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Persistence of auxinic herbicides applied on pasture and toxicity for succeeding crops*

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ABSTRACT

The aim of this work was to determine the persistence of auxinic herbicides applied on tropical pasture and toxicity for succeeding crops. The herbicides were applied in an area of dystrophic red–yellow latosol with pasture infested of weeds. At 40, 80, and 280 days after application of herbicide, the soil samples were collected at depths of 0 to 20 cm. Soil with residues of 2,4-D, 2,4-D + picloram, triclopyr, and a soil without herbicide application were analyzed with six replicates. Seven crops were cultivated in these soils: cucumber (*Cucumis sativus* L.), velvet bean [*Mucuna pruriens* (L.) DC.], pigeon pea [*Cajanus cajan* (L.) Millsp.], alfalfa (*Medicago sativa* L.), lablab bean [*Lablab purpureus* (L.) Sweet], corn (*Zea mays* L.), and sorghum [*Sorghum bicolor* (L.) Moench]. The plants of cucumber, pigeon pea, and alfalfa were the most susceptible to the auxinic herbicide residues. However, the lablab bean was the only one among the dicot evaluated that showed tolerance to the 2,4-D + picloram residual when cultivated in soils at 280 days after application of herbicide. Corn and sorghum showed lower chlorophyll content in soils with 2,4-D + picloram residual up to 80 days after application of herbicide.

Key words: growth regulator, herbicide residue, 2,4-D, picloram, triclopyr.

INTRODUCTION

Auxinic or auxin–mimetic herbicides represent the oldest and possibly the most widely used herbicide to control weeds in pastures (MacDonald et al. 2013). However, some of these herbicides have residual activity in the soil and can become undesirable, because they can result in damage to succeeding crops, contamination of water sources by leaching, and toxicity to non–target organism (Braga et al. 2016).

The herbicides 2,4-D, picloram, and triclopyr are synthetic auxins which have the highest number of commercial products registered in Brazil (MAPA 2015). In addition, these herbicides are acidic and have great mobility in soil solution. However, mainly the soil characteristics and the rainfall after application will determine the leaching index of these herbicides (D'Antonino et al. 2009a).

The soil persistence of 2,4-D and triclopyr, when applied in commercial dosages, is around

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four weeks in clayey soils and warm climate. While the picloram has a long persistence in the soil, it can be found up to two or three years after application depending on soil and environmental conditions (Deubert and Corte-Real 1986, Santos et al. 2006). However, there are few studies addressing the soil persistence of these herbicides and the intoxication of plants in systems with succession of crops. Knowledge about crop responses to herbicide residual enables the development of strategies for more intensive use of the area, in addition to the possibility of discovering crops with potential phytoremediation, bioindicators, and the best variables for evaluating crops in soils contaminated by herbicides.

Therefore, based on the hypothesis that the herbicide persistence in the soil depends on several factors and the crops have a differentiated sensitivity to the auxinic herbicides. Thus, was proposed the present work with the objective of evaluating the persistence of auxinic herbicides applied on tropical pasture and toxicity for succeeding crops.

MATERIALS AND METHODS

The experiment was carried out in two stages. In the first stage, the herbicides were applied in a pasture of bahia grass (*Paspalum notatum* Flüggé) infested with siamweed [*Chromolaena odorata* (L.) King & H.E. Robins] and sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] in "Couto de Magalhães de Minas", MG, Brazil. The herbicide was applied under suitable environmental conditions by using 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}. At 40, 80, and 280 days after application of herbicides (DAA), the soil samples were collected at depths of 0 to 20 cm, in representative sites of the area and in an area without herbicide application (control).

The second stage of the experiment was conducted in a greenhouse at the "Universidade Federal dos Vales do Jequitinhonha e Mucuri" in Diamantina, MG. The soil was classified as dystrophic red–yellow latosol with clay-sandy texture and the chemical characterization described in Table I. The rainfall and temperature averages during the 280 DAA is represented in Figure 1.

Four treatments with six replicates were analyzed; soil with residues of 2,4-D (1340 g a.e. ha^{-1}), 2,4-D + picloram (720 + 192 g a.e. ha^{-1}), triclopyr (960 g a.e. ha^{-1}), and a soil control without herbicide application. Seven crops were cultivated in these soils: cucumber (*Cucumis sativus* L.), velvet bean [*Mucuna pruriens* (L.) DC.], pigeon pea [*Cajanus cajan* (L.) Millsp.], alfalfa (*Medicago sativa* L.), lablab bean [*Lablab purpureus* (L.) Sweet], corn (*Zea mays* L.), and sorghum [*Sorghum bicolor* (L.) Moench]. The evaluations were performed at 20 and 40 days after sowing (DAS).

The soil was sieved and the equivalent to 3 t ha^{-1} of dolomitic limestone was applied. The substrates were fertilized with equivalent to 400 kg ha^{-1} formulated at 8–28–16 ($N–P_2O_5–K_2O$) and 100 mg dm^{-3} of urea 20 days after plant emergence. Ten seeds per pot of each crop species were sown and then later thinned and two plants left per pot. Watering was done daily to keep the soil moisture with 80% field capacity. The crops grew for a period of 40 days in polyethylene pots of 7 L volume, coated with polyethylene film to avoid herbicide loss by leaching.

