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Action of *Canavalia ensiformis* in remediation of contaminated soil with sulfentrazone

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ABSTRACT: This study evaluated the jack bean (*Canavalia ensiformis*) as a potential remediator of sulfentrazone in the soil. The experiment was conducted under field conditions in a complete randomised block design. The treatments consisted of soils with and without herbicide application as well as the absence and presence of *C. ensiformis* cultivation associated with incorporation into the soil or the removal of shoots of *C. ensiformis*. Sorghum was planted as a bioindicator to evaluate the remediation efficiency of jack bean. Sulfentrazone application in areas without *C. ensiformis* cultivation decreased plants stands, productivity, and height of sorghum compared to treatments where *C. ensiformis* was cultivated.

Sorghum cultivated in succession to *C. ensiformis* in areas contaminated with sulfentrazone resulted in dry matter production, plants numbers, productivity, and height of sorghum equivalent to uncontaminated areas. The results of this research indicate that the use of jack bean for the bioremediation of sulfentrazone treated soils would provide greater security in the planting of susceptible species in areas where this herbicide has been applied. The cultivation of *C. ensiformis* in contaminated areas may reduce the risk of environmental impacts caused by sulfentrazone.

Key words: bioremediation, soil decontamination, herbicides, residue, contamination.

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INTRODUCTION

Herbicides that persist for long periods in the soil are effective because they ensure the productivity of cultivated plants, especially those with long periods of growth, which need prevention from weed interference. However, by the end of the total period of interference (PTPI) — which usually occurs simultaneously with the closing of the crop canopy —, the presence of the herbicide in the soil may be undesirable (Belo et al. 2007; Dan et al. 2010). Undesirable herbicide residues in the soil, after the period of effective use for weed management, may result in contamination of the groundwater by leaching, or in contamination by surface runoff (Krutz et al. 2005), and may impact non-target organisms (Boutin et al. 2012; Larras et al. 2013).

A solution to reduce the problems caused by herbicides with long persistence is the cultivation of plants that can accelerate degradation of the herbicide in the environment. Some studies have proved the efficiency of this technique in soil remediation, in diverse ecosystems contaminated with organic and inorganic compounds (Mitter et al. 2013; Mitton et al. 2014; Oliveira et al. 2014). However, studies in tropical soils for the identification of species that may be effective in phytoremediation schemes are scarce. Studies such as this, aiming to identify prospective bioremediation species, have been conducted in other countries (Fernandez et al. 2012; Ibrahim et al. 2013; Merini et al. 2009).

Sulfentrazone is an herbicide widely used in Brazil and has long persistence in soils (Blanco and Velini 2005; Vivian et al. 2006). It is an aryl-triazolinone herbicide used for weed control in sugarcane, soybean, and reforestation areas. This herbicide has a molecular weight and molecular formula equivalent to 387.18 g·mol⁻¹ and C₁₁H₁₀CL₂F₂N₄O₃S, respectively. Sulfentrazone is highly persistent and highly mobile with a mean partitioning coefficient K_{oc} = 43 and mean sorption coefficient K_d < 1 (Nalini et al. 2016). Due to these characteristics, sulfentrazone has a high leaching potential. Several studies in Brazilian soils about the persistence (Blanco and Velini 2005; Blanco et al. 2010), sorption, and leaching (Monquero et al. 2010) of this herbicide have shown its potential for the injury of successive crops, as well as the contamination of surface and groundwater.

Therefore, it is necessary to develop alternatives that reduce the persistence of this compound in the

soil. Research has identified the jack bean (*Canavalia ensiformis*) as an efficient species for the remediation of sulfentrazone, under controlled conditions (Madalão et al. 2012; Madalão et al. 2013).

The environmental decontamination of pesticides is aided by soil microbes, and agronomic practices, if implemented together, may help remove, immobilise or neutralise the contaminants from the ecosystem. Although the efficiency of jack bean has been shown for the remediation of sulfentrazone, there is a lack of information about the most effective management methods to be used as part of the soil remediation process.

Some crops such as jack bean that have been proposed as candidates for soil remediation may thus help to maintain economic stability and soil fertility in the farm. The aim of this research was to evaluate the potential of *C. ensiformis* and the effect of its management on the effectiveness for the remediation of contaminated soils with sulfentrazone.

MATERIAL AND METHODS

Location experiment

The experiment was conducted during the period from September 2012 to May 2013, in an Ultisol soil with a loamy-clay-sandy texture. The experiment was carried out in a field at Diogo Alves de Melo (lat 20°46'21"S and long 42°52'15"W) with an altitude of 650 m in Minas Gerais State. The area where the experiment was conducted had not been previously cultivated. The climate is characterised as humid, subtropical, with a dry winter and hot summer, according to the Köppen-Geiger classification. The average annual temperature and annual precipitation was 21 °C and 1,200 mm, respectively. The climatic data collected in the experimental area during the research is shown in Fig. 1 and chemical characteristics and granulometric analysis of soil are presented in Table 1.

