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Phytotoxicity and leaf anatomy of young coffee plants subjected to herbicides exclusively and in associations

Rafael Jorge Almeida Rodrigues[1], Adenilson Henrique Gonçalves[1], Pedro Menicucci Netto[1], Arthur Henrique Cruvinel Carneiro[1], Dalyse Toledo Castanheira[2], Rubens José Guimarães[1], Gladyston Rodrigues Carvalho[3]

[1]Universidade Federal de Lavras/UFLA, Departamento de Agricultura/DAG, Lavras, MG, Brasil
[2]Universidade Federal de Viçosa/UFV, Viçosa, MG, Brasil
[3]Empresa de Pesquisa Agropecuária de Minas Gerais/EPAMIG, Lavras, MG, Brasil
Contact authors: rjragro@gmail.com, adenilsonhg@ufla.br, pedromenicucci2010@hotmail.com, arthurhcruvinel@hotmail.com, dalysecastanheira@hotmail.com, rubensjg@ufla.br, carvalho@epamig.ufla.br
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ABSTRACT

The lack of work force and the damage that weeds can cause to coffee plants are the causes of the growing demand for selective herbicides to be used in coffee farming. Thus, the objective of this study was to evaluate the phytotoxicity symptoms and leaf anatomical characteristics of young coffee plants submitted to application isolated herbicides and also in associations. An experiment was carried out in a protected environment in randomized blocks: four replicates with coffee seedlings (*Coffea arabica* L.) cultivar "Topázio MG-1190", grown in pots with a capacity of 11 liters of substrate. The herbicides applied, in isolation, were: pyrazosulfuron-ethyl (0.015 kg ha^{-1}), saflufenacil (0.049 kg ha^{-1}), imazetaphyr (0.1 kg ha^{-1}), iodosulfuron-methyl (0.0035 kg ha^{-1}), chlorimuron-ethyl (0.015 kg ha^{-1}) and sethoxydim (0.184 kg ha^{-1}). The latter was used in associations with the others. In addition, a control without herbicides was used. Phytotoxicity symptoms were evaluated up to 49 days after application (DAA) and anatomical characteristics at 65 DAA. Saflufenacil exclusively and inassociation with sethoxydim caused visual phytotoxicity symptoms in the leaves and negatively influence in the characteristics of the epidermis thickness of the adaxial face (EAD), thickness of the palisade parenchyma (PAP), thickness of the spongy parenchyma (SPP) and thickness of the mesophyll (MES). The other herbicides, isolated or in associations, didn't cause phytotoxicity symptoms, but had negative influence in the anatomical parameters of the leaf blade. However they did not interfere with the paradermic parameters and the vascular bundle.

Key words: *Coffea arabica*; leaf; selectivity; symptoms.

1 INTRODUCTION

Brazilian coffee farming has been suffering a big transformation process, with the professionalization of producers and the adoption of new technologies, from the crops implantation to the consumer's cup due to its great socioeconomic importance for Brazilian agribusiness. The main impetus for modernizing coffee farming was the need to mechanize phytosanitary control because the labor shortage. Mechanization provided agility and precision in crop management, even though, there was need to increase planting spacing due to machine path. This practice created ideal conditions for the establishment of weeds.

In the first two years after planting, the coffee grows slowly and leaves the soil exposed to light. In that sense, the weed infestation and development is favored, consequently, coffee tree growth can be impaired if control is not carried out in a timely manner, especially in the planting line (Fialho et al., 2010; Ronchi; Terra; Silva, 2007).

Weed control in the young coffee trees on the field, historically, is performed by the traditional method of manual weeding, since few herbicides are efficient in the control and, at the same time, are selective to the crop. Currently, with labor increasingly scarce, disabled and costly, producers have intensified the application of herbicides, often combining two or more products, without knowing the risks that this practice can result, such as: physical incompatibility or potentiation of phytotoxicity (Maciel et al., 2013; Trezzi et al., 2016).

Selective herbicides are those that have the ability to kill or retard the growth of plants of one or more species without harming other plants of commercial interest. Selectivity cannot be determined by simply checking visual symptoms of phytointoxication. There are known examples of herbicides that can decrease crop yields with mild symptoms of intoxication. There are also examples of herbicides that cause marked damage, but that allow to express productive potentials (Silva et al., 2017a; Yu; Powles, 2014).

The characteristics of the leaf surface can indicate the mechanisms that give the plant species its sensitivity, tolerance or resistance when exposed to an herbicidal molecule, also serving to understand the description of phytotoxicity symptoms (Costa et al., 2012). Thus, the anatomical study of leaves can improve knowledge about the barriers that each species imposes on the absorption and retention of herbicides. According to Livramento (2010), the leaves of the coffee tree, for example, are covered by a cuticle layer, which stops the absorption of solutions, giving advantages to the mechanisms of differential absorption.

