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# Selection of species tolerant to the herbicide sulfentrazone with potential for phytoremediation of contaminated soils

# Seleção de espécies tolerantes ao herbicida sulfentrazone com potencial para a fitorremediação de solos contaminados

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## Abstract

This paper aimed to select species with potential for phytoremediation of soils contaminated with the herbicide sulfentrazone. Eight species were evaluated: *Arachis pintoi, Eleusine coracana*, *Crotalaria spectabilis*, *Crotalaria ochroleuca*, *Cajanus cajan*, *Leucaena leucocephala*, *Stizolobium cinereum*, and *Raphanus sativus*. The experiment was set-up inside a greenhouse, using pots with a capacity of $6dm^3$ filled with soil samples collected at a depth of 0-20cm. The experimental design was arranged into randomised blocks in a factorial scheme (8 × 5) with four replications, which consisted of the combination between the species and five doses of sulfentrazone (0, 200, 400, 800, and 1,600g $ha^{-1}$). The herbicide phytotoxicity, plant heights, and dry masses of shoots and roots were evaluated. The species *Cajanus cajan* and *Leucaena leucocephala* had a higher tolerance to sulfentrazone up to a dose of 400g $ha^{-1}$, showing minor symptoms of phytotoxicity and smaller decreases in plant heights and in dry matter accumulation, both in the shoots and roots, when compared to the control treatment, indicating, thus, the potential to be used for further studies on phytoremediation of sulfentrazone in soil.
**Key words:** Soil decontamination, residual effect, green manures, selectivity

## Resumo

Este trabalho teve como objetivo selecionar espécies com potencial para a fitorremediação de solos contaminados com o herbicida sulfentrazone. Foram avaliadas oito espécies: *Arachis pintoi, Eleusine coracana*, *Crotalaria spectabilis*, *Crotalaria ochroleuca*, *Cajanus cajan*, *Leucaena leucocephala*, *Stizolobium cinereum* e *Raphanus sativus*. O experimento foi instalado em casa de vegetação, com a utilização de vasos com capacidade para 6 $dm^3$ preenchidos com porções de solo coletadas na profundidade de 0-20 cm. O delineamento experimental foi o de blocos casualizados em esquema fatorial 8 × 5, com quatro repetições, composto pela combinação entre as espécies e cinco doses do sulfentrazone (0, 200, 400, 800 e 1.600 g $ha^{-1}$). Foram avaliadas a fitotoxicidade do herbicida, a altura de plantas e a massa da matéria seca da parte aérea e de raízes. As espécies *Cajanus cajan* e *Leucaena leucocephala* apresentaram maior tolerância ao sulfentrazone até a dose de 400 g $ha^{-1}$, mostrando menores sintomas de fitotoxicidade e menores reduções na altura de plantas e no acúmulo de matéria seca, tanto na parte aérea como nas raízes, em relação ao tratamento controle, indicando, com isso, potencial de utilização para posteriores estudos de fitorremediação de sulfentrazone em solo.
**Palavras-chave:** Descontaminação do solo, efeito residual, adubos verdes, seletividade

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## Introduction

The application of herbicides with long residual effects on soils allows, in many cases, an effective control of weeds over a period of time sufficient for the entire space to be occupied by crops, which is something which would not be possible with a single application of herbicides acting only in a post-emergence basis. The use of these herbicides with prolonged action in the soil represents a reduction in the cost of chemical control of weeds, especially in crops with long periods of total interference prevention (PTIP), such as cane sugar. On the other hand, the occurrence of phytotoxicity to sensitive crops (carryover) seeded after using herbicides has been observed in some situations (DAN et al., 2011). The residual effect of these herbicides on soil varies from a few months to more than three years (BOVEY; MEYER; HEIN JUNIOR, 1982). Besides the harmful residual effect, there is also the environmental problem caused by leaching of original molecules of herbicides or their metabolites into deeper layers in the soil profile, which can reach underground aquifers, to consider (DORNELAS DE SOUZA et al., 2001).

Among the herbicides which have a high potential for contamination of soils, sulfentrazone (N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl] methanesulphonamide) stands out, as it has a long residual effect on soil (VIVIAN et al., 2006; MELO et al., 2010; MONQUERO et al., 2010), and can cause damage to subsequent susceptible crops, such as millet, black oats, and sorghum (PEREIRA; ALVARENGA; OTUBO, 2000; DAN et al., 2011).

In addition to being persistent, sulfentrazone is classified as mobile and it has a high potential for leaching both vertically (to groundwater) and horizontally (PARAÍBA et al., 2003; EPA, 2003), mainly due to its low affinity for organic matter.

Another aspect to consider is that the presence of sulfentrazone in tillage is important until the end of PTIP of cane sugar, as after this period, which coincides with the canopy closing, the crop itself is able to prevent infestation with weed species. As a result, the presence of sulfentrazone becomes undesirable, due to the potential for contaminating underground water sources by leaching and/or superficial water sources by runoff (BARRA et al., 1999; PALMA et al., 2004; KRUTZ et al., 2005), the impossibility of cultivating susceptible species in succession (BELO et al., 2007; DAN et al., 2011), and the toxicity to non-target organisms (ROUSSEAUX et al., 2003).

Seeking alternatives to remove and/or reduce this problem, the use of phytoremediation has been studied with a greater emphasis in recent years, i.e. plant species able to remove and/or degrade xenobiotics in soil (PIRES et al., 2003b), and, therefore, allow the subsequent cultivation of susceptible species in the area, eliminating the carryover effect.

The herbicide sulfentrazone has not been well studied, although it is a compound with some environmental risk and the potential to be phytoremediable. Few studies aimed at the phytoremediation of environments contaminated with this herbicide are currently available for consultation.

