PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

SBCPD

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Tolerance of Annual Winter Species to Protoporfirinogen Oxidase Inhibiting Herbicides (Protox)

Tolerância de Espécies Anuais de Inverno a Herbicidas Inibidores da Protoporfirinogênio Oxidase (Protox)

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ABSTRACT - The use of plant species for the phytoremediation of soils contaminated with herbicides is an alternative that has been emphasized to minimize the effects of the persistence of agrochemicals in the environment. The objective of this study was to evaluate the tolerance potential of winter species in soils contaminated with sulfentrazone and fomesafen. The experiment was in a completely randomized design with four replications. Doses of 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 during the pre-emergence of phytoremediate species (black oat, vetch, birdsfood trefoil, radish and lupin). Forty five days after the emergence of the species, the phytotoxicity (%), leaf area (cm^2), stalk and/or stem diameter (mm), height (cm) and dry matter (g) variables of the plants were evaluated. Data were submitted to analysis of variance by F test; when significant, linear or non linear regressions were applied to evaluate the effect of herbicide doses on the studied species. Birdsfood trefoil was the less tolerant species to fomesafen and sulfentrazone. Black oat was less affected by the application of fomesafen doses, but it was highly susceptible to sulfentrazone. Radish presented tolerance only up to the fomesafen dose of 0.25 kg ha^{-1}; as for sulfentrazone, the species showed tolerance. The most tolerant species to fomesafen and sulfentrazone, regardless of the dose, was the lupine, which is a possible alternative for the phytoremediation of soils contaminated with these herbicides.

Keywords: sulfentrazone, fomesafen, phytoremediation, *Lupineus albus*.

RESUMO - O emprego de espécies vegetais para fitorremediação de solos contaminados com herbicidas é uma alternativa que vem ganhando destaque para minimizar os efeitos da persistência de agrotóxicos no ambiente. Objetivou-se com este trabalho avaliar o potencial de tolerância de espécies de inverno em solo contaminado com sulfentrazone e fomesafen. O experimento foi montado em delineamento inteiramente casualizado com quatro repetições. Foram aplicadas doses dos herbicidas fomesafen (0,0, 0,125, 0,250 e 0,5 kg ha^{-1}) e de sulfentrazone (0,0, 0,3, 0,6 e 1,2 kg ha^{-1}) em pré-emergência das espécies fitorremediadoras (aveia-preta, cornichão, ervilhaca, nabo e tremoço). Aos 45 dias após a emergência das espécies, foram avaliadas as variáveis fitotoxicidade (%), área foliar (cm^2), diâmetro de colmo e/ou caule (mm), altura (cm) e massa seca (g) das plantas. Os dados foram submetidos à análise de variância pelo teste F; havendo significância, aplicaram-se regressões lineares ou não lineares para avaliar o efeito das doses dos herbicidas sobre as espécies estudadas. O cornichão foi a espécie menos tolerante aos herbicidas fomesafen e sulfentrazone. A aveia-preta foi pouco afetada pelas doses de fomesafen, porém foi altamente suscetível ao sulfentrazone. O nabo

Received: January 19, 2017
Approved: April 23, 2017

Planta Daninha 2018; v36:e018174651

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Doi: 10.1590/S0100-83582018360100089

tolerou o fomesafen apenas até a dose de 0,25 kg ha⁻¹; em relação ao sulfentrazone, a espécie foi tolerante. A espécie mais tolerante ao fomesafen e ao sulfentrazone, independentemente da dose, foi o tremoço, o qual se apresenta como uma possível alternativa para a fitorremediação de solos contaminados com esses herbicidas.

Palavras chave: sulfentrazone, fomesafen, fitorremediação, *Lupineus albus*.

INTRODUCTION

In recent years, Brazil has become the world's largest consumer of agricultural pesticides; herbicides account for about 69% of that total (SINDIVEG, 2016). Due to the great use of herbicides, there is great concern about the contamination of surface and groundwater (Celis et al., 2005). Some herbicides can contaminate the soil, hindering crops in succession to their application, or they may even reach water by leaching. This leads to serious environmental problems and, according to the soil composition, the physicochemical characteristics of the contaminant, the climatic conditions (Santos et al., 2007; Belo et al., 2011; Santos et al., 2013), and the cultural management and practices adopted, may prevent the use of a certain area.