The demand for selective herbicidal molecules by the coffee community has been drive research. Acetolactate

Synthase (ALS) inhibiting herbicides have been used more frequently in coffee growing due to their effectiveness: low recommended doses, low toxicity to mammals and selectivity to various cultures. Acetyl-CoA carboxylase (ACCase) inhibiting herbicides appear as an alternative to glyphosate, which is a non-selective full-action herbicide for coffee trees, as well as the possibility of association with ALS-inhibiting herbicides. In this context, Castanheira et al. (2019a) and Voltolini et al. (2019) observed that the herbicides chlorimuron-ethyl (ALS) and fluazifop-p-butyl (ACCase) did not cause significant changes in growth and physiological characteristics and morphological characteristics of coffee seedlings even submitted to high doses of these herbicides.

In direct contact with the leaves, the protoporphyrinogen oxidase (PPO) inhibiting herbicides are few selective (Oliveira Junior, 2011). Some PPO-inhibiting herbicides, alone and in association, can cause damage such as reduced growth in young coffee trees (Carvalho et al., 2014; Silva et al., 2017a). Gonçalves et al. (2016) showed the selective potential of saflufenacil alone and in association with glyphosate when applied to the ground.However, the heterogeneity and the difficulty in controlling the weed species found in coffee farming drive the need for selectivity research on more herbicide molecules and associations between them. Thus, the objective of this work was to evaluate the symptoms of phytotocity and the leaf anatomical characteristics of young coffee plants submitted to the application of herbicides isolated and in associations.

2 MATERIAL AND METHODS

The experiment was carried out in the Department of Agriculture, cafeiculture sector, Federal University of Lavras (UFLA), Lavras- Minas Gerais, Brazil,located at latitude 21°13'36.24"S and longitude 44°58'10.41"W , in the period from April to December 2016. The experiment was conducted over three masonry benches, screened 1.2 meters high, 10 meters long, 50% shading and with side screens with sombrite screen. The experimental design used was a randomized block with 4 replicates. Each plot consisted of three seedlings aged six months of *Coffea arabica* L., cultivar Topázio MG 1190 planted in polyethylene pots with a capacity of 11 dm^3. Twelve treatments were applied, 11 with herbicides alone or in mixture and 1 control (treatment with water), totaling 144 experimental units.

The climate of the experiment site is named according to the Köeppen classification as being of the Cwb type (humid temperate climate with dry winter and temperate summer), with an average temperature between 16 °C and 23 °C and rainfall below 2000 mm annually (Reibota et al., 2015). The average monthly temperature (C°), precipitation (mm) and relative humidity (%) corresponding to the period between January and December 2016 were 21.1 °C, 1253.6 mm and 70.9% respectively (Figure 1),this information was obtained through the climatological station of the Engineering Department (DEG - UFLA).

The treatments to evaluate the phytotoxicity symptoms and anatomical leaf characteristics of coffee seedlings submitted to the application of isolated herbicides and in combinations are shown in Table 1. The doses of the herbicides followed the recommendation described in the respective package inserts. The herbicide applications on the seedlings occurred on August 20, 2016, according to the DEG - UFLA weather station, on that day the maximum, minimum and average temperature was 26.8; 15.4 and 20.8 °C respectively, relative humidity of 71.3%. The applications were carried out with a backpack sprayer of constant working pressure (310kPa) based on $CO_2$with a single lance also equipped with a single spray nozzle of type XR 80.03, providing an application volume equivalent to 300 L ha^{-1} of syrup.

Phytotoxicity symptoms were evaluated (means of three evaluating researchers) at 7, 14, 21, 28, 35, 42 and 49 days after application (DAA) of the treatments taking into account the parameter of scores, in which, "1" corresponds to no phytointoxication caused by the herbicide in the plant and "9" corresponding to the death of the entire plant, according to the EWRC scale (European Weed Research Council – EWRC, 1964).

The evaluation of leaf anatomical characteristics was performed 65 days after application of herbicide treatments. Leaves without symptoms were collected in the second node of the plants plagiotropic branch. The plant material was dehydrated in an increasing sequence of ethyl alcohol (10%, 20%, 30%, 40%, 50%, 60%, 70%), remaining 30 minutes in each stage. After dehydration, it was included in methacrylate and sectioned about 8 μm thick, with the help of a rotating microtome, obtaining cross sections of the leaves. The sections obtained were stained with toluidine blue (O'brien; Feder; Mccully, 1964) and the slides were mounted, using Entelan® as the mounting method. The paradermic leaf sections were obtained by printing the epidermis using the universal instant adhesive (cyanoacrylate ester) printing method (Segatto et al., 2004).

The slides were observed and photographed in an optical microscope, model Olympus BX 60, coupled to the Canon A630 digital camera to capture images. For each repetition of treatments, twelve photographs were taken, nine of slides containing transverse sections (three images of the main vein, three of the leaf blade and three of the cuticle of the epidermis of the adaxial face) and three of slides with paradermic sections, always of sections many different.

