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Article

Selection of Species with Soil Phytoremediation Potential After the Application of Protox-Inhibiting Herbicides

Seleção de Espécies com Potencial Fitorremediador de Solo Tratado com Herbicidas Inibidores da Protox

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ABSTRACT - The objective of this work was to evaluate the phytoremediation potential of summer-grown species planted in soils contaminated with the herbicides fomesafen or sulfentrazone. In a greenhouse, doses of fomesafen (0.0, 0.125, 0.25 and 0.5 kg ha^{-1}) and or sulfentrazone (0.0, 0.3, 0.6 and 1.2 kg ha^{-1}), were applied in the pre-emergence of brown hemp, millet, velvet bean and sorghum. On the field, the recommended doses of fomesafen (0.250 kg ha^{-1}) and sulfentrazone (0.600 kg ha^{-1}) were used in the pre-emergence of brown hemp, velvet bean and sorghum, plus a treatment without cultivation. These species, before planting canola (bioindicator species), were submitted to mowing and burndown. In greenhouse trials, velvet bean, millet and sorghum tolerated fomesafen up to the recommended dose; sulfentrazone caused the total death of these plants. In the field experiments, the cultivation of velvet bean and brown hemp, in general, were the best alternatives preceding canola, in soil contaminated with fomesafen, and mainly velvet bean for sulfentrazone-contaminated areas. The number of siliques per plant of canola and its productivity were superior when using velvet bean as cover crop for both herbicides, regardless of the adopted management. It is possible to conclude that brown hemp and velvet bean were the species that best phytoremediated soils treated with fomesafen or sulfentrazone, and could be used in the decontamination of soils treated with these herbicides, regardless of the adopted management.

Keywords: *Brassica oleracea* var. *oleifera*, *Crotalaria juncea*, *Mucuna pruriens*.

RESUMO - Objetivou-se com este trabalho avaliar o potencial fitorremediador de espécies cultivadas no verão para fitorremediação de solo contaminado com os herbicidas fomesafen e sulfentrazone. Em casa de vegetação, foram aplicadas doses de fomesafen (0,0; 0,125; 0,25 e 0,5 kg ha^{-1}) e/ou de sulfentrazone (0,0; 0,3; 0,6 e 1,2 kg ha^{-1}), em pré-emergência de crotalária, milheto, mucuna-preta e sorgo. Em campo, foram usadas as doses recomendadas de fomesafen (0,250 kg ha^{-1}) e de sulfentrazone (0,600 kg ha^{-1}), aplicados em pré-emergência de crotalária, mucuna-preta e sorgo, e um tratamento sem cultivo. Essas espécies, antes da semeadura em campo da canola (bioindicadora), foram submetidas aos manejos de roçada e dessecadas. Nos ensaios de casa de vegetação, a mucuna-preta, o milheto e o sorgo toleraram o fomesafen até a dose recomendada; já o sulfentrazone ocasionou a morte total dessas plantas. Nos experimentos de campo, o cultivo de mucuna-preta e de crotalária, de maneira geral, foram as melhores alternativas como culturas antecessoras à canola, em solo contaminado com fomesafen e principalmente a mucuna-preta para o sulfentrazone. O número de síliquas por planta e a produtividade de grãos da canola foram melhores ao se usar a

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mucuna-preta como planta de cobertura ao se aplicar os dois herbicidas, independentemene do manejo adotado. Conclui-se que a crotalária e a mucuna-preta foram as espécies que fitorremediaram o solo tratado com fomesafen e sulfentrazone, podendo ser utilizadas na despoluição de solo em que se aplicou esses herbicidas, independentemente do manejo adotado.

Palavras-chave: *Brassica oleracea* var. *oleifera*, *Crotalaria juncea, Mucuna pruriens*.

INTRODUCTION

Currently, the control of weeds of agricultural crops becomes mandatory to achieve a high productivity and also to obtain the quality of the harvested products. However, in order to control these weed species, herbicides have been used as the main management method, because of their efficiency, practicality and lower cost, compared to other forms of control. However, many of these products can cause soil contamination, because of their high persistence or even due to the physicochemical characteristics of their molecules.

In this context, PROTOX inhibiting herbicides are widely used in the weed control of soybean, beans, sugarcane, forests, and other crops (Rodrigues and Almeida, 2011). One of the main characteristics of these herbicides is that they can remain in the soil until the next growing season, at concentrations that are capable of causing damages to crops sown in succession (Rodrigues and Almeida, 2011). This persistence in the soil can last a few months or even years (Pires et al., 2003), depending on the herbicide applied to the crop.

When the molecule of an herbicide reaches the soil, it can be degraded by chemical and/or biological processes, be sorbed to colloids or even leached (Belo et al., 2007). The sorption and degradation of the herbicide will define the availability of the product in the soil solution to be absorbed by plants or leached to the subsurface layers of the soil; it may also reach groundwater (Prata and Lavorenti, 2000).

Phytoremediation has appeared as a promising technique for the decontamination of soil contaminated with various herbicides, such as tebuthiuron (Pires et al., 2005), trifloxysulfuron-sodium (Santos et al., 2007), sulfentrazone (Madalão et al., 2012), imazethapyr + imazapic and imazapyr + imazapic (Galon et al., 2014; Berlamino et al., 2012), among others.

In order to adopt phytoremediation, it is necessary to identify species that have the capacity to decontaminate soil and that show selectivity or natural tolerance when in contact with certain herbicides applied to the soil. This tolerance may result from processes such as the differential translocation of organic compounds to other tissues, with subsequent volatilization and/or partial or complete degradation into less toxic compounds (Pires et al., 2003). Another possibility is phytostimulation, in which, due to the release of root exudates, there is a stimulus to microbial activity, which acts degrading the contaminant compound in the soil (Santos et al., 2007).

Studies recommend that some characteristics should be considered when choosing the plant species to be used in remediation programs of areas (when on the field) contaminated by herbicides, including: a) absorption capacity, concentration and/or metabolization and tolerance to the herbicide; b) retention of the herbicide in the roots, in case of phytostabilization, as opposed to the transfer to the shoot, avoiding its manipulation and disposal; c) deep and dense root system; d) high growth rate and biomass production; e) high transpiratory capacity, especially in perennial trees and plants; f) easy harvesting, when it is necessary to remove the plant from the contaminated area; g) high rate of root exudation; h) resistance to pests and diseases; i) easy acquisition or multiplication of propagules; j) easy control or eradication; and k) ability to develop well in different environments (Newman et al., 1998; Vose et al., 2000, Pires et al., 2003; Santos et al., 2007; Accioly and Siqueira, 2008). It would be ideal to gather all these characteristics in a single plant, but the selected one must gather the largest number of them (Santos et al., 2007).

