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Water use efficiency by coffee arabica after glyphosate application

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ABSTRACT. Many coffee growers apply glyphosate in directed applications, but some phytotoxicity has been noted. It is believed some herbicides can exert a direct or indirect negative effect on photosynthesis by reducing the metabolic rate in a way that can affect the water use efficiency. The objective of this study was to investigate the variables related to water use among coffee cultivars subjected to the application of glyphosate and the effects of each dose. The experiment was conducted in a greenhouse using three varieties of coffee (*Coffea arabica*), Acaiá (MG-6851), Catucaí Amarelo (2SL) and Topázio (MG-1190), and three doses of glyphosate (0.0, 115.2 and 460.8 g acid equivalent ha^{-1}), in a factorial 3 x 3 design. At 15 days after application, a reduction in stomatal conductance was observed, and smaller transpiration rate and water use efficiency were found in the fourth leaf at 15 days after application. There was a decrease in the transpiration rate at 45 DAA, with the Acaiá cultivar showing reductions with 115.2 g ha^{-1}. There was transitory reduction in water use efficiency with glyphosate application, but can affect the growth and production. The Acaiá cultivar showed the highest tolerance to glyphosate because the water use efficiency after herbicide application.

Keywords: herbicide, EPSPs, *Coffea arabica*, water use efficiency, transpiration.

Eficiência do uso da água pelo cafeeiro arábica após aplicação de glyphosate

RESUMO. Muitos cafeicultores utilizam o glyphosate em aplicações dirigidas. Nessas aplicações são constatados intoxicações. Através do efeito negativo direto na fotossíntese, ou indiretamente, reduzindo da taxa metabólica da planta, acredita-se que possam influenciar a eficiência do uso da água. Objetivou-se no trabalho averiguar as variáveis relacionadas ao uso da água entre cultivares de café submetidos a aplicação de subdoses de glyphosate e os efeitos de cada dose. O experimento foi conduzido em casa de vegetação utilizando-se três cultivares de cafeeiro (*Coffea arabica*): Acaiá (MG-6851), Catucaí Amarelo (2 SL) e Topázio (MG-1190) e, três subdoses do glyphosate (0,0; 115,2 e 460,8 g ha^{-1} do equivalente ácido), em esquema fatorial 3 x 3. Aos 15 DAA observou-se redução da condutância estomática. Constatou-se menor taxa de transpiração e eficiência do uso da água na quarta folha aos 15 dias após a aplicação. 45 dias após aplicação ocorreu queda da transpiração, sendo que, o Acaiá apresentou redução com aplicação de 115,2 g ha^{-1}. O glyphosate reduziu a eficiência do uso da água, apresentando efeitos transitórios, mas que podem causar atraso no crescimento e produção do cafeeiro. O cultivar Acaiá foi o mais tolerante ao glyphosate, não alterando sua eficiência do uso da água com aplicação do herbicida.

Palavras-chave: herbicida, EPSPs, *Coffea arabica*, eficiência do uso da água, transpiração.

Introduction

The occurrence of weeds in coffee plantations can result in competition for such resources as water, nutrients and light, thus requiring that decisions be made regarding control of the weeds. For economic and environmental reasons, the control method utilized by many coffee growers is the use of glyphosate in direct applications to guarantee crop selectivity. However, due to the dispersion of the droplets to non-target plants, a phenomenon known as drift, intoxication in coffee plants was noted after the direct application of glyphosate to weeds (RODRIGUES et al., 2003). In addition to visual symptoms of intoxication, it has been reported that there may be damage to the growth and nutrient uptake of the coffee plants (FRANÇA et al., 2010a and b).

When in contact with leaves, glyphosate penetrates quickly across the cuticle and then is absorbed via the symplastic route (ZABLOTOWICZ; REDDY, 2007). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase

(EPSPs), an enzyme in the shikimate pathway, which is responsible for the biosynthesis of aromatics amino acids, defense compounds and many phenolic compounds (ZOBIOLE et al., 2010). Although glyphosate does not directly affect the photosynthetic pathway, secondary effects on plant metabolism can occur, with consequent effects on the water use efficiency. However, there is no work to date that demonstrates these effects.

It is believed that the negative direct effects of some herbicides on photosynthesis, transpiration and stomatal conductance, or indirectly by a reduction of the plant metabolic rate, can influence the water use efficiency of plants. Plants with a higher water use efficiency are those that fix more CO_2 per volume water transpired. In this way, the water use efficiency of a crop can be characterized as the production of dry matter through the consumption of evapotranspired water (SILVA; SILVA, 2007). This efficiency is directly correlated with stomatal opening; therefore, although the plant acquires CO_2 for photosynthesis, water is also lost by transpiration, and these exchanges vary with the gradient of water potential between the leaf and atmosphere (LARCHER, 2004).

Despite the wide importance and utilization of glyphosate in weed control for coffee, few studies are available that describe the effects of glyphosate on the physiology of coffee, particularly those parameters related to water use. Indeed, a deeper understanding of these effects can lead to a greater caution by growers with regard to herbicide use. Therefore, this study aimed to examine the variables of water use in coffee cultivars subjected to glyphosate subdosage application.

Material and methods

The experiment was conducted in a greenhouse using three coffee (*Coffea arabica*) cultivars, including Acaiá (MG-6851), Catucaí Amarelo (2 SL) and Topázio (MG-1190), treated with three subdoses of glyphosate. The seedlings of the coffee cultivars were produced by direct sowing in polyethylene bags. At the stage of five pairs of completely expanded leaves, the plants were transplanted to pots containing 10 dm^3 of substrate composed of a sample of sifted soil and aged manure (3:1). The soil sample was obtained from a typical dystrophic Red-Yellow Latosol with a sandy clay texture. The chemical analysis presented the following results: a pH (water) of 4.7; an organic matter rate of 2.4 dag kg^{-1}; P and K contents of 2.3 and 48 mg dm^{-3}, respectively; and Ca, Mg, Al, H+Al and $CTC_{effective}$ values of 1.4, 0.4, 0.6, 6.27 and 2.52 cmol dm^{-3}, respectively. Simple superphosphate (100 g per pot) was used to supply P_2O_5, and dolomitic limestone was used to increase the saturation per base to 60%. After transplanting the seedlings, the pots remained in the greenhouse with a irrigation system until the treatments were applied. Potassium chloride (31.48 g per pot) and urea (10 g per pot) were added at 30 and 60 days after transplantation.

The experiment was arranged in a 3 x 3 factorial scheme. The first factor corresponded to the coffee cultivar, and the second factor corresponded to the subdose of glyphosate. The experiment was set up in a randomized block design with four replications. Subdoses of 0.0, 115.2 and 460.8 g acid equivalent of glyphosate ha^{-1} were