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Article

# Leaf Morphoanatomy and Biochemical Variation on Coffee Cultivars Under Drift Simulation of Glyphosate

*Modificações Morfoanatômicas e Histoquímicas de Cultivares de Café Submetidos à Deriva Simulada de Glyphosate*

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ABSTRACT - The most widely used herbicide in coffee crop is glyphosate, it inhibits the biosynthesis of secondary metabolites, responsible for structures and substances of plant defense. This work aimed to evaluate the morphoanatomy, total phenols, flavonoids and caffeine in leaves of Arabica coffee submitted to different sub-doses of glyphosate. The treatments were combinations of three coffee cultivars (MGS Travessia, Oeiras MG 6851 and Catuaí IAC 144) and five sub-doses of glyphosate (0.0, 57.6, 115.2, 230.4 and 460.8 g a.e. ha$^{-1}$). The total thickness of the leaf blade, thickness of adaxial and abaxial epidermis, palisade and spongy parenchyma, in addition to the total phenol, total flavonoids and caffeine were evaluated 30 days after spraying. Catuaí showed a reduction in the total leaf thickness, while the others cultivars had an increase. The thickness of adaxial and abaxial epidermis and palisade parenchyma was reduced, and the spongy parenchyma increased with increasing doses of glyphosate, regardless of the cultivars. There was an increase on the total phenols up to 115.2 g ha$^{-1}$ glyphosate, and above this dose, the concentrations of phenols were reduced. The Travessia and Oeiras cultivars obtained a reduction of flavonoids up to 115.2 g ha$^{-1}$; and above this dose, the concentration of flavonoids increased. The Catuaí cultivar showed an opposing behavior of the concentration of flavonoids. Oeiras and Catuaí showed a decrease of caffeine up to 115.2 g ha$^{-1}$ and an increase at higher doses. Travessia showed an increase of caffeine in sub-doses above 115.2 g ha$^{-1}$. The three cultivars showed a higher concentration of phenols in the palisade parenchyma after the application of glyphosate drift. Glyphosate reduces the thickness of the adaxial and abaxial epidermis and palisade parenchyma, and increases the thickness of spongy parenchyma. Doses of glyphosate above 230.4 g ha$^{-1}$ result in the reduction of total phenols. The concentration of flavonoids and caffeine has varying effects with glyphosate application.

**Keywords:** herbicide drift, *Coffea arabica*, epidermis, parenchyma, caffeine, flavonoid.

*RESUMO - O glyphosate, herbicida mais utilizado na cultura do café, inibe indiretamente a rota de biossíntese de metabólitos secundários, responsáveis por estruturas e substâncias de defesa das plantas. Objetivou-se com este estudo avaliar a morfoanatomia foliar, fenóis totais, flavonoides e cafeína em folhas de café arábica submetidas a diferentes subdoses de glyphosate. Os tratamentos consistiram da combinação de três cultivares de café (MGS Travessia, Oeiras MG 6851 e Catuaí IAC 144) e cinco subdoses de glyphosate (0,0; 57,6; 115,2; 230,4 e 460,8 g e.a. ha$^{-1}$). Aos 30 dias após a pulverização, avaliou-se a espessura total da lâmina foliar, espessura das epidermes adaxial e abaxial, parênquima paliçádico e lacunoso, além das concentrações de fenóis totais, flavonoides totais e cafeína.*

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*O cultivar Catuaí teve redução na espessura foliar total, enquanto os demais cultivares tiveram incremento. As epidermes adaxial e abaxial e o parênquima paliçádico reduziram em espessura e o parênquima lacunoso aumentou com o incremento das doses de glyphosate, independentemente do cultivar. Houve aumento de fenóis totais até 115,2 g ha$^{-1}$ de glyphosate; acima desta dose as concentrações de fenóis foram reduzidas. Verificou-se redução de flavonoides até 115,2 g ha$^{-1}$ nos cultivares Travessia e Oeiras; acima desta dose, a concentração de flavonides aumentou. O cultivar Catuaí teve comportamento contrário da concentração de flavonoides. Oeiras e Catuaí tiveram diminuição da cafeína até 115,2 g ha$^{-1}$ de glyphosate e aumento em doses superiores. Travessia mostrou aumento de cafeína a partir de 115,2 g ha$^{-1}$. Os três cultivares apresentaram maior concentração de fenóis no parênquima paliçádico após a aplicação da deriva de glyphosate. O glyphosate reduz a espessura das epidermes adaxial e abaxial e do parênquima paliçádico e aumenta a espessura de parênquima lacunoso. Doses acima de 230,4 g ha$^{-1}$ de glyphosate resultam em redução da concentração de fenóis totais. A concentração de flavonoides e cafeína possui efeitos variados com aplicação de glyphosate.*

**Palavras-chave:** deriva de herbicida, *Coffea arabica*, epiderme, parênquima, cafeína, flavonoides.

## INTRODUCTION

On coffee culture, the interference imposed by weeds is an ongoing issue for the management of the crop. The competition of these plants with coffee may create irreparable damages, mainly during the initial growth phase of the culture, when it has a lower competitive ability, and its growth and nutrition may be affected by weeds (Fialho et al., 2012). The low soil covering promoted by the Young plant favors enough light incidence for the germination, growth and reproduction of weeds (Silva et al., 2008), enabling a high occurrence and intensity of the competition. Therefore, the control is necessary in order to minimize the negative effects on the growth and production of coffee.

Weeds may be controlled in several ways; Chemical control stands out due to its efficiency and low use of labor. Glyphosate is among the most used herbicides for this purpose (Mesquita et al., 2012).

Glyphosate has a complete action and, if it is sprayed on the coffee leaf, even on low amounts, it may intoxicate the plants (França et al., 2010; Carvalho et al., 2013). On the coffee culture, this herbicide must be applied in such a way that avoids the contact with the culture leaves. However, climatic variations or failures on the application technology may cause a drift effect (Costa et al., 2007) – which is a phenomenon in which sprayed herbicide droplets drift to non-target locations.