Another aspect to be observed is that although most tests evaluate isolated plants, several species may, as suggested by Miller (1996), be used in one place at the same time or subsequently, in order to promote greater decontamination. Essentially, the ideal plant species to remediate a soil contaminated by herbicides would be one with high biomass production, and which can both tolerate and accumulate the product. Thus, the choice of herbicide-tolerant plants is the first step in the selection of potentially phytoremediation species.

Therefore, it is remarkable that the persistence of herbicide residues in the soil may intoxicate crops sown in succession, which is not desirable in the agricultural activity. Because of this, it is possible and advisable to use plant species that can phytoremediate soils contaminated with herbicides (Pires et al., 2003; Galon et al., 2014).

Therefore, the objective of this work was to evaluate the tolerance potential of species cultivated in the summer for the phytoremediation of soil after the application of the herbicides fomesafen and sulfentrazone.

MATERIAL AND METHODS

Four experiments were conducted, two in a greenhouse and two on the field, both at the Universidade Federal da Fronteira Sul, Erechim-RS, Campus, in 2015 and 2016.

Greenhouse tests

Two greenhouse experiments were conducted, one with the herbicide fomesafen and another one with sulfentrazone, from November 2015 to January 2016. The used summer species with phytoremediation potential were: brown hemp (*Crotalaria juncea*), millet (*Pennisetum glaucum*), velvet bean (*Mucuna pruriens*) and sorghum (*Sorghum bicolor* cv. Sudanense). These species were selected because they are of agricultural importance when used as pasture, soil cover or for grain production.

The experiments were installed in a randomized block design with four replications; the first one was installed with the herbicide fomesafen and the second one with sulfentrazone, trademarks Flex® and Boral 500®, respectively. Fomesafen (0.0, 0.125, 0.250 and 0.5 kg ha^{-1}) and sulfentrazone (0.0, 0.3, 0.6 and 1.2 kg ha^{-1}) were applied after sowing the species with phytoremediation potential.

Species were cultivated in polyethylene pots with a capacity of 8 dm^3, filled with humic aluminoferric Red Latosol (EMBRAPA, 2013). Soil was collected at depths from 0 to 20 cm, in an area with no history of herbicide application, and physically and chemically characterized. This characterization served as a basis for pH correction and for fertilization, according to the technical recommendations for the crops (ROLAS, 2004). The chemical and physical characteristics of the soil were: pH in water 4.8; MO = 3.5%; P = 4.0 mg dm^{-3}; K = 117.0 mg dm^{-3}; Al^{3+} = 0.6 $cmol_c$ dm^{-3}; Ca^{2+} = 4.7 $cmol_c$ dm^{-3}; Mg^{2+} = 1.8 $cmol_c$ dm^{-3}; CTC(t) = 7.4 $cmol_c$ dm^{-3}; CTC(TpH = 7.0) = 16.5 $cmol_c$ dm^{-3}; H+Al = 9.7 $cmol_c$ dm^{-3}; SB = 6.8 $cmol_c$ dm^{-3}; V = 41%; and clay = 60%.

The sowing of species with phytoremediation potential was performed one day before the application of the herbicides. They were applied using a precision backpack sprayer, equipped with two spray nozzles from the TT 110.02 series, with a spraying volume of 150 L ha^{-1}. After the germination of the species, thinning was performed, leaving 10 plants per pot. The irrigation of plants was carried out whenever necessary.

The variables analyzed 30 days after plant emergence (DAE) were: phytotoxicity, height, stalk and/or stem diameter, leaf area and shoot dry matter of the plants. Phytotoxicity was determined visually by two evaluators, giving scores of zero (no injury) and 100% (complete death of plants) according to the SBCPD methodology (1995). Plant height was measured with a ruler graduated in centimeters, measuring from the soil up to the apex of the last fully developed leaves. The stem diameter was determined with a digital caliper at 5 cm from the ground. The leaf area was measured with a portable leaf measuring device, model CI-203, by BioScence, quantifying all the plants found in each treatment. After determining the leaf area, plants were placed in kraft paper bags and put in a forced air circulation oven at a temperature of 60±5 ^oC until reaching constant weight, in order to measure the dry matter of the shoot.

Data were submitted to analysis of variance by F test; when significant, linear or non-linear regressions were applied to evaluate the effect of the herbicide doses on the studied species. It is worth highlighting that comparisons were not made for the tested variables among the species, because they naturally present differences between them. All tests were performed at p>0.05.

Field tests

In the experimental area of the Universidade Federal da Fronteira Sul (UFFS), Erechim campus, two experiments were installed, one with fomesafen and another one with sulfentrazone, using sorghum, brown hemp, velvet bean and a treatment without cultivations preceding the sowing of canola.

The chemical and physical characteristics of the soil were: pH in water 4,8; MO = 3.0%; P = 5.2 mg dm^{-3}; K = 118.0 mg dm^{-3}; Al^{3+} = 0.3 $cmol_c$ dm^{-3}; Ca^{2+} = 5.5 $cmol_c$ dm^{-3}; Mg^{2+} = 3.0 $cmol_c$ dm^{-3}; CTC(t) = 9.10 $cmol_c$ dm^{-3}; CTC(TpH = 7.0) = 16.5 $cmol_c$ dm^{-3}; H+Al = 7.7 $cmol_c$ dm^{-3}; SB = 8.8 $cmol_c$ dm^{-3}; V = 53.33%; and clay = >60%.

The experimental design was a randomized block one, arranged in subdivided plots, with four replications. In the plots, the summer coverings were allocated (velvet bean, brown hemp, sorghum and an additional treatment without cultivation) and, in the subplots, the managements were arranged (burndown or mowing). One day before sowing the pre-emergence summer coverings, herbicide doses were 250 and 400 g ha^{-1} of fomesafen and sulfentrazone, respectively, applied with a CO^2 pressurized backpack sprayer. Together with this, there was a bar with four spray jet spray nozzles, model TT 110.02, spraying a volume of 150 L ha^{-1}.

