Leaching potential of the herbicide mixture 2,4-D + picloram

Potencial de lixiviação da mistura dos herbicidas 2,4-D + picloram

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Abstract

Weed control strategies are fundamental to ensure pasture productivity and quality in Brazil. However, care must be taken in the use of long residual herbicides, which can increase the risk of leaching. From this context, the present study evaluates the leaching potential of the commercial mixture of herbicides 2,4-D + picloram. Leaching columns filled with loam-clay-sandy soil were used. The columns were 0.6 m in length and 0.2 m in diameter. The experimental design was randomized blocks with eight replications. Treatments were arranged in a 5x2 factorial scheme, in which factor A corresponds to herbicide doses (0, 1, 2, 3 and, 4 L ha^{-1}) and factor B corresponds to the position of herbicides in the columns (0-30 cm and 30-60 cm depth). After herbicide application, weekly rainfall of 60 mm was simulated for four weeks at the top of the columns. The columns were sectioned vertically giving rise to two section, which were separated into two portions (0-30 cm and 30-60 cm). Then, cucumber was sown as a bioindicator in the 0-30 cm and 30-60 cm layers. At 45 days after sowing, the following parameters were determined: chlorophyll *a* fluorescence transient, shoot length, root length, and total dry weight of plants. Both the chlorophyll fluorescence data and the biometric data of the bioindicator plant showed the presence of the commercial mixture only in the first 30 cm of depth. It is concluded that 2,4-D + picloram remained in the 0-30 cm deep layer of the loam-clay-sandy soil after 240 mm rainfall.

Additional keywords: auxin mimics; bioindicators; carryover*; Cucumis sativus.*

Resumo

Para assegurar a produtividade e a qualidade das pastagens no Brasil é fundamental a adoção de estratégias de controle da comunidade de plantas daninhas. No entanto, cuidados devem ser tomados quanto ao uso de herbicidas com longo período residual, que pode aumentar o risco de lixiviação. A partir deste contexto, o objetivo deste trabalho foi avaliar o potencial de lixiviação da mistura comercial dos herbicidas 2,4-D+picloram. Foram utilizadas colunas de lixiviação, com 0,6 m de comprimento e 0,2 m de diâmetro, preenchidas com solo de textura franco-argilo-arenosa. O delineamento experimental foi em blocos casualizados, com 8 repetições. Os tratamentos foram dispostos em esquema fatorial 5x2, sendo o fator A as doses dos herbicidas (0, 1, 2, 3 e 4 L ha^{-1}) e o fator B as posições na coluna (0-30 cm e 30-60 cm de profundidade). Após a aplicação do herbicida, simulou-se a precipitação de 60 mm semanais, durante quatro semanas, no topo das colunas. As colunas foram seccionadas verticalmente originando duas canaleta, as quais foram separadas em duas porções, semeando-se pepino como bioindicadora nas camadas de 0-30 cm e 30-60 cm. Determinou-se parâmetros de transiente da fluorescência da clorofila *a*, comprimento da parte aérea, comprimento das raízes e massa seca total das plantas 45 dias após a semeadura. Tanto os dados de fluorescência da clorofila *a* quanto os dados biométricos da planta bioindicadora evidenciaram a presença da mistura comercial apenas nos primeiros 30 cm de profundidade. Conclui-se que o 2,4-D+picloram permaneceu na camada de 0-30 cm de profundidade do solo franco-argilo-arenoso após 240 mm de precipitação.

Palavras-chave adicionais: bioindicadoras; *carryover; Cucumis sativus*; mimetizadores de auxina.

Introduction

Livestock is one of the most important activities in Brazilian agribusiness, influencing gross domestic product (GDP) and job generation. However, a large part of the pastures in the country are in some stage of degradation, with weed infestations being commonly noticed, which results in low quality products and yield loss in these areas (Pellegrini et al., 2010; Mendes et al., 2016).

There are different management possibilities to minimize weed interference in pasture areas. Chemical management is highlighted in this context due to the ease of use of its main tool, herbicides. Among the herbicides registered for pasture, the 2,4-D + picloram mixture is one of the most used. These two molecules have the mechanism of action of auxin mimics (Franco et al., 2014; Santos et al., 2015).