However, before inferring the phytoremediation capacity of a certain species, there is a need, first, to demonstrate its tolerance to xenobiotics. Since not all plant species are developed in contaminated environments, as observed by Jesus et al. (2009) when evaluating the phytoremediation capacity of metals by *Cyperus rotundus* plants collected in an area where there was discharge of industrial wastes, the first step is the identification of species which are both tolerant to the contaminant and appropriate to local conditions (MARQUES; AGUIAR; SILVA, 2011). Notwithstanding, the selection of plant species for phytoremediation of herbicide compounds presents limitations when compared to programs aimed at the remediation of other contaminants. This is due to the fact that herbicides are contaminants with molecular diversity and a higher complexity for analysis given the constant changes that they are subject to (PIRES et al., 2003b).

The herbicide sulfentrazone acts by inhibiting the enzyme protoporphyrinogen oxidase (PROTOX). The selectivity basis of herbicides inhibiting PROTOX in tolerant species may be attributed to the minimal absorption and translocation of herbicide, herbicide sequestration, the increased concentration of the mitochondrial PROTOX enzyme which acts as a reducer for the excess of protoporphyrinogen in the cytoplasm (HIGGINS et al., 1988; MATSUMOTO; KASHIMOTO; WARABI et al., 2001) and rapid metabolism (VAUGHN; DUKE, 1991). Tolerance or resistance by metabolism or detoxification of the herbicide in the plant is due to the presence of monooxygenase enzymes ($P_{450}$) and glutathione-s-transferase, which are responsible for antioxidant reactions and herbicide conjugation, respectively (VIDAL, 2002). Velini et al. (2005) found that configurations of the PROTOX enzyme or promoters which might enable different expression levels are able to provide genotypes that are more tolerant to the herbicides acting to inhibit this enzyme.

Considering the long persistence of sulfentrazone and the problems caused by its carryover effect, as well as the contamination of underground aquifers, this paper aimed to select tolerant species with potential for the phytoremediation of soils contaminated with the herbicide sulfentrazone.

## Material and Methods

This trial was carried out to determine the tolerance of plant species to sulfentrazone and, thereafter, infer on the potential of this herbicide for application in phytoremediation programs. Eight species previously selected were used, based on information gathered from the literature on tolerance to the herbicide and/or species previously reported as being phytoremediators of other herbicides. Besides the main feature (i.e. tolerance), species with agricultural/economic value were preferentially tested, aiming to promote a double benefit: phytoremediation and its subsequent use in agriculture or livestock, depending on the degree of detoxification promoted by the tested plant. The species tested were: Pinto peanut (*Arachis pintoi* Krap. & Greg.), African finger millet (*Eleusine coracana* (L.) Gaertn.), crotalaria spectabilis (*Crotalaria spectabilis* L.), crotalaria ochroleuca (*Crotalaria ochroleuca* G. Don), pigeon pea (*Cajanus cajan* (L.) Millsp.), leucaena (*Leucaena leucocephala* (Lam.) De Wit.), grey mucuna (*Stizolobium cinereum* Piper & Tracy), and oilseed radish (*Raphanus sativus* L.).

The experiment was installed in a greenhouse located in the campus of the Northern Espirito Santo University Centre (CEUNES) of Universidade Federal do Espirito Santo (UFES), located in Sao Mateus, Espirito Santo, Brazil. Pots with a capacity of $6dm^3$ were filled with soil samples collected at a depth of 0-20cm, classified as typical eutric yellow argisol (EMBRAPA, 2006), with a medium texture, characteristic of the region, that had been sifted through a sieve with a diameter of 0.004m, and chemically and physically analysed afterwards (Table 1).

The treatments consisted of combinations between the 8 plant species already mentioned and 5 doses of sulfentrazone: 0 (zero), 200g $ha^{-1}$ (¼ commercial dose rate), 400g $ha^{-1}$ (½ commercial dose rate), 800g $ha^{-1}$ (full commercial dose rate), and 1,600g $ha^{-1}$ (2× full commercial dose rate), totalling 40 treatments. The experimental design was randomised blocks in a factorial scheme (8 × 5), with four replications.

**Table 1.** Physicochemical composition of the topsoil (0-20cm) used in the experiment.

| Grain-size analysis g kg$^{-1}$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Clay | | Silt | | Sand | | Textural classification | | | |
| 220 | | 30 | | 750 | | Sandy clay loam | | | |
| Chemical analysis | | | | | | | | | |
| pH | P | K | $Ca^{2+}$ | $Mg^{2+}$ | H+Al | $Al^{3+}$ | CTC | V | C |
| $H_2O$ | mg dm$^{-3}$ | ----------------------- cmol$_c$ dm$^{-3}$ ----------------------- | | | | | | % | g kg$^{-1}$ |
| 5.4 | 3.0 | 0.18 | 1.8 | 1.0 | 2.6 | 0.1 | 5.6 | 53.9 | 22.8 |

**Source**: Elaboration of the authors.

After filling in the pots, fertilisation at planting was performed using a soil chemical analysis as a basis. The herbicide sulfentrazone was applied in a pre-emergence basis, using a pressurised sprayer with $CO_2$, adjusted to apply a spray volume equivalent to 200L h$^{-1}$. After 20 days of herbicide application, the sowing of plant species was performed. After plant emergence, a thinning was done, leaving two plants per pot. All pots were watered three times a day to maintain the soil moisture.

The following evaluations were made: plant height (cm) 30 and 60 days after sowing (DAS), taking as a basis for measurement the apical meristem in dicots and the edge of top leaf in the other species; toxicity was visually evaluated (%) 30 and 60 DAS, assigning grades according to the symptoms of intoxication in the shoot, using a scale from 0 to 100, for the absence of symptoms and plant death, respectively; and root dry biomass (g) and shoot dry biomass (g) 60 DAS, making up the drying of material collected in a greenhouse in a forced-circulation mode (70 ± 2°C) for 72 h. To allow the comparison between different species, which have an intrinsic morphological potential, data on height and biomass were transformed into an index with regard to the average values obtained by the respective control of each species for each dose, which received a numerical value equal to 1 (one).