The use of herbicides for grain production becomes fundamental, especially when crops are infested with a great diversity of herbaceous, annual or perennial weeds. Many of these weed species cause considerable losses to the productivity and quality of the harvested product. Among control methods, chemical control becomes an indispensable practice for large-scale agriculture, due to its less dependence on labor, efficiency and speed in weed management, compared to other control methods (Santos et al., 2013).

Among herbicides that inhibit protoporphyrinogen oxidase (PROTOX), used to control weeds in soy, beans, sugarcane and citrus crops, fomesafen and sulfentrazone can lead to carryover problems. Although herbicides belonging to this mechanism of action show high efficacy in controlling weeds, they present physicochemical characteristics that allow them to persist in the environment for a prolonged period. These herbicides are also subject to the leaching of molecules or their metabolites to deeper layers in the soil profile, thus being able to reach the water table (Procópio et al., 2004).

In order to relieve the problems caused by the high persistence of herbicides in the soil, phytoremediation has been used as a decontamination strategy (Santos et al., 2007). This technique consists of the decontamination of soil or water using plants and their associated microbiota (Santos et al., 2007). However, soils contaminated with herbicides present limitations to the selection of remediation plant species, since they are often toxic to them, especially where broad-spectrum herbicides are present. Bioremediation consists in managing over time the degradation of contaminants (herbicides) occurring through natural processes, and has proved viable in cases where there are biogeochemically favorable conditions.

There are only few studies about the phytoremediation of soils contaminated by herbicides, especially in the state of Rio Grande do Sul. Some studies have already demonstrated that the species *Stizolobium aterrimum*, *Canavalia ensiformis*, *Dolichos lablab* and *Eleusine coracana* are demonstrably efficient in the decontamination of areas treated with trifloxysulfuron-sodium, tebuthiuron, picloram and sulfentrazone (Santos et al., 2007; Belo et al., 2011; Madalão et al., 2012), *Vicia sativa* and *Lolium multiflorum* to clean soils contaminated with imazethapyr + imazapic and imazapyr + imazapic (Galon et al., 2014), commercial mixtures used for the weed management of irrigated rice. It is known that the probable mechanism involved in soil decontamination by these species occurs through the interaction of phytostimulation and phytodegradation. Thus, it is necessary to conduct studies that aim at selecting species capable of degrading or immobilizing herbicides in soil and/or plants.

The hypothesis of this work is that the species black oat, vetch, birdsfood trefoil, radish and lupine present the potential to decontaminate soil contaminated with sulfentrazone and fomesafen, which is why the objective was to evaluate the tolerance of winter species cultivated in soil contaminated with sulfentrazone and fomesafen, (PROTOX inhibiting herbicides).

MATERIAL AND METHODS

The experiments were conducted in a greenhouse at the Federal University of Fronteira Sul (UFFS), Campus Erechim - Rio Grande do Sul state. Different species of plants were used to identify which one has potential to tolerate fomesafen and sulfentrazone for later use as phytoremediation in areas contaminated by the herbicides. The used species were: black oat (*Avena sativa*), vetch (*Vicia sativa*), radish (*Raphanus sativus*), birdsfood trefoil (*Lotus corniculatus*) and lupine (*Lupineus albus*). They were selected because they have agricultural importance for the State of Rio Grande do Sul, where they are used as pasture, soil cover or for the production of grains.

Two experiments were installed in a completely randomized design with four replications; the first one was installed with fomesafen and the second one with sulfentrazone, trademark Flex® and Boral 500®, respectively. It is worth mentioning that both herbicides are widely used in weed control for tobacco, soybean, bean, sugarcane and citrus crops. 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 in the pre-emergence of black oat, birdsfood trefoil, vetch, radish and lupine, during the pre-emergence of these winter coverings.

The experimental units were composed of polyethylene planters, base-perforated, with a capacity of 8 dm^3, filled with humic Aluminoferric Red Latosol (Embrapa, 2013) coming from an herbicide free area. Soil fertility correction was performed based on its chemical analysis and in accordance with the technical recommendations for the cultures involved in the experiments (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}; CEC(t) = 7.4 $cmol_c$ dm^{-3}; CEC(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%.

Fifteen seeds per pot were sown, which, after emergence and thinning, resulted in 10 plants per pot. One day after sowing the species, fomesafen and sulfentrazone were applied using a precision backpack sprayer equipped with two TT 110.02 spraying tips, with a spraying volume of 150 L ha^{-1}. Irrigation was controlled daily in the experimental units, maintaining humidity around 80% of the field capacity.

