Tolerance of cowpea cultivars to pre-emergence application of sulfentrazone

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ABSTRACT

Improved methods of weed control are required to support expansion of large-scale cowpea cultivation in mid-west Brazil. With the aim of testing our hypothesis that the tolerance of cowpea cultivars to sulfentrazone is dose- and genotype-dependent, we assessed the effects of increasing doses (0, 250, 500 and 1,000 g ha^{-1} of sulfentrazone) on the cultivars BRS Imponente, BRS Novaera, BRS Tumucumaque and BRS Itaim. The phytotoxic effects of sulfentrazone varied according to the dose of herbicide applied, although the symptoms were mild and only observable at the initial stages of development and at the highest dose tested. No statistically significant interactions were detected between cultivars and doses, and there were no significant differences between doses regarding population density, plant height, yield components and grain yield. Our results demonstrate that sulfentrazone is highly selective and can be applied to the studied cultivars without affecting growth or yield.

Keywords: *Vigna unguiculata*; selective residual herbicide; weed control; pre-emergence; phytotoxicity.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is cultivated extensively in north and northeastern Brazil because of its capacity to withstand the shortage of water and poor soil fertility (EMBRAPA, 2017). In these regions, however, cowpea crops are grown mainly by family farmers who employ traditional technology and produce low yields of grain. During the last decade, breeding programmes have led to the selection of new erect or semi-erect cowpea cultivars such as BRS Imponente, BRS Novaera, BRS Tumucumaque and BRS Itaim that combine high yield potential with a short production cycle (60 to 70 days) (Freire-Filho, 2011). These cultivars have been made available to high-tech large-scale growers in the mid-west of Brazil, most especially in the state of Mato Grosso where cowpea is typically planted in succession with soybean.

The main obstacle to the expansion of cowpea crops in the mid-west region is the lack of effective weed management, particularly during the early phase of development since weed infestation during this period can result in reductions in yield of up to 90% (Oliveira *et al*., 2010). The most efficient and economical solution to this problem, especially in large high-tech cropland areas, is the pre-emergence application of herbicide (Ugbe *et al*., 2016). Among the various herbicides applied pre-emergence with potential use in cowpea crop, the triazole sulfentrazone appears to be an eminently suitable candidate because it has a long residual activity in the soil (half-life of 180 days) and may contribute to weed control for a prolonged period (Melo *et al*., 2010; Rodrigues & Almeida, 2018). This characteristic is especially helpful in the case of upright cultivars such as BR 16 that have

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delayed canopy closure and a total *period of interference prevention (TPIP)* ranging from 35 to 53 days (Freitas *et al*., 2009; Corrêa *et al*., 2015). Furthermore, sulfentrazone is active against a broad spectrum of weed species that are predominant in cowpea cultivation, including *Tridax procumbens* (L.) L., *Emilia sonchifolia* (L.) DC. ex DC., *Solanum americanum* Mill. and the grasses *Eleusine indica* (L.) Gaertn. and *Digitaria horizontalis* Willd. (Ikeda & Vivian, 2012).

Although information about the tolerance of cowpea cultivars towards sulfentrazone is important in assessing the feasibility of applying the molecule, few studies have focused on this aspect and the reports that are available generally relate to a single dose of herbicide or to a single cultivar (Fontes *et al*., 2013). With the aim of testing the hypothesis that the tolerance of cowpea cultivars to sulfentrazone is dose- and genotype-dependent, we have assessed the effects of increasing doses of sulfentrazone on four different cowpea cultivars recommended to mid-west of Brazil.

MATERIALS AND METHODS

The experiment was set up in the municipality of Sorriso, Mato Grosso state, Brazil. Precipitation levels and temperatures during the experimental period are represented in Figure 1. The climate in the area is tropical monsoon (Am) according to *Köppen's climate classification* (Alvares *et al*., 2013), while the soil is *dystrophic Red Yellow Latosol* (oxisol) of medium texture and flat relief (Santos *et al*., 2018; SEPLAN, 2011). The physicochemical characteristics of soil in the 0 to 0.2 m layer were: 292 g kg^{-1} clay; 58 g kg^{-1} silt; 650 g kg^{-1} sand; 5.8 pH in H_2O; 12.1 mg dm^{-3} P (Mehlich extraction); 0.09 cmol$_c$ dm^{-3} K; 1.9 cmol$_c$ dm^{-3} Ca; 0.5 cmol$_c$ dm^{-3} Mg; 0.1 cmol$_c$ dm^{-3} Al; 3.50 cmol$_c$ dm^{-3} H + Al; 3.2 dag kg^{-1} organic matter; 5.96 cmol$_c$ dm^{-3} cation exchange capacity; and 41.3% base saturation.

