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Effect of growing *Brachiria brizantha* on phytoremediation of picloram under different pH environments

Renan Rodrigues Braga[a], José Barbosa dos Santos[b], José Cola Zanuncio[c,*], Camila Silva Bibiano[d], Evander Alves Ferreira[b], Maxwel Coura Oliveira[e], Daniel Valadão Silva[g], José Eduardo Serrão[f]

[a] Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570-900 Viçosa, Minas Gerais, Brazil
[b] Departamento de Agronomia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39100-000 Diamantina, Minas Gerais, Brazil
[c] Departamento de Entomologia/BIOAGRO, Universidade Federal de Viçosa, 36570-000 Viçosa, Minas Gerais, Brazil
[d] Departamento de Fitotecnia, Universidade Federal de Lavras, 37200-000 Lavras, Minas Gerais, Brazil
[e] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, 68503 Lincoln, NE, United States of America
[f] Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-900 Viçosa, Minas Gerais, Brazil
[g] Departamento de Ciências Vegetais, Universidade Federal Rural do Semi-Árido, 59625-574 Mossoró, Rio Grande do Norte, Brazil

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A B S T R A C T

Picloram is used in the management of *Brachiaria brizantha* (Poaceae) in pasture. The persistence of this herbicide varies with climate and soil characteristics, such as texture and pH. The aim was to determine the capacity of *B. brizantha* (Hochst.) Stapf. cv. Marandu to remediate soils of different pH levels contaminated with picloram. The experiment had a randomized block design with four replications. The remedial species was grown for 60 days after cucumber [*Cucumis sativus* L. (Cucurbitaceae)] was cultivated as an indicator of the presence of the herbicide in the soil. This plant reduced the picloram concentration in the soil layer surface, which can be attributed to its ability to degrade, to absorb, and/or to exude herbicides. The picloram has greater leaching potential in higher pH soils, in the absence of *B. brizantha*. Soils with lower pH tend to have higher sorption and concentration of this herbicide in the intermediate layers. *B. brizantha* can remediate soils contaminated with picloram and reduce leaching, which is higher in soils treated with limestone.

1. Introduction

Hormone herbicides have been used mainly for weed control in pasture, but they have longer periods and persistence in the environment (Santos et al., 2006; Seefeldt et al., 2014).

The main herbicides for weed control in pastures contain 2.4-D or picloram (MAPA, 2015). The latter one has longer residual activity period in soils that can reduce the cultivation in short and medium-terms (Santos et al., 2006). This molecule has a high retention in the soil and persistence in the environment (Santos et al., 2006; Seefeldt and Conn, 2011). Furthermore, it can have low sorption (Point-Brown et al., 2014), and its solubility in water facilitates reaching underground aquifers depending on soil characteristics, such as pH (Fast et al., 2010).

The excavation, incineration, solvent extraction, or oxidation–reduction can recover contaminated areas by herbicides, but they are costly methods and difficult to use (Oliveira et al., 2014). *In situ* methods, including phytoremediation is a strategy for the removal of toxic compounds from the environment using plants. This technique is recommended because it is economically viable and provides lower environmental impacts (Ali et al., 2013).

The objective of this study was two-fold: In soil contaminated to picloram, (1) determine the *Brachiaria brizantha* potential to prevent herbicide leaching and (2) evaluate the potential phytoremediator of *B. brizantha* for different soil pH and depth.

2. Material and methods

2.1. Experimental approach

Three assays were carried out in a greenhouse of the "Departmento de Pós-Graduação em Produção Vegetal" of the "Uni-

* Corresponding author.
E-mail addresses: granderenan@gmail.com (R.R. Braga), jbarbosasantos@yahoo.com.br (J.B. dos Santos), zanuncio@ufv.br, zanuncio@pq.cnpq.br (J.C. Zanuncio), camila.bibiano@yahoo.com.br (C.S. Bibiano), evanderalves@yahoo.com.br (E.A. Ferreira), maxwelco@gmail.com (M.C. Oliveira), danielvaladaos@yahoo.com.br (D.V. Silva), jeserrao@ufv.br (J.E. Serrão).