Phitotoxicity (%), plant height (cm), stalk and/or stem diameter (mm), leaf area (cm^2 pot^{-1}) and shoot dry matter (g pot^{-1}) were evaluated 45 days after plant emergence (DAE). In order to evaluate the phytotoxicity of the herbicides, percentage marks were attributed, with zero (0%) for treatments with no injuries and 100 (100%) for the complete death of plants, according to the methodology proposed by SBCPD (1995). Plant height was measured with a ruler graduated in centimeters, and it was measured from ground level to the apex of the last fully expanded leaf. The diameter of stalks and/or stems was determined with the aid of a digital caliper at 5 cm from the ground. The leaf area was measured with a portable leaf area meter, model CI-203 BioScence, on five plants from each treatment. After determining the leaf area, plants were packed in paper bags, placed in a forced air circulation drying oven, at a temperature of 60 ± 5 ^{o}C, until the material reached constant weight, in order to measure the shoot dry matter of the species.

Data were submitted to analysis of variance by F test; when significant, linear and non-linear regressions were adjusted to evaluate the effect of herbicide doses on the studied species. It is worth mentioning that for the tested variables, comparisons were not made among the species, because they naturally present differences. All tests were conducted at 5% probability.

RESULTS AND DISCUSSION

A significant effect of the doses of sulfentrazone and fomesafen on the variables tested for black oat, vetch, radish, birdsfood trefoil and lupine was observed, evaluating the results presented in Figures 1, 2, 3, 4 and 5.

The results obtained for the phytotoxicity of fomesafen, when applied on radish, demonstrated a 26% increase at the highest applied dose, in relation to half the dose, causing the complete death of plants. Vetch plants presented significant differences as for phytotoxicity, increasing the symptoms according to the dose increase, but injuries were not as pronounced, with a maximum of 20%. There was no difference in applying fomesafen on black oat and lupine, with averages of 4 and 10%, respectively (Figure 1A).

Vetch, radish and lupine were the species that showed the smallest symptoms of phytotoxicity, with a linear increase as the doses of sulfentrazone increased (Figure 1B). In a study conducted by Galon et al. (2014), vetch presented the lowest phytotoxicity when it was submitted to the application of imazapic + imazapyr; this resembles the results found in this study in relation to sulfentrazone.

Birdsfood trefoil and black oat had high phytotoxicity values, with the death of the former and injuries near 80% for the latter at the highest dose of sulfentrazone (1.2 kg ha^{-1}). Galon et al. (2014) observed severe phytotoxicity symptoms of imazethapyr + imazapic and imazapyr + imazapic on wild oat and radish when using these species as potential soil phytoremediation agents. Krenchinski et al. (2013), when evaluating the phytotoxicity of two different glyphosate formulations (Zapp QI® - ZQ e Glyphosate 480 Agripec® - GA), observed that four days after the application of ZQ, the species with the highest phytotoxicity was the white oat. Sixteen days after the application of GA, radish presented greater phytotoxicity, differing significantly from ryegrass.

The increase in phyto-intoxication signals occurs in the chloroplasts of plants submitted to herbicides inhibiting the Protox enzyme, which is responsible for the synthesis of chlorophyll and heme group, inhibiting the transformation of protoporphyrinogen into protoporphyrin. Protoporphyrinogen accumulates in the cytoplasm of cells as protoporphyrin. In photostetic cells and with the presence of light and O_2, protoporphyrin helps the formation of singlet oxygen, which acts as a free radical, causing the lipid peroxidation of cell membranes, and leading to chlorosis and cell death (Lee et al., 1993; Dayan and Weete, 1996; Hulting et al., 2001).

Results showed that radish presented a significant decrease in plant height with increasing fomesafen doses; most plants died (Figure 2A). Black oat, vetch, birdsfood trefoil and lupine did not show significant differences in growth in relation to the control treatment without herbicide (0 g ha^{-1}), with mean values of 36.11, 16.61, 6.09, and 27.41 cm, respectively.