When in contact with the leaves, glyphosate penetrates through the cuticle, reaches the symplast inside the leaf and, then, follows the route of photoassimilates on the plant, promoting biochemical and anatomical changes (Tuffi Santos et al., 2007). Glyphosate Works by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) enzyme and prevents the production of aromatic amino acids, such as phenylalanine, tyrosine and tryptophan. These amino acids are the precursors of secondary metabolites that are essential to the growth of the plant (Trezzi et al., 2001).

With the drift, a low concentration of the herbicide gets in contact with the plant, and it may inhibit the shikimate route and promote variations on its concentration and the concentration of secondary metabolites (Silva et al., 2009). Therefore, the aim of this study was to evaluate the effects of a simulated glyphosate drift on the leaf morphoanatomy and the production of compounds on the secondary metabolism of Arabica coffee cultivars.

## MATERIAL AND METHODS

The experiment used a 3 x 5 factorial, with three coffee cultivars and five glyphosate doses, on a randomized block design with four replicates. The cultivars used were MGS Travessia, Oeiras MG 6851 and Catuaí IAC 144; and the tested doses were: 0.0; 57.6; 115.2; 230.4; and

460.8 g $ha^{-1}$ of an acid equivalent of glyphosate (isopropylamine salt). The experimental plot was constituted by a pot containing one plant.

The seedlings were produced on polyethylene bags and transplanted, when they showed six pairs of leaves and two reproductive branches, for 10 $dm^{-3}$ pots, filled with sifted soil and cattle manure (3:1). At the time of the transplantation of the seedlings, simple superphosphate was offered to the plants, as a $P_2O_5$ source (400 g $pot^{-1}$), according to the soil analysis (Table 1). The $K_2O$ and N supplementation was conducted on three biweekly applications of potassium chloride (10 g $pot^{-1}$) and ammonium sulphate (2.5 g $pot^{-1}$), starting after 30 days of the transplantation.

At 120 after the seedlings had been planted, when the plants had approximately 21 pairs of developed leaves and 6 reproductive branches, glyphosate was applied in such a way not to reach the upper third of the coffee plants, using a pressurized backpack sprayer, calibrated at a 300 kpa pressure, equipped with one bar and two fan-type spraying nozzles (TT 11002), 1 meter away from each other, offering an application of 200 L $ha^{-1}$ of the product. At the time of the application, he temperature reached 26.4 °C (±1), the relative humidity of the air was of 78% (±3), and the wind speed reached 1.8 km $h^{-1}$.

The evaluations were conducted on samples constituted by eight recently expanded leaves from the last reproductive branch of the coffee plant, which were removed 30 days after the application (DAA) of glyphosate. The leaves were divided into two samples per treatment: one part was immediately used on a morphoanatomical analysis, and the other part was maintained at 5 °C up to the beginning of the laboratory biochemical analyses.

From the fragment samples with approximately 100 $mm^2$ of the median region of the leaf blade, four leaves were subjected to morphoanatomy analysis, and they were firstly fixed in $FAA_{50}$ and stored in ethanol 70%. Then, the material was dehydrated on an ethylic series, followed by the inclusion on methacrylate (Historresina, Leica®, Heidelberg, Germany). With the help of a table rotary microtome with automatic advance (RM 2155 – Leica® Microsystems Inc., Deerfield, USA) transversal sections with 5 μm of thickness were obtained, stained with toluidine blue at pH 4 (O'Brien and McCully, 1981), and then, placed on blades.

The measurement of the leaf morphoanatomy of the coffee plants treated with glyphosate started with the production of three blades, containing 15 cuts each; from each blade, three digital images were obtained, using a camera attached to an optical microscope. Three measurements for each functional character were taken, knowingly: total leaf blade thickness (μm), adaxial epidermis thickness (μm), abaxial epidermis thickness (μm) and thickness of the palisade and spongy parenchyma (μm). To measure the functional characters, the image analysis program Image-Pro Plus version 4.1 for Windows® (Media Cybernetics, Silver Spring, MD, USA) was used.

The phenol histochemical analyses began with the quantification of total phenolics using the Folin-Ciocauteau method. On the total flavonoid analysis, the stratum prepared on the quantification of total phenols conducted on methanol 80% was used, according to Zhishen et al. (1999), with pyrocatechin as the standard (mg/100 g), and the quantification was conducted by spectrophotometry. The caffeine concentration was determined according to the methodology described by Instituto Adolfo Lutz (Pregnolatto and Pregnolatto, 1985), by spectrophotometry.

***Table 1*** - Physical and chemical characteristics of the Red Latosol used on the experiment

| Sand | Silt | Clay | Texture class | Organic matter |
|---|---|---|---|---|
| 38 | 6 | 56 | Clayish | 1.0 |

| Chemical analysis | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | P | K | Ca | Mg | Al | H+Al | SB | t | T | m | V |
| ($H_2O$) | (mg $dm^{-3}$) | | ($cmol_c$ $dm^{-3}$) | | | | | | | (%) | |
| 6.1 | 0.7 | 25 | 1.7 | 0.5 | 0.0 | 3.7 | 2.3 | 2.3 | 6.0 | 2 | 38 |

The data were subjected to the analysis of variance, using the F test ($p \leq 0.05$). Then, the analysis of the significant interaction was conducted, using Tukey's test at 5% probability in the comparison of cultivars and the regression analysis of the glyphosate doses, choosing the models based on their significance, in the biological phenomenon and the determination coefficient.

## RESULTS AND DISCUSSION

### Morphoanatomy

The intoxication symptom shown by the plants treated with glyphosate was the narrowing and chlorosis of recently expanded leaves from the seventh day after the application on (DAA), and these were the most evident effects on plants treated with doses above 230.4 g $ha^{-1}$, regardless of the cultivar (Figure 1). The absorption of glyphosate is slow and it occurs via symplast, then, the translocation occurs on the plant, in the direction of photoassimilates; due to this reason, the first symptoms, such as chlorosis, leaf narrowing and necrosis, are seen on the growth demand zones of the plant.