Physicochemical characteristics of picloram include a 2.3 pKa (electrolytic dissociation constant). Therefore, this herbicide predominates in its dissociated form, which favors low sorption in most Brazilian soils (Senseman, 2007). Moreover, it is very soluble in water, which increases its leaching potential (Chen et al., 2010; Maciel et al., 2013; Marco-Brown et al., 2014). This may cause groundwater contamination and prevent rotation of crops sensitive to synthetic auxins.

Regarding environmental dynamics, despite being potentially mobile in the soil, 2,4-D presents rapid degradation, mainly by microorganisms, which minimizes its leaching. The half-life of 2,4-D is approximately 10 days (Senseman, 2007; Peres-Oliveira et al., 2017), but drift problems are commonly associated with this herbicide.

Molecules that have long persistence in the soil, such as picloram, can lead to a phenomenon called carryover, in which its residues, in addition to affecting sensitive successive crops, also increase the risks of leaching and groundwater contamination due to the permanence of the product in the soil for a longer period of time than desired (Franco et al., 2015).

Bioassays with indicator plants are used to identify the activity of herbicidal molecules in the soil (Peres-Oliveira et al., 2017). Cucumber (*Cucumis*

sativus L.) is a bioindicator of contamination caused by auxinic herbicides, and shows easy cultivation, fast growth, and high sensitivity to products from this group (Santos et al., 2015). Symptoms of intoxication such as epinasty, shortening of internerve tissue, growth arrest, among others, are easily identified in cucumbers when exposed to this type of residues in the soil (Santos et al., 2015; Christoffoleti et al., 2015).

In this context, the present study evaluates leaching after application of the commercial mixture of herbicides 2,4-D + picloram, using cucumber plants as bioindicators.

Material and methods

The experiment was conducted in a greenhouse, in a randomized block design with eight replications arranged in a 5x2 factorial scheme, with factor A corresponding to doses (1, 2, 3, and 4 L ha^{-1}) of the commercial mixture 2,4-D (240 g a.e. L^{-1}) + picloram (64 g a.e. L^{-1}) (commercial mixture-Tordon$^®$) plus the control without application, and factor B corresponding to the position of herbicides in the columns (0-30 cm and 30-60 cm depth).

Experimental units were leaching columns made of PVC, with 0.60 m in length and 0.20 m in diameter. The columns were filled using sieved Latosol without history of herbicide use. Chemical and physical characteristics are shown in Table 1. Screens were placed in the basal portion of the columns to retain the soil and allow water to drain, since the tubes were kept in a vertical position, inside polyethylene pots filled with gravel. After being filled with the soil samples, they were saturated with water.

Table 1 - Chemical and granulometric analysis of the soil used in the experiment.

pH	Al^{3+}	(H$^+$+Al^{3+})	Ca^{2+}	Mg^{2+}	K$^+$	P (mg dm^{-3})	V	m
		---------------------------- (cmol$_c$ dm^{-3}) ----------------------------					-------- (%) --------	
5.7	0.4	4.4	3.0	1.4	70.0	48.0	57.0	7.8

Coarsesand	Thinsand	Silt	Clay	Textural class
----------------------------------- (dag kg^{-1}) -------------------------------------				Sandy Clay Loam
55	18	4	23	

The herbicide mixture was applied on the surface of columns using a CO_2-pressurized backpack sprayer equipped with four TeeJet XR110020 flat-fan nozzles (TeeJet Technologies), which delivered 150 L ha-1 of spray solution at 2.8 bar.

All columns received a weekly rainfall of 60 mm, totaling 240 mm of rainfall until the opening of columns. At 32 days after application (DAA) of the commercial mixture of herbicides 2,4-D + picloram, the columns were opened vertically, giving rise to two section and, consequently, eight replications of each treatment. The soil inside the section was divided with a plastic sheet into two parts: the first (0-30 cm) and the second portion (30-60 cm) of the column. Then, cucumber (*Cucumis sativus* L.) was sown at 0-30cm and 30-60cm depth,

corresponding to the first and second portion of the column, respectively.

Transient chlorophyll *a* fluorescence was evaluated at 45 days after cucumber sowing using a portable fluorometer (HandyPEA, Hanstech, King's Lynn, Norfolk, UK). The clips used for these measurements were placed on the middle third of fully expanded young leaves in the morning. Measurements were made 20 min after the leaves were adapted to the dark. Fluorescence emission was induced in an area of 4 mm in diameter by exposing the sample to a pulse of saturating light at an intensity of 3,000 µmol m^{-2} s^{-1}. From the transient fluorescence curve obtained after the pulse, the intensities determined at 50 µs (initial fluorescence - F0), 100 µs, 300 µs, 2 ms (FJ), 30 ms

(FI), and FM (maximum fluorescence) were used to calculate the parameters established by the JIP Test (Strasser & Strasser, 1995).