The experimental area was treated with a mixture of glyphosate and carfentrazone-ethyl (720 and 20 g ha^{-1}, respectively) for weed burndown. The experiment was of randomized block design and conducted in strips according to a 4 x 4 factorial scheme with four replications. Four cowpea cultivars (BRS Imponente, BRS Novaera, BRS Tumucumaque and BRS Itaim) were sown in the row strips and four herbicide treatments (0, 250, 500 and 1,000 g ha^{-1} of sulfentrazone) were applied in the column strips. Each subplot occupied 4 x 3 m and for evaluations were disregard 0.5 m border on each end and one border row on each side.

The row spacing of cowpeas was 0.5 m and the number of seeds per linear meter was adjusted to 10 by seed germination test for each cowpea cultivar. Sowing fertilization was performed by applying 180 kg ha^{-1} of NPK (04-14-08). The sulfentrazone treatments were applied immediately after seed sowing, and before the emergence of weeds, with a CO_2 pressurized backpack sprayer fitted with a spray boom fitted with six flat-jet nozzles, *model XR 110.02,* at 0.5 m spacing and calibrated for an application

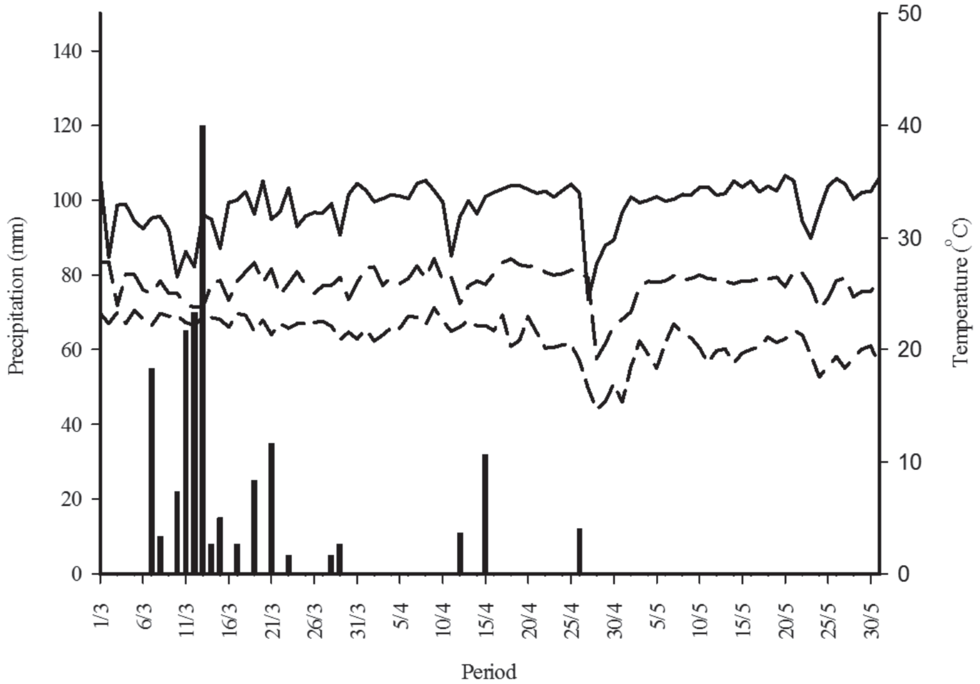

Figure 1: Precipitation[a] (▌) and maximum (—), mean (— — —) and minimum (- - - -) temperatures[b] during the experimental period. [a] Data recorded at Instituto Mato-grossense do Algodão, Sorriso, Mato Grosso, Brazil. [b] Data recorded at Embrapa Agrossilvipastoril, Sinop, Mato Grosso, Brazil.

volume of 200 L ha^{-1}. The subplots were hand-weeded throughout the entire crop cycle when necessary. Side-dressing fertilization was performed 15 days after the emergence of cowpea seedlings by applying 120 kg ha^{-1} of ammonium dihydrogen phosphate and 60 kg ha^{-1} of K_2O. The imidacloprid, cypermethrin and acetamiprid + alfa-cypermethrin were also applied to pest control after cowpea emergence.

