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Article

Anatomical Changes on the Stem and Leaves of *Solanum lycopersicum* Caused by Different Concentrations of Picloram + 2,4-D, in Two Different Types of Soil

*Modificações Anatômicas no Caule e na Folha de **Solanum lycopersicum** Provocadas por Diferentes Concentrações de Picloram + 2,4-D em Dois Tipos Distintos de Solo*

BATISTÃO, A.C.[1]
YAMASHITA, O.M.[2]
SILVA, I.V.[2]
ARAÚJO, C.F.[2]
LAVEZO, A.[3]

ABSTRACT - Contamination by herbicides with a prolonged effect on the soil can cause anatomical changes in sensitive plants. Thus, this study aimed at verifying the anatomical changes of tomato stem and leaves caused by different concentrations of picloram in two classes of soil from the Amazon region. The study was developed at UNEMAT, Alta Floresta - Mato Grosso state, in a CRD, in a 2 x 5 factorial arrangement, with four replications. A clayey Rhodic Hapludox (LVAw) and a sandy clay loam Typic Ustipsamments (RQo) were contaminated with 0, 1, 2, 3, and 4 L ha^{-1} of Tordon®, leaving the soil exposed to weathering. One-hundred and twenty days after the application of the herbicide, 10 tomato seeds were sown in samples of both soils. Thirty days after sowing, cross sections of stem and leaf were fixed in FAA$_{50}$, immersed in methacrylate, cut into a rotary microtome and stained with toluidine blue. The thickness of stem and leaf tissues was analyzed. Data were submitted to analysis of variance and regression analysis by the statistical program Sisvar. The increase in the concentration of picloram caused an increase in the thickness of the leaf blade and in the vascular bundle of the leaf in both soils, with greater effect in the LVAw, where there was tissue disorganization, with irregular and quite collapsed lacunar parenchyma cells and large intercellular spaces. There was also an increase in the diameter of the cortex and in the vascular cylinder of the stem up to the concentration of 2 L ha^{-1}, but in the RQo, plants had more flattened cells with conspicuous intercellular spaces. The anatomical structures of the leaf were more affected by this herbicide.

Keywords: leaf blade, synthetic auxin, Oxisol, Entisol, tomato.

RESUMO - A contaminação por herbicidas de efeito prolongado no solo pode provocar alterações anatômicas de plantas sensíveis. Assim, este trabalho objetivou verificar as modificações anatômicas de caule e folha de tomateiro provocadas por diferentes concentrações de picloram em duas classes de solo da região amazônica. O trabalho foi desenvolvido na UNEMAT, em Alta Floresta-MT, em DIC, em esquema fatorial 2 x 5, com quatro repetições. Contaminaram-se um Latossolo Vermelho-Amarelo Ácrico (LVAw) e Neossolo Quartzarênico Órtico (RQo) com 0, 1, 2, 3 e 4 L ha^{-1} de Tordon®, deixando o solo exposto às intempéries climáticas. Aos 120 dias após a aplicação do herbicida, foram semeadas 10 sementes de tomateiro em amostras de ambos os solos. Aos 30 dias após a semeadura, secções transversais do caule e folha foram fixadas em FAA$_{50}$, emblocadas em metacrilato,

*** Corresponding author:**
<yama@unemat.br>

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[1] Universidade Federal de Santa Maria, Santa Maria-RS, Brazil; [2] Universidade do Estado de Mato Grosso, Alta Floresta-MT, Brazil; [3] Grupo AMaggi Agropecuária, Querência-MT, Brazil.

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cortadas em micrótomo rotativo e coradas com azul de toluidina. Analisou-se a espessura dos tecidos do caule e folha. Os dados foram submetidos à análise de variância e regressão pelo programa estatístico Sisvar. O aumento da concentração de picloram provocou incremento na espessura do limbo foliar e do feixe vascular da folha em ambos os solos, com maior efeito em LVAw, onde ocorreu desorganização nos tecidos, com células do parênquima lacunoso irregulares e bastante colapsadas, com amplos espaços intercelulares. Também houve aumento no diâmetro do córtex e do cilindro vascular do caule até concentração de 2 L ha^{-1}, mas em RQo as plantas apresentaram células mais achatadas e com espaços intercelulares conspícuos. As estruturas anatômicas da folha foram mais afetadas pelo herbicida.

Palavras-chave: limbo foliar, auxina sintética, Latossolo, Neossolo, tomate.

INTRODUCTION

The use of herbicides to control invasive plants in pastures has contributed substantially to the expansion of cattle production, since it is an effective and economically viable alternative. Among the herbicides registered for pastures in Brazil, pre-formulated mixtures of herbicides from the synthetic auxin chemical group, such as picloram + 2,4-D and picloram + fluroxypyr, stand out (Rodrigues and Almeida, 2005).

These herbicides show a structural similarity with the natural auxins found in plants (Oliveira Jr, 2011) and are commonly used on several annual and perennial crops for an efficient control of woody and climbing plants (Rodrigues and Almeida, 2005).

Picloram presents a long residual effect in the soil, having a marked influence on the efficiency in controlling weeds, but it can cause problems to the development of sensitive species (Roman et al., 2007), such as tomato, lettuce and cucumber (Nascimento and Yamashita, 2009), cotton, grape, and tobacco (Constantin et al., 2007a,b; Oliveira Jr et al., 2007), mainly when they are cultivated in area that were treated with this product (Silva et al., 2007).