The species were chosen because they present phytoremediation potential in previous works conducted in a greenhouse. Velvet bean, brown hemp and sorghum plants were cultivated for 55 days after emergence, when half of the experimental unit was sectioned close to the soil, using a costal mower; their shoot was left on the soil surface, and the other part of the plot was desiccated with glyphosate (3 L ha^{-1}). These operations were carried out because they are the most common ones that rural producers can adopt in their farming. The treatment without cultivation was composed only of spontaneous vegetation, which germinated after the application of fomesafen and/or sulfentrazone, exclusively composed by alexandergrass (*Urochloa plantaginea*), which was mowed or dried before the cultivation of canola.

The sowing of the bioindicator culture, Hyola 61 hybrid canola, was performed with a sewer/fertilizer at a spacing of 0.17 m, at the density of 40 plants m^{-2}, which was equivalent to 3 kg ha^{-1} of seeds. Two hundred kg ha^{-1} of the N-P-K 05-30-15 formula was used as base fertilizer. The other management and cultural treatments carried out for canola were all recommended by the research (ROLAS, 2004).

The variables related to plant physiology, such as: chlorophyll index, stomatal conductance of water vapors, transpiration rate, photosynthetic activity, water use efficiency, and shoot dry matter (g m^{-2}) were evaluated 60 days after the emergence (DAE) of canola, on 09/05/2016. To measure the chlorophyll index, a portable chlorophyllometer, model SPAD 502 - Plus, was used, determining the measurements at five points of each plant in the lower, middle and upper leaves of the canopy. The stomatal conductance of water vapors (GS - mol m^{-1} s^{-1}), transpiration rate (E - mol H_2O m^{-2} s^{-1}), photosynthetic activity (E - mol H_2O m^{-2} s^{-1}) and water use efficiency (EUA - mol CO_2 mol H_2O^{-1}) were determined in the middle third of the first fully expanded leaf of plants. For this, an infrared gas analyzer (IRGA), ADC brand, model LCA PRO (Analytical Development Co. Ltd, Hoddesdon, UK), was used from 8 to 10 o'clock a.m. The dry matter was measured after drying the plants in a forced air circulation oven at a temperature of 60±5 ^oC, until reaching constant weight.

During the pre-harvesting of canola, the number of siliques per plant was determined in 10 plants harvested randomly in each experimental unit, and grain yield was determined during the harvest. The determination of the grain yield of canola was obtained by harvesting the plants in a 3 x 1.5 m (4.5 m^2) usable area, in each experimental unit, when the grain moisture content reached approximately 16%. After weighing the grains, their moisture content was determined, and the grain mass were corrected to 12% moisture, extrapolated to kg ha^{-1}.

The results were submitted to analysis of variance by F test; when significant, the Tukey's test was applied. All tests were performed at p>0.05.

RESULTS AND DISCUSSION

Greenhouse tests

The results show that brown hemp, millet, velvet bean and sorghum were negatively affected when exposed to all doses of sulfentrazone, where the emergence of plants did not occur, that is, they were completely killed (data not shown). Madalão et al. (2013), applying 800 and 1,600 g ha^{-1} of sulfentrazone on two sorghum species, *Sorghum deeringianum* and *S. aterrimum*, also reported the elimination of all plants, which corroborates the results found in this study.

It was observed that all species exposed to fomesafen showed a significant increase of phytotoxicity according to the increase of the doses (Figure 1). The application of half the dose and twice the recommended dose (0.25 and 0.5 kg ha^{-1}, respectively) of fomesafen caused a high phytotoxicity to the species, except for the velvet bean, which presented injuries below 20%. Thus, it was observed that the velvet bean shows the ability to tolerate fomesafen, whereas the other species presented phytotoxicity above 90% at the dose of 0.5 kg ha^{-1} (Figure 1).

Figure 2 shows a significant effect on plant height according to the increment of fomesafen doses only for brown hemp, with the highest herbicide dose applied (0.5 kg ha^{-1}). For the other species, there was no effect from the increment of the herbicide doses; even using twice the recommended dose, they did not show the severe effects of fomesafen. Procópio et al. (2005), while working on bean cultures, observed that the use of fomesafen + chlorimuron-ethyl and fomesafen + chloransulam-methyl caused a reduction in plant height for the BRS Grafite and BRS Pérola cultivars; this is similar to the results found in this research.

The results demonstrate that the use of fomesafen at different doses did not present data fitting to the stem diameter models of the evaluated plants (Figure 3). Some herbicides, when applied to plants, cause an increase in the stem diameter in order to try and protect themselves from their phytotoxic effect, as was also observed by Galon et al. (2016), on the diameter of sorghum stalks after the application of atrazine, which is a FS II-inhibiting herbicide. Meschede et al. (2008) reported an increase in the diameter of the roots of *Commelina benghalensis* after the application of glyphosate.

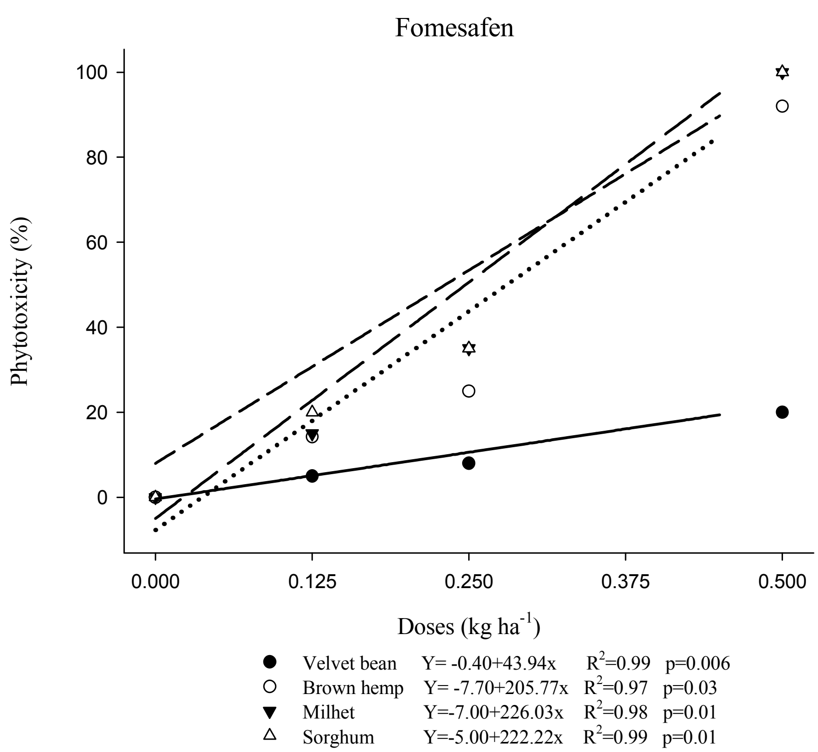

Figure 1 - Phytotoxicity (%) of the species cultivated in summer (vevet bean, brown hemp, millet and sorghum), 30 days after emergence, according to the pre-emergence application of fomesafen doses.