The images were analyzed with the specific software for image analysis UTHSCSA-Imagetool, version 3.0. The

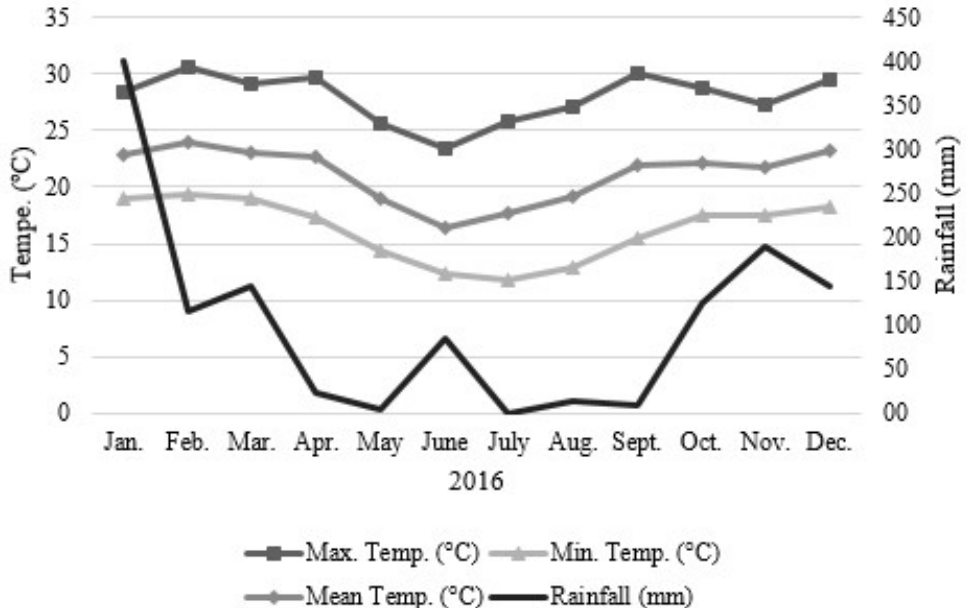

Figure 1: Climatic data of maximum, medium and minimum temperature (°C) and precipitation (mm), between January and December 2016. Lavras – Minas Gerais, Brazil, 2016.
Source: DEG – UFLA

Table 1: Herbicidal treatments tested in the selectivity evaluation of young coffee plants of the cultivar Topázio MG 1190 and their respective doses. Lavras – Minas Gerais, Brazil, 2016.

Herbicide	Mechanism of action	Dose c. p. (L kg ha^{-1})	Dose a. i. (kg ha^{-1})
1. Pyrazosulfuron-ethyl	ALS	0.06	0.015
2. Saflufenacil	PPO	0.07	0.049
3. Imazethapyr	ALS	1.0	0.1
4. Iodosulfuron-methyl	ALS	0.07	0.0035
5. Chlorimuron-ethyl	ALS	0.07	0.015
6. Sethoxydim	ACCase	1.0	0.184
7. Pyrazosulfuron-ethyl + Sethoxydim	ALS+ACCase	0.06+ 1.0	0.015 + 0.184
8. Saflufenacil + Sethoxydim	PPO+ACCase	0.07 + 1.0	0.049 + 0.184
9. Imazethapyr + Sethoxydim	ALS+ACCase	1.0 + 1.0	0.1 + 0.184
10. Chlorimuron-ethyl + Sethoxydim	ALS+ACCase	0.07 + 1.0	0.015 + 0.184
11. Iodosulfuron-methyl + Sethoxydim	ALS+ACCase	0.07 + 1.0	0.0035 + 0.184
12.No Herbicide	-	-	-

characteristics evaluated, in the cross sections of the leaf blade, were: thickness of the epidermis of the adaxial face (EAD- µm), thickness of the palisade parenchyma (EPP- µm), thickness of the spongy parenchyma (EPE- µm), thickness of the mesophile (MES - µm) and thickness of the epidermis of the abaxial face (EAB- µm), of the vascular bundle, the number of xylem vessels (NVX), xylem vessel diameter (DVX- µm) and phloem thickness (EFL- µm) were analyzed . Stomatal density (DEN - number of stomata / mm^2) and stomatal functionality (FUN- polar diameter / equatorial diameter of stomata) were analyzed for the paradermic sections.

The data obtained by phytotoxicity symptoms scale (previously transformed according to the equation $y=(x+1.0)^{0.5}$) and leaf anatomical characteristics were subjected to analysis of variance by the F test at 5% probability, and when significant, compared by the Skott-Knott algorithm at 5% probability of error ($p> 0.05$), using the statistical analysis system SISVAR (Ferreira, 2011).