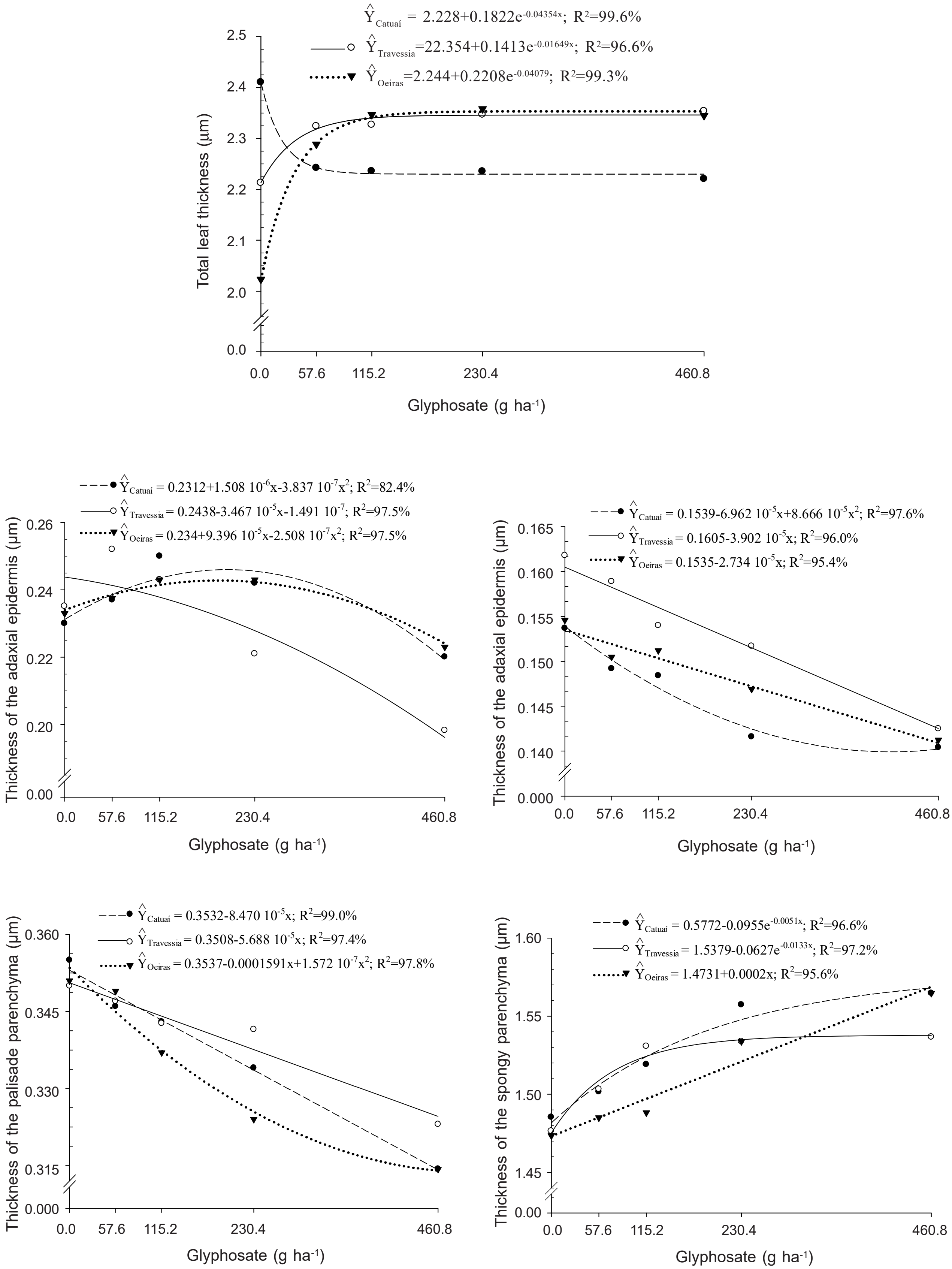
The leaf narrowing and chlorosis, also observed by França et al. (2010), occur from the seventh day after application of glyphosate on. Chlorosis may be explained by several effects caused by glyphosate on the synthesis of aminolevulinic acid (ALA), precursor of the chlorophyll biosynthesis, and glyphosate may reduce the concentration of chlorophyll or degrade it. However, studies with eucalypt showed more severe symptoms, such as necrosis, leaf withering, proliferation due to the death of apical meristems and death of the plants (Tuffi Santos et al., 2007).

When not subjected to glyphosate, the Catuaí cultivar had a higher total leaf thickness value than the Travessia and Oeiras cultivars, with lower differences of approximately 0.19 µm (7.9%) and 0.38 µm (15.8%), respectively. However, with the application of the herbicide, the Catuaí cultivar showed an exponential reduction of the leaf thickness of 0.17 µm (6.97%) up to the dose of 57.6 g $ha^{-1}$ of glyphosate, with linear stabilization of the leaf thickness on higher doses. However, with the increase of the glyphosate doses, Oeiras and Travessia showed an exponential increase of the total leaf thickness of 0.24 µm (11.88%) and 0.09 µm (4.03%), up to the dose of 57.6 g $ha^{-1}$ of glyphosate, respectively, with a tendency for thickness stabilization as the glyphosate dose increased (Figure 2A).

The variation on the total leaf thickness may also be visually observed according to the increase of the concentration of glyphosate applied on the three cultivars (Figure 3).