The increase in the sulfentrazone dose caused a shorter height in birdsfood trefoil plants; the crop died when 1.2 kg ha^{-1} of the herbicide were applied, that is, at the highest applied dose. For black oat, vetch, radish and lupine, there was no significant difference in plant height with increasing doses of sulfentrazone (Figure 2B). Madalão et al. (2013), when evaluating plant of *Calopogonium mucunoides*, sorghum hybrids (*Sorghum bicolor* x *S. sudanense*), *Crotalaria breviflora*, *C. juncea*, *Canavalia ensiformis*, *Dolichos lablab*, *Stizolobium deeringianum* and *S. aterrimum* 60 days

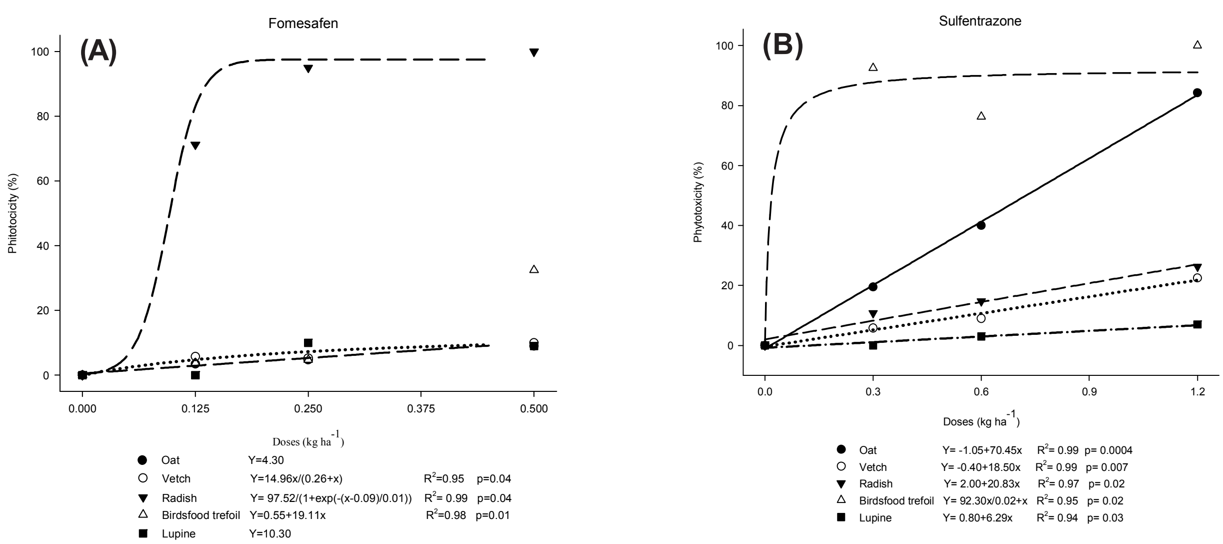

Figure 1 **-** Phytotoxicity (%) of herbicides to black oat, vetch, radish, birdsfood trefoil and lupine species, 45 days after emergence, according to the application of fomesafen (A) and sulfentrazone (B).

after emergence, observed a reduction in plant height with increasing doses of sulfentrazone. In this same study, *C. ensiformis* showed a lower reduction of height, and *S. deeringianum* and *S. aterrimum*, the highest reductions.

Results about stalk and/or stem diameter showed that there was a significant decrease when fomesafen was applied to the radish crop, with a 38% reduction in the 0.25 kg ha^{-1} dose compared to the zero dose (Figure 3A). Black oat, vetch, birdsfood trefoil and lupine did not show significant differences when submitted to increasing doses of fomesafen. As for sulfentrazone, birdsfood trefoil presented a smaller diameter with the increase of the applied doses. Black oat, lupine, vetch and radish did not present significant differences with the application of sulfentrazone doses (Figure 3B). Simplício et al. (2016), when evaluating the effects of quizalofop-p-ethyl and linuron doses, did not observe differences in the stem size of cowpea, since it was not affected by the doses of the applied herbicides.