Experimental design and treatments

The experimental design was a randomised block with 4 replications. The treatments consisted of soils with and without application of herbicide in the presence or absence of *C. ensiformis*. Two management methods were used for the treatments with remediator plants:

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cultivation plus the incorporation of shoots in the soil and cultivation plus the removal of shoots from the plots, both conducted during the flowering period.

Each plot had dimensions of 3.0 m × 6.0 m. The useful area of each plot corresponded to 4 central rows, 2 m wide and 4 m long, for a total of 8 m² per plot.

Herbicide application

The maximum commercial dose of sulfentrazone used in commercial fields is 700 g a.i.·ha⁻¹. However, other research has shown that jack bean can promote the phytoremediation of soil contaminated with sulfentrazone at doses of 800 g a.i.·ha⁻¹ (Belo et al. 2016; Belo et al. 2011). In this work, sulfentrazone was applied at a dose of 1,000 g a.i.·ha⁻¹ before the planting of jack bean with a pressurised CO₂ sprayer and nozzle 110.02 T/T, with a spray volume of 150 L·ha⁻¹. At the time of application, the average air temperature, relative humidity, and average wind speed were 15.7 °C, 86%, and 1.18 m·s⁻¹, respectively.

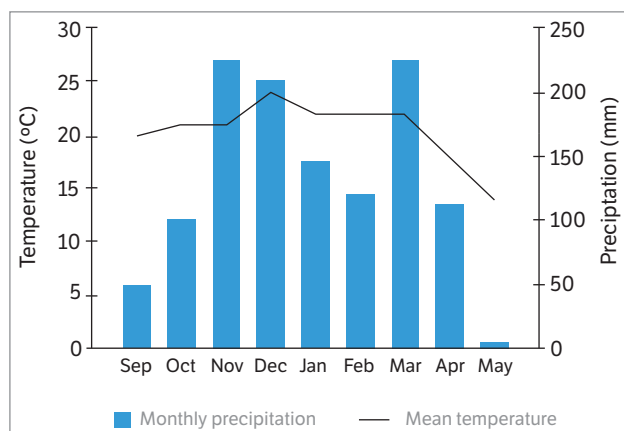


Figure 1. Rainfall and average monthly temperatures during the experiment (from September 2012 to May 2013). Source: Meteorological Station of the Department of Agricultural Engineering of the Federal University of Viçosa (UFV).

Phytoremediator planting

After 30 days of herbicide application, the jack bean was sowed manually at a density of 140,000 plants·ha⁻¹, with a spacing of 0.5 m between the rows. The plants were desiccated 75 days after sowing, with the plants at full flowering. Desiccation was carried out using a mixture of glyphosate (1.080 g·ha⁻¹) + 2,4-D (335 g·ha⁻¹) in all of the plots. After desiccation, the dry matter of plants was managed according to the respective treatments — incorporation or removal of shoots.

Bioindicator planting

The bioindicator species (*Sorghum bicolor*) was sown mechanically, with spacing of 0.50 m between rows, at a population of 120,000 plants·ha⁻¹. Fertilisation was performed using 12; 42 and 24 kg·ha⁻¹ of nitrogen (N), phosphorus (P), and potassium (K), respectively. *S. bicolor* was chosen as an indicator for the presence of herbicide residues due its high sensitivity to sulfentrazone in soil (Vivian et al. 2006; Belo et al. 2011).

Data collection

Sorghum plant height (5 plants) was determined at 20; 40 and 60 days after planting (DAP). At 120 DAP, dry matter was determined by harvesting the top-growth above ground level of 5 plants per plot and drying in a forced circulation oven at 72 °C until constant weight. The number of plants·ha⁻¹ was determined after harvesting 2 lines of 3 m in each plot. Productivity of fresh weight of sorghum (plants·ha⁻¹) was calculated by multiplying the fresh biomass of plants harvested by the number of plants·ha⁻¹ previously estimated.

Table 1. Physical and chemical attributes of the surface soil (Ultisol, 0 – 20 cm) of the experimental area.

Granulometric analysis (%)												
Gravel			Sand		Silt		Clay		Textural classification			
32			16		19		33		Loam-Clay-Sand			
Chemical analysis												
pH	P	K	Ca ²⁺	Mg ²⁺	H + Al	Al ³⁺	BS	(t)	T	V	m	OM
H ₂ O	Mg·dm ⁻³		cmol _c ·dm ⁻³							%		%
6.51	171	104	4.12	0.87	2.1	0.0	5.26	7.36	5.26	71.5	0.0	4.06

Extractors: pH — H₂O; P and K — Mehlich¹; Ca²⁺ and Mg²⁺ — HCl 1 mol·L⁻¹; H + Al — Ca (OAc)₂ 0.5 mol·L⁻¹; BS = Base Saturation; (t) = effective cation exchange capacity; T = Cation exchange capacity; m = Aluminium saturation; OM = Organic matter.