Among the visual symptoms caused by picloram residues in the soil, it is possible to highlight a drastic reduction in seedling height, stem twisting and an abnormal leaf growth in *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (tomato), as well as a gradual yellowing of the leaves in *Lactuca sativa* (lettuce) (Nascimento and Yamashita, 2009), shriveling and bending of the leaf blade edges, with later chlorosis and necrosis of leaves and stem, in *Beta vulgaris* (beet), *S. lycopersicum* and *C. sativus* (Santos et al., 2013).

In Brazil, the influence of herbicides on plant morphology has been studied, such as the application of trifuralin, pedimentalin and 2,4-D on *Euphorbia heterophylla* (Deuber et al., 1977), of lactofen on soybean (Damião Filho et al., 1992), of nicosulfuron on maize (Môro and Damião Filho, 1999), of glyphosate on eucalyptus (Santos et al., 2008), of 2,4-D, diquat and glyphosate on *Polygonum lapathifolium* (Costa et al., 2011), of quinclorac on junglerice (Ferreira et al., 2012), of diquat, glyphosate and imazapyr on the leaf anatomy of *Brachiaria subquadripara* (Belo et al., 2011), among others.

Even in apparently healthy plants, anatomical studies allow the best evaluation of the injuries caused by herbicidal residues, since they provide important information about the structural changes in the proportion of plant tissues.

Thus, the objective of this work was to verify the anatomical changes in the stem and leaf of tomato caused by increasing concentrations of picloram + 2,4-D in two soil classes found in the Amazon region.

MATERIAL AND METHODS

The study was conducted in Alta Floresta, located in the extreme north of the State of Mato Grosso, at the coordinates of 09°52’18" south latitude and 56°06’4" west longitude, with an altitude of 280 m. The climate is Awi-type, according to the Köppen classification, rainy tropical with a clear dry season. The temperature varies between 20 and 38 °C, with a mean of 26 °C. Rainfall is around 2,400 mm year^{-1}, with an average annual relative humidity of 70% (Ferreira, 2001).

The study was conducted in an experimental area belonging to the Universidade do Estado de Mato Grosso, Campus Universitário de Alta Floresta, where, in different soil classes, the persistence of increasing doses of the herbicide Tordon®, whose formulation contains picloram, was evaluated.

The analysis of the herbicide interference on the anatomy of the stem and leaf of tomato was carried out in two different soil classes: a clayey Rhodic Hapludox (LVAw) and sandy clay loam Typic Ustipsamments (RQo), whose chemical and textural analyses are found in Table 1. Thus, the experimental design was completely randomized (CRD), in a 2 x 5 factorial arrangement (2 classes of soil and 5 herbicide doses), with four replications.

Table 1 - Chemical and textural analysis of the used Rhodic Hapludox (LVAw) and Typic Ustipsamments (QRo) (RQo). Alta Floresta - Mato Grosso, 2014

Sample description	pH	MO	P (mehl)	K	Ca	Mg	Al	H+Al
	(H_2O)	(dag kg^{-1})	(mg dm^{-3})	($cmol_C$ dm^{-3})				
RQo	4.9	1.87	1.2	0.03	0.4	0.2	0.8	4.6
LVAw	5.7	1.93	3.3	0.06	1.5	0.6	0.0	2.9
Sample description	SB	t	T	V	m	Areia	Argila	Silte
	($cmol_C$ dm^{-3})			(%)		(g kg^{-1})		
RQo	0.60	1.43	5.2	12.3	51	690	244	66
LVAw	2.20	2.16	5.0	43.2	0	273	578	149

SB = Sum of bases; t = Effective CTC; T = CTC pH 7.0; V = Base Sat.; m = Aluminum Sat.; P, K extracted by: HCl 0.05 mol L^{-1} + H_2SO_4 0.025 mol L^{-1}; Ca, Mg, Al extracted by KCL 1 mol L^{-1}; M.O. = Colorimetric method; H + Al extracted by: SMP buffer solution at pH 7.5.

Both soils were collected in forest areas, free of herbicide application. The soil surface was cleaned, removing the vegetation and a layer up to 5 cm of depth. Afterwards, the soil was removed using shovel and hoe and it was placed in plastic containers with a volume of 20 L.

Subsequently, the soils were contaminated with four herbicide concentrations, proportionally to the recommendation for weed control in pastures (4.0 L ha^{-1} of Tordon®), whose formulation contains 64 g L^{-1} (6.40% m^{-1}) of the acid equivalent of picloram. Concentrations were 25% (1.0 L ha^{-1}), 50% (2.0 L ha^{-1}), 75% (3.0 L ha^{-1}) and 100% (4.0 L ha^{1}), in addition to the soil without contamination (control sample).