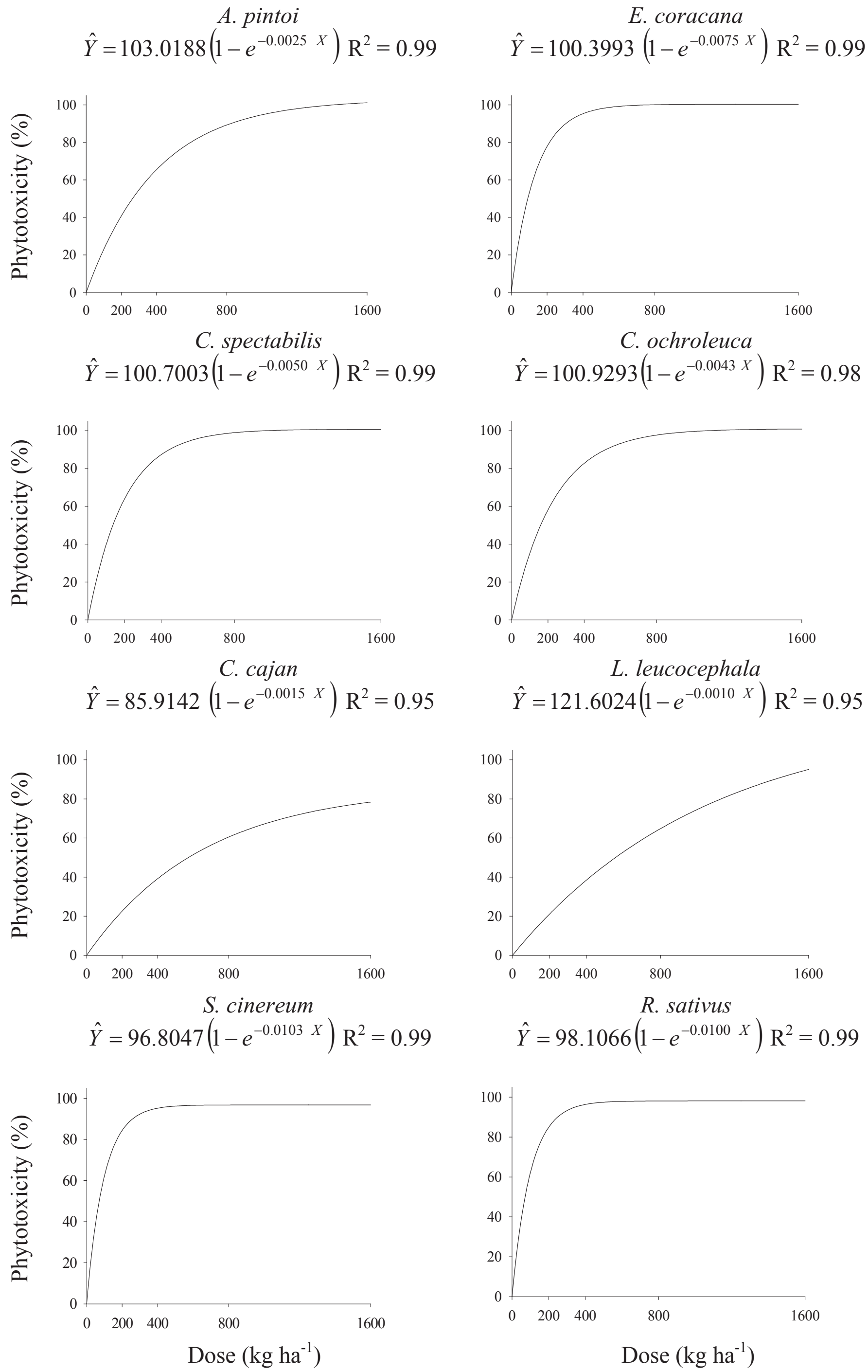
After collecting and tabulating data, variance analysis and F test, at 5% significance level, were performed. The effect of sulfentrazone doses was studied through regression at 5%.

## Results and Discussion

The plant species under analysis differed in tolerance to the herbicide sulfentrazone in the soil. With the application of the lowest dose of the herbicide (200g ha$^{-1}$), the species found to be more susceptible in the evaluation carried out 30 DAS were *S. cinereum* and *R. sativus*, both of which presented an average phytotoxicity of 86% due to the effects of this herbicide (Figure 1). At the same time, analysing the effects of sulfentrazone in this evaluation and in the same level of soil contamination, it was found that *L. leucocephala*, *C. cajan*, and *A. pintoi* presented the best results with regard to tolerance (Figure 1).

With the application of 400g ha$^{-1}$, which corresponds to half of the commercial dose rate, in the assessment carried out 30 DAS, *L. leucocephala* and *C. cajan* were found to be the species presenting the highest levels of tolerance, with 26% and 36% of phytotoxicity, respectively. The species *A. pintoi* presented 70% of phytotoxicity, which is considered a high level, and the species *R. sativus*, *S. cinereum*, *C. ochroleuca*, *E. coracana*, and *C. spectabilis* were the most sensitive species at this dose, with injury levels higher than 88%. With the application of 800g ha$^{-1}$, corresponding to the full commercial dose, *C. cajan* was the species presenting the best result, with 55% of intoxication symptoms, followed by *L. leucocephala*, with 75%. The other species hardly emerged when exposed to such amounts of the product. At a dose of 1,600g ha$^{-1}$, corresponding to twice the full commercial dose rate, no species were found to be tolerant to sulfentrazone, highlighting the broad action spectrum of this herbicide, something which complicates the selection of species that are tolerant and of agronomic interest, which are desirable characteristics in a phytoremediation plant. Similar results were also found by Pires et al. (2003c) when selecting plants able to phytoremediate soils contaminated with the herbicide tebuthiuron.