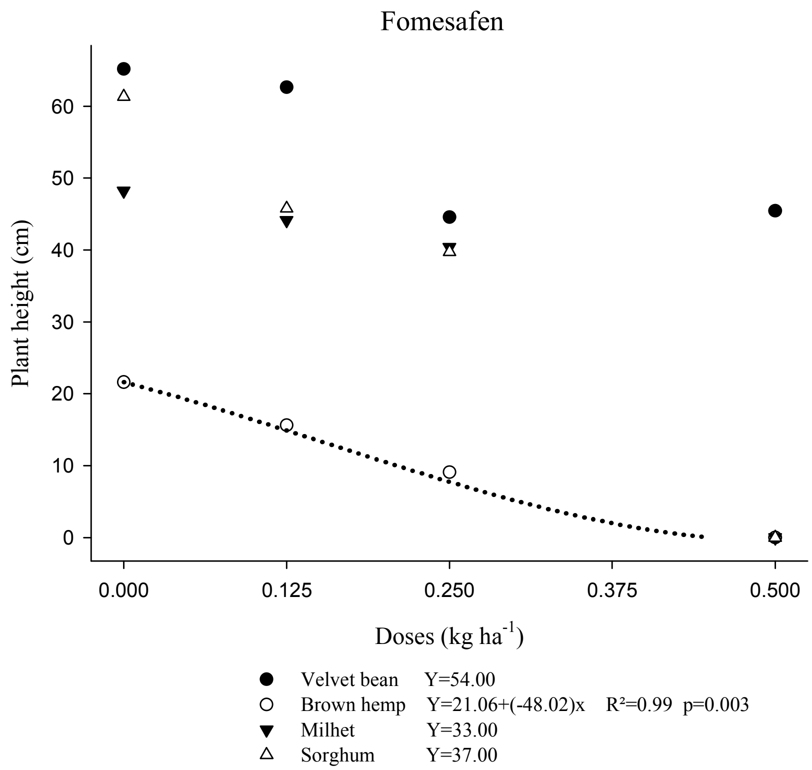

Figure 2 **-** Plant height (cm) of velvet bean, brown hemp, millet and sorghum species 30 days after emergence, according to the pre-emergence application of fomesafen doses.

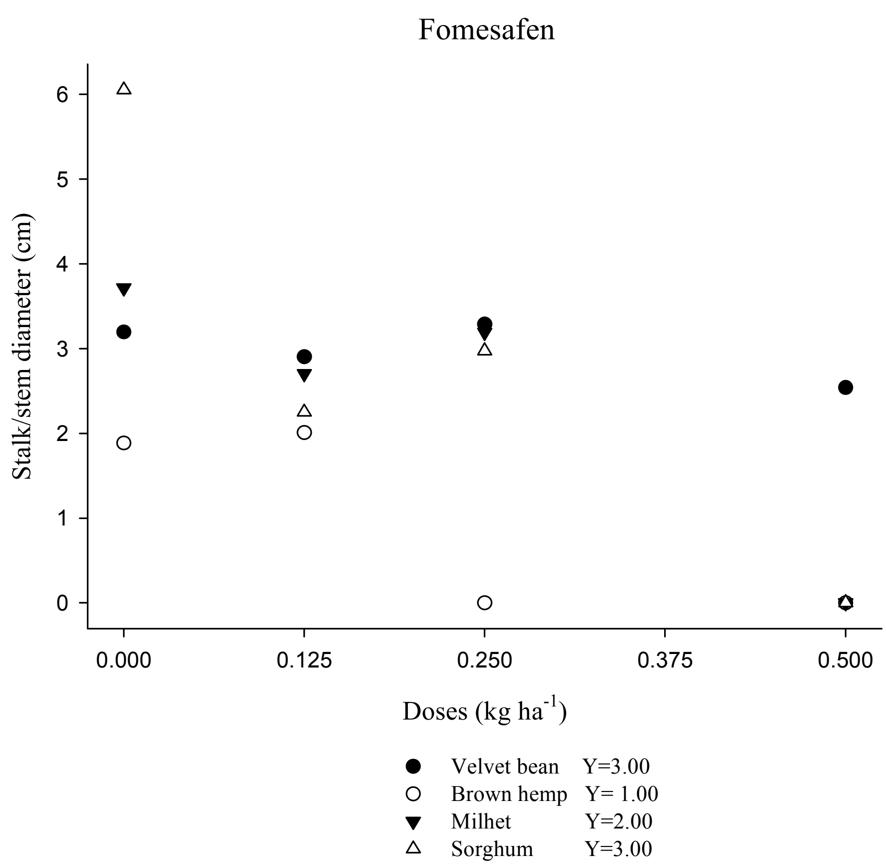

Figure 3 **-** Stalk and/or stem diameter (cm) of velvet bean, brown hemp, millet and sorghum 30 days after emergence, according to pre-emergence doses of fomesafen.

It was observed that the leaf area values of three species were adjusted to equations, linear ones (millet) and non-linear ones (sorghum and brown hemp) (Figure 4). The highest leaf area reduction was for sorghum, with about 63%, compared to the absence of application, 0 dose, of the herbicide with the recommended dose (0.25 kg ha^{-1}). For millet and brown hemp, the greatest injuries were already found with the use of half the recommended dose, occurring death when they were exposed to twice the recommended dose. For millet, a linear reduction of the leaf area occurred with the increase of the applied doses.