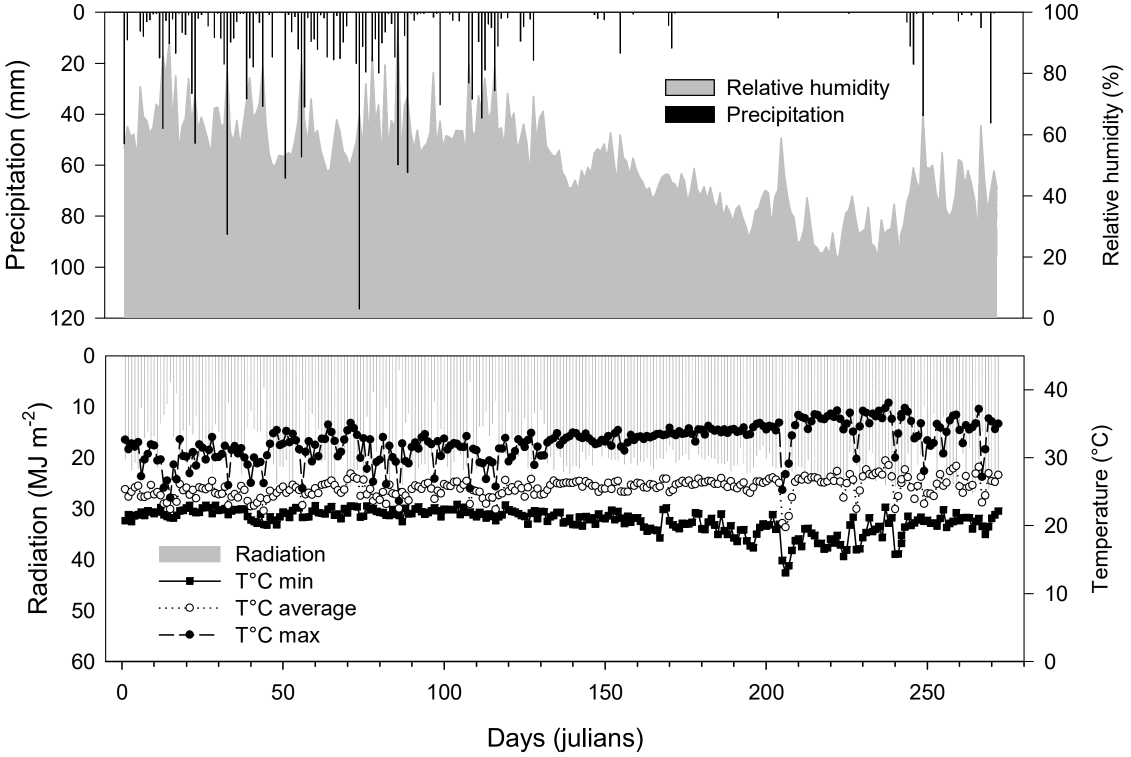
In the application of the herbicide, a CO_2 pressurized backpack sprayer was used, with a constant pressure of 1.41 kgf m^{-2}, equipped with an 80.50 fan-type nozzle, providing a spraying volume of 200 L ha^{-1}. This volume of syrup was manually mixed for herbicide incorporation immediately after spraying in each soil class.

The contaminated soil classes were exposed to natural conditions of climatic variation, and submitted to all weathering occurred in the experimental period. Temperature variation, humidity and rainfall are presented in Figure 1. One-hundred and twenty days after application (DAA), soil samples were removed, and they were used to fill plastic pots with a capacity of 0.5 L. Each pot represented a sample unit.

In each pot, 10 tomato seeds (*Solanum lycopersicum*) were sown at a depth of 0.01 m, and were then kept in a protected environment for germination and seedling development. Seeds were previously submitted to a standard germination test, according to the methodology described by Brazil (2009), presenting a mean germination of 96%.

Thirty days after sowing (DAS), one plant per replication was randomly removed in each treatment; it was fixed in FAA_{50} (formaldehyde, glacial acetic acid and 50% ethanol; 5:5:90 v/v) for 48 hours and placed in 70% ethanol (Johansen, 1940).

In the anatomical analyses, cross sections were made in the middle third area of the stem and in the middle leaf are, by including the samples in methacrylate (Meira and Martins, 2003).



Source: UNEMAT Meteorological Station, Campus Universitário de Alta Floresta.

Figure 1 - Climatic characterization of Alta Floresta - Mato Grosso state, in the period from January to June 2014.

The blocks were cut crosswise in an automatic advance rotary microtome, with the use of disposable steel knives. The sections, which were 8 μm thick, were stained with toluidine blue. The blades were mounted between blades and coverslips in Permount synthetic resin.

The blades were analyzed with a Leica ICC50 trinocular photonic microscope (lens: 4x and 10x) connected to a computer and they were examined with the LAZ EZ V1.7.0 software. Photomicrographs of the blades were made, from which they were listed and shown on boards. Stem and leaf tissues were measured five times in five different cross sections of each individual, with the help of the Anati Quanti 2® UFV program (Aguiar et al., 2007).

The variables measured from the cross sections of the stem were: epidermal thickness, cortex thickness and vascular cylinder diameter. As for leaf cross sections, the thickness of the leaf blade in the median leaf area and the diameter of the central vein vascular bundle were analyzed. In addition, a qualitative and quantitative visual analysis of the anatomical structures was performed through micrographs.

For all measured variables, normality tests were performed for the residues (Shapiro and Wilk, 1965) and the homogeneity of variances (Levene, 1960). The analysis of variance by F test was also performed, and, where significant, tests of mean comparison were applied by the Tukey's test with a significance level of 5% for the qualitative factors, and a regression analysis was performed for the quantitative treatments, according to Ferreira (2011).

RESULTS AND DISCUSSION

There was a significant effect of the interaction between soil and herbicide dose factors as for cortex thickness and stem vascular cylinder diameter, as well as for leaf blade and vascular bundle thickness. There was no significance for the isolated factors or for the interaction between the factors in terms of epidermal thickness of the stem (Table 2).