Statistical analysis

Data were submitted to analysis of variance using the statistical software SISVAR[®] version 5.6 to determine significance between treatments using the Tukey's test at 5% probability.

RESULTS AND DISCUSSION

Sorghum height

By 20 DAP, sorghum plant height was not affected by the planting of jack bean in sulfentrazone contaminated soils (Table 2). However, by 20 DAP in non-contaminated soils, sorghum plant height was the greatest in treatments with jack bean and shoots removed, as compared to fallow treatments without jack bean. When jack bean was cultivated and removed from the plot at 20 DAP, the

Table 2. Height (cm) of cultivated sorghum plants in contaminated and non-contaminated soil with sulfentrazone under different management strategies with the remedial species (*C. ensiformis*).

Treatments	Sulfentrazone	
	Contaminated soil	Nonw-contaminated soil
<i>C. ensiformis</i>	20 DAP	
Without cultivation	20.27 aA'	19.85 bA
Shoots incorporated in soil	23.52 aA	23.02 abA
Shoots removed from area	20.75 aB	23.77 aA
CV (%)	8.67	
<i>C. ensiformis</i>	40 DAP	
Without cultivation	77.83 bA	86.08 bA
Shoots incorporated in soil	95.50 aA	93.41 bA
Shoots removed from area	91.83 aB	109.33 aA
CV (%)	6.57	
<i>C. ensiformis</i>	60 DAP	
Without cultivation	150.54 aA	162.15 aA
Shoots incorporated in soil	164.65 aA	174.10 aA
Shoots removed from area	171.35 aA	175.65 aA
CV (%)	7.63	

*Means followed by the same letter, uppercase on the row and lowercase in the column, do not differ by Tukey's test at 5% probability. DAP = Days after planting; CV = Coefficient of variation.

sorghum plant height was lower in the plots with herbicide application (Table 2). Cultivating and incorporating jack bean, as well as its absence, did not affect the height of sorghum plants. When sulfentrazone was not applied, by 20 DAP, the absence of jack bean cultivation resulted in lower sorghum height as compared to jack bean cultivation with shoot removal.

By 40 DAP, the planting of jack bean, with or without shoot removal, resulted in increased sorghum height in sulfentrazone contaminated soils (Table 2). However, by 60 DAP, no difference in plant height was observed among the different treatments.

Organic material such as green manure can increase nutrient content, cation-exchange capacity (CEC), infiltration, and water retention in the soil; besides, it can improve conditions for the growth of beneficial microorganisms to plants (Costa et al. 2004; Jaramillo-Botero et al. 2008). Possibly, this effect was observed with jack bean, resulting in high initial sorghum growth, as observed in areas pre-sowed with the legume. The fast initial growth is important to allow rapid canopy closure of the crop. Therefore, the jack bean may be beneficial in the rotation for weed management of the following crop by minimising competition for water, light, and nutrients (Gustafson et al. 2004).

Sorghum dry matter

The lowest shoot dry matter for sorghum was observed in soil contaminated with sulfentrazone and without phytoremediation with *C. ensiformis* (Table 3). However, differences were not observed between the incorporation

Table 3. Dry biomass (g) of sorghum shoots grown in contaminated and non-contaminated soils with sulfentrazone under different top growth managements of the remedial species (*C. ensiformis*).

Treatments	Sulfentrazone	
	Contaminated soil	Non-contaminated soil
<i>C. ensiformis</i>		
Without cultivation	349.81 bA'	424.35 aA
Shoots incorporated in soil	484.19 aA	468.41 aA
Shoots removed from area	506.82 aA	433.12 aA
CV (%)	15.61	

*Means followed by the same letter, uppercase on the row and lowercase in the column, do not differ by Tukey's test at 5% probability. CV = Coefficient of variation.

and non-incorporation of jack bean residues for this variable. None of the treatments affected the dry matter production of sorghum in soil not contaminated with sulfentrazone. According to Pires et al. (2005), in the phytoremediation of herbicides, leguminous species provide a double environmental benefit: soil remediation and improved soil fertility. Thus, sorghum may have been benefited by the pre-cultivation of jack bean, as observed by Ceccon et al. (2013) with corn grown in association with this species.

Number of sorghum plants

Sulfentrazone contaminated soils reduced the number of sorghum plants as compared to the controls with either no herbicide or without *C. ensiformis*, when compared to 2 management methods: incorporation or removal of jack bean shoots from the area (Table 4).