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versidade Federal dos Vales do Jequitinhonha e Mucuri" (UFVJM) in Diamantina, Minas Gerais State, Brazil. Red latosol (oxisol) samples from Diamantina, Minas Gerais State, Brazil, characterized as sandy clay loam with 23, 12 and 65% of clay, silt and sand, respectively, were used. Chemical analysis showed pH (in water) of 4.5; organic matter content of 8.7 dag kg^{-1}; P, K, Ca, Mg, Al, H + Al (potential acidity) and $CTC_{effective}$ of 3.0 and 39.0 mg dm^{-3}; 1.1, 0.3, 1.4, 15.7 and 2.9 cmolc dm^{-3}, respectively. The soil was not corrected in the first assay, but the equivalent to 2.6 and 5.2 t ha^{-1} of dolomitic limestone (PRNT = 80%) was applied in the second and third assays. The soil samples were moistened and incubated for 40 days for reaction with the corrective.

The substrates were fertilized with 2.7 g dm^{-3} formulated at 4-14-8 (N-P_2O_5-K_2O) and 100 mg dm^{-3} of urea 15 days after plant emergence. Watering was done daily to keep the soil moisture with 80% field capacity.

The container used for cultivation composed of four 100 mm high and 150 mm diameter rings each of polyvinyl chloride (PVC) tube internally lined with paraffin to reduce water seepage through the walls of the columns. These rings were mounted with the base closed by gauze and filter paper and supported on plates to prevent water and herbicide losses. After preparation, the columns were wetted, and the herbicide was applied with a sprayer with constant pressure coupled to a bar with nozzle-type range TT11002 with 3 bar pressure and volume sprayed of 150 L ha^{-1}.

2.2. Estimated phytoremediation

The assays were divided into two stages: in the first, the bioremediation was tested and in the second, the concentration of picloram was evaluated with a plant bioindicator. The experimental design was a randomized block with four replications. The treatments were arranged in a split plot design in 2 × 2 × 4 scheme; the first level with *B. brizantha* or not, the second with the herbicide doses (120 and 240 g ha^{-1} of picloram in Padron® formulation), and the third with the depths evaluated (0–10, 10–20, 20–30 and 30–40 cm).

Brachiaria brizantha (Hochst. ex. A. Rich) Stapf. belongs to the Poaceae family and it is native to the African savanna. *Brachiaria* is commonly used as forage grass in tropical rangelands worldwide. In addition, it is generally used as phytoremediator of contaminated soils. Six seeds of *B. brizantha* were sown per column 1 day after the herbicide application, later thinned and two plants left per plot. These plants were grown until the beginning of flowering at 60 days after their emergence. The rings were removed from the column when the soil layers and the roots were removed individually and the soil homogenized.

A total of 80 g of soil from each plot was weighed and placed in plastic cups to estimate the herbicide concentration in the soil. Three seeds of the plant bioindicator, *Cucumis sativus* L. (Cucurbitaceae) were placed per sample. The poisoning of the plants was evaluated at 30 days after sowing by Visual Intoxication Index. The degree of intoxication was set on a scale in percentage with 0% representing no injury and 100% plant death (EWRC, 1964).

2.3. Dose-response curve

Three dose-response curves were plotted per soil pH, estimating the relationship between the concentration of the herbicide in the soil and the effects on the plant bioindicator. Ten herbicide doses corresponding to 0, 0.5, 0.9, 1.9, 3.8, 7.5, 15.0, 30.0, 60.0 and 120.0 g ha^{-1} of picloram were applied. The design was a randomized block with four replications and evaluated as in the bioindicator test.

2.4. Statistical analyses

The variables were subjected to analysis of variance (ANOVA) when the significant interactions were deployed and the means compared by Tukey's test at 5% significance level. The depth factor data and the dose-response curve were subjected to linear and non-linear regression analysis ($p < 0.05$). Adjusted equations were compared by the model identity test to verify the null hypothesis. The models were grouped and represented by the same trend line in case of no differences (Regazzi and Silva, 1999).

3. Results

The triple interaction was significant only for soil with pH 4.5 and 5.6. *B. brizantha* reduced the concentration of the herbicide picloram at the surface layer of soil with pH 4.5 and increased it between 20 and 30 cm deep (Table 1).

The herbicide concentration was lower in the superficial and the deepest soil layers with the highest herbicide dose with remedial species cultivated in soil with pH 5.6 (Table 1).

The increase in picloram doses increased the cucumber plant's intoxication in soil with pH 4.5 from all depths with *B. brizantha* (Table 1). Plant poisoning increased in soils from 0 to 10 cm depth without previous cultivation of *B. brizantha*.