At the recommended dose of fomesafen, there was a 27% reduction; however, unlike the other species, with twice the dose there was no death of the velvet bean plants. It is worth mentioning that the results related to the leaf area (Figure 4) are in agreement with those observed for phytotoxicity (Figure 1), that is, the greatest injuries caused by the herbicide expressed the smallest accumulations of leaf areas. Some studies report that normally, when herbicides present low phytotoxicity, in tests related to phytoremediation, the plants used for this technique demonstrate a larger leaf area. This was verified by Belarmino et al. (2012) when they evaluated the species *Lolium multiflorum*, *Lotus corniculatus*, *Trifolium repens*, *Festuca arudinacea*, *Vicia sativa* and *Brassica napus*, when exposed to the application of imazapic + imazapyr at doses of 140 and 280 g ha^{-1}.

The accumulation of shoot dry matter diminishes with the increment of the doses of fomesafen for brown hemp and sorghum (Figure 5). As for the other species, there was no significant effect from the herbicide doses on this variable. It was observed that only velvet bean tolerated twice the dose of fomesafen; in the other species, all plants died. Procópio et al. (2005), when working with phytoremediation after the application of trifloxysulfuron-sodium, verified that there was a reduction in the dry matter accumulation of the shoot of beans, proportional to the increase of the herbicide dose and to the variation in relation to the species *C. mucunoides*, *C. juncea*, *C. cajan*, *P. glaucum*, *S. guianensis*, *M. cinereum*, *M. aterrima*, *R. sativus* and *L. albus.*

Similarly to what occurred with the leaf area, the dry matter also showed lower accumulation in the species that had the highest phytotoxicity indices (Figures 1, 4 and 5). Madalão et al. (2012) also reported the occurrence of reduced dry matter accumulation according to an increased phytotoxicity when cultivating *Pennisetum glaucum* in soils contaminated with sulfentrazone. The increase in the morphological characteristics and the low phytotoxicity, even with the

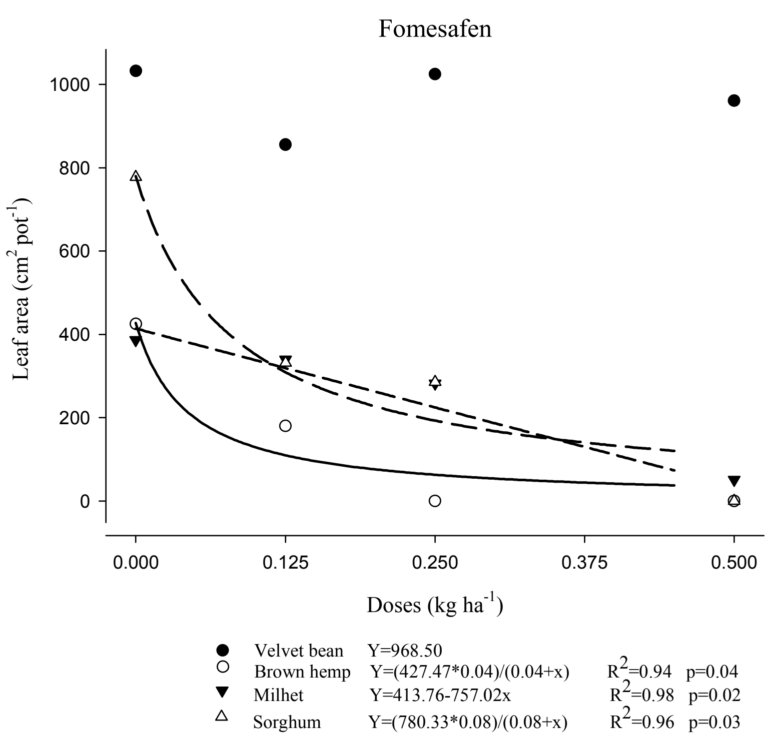

Figure 4 **-** Leaf area (cm² pot^{-1}) of velvet bean, brown hemp, millet and sorghum plants, 30 days after emergence, according to the application of pre-emergence doses of fomesafen.

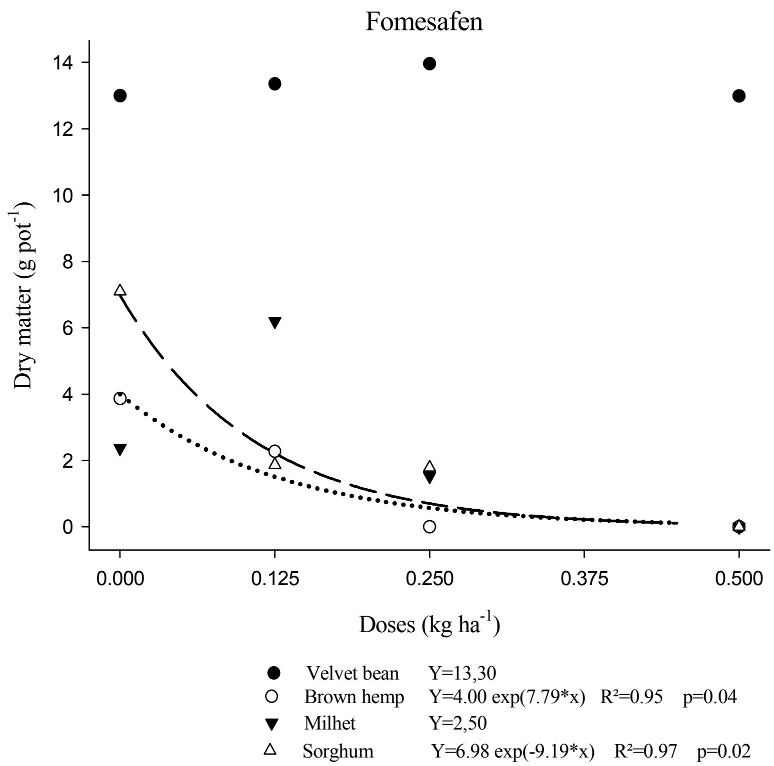

Figure 5 - Shoot dry matter (g pot^{-1}) of the species velvet bean, brown hemp, millet and sorghum, 30 days after emergence, according to the application of pre-emergence doses of fomesafen.

increase of fomesafen doses, demonstrate that the velvet bean has the capacity to be used as a phytoremediation of the herbicide.