The symptoms of injuries were visually estimated at 7, 14 and 21 days after application (DAA) of herbicide using the scale proposed by the European Weed Research Council (EWRC, 1964). The heights above ground of 10 random plants in each subplot were determined at 14 and 28 DAA, and population density was established at 28 DAA considering four 1 m rows of each subplot. Plants in four 2 m rows per subplot were harvested at 87 DAA and pods collected manually to estimate grain yield. Population density at harvest was established considering three of these four 2 m rows, and the number of pods per plant determined for 10 randomly selected plants per subplot. At the laboratory, the numbers of beans per pod in 10 randomly selected pods per subplot were evaluated and the pods were threshed manually to establish the mass of 100 beans for each subplot. Grain yields and masses of 100 beans were adjusted to 13% moisture (Ministério da Agricultura, Pecuária e Abastecimento, 2009). After harvesting, the straw in the experiment was collected from 0.25 x 0.25 m quadrats placed randomly in each block, transferred to paper bags, and dried at 65 ºC in a forced-air circulation oven to constant weight. The straw dry matter was estimated of 0.5 t ha^{-1}.

Data that satisfied the assumptions of normality, homoscedasticity and independence of residues were analyzed directly by analysis of variance (ANOVA) and the F-test using SAS software version 9.1. Data relating to the mass of 100 of beans were subjected to log_{10} transformation prior to analysis. When significantly different, the data were compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Evaluation of phytointoxication at 7 DAA revealed that the highest dose of sulfentrazone (1,000 g ha^{-1}) induced leaf curling varying from slight (BRS Itaim) to medium (BRS Imponente, BRS Novaera and BRS Tumucumaque), while lower doses generated only slight symptoms in all four cultivars (Table 1). At 14 DAA, the symptoms caused by the lowest and the highest doses of sulfentrazone had subsided in all cultivars, with the exception of BRS Itaim in which leaf curl had progressed to medium at the highest dose. At 21 DAA, no effects could be detected in cultivars treated with the lower doses of herbicide while at the highest dose BRS Imponente

Table 2: Effects of sulfentrazone doses on the population density (PD), plant height (PH), yield components and grain yield (GY) of cowpea cultivars

Cultivar	14 DAA	28 DAA	Harvest (87 DAA)					
	PH (cm)	PD (plants m^{-1}) [plants ha^{-1}]	PH (cm)	PD (plants m^{-1}) [plants ha^{-1}]	No. of pods plant^{-1}	No. of beans pod^{-1}	M100 (g)	GY (kg ha^{-1})
BRS Imponente	17.2	10.7 [218,000]	24.9	9.3 [186,000]	3.4	5.9 ^{b}	34.2 ^{a}	925.5
BRS Novaera	17.0	13.1 [266,000]	25.3	11.3 [224,000]	4.0	7.3 ^{ab}	25.5 ^{b}	868.9
BRS Tumucumaque	17.4	12.9 [256,000]	25.4	12.9 [258,000]	3.4	8.5 ^{a}	23.9 ^{c}	946.8
BRS Itaim	17.4	12.5 [252,000]	25.1	13.1 [262,000]	3.3	7.4 ^{ab}	22.5 ^{c}	859.5
F_{block}	2.02 ^{ns}	0.12 ^{ns}	2.23 ^{ns}	0.02 ^{ns}	0.74 ^{ns}	0.56 ^{ns}	4.34 *	1.57 ^{ns}
$F_{cultivar}$	2.14 ^{ns}	0.85 ^{ns}	0.11 ^{ns}	2.86 ^{ns}	0.73 ^{ns}	5.83 *	47.67 **	0.19 ^{ns}
$F_{Block*cultivar}$	0.83 ^{ns}	3.88**	1.83 ^{ns}	9.04 **	5.40 ^{ns}	2.33 *	7.68 **	26.70 **
F_{doses}	3.13 ^{ns}	0.16 ^{ns}	0.28 ^{ns}	0.35 ^{ns}	0.82 ^{ns}	0.08 ^{ns}	2.75 ^{ns}	0.54 ^{ns}
$F_{Blockxdose}$	1.68 ^{ns}	3.39**	8.77 **	5.93 **	1.37 ^{ns}	2.58 *	0.76 ^{ns}	3.08 *
$F_{cultivars\ x\ doses}$	1.22 ^{ns}	1.47 ^{ns}	0.83 ^{ns}	0.95 ^{ns}	0.78 ^{ns}	1.5 ^{ns}	1.32 ^{ns}	0.85 ^{ns}
CV (%)	3.5	19.4	6.8	10.9	17.1	14.5	1.2	7.4