3 RESULTS

3.1 Phytotoxicity assessment

Table 2 shows the results of the evaluations of the phytotoxicity scale caused by herbicides in young coffee trees. The isolated application of saflufenacil (0.049 kg ha^{-1}) caused the appearance of circular chlorotic injuries, deformations and yellowing of the leaves in all plants at 7, 14 and 21 days after application (DAA) (Figure 2 – A1). At28 and 35 DAA, the phytotoxicity scale decreased (Figure 2 – A2),later,some new shoots showed deformations at 42 DAA, then, at the end of the evaluation the young coffee trees began to develop new leaf buds, indicating recovery from the injuries (Figure 2 –A3). The association saflufenacil + sethoydim (0.049 + 0.184 kg ha^{-1}) caused symptoms that varied between necrotic, deformationsand yellowing in some leaves on all plants at 7, 14, 21, 28, 35 and 42DAA (Figure 2 – B1 and B2). In the end of the 49 DAA, yellowing,circular chlorotic injuries andmarginal necrosis of the new shoots and limbus in old leaves were observed (Figure 2 – B3), but without increasing or decreasing symptoms.

The herbicide imazethapyr (0.100 kg ha^{-1}) used alone, caused small deformations in some plants at 7 DAA and after the second evaluation, symptoms decreased. However, the association imazethapyr and sethoxydim, caused discoloration and deformations on the leaves of some plants appeared at 49 DAA (Figure 3A). Iodosulfuron-methyl (0.0035 kg ha^{-1}) alone and in association with sethoxydim, caused small deformations on the leaves of some plants from 7 DAA to 28 DAA and at 14, 28, 35 and 42 DAA, respectively (Figure 3B). In the isolated and in association application of chlorimuron-ethyl (0.015 kg ha^{-1}), small deformation were observedin all evaluations (Figure 3C). The herbicides pyrazosulfuron-ethyl and sethoxydim and their association did not cause significant symptoms of phytotoxicity in young coffee plants.

3.2 Anatomical features

The values and images of the anatomical characteristics of the leaf limbs of coffee plants submitted to the application of herbicides alone and in associations are shown in Figures 4 and 5. It can be observed that there was a significant difference for the characteristics: thickness of the epidermis of the adaxial face (EAD), palisade parenchymathickness (PAP), spongy parenchymathickness (SPP) and mesophyll thickness (MES).

In foliar applications, the herbicides cross the cuticular barrier reaching the epidermal tissues, thus, most herbicides reduced the thickness epidermis thickness of the adaxial face (EAD). These herbicides are: saflufenacil (0.049 + 0.184 kg ha^{-1}) isolated and its association with sethoydim (0.049 + 0.184 kg ha^{-1}), imazethapyr (0.1 kg ha^{-1}) isolated and its association with sethoxydim (0.1 + 0.184 kg ha^{-1}), iodosulfuron - methyl (0.0035 kg ha^{-1}) isolated, chlorimuron-ethyl (0.015 kg ha^{-1}) isolated, sethoxydim (0.184 kg ha^{-1}), and the association of pyrazosulfuron-ethyl with sethoxydim (0.015 + 0.184 kg ha^{-1})) (Figure 4).

Table 2: Average values (unprocessed data) of phytotoxicity of young coffeeplantscultivar Topázio MG 1190 according to the application of isolated herbicides and in associations. Lavras –Minas Gerais, Brazil, 2016.

Herbicide	Phytotoxicity scale(1)						
	7 DAA	14 DAA	21 DAA	28 DAA	35 DAA	42 DAA	49 DAA
Chlorimuron-ethyl	1.50 dA	1.15 dA	1.20 bA	1.28 cA	1.00 aA	1.19 cA	1.63 cA
Chlorimuron-ethyl + Sethoxydim	1.4 dA	1.00 dA	1.00 bA	1.29 cA	1.08 dA	1.00 cA	1.21 dA
Imazethapyr	2.00 cA	1.00 dB	1.00 bB	1.00 cB	1.00 aB	1.21 cB	1.08 dB
Imazethapyr + Sethoxydim	1.07 dB	1.00 dB	1,00 bB	1.17 cB	1.16 dB	1.22 cB	1.80 cA
Iodosulfuron-methyl	2.14 cA	1.50 cB	1.53 bB	1.24 cB	1.00 dB	1.13 cB	1.23 dB
Iodosulfuron-methyl + Sethoxydim	1.18 dB	1.65 cA	1.29 bB	1.47 cA	1.73 cA	1.88 bA	1.05 dB
No Herbicide	1.00 dA	1.00 dA	1.00 bA	1.00 cA	1.00 dA	1.00 cA	1.03 dA
Pyrazonsulfuron-ethyl	1.24 dA	1.00 dA	1.00 bA	1.03 cA	1.00 dA	1.00 cA	1.00 dA
Pyrazonsulfuron-ethyl + Sethoxydim	1.05 dA	1.00 dA	1.00 bA	1.08 cA	1.00 dA	1.00 cA	1.05 dA
Saflufenacil	3.65 bA	4.08 bA	4.17 aA	3.51 bB	3.12 bB	3.96 aA	2.98 bB
Saflufenacil + Sethoxydim	4.64 aA	5.02 aA	4.26 aA	4.43 aA	4.47 aA	4.50 aA	4.47 aA
Sethoxydim	1.29 dA	1.00 dA	1.00 bA	1.08 cA	1.00 dA	1.00 cA	1.00 dA
Average	1.85	1.7	1.62	1.63	1.55	1.67	1.63
CV (%)(2)	6.98	5.02	9.61	7.31	5.44	9.19	11.60

(1)Phytotoxicity scale (EWRC, 1964) evaluated at 7, 14, 21, 28, 35, 42 and 49 days after application (DAA)/Averages followed by different letters, lower case in columns and upper case in rows, differ significantly by the Skott-Knott test ($p \leq 0.05$)/ (2)CV (%): Coefficient of variation. Means obtained from a value n = 4.