The capacity of leguminous species to tolerate or present phytoremediation of the soil can be directly related to its association with microorganisms in the soil, through nitrogen fixing bacteria. Santos et al. (2007) reported that the cultivation of *Stizolobium aterrimum* until its complete flowering favored the accumulation of exudates in the root region, allowing the growth and development of a greater number of groups of microorganisms when the soil was treated with trifloxysulfuron-sodium.

Velvet bean and brown hemp presented the lowest changes in the tested variables when submitted to twice the dose of fomesafen. Velvet bean, millet and sorghum tolerated fomesafen up to the recommended dose (0.25 kg ha^{-1}). Among the tested herbicides, sulfentrazone had a higher toxic effect on the species, causing the death of plants even with the application of half the recommended dose.

Field tests

Two field tests were installed in the experimental area of the Universidade Federal da Fronteira Sul (UFFS), Erechim campus, to evaluate the phytoremediation potential of species that were previously cultivated under greenhouse conditions. In test 1, the herbicide fomesafen was used and, in test 2, sulfentrazone was used; as a bioindicator of the presence or absence of herbicides in the soil, canola was sown, Hyola 61 hybrid.

Experiment with fomesafen

There was an interaction between the adopted managements (mowing or burndown) of sorghum, brown hemp, and velvet bean cultivations, and the treatment without cultivation, for the variables chlorophyll index, shoot dry matter, number of siliques and grain yield of canola, when using fomesafen before cultures with phytoremediation potential (Table 1).

Table 1 - Effect of management methods (mowing or burndown with glyphosate) and plant species with phytoremediation potential of fomesafen-treated soil on the chlorophyll index, shoot dry matter, number of siliques per plant and grain yield of the canola hybrid Hyola 61

Management method	Phytoremediation species			
	Sorghum	Brown hemp	Velvet bean	No cultivation
Canola plant chlorophyll index - SPAD				
Mowing	46.43 aB	52.23 aA	49.48 aAB	46.40 aB
Burndown	44.25 aA	45.08 bA	48.08 aA	47.85 aA
VC (%)	4.74			
Canola plant shoot dry matter (g per plant)				
Mowing	3.24 aA	2.30 bA	2.13 aA	3.28 aA
Burndown	3.15 aA	3.46 aA	2.81 aA	2.42 aA
VC (%)	23.42			
Number of siliques per canola plant				
Mowing	77.62 aC	100.06 aB	121.04 aA	81.05 bBC
Burndown	73.39 aB	87.29 aAB	94.29 bA	99.70 aA
VC (%)	11.34			
Produtividade de grãos de canola (kg ha^{-1})				
Mowing	323.25 aC	629.40 aAB	802.54 aA	456.58 aBC
Burndown	344.06 aB	628.33 aA	565.32 bAB	577.57 aAB
VC (%)	23.13			

Means followed by the same letters, lower case letters in the column and upper case in the line, do not differ by Tukey's test at p≤0,05.

When comparing the different managements among them, it was observed that mowing showed a better performance than burndown for the chlorophyll index of canola when using brown hemp as a covering with phytoremediation potential (Table 1). This can derive from the nitrogen input that brown hemp provided to the subsequent crop (canola), because nitrogen presents a positive correlation with the chlorophyll index of plants (Reinbothe et al., 2010; Leonardo et al., 2013).

The use of brown hemp and velvet bean covering on the mowing management showed the best chlorophyll indices of canola compared to sorghum and to the non-cultivated treatment (Table 1). Treatments did not differ when desiccating with glyphosate the species with phytoremediation potential. Possibly, a better performance of mowing for brown hemp and velvet bean is due to the rapid decomposition of these coverings, since they are leguminous and present a lower C/N ratio compared with sorghum, which is a grass with a higher C/N. In the burndown management, there were no differences between the treatments, since glyphosate presents a high control of the species used as phytoremediation agents: sorghum, brown hemp and velvet bean.

The shoot dry matter of canola in soils submitted to the application of fomesafen and the use of coverings with phytoremediation potential did not influence the plant species in each adopted management. There was a 50.4% increase in the variable when the burndown of the crop was carried out, compared to the mowing management, only for brown hemp. However, this higher shoot dry matter production in the burndown management did not influence the productivity of canola (Table 1).

The number of canola siliques showed variation according to the species that was previously sown in the soil that received the application of fomesafen. The cultivation of velvet bean before the sowing of canola caused the greatest production of siliques in the mowing management. In the burndown management, the cultivation of velvet bean was statistically equal to that of the brown hemp and the treatment without cultivation (Table 1). In an experiment conducted by Kaefer et al. (2014), the production of siliques per plant increases according to the availability of N; since the velvet bean is a plant that fixes this element, the canola crop was possibly benefited by this extra contribution of N. As for the treatment without cultivation, since there was no cultivation of the species, there was greater availability of nutrients for the canola crop; this was not observed in the other treatments, due to the cultivation of coverings that use a greater amount of nutrients. However, it is worth highlighting that the plants used as coverings, as well as allowing the possibility of phytoremediation, they can help controlling erosive processes, avoiding the loss of water by evaporation and the loss of nutrients by leaching, helping the maintenance of the constant temperature of soil and preventing the germination of many weed species that could infest the crops sown in succession (Moosavi and Seghatoleslami, 2013).

Velvet bean in the mowing management showed a higher number of siliques than the burndown management, and the increase of this variable resulted directly in the yield of canola grains, as it is possible to observe in Table 1. The treatment without cultivation in the mowing management presented the production of smaller number of siliques compared to the burndown one; this could be attributed to the possible regrowth effect of some weed found in the area, and which may have competed with canola at the beginning of the cycle, possibly affecting the expression of the number of siliques per plant. The number of siliques per plant is considered to be an important variable that is part of the productivity components of canola and tends to increase as nitrogen availability increases (El-Nakhlawy and Bakhashwain, 2009) - something that was also found in this study.

The yield of canola grains (Table 1), generally speaking, showed a significant reduction when it was sown on the sorghum crop and the treatment without cultivation, in both managements (mowing and desiccating). Again, the nitrogen availability may explain the increase of this variable, as well as the phytoremediation potential of these species, which was verified in the greenhouse experiment for brown hemp and velvet bean, with canola being highly responsive to the availability of nitrogen in the soil (Lucas et al., 2013).