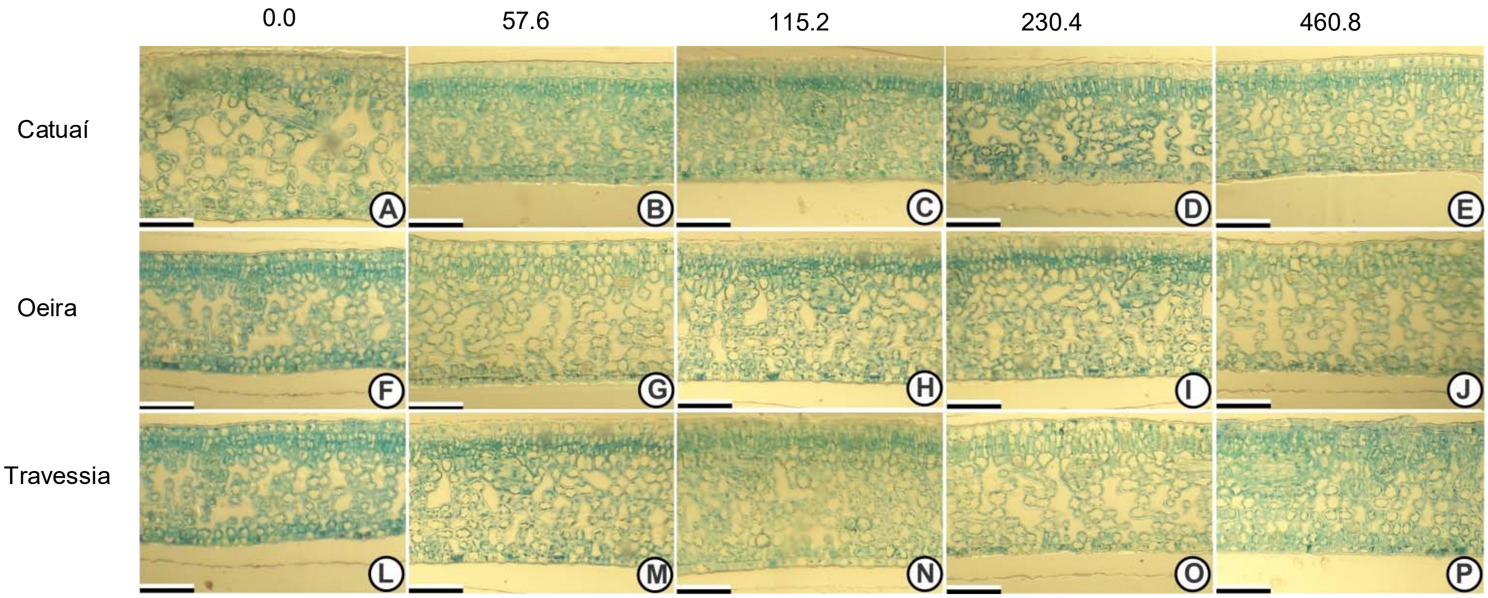
When exposed to low doses of glyphosate, the plants showed effects related to the stress promoted by the herbicide. This reaction may be observed by the increase on the total leaf thickness of the Travessia and Oeiras cultivars. The cell loosening may be associated to the increase of the leaf thickness, also observed by Tuffi Santos et al. (2008), on eucalypts. The cell

***Figure 1 -*** Intoxication symptoms on coffee plants subject to simulated glyphosate drift, 30 days after the application. Red arrows indicate leaf narrowing, and yellow arrows, chlorosis.




(A) total thickness. (B) thickness of the abaxial epidermis. (C) thickness of the adaxial epidermis. (D) thickness of the palisade parenchyma. (E) thickness of the spongy parenchyma.

***Figure 2 -*** Leaf thickness of Arabica coffee cultivars subjected to simulated drift of glyphosate, 30 days after the application.




The (–) scale on the figure corresponds to 100 μm.

***Figure 3*** - Leaf thickness of Arabica coffee cultivars Catuaí (A-E), Oeiras (F-J) and Travessia (L-P), subjected to five doses of glyphosate: 0.0 (A, F and L), 57.6 (B, G and M), 115.2 (C, H and N), 230.4 (D, I and O) and 460.8 g ha$^{-1}$ (E, J e P), 30 days after the application.

loosening may be promoted by the reduction on the ethylene concentration caused by the interruption on the shikimate route, since ethylene inhibits the cell division and expansion (Campos et al., 2009).

Glyphosate also interrupts the synthesis of indole-acetic acid (IAA), and the lack of it may cause a reduction on the total thickness observed on the Catuaí cultivar, considering that the evaluations were made on new leaves, expanded under the effect of glyphosate.

Analyzing the thickness of the adaxial epidermis (Ed), it was observed that all tested cultivars showed a reduction of this variable with the increase of the herbicide dose (Figure 2B). A reduction of 0.03 μm (15.42%), 0.0009 μm (4.22%) and 0.0009 μm (4.18%) was observed on the Travessia, Catuaí and Oeiras cultivars, respectively, when a dose of 460.8 g ha$^{-1}$ of glyphosate was received.

The increase on the glyphosate dose influenced the thickness of the abaxial epidermis (Eb) on the three coffee cultivars (Figure 2C). Travessia and Oeiras obtained a linear reduction on the thickness of the abaxial epidermis (Eb) with the increase of the glyphosate doses. The Catuaí cultivar followed a quadratic reduction trend on the Eb thickness with the increase of the glyphosate doses.

The glyphosate doses influenced the three coffee cultivars on a similar manner regarding the thickness of the palisade parenchyma (PP), and a reduction of 0.027 (7.73%), 0.040 (11.43%) and 0.037 (10.62%) (Figure 2D), on the Travessia, Oeiras and Catuaí cultivars, respectively, occurred in relation to the control at the dose of 460.8 g ha$^{-1}$ of glyphosate.

With the increase on the glyphosate doses, the Travessia and Catuaí cultivars showed an exponential increase on the thickness of the spongy parenchyma (Pl). However, on the Oeiras cultivar, a linear increase on the Pl thickness was observed according to the glyphosate doses (Figure 2E).