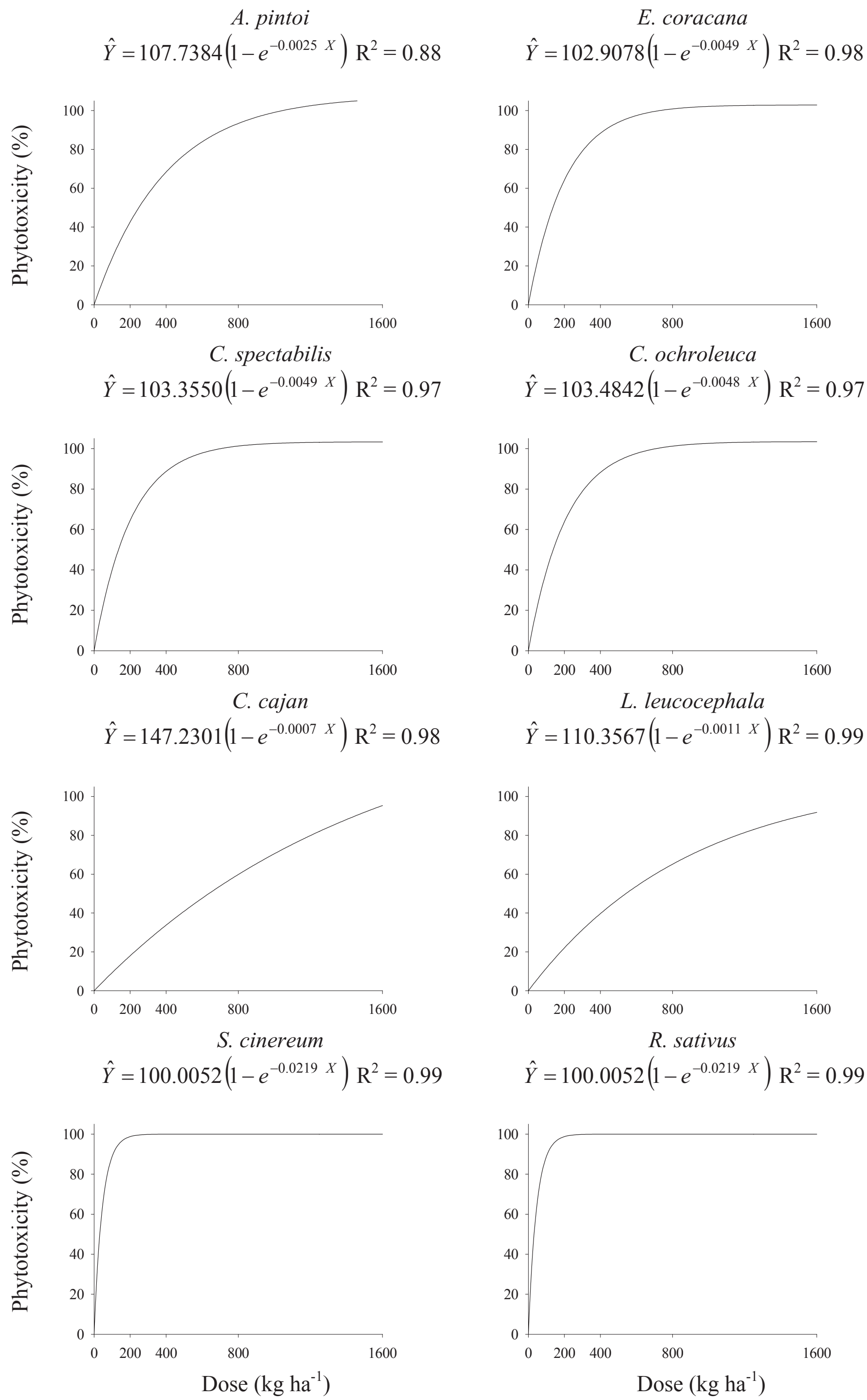
**Figure 1**. Phytotoxicity of plants (%) 30 days after sowing, depending on the dose of the herbicide sulfentrazone.




**Source**: Elaboration of the authors.

When the second evaluation was performed at 60 DAS (Figure 2), it was noticed that there was a slight increase in the phytotoxicity symptoms, evidenced by the overall averages, except for the dose of 200g ha$^{-1}$. In this study, *A. pintoi*, *C. cajan*, and *L. leucocephala* showed minor symptoms of injury. In the remaining doses, *C. cajan* and *L. leucocephala* were always the most tolerant species.

**Figure 2.** Phytotoxicity of plants (%) 60 days after sowing, depending on the dose of the herbicide sulfentrazone.




**Source**: Elaboration of the authors.

The evolution of phytotoxicity symptoms 30 and 60 DAS caused by doses of the herbicide in the eight species tested can be observed in Figures 1 and 2, respectively. Note that there is a different behaviour between species with increased doses of sulfentrazone. It is clearly shown that *S. cinereum* and *R sativus* are clearly the most susceptible species, contrary to what was observed with regard to *C. cajan* and *L. leucocephala*. Notwithstanding, in all species, an increase in the dose led to a significant increase in phytotoxicity.