The following were also determined at 45 days after cucumber sowing: shoot length (SL), root length (RL), and total dry weight (TDW) of plants. The shoot and root length of bioindicator plants were measured from the ground to the apex of shoots and roots, respectively. After collecting the plants, shoots and roots were packed together in paper bags and placed in a forced-air circulation oven at 65 ± 5 °C until constant weight to obtain TDW a precision balance.

Data were submitted to analysis of variance ($p \leq 0.05$) and tested by polynomial regression models when significant. The choice of models was based on statistical significance (F test), adjustment of the coefficient of determination (R^2), and biological significance of the model.

Results and discussions

There was no interaction between the herbicide dose and herbicide position in the leaching column. However, in isolation, these factors were significant for all variables evaluated (Table 2).

Table 2 - Means of shoot length (SL; cm), root length (RL; cm) and total dry mass (TDM; g) of cucumber plants (*Cucumis sativus*) grown in two positions (top and bottom) of leach columns containing soil with the commercial mixture of 2,4-D + picloram herbicides.

Depth	SL	RL	TDM
0-30 cm	3.86	1.6	0.14
30-60 cm	6.73*	2.3*	0.25*

* significant by F test ($p \leq 0.05$).

Cucumis sativus plants showed lower SL, RL, and TDW in the first 30 cm depth compared to the 30-60 cm depth, regardless of the applied dose. Retention of molecules of the mixture in the first centimeters of depth (0-30 cm) indicates that the rainfall volume of 240 mm during the 32 days before the opening of columns was not sufficient to leach a significant amount of the commercial mixture to depths greater than 30 cm, which would cause toxicity to cucumber plants (Table 2). These findings contradict the predictions for auxinic herbicides such as picloram and 2,4-D, which are known as molecules with high leaching potential in different soil types (D'Antonino et al., 2009; Franceschi et al., 2015). Picloram is characterized by low sorption in soil colloids (D'Antonino et al., 2009), with a half-life of 90 days, and lasting up to 360 DAA (Santos et al., 2006). As well as picloram, 2,4-D has high solubility, 2.8 pKa, and an average Koc of 20 mL g^{-1} soil. Notwithstanding, 2,4-D has low persistence in the soil, with a half-life of 10 days (Senseman, 2007).

The permanence of herbicides in the 0-30 cm layer is due to the low pKa of both herbicides associated with soil pH of 5.7 and loam-clay-sandy texture, indicating less adsorption of the herbicide to colloids and greater availability in the soil solution. Herbicide leaching is highly dependent on rainfall, among other characteristics (Monquero et al., 2008). Picloram was shown to persist in the first 10 cm of depth in clay-sandy soil (Santos et al., 2006), and leached up to depth of 32 cm in dystrophic Red-Yellow Latosol (Franceschi et al., 2015).

The variables SL, RL, and TDW decreased with increasing doses of the commercial mixture, regardless of the position in the column. Shoot length (SL) decreased by 29, 45, 61, and 63% with the doses of 1, 2, 3, and 4 L ha^{-1}, respectively, compared to the control (untreated) (Figure 1A). Root length (RL) decreased by 35, 34, 70, and 72% with the doses of 1, 2, 3, and 4 L ha^{-1} respectively, compared to the control (Figure 1B). The reduction in SL and RL is related to the sensitivity of cucumber to auxinic herbicides (Christoffoleti et al., 2015).

Total dry weight (TDM) decreased by 24, 24, 55, and 59% with the doses of 1, 2, 3, and 4 L ha^{-1} of the commercial mixture of herbicides 2,4-D + picloram, respectively, compared to the control (Figure 1C). This may be related to the action of synthetic auxins, which in addition to promoting characteristic symptoms such as leaf epinasty and growth paralysis, can disturb conducting vessels, hindering water transport and redistribution of photoassimilates (Christoffoleti et al., 2015; Peterson et al., 2016). D'Antonino et al. (2009) observed similar results, where crops sensitive to auxin-mimicking herbicides and grown in loamy-clayey soils subject to the application of herbicides belonging to this mechanism of action also had a decrease in dry weight accumulation.