The compaction of parenchymatic cells and reduced intercellular spaces confer resistance to the plant against phytopathogens, due to a more difficult colonization by microorganisms (Jerba et al., 2005; Silva et al., 2005; Smith et al., 2006).

Tuffi Santos et al. (2008) did not observe a variation on the epidermal thickness with glyphosate doses on the eucalypt clones. However, a difference was observed on the thickness of the abaxial and adaxial epidermis on this paper.

Differently from the variations observed on this paper, non-significant variations were observed when comparing eucalypt clones subjected to sub-doses of glyphosate regarding the thickness of the epidermis (Tuffi Santos et al., 2008).

By using glyphosate doses on three different clones of eucalypt, a different behavior was observed regarding the thickness of the palisade parenchyma across the clones 15 days after the application (Tuffi Santos et al., 2009). The differentiated tolerance of plants may be explained by the variation on the capacity to absorb glyphosate, by the phenological state and due to differences on the interception and absorption of the product, by the sensitivity of the target enzyme (place of action) and by the ability of the species to get detoxified (Reddy et al., 2008; Carvalho et al., 2009). The thickness of the palisade parenchyma is an important characteristic related to the protection against high light intensity; upon the reduction on the thickness, a photosynthetic stress may occur, caused by luminous stress (Mantuano et al., 2006; Terashima et al., 2006; Taiz and Zeiger, 2013).

On this study, with the increase on the glyphosate doses, a reduction on the total leaf thickness for the Catuaí cultivar and an increase for the Oeiras and Travessia cultivars were observed. The reduction on the total leaf thickness may be explained by the reduction of the palisade parenchyma. However, since the other two cultivars had an increase of the total thickness and a reduction of the palisade parenchyma with the increase of the glyphosate doses, the increase on the total thickness of the Oeiras and Travessia cultivars may be due to the increase on the thickness of the spongy parenchyma.

Different behaviors across the cultivars, as occurred in relation to the palisade parenchyma, are expected, since, even being genetically close, the constitutions of the three cultivars count on the presence of the cross with the Caturra cultivar, in addition to other characteristics, such as leaf color, resistance to diseases and architecture and leaf aspects that allow them to be categorized as different cultivars; the crosses are the following: Catuaí - Mundo Novo x Caturra; Travessia – Catuaí (Mundo Novo x Caturra) x Mundo Novo; Oeiras – Caturra x Híbrido do Timor.

There are several defense mechanisms common to all coffee cultivars, which work conferring structural resistance to the host against the infection by pathogens, which may be divided into pre-formed or constitutive, or post-formed, which originates after the contact with the pathogen-host (Pascholati and Leite, 1995; Agrios, 2005). A higher proportion of palisade parenchyma and lower proportion of spongy parenchyma are considered a pre-formed mechanism, since they increase the resistance of the plant to the attack and initial development of the pathogen (Smith et al., 2006). On this study, it was observed that the relationship became unfavorable to the resistance of the plants, since, the higher the dose of glyphosate, the lower as the proportion of the palisade parenchyma and the higher was the proportion of the spongy parenchyma. It is pointed out that studies that use the inoculation of phytopathogen agents on plants subjected to the herbicide must be conducted to attest the change on the resistance to pathogens.

**Biochemistry**

With the increase on the glyphosate dose, the total phenol concentrations reduced the three cultivars, displaying a sigmoid behavior. When compared to the control, reductions of 3.90 μg 100 $g^{-1}$ (22.54%), 4.00 μg 100 $g^{-1}$ (21.96%) and 2.34 μg 100 $g^{-1}$ (12.50%) were observed on the Travessia, Catuaí and Oeiras cultivars, respectively, at a dose of 460.8 g $ha^{-1}$ of glyphosate (Figure 4). Despite the reduction on the total phenol concentration (Figure 4), a higher phenol concentration was observed on the palisade parenchyma (Figure 5).

Glyphosate inhibits EPSPs, blocking the synthesis of some aromatic amino acids and the phenols derived from them. Some studies indicate that glyphosate may increase the action of phenylalanine ammonia-lyase (PAL), at the same time, preventing the production of secondary compounds, thus, not allowing the phenol concentrations to be expressively reduced or increased after the application of low doses of the herbicide (Silva et al., 2009). Therefore, expressive variations on the phenol concentration were not found on this study and on other studies (Eker et al., 2006; Moldes et al,. 2008; Zobiole et al., 2010).