The intoxication of cucumber plants was higher with increasing picloram doses in the superficial soil layer with pH 5.6 with or without previous cultivation of *B. brizantha* and in those of greater depth without this plant (Table 1).

The regression for herbicide analysis throughout the soil depth is suited to a second degree with upward concavity (Fig. 1). The slope of the curve increased in soil with the highest herbicide doses without plant remedial, independently of the pH.

The value of "*b*" was lower in the equation with the lowest dose of the herbicide and with *B. brizantha* in soil with pH 5.6 (Fig. 1).

Table 1
Plant poisoning (%) of cucumber, *Cucumis sativus* (Cucurbitaceae) grown in soil pH 4.5 and 5.6 taken at different depths after cultivation (yes) or not (not) with *Brachiaria brizantha* (Poaceae) under two picloram doses.

pH	Picloram (g i.a. ha^{-1})	Depth (cm)							
		0–10		10–20		20–30		30–40	
		yes	not	yes	not	yes	not	yes	not
4.5	120	12.1Bb	16.3Ab	7.9Ab	10.2Aa	5.8Ab	9.5Aa	15.4Ab	14.0Aa
	240	23.2Ba	29.4Aa	15.2Aa	13.6Aa	20.7Aa	11.7Ba	19.9Aa	17.7Aa
	Coefficient of variation: CV[1] = 16.78, CV[2] = 28.67, CV[3] = 29.12								
5.6	120	25.6Bb	42.9Ab	6.3Aa	6.6Aa	5.8Aa	6.0Aa	13.3Aa	10.6Ab
	140	46.0Ba	51.9Aa	4.8Aa	6.8Aa	6.5Aa	5.8Aa	8.6Bb	16.8Aa
	Coefficient of variation: CV[1] = 23.87, CV[2] = 24.82, CV[3] = 19.91								

Means followed by the same uppercase per line or lowercase letters per column do not differ by F test at 5% significance. [1/]presence or absence of *B. brizantha*; [2/]picloram dose; [3/]depths.