Figure 2: Phytotoxicity symptoms of saflufenacil alone (A) and their association with sethoxydim (B) after 14 (A1 and B1), 28 (A2 and B2) and 49 days after application (A3 and B3). Lavras – Minas Gerais, Brazil, 2016.

Figure 3: Phytotoxicity symptoms of the herbicides imazethapy + sethoxydim (A), iodosulfuron-methyl + sethoxydim (B) and chlorimuron-ethyl (C), 49 days after application. Lavras – Minas Gerais, Brazil, 2016.

Greater thicknesses of the spongy parenchyma and mesophyll were obtained by the control without herbicide application and by the associations between iodosulfuron + sethoxydim (0.0035 + 0.184 kg ha^{-1}), imazethapyr + sethoxydim (0.1 + 0.184 kg ha^{-1}) (Figure 5). The coffee plants treated with the herbicides pyrazosulfuron-ethyl (0.015 kg ha^{-1}) isolated and associated with sethoxydim (0.015 + 0.184 kg ha^{-1}), association of chlorimuron-ethyl + sethoxydim (0.015 + 0.184 kg ha^{-1}) and isolated sethoxydim (0.184 kg ha^{-1}) did not show visual symptoms of leaf poisoning (Table 1), however, they influenced the anatomical characteristics of the leaf blade.

Unlike to the anatomical characteristics of the leaf limbs, there were no statistical differences ($p>0,05$) between the characteristics paradermic cross sections and the vascular bundle of the stomatal function (FUN), stomatal density (DEN), number (NVX) and diameter (DVX) of the xylem vessels and phloem thickness (FLT) (Table 3).

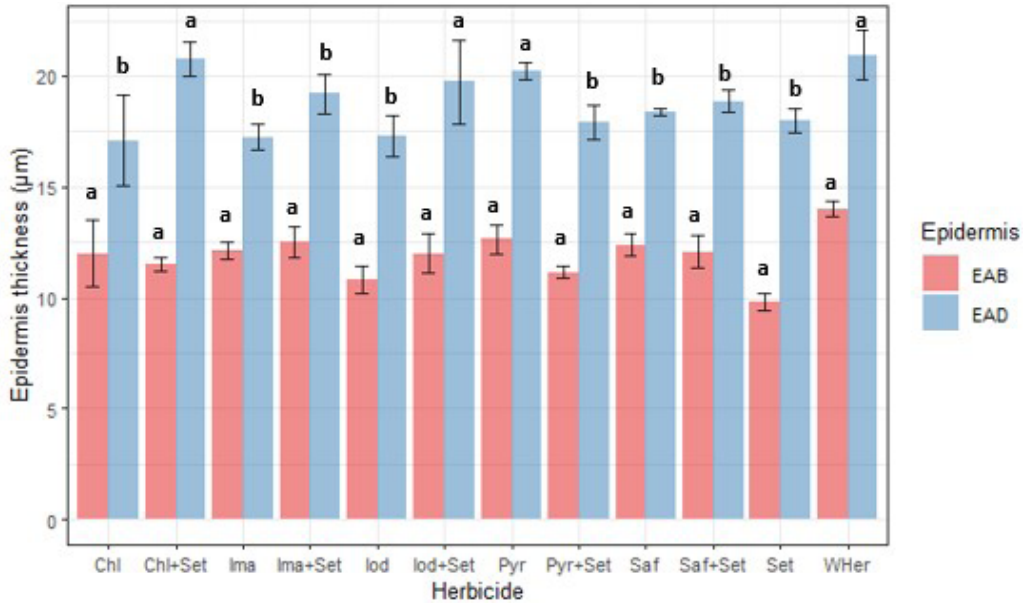

Figure 4: Average and standard deviation of the thickness (µm) of the abaxial (EAB) and adaxial (EAD) epidermis of the leaf blade of coffee seedlings submitted to the isolated and associated chlorimuron-ethyl (Chl) Herbicides (Chl + Set), imazethapyr (Ima) isolated and associated (Ima + Set), iodosulfuron-methyl (Iod) isolated and associated (Iod + Set); pyrazosulfuron-ethyl (Pyr) isolated and associated (Pyr + Set), saflufenacil (Saf) isolated and associated (Saf + Set), Sethoxydim (Set) and control without herbicide application (WHer). Lavras – Minas Gerais, Brazil, 2017.