Generally speaking, the middle third of the stem of tomato seedlings presented a layer of flattened and juxtaposed epidermal cells, a cortex with six layers of rounded parenchyma cells

Table 2 - Mean square, P-value and variation coefficient for epidermal thickness (EpT), cortex diameter (CoD) and vascular cylinder diameter (VCD) of cross-sections of the stem and leaf blade thickness (LBT) and central vascular bundle diameter (CVBD) of cross sections of the leaf of Solanum lycopersicon cv. Ângela Gigante cultivated on two distinct types of soil contaminated with picloram + 2,4-D. Alta Floresta - Mato Grosso, 2014

FV	GL	EpT		CoD		VCD		LBT		CVBD	
		MS	P-value	MS	P-value	MS	P-value	MS	P-value	MS	P-value
Soil (S)	1	4039.20	0.23	62878.38	<0.01	223106.65	<0.01	285455.10	<0.01	48198.91	<0.01
Dose (D)	4	3177.95	0.32	11737.43	0.01	28905.65	<0.01	54219.10	<0.01	8181.49	<0.01
(S) x (D)	4	2761.08	0.42	17198.25	<0.01	74177.83	<0.01	53710.37	<0.01	6309.09	<0.01
Error	40	3001.03	-	3240.46	-	390.66	-	1369.79	-	315.97	-
VC (%)		36.51		18.21		3.37		11.77		17.08	

and with an intercellular space and vascular cylinder with four isolated collateral bundles, separated by parenchyma cells and perivascular fibers, initiating wall thickening (Figure 2A-J, L).

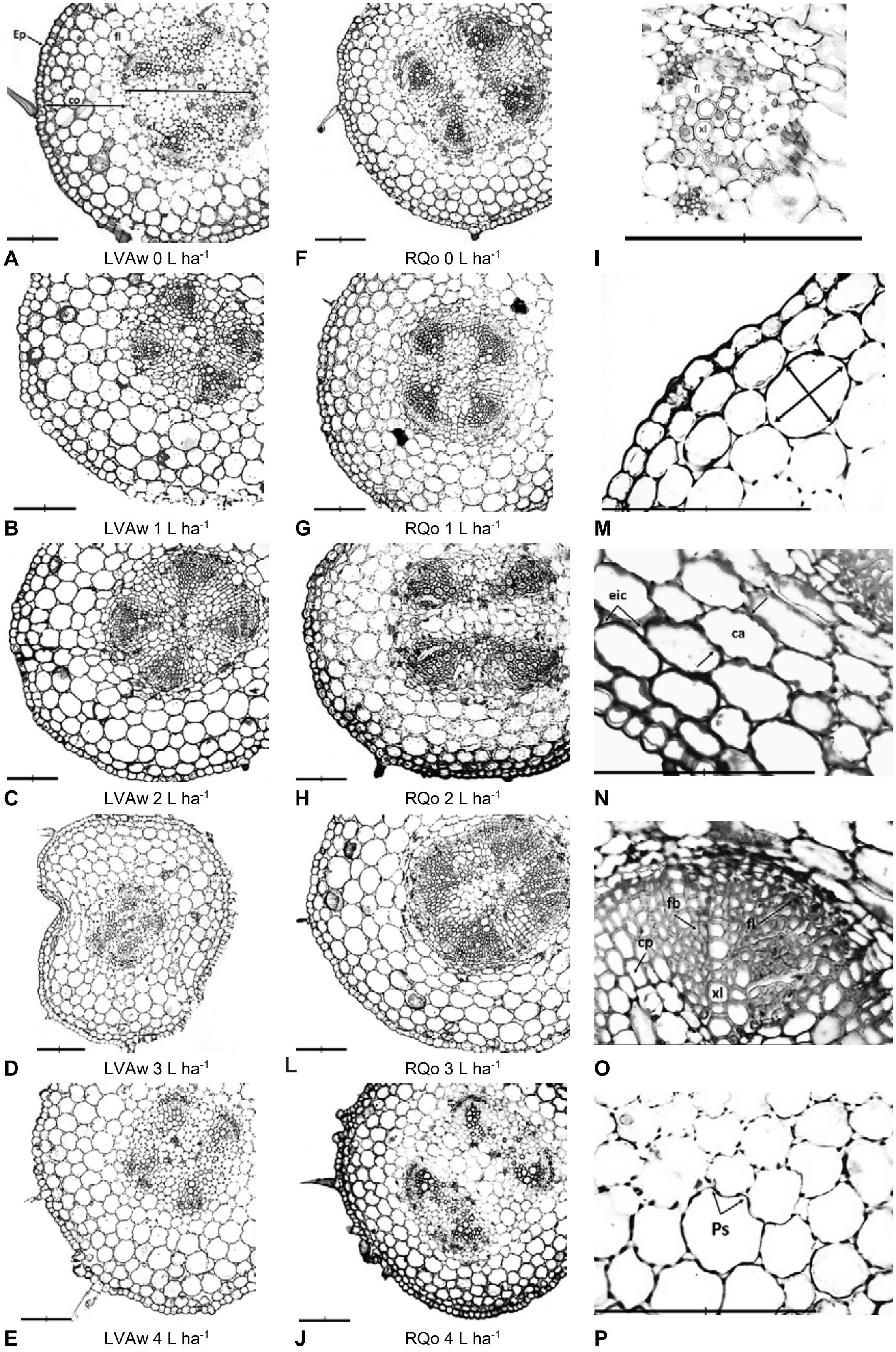
There was a 34.8% increase in the thickness of the stem cortex of tomato seedlings cultivated on a LVAw contaminated with a concentration of 1.93 L ha^{-1}, reaching a maximum thickness of 403.6 µm, with a subsequent 29.5% reduction until the highest concentration. The same behavior was observed in seedlings cultivated on an RQo, where they increased by 28.7%, reaching a thickness of 315.6 µm at the recommended concentration of 1.9 L ha^{-1}, from which there was a decrease to 26.3%, reaching 232.6 µm at the highest concentration. Also, the cortex thickness of plants grown on a LVAw was higher at all herbicide concentrations than those grown on an RQo (Figure 3A).