In plants under stress conditions, chlorophyll *a* fluorescence analysis can be used to understand the mechanisms of photosynthesis and to assess changes in photosynthetic capacity by different types of stress (Strasser et al., 1995; Dayan and Zaccaro, 2012). The evaluation of fluorescence emission kinetics is a fast, simple, and non-invasive method. Although photosynthesis is not considered a primary target for auxin-herbicides, it is possible to detect changes in fluorescence responses in plants treated with these herbicides (Dayan & Zaccaro, 2012).
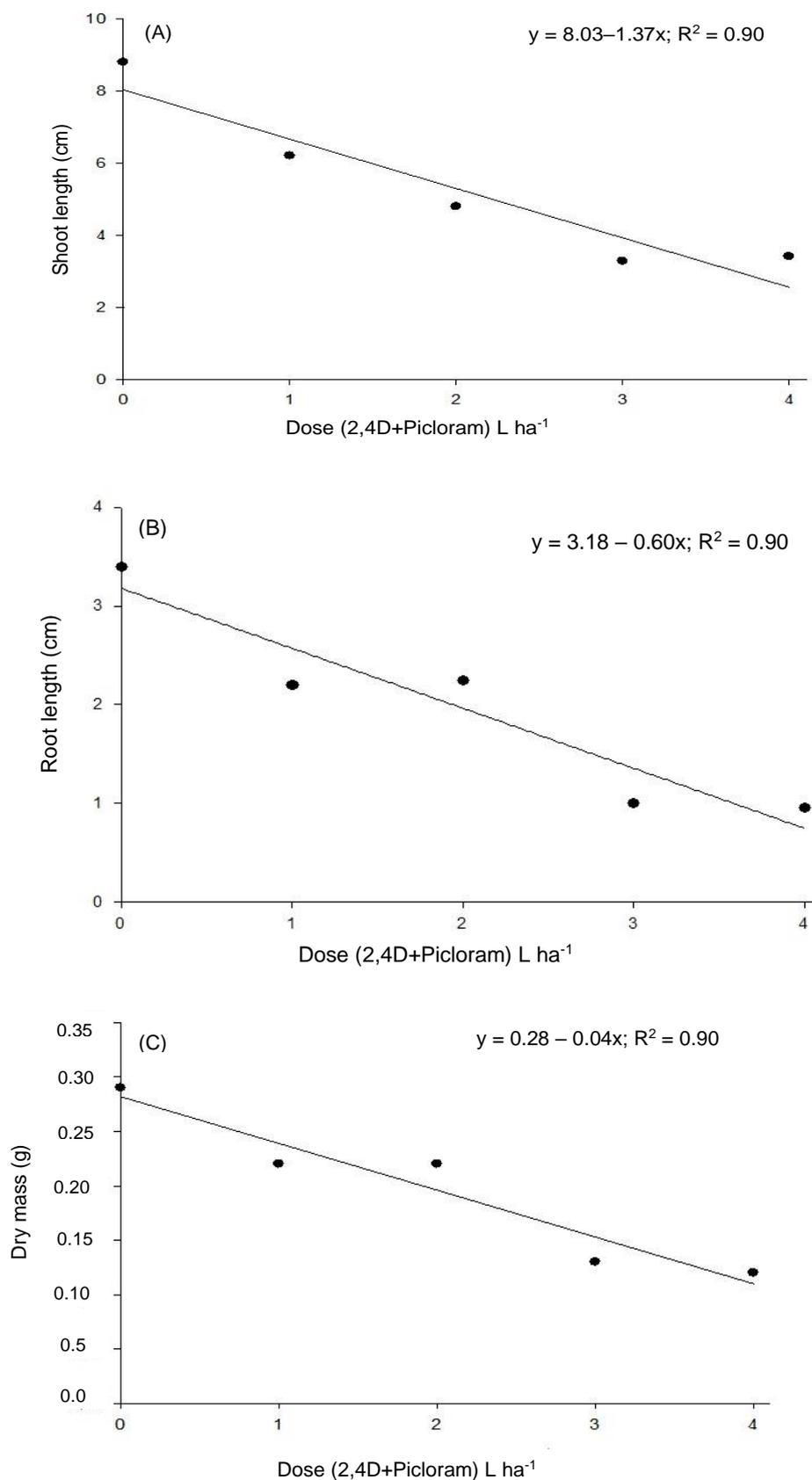
Figure 1 - Shoot length (A), root length (B) and total dry mass (C) of cucumber plants (*Cucumis sativus*) sown in leach columns 32 days after application of commercial mixture of herbicides 2,4-D + picloram and evaluated 45 days after sowing.