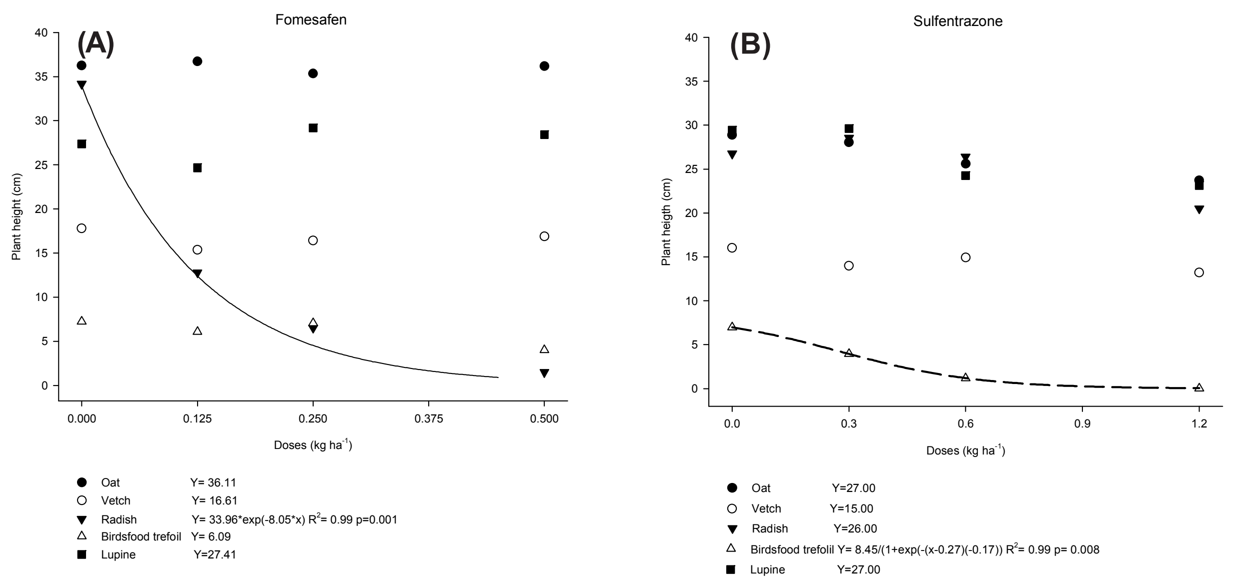

Figure 2 **-** Plant height (cm) of black oat, vetch, radish, birdsfood trefoil and lupine at 45 days after emergence, according to the application of doses of fomesafen (A) and sulfentrazone (B).

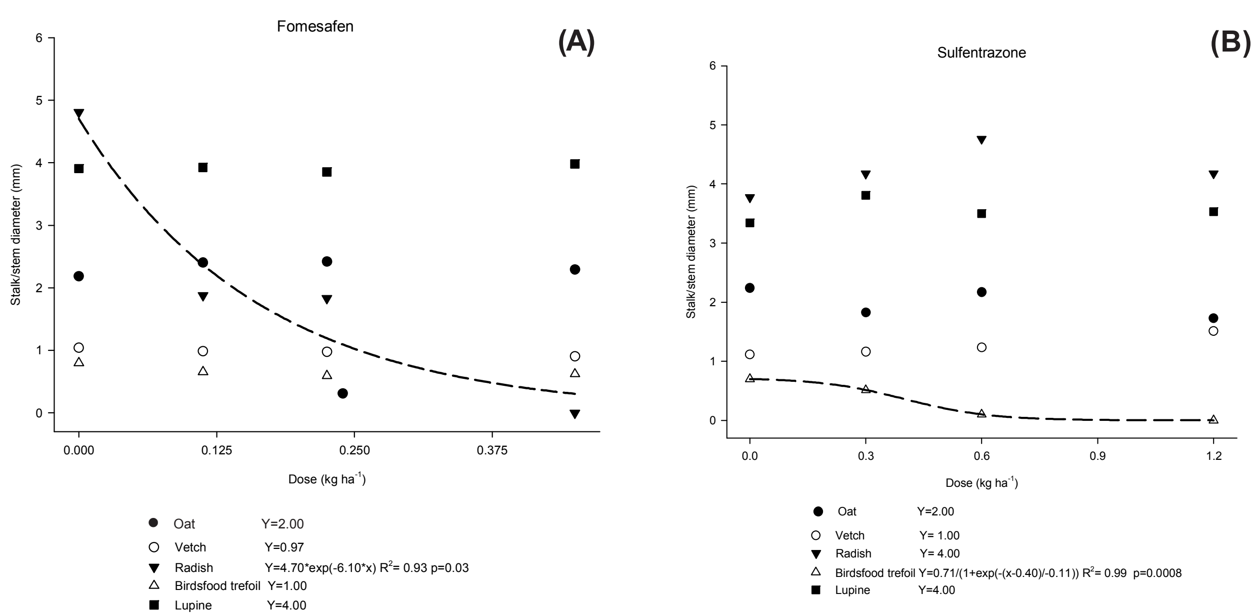

Figure 3 **-** Diameter of stalks and/or stems (mm) of black oat, vetch, radish, birdsfood trefoil and lupine species, 45 days after emergence, according to the application of fomesafen (A) and sulfentrazone (B).