At 20 and 40 DAS the plants intoxication were visually evaluated using a scale of 1 to 4 (EWRC 1964). Where 1 represents no toxicity (normal plants), 2 mild intoxication (intoxication evident only in the leaf blade, with folds at the edges), 3 moderate intoxication (plants with more intense folds, increase of growth, and epinastia), and 4 severe intoxication (reduction of leaf area with intense folds, epinasty, thickening of terminal buds, and reaching death). Concurrently, the plant heights were measured using graduated ruler, with reference to the apical meristem to the ground

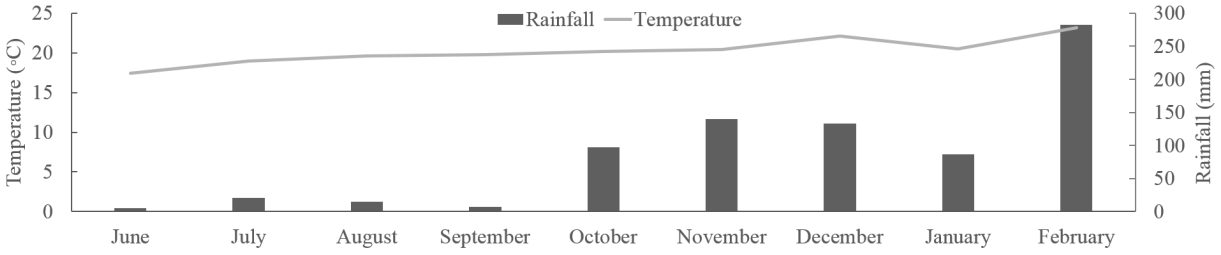

Figure 1 - The monthly rainfall and air temperature averages of experimental period.

TABLE I
Chemical analysis of the soil in the 0–20 cm depth layer.

pH (H_2O)	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	BS[a]	OM[b]	Clay	Sand
	mg dm^{-3}				cmolc dm^{-3}			dag kg^{-1}	%	
5.12	1.02	25.3	0.40	0.20	0.76	5.20	0.66	0.70	47	53

[a] BS = base sum;
[b] OM = organic matter.

level. It was also determined the total chlorophyll content of the newest fully developed leaf, in the central part of the limbus (grass) or central leaflet (legumes), avoiding the ridges. This evaluation were performed between 08:00 and 10:00 h by using a portable chlorophyllometer (SPAD–502, Minolta, Osaka, Japan).

At 40 DAS, after all evaluations, the plants were harvested and shoots and roots were separated. The samples were weighed and then taken to a forced circulation oven at 60°C until reaching constant mass.

STATISTICAL ANALYSIS

Intoxication data were discussed based on the visual scale of symptoms on the crops, without statistical analysis. All others data were submitted to ANOVA and the means were compared by using the Tukey test at 5% significance in SAS (Statistical Analysis Software, Inc., Cary, NC).

RESULTS AND DISCUSSION

CUCUMBER RESPONSES

In soils at 40 DAA, in the first evaluation at 20 DAS, the plants showed moderate intoxication for 2,4-D + picloram residual and mild intoxication for 2,4-D and triclopyr residues. However, at the same evaluation date, the chlorophyll content was not affected, although the 2,4-D + picloram residual increased the plant height (Table II).

In the second evaluation in the soils at 40 DAA, at 40 DAS, severe intoxication on the plants by 2,4-D + picloram residual was observed, however, no intoxication for the others herbicides. The plants died due to severe intoxication by the 2,4-D + picloram residual, so the chlorophyll content, height, and dry mass of shoot and roots of plants were null. In this same evaluation date, the 2,4-D and triclopyr residues did not affect the chlorophyll content in relation to the control; while that the plants height was smaller for the 2,4-D residual compared to the control and for the triclopyr an intermediate height was observed. Despite of the lower plant height for the 2,4-D residual, the dry mass of shoot and roots of the plants were not affected.

Thus, it is possible to infer that the cucumber plants were sensitive to 2,4-D residual even at 40 DAA, so it is one of the plants most used as an indicator of soil herbicide residues. The cucumber is an indicator of soil contamination with auxinic herbicides because the activity residual of picloram,