CV: Coefficient of variation; ^{ns} not significant, * significant at 5 ≥, ** significant at 1% according to *F* test. The mass of 100 g (M100) was submitted to log_{10}.

and BRS Novaera showed only slight symptoms although leaf curl in BRS Itaim remained medium. In general, BRS Itaim was less tolerant to high doses of sulfentrazone in comparison with the other cultivars.

The divergence in tolerance of cowpea cultivars towards sulfentrazone was as expected (Araújo *et al*., 1984) since this trait is a function of genotypic variation (Dayan *et al*., 1997) and the toxic effects of sulfentrazone could vary from very slight to severe depending on the genotype and doses applied, while some cultivars exhibit no symptoms at all. Burgos *et al.* (2007), for example, treated six American cowpea cultivars and four advanced lines with sulfentrazone in the range 0.5- to 2-times the recommended dose of 420 g ha^{-1} and reported that cultivars AR Blackeye, CT Pinkeye and Early Scarlet and line 00-855 showed the least growth reduction (18-23%) at 14 DAA. Stunting averaged over all cultivars was around 10% at the recommended rate and 52% at 840 ha^{-1}, while yields of cultivars Early Acre and CT Pinkeye, along with lines 92-551, 01-1764 and 01-243, were not affected by the herbicide applied at the recommended rate. In a similar manner, Fontes *et al.* (2013) reported the absence of visual symptoms in the Brazilian cultivar BRS Guariba at 14 and 28 DAA of sulfentrazone at 500 g ha^{-1}. On the other hand, Costa *et al.* (2017) reported that cowpea cultivar IPA 207 exhibited relatively strong symptoms of phytotoxicity at 7 DAA of sulfentrazone at 600 g ha^{-1} although the symptoms had subsided markedly at 21 DAA.

Symptoms of intoxication of plants susceptible to sulfentrazone are expected upon emergence and exposure to light (Rizzardi *et al*., 2008). Typically, dark green spots appear on the leaves and subsequently progress to tissue necrosis and death within two to three days of herbicide application. Sulfentrazone inhibits protoporphyrinogen oxidase in the chlorophyll biosynthetic pathway leading to the accumulation of harmful intermediates which, in the presence of light and molecular oxygen, generate singlet oxygen that reacts rapidly with cellular macromolecules causing membrane disruption (Hao *et al*., 2011). However, such symptoms were not observed in the cowpea cultivars studied herein, probably because of their high tolerance.

Although there is no information available concerning the possible mechanisms of sulfentrazone tolerance in cowpea, some studies involving cultivars of soybean [*Glycine max* (L.) Merr.] suggest that the basis for the differential response could be the dissimilar absorption/ translocation of herbicide during the earliest stages of development, conjugation of sulfentrazone with glutathione, divergent metabolic rates of the herbicide mediated by cytochrome P450 monooxygenases, or disparate sensitivity to peroxidative stress (Dayan *et al*., 1997; Li *et al*., 2000; Rodrigues & Almeida, 2018).

The mild toxic effects of sulfentrazone observed (Table 1) may result from factors such as air temperature, humidity, reduced soil compaction and edaphoclimatic conditions characteristic of the study area (Figure 1) and the Brazilian Cerrado. In this region, cowpea is usually grown after the main soybean-harvesting season when rainfall diminishes, and average air temperatures start to increase. These conditions differ markedly from those reported to potentiate herbicide damage in less tolerant cultures (Swantek *et al*., 1998; Li *et al*., 2000) such as lower temperatures, which lead to a longer exposure time of seedlings to the herbicide, and soils with high moisture content.