Visually, there was an increase in the diameter of the cortex up to the herbicide concentration of 2 L ha^{-1} in the stem of seedlings grown on both soils, with a reduction in size at subsequent concentrations (Figure 2A-E, F-J). It is also possible to observe that plants grown on a LVAw showed larger cortical cells at all concentrations (Figure 2M) compared to those grown on an RQo, whose cells were flattened and with conspicuous intercellular spaces (Figure 2N). At the highest concentration, there was a reduction in the parenchyma cells, which showed sinuous cell walls, with more intercellular spaces (Figure 2P).

The increased size of the cortical cells caused by the herbicide is verified in the thickness increase of the cortex, which increased by 34.8% in tomato seedlings grown on a LVAw contaminated with the 2 L ha^{-1} concentration of picloram, reaching a maximum thickness of 403.6 µm, with a subsequent reduction of 29.5% until the highest concentration. The same behavior was observed for seedlings grown on an RQo, where they increased by 28.7%, reaching a thickness of 315.6 µm at half the recommended concentration, from which it decreased by 26.3%, reaching 232.6 µm at the highest concentration. Moreover, the cortex thickness of plants grown on a LVAw was higher at all herbicide concentrations than those grown on an RQo (Figure 3A).

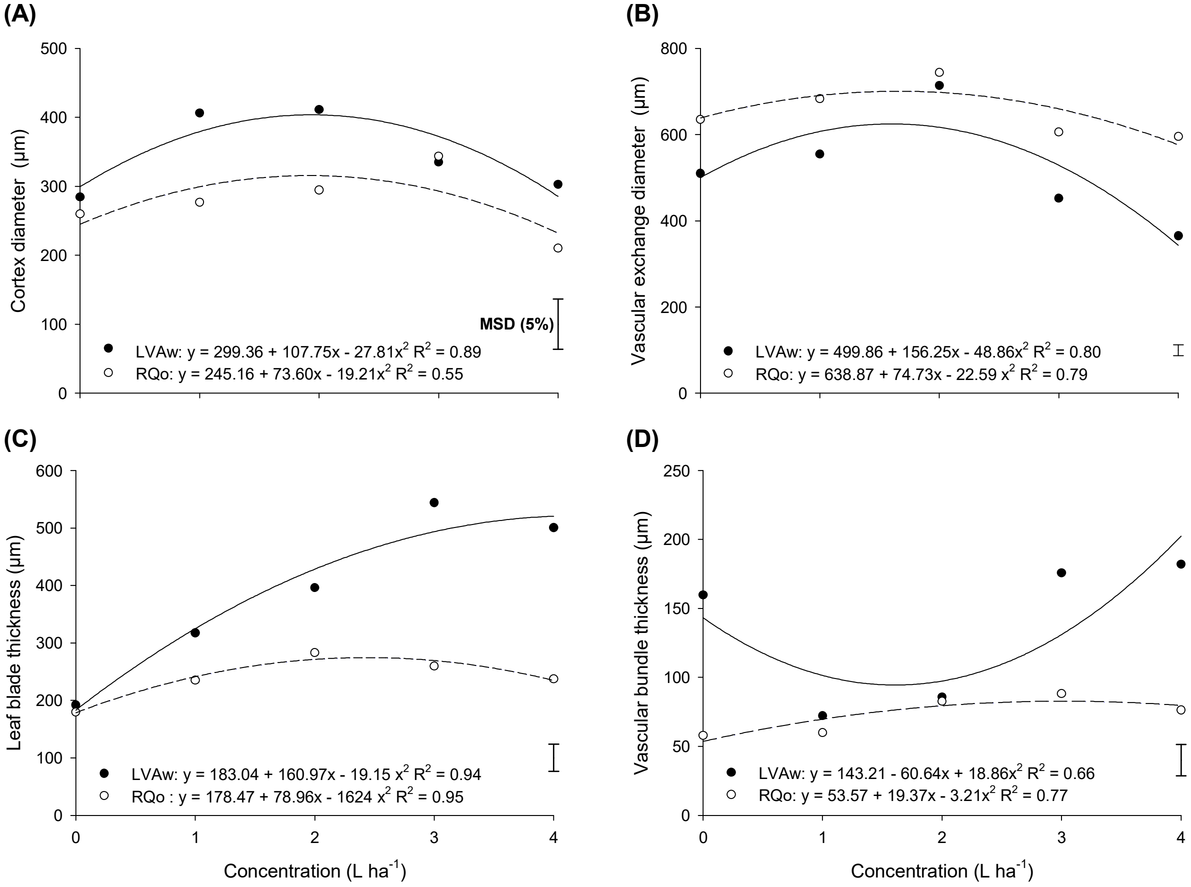
Analyzing the influence of picloram concentrations on the anatomical aspects of the vascular cylinder, it is possible to observe that, up to the herbicide concentration of 1.6 L ha^{-1}, there was a 25% and 9.7% increase in the vascular exchange diameter of plants cultivated on LVAw and RQo, respectively, reaching a maximum diameter of 624.75 and 700.69 µm. At concentrations above 1.6 L ha^{-1}, there was a reduction in the diameter of the vascular cylinder by 45% and 17%, respectively. Moreover, plants cultivated on an RQo had a thicker vascular cylinder and a smaller change in their diameter, compared to those grown on a LVAw (Figure 3B).