TABLE II
Cucumber plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication	Intoxication scale[a]					
2,4-D + picloram	3	4	3	4	2	4
2,4-D	2	1	1	1	1	1
Triclopyr	2	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll	SPAD[b]					
2,4-D + picloram	36.5	0 b	45.3	0 b	39.1	0 b
2,4-D	40.5	40.2 a	43.6	42.0 a	38.7	48.8 a
Triclopyr	40.8	40.7 a	43.2	41.6 a	38.5	49.0 a
Control	44.3	46.4 a	44.9	42.4 a	38.9	49.7 a
CV[c] %	13.1	14.3	6.11	11.2	6.71	8.53
Height	cm					
2,4-D + picloram	8.02 a	0 c	8.82 a	0 b	11.1	0 b
2,4-D	6.05 b	18.6 b	6.80 b	20.1 a	11.2	29.4 a
Triclopyr	6.10 b	19.1 ab	6.70 b	19.3 a	11.8	29.3 a
Control	6.55 b	21.4 a	6.72 b	21.4 a	11.0	29.0 a
CV[c] %	11.2	10.2	11.2	9.77	15.4	9.87
Shoot dry weight	$g\ pot^{-1}$					
2,4-D + picloram	0 b		0 b		0 b	
2,4-D	19.3 a		23.3 a		28.3 a	
Triclopyr	21.4 a		25.0 a		28.5 a	
Control	21.1 a		23.4 a		27.5 a	
CV[c] %	11.6		8.86		11.4	
Roots dry weight	$g\ pot^{-1}$					
2,4-D + picloram	0 b		0 b		0 b	
2,4-D	8.25 a		11.1 a		14.8 a	
Triclopyr	9.85 a		10.1 a		15.0 a	
Control	9.67 a		12.4 a		15.1 a	
CV[c] %	17.0		19.3		17.7	

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr, 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

fluroxypyr, triclopyr, and aminocyclopyrachlor poison it (Christoffoleti et al. 2015, Braga et al. 2016). In addition, Santos et al. (2006) observed effect of triclopyr residual in the soil at 10 DAA, but no plant intoxication was observed for the treatments from 40 DAA. However, these same authors observed residual effects in the soil of mixtures containing picloram up to 360 DAA. Norris et al. (1987) and Johnson et al. (1995) also reported the short persistence of triclopyr in soil. In our study, the herbicide triclopyr showed a lower residual period in the soil, therefore considering the need to preserve the environment and to allow the crop succession, this herbicide may be more appropriate.

In soils at 80 and 280 DAA, in the evaluations at 20 and 40 DAS, no effects of 2,4-D and triclopyr residues were observed for analyzed variables. However, at 40 DAS, for the 2,4-D + picloram residual, all plants showed severe intoxication and died up to 280 DAA. The cucumber plants before dying showed the typical symptoms of auxinic herbicides, that is, a higher plant height due to intense cell division, leading to disordered cell growth, leaf epinasty, and twisting of the stem (Christoffoleti et al. 2015).

Santos et al. (2006) evaluating the residual toxicity of 2,4-D + picloram from soil samples taken at depths of 0–10 cm and 10–20 cm, obtained severe intoxication up to 180 DAA in cucumber seedlings. However, D'Antonino et al. (2009b) observed persistence of 2,4-D + picloram (960 + 256 g ha^{-1}) up to 150 DAA. These authors state that the persistence of the herbicide was related to the rainfall and soil characteristics, mainly organic matter content, texture and pH.

VELVET BEAN RESPONSES

Plants grown in soils at 40 DAA, evaluated at 20 DAS, showed severe intoxication by 2,4-D + picloram and mild intoxication by 2,4-D residual in the soil. In addition, a lower chlorophyll content was observed for these two residues compared to the triclopyr residual and the control. On the same evaluation date, a lower plant height was observed for 2,4-D + picloram residual in the soil compared to the others treatments. At 40 DAS, a severe intoxication and plant death was observed caused by 2,4-D + picloram residual and the others treatments did not affect the analyzed variables. Although the 2,4-D residual reduced the chlorophyll content at 20 DAS, the plants were able to recover from the injury and in the subsequent evaluation this reduction was not observed (Table III).

The results found in our study corroborate with Carmo et al. (2008) who concluded that with the application of a picloram subdose (80 g ha^{-1}) in the soil, evaluated at 34 DAA, the velvet bean plants were susceptible with 86.8% intoxication. In addition, these authors observed, at 84 DAA, a maximum intoxication at doses from 160 g ha^{-1}.

Our results were similar for velvet bean plants grown in soils at 80 and 280 DAA. At 20 DAS, moderate intoxication, lower chlorophyll content, and lower plant height were observed in the soils with 2,4-D + picloram residual compared to the others treatments. At 40 DAS, for the 2,4-D + picloram residual in the soil, severe intoxication and plant death were observed; for the others treatments no effect on the analyzed variables was observed.