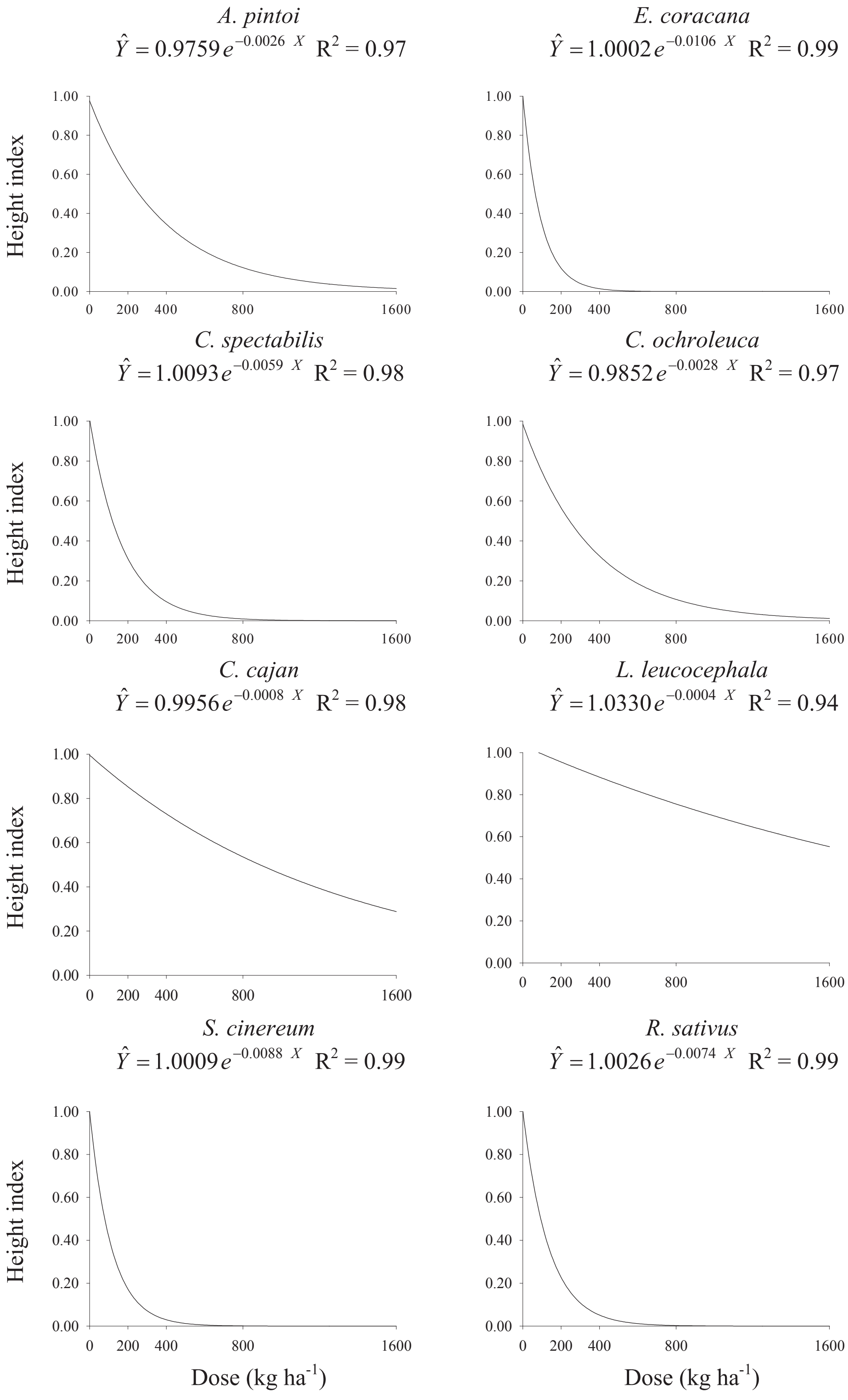
Regarding the plants height, in the first evaluation 30 DAS, as shown in Figure 3, *L. leucocephala* was the species with the lowest decrease when compared to the control (dose 0). When exposed to ¼ of the commercial dose, this species had no decrease in height, and at ½ of the commercial dose it had a decrease that reached 53% of the height of control at twice the full commercial dose rate. Another species which also showed good results was *C. cajan*, with a linear decrease pattern, presenting 81% of the height of control when exposed to a dose of 200g $ha^{-1}$ and 24% when exposed to a dose of 1,600g $ha^{-1}$.

In the height evaluation performed 60 DAS (Figure 4), the effect of doses became more evident due to the progressive poisoning of the plant and its consequently lower height. Data show that when the soil was exposed to 200g $ha^{-1}$, the best results were presented by the species *A. pintoi*, *C. cajan*, and *L. leucocephala*, which presented 89%, 84%, and 77% of the height when compared to the control, respectively. At doses of 400g $ha^{-1}$ and 800g $ha^{-1}$, *C. cajan* and *L. leucocephala* presented lower decreases in height when compared to the other species evaluated.

Dry matter accumulation of shoots and roots corroborated the evidence of increased tolerance shown by the species *L. leucocephala* and *C. cajan*, followed by *A. pintoi* (Figures 5 and 6), indicating that these species are potentially interesting for phytoremediation of the herbicide sulfentrazone under lower levels of this herbicide in soil. The greatest accumulation of biomass is an interesting feature for phytoremediation species, because if the plant uses a tolerance mechanism involving absorption of the active principle concerned, it may correspond to a greater capacity to absorb the herbicide through the roots (NEWMAN et al., 1998) and greater accumulation and/or degradation of the xenobiotic in the shoot. On the other hand, the more sensitive plants with the smallest increase in biomass may indicate increased uptake and degradation of a larger amount of the herbicide (NASCIMENTO; XING, 2006). However, when the plant exhibits high sensitivity to the molecule, it can be fully intoxicated within a few weeks, leading to a complete suppression (death) of the plant, something which, in this case, would hinder its growth and, consequently, cause a significant phytoremediation action. Based on this, a desirable behaviour would be that plants absorb a large amount of herbicide, and retain their metabolic activities without any severe damage. It is therefore essential for further studies to assess whether the plants found to be more tolerant are able to remediate soils contaminated with sulfentrazone.