Average followed by different letters are significantly distinguished by the Skott-Knott test (p≤0.05).Means obtained from a value n = 4.

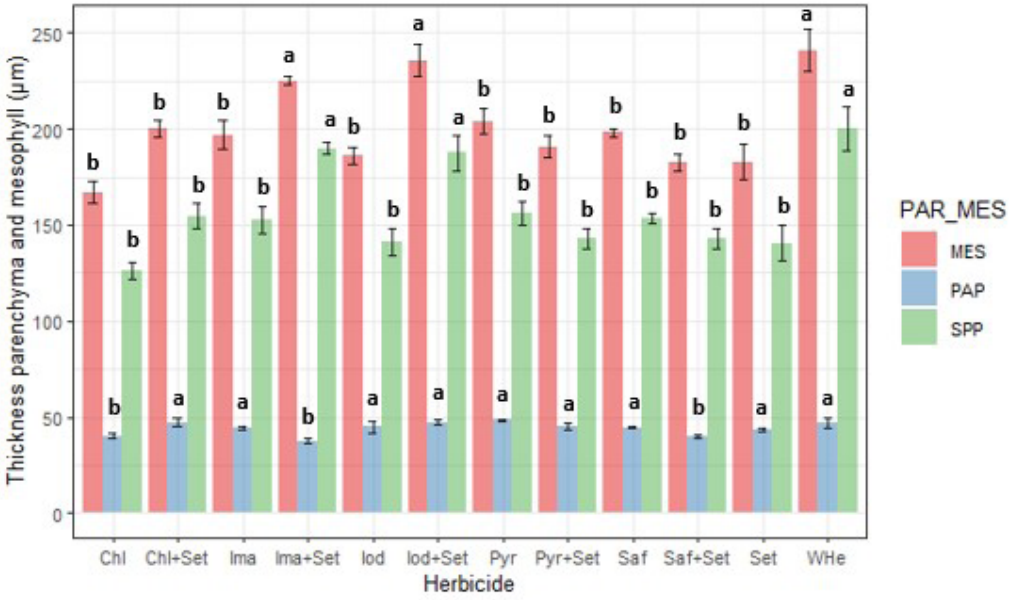

Figure 5: Average and standard deviation of the thickness (µm) of palisade (PAP) and spongy parenchyma (SPP) and mesophilic (MES) of leaf blade of coffee seedlings submitted to the application of the herbicides, chlorimuron-ethyl (Chl) Isolated and associated (Chl + Set), imazethapyr (Ima) isolated and associated (Ima + Set), iodosulfuron-methyl (Iod) isolated and associated (Iod + Set); pyrazosulfuron-ethyl (Pyr) isolated and associated (Pyr + Set), saflufenacil (Saf) isolated and associated (Saf + Set), Sethoxydim (Set) and control without herbicide application (WHer).Lavras – Minas Gerais, Brazil, 2017.

Average followed by different letters are significantly distinguished by the Skott-Knott test (p≤0.05).PAR_MES (Parenchyma and Mesophilic)/ Means obtained from a value n = 4.

Table 3: Average values and standard deviation of the parademic cross sections and the vascular bundle, stomatal function and stomatal density (FUN and DEN), number and diameter of the xylem vessels (NVX and DVX) and phloem thickness (PLT), in Arabica coffee seedlings cultivar Topázio MG 1190 depending on the application of isolated herbicides and in associations. Lavras –Minas Gerais, Brazil, 2017.

Herbicide	FUN	DEN	NVX	DVX	PLT
	Means ± Standard deviation				
Chlorimuron-ethyl	1.6 ± 0.06 a	185.1 ± 32.1 a	139.6 ± 16.3 a	11.7 ± 0.5 a	36.6 ± 5.1 a
Chlorimuron-ethyl + Sethoxydim	1.6 ± 0.03 a	225.3 ± 16.0 a	147.7 ± 19.4 a	11.8 ± 0.6 a	37.9 ± 2.5 a
Imazethapyr	1.7 ± 0.1 a	203.1 ± 6.2 a	168.1 ± 4.4 a	12.8 ± 1.0 a	38.7 ± 6.2 a
Imazethapyr + Sethoxydim	1.7 ± 0.01 a	203.1 ± 21.6 a	140.6 ± 5.9 a	11.8± 0.2 a	35.4 ± 3.6 a
Iodosulfuron-methyl	1.7 ± 0.07 a	211.5± 12.0 a	158.8 ± 9.4 a	12.1 ± 0.9 a	37.6 ± 1.0 a
Iodosulfuron-methyl + Sethoxydim	1.6 ± 0.08 a	188,8± 4.7 a	149.7 ± 2.6 a	11.4 ± 0.3 a	38.7 ± 1.8 a
Pyrazosulfuron-ethyl	1.6 ± 0.02 a	218.4 ± 5.8 a	152.7 ± 13.1 a	12.5 ± 0.3 a	38.3 ± 2.2 a
Pyrazosulfuron-ethyl + Sethoxydim	1.6 ± 0.08 a	199.9± 22.2 a	153.7 ± 5.3 a	12.1 ± 0.5 a	38.6 ± 2.6 a
Saflufenacil	1.6 ± 0.03 a	196.21± 23.0 a	175.6 ± 9.6 a	12.4 ± 0.6 a	40.5 ± 1.7 a
Saflufenacil + Sethoxydim	1.6 ± 0.06 a	206.3 ± 21.7 a	156.6 ± 18.2 a	11.5 ± 0.3 a	39.1 ± 4.3 a
Sethoxydim	1.6 ± 0.05 a	211.5 ± 14.2 a	158.1 ± 12.2 a	12.4 ± 0.5 a	42.0 ± 3.5 a
No Herbicide	1.7 ± 0.08 a	221.5 ± 12.9 a	156.0 ± 14.8 a	12.1 ± 0.4 a	40.5 ± 0.5 a
Average	1.6	205.9	154.8	12.1	38.7
CV (%)	4.34	8.09	8.12	4.7	7.5