It is known that depending on the soil and climatic conditions the picloram may presents long persistence in the environment, with half-life of 20 to 300 days, being able to be found in the soil up to three years after its application (Deubert and Corte-Real 1986). Santos et al. (2006) found picloram residual at commercial doses in tropical pasture up to 360 DAA. While for 2,4-D, the soil persistence is short to medium and, at commercial doses, the residual activity does not exceed four weeks in clayey soils and warm weather (Peres-Oliveira et al. 2016). According to Carmo et

TABLE III
Velvet bean plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication	Intoxication scale[a]					
2,4-D + picloram	4	4	3	4	3	4
2,4-D	2	1	1	1	1	1
Triclopyr	1	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll	SPAD[b]					
2,4-D + picloram	32.6 b	0 b	26.2 b	0 b	25.5 b	0 b
2,4-D	33.2 b	40.9 a	36.8 a	39.4 a	39.9 a	40.4 a
Triclopyr	39.7 a	42.3 a	36.6 a	39.7 a	40.2 a	41.0 a
Control	42.1 a	41.5 a	39.0 a	39.1 a	39.8 a	39.7 a
CV[c] %	6.22	9.19	5.56	5.37	9.32	7.36
Height	cm					
2,4-D + picloram	3.95 b	0 b	6.82 b	0 b	19.1 b	0 b
2,4-D	13.4 a	49.1 a	14.5 a	45.3 a	25.3 a	51.6 a
Triclopyr	13.6 a	49.4 a	13.7 a	45.8 a	26.1 a	53.0 a
Control	12.9 a	49.2 a	13.8 a	45.8 a	24.9 a	52.1 a
CV[c] %	12.3	9.77	7.66	12.2	12.3	12.2
Shoot dry weight	$g\ pot^{-1}$					
2,4-D + picloram		0 b		0 b		0 b
2,4-D		26.4 a		31.5 a		34.9 a
Triclopyr		26.7 a		31.0 a		34.4 a
Control		27.8 a		31.3 a		34.0 a
CV[c] %		8.43		9.13		9.65
Roots dry weight	$g\ pot^{-1}$					
2,4-D + picloram		0 b		0 b		0 b
2,4-D		14.2 a		16.9 a		15.2 a
Triclopyr		15.1 a		16.3 a		15.7 a
Control		15.2 a		16.8 a		14.9 a
CV[c] %		16.9		9.34		16.4

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

al. (2008), the plants of velvet bean, jack bean [*Canavalia ensiformis* (L.) DC.], and palisade signal grass [*Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf.] were the most sensitive plants in soil that received 160 g ha^{-1} of picloram at 34 DAA, presenting plant intoxication above 85%. These authors also reported that the jack bean plants were more tolerant to activity of picloram in the soil in relation to the velvet bean, although the tolerance was restricted to the contamination level of 132.4 g ha^{-1}. Therefore, it is possible to infer that, because of the high susceptibility to 2,4-D + picloram residual in the soil, the velvet bean can be used as a plant indicative of the presence of this herbicide in organic compounds.

PIGEON PEA RESPONSES

Plants grown in soils at 40 DAA, evaluated at 20 DAS, showed a moderate intoxication by 2,4-D + picloram residual, mild intoxication by 2,4-D residual, and no intoxication by triclopyr residual in the soil. On the same evaluation date, a lower chlorophyll content for the plants grown in the 2,4-D + picloram and intermediate values of chlorophyll content for the 2,4-D and triclopyr residues compared to the control were observed. However, at the same evaluation date, the plant height was affected only by the 2,4-D + picloram residual in the soil, which resulted in the lowest plant height in comparison with the others treatments (Table IV). Peres-Oliveira et al. (2016) found similar results under tropical conditions. These authors reported residual activity of 2,4-D in the first weeks after its application at commercial dosage.

In our study, the plants grown in soils at 40 DAA, evaluated at 40 DAS, showed a severe intoxication by 2,4-D + picloram residual and mild intoxication by 2,4-D residual. Plant intoxication by 2,4-D + picloram residual resulted in plants death and null values for all others variables analyzed. At the same evaluation date, the triclopyr residual in the soil resulted in lower chlorophyll content. While for the plants grown in soil with 2,4-D residual showed intermediate values when compared to the control. However, the plant height and dry mass of shoot and roots were not affected by 2,4-D and triclopyr residues. An interesting result was observed about the chlorophyll content of pigeon pea, since this variable was the only one to be affected by triclopyr residual. Thus, the chlorophyll content is an important variable to monitoring of pigeon pea plants when grown in soils with a possible presence of auxinic herbicide residues.

The plants grown in the soils at 80 and 280 DAA had similar results; in the evaluations at 20 DAS, the plants showed a moderate intoxication and lower chlorophyll content by the 2,4-D + picloram residual compared to the other treatments. However, in the evaluations at 40 DAS, the plants showed a severe intoxication and died, resulting in null values of chlorophyll content, height and dry mass. At the same evaluation date, the other treatments (2,4-D and triclopyr) did not affect the analyzed variables.

The results of the present work confirm that the effects of 2,4-D and triclopyr residues can be observed in the plants only in the first weeks after the herbicide application. In addition, the importance of monitoring the chlorophyll content in the pigeon pea plants for the detection of auxinic herbicide residual in the soil.