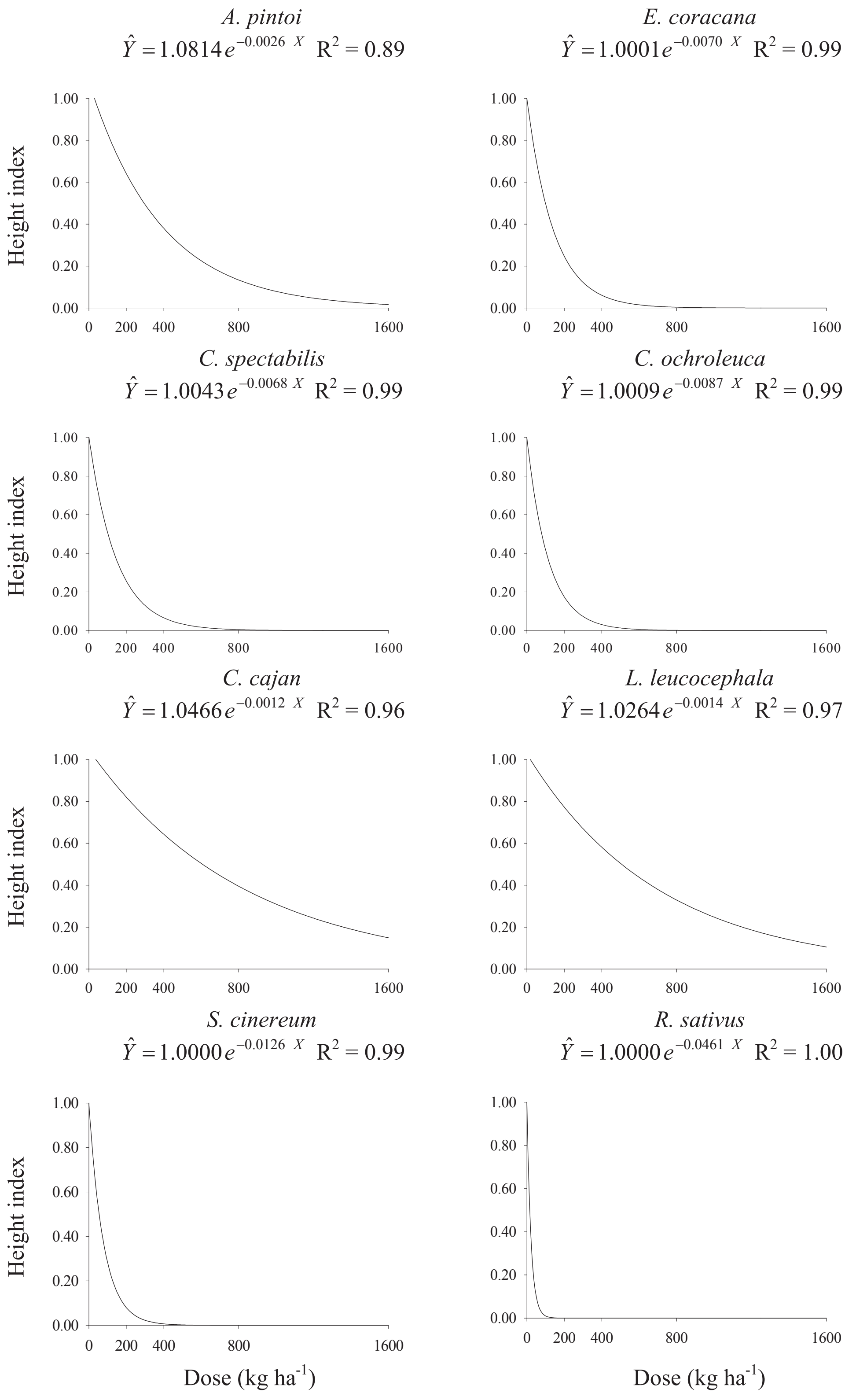
Soils with high sand content are usually poorer in nutrients and have lower CEC, so the use of green manures in these soils, besides providing the phytoremediation of the herbicide, may favour an increase in CEC and fertility. Faria, Soares, and Leão (2004) found that legumes provide higher values for the cation exchange capacity (CEC) and the levels of organic matter (OM) and exchangeable Ca in the layer of soil at 0-10cm depth for a eutric yellow argisol, which presented in the layer at 0-20cm depth 800g $kg^{-1}$ of sand, 100g $kg^{-1}$ of silt, and 100g $kg^{-1}$ of clay. Furthermore, inferences on the results obtained in this kind of soil highlight a behaviour which involves the high availability of the herbicide to the plants and low adsorption. This indicates that the species were cultivated under a condition that favoured phytoremediation via the phytoextraction mechanism. On the other hand, a lower remediation performance could be expected when cultured in soils containing more clay, in which lower levels in the soil solution available to be absorbed by plants are expected (PIRES; EGREJA FILHO; PROCÓPIO, 2009).

**Figure 3**. Plant height index (obtained with regard to average values of the respective control of each species within each dose, which received a numerical value equal to 1) 30 days after sowing, depending on the dose of the herbicide sulfentrazone.