Table 4. Stand establishment (number of plants per hectare) of sorghum grown in contaminated and non-contaminated soils with sulfentrazone under different managements of remedial species (*C. ensiformis*).

Treatments	Sulfentrazone	
<i>C. ensiformis</i>	Contaminated soil	Non-contaminated soil
Without cultivation	60,000.00 bB*	78,750.00 aA
Shoots incorporated in soil	97,916.66 aA	93,333.33 aA
Shoots removed from area	94,166.67 aA	89,750.00 aA
CV (%)	12.27	

*Means followed by the same letter, uppercase on the row and lowercase in the column, do not differ by Tukey's test at 5% probability. CV = Coefficient of variation.

These data confirm the detrimental effect of sulfentrazone residues in the soil, as shown with the lower plant stands in sorghum. These results also indicate that natural soil mechanisms alone were not capable of reducing the sulfentrazone concentrations. This indicates the importance of phytoremediation processes to accelerate the herbicide degradation process, reducing the time for which the herbicide remains available in the soil.

Procópio et al. (2005) and Santos et al. (2004) evaluated the potential of various species to decontaminate herbicide treated soils. These authors concluded that *C. ensiformis* was one of the most efficient species in remediation of

the herbicide trifloxysulfuron-sodium. Pires et al. (2005) and Pires et al. (2006) found that the same species was effective for phytoremediation of tebuthiuron in Haplic Plintossol and Argisol soils, respectively. Pires et al. (2008) also evaluated the phytoremediation of Argisol Red-Yellow soils contaminated with different tebuthiuron levels, using *Crotalaria juncea* as an indicator species for the presence of this herbicide. The authors concluded that *C. ensiformis* and *Cajanus cajan* showed the best results for remediation of soil contaminated with tebuthiuron up to a dose of 1,000 g·ha⁻¹. All of these studies showed the versatility of jack bean for phytoremediation of different herbicides in Haplic Plintossol, Argisol Red-Yellow, and Latosol Red-Yellow soils.

Top-growth fresh weight productivity of sorghum

Top-growth fresh weight productivity of sorghum had the same trend as the data on plant stands. In the treatments with herbicide application, high sorghum productivity was obtained in the plots with jack bean, independently of the management method, involving the incorporation or removal of shoots (Table 5). These results confirm the potential beneficial effects of jack bean for soil remediation. The productivity of these treatments ranged between 59.19 and 62.86 t·ha⁻¹; however, in the no-phytoremediation areas where the herbicide was applied, the productivity was only 32.08 t·ha⁻¹. It was also observed that, in treatments with no herbicide application, the sorghum productivity was higher (47.71 t·ha⁻¹) compared to treatments where the herbicide was applied and without remediation. This information is important because it indicates that the higher productivity in remediated areas occurred due not only to the nutrient inputs provided by the legume, but also to the remedial effect of the species.

For the phytoremediation to be effective, it is not necessary to remove the plant residues from the field. Residue removal may increase the system costs, discouraging the producer from adopting phytoremediation. In this study, there was no difference between the types of management (removal or incorporation) or between treatments with or without herbicide, indicating that the jack bean may absorb, degrade, or facilitate sulfentrazone degradation in their own rhizospheric environment. Thus, it is not necessary to remove remedial plants from the area before planting a crop susceptible to the herbicide.



Herbicide degradation depends on various factors; for example, plants may absorb herbicide molecules and, through specific metabolic routes, degrade and/or store these agrochemicals in organelles (Vail et al. 2015; Han et al. 2013; Rojano-Delgado et al. 2012). Beyond these mechanisms, certain plants — principally legume species — stimulate microbial activity in the soil, accelerating the degradation process. Studies have shown that *C. ensiformis* plants can promote rhizospheric remediation because flavonoids and other compounds released by the roots can stimulate the growth and activity of degrading microorganisms, or even react with the herbicide, thus immobilising it (Chaudhry et al. 2005; Leigh et al. 2002; Leigh et al. 2006). In addition, the nitrogen fixation ability of jack bean provides a source of nitrogen, which may stimulate microorganism activity in the rhizosphere. Additionally, the growth and death of roots promote soil aeration, which can increase the oxidative degradation of organic compounds (Kuiper et al. 2004; Leigh et al. 2002).

CONCLUSION

C. ensiformis was able to remediate areas contaminated with sulfentrazone, with or without shoot removal from the remediated areas. In practice, this provides greater security for the planting of susceptible species in areas where this herbicide was applied in the previous crop. In addition, the cultivation of *C. ensiformis* in contaminated areas may reduce the risk of environmental impacts caused by sulfentrazone.

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