ALFALFA RESPONSES

Plants grown in soils at 40 DAA, evaluated at 20 DAS, showed a severe intoxication by 2,4-D + picloram residual and mild intoxications by 2,4-D and triclopyr residues in the soil. In addition, the 2,4-D + picloram residual resulted in lower chlorophyll content and for the 2,4-D and triclopyr residues resulted in intermediate values compared to the control. While the 2,4-D + picloram residual

TABLE IV
Pigeon pea plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication			Intoxication scale[a]			
2,4-D + picloram	3	4	3	4	3	4
2,4-D	2	2	1	1	1	1
Triclopyr	1	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll			SPAD[b]			
2,4-D + picloram	22.9 c	0 d	21.5 b	0 b	19.8 b	0 b
2,4-D	30.4 b	31.2 b	34.3 a	35.3 a	44.9 a	43.2 a
Triclopyr	30.7 b	29.9 c	33.4 a	35.7 a	44.7 a	43.7 a
Control	36.1 a	35.8 a	36.2 a	35.0 a	47.0 a	43.5 a
CV[c] %	6.16	8.02	7.94	6.60	12.60	8.38
Height			cm			
2,4-D + picloram	2.97 b	0 b	12.9	0 b	15.9	0 b
2,4-D	13.3 a	30.6 a	14.2	29.7 a	16.1	43.9 a
Triclopyr	13.9 a	31.6 a	14.1	29.1 a	16.6	44.1 a
Control	13.4 a	31.7 a	13.6	28.5 a	17.2	42.9 a
CV[c] %	8.78	11.2	8.78	10.2	10.2	9.87
Shoot dry weight			g pot^{-1}			
2,4-D + picloram		0 b		0 b		0 b
2,4-D		17.4 a		20.9 a		24.7 a
Triclopyr		19.1 a		19.0 a		25.0 a
Control		20.1 a		19.7 a		25.2 a
CV[c] %		9.96		9.26		9.76
Roots dry weight			g pot^{-1}			
2,4-D + picloram		0 b		0 b		0 b
2,4-D		9.87 a		10.7 a		13.2 a
Triclopyr		10.9 a		11.5 a		14.6 a
Control		10.7 a		11.9 a		13.2 a
CV[c] %		7.42		13.7		17.3

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

in the soil resulted in lower plant height compared to the other treatments (Table V).

In the soils at 40 DAA, evaluated at 40 DAS, the plants showed a severe intoxication by the 2,4-D + picloram residual that resulted in plants death and the values of chlorophyll, height and dry mass of shoot and roots of the plants were null. At the same evaluation date, the plants showed a mild intoxication by the 2,4-D residual in the soil, while the chlorophyll content were lower for the 2,4-D and for the triclopyr residues when compared to the control. However, the plants height and the dry mass of shoot and roots were not affected by 2,4-D and triclopyr residues. As observed in the present study for pigeon pea, the chlorophyll content of alfalfa was an important variable in the detection of 2,4-D and triclopyr residues in soil at 40 DAA, because in the evaluation at 40 DAS, despite of lower chlorophyll content, no other effect was observed for the triclopyr residual.

Plants grown in soils at 80 DAA, evaluated at 20 DAS, an moderate toxicity, lower chlorophyll content, and smaller plants was observed for the 2,4-D + picloram residual when compared to other treatments. In the evaluations at 40 DAS, the 2,4-D + picloram residual resulted in a severe intoxication and plant death, while the 2,4-D and triclopyr residues did not affected the variables analyzed.

Plants grown in soils at 280 DAA, evaluated at 20 DAS, a mild intoxication for the 2,4-D + picloram residual was observed, although the chlorophyll content and the plants height were not affected by the treatments. In the evaluations at 40 DAS, an severe intoxication and plant death were observed for 2,4-D + picloram residual, whereas that the 2,4-D and the triclopyr residues did not affect the analyzed variables.

Kniss and Lyon (2011), working with aminocyclopyrachlor, did not observed intoxication on common wheat (*Triticum aestivum* L.) growing in soils that received, 15 days before sowing, 20 g ha^{-1} of aminocyclopyrachlor. However, these authors, at the time of harvest, observed a reduction of 50% in grain yield. Conklin and Lym (2013) reported that the degradation of aminocyclopyrachlor might be slower in soils with low organic matter content and higher clay content. The higher microorganisms population in the soil with higher content of organic matter can accelerate the degradation of the herbicide molecule. In addition, the soils with high clay content and low pH, can result in higher adsorption rate and lower mobility of this herbicide. In our study, the high persistence of 2,4-D + picloram in the soil is related to the low rainfall, as well as the slightly acidic pH, low organic matter content, and moderate clay content of soil (Table I and Figure 1).

Silva et al. (2011) verified in their studies with soybean [*Glycine max* (L.) Merr.], a reduction in the plants height when submitted to increasing doses of 2,4-D residual in the soil, until 15 days before sowing. Hansen and Grossmann (2000) explain that the auxin compounds affect the plant biomass production, because there is a release of excess ethylene causing the synthesis of abscisic acid that when metabolized by the plant results in the stomatal closure which limits the carbon assimilation.