No productivity differences were observed for all treatments within the managements, except when desiccating the velvet bean, where canola produced less grains than when mowing the covering with phytoremediation potential for soil treated with fomesafen (Table 1). This may be conditioned by the rapid decomposition of the velvet bean straw after burndown and, thus, the emergence of weeds along with canola occurs, which would lead to the competition for the resources of the medium, such as water, light and nutrients, and the decrease in crop productivity. Kozlowski (2002) reports that the effects of interference are irreversible, and there is no recovery of crop development or grain yield after the withdrawal of the stress caused by the presence of weeds, being necessary to avoid the competition between plants already at the early stages of development.

There was a significant effect only for the use of cover crops, sorghum, brown hemp, velvet bean, and the treatment without cultivation for the variables stomatal conductance, transpiration rate, photosynthetic activity and water use efficiency when using fomesafen before the crops with soil phytoremediation potential (Table 2).

The stomatal conductance, transpiration rate, photosynthetic activity and water use efficiency of canola plants presented similar behaviors to the treatment without cultivation, that is, the best results were observed in this treatment compared with the others (Table 2). The lower the stomatal opening, the greater the stomatal resistance, decreasing the transpiration rate, as the stomatal conductance is responsible for the CO_2 inlet flow and water outflow through stomata (Taiz et al., 2017). Among soil covering, sorghum was the one that had the worst results in

Table 2 - Effect of previous cultivations of plant species with phytoremediation potential for fomesafen-treated soil on stomatal conductance of water vapors, transpiration rate, photosynthetic activity, and water use efficiency of Hyola 61 canola hybrid

Phytoremediation species	Stomatal conductance (mol m^{-1} s^{-1})	Transpiration rate (mol H_2O m^{-2} s^{-1})	Photosynthetic activity (µmol m^{-2} s^{-1})	Water use efficiency (mol CO_2 mol H_2O^{-1})
Sorghum	0.48 c	1.09 b	5.38 c	4.92 c
Brown hemp	0.58 ab	1.48 a	9.09 b	6.24 ab
Velvet bean	0.52 bc	1.51 a	6.87 c	5.36 bc
No cultivation	0.63 a	1.63 a	11.69 a	6.69 a
VC (%)	9.97	9.53	14.16	12.95

Means followed by the same letters, lowercase in the column and uppercase on the line, do not differ by Tukey's test at $p \leq 0.05$.

relation to the physiological variables of canola, possibly because it did not cause the phytoremediation of the fomesafen found in the soil.

The transpiration rate of canola was only affected when in succession to sorghum cultivation. This reduction was 26.3, 27.8 and 33.1%, compared to brown hemp, velvet bean and the non-cultivated treatment, respectively (Table 2). Vercampt et al. (2016), when applying s-metolachlor (1.0 kg ha^{-1}) found a reduction of 67, 88, and 81% in the physiological variables transpiration rate, stomatal conductance and photosynthetic activity of canola, respectively.

Canola sown on soil contaminated with fomesafen without previous cultivation increased by about 6.31, 2.6 and 4.82 µmol CO_2 m^{-2} s^{-1} its photosynthetic activity compared with sorghum, brown hemp and velvet bean, respectively (Table 2). Photodegradation is one of the main mechanisms of fomesafen degradation in the soil (Rodrigues and Almeida, 2011); possibly, the lack of a previous cultivation (bare soil) favored the degradation of the herbicide by light. For Silva et al. (2014), the persistence of fomesafen in the soil depends on the performed planting system and the applied dose: the lower the soil mobilization, the lower its persistence. The uncultivated system may be beneficial for the decrease of fomesafen in the soil, but it did not result in a higher yield of canola grains.

The water use efficiency in canola plants in succession to sorghum and velvet bean crops was respectively 26.5 and 19.9% lower compared to the treatment without previous cultivation. This fact is justified by the lower stomatal conductance and lower transpiration rate observed in canola plants sown over sorghum and velvet bean cultivars (Table 2), and due to the photodegradation of the herbicide in the treatment without cultivation, as already explained.

As observed in the experiments that were installed in a greenhouse, the best results on the field for all variables were obtained when using the covering species with phytoremediation potential, brown hemp and velvet bean, when applying fomesafen, highlighting the number of siliques and grain yield, which are the main yield components of canola; this will directly reflect on the profitability of producers when trying to clean the soil treated with such herbicide.

Experiment with sulfentrazone

There was an interaction between sorghum, brown hemp, and velvet bean cultures and the treatment without cultivation, according to the adopted management (mowing and burndown) for the following variables: chlorophyll index, photosynthetic activity, water use efficiency, number of siliques and grain yield of canola when using sulfentrazone before the cultivation of soil phytoremediation species (Table 3). As for the treatment without cultivation, the different management methods (mowing or burndown) did not differ statistically from each other when evaluating the chlorophyll index, photosynthetic activity, water use efficiency, number of siliques and grain yield of canola.

The chlorophyll index of canola was not influenced by the covering sown before the crop in the mowing management; on the other hand, in the burndown management, there was an influence only by velvet bean, which was lower than the other cover crops used as phytoremediation for the soil. In the comparison between the managements in each covering, mowing showed a higher chlorophyll index than the burndown one when using velvet bean; on the other hand, for the other cover crops, there was no significant difference between mowing or desiccating (Table 3).

It was observed that, in relation to the photosynthetic activity of canola cultivated after the use of species with phytoremediation potential of the herbicide sulfentrazone, in the mowing management, sorghum proved to be better than the other species, and did not differ statistically from the treatment without cultivation. In the desiccating management, the treatment without cultivation did not differ statistically from the brown hemp (9.67 µmol m^{2} s^{-1}), being superior to all the others. It is worth highlighting that sulfentrazone indirectly inhibits the photosynthetic activity, especially with the increase of the herbicide dose (Belo et al., 2016).

When comparing the management of each species, it was observed that the photosynthetic activity was better when sorghum and velvet bean were used, mowing the vegetation, and that burndown presented better results only for brown hemp (Table 3).

