazone plus sulfentrazone was the best treatment for bermudagrass control and yield of sugarcane. Other options involved clomazone plus sulfentrazone preceded by imazapyr or indaziflam also provided control and selectivity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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