However, Yamashita et al. (2009) evaluated the influence of herbicide mixture (glyphosate + 2,4-D) on the initial development of forest essences, and verified for kapok tree [*Ceiba pentandra* (L.) Gaertn.], that the treatments influenced significantly the root length, although this pattern was not observed in our study for the 2,4-D residual. According to Nascimento and Yamashita (2009), the symptoms in the root caused by auxin compounds are intense cell proliferation, hypertrophy, lateral thickening, and reduction of dry mass.

TABLE V
Alfalfa plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication	Intoxication scale[a]					
2,4-D + picloram	4	4	3	4	2	4
2,4-D	2	2	1	1	1	1
Triclopyr	2	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll content	SPAD[b]					
2,4-D + picloram	25.5 c	0 c	26.4 b	0 b	36.8	0 b
2,4-D	28.3 b	31.0 b	34.3 a	32.5 a	39.2	43.1 a
Triclopyr	28.7 b	28.7 b	33.9 a	32.7 a	37.7	42.4 a
Control	32.8 a	33.9 a	35.1 a	33.2 a	39.0	43.5 a
CV[c] %	7.18	6.82	6.95	7.42	6.50	8.81
Height	cm					
2,4-D + picloram	0.95 b	0 b	0.90 b	0 b	3.90	0 b
2,4-D	2.45 a	15.4 a	2.55 a	14.4 a	4.12	17.1 a
Triclopyr	2.57 a	15.5 a	2.57 a	15.7 a	3.97	16.6 a
Control	2.45 a	16.2 a	2.35 a	14.9 a	4.22	17.1 a
CV[c] %	8.87	11.2	14.2	11.2	9.65	15.2
Shoot dry weight	g pot^{-1}					
2,4-D + picloram	0 b		0 b		0 b	
2,4-D	12.9 a		14.2 a		16.8 a	
Triclopyr	14.8 a		15.1 a		17.5 a	
Control	14.6 a		13.7 a		16.8 a	
CV[c] %	18.4		18.4		13.3	
Roots dry weight	g pot^{-1}					
2,4-D + picloram	0 b		0 b		0 b	
2,4-D	8.20 a		8.50 a		9.01 a	
Triclopyr	8.62 a		8.87 a		9.34 a	
Control	8.32 a		8.10 a		8.93 a	
CV[c] %	6.21		18.0		21.5	

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

LABLAB BEAN RESPONSES

Plants grown in soils at 40 DAA, evaluated at 20 DAS, a severe intoxication and plant death was observed for the 2,4-D + picloram residual, while for the 2,4-D residual a mild intoxication. At the same evaluation date, a lower chlorophyll content was observed for the 2,4-D and triclopyr residues compared to the control, however, the plants height was not affected. In the soils at 40 DAA, evaluated at 40 DAS, only the 2,4-D + picloram residual caused a severe intoxication and plant death, while the others treatments did not affect the analyzed variables (Table VI).

Plants grown in soils at 80 DAA, evaluated at 20 DAS, a moderate intoxication and lower chlorophyll content was observed for the 2,4-D + picloram residual, although without affecting the plant height compared to others treatments. In the evaluations at 40 DAS, the 2,4-D + picloram residual in the soil resulted in a severe intoxication and plant death and the others treatments (2,4-D and triclopyr) did not affect the variables analyzed.

Plants grown in soils at 280 DAA, evaluated at 20 DAS, a mild intoxication and lower chlorophyll content was observed for the 2,4-D + picloram residual, however, without affecting the plants height; while the other treatments did not affect the analyzed variables. In the evaluations at 40 DAS, a moderate intoxication, lower chlorophyll content, lower plant height, and lower shoot dry mass was observed for the 2,4-D + picloram residual compared to the others treatments, without affecting the roots dry mass. At the same evaluation date, the others treatments did not affect the analyzed variables. Among all the legumes evaluated, the lablab bean was the only one that, despite the injuries observed, were able to develop in soil with 2,4-D + picloram residual. Possibly, this occurs due to the tolerance mechanism of this species to picloram. According to Carmo et al. (2008) and Madalão et al. (2012), the plants that grow on soils contaminated by herbicide residual are potential for use as phytoremediation of contaminated soil. Plant species used for phytoremediation may have several mechanisms for their development and soil decontamination. For development, the plants may simply reduce the absorption of the herbicide and this can remain in the soil. On the other hand, the decontamination mechanism may be related to increased plant metabolism and thus, may increase the rate of degradation of the herbicidal molecule (Madalão et al. 2012, Braga et al. 2016).

CORN RESPONSES

Plants grown in soils at 40 DAA, evaluated at 20 DAS, showed a mild intoxication by the 2,4-D + picloram residual, resulting in lower chlorophyll content, without affecting the height and dry mass of the plants. The others treatments did not affect the analyzed variables. In the evaluations at 40 DAS, although no intoxication symptoms were observed, the 2,4-D + picloram residual resulted in lower chlorophyll content compared to the others treatments, without affecting the height and dry mass of the plants. However, 2,4-D and triclopyr residues did not affect the analyzed variables (Table VII).