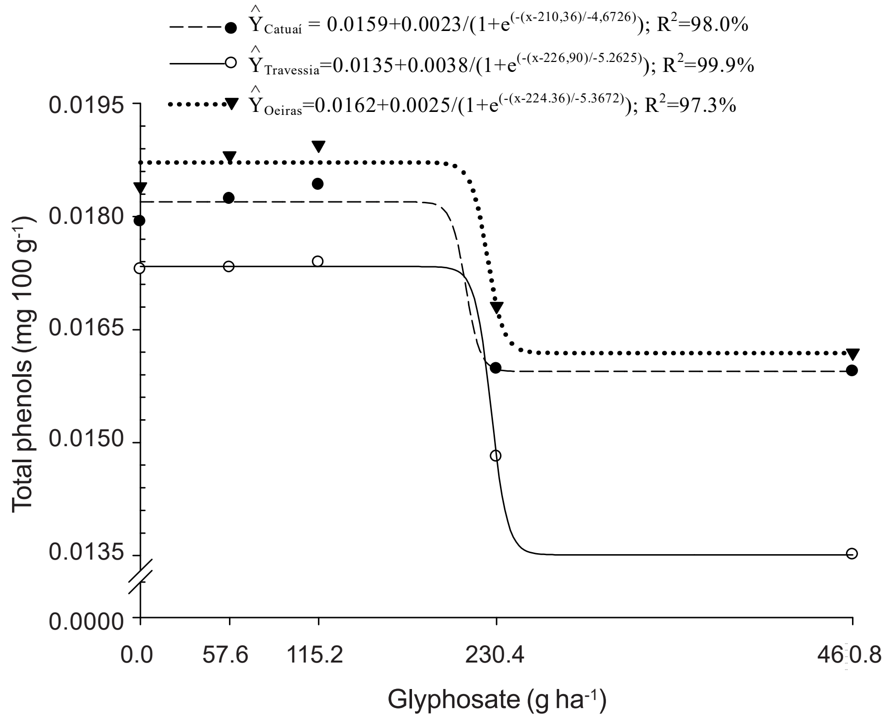
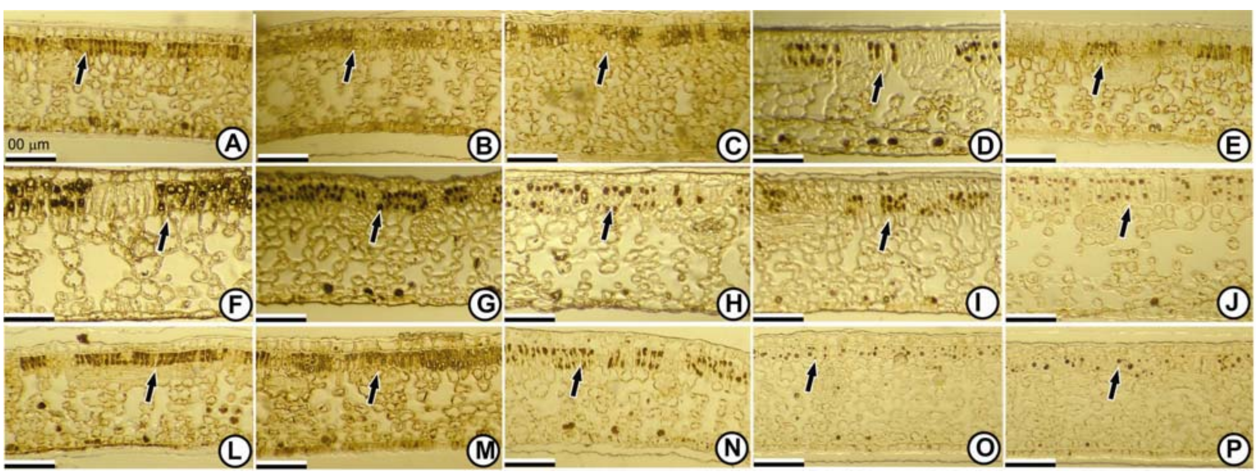



***Figure 4*** - Total phenol concentration on leaves from the coffee cultivars subjected to different doses of glyphosate applied on a simulated drift.




The scale (–) on the figure corresponds to 100 µm.

***Figure 5*** - Histochemical test for phenols on a transversal cut of a leaf from the Catuaí cultivar subjected to five doses of glyphosate: A (0.0 g ha$^{-1}$), B (57.6 g ha$^{-1}$), C (115.2 g ha$^{-1}$), D (230.4 g ha$^{-1}$) and E (460.8 g ha$^{-1}$).

It is observed on the literature that, when subjected to varied types of stress, in general, the plants produce a higher concentration of total phenols (Peixoto et al., 2007; Formiga et al., 2009; Mendes et al., 2011). However, glyphosate inhibits the secondary metabolism of the plants, and phenols are substances that are part of the large group that encompasses the secondary metabolites. They constitute a rather chemically heterogeneous group, constituted by over 10,000 compounds with several functions, whose main function is the defense of the plants against plagues, pathogens and environmental stress (Taiz and Zeiger, 2013).

The total flavonoid concentrations followed a quadratic trend as the glyphosate doses increased on the Travessia e Oeiras cultivars; at lower doses, a reduction and a posterior increased occurred, after the doses of 124.07 and 157.50 g ha$^{-1}$ of glyphosate, respectively (Figure 6). The Catuaí

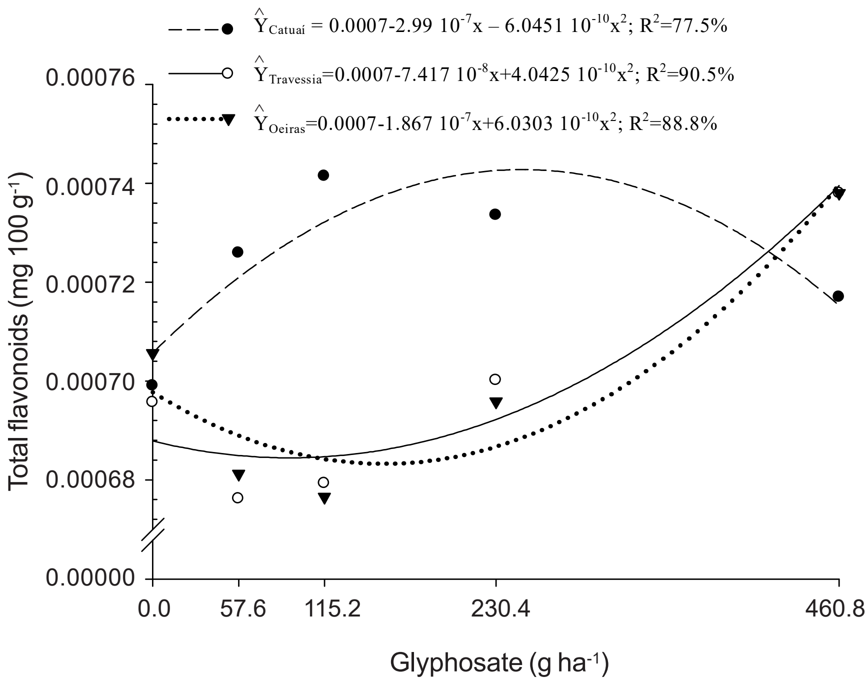



***Figure 6*** - Concentration of total flavonoids on the leaves of three coffee cultivars subjected to different doses of glyphosate applied on a simulated drift.

cultivar showed an increase on the total flavonoid concentration and a posterior reduction from the dose of 252.91 g $ha^{-1}$ of glyphosate. When comparing the flavonoid concentration after the application of 460.8 g $ha^{-1}$ of glyphosate, an increase of 0.039 μg 100 $g^{-1}$ (5.67%), 0.002 μg 100 $g^{-1}$ (0.28%) and 0.031 μg 100 $g^{-1}$ (4.47%) was observed on the Travessia, Catuaí and Oeiras cultivars, respectively.