The chlorophyll *a* fluorescence transient corroborate with biometric data presented previously. At the 0-30 cm depth (Figure 2A), reductions of 20% (doses of 1 and 2 L ha^{-1} of the herbicide) and 40% (doses of 3 and 4 L ha^{-1} of the herbicide) were observed in photosynthetic indexes (PI_{TOTAL} and PI_{ABS}). This is due to the energy conservation of the captured exciton to reduce the final electron acceptor of the intersystem (PI_{ABS}) and PSI (PI_{TOTAL}). The energy absorbed by the plant during photosynthesis has three destinations that compete against each other: photochemistry (energy production and reducing power); energy dissipated in the form of heat; and fluorescence (Krause and Weis,

1991), so that any increase in the efficiency of one will decrease the performance of the others. Thus, the observed decline in the photosynthetic performance of cucumber plants in the 0-30 cm layer reflects an increase in energy dissipation in the form of heat ($\varphi D0$ and $DI0/RC$) by approximately 20% at a dose of 4 L ha^{-1}. This demonstrates the inability of the plant to use this energy for photosynthesis, which may impair plant growth. Fluorescence is a potential indicator of stress and can be considered as an indicator of physiological disorders before the appearance of visible signs of stress (Oukarroum et al., 2007).
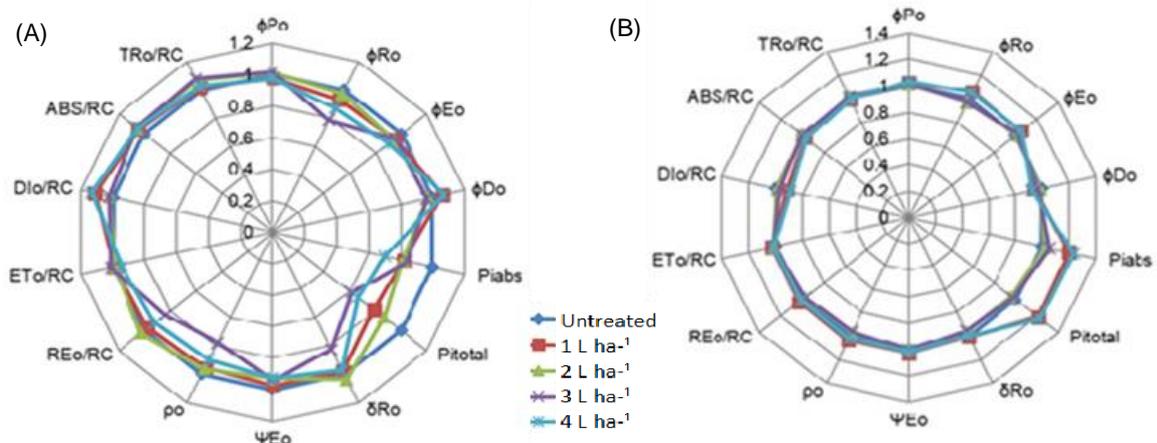

Figure 2 - Residual effect of the commercial mixture of 2,4-D + picloram herbicides on cucumber plants (*Cucumis sativus*) cultivated in the 0-30 cm (A) and 30-60 cm (B) portions of the leaching columns at 45 days after sowing, under the transient fluorescence parameters of chlorophyll *a*, obtained by the JIP test (center radar plot = 0.0, maximum = 1.4) in relation to the control - behavior pattern (full line = 1.0).

At the 30-60 cm depth, cucumber plants grown in soil subject to the commercial mixture of herbicides 2,4-D + picloram did not differ from the control regarding transient chlorophyll *a* fluorescence (Figure 2B), except in the dose of 1and 4 L ha^{-1} of the herbicide, which caused a 20% increase in photosynthetic parameters. This increase in photosynthetic performance is probably associated with plant recovery from stress, not reflecting differences in plant height and biomass, as can be seen in biometric data.

Conclusion

The commercial mixture of herbicides 2,4-D + picloram remained in the first 30 centimeters of depth of a loam-clay-sandy soil when subject to 240 mm rainfall.

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