Plants grown in soils at 80 DAA, evaluated at 20 DAS, showed no intoxication symptoms by herbicide residues, although the 2,4-D + picloram residual resulted in lower chlorophyll content compared to the others treatments. However, in the evaluations at 40 DAS, no effect of the treatments on the analyzed variables was observed.

Plants grown in soils at 280 DAA, in both evaluations, the analyzed variables were not affected by the treatments.

D'Antonino et al. (2009b) observed a sensitivity of corn plants to the 2,4-D + picloram residual, however, although the herbicide adversely affected the mass yield of plants, the final grain yield was not affected. In addition, Belo et al.

TABLE VI
Lablab bean plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication	Intoxication scale[a]					
2,4-D + picloram	4	4	3	4	2	3
2,4-D	2	1	1	1	1	1
Triclopyr	1	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll	SPAD[b]					
2,4-D + picloram	0 c	0 b	29.5 b	0 b	35.1 b	17.5 b
2,4-D	32.0 b	44.9 a	43.7 a	42.2 a	46.3 a	44.1 a
Triclopyr	34.2 b	44.6 a	44.0 a	44.3 a	45.9 a	43.9 a
Control	42.4 a	45.3 a	42.5 a	43.0 a	46.0 a	43.1 a
CV[c] %	5.57	4.53	3.74	10.3	11.5	8.91
Height	cm					
2,4-D + picloram	0 b	0 b	13.02	0 b	15.90	26.15 b
2,4-D	13.30 a	26.22 a	13.17	26.42 a	16.45	31.52 a
Triclopyr	13.40 a	25.37 a	13.17	26.30 a	16.57	31.95 a
Control	13.07 a	27.07 a	13.80	27.27 a	16.37	32.62 a
CV[c] %	10.23	8.78	17.54	9.65	14.22	9.78
Shoot dry weight	g pot^{-1}					
2,4-D + picloram		0 b		0 b		16.6 b
2,4-D		20.7 a		23.8 a		26.4 a
Triclopyr		20.4 a		25.3 a		25.7 a
Control		20.5 a		26.7 a		27.3 a
CV[c] %		9.61		9.52		10.4
Roots dry weight	g pot^{-1}					
2,4-D + picloram		0 b		0 b		10.3
2,4-D		10.3 a		12.7 a		13.7
Triclopyr		10.0 a		13.4 a		13.7
Control		10.7 a		12.1 a		13.2
CV[c] %		15.2		19.5		15.1

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

TABLE VII
Corn plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication	Intoxication scale[a]					
2,4-D + picloram	2	1	1	1	1	1
2,4-D	1	1	1	1	1	1
Triclopyr	1	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll	SPAD[b]					
2,4-D + picloram	14.2 b	23.5 b	17.0 b	28.9	43.2	46.4
2,4-D	32.9 a	30.9 a	30.4 a	29.5	42.0	43.1
Triclopyr	30.5 a	30.7 a	29.3 a	30.1	43.7	44.8
Control	34.7 a	32.1 a	30.1 a	30.3	43.4	43.4
CV[c] %	18.8	8.50	10.1	5.08	7.88	5.10
Height	cm					
2,4-D + picloram	21.4	53.1	21.6	49.7	26.1	55.2
2,4-D	21.8	50.8	21.0	49.3	26.7	54.5
Triclopyr	20.2	50.2	21.6	49.1	27.0	55.1
Control	22.9	51.9	23.0	48.6	26.7	55.9
CV[c] %	10.2	9.44	9.76	7.65	9.76	9.87
Shoot dry weight	$g\ pot^{-1}$					
2,4-D + picloram		31.9		37.0		39.2
2,4-D		31.0		35.0		40.3
Triclopyr		31.1		35.9		40.8
Control		30.5		36.4		39.8
CV[c] %		7.22		6.89		7.01
Roots dry weight	$g\ pot^{-1}$					
2,4-D + picloram		17.2		19.4		23.0
2,4-D		16.5		18.2		20.4
Triclopyr		17.1		18.1		20.3
Control		16.6		18.5		21.4
CV[c] %		12.3		6.33		9.75

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

(2011) evaluated the effects of picloram on the photosynthetic characteristics of corn plants and observed that this species was tolerant to the herbicide at the dose of 160 g ha^{-1} applied in pre-emergence.

Furthermore, Strachan et al. (2011) concluded that monocot species as corn, oat (*Avena sativa* L.), rye (*Lolium perenne* L.), and barley (*Hordeum vulgare* L.) are tolerant to most of the auxin mimic herbicides. However, the plant tolerance may be related to several factors, such as the vascular tissue arrangement in bundles, the presence of intercalary meristems, besides of the metabolization and exudation via the root system. In addition, the plants that have a restricted auxins translocation between the tissues generally also present a high metabolization of the herbicidal molecule (Flessner et al. 2011). Carmo et al. (2008) observed that corn plants were tolerant to picloram residual in the soil at the dose of 640 g ha^{-1} and the phytotoxicity values were 5% and 22% in the evaluation at 34 and 84 DAA, respectively. This represents besides of the continuity of phytoremediation studies, less risk of yield losses due carryover by applications of picloram on farms with crop and livestock integration.