The role of the flavonoids located on the leaves, stems and flowers is to protect the cells against UV-B rays (280-320 nm), which are intensively absorbed by these substances, allowing the light on the photosynthetically active length, the visible length (400-700 nm), to be captured (Taiz and Zeiger, 2013). Flavonoids are phenolic compounds produced from phenylalanine, an amino acid produced by the shikimate route – precisely the route that is interrupted by the action of glyphosate. However, other cultures also showed no reduction of the mycorrhizal colonization, which is positively influenced by the production of flavonoids (Malty et al., 2006).

The variation on the photosynthesis activity (*A* - mmol $m^{-2}$ $s^{-1}$), stomatal conductance (*gs* - mol $m^{-1}$ $s^{-1}$), transpiration (*E* - mol $H_2O$ $m^{-2}$ $s^{-1}$) and water use efficiency (WUE - mol $CO_2$ mol $H_2O^{-1}$) showed that low glyphosate doses that reach the plant, due to incorrect spraying, may harm the photosynthesis activity of the coffee plant and, consequently, its growth, which was attested by Carvalho et al. (2013, 2014). Cedergreen and Olesen (2010) observed an increase on the photosynthesis rate when glyphosate is applied at low doses. These variations associated to the increase of the flavonoid concentrations, observed on this study, may represent the hormesis effect regarding the vegetal production on the Travessia and Oeiras cultivars.

Another effect on the photosynthesis activity caused by the action of glyphosate is the reduction on the chlorophyll concentrations (Tan et al., 2006); if associated to the reduction of the flavonoid concentrations, observed on this paper with the two lowest doses (57.6 and 115.2 g $ha^{-1}$ of glyphosate) on the Oeiras and Travessia cultivars, it may lead to an increase of the chances of plants showing a photo-oxidative stress.

With the application of glyphosate, there was a decrease on the caffeine concentration on the leaves of the Oeiras and Catuaí cultivars up to the dose of 230.4 g $ha^{-1}$ of glyphosate, with a posterior increase for higher doses of glyphosate. The Travessia cultivar showed an increase on the caffeine concentration up to the dose of 230.4 g $ha^{-1}$ of glyphosate, with a posterior trend to reach stability on higher doses. If compared to the caffeine concentrations with the application of 460.8 g ha-1 of glyphosate, an increase of approximately 0.138 µm 100 g-1 (4.6%) was observed on the Travessia cultivar, as well as a decrease of 0.005 µm 100 g-1 (1.56%) and 0.005 µm 100 g-1 (1.5%) on the Catuaí and Oeiras cultivars (Figure 7).

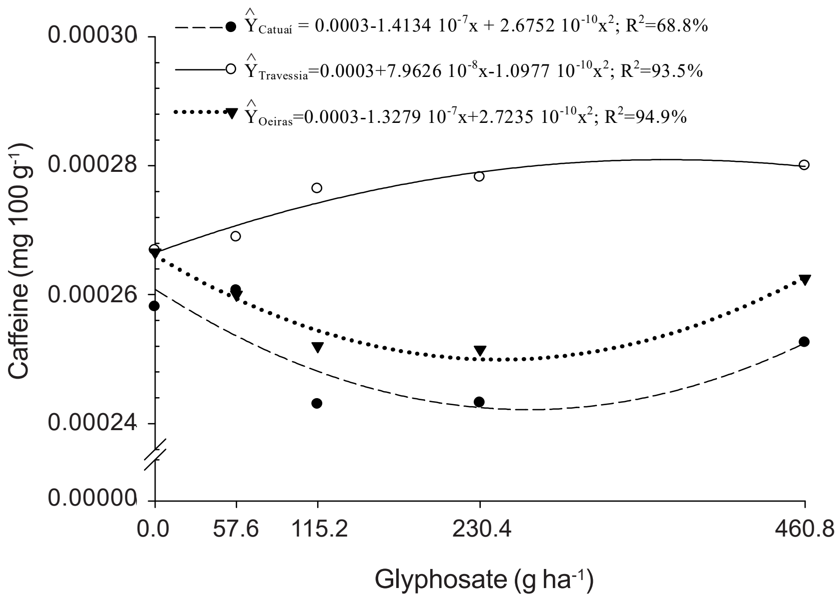



***Figure 7 -*** Total caffeine concentration on the leaves of three coffee cultivars subjected to different doses of glyphosate applied on a simulated drift.

Caffeine is a purine-type alkaloid, it has a heterocyclic ring and it is part of the large group of secondary metabolites, produced from aromatic and aliphatic amino acids, considering that the aromatic amino acids are formed through the shikimate route (Taiz and Zeiger, 2013). Caffeine is a bioactive constituent, with an anti-oxidative function that may show defense actions. The change on this compound may affect the defense of the plant against pathogens (Kuranda et al., 2006).

The glyphosate drift causes morphoanatomical leaf changes, with responses that vary according to the cultivar and the amount of product that reaches the plant. The contact of the herbicide with the coffee leaves may also lead to biochemical changes on total phenols, total flavonoids, and caffeine. These changes may be irreversible on plants and cause other damages.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## REFERENCES


Agrios G.N. **Plant pathology**. 5th. ed. New York: Elsevier Academic, 2005. 922p.