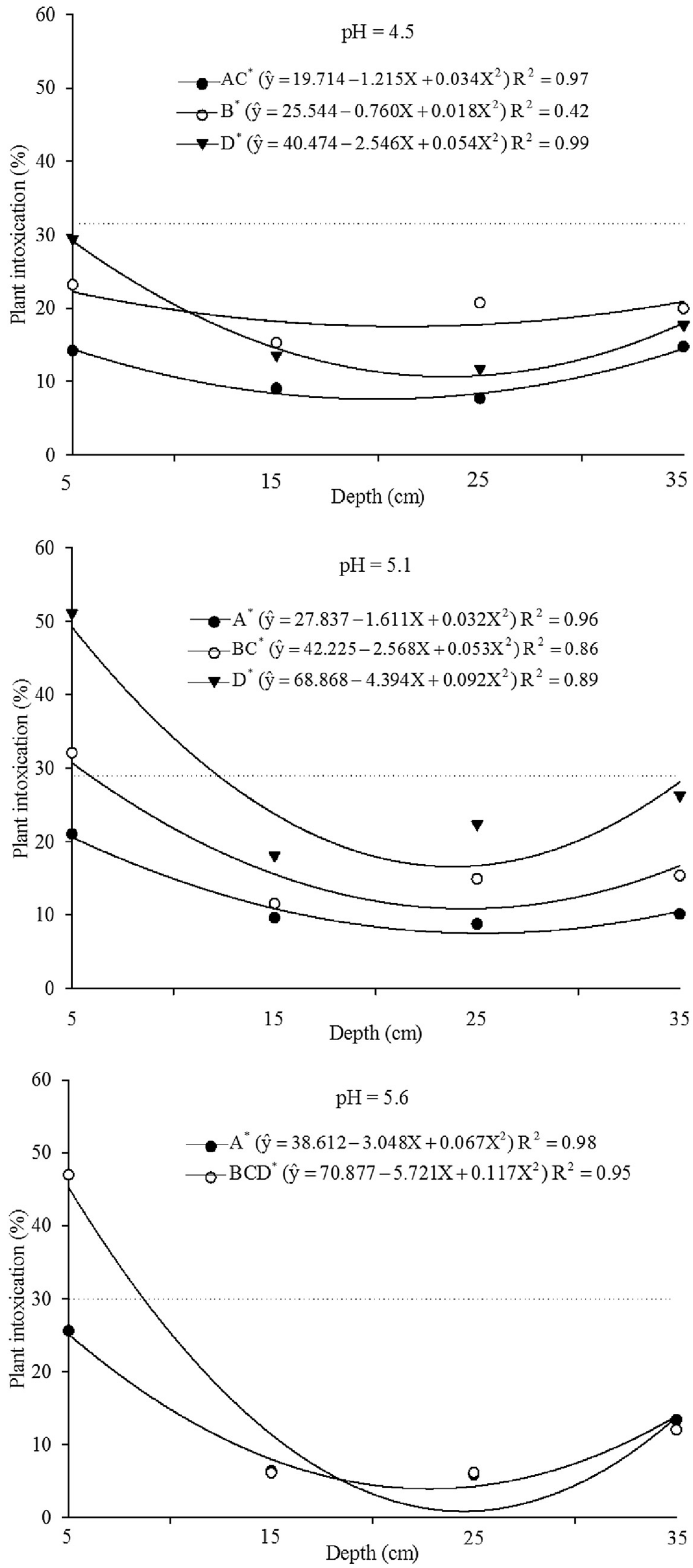

Fig. 1. Poisoning of cucumber, *Cucumis sativus* (Cucurbitaceae), plants on soils with pH 4.5, 5.1, and 5.6 from different depth. A-Soil with *Brachiaria brizantha* (Poaceae) and application of 120 g ha^{-1} of picloram; B-Soil with *B. brizantha* and application of 240 g ha^{-1} of picloram; C-Soil without *B. brizantha* and application of 120 g ha^{-1} of picloram; D-Soil without *B. brizantha* and application of 240 g ha^{-1} of picloram. *Significant at 5%.

The lower picloram doses, 0.5, 0.9, 1.9, and 3.8 g ha^{-1} poisoned cucumber plants as shown by the dose-response curve (Fig. 2). The intoxication of this plant increased in soils with higher pH and tended to stabilize faster with increasing herbicide doses.

4. Discussion

The lowest concentration of the picloram in the upper soil layers with pH 4.5 with *B. brizantha* can be due to the ability of this plant to phytodegrade or phytostimulate the picloram degradation (Adki et al., 2014) by metabolizing or stimulating soil microbiota due to root exudate release (Spohn et al., 2013). However, part of the herbicide absorbed by the plant can be exudated (Brunetti et al., 2011) at deeper soil layers, which would explain its highest concentration at 20–30 cm deep. This plant can absorb the herbicide and degrade, vaporize, combine it into less toxic molecules, and/or accumulate it in its tissues, such as the vacuoles of meristematic tissues (Susarla et al., 2012). This latter situation is important because of the high stability of the picloram in the plant (Gibbs and Sterling, 2004).

The reduction in the concentration of the picloram in the upper soil layers with pH level 5.6 is due to the ability of *B. brizantha* to remediate this product, as shown for grasses as *Eleusine coracana* Gaertn. (Poaceae) (Assis et al., 2010a; Silva et al., 2012) and *Panicum maximum* L. (Poaceae) (Assis et al., 2010b). Moreover, this plant can remediate soil at greater depths by increasing degradation of herbicide molecules (Lojková et al., 2014), besides microorganism's presence which are the main responsible for pesticide degradation (Olchanheski et al., 2014; Rodriguez-Campos et al., 2014). However, the action of microorganisms to degrade this compound depends on its availability in the soil, common in those with higher pH levels (Maciel et al., 2013).

The high-leaching potential of picloram in soil with pH 5.6, even at the lowest dose and without *B. brizantha* or with the highest dose with this plant, shows that this herbicide leaching depends on soil pH. This is due to the fact that around 95–100% of picloram molecules remain in the anionic state at pH above 5.0 (Cheung and Biggar, 1974) thereby reducing the adsorption of its molecules by clay and organic matter particles and leaving it in the soil solution and thus more susceptible to leaching. This helps in understanding the dynamics of the herbicide and agrees with the fact that liming in pasture with picloram applications can increase leaching of this herbicide, as reported in different soil and climatic regions, soil pH of 6.3 and 7.3 in Europe (Celis et al., 2005).