In the photomicrographs of the stem, at low herbicide concentrations, there was an increase in the perivascular fibers separating the vascular bundles in LVAw (Figure 2A-C, O), whereas at high concentrations, there is a decrease in these fibers, as well as in the amount of vascularization elements (Figure 2D-E). In the RQo, there is a clear separation of the vascular bundles, with an increase in the size of the parenchyma cells filling and multiplication of the fibers, up to the herbicide concentration of 2.0 L ha^{-1} (Figure 2F-H, O), with the collapse of the xylem and phloem conductance cells (Figure 2H). At the highest concentrations, there was a decrease in the amount of conductance cells, a decrease in the amount and a smaller size of cells from the fundamental tissue that fill the vascular cylinder (Figure 2I-J).



ca: flat cell; vascular exchange; co: cortex; cv: cp: parenchyma cells; eic: intercellular space: Ep: epidermis; fb: fibers; fl: phloem; Ps: sinuous cell wall. xl: xylem; FV: vascular bundle. Bar = 200 µm.

Figure 2 - Cross section of the middle third of the stem of tomato seedlings sown 120 days after substrate contamination with picloram + 2,4-D concentrations.



MSD: minimum significant difference of the test of mean comparison by the Tukey's test at 5% of probability.

Figure 3 - Diameter of the cortex (A) and vascular exchange (B) of the stem, thickness of the leaf blade (C) and vascular bundle (D) of the leaves of tomato seedlings according to the concentration of picloram + 2,4-D in LVAw and RQo. Alta Floresta - Mato Grosso, 2014.

Comparing the stem of tomato seedlings cultivated on both soil classes, it is possible to observe a cortical cell flattening in the RQo; this effect is observed mainly in cells that are close to the vascular cylinder (Figure 2H-J), as well as the distancing of the vascular bundles, due to the greater number and size of the fundamental filling parenchyma cells (Figure 2A-E, F, J).

These anatomical changes in the stem of tomato seedlings may be related to the action mechanism of hormonal herbicides that affect plant growth in a similar way as natural auxins, but that are more persistent and active (Oliveira Jr, 2011), with a variation of the symptoms according to the concentration (Nascimento and Yamashita, 2009).

Auxin mimic herbicides, such as picloram, may cause the acidification of the cell membrane through the stimulation of ATPases, which will reduce the pH of the apoplast, inducing elongation by increasing the activity of the enzymes related to the decrease in the physical resistance of the cell wall. In addition, low concentrations of these herbicides may stimulate RNA polymerase, resulting in increased RNA and DNA and protein biosynthesis, which will consequently promote the synthesis of auxins and gibberellins (Oliveira Jr, 2011).

These hormones are important for cell stretching, lateral growth control and cell formation (Marchi et al., 2008). They may also stimulate the synthesis of proteins, DNA and RNA, promote accelerated cell division, provide plant growth (Tu et al., 2001), causing cell elongation due to loss of cell wall stiffness, because of the increased cellulase enzyme synthesis and the reduction of the cell osmotic potential caused by the accumulation of proteins (Silva et al., 2007), especially in the new parts of plants (Oliveira Jr, 2011). These effects on plant growth can be noticed at very low doses, especially on sensitive plants (Oliveira Jr, 2011), such as tomato, lettuce and cucumber (Nascimento and Yamashita, 2009), cotton, grapes and tobacco (Constantin et al. 2007a, b; Oliveira Jr et al., 2007).

Currently, the effects on cell growth are observed on the cells of the cortex and of the vascular cylinder of tomatoes cultivated on both soils contaminated with picloram, since, with the increase of herbicide concentration, there was an increase in the cortical cell size and an intense division of the filling parenchyma cells (Figures 2A-E; 3F-J). However, at high concentrations, there was a reduction in size and a disordered cell division in the vascular cylinder cells (Figure 2A-J), with a collapse of the vascularization elements (Figure 2H).

The leaves of seedlings, in general, have a uniseriate epidermis, with juxtaposed and flattened cells on both sides. However, the epidermal cells of the abaxial side increase in size, acquiring a rounded shape near the central vein (Figure 4M). The mesophyll is dorsiventral, with a compacted palisade parenchyma and a lacunar parenchyma with little intercellular space. In the central vein, there is a single collateral bundle, surrounded by sclerenchyma cells (Figure 4L). One or two layers of colenchyma are observed close to the abaxial epidermis (Figure 4M); this number is variable, depending on the contamination dose (Figure 4-J).

The thickness of the leaf blade of tomato plants submitted to herbicide-contaminated LVAw increased according to the concentration of picloram, with a 184% increase in leaf thickness, reaching 521 μm (Figure 3D). However, there was also disorganization in the leaf blade tissues, with lacunar parenchyma cells presenting irregular and quite collapsed forms, with large intercellular spaces (Figure 4N-P). There was also a disruption of the palisade parenchyma, with uneven cells, collapsed, with rupture of the cell wall, losing the compacted characteristic of the tissue (Figure 4N).