Averages followed by different letters in the columns differ significantly by the Skott-Knott test (p≤0.05).Means obtained from a value n = 4.

4 DISCUSSION

4.1 Phytotoxicity assessment

As they are young plants, the initial development stage, contributed to the resultsof the applications of saflufenacil alone and in association. Considering that plants with 2 - 6 pairs of leaves are more sensitive to PPO-inhibiting herbicides, due to the greater leaf wetness and greater penetration due to the lower deposition of cuticular wax (Carvalho; Netto, 2016; Gonçalves; Carvalho, 2017). According to Oliveira Junior (2011); Silva et al. (2017a), plants considered tolerant to PPO inhibitors, can present injuries, from moderate to severe, and that drift with small drops cause the appearance of small white spots on the leaves after application in post-emergence. In addition, due to the contact effect of PPO-inhibitors, even if injuries occur, new leaves are not affected after application (Oliveira Junior, 2011; Silva et al., 2019), which explains the recovery of coffee plants to the application saflufenacil alone at 49 DAA

The association of saflufenacil with other herbicides is necessary to increase the spectrum of weed control (Agostineto et al., 2016; Grossmann et al., 2011; Jhala; Ramirez; Singh, 2013). Although there may be antagonisms between PPO-inhibiting and ACCase- inhibiting (Rodrigues; Almeida, 2018; Rustom et al., 2019), our results showed that sethoxydim potentiated the effect of saflufenacil, when compared to the isolated application, In addition, sethoxydim with systemic translocation and action in meristematic regions, prolonged the effect of saflufenacil, configuring a harmful factor to growing coffee trees.In the same way Carvalho et al (2014), observed that coffee seedlings showed symptoms of phytotoxicity to fomesafen (PPO) mixed with flauzifop-p-buthyl (ACCase), the same effect not occurring in isolated applications of these herbicides.

Some herbicides have the ability to cause phytotoxic effects later (Rodrigues; Almeida, 2018), it can be stated that this characteristic explains the observed symptoms of the association between imazethapyr and sethoxidym.This suggests that coffee seedlings are able to metabolize the molecules imazethapyr and iodosulfuron-methyl more quickly in isolation than in combination with ACCase inhibitors. Karpinsk et al. (2018) have shown the ability to cause late negative effects towheat and barley crops by associating ALS-inhibiting herbicides with ACCase inhibitors.

One of the main mechanisms that confer the natural tolerance of some plant species to ALS-inhibiting herbicides is metabolism at the non-target site (Yu; Powles, 2014), which explains the deformity of the leaves of coffee trees when treated with this group of herbicides, showing that phytotoxic effects occur at the application site. Castanheira et al. (2019a) reported deformations and fissures in the leaves of coffee seedlings 120 days after the application of chlorimuron-ethyl. In the same way, Ronchi and Silva (2003) observed mild symptoms that appeared late in newly planted coffee trees after simulated drift of this herbicide.

In this study, we showed that the herbicides pyrazosulfuron-ethyl and sethoxydim and the association did not cause symptoms of phytotoxicity in young coffee plants, with the potential to be used in the management of weeds. However, only sethoxydim is registred for use in coffee trees (Rodrigues; Almeida, 2018). Even though it does not differ from the control without herbicides, chlorimuron-ethyl (registered for the crop), when used in combination, needs attention since its isolated use caused symptoms that were later noticeable, according to symptoms observed at 49 DAA (Table2). Thus, it is necessary to analyze other parameters, such as leaf anatomy, to certify the safety of the use of these herbicides, especially when used in associations.