It was observed that there were no significant differences between the 0 dose and the application of the highest dose of fomesafen to the leaf area of black oat and lupine. Vetch presented a 7% increase when 0.5 kg ha^{-1} of fomesafen were applied, compared to the control treatment (0 kg ha^{-1}) (Figure 4A). Birdsfood trefoil did not present leaf area values, that is, the death of all plants occurred with the use of fomesafen. Radish showed a significant decrease in its leaf area with increasing doses of fomesafen; at the dose of 0.5 kg ha^{-1}, plants died. As for sulfentrazone, vetch had a 285 decrease in its leaf area with increasing herbicide doses (Figure 4B). The opposite was observed by Belarmino et al. (2012), when working with *Lolium multiflorum*, *Lotus corniculatus*, *Trifolium repens*, *Festuca arudinacea*, *Vicia sativa*, and *Brassica napus* exposed to doses of imazapic + imazapyr, where there was greater leaf area development in the species that presented lower phytotoxicity. Linhares et al. (2014), when evaluating the effect of fomesafen on the leaf area of cowpea, observed severe phytotoxicity caused by this herbicide, resulting in a reduction of the leaf area 10 days after emergence. However, with the emission of new leaves, the accumulation of leaf area occurred around 19 days after emergence, with a similar behavior to the treatment without the application of herbicides, with a delay of approximately seven days.

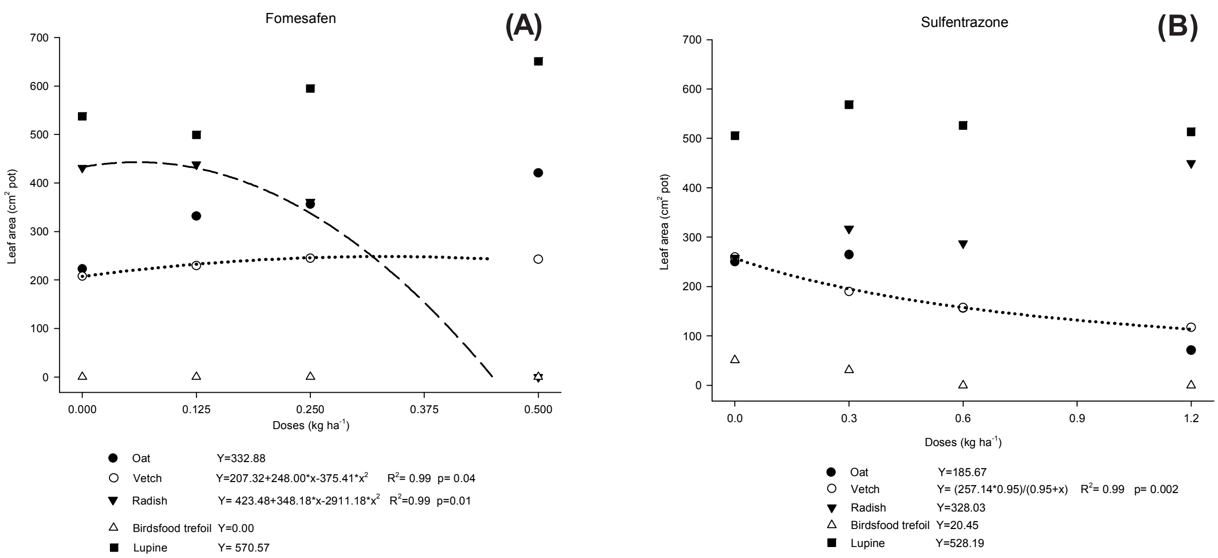

Figure 4 - Leaf area (cm² pot^{-1}) of the species black oat, vetch, radish, birdsfood trefoil and lupine, 45 days after emergence, according to the application of fomesafen (A) and sulfentrazone (B).

These results demonstrate that the leaf area is correlated with the phytotoxicity of plants, since as the injuries caused by the herbicide increased, there was a decrease in the leaf area. When testing compaction levels and the presence of sulfentrazone in the soil for millet plants, Zobiole et al. (2007) observed that the leaf area was affected by the interaction between soil compaction levels with the presence or absence of the herbicide.