SORGHUM RESPONSES

Plants grown in soils at 40 DAA, evaluated at 20 DAS, showed a mild intoxication and lower chlorophyll content by the 2,4-D + picloram residual, without affecting the plant height compared to the others treatments. At 40 DAS, although the plant intoxication was not observed, the 2,4-D + picloram residual resulted in lower chlorophyll content, but the others variables were not affected by the treatments (Table VIII). It is important to note that the low residual period of triclopyr and 2,4-D herbicides is of great interest, because it reduces potential risks of environmental contamination and makes the area available in relatively short periods.

Plants grown in soils at 80 DAA, the results of both evaluations were similar, at 20 and 40 DAS; only the chlorophyll content was lower for the 2,4-D + picloram residual compared to the others treatments. The sorghum plants, like the most of monocot plants, presents a greater tolerance to the auxinic herbicides in relation to the dicot, probably due to the arrangement of the tissues (Strachan et al. 2011).

Plants grown in soils at 280 DAA, in both evaluations, at 20 and 40 DAS, the analyzed variables were not affected by treatments. Carmo et al. (2008) evaluated the intoxication of crops grown in soils with different doses of picloram and concluded that corn and sorghum hybrids did not show susceptibility to the picloram at the dose of 160 g ha^{-1}. In addition, D'Antonino et al. (2009b) evaluated the persistence of the 2,4-D (960 g ha^{-1}) and the 2,4-D + picloram (256 + 960 g ha^{-1}). These authors concluded that sorghum was not influenced by herbicides applied in the soil, one day after sowing.

CONCLUSIONS

The plants of cucumber, pigeon pea, and alfalfa were affected by the 2,4-D and triclopyr residues at 40 DAA, therefore, were the species most susceptible to the auxinic herbicide residues. For all dicot species, the 2,4-D + picloram showed residual effect up to 280 DAA. However, the lablab bean was the only one among the dicot evaluated that showed tolerance to the 2,4-D + picloram residual when cultivated in soils at 280 DAA.

The plants of corn and sorghum showed lower chlorophyll content in soils with 2,4-D + picloram residual up to 80 DAA. However, these species were not affected by the auxinic herbicide residues in the soil in relation to the height and biomass production of the plants. In addition, monitoring of chlorophyll content was an important variable in the detection of auxinic herbicides residues in the soil.

TABLE VIII
Sorghum plants grown on soils with auxinic herbicide residues at 40, 80, and 280 days after application of the herbicide (DAA) and evaluated at 20 and 40 days after sowing (DAS).

	40 DAA		80 DAA		280 DAA	
	20 DAS	40 DAS	20 DAS	40 DAS	20 DAS	40 DAS
Plant intoxication	Intoxication scale[a]					
2,4-D + picloram	2	1	1	1	1	1
2,4-D	1	1	1	1	1	1
Triclopyr	1	1	1	1	1	1
Control	1	1	1	1	1	1
Chlorophyll content	SPAD[b]					
2,4-D + picloram	19.7 b	19.9 b	20.2 b	19.5 b	39.2	35.0
2,4-D	24.9 a	25.8 a	23.8 a	23.7 a	40.7	35.6
Triclopyr	26.0 a	26.5 a	24.0 a	25.1 a	41.1	35.4
Control	25.7 a	28.1 a	24.2 a	24.4 a	41.3	36.6
CV[c] %	4.17	11.0	4.03	9.80	9.10	12.0
Height	cm					
2,4-D + picloram	16.2	42.0	16.4	33.9	27.2	54.2
2,4-D	16.5	41.5	16.0	32.3	28.3	55.5
Triclopyr	16.2	40.1	16.1	32.0	27.7	53.7
Control	16.4	42.9	16.1	32.5	28.7	54.9
CV[c] %	6.76	8.76	9.87	9.80	6.79	9.88
Shoot dry weight	g pot^{-1}					
2,4-D + picloram		24.9		31.1		34.8
2,4-D		28.4		28.6		35.2
Triclopyr		28.3		28.7		35.5
Control		28.2		29.5		34.8
CV[c] %		14.2		11.7		8.91
Roots dry weight	g pot^{-1}					
2,4-D + picloram		13.1		16.3		18.8
2,4-D		15.0		16.4		18.8
Triclopyr		15.6		16.3		18.4
Control		15.4		17.1		18.9
CV[c] %		11.6		6.98		6.03

[a] Intoxication scale without statistical analysis: 1 = absence, 2 = slight, 3 = moderate, and 4 = severe.
[b] Soil plant analysis development.
[c] Coefficient of variation.
Doses applied: 2,4-D + picloram, 720 + 192 g a.e. ha^{-1}; 2,4-D, 1.340 g a.e. ha^{-1}; triclopyr 960 g a.e. ha^{-1}.
Means followed by the same letters in the columns do not differ by Tukey test at 5% significance.

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