4.2 Anatomical features

The first barrier to the penetration of herbicides in foliar applications is the cuticular layer. The anatomical characteristics of coffee trees can differ according to the genotype and undergo adaptations in response to environmental conditions (Batista et al., 2010; Castanheira et al., 2016; Queiroz-Voltan et al., 2014).The higher thicknesses of EAD observed in plants without herbicides, in the applications of isolated pyrazosulfuron-ethyl, chlorimuron-ethyl and iodosulfuron-ethyl associated with sethoxydim, proved that the results were due to the effects of the herbicides, instead of adaptive strategies.

The deleterious effects of the herbicides imazethapyr and sethoxydim are associated with their potential toxic effects, such as the ability to affect the metabolism of sugar, starch, lipids and photosynthesis, thus being able to influence the morphological characteristics of coffee trees (Pereira et al., 2017; Qian et al., 2015; Qian et al., 2013). Protoporphyrinogen oxidase (PPO) inhibiting herbicides contribute to the low translocation of the herbicides to the other parts of the plant (Frihauf; Stahlman; Al-Khatib, 2010; Grossmann et al., 2011) because of the damage caused to the leaf structure in a short period of time. This is demonstrated by the lower interference of the epidermis of the abaxial face (EAB). Silva et al. (2017b) observed in cassava seedlings (*Manihot esculenta*) submitted to applications of fluzifop-p-butyl (ACCase inhibitor), this herbicide selective for species of dicotyledonous plants, nicosulfuron (ALS inhibitor) and fomesafen (PPO-inhibitor), provided smaller thicknesses of the adaxial epidermis, palisade parenchyma, spongy parenchyma and leaf blade thickness, without any visible symptoms of phytotoxicity

Greater thicknesses of the mesophyll and spongy parenchyma in coffee trees favor the accumulation and storage of CO_2, necessary for photosynthesis, helping the plant to withstand adverse conditions such as high temperatures and radiation (Castanheira et al., 2016; Terashima et al. 2011). In addition, the presence of weeds that have greater capacity for extracting water and nutrients, added to periods of water deficit, results in restrictions on the assimilation of CO_2, in which, the oxidative stress will certainly limit growth patterns (Fialho et al., 2010; Fialho et al., 2011; Matos et al., 2013).Therefore, caution is required at the time of application, considering that young coffee trees treated with most herbicides had less thickness of mesophyll and spongy parenchyma, especially for treatments with isolated Chlorimuron-ethyl and associated saflufenacil, which also influenced cells of the palisade parenchyma. For saflufenacil + sethoxydim, the phytotoxicity caused (Table 2) should be included as a way to avoid direct contact between the spray drift and the coffee leaves.

Stomatal density is related to CO_2 absorption and water loss through transpiration. The thickness and number of conducting vessels, on the other hand, favor the transport of photoassimilates, mineral salts and hydraulic conductance, optimizing photosynthesis, plant growth and development (Queiroz-Voltan et al., 2014; Sack; Holbrook, 2006; Shimazaki et al., 2007). Even though it can translocate, the rapid contact action of saflufenacil, causing the death of cells (Dalazen et al., 2015; Grossmann et al., 2010), prevented its effect on the coffee tree's paradermic and vascular bundle characteristics. The results of ALS and ACCase-inhibitorscan be explained by the crop tolerance mechanism, where their potential for damage is minimized (Castanheira et al., 2019a; Yu; Powles, 2014).

As well as the symptoms of phytotoxicity, the time factor is also crucial for observing changes in anatomical leaf characteristics in coffee plants submitted to herbicide application. In this work, changes in anatomical leaf characteristics were identified at 65 DAA, despite that, Voltolini et al. (2019) and Castanheira et al. (2019a) did not find influence of these characteristics at 120 DAA of the herbicides fluazifop-p-butyl and chlorimuron-ethyl respectively.

In this way, the anatomical results and the symptoms of phytotoxicity are indicative of the recovery capacity of the coffee trees when applying herbicides, which corroborate the work of Castanheira et al. (2019b) and França et al. (2010) when using the glyphosate herbicide.In this context, the mechanisms that elucidate this survival capacity, with a focus on translocation and metabolism, associated with the phytotoxicity symptoms identification, are necessary for that the choice of chemical management of weeds is make it safe, efficient and sustainable.

5 CONCLUSIONS

The herbicide saflufenacil exclusively causes circular chlorotic injuries, deformations and yellowing of the leaves in all plants, with stagnation of phytotoxicity symptoms and emission of new sprouts by coffee trees after 42 DAA. In association with sethoxydim, the symptoms of necrosis and deformation were more intense and remained constant

until 49DAA. These treatments negatively influenced the characteristics of the thickness of the epidermis of the adaxial face (EAD), thickness of the palisade parenchyma (PAP), thickness of the spongy parenchyma (SPP) and thickness of the mesophyll (MES).

The other herbicides, exclusively or in associations, even without causing symptoms of phytotoxicity, had a negative influence on the anatomical parameters of the leaf blade, without interfering in the parameters of the and the vascular bundle. Thus, the use of these herbicides (when registered for the crop) should be cautious, applied in a jet directed between the planting lines in the post-emergence of weeds.

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