Sulfentrazone is recommended mainly to apply pre-emergence, hence it is important to consider its adsorption onto soil particles and its bioavailability in soil solution. There is evidence that clay soils with a high content of organic matter and low pH tend to reduce herbicide phytotoxicity owing to increased adsorption to soil particles and lower herbicide availability, while soils containing low levels of organic matter and with high pH enhance phytotoxicity (Grey *et al*., 2000; Szmigielski *et al*., 2009, 2012). The Brazilian Cerrado soils generally contain high levels of organic matter (3.2 dag kg^{-1} soil in the present study) and exhibit pH values below 6.56 [the pKa of sulfentrazone (Rodrigues & Almeida, 2018)]. In this condition, weakly acidic herbicides as sulfentrazone exist in the non-ionic form in soils (Grey *et al*., 1997, 2000; Ohmes & Mueller, 2007; Szmigielski *et al*., 2012). So, non-ionic sulfentrazone molecules cannot be absorbed by plants, despite their high lipophilicity and permeability to cell membranes (Ferrell *et al*., 2003), and generate only mild intoxication symptoms.

As shown in Table 2, no significant interactions were detected between cultivars and doses, and there were no significant differences between doses regarding the variables population density, plant height, yield components and grain yield, thus demonstrating tolerance of the cultivars to sulfentrazone. In this context, Fontes *et al.* (2013) reported a grain yield of 785.1 kg ha^{-1} for plants of BRS Guariba that had been exposed to 500 g ha^{1} sulfentrazone, a value that was similar to that (843 kg ha^{-1}) produced by plants subjected to hand-weed control.

The potential productivity of cultivar BRS Imponente is over 2,000 kg ha^{-1} (EMBRAPA, 2016), while those of BRS Itaim, BRS Novaera and BRS Tumucumaque are 1,895, 2,020 and 1,924 kg ha^{-1}, respectively (Souza *et al*., 2018). These elevated potential values may be explained by high levels of accumulated rainfall and favorable conditions during the crop cycle (Almeida *et al*., 2017) and by soil tillage (Cardoso *et al*., 2017). Such circumstances are unlikely to occur in mid-west Brazil, however, because

Table 1: Phytotoxicity of sulfentrazone (EWRC scale – notes 1-9) towards cowpea cultivars as a function of dose and days after application (DAA)

Cultivar	Dose of herbicide (g ha^{-1})											
	0	250	500	1000	0	250	500	1000	0	250	500	1000
	7 DAA				14 DAA				21 DAA			
BRS Imponente	1	2	2	3	1	1	1	2	1	1	1	2
BRS Novaera	1	2	2	3	1	1	2	2	1	1	1	2
BRS Tumucumaque	1	2	2	3	1	1	2	2	1	1	1	1
BRS Itaim	1	2	2	2	1	1	2	3	1	1	1	3

cowpea is grown in succession to soybean in this region and sowing in no-tillage soil. Certainly, the yields recorded herein for the four studied cultivars were lower than the potential yields, but this was probably caused by other interfering factors and not by the application of sulfentrazone. In addition, despite the germination tests performed prior to the study and the consequential adjustment to obtain 10 plants per linear meter, the population densities for all cultivars were higher at 28 DAA (Table 2), and this probably contributed to the lower yields observed (Bezerra *et al*., 2014).

As shown in Table 2, the study cultivars differed significantly only with respect to the variables beans per pod and mass of 100 beans. The first mentioned variable probably varies depending on the number of pods per plant. According to Almeida *et al.* (2017), a larger number of pods per plant were obtained for BRS Itaim, BRS Tumucumaque and BRS Novaera compared with those reported here, although the numbers of beans per pod were smaller (6, 7 and 6, respectively), suggesting that one variable may compensate for the other. Usually, the mass of 100 beans suffers little variation, so that the results obtained in the present study (Table 2) were close to those reported for BRS Itaim, BRS Tumucumaque, BRS Novaera and BRS Imponente (Almeida *et al.*, 2017; EMBRAPA, 2016).

Our results demonstrate that the studied cowpea cultivars were tolerant to doses of sulfentrazone up to 1,000 g ha^{-1}, which is equivalent 4-times the average employed in soybean cultivation in light textured soils and almost twice the recommended value of 600 g ha^{-1} for soybean grown in heavy textured soils. Even at the maximum concentration tested, the grain yields of the four cultivars were not negatively affected and only mild intoxication symptoms were observed in the emerging seedlings.

CONCLUSIONS

Our study demonstrated that sulfentrazone is highly selective and can be applied to the studied cultivars without affecting crop growth and yield.

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