The higher intoxication of cucumber plants with increasing herbicide doses is expected due to changes in the plasticity of the cellular walls and increasing production of proteins and ethylene, which causes uncontrolled growth, interruption of flow sap through the xylem, leaf withered, and eventually, plant death (Song, 2014). In addition, cucumber plant has been reported as highly sensitive to picloram residues in soils (Assis et al., 2010a,b). The similar plant intoxication with different picloram doses without *B. brizantha* can be attributed to the non-distribution of this herbicide into the deeper soil layers and the ability of this plant to absorb and exudate herbicides throughout the soil layers (Sterling and Hall, 1997) as reported for glyphosate by *Brachiaria decumbens* Stapf. (Poaceae) (Tuffi Santos et al., 2008).

The highest plant intoxication with the highest picloram dose in soils with pH 5.6 at distal layers of the columns is justified by its higher concentration. The absence of differences in the other soil layers can be due to low herbicide doses because the accuracy of *B. brizantha* as a bioindicator is low as observed in the dose-response curve for this work and also on a study evaluating the contamination of Yangtze river by hormonal herbicides in Chongqing City, China (Zhang et al., 2014).

The highest quantity of the picloram at the first and last soil layers is due to the mobility of this herbicide and to physical limitations of the container used, respectively. The last layer represents the leaching potential of picloram beyond the 30 cm depth and not its concentration. Larger values of "*b*" without plant remediation with the highest picloram doses indicate that part of this herbicide accumulates in the superficial soil depth (0–10 cm) and higher leaching occurs in layers deeper than 30 cm. This may indicate the potential of contamination of the water table, and that *B. brizantha* act as a hydraulic barrier, creating an upward water flow and reducing the picloram leaching. This plant stands out for its high density of root system with effective depth up to 50 cm (Cunha et al., 2010).

Intoxication of cucumber, presented in the dose-response curve with the lowest picloram doses, shows the high sensitivity of this plant to the herbicide as reported with the hormone herbicide 2.4-D (Nascimento and Yamashita, 2009). The higher plant intoxication

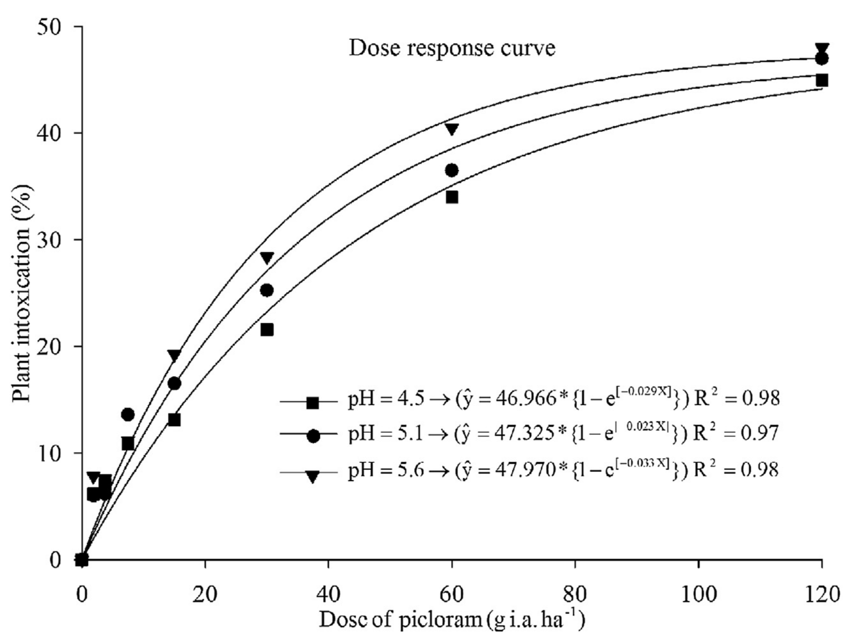

Fig. 2. Dose-response curve for the cucumber, *Cucumis sativus* (Cucurbitaceae) assay.

with increasing soil pH level is due to the fact that picloram behaves as a weak acid, when its molecular form donates protons to produce negative ions with increasing soil pH level (Belo et al., 2011). This results in lower sorption of its molecules by colloids (D'Antonino et al., 2009) and higher concentrations of herbicide in the soil solution.

5. Conclusions

Brachiaria brizantha can remediate contaminated soils with picloram. The cultivation of *B. brizantha* reduces picloram leaching. Soils with pH levels near neutrality tend to have higher picloram losses by leaching.

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