Campos M.F. et al. Desenvolvimento da parte aérea de plantas de soja em função de reguladores vegetais. **Rev Ceres**. 2009;56:74-9.

Carvalho F.P. et al. Water use efficiency by coffee arabica after glyphosate application. **Acta Sci Agron.** 2014;36:373.

Carvalho F.P. et al. Photosynthetic activity of coffee after application of glyphosate subdoses. **Acta Sci Agron**. 2013;35:109-15.

Carvalho S.J.P. et al. Herbicide selectivity by differential metabolism: considerations for reducing crop damages. **Sci Agríc.** 2009;66:136-42.

Cedergreen N., Olesen C.F. Can glyphosate stimulate photosynthesis? **Pest Biochem Physiol.** 2010;96:140-8.

Costa A.G.F. et al. Efeito da intensidade do vento, da pressão e de pontas de pulverização na deriva de aplicações de herbicidas em pré-emergência. **Planta Daninha.** 2007;25:203-10.

Eker S. et al. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower *(Helianthus annuus L.)* plants. **J Agric Food Chem**. 2006;54:10019-25.

Fialho C.M.T. et al. Teor foliar de nutrientes em plantas daninhas e de café cultivadas em competição. **Planta Daninha**. 2012;30:65-73.

Formiga A.T. et al. Relações entre o teor de fenóis totais eo ciclo das galhas de Cecidomyiidae em Aspidosperma spruceanum Müll. Arg.(Apocynaceae). **Acta Bot Bras.** 2009;23:93-9.

França A.C. et al. Crescimento de cultivares de café arábica submetidos a doses do glyphosate. **Planta Daninha**. 2010;28:599-607.

Jerba V.F. et al. Relação entre a estrutura foliar de feijoeiro e a pré-infecção por *Glomerella cingulata f.sp. phaseoli*. **Pesq Agropec Bras**. 2005;40:217-23.

Kuranda K. et al. Investigating the caffeine effects in the yeast Saccharomyces cerevisiae brings new insights into the connection between TOR, PKC and Ras/cAMP signalling pathways, **Molec Microbiol.** 2006;61:1147-66.

Malty J.S. et al. Efeitos do glifosato sobre microrganismos simbiotróficos de soja, em meio de cultura e casa de vegetação. **Pesq Agropec Bras.** 2006;41:285-91.

Mantuano D.G. et al. Leaf anatomy variation within and between three “restinga” populations of *Erythroxylum ovalifolium* Peyr: (Erythroxylaceae) in Southeast Brazil. **Rev Bras Bot.** 2006;29:209-15.

Mendes B.S.S. et al. Mecanismo fisiológicos e bioquímicos do abacaxi ornamental sob estresse salino. **Rev Caatinga**. 2011;24:71-7.

Mesquita H.C. et al. Riscos toxicológicos do herbicida glyphosate. **Agropec Cient Semiárido**. 2012;7:1-5.

Moldes C.A. et al. Biochemical responses of glyphosate resistant and susceptible soybean plants exposed to glyphosate. **Acta Physiol Plant**. 2008;30:469-79.

O’Brien T.P., Mccully M.E. **The study of plant struture. principles and selected methods**. Melborne: Termarcarphi, 1981. 352p.

Pascholati S.F., Leite B. Hospedeiro: mecanismos de resistência. In: Bergamini Filho A., Kimati H., Amorim L. **Manual de fitopatologia**; princípios e conceitos. 3ª. ed. São Paulo: Agronômica Ceres, 1995. p.417-53.

Peixoto P.H.P. et al. Alterações morfológicas e acúmulo de compostos fenólicos em plantas de sorgo sob estresse de alumínio. **Bragantia**. 2007;66:17-25.

Pregnolatto W., Pregnolatto N.P. **Normas analíticas, métodos químicos e físicos para análise de alimentos**. 3ª. ed. São Paulo: Instituto Adolfo Lutz, 1985. v.1. p.190-2.

Reddy K.N. et al. Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. **J Agric Food Chem**. 2008;56:2125-30.

Silva A.A. et al. Manejo integrado de plantas daninhas em lavouras de café. In: Silva L.M. et al. Inter-relações entre a anatomia vegetal e a produção vegetal. **Acta Bot Bras.** 2005;19:183-94.

Silva M.A. et al. Efeito hormético de gliphosate no desenvolvimento inicial de cana-de-açúcar. **Bragantia**. 2009;68:973-8.

Smith A.H. et al. Anatomical variation and defence responses of juvenile *Eucalyptus nitens* leaves to Mycosphaerella leaf disease. **Austr Plant Pathol.** 2006;35:725-31.

Taiz L., Zeiger E. **Fisiologia vegetal**. 5ª. ed. Porto Alegre: Artmed, 2013. 954p.

Tan S. et al. Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops. **Amino Acids**. 2006;30:195-204.

Terashima I. et al. Why are sun leaves thicker than shade leaves? Consideration based on analyses of $CO_2$ diffusion in the leaf. **J Plant Res.** 2006;114:93-105.

Trezzi M.M. et al. Inibidores de EPSPS. In: Vidal R.A., Merotto Jr. A. **Herbicidologia**. 2001. p.37-45.

Tuffi Santos L.D. et al. Crescimento e morfoanatomia foliar de eucalipto sob efeito de deriva do glyphosate. **Planta Daninha**. 2007;23:133-42.

Tuffi Santos L.D. et al. Danos visuais e anatômicos causados pelo glyphosate em folhas de *Eucalyptus grandis*. **Planta Daninha**. 2008;26:9-16.

Tuffi Santos L.D. et al. Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate. **Braz J Biol.** 2009;69:129-36.

Zhishen J. et al. The determination of flavonoid contents in mulberry and the scavenging effects on superoxide radicais. **Food Chem.** 1999;64:555-9.

Zobiole L.H.S. et al. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. **Plant Soil**. 2010;328:57-69.