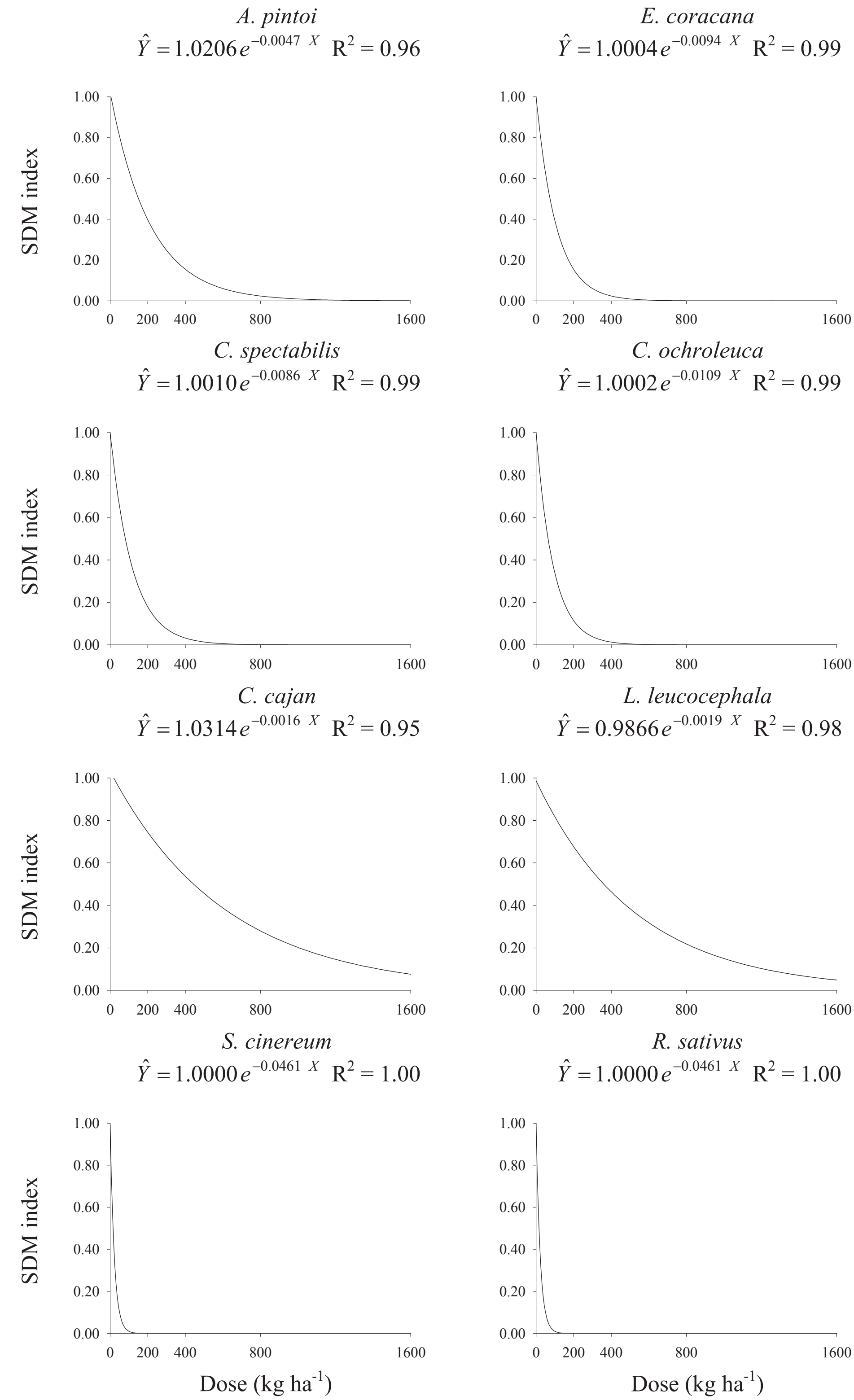

**Source**: Elaboration of the authors.

**Figure 4.** Plant height index (obtained with regard to the average values of the respective control of each species within each dose, which received a numerical value equal to 1) 60 days after sowing, depending on the dose of the herbicide sulfentrazone.



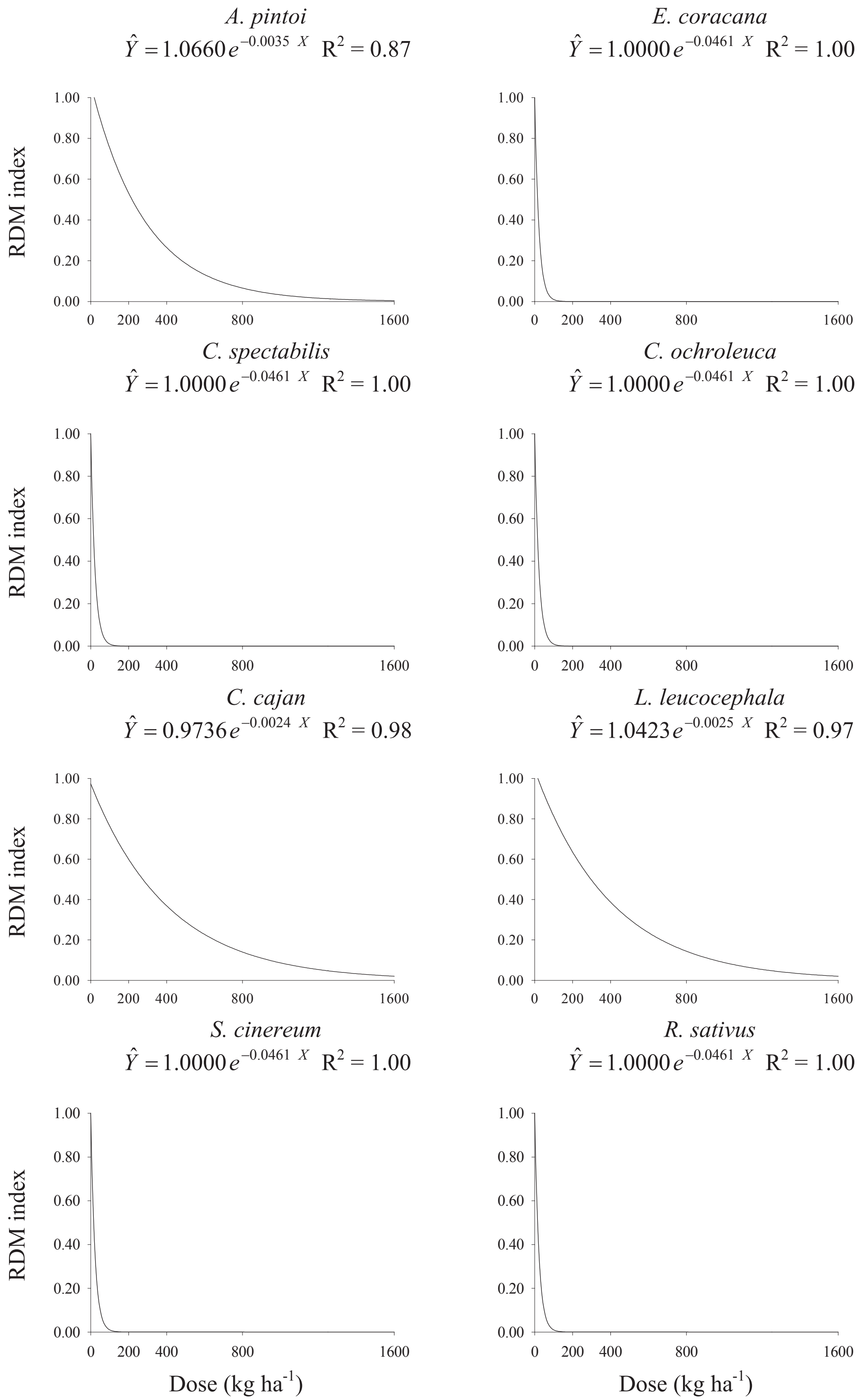

**Source**: Elaboration of the authors.

**Figure 5**. Shoot dry matter (SDM) index (obtained with regard to average values of the respective control of each species within each dose, which received a numerical value equal to 1), evaluated 60 days after sowing, depending on the dose of the herbicide sulfentrazone.