Plants cultivated on the RQo had a maximum increase point near the herbicide concentration of 2.43 L ha^{-1}, reaching a leaf blade thickness of 274 μm, with a subsequent reduction of approximately 15% until the highest concentration (Figure 3D). It was possible to observe that mesophyll cells had a larger size, with the appearance of intercellular spaces, compared to the mesophyll cells of plants grown on uncontaminated soils. However, unlike seedlings cultivated on the LVAw, there was no disorganization of the leaf blade tissue, with very few collapsed parenchyma cells (Figure F-J).

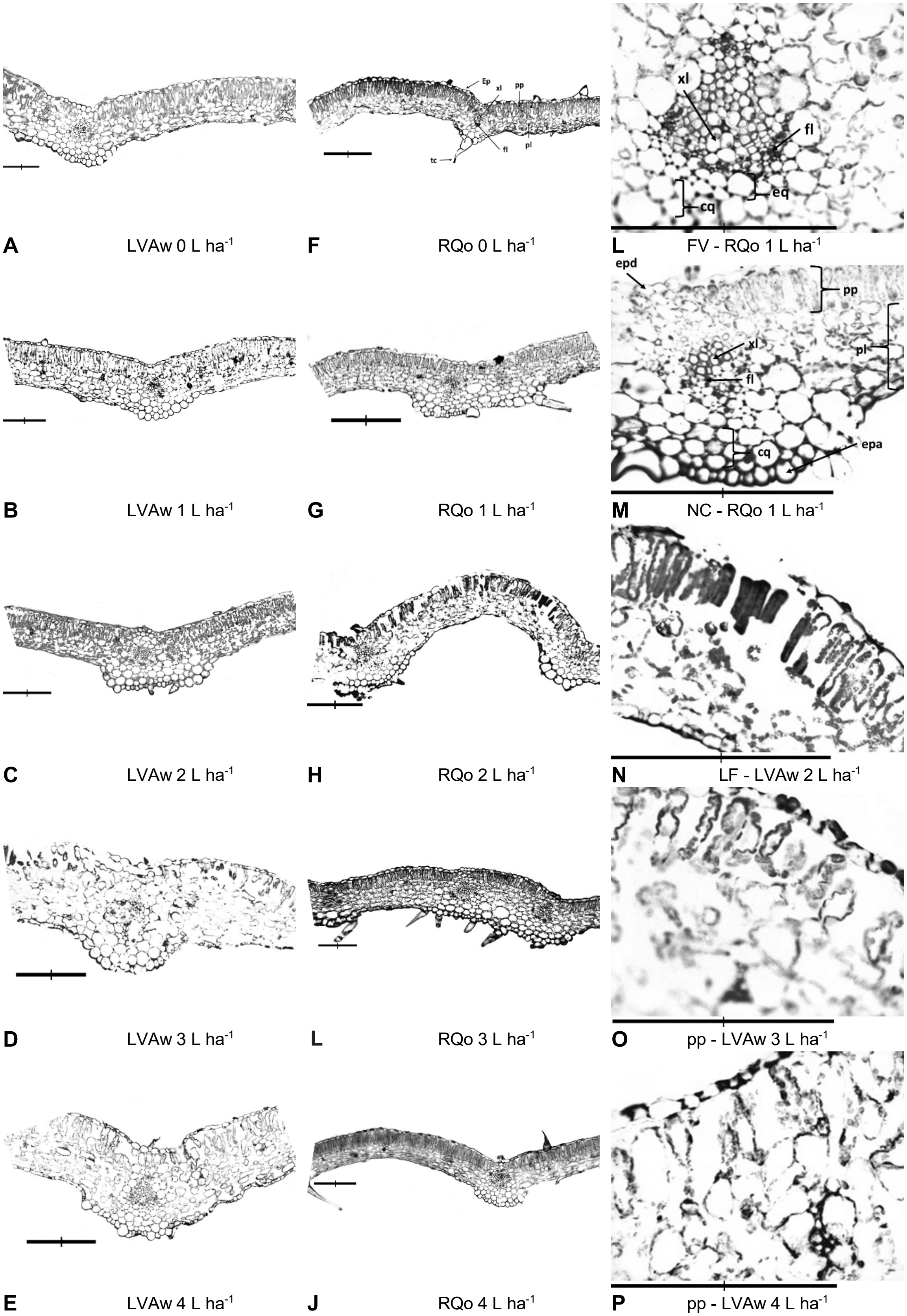
The vascular bundle thickness in the middle leaf area of tomatoes decreased by 34% until the picloram concentration of 1.6 L ha^{-1}, increasing by 111% until the highest concentration; then, seedlings presented vascular bundles that were 39.4% thicker in relation to the control sample, reaching 199 μm (Figure 3C). In the RQo, there was a proportional increase of 49.5% in the diameter of the vascular bundles, reaching only a 80.1 μm thickness at the highest concentration.

As an anatomical alteration of the vascular bundle, it is possible to visually observe an increase in the amount of xylem and phloem conduction elements and fibers, as well as greater spacing between vascularization elements in plants grown on the LVAw (Figure 4A-E), and a higher diameter of the vascular bundles of plants grown on the RQo (Figure 4F-J).