Black oat, vetch, radish, birdsfood trefoil and lupine did not show significant dry matter differences when submitted to doses of fomesafen. As for the shoot dry matter of the plants from all the winter covers tested, there was no data adjustment to the statistical models, except for black oat, when sulfentrazone was applied (Figure 5A and B). There was a significant reduction in the dry matter accumulation of black oat when submitted to sulphentrazone application, by 27% and 55% with doses of 0.6 and 1.2 kg ha^{-1}, respectively, in relation to plants without herbicide application (Figure 5B). Ferraço et al. (2017) observed that, as sulphentrazone doses increased, the dry matter in millet plants decreased, similarly to what happens in this study with black oat, possibly because they belonged to the same family (Poaceae), thus demonstrating low selectivity to the herbicide. Madalão et al. (2012) also report the reduction of dry matter accumulation as a result of increased phytotoxicity on *Pennisetum glaucum* in soil contaminated with sulfentrazone. The decrease in height, leaf area and stem diameter of the species is closely related to herbicide phytotoxicity, demonstrating the tolerability of each species to fomesafen and sulfentrazone.

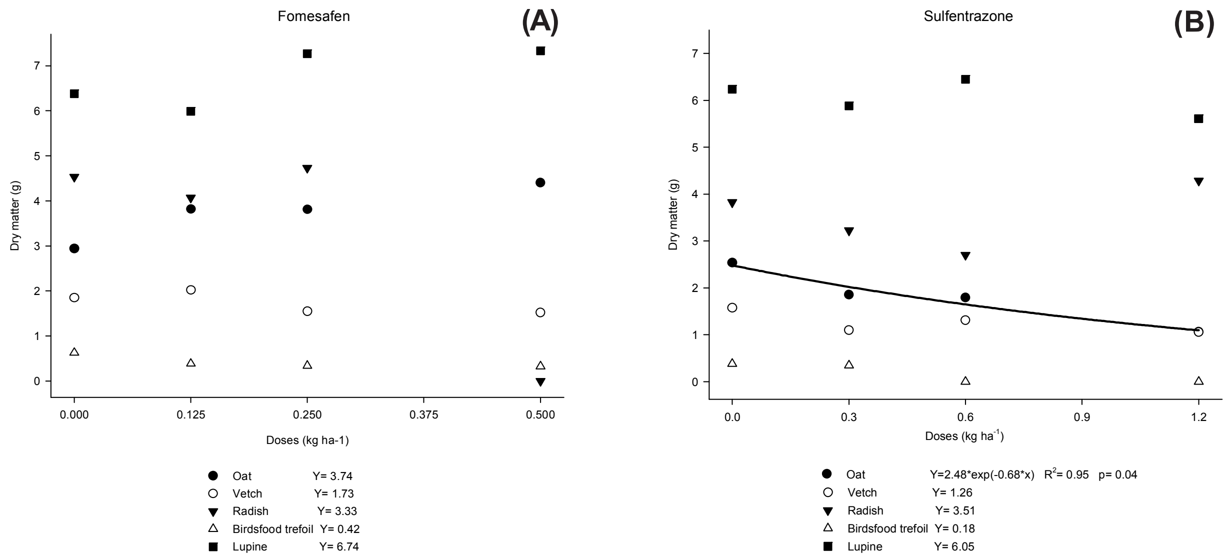

Figure 5 **-** Shoot dry matter (g pot^{-1}) of black oat, vetch, radish, birdsfood trefoil and lupine species, 45 days after emergence, according to the application of fomesafen (A) and sulfentrazone (B).

The evaluated species present differences as for their tolerance to fomesafen and sulfentrazone, and also to the applied dose of these herbicides in pre-emergence. Birdsfood trefoil was less tolerant to the herbicides and, therefore, does not present potential for the phytoremediation of soils treated with sulfentrazone and fomesafen. Vetch and black oat were at intermediate levels of herbicide tolerance. Radish presented tolerance only up to the 0.25 g ha^{-1} dose of fomesafen; however, for sulfentrazone, the species showed tolerance at all tested doses. The most tolerant species to both herbicides was lupine, which may be indicated for later studies about phytoremediation in areas that received the application of fomesafen and sulfentrazone.