**Source**: Elaboration of the authors.

**Figure 6**. Root dry matter (RDM) index (obtained with regard to average values of the respective control of each species within each dose, which received a numerical value equal to 1), evaluated 60 days after sowing, depending on the dose of the herbicide sulfentrazone.




**Source**: Elaboration of the authors.

From the results obtained, it can be seen that most species are very susceptible to sulfentrazone and that nearly all of them are killed by this herbicide at doses above 800g $ha^{-1}$. As the growing period increases, the general symptoms were intensified in the plants, something which was observed even in species with a higher tolerance, as also observed by Pires et al. (2003a, 2003c). Notwithstanding, it does not hinder further study. Generally, it is expected that plants tolerate the herbicide and keep up with their physiological and metabolic activities (MARQUES; AGUIAR; SILVA, 2011), but not at the same level of a non-poisoned plant, i.e. the plant is not in a detoxification process, or even protecting itself by adopting another mechanism, the deleterious effects of the herbicide in the soil. These hypotheses are supported by the results obtained by D'Antonino et al. (2009), who concluded that the growth of corn plants was lower in soils with picloram residues, but that the final grain production was not affected.

The plants defence mechanism providing resistance to sulfentrazone may be related to sequestration of the herbicide, where the molecule is either conjugated to the plant metabolites, becoming inactive, or is removed from the metabolically active parts of the cell and stored in inactive locations, such as the vacuole (KISSMANN, 1996). The resistance may be related to the increased concentration of the PROTOX mitochondrial enzyme, which acts as a reducer of the excess protoporphyrinogen in the cytoplasm; or to the minimal absorption and translocation of the herbicide (HIGGINS et al., 1988; MATSUMOTO; KASHIMOTO; WARABI et al., 2001). In the latter case, the lowest sensitivity of *L. leucocephala* and *C. cajan* may be due to the exclusion mechanism by which the plant blocks the absorption of the herbicide molecules, preventing them from being carried to the photosynthesising tissues, but this does not fulfil the desired phytoremediation effect.

For phytoremediation, it is interesting that plants absorb the herbicide and use some mechanisms to promote its degradation. If the resistance mechanism of selected plants is related to the minimal absorption of the herbicide, phytoremediation may be compromised. On the other hand, even if the plants are not absorbing the herbicide, green manures can favour the presence of a greater number of microorganisms, which can contribute to the degradation of the pesticide, since the interaction between the soil and roots stimulates the proliferation of the microbial community in the rhizosphere region due to the exudation of amino acid and polysaccharide nutrients of the plant (ARTHUR et al., 2000).

The selected species with higher tolerances to the herbicide sulfentrazone were *C. cajan* and *L. leucocephala*. However, *C cajan*, due to its growth habit (erect shrubs) and annual cycle, as well as rapid growth, becomes more interesting because of the ease of management in systems involving a succession of annual crops. On the other hand, if the goal is the restoration of degraded natural ecosystems, *L. leucocephala* is the species which arouses interest, especially with regard to the indirect effects. This species, despite presenting a slower initial growth, can reach up to 15m height, something which can be a positive feature, as larger plants with greater root coverage have a greater capacity to extract and accumulate contaminants. With this in mind, tree species were studied with regard to their selectivity to sulfentrazone, and *Enterolobium contortisiliquum*, *Ceiba speciosa*, and *Luehea divaricata* presented tolerance to this herbicide (MONQUERO et al., 2011).

Most species studied are annual and have a relatively short life cycle, which is interesting when a phytoremediation program involving crop rotation is considered, as observed with regard to the herbicide *trifloxysulfuron sodium*, with black mucuna and Jack-bean (PROCÓPIO et al., 2007). Notwithstanding their relatively short cycle, these species can produce up to 8 tons of dry matter per hectare, which is another desirable feature for phytoextraction of undesirable compounds in the soil (PIRES et al., 2003b). Moreover, the easy management and low height encourage its use in agriculture, with the exception of leucaena.

In previous studies carried out with the purpose of selecting species for herbicides phytoremediation programs, some green manures also exhibited a better performance. *Canavalia ensiformis* and *Stizolobium aterrimum* were the species that stood out with regard to selectivity to the herbicide tebuthiuron (PIRES et al., 2003a, 2003c). Procópio et al. (2004a) and Santos et al. (2004) also selected *Stizolobium aterrimum* and *Canavalia ensiformis* as the most tolerant species to the herbicide *trifloxysulfuron sodium*. Recent research has shown the tolerance of *E. coracana* to picloram, selected from more than 20 species, and its potential ability to reduce levels of picloram in soil (CARMO et al., 2008). All of these studies showed that species which tolerate herbicides in the soil also had, later on, a satisfactory performance as phytoremediators. Thus, in subsequent steps, the species selected in this study will undergo evaluations on the ability to extract sulfentrazone from the soil at levels which allow the introduction of susceptible crops of economic interest into schemes of succession.

In the environmental conditions in which the experiment was carried out, it can be concluded that among the species evaluated *C. cajan* and *L. leucocephala* showed a higher tolerance to sulfentrazone up to the dose of 400g $ha^{-1}$, showing less phytotoxicity symptoms and higher values for plant height and dry matter accumulation, both in shoots and roots with regard to the control treatment, indicating a potential to be used for further studies on the phytoremediation of sulfentrazone in soil.

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