These results are similar to those found by Martins and Castro (1999), where growth regulators containing auxin (100 mg L^{-1} of 20% NAA) caused an increase in the leaf blade thickness, providing greater height and width to the vascular bundle of the main vein of the middle third of the lateral leaflets of *S. lycopersicum* leaves.

These results also corroborate those found by Santos et al. (2008) when studying the effect of four glyphosate formulations on the leaf structure of six *Eucalyptus grandis* clones. These authors verified that, although visually healthy, the sampled leaves showed structural changes in the middle vein and middle leaf area, such as collapsed lacunar parenchyma, rupture of the external periclinal wall of the epidermis, collapse of more internal tissues, such as subepidermal colenchyma, hypertrophy, hyperplasia and plasmolysis.

The increase in leaf blade thickness is due to changes in the epidermal thickness, mesophilic cell volume, number of mesophyll layers, number of intercellular spaces, thickness of the veins or cell wall thickness (Meziane and Shipley, 1999). In this study, there was an increase in the thickness of the leaf blade, mainly due to the larger size of lacunar parenchyma cells and the greater space between parenchyma cells, caused by the herbicide residue in the soil, since any type of biotic or abiotic stress caused by environmental changes, such as soil type or substrate contamination by agrochemicals, can modify the anatomical characteristics of the leaves of the cultivated species (Figueiredo et al., 2013).



cq: colenchyma; epa: abaxial epidermis; epd: adaxial epidermis; eq: sclerenchyma; fl: phloem; FV: vascular bundle; LF: leaf blade; NC: central vein; pl: lacunar parenchyma; pp: palisade parenchyma; xl: xylem. Bar = 200 μm.

Figure 4 - Cross section of the middle third in the region of the central vein of the leaf of tomato seedlings sown 120 days after the contamination of the substrate with concentrations of picloram + 2,4-D.

Generally speaking, the application of picloram caused anatomical changes in the stem and leaves of tomato in both soil classes, proving the sensitivity of the species to this herbicide. However, the effects were more severe in plants grown on the LVAw.

The less expressive changes of the herbicide verified in RQo may be related to the high solubility of picloram in water. RQo's are sandy or loamy-sandy soils with low particle aggregation capacity, conditioned by low clay and organic matter content; in addition, they have relatively large pores (Brady and Weil, 2013), which facilitates mass flow. They also have low cationic exchange capacity and, consequently, they have limitations in the retention and storage of water, nutrients and polar molecules (Embrapa, 2013).

Moreover, picloram molecules present low sorption, high water solubility and high leaching potential, and they can reach underground waters (Bovey and Richardson, 1991; Pang et al., 2000; Close et al., 2003; Silva et al. 2007; D'Antonino et al., 2009).

In the experimental period, high precipitations occurred (Figure 1), leaching part of the picloram in this soil class, thus reducing the availability of the herbicide for root absorption and providing less phytointoxication to plants.

Berisford et al. (2006) reported that picloram presented high lateral and vertical mobility, as well as high persistence in clayey soils, such as LVAw's, promoting greater plant phytointoxication. This may explain the more drastic changes occurred to the leaf and steam tissues of tomato seedlings grown on a LVAw contaminated with the herbicide.

Due to the low sorption of the herbicide and the low retention of the particles in the RQo's, together with the high rainfall of the region, the availability of this herbicide molecule for plant absorption at 120 DAA was lower in this soil class, reducing the phytointoxication of the seedlings and, consequently, leading to less visible anatomical changes.

Moreover, the major changes observed on leaf tissues may be related to the processes of the herbicide in the plant. Picloram acts as a synthetic auxin, causing unequal and uncontrolled growth in susceptible plants (Tu et al., 2001); this causes disturbances in the metabolism of nucleic acids, increasing the enzymatic activity and, consequently, destroying the phloem. It also causes cell stretching, turgescence and cell disruption (Machado et al., 2006), as well as inducing stomatal closure and causing changes in the ionic balance of cells, which, in turn, causes disturbances to the photosynthetic activity and respiration (Pemadasa and Jeyaseelan, 1976).

Another process caused by auxinic herbicides is the stimulation of ethylene production in plants, stimulating the synthesis of abscisic acid, which initially accumulates in the leaves and is then translocated by the plant, influencing the stomatal closure (Mercier, 2004) and reducing the inflow of CO_2 into the leaves. Ethylene promotes the formation of cellulase, reducing the physical resistance of the cell wall, and providing cell elongation by increasing the internal pressure of the cell by water, conditioned by the accumulation of calcium in the cytoplasm (Mercier, 2004; Machado et al., 2006). Thus, with the increase in cell size, phloem obstruction may occur; this reduces the translocation of water and photoassimilated compounds (Belo et al., 2011).

Thus, the anatomical changes of the stem depend on the processes by which the herbicide molecule is translocated to the leaf, characterizing more severe anomalies in the leaf blade tissues. This happens because picloram molecules, when in solution, are rapidly absorbed and translocated to the entire plant, especially to growth points. In addition to this, as the concentration of the herbicide in the soil solution decreases, the concentration of picloram also decreases in the roots and photosynthetic leaves, concentrating in the meristematic regions of the plant (Silva and Silva, 2007), and causing greater toxicity to these parts.

It is worth highlighting that it is still not possible to state that the changes observed in the stem and leaf anatomy are detrimental to the development and production of tomato, since the analysis was conducted at the seedling stage, not allowing them to complete their cycle. Thus, it is interesting to conduct studies at other stages of the plant life cycle to verify if the anatomical changes caused by picloram are harmful.

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