Table 3 - Effect of management methods (mowing or burndown with glyphosate) and plant species with phytoremediation potential for soils treated with sulfentrazone on the chlorophyll index, photosynthetic activity, water use efficiency, number of siliques per plant and grain productivity of Hyola 61 canola hybrid

Management method	Phytoremediation species			
	Sorghum	Brown hemp	Velvet bean	No cultivation
Canola plant chlorophyll index - SPAD				
Mowing	48.7 aA	48.48 aA	51.50 aA	51.63 aA
Burndown	49.33 aAB	50.13 aA	44.23 bB	47.55 aAB
VC (%)	5.99			
Canola plant photosynthetic activity (µmol m^{-2} s^{-1})				
Mowing	11.60 aA	6.77 bC	8.85 aBC	10.07 aAB
Burndown	7.86 bB	9.67 aAB	5.49 bC	10.62 aA
VC (%)	13.20			
Efficient water use by canola plants (mol CO_2 mol H_2O^{-1})				
Mowing	10.14 aA	4.40 bB	5.90 aB	5.36 aB
Burndown	6.87 bA	6.02 aAB	3.26 bC	4.77 aBC
VC (%)	13.20			
Number of siliques per canola plant				
Mowing	93.02 aAB	95.02 aAB	117.52 aA	91.11 aB
Burndown	110.81 aA	73.78 bB	90.80 bAB	86.73 aAB
VC (%)	13.38			
Canola grain productivity (kg ha^{-1})				
Mowing	552.53 aA	503.54 aA	615.92 aA	352.74 aB
Burndown	422.88 bB	407.63 aB	615.10 aA	442.20 aB
VC (%)	13.59			

Means followed by the same letters, lowercase in the column and upper case in the row, do not differ by Tukey's test $p \leq 0.05$.

It is worth highlighting that the cultivation of sorghum before canola, in the mowing management, showed higher efficiency in water use (Table 3). Similarly to what was observed in the photosynthetic activity, water use efficiency was better when using sorghum and velvet bean as phytoremediation species in the method of the mowing management. The only treatment in which burndown proved to be more beneficial as for water use efficiency was for the brown hemp species. Crop management directly influences the efficiency of water use by canola when sown under sulfentrazone-contaminated area; the efficiency of plants is to reduce water loss, while CO_2 absorption is enough to maintain the photosynthesis (Taiz et al., 2017).

The number of canola siliques was higher when using a velvet bean covering in the mowing management; this treatment is different from the no-crop and equal to the use of sorghum and brown hemp (Table 3). However, for the desiccating management, significant differences were observed only for the cultivation of sorghum (110.81 mol CO_2 mol H_2O^{-1}) and brown hemp (73.78 mol CO_2 mol H_2O^{-1}). When comparing the two managements (mowing and desiccating), the greatest number of canola siliques were found when the crops were mowed, for brown hemp and velvet bean; the use of sorghum and the treatment without cultivation did not cause differences in the management.

The results showed that the productivity of canola grains was higher when using velvet bean in the two management methods and that there were no differences between the used methods (Table 3); this was not directly related to the higher values of the physiological variables (Tables 3 and 4). When mowing cover crops, the worst treatment was the non-cultivated one, in comparison to the others, while in the desiccating management the best productivity was observed when using the velvet bean. There were differences between the vegetation management methods only when using sorghum as a possible soil phytoremediator; the dried vegetation was the one providing the lowest grain yield of canola. Maladão et al. (2012), when working with the phytoremediation of soils contaminated with sulfentrazone, concluded that the brown hemp was, out of the tested species, the one presenting the greatest potential to clean the soil.

Table 4 - Effect of previous cultures of plant species phytoremediation potential for sulfentrazone-treated soil on the stomatal conductance of water vapors, transpiration rate and shoot dry matter of Hyola 61 canola hybrid

Phytoremediation species	Stomatal conductance (mol m^{-1} s^{-1})	Transpiration rate (mol H_2O m^{-2} s^{-1})	Dry matter (g per plant)
Sorghum	0.53 b	1.15 c	2.08 b
Brown hemp	0.54 ab	1.54 b	2.35 b
Velvet bean	0.65 a	1.62 b	3.57 a
No cultivation	0.65 a	2.00 a	2.58 b
VC (%)	13.78	8.37	21.10

Means followed by the same letters, lowercase in the column, do not differ by Tukey's test at $p \leq 0.05$.

The increase in the grain yield of canola in succession to velvet bean may be related to the reduction of the amount of herbicide in the soil, the increase of N availability and the higher decomposition rate of plants. Thus, this species may also help the action of sulfentrazone-degrading microorganisms in the soil (Rodrigues and Almeida, 2011). The degradation rate of this herbicide may have been higher in the mowing management for sorghum, which favored higher productivity compared to the desiccating management (Table 3).

Statistical differences were observed in stomatal conductance, transpiration rate and shoot dry matter only for the use of cover crops with a phytoremediation potential of sulfentrazone - sorghum, brown hemp, velvet bean and the treatment without cultivation, with no significance for the managements - mowing and desiccating (Table 4).

It was observed again, as verified in the experiment with fomesafen, that the use of sorghum as a phytoremediator caused less stomatal conductance and transpiration in canola plants and was also one of the treatments that showed lower dry matter production (Table 4). The brown hemp, velvet bean and the treatment without cultivation caused greater stomatal conductance of canola, and this was also reflected in the treatments where the culture presented greater perspiration. The highest dry matter production of canola was caused by the use of the velvet bean; the others were statically lower than this species and equal to each other. This may be related to a greater soil decontamination provided by the phytoremediation species or to the nitrogen supply that the species provided to the crop after canola. Research reports that the velvet bean can accumulate about 50 kg ha^{-1} of N in their tissues, much of which is available to later crops (Silva et al., 2011).

It is possible to notice that these variables do not present a direct relation with the shoot dry mass; this was also reported by Belo et al. (2016). These authors found a reduction in the physiological variables with the application of sulfentrazone, but this reduction did not affect the shoot dry mass of *Helianthus annus*, *Canavalia ensiformis*, *Dolichos lab lab* and *Arachis hypogaea*.

The physiological variables of canola depended on the adopted management and the previously cultivated species. It is worth mentioning that the velvet bean cultivation was the best alternative to precede the cultivation of canola on soil contaminated with sulfentrazone.

Generally speaking, it is possible to conclude that brown hemp and velvet bean had the best results for the phytoremediation of soil treated with fomesafen and sulfentrazone, both in a greenhouse and under field conditions. Mowing the species or desiccating them with glyphosate did not demonstrate significant changes in the vast majority of the variables evaluated on the field.

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