ACKNOWLEDGMENTS

CNPq, FAPERGS and FINEp, for granting the financial aid and for the awarded scholarships.

REFERENCES

Belarmino J.G. et al. Seleção de espécies vegetais para fitorremediar solo contaminado com imazapic + imazapyr. In: Anais do 28º Congresso Brasileiro da Ciência das Plantas Daninhas. Campo Grande: CSBCPD, 2012. p.158-62.

Belo A.F. et al. Potencial de espécies vegetais na remediação de solo contaminado com sulfentrazone. **Planta Daninha**. 2011;29:821-8.

Celis R. et al. Sorption and leaching behaviour of polar aromatic acids in agricultural soils by batch and column leaching tests. **Eur J Soil Sci.** 2005;56:287-97.

Dayan F.E., Weete J.D. Mechanism of tolerance to a novel phenyl triazolinone herbicide. **Plant Physiol.** 1996:111-9.

Empresa Brasileira de Pesquisa Agropecuária – Embrapa. **Sistema Brasileiro de Classificação de Solos**. 3ª.ed. Rio de Janeiro: Embrapa Solos, 2013. 353p.

Ferraço M. et al. Efeito da densidade populacional de *Canavalia ensiformis* na fitorremediação de solo contaminado com sulfentrazone**. Rev Ci Agron.** 2017;48:32-40.

Galon L. et al. Potential of plant species for bioremediation of soils applied with imidazolinone herbicides. **Planta Daninha**. 2014;32:719-26.

Hulting A.G. et al. Soybean (*Glycine max* (L.) Merr.) cultivars tolerance to sulfentrazone. **Crop Prot.** 2001;20:679-83.

Lee H.J., Duke S.O., Duke M.V. Cellular localization of protoporphyrinogen-oxidizing activities of etiolated barley (*Hordeum vulgare* L.) leaves. **Plant Physiol.** 1993;102:881-9.

Linhares C.M.S. et al. Crescimento do feijão-caupi sob efeito dos herbicidas fomesafen e bentazon+imazamox**. Rev Caatinga**. 2014;27:41-9.

Krenchinski F.H. et al. Dessecação de espécies de cobertura do solo com formulações de glyphosate. **Rev Bras Herb.** 2013;12:104-11.

Madalão J.C et al. Susceptibilidade de espécies de plantas com potencial de fitorremediação do herbicida sulfentrazone**. Rev Ceres**. 2013;60:111-21.

Madalão J.C. et al. Uso de leguminosas na fitorremediação de solo contaminado com sulfentrazone. **Pesq Agropec Trop.** 2012;12:390-6.

Procópio S.O. et al. Seleção de plantas com potencial para fitorremediação de solos contaminados com o herbicida trifloxysulfuron sodium. **Planta Daninha**. 2004;22:315-22.

Rede Oficial de Laboratórios de Análise de Solo e de Tecido Vegetal – ROLAS. **Manual de adubação e calagem estados do Rio Grande do Sul e Santa Catarina**. 10ª.ed. Porto Alegre: Sociedade Brasileira de Ciência do Solo, 2004.

Santos D.P. et al. Determinação de espécies bioindicadoras de resíduos de herbicidas auxínicos. **Rev Ceres**. 2013;60:354-62.

Santos E.A. et al. Fitoestimulação por *Stizolobium aterrimum* como processo de remediação de solo contaminado com trifloxysulfuron-sodium. **Planta Daninha**. 2007;25:259-65.

Sociedade Brasileira da Ciência das Plantas Daninhas – SBCPD. **Procedimentos para instalação, avaliação e análise de experimentos com herbicidas**. Londrina: 1995. 42p.

Simplício S.F. et al. Características de crescimento e produção do feijão-caupi (*Vigna unguiculata* L. Walp.) sob aplicação de herbicidas. **Rev AGROTEC.** 2016;37:55-62.

Sindicato Nacional da Indústria de Produtos para Defesa Vegetal – SINDIVEG. **Importações de defensivos agrícolas têm aumento no primeiro semestre de 2016**. [acessado em: 01 de set. 2016. Disponível em: http://sindiveg.org.br/importacoes-de-defensivos-agricolas-tem-aumento..

Zobiole L.H.S. et al. Efeito da compactação do solo e do sulfentrazone sobre a cultura da soja em duas condições de água no solo. **Planta Daninha**. 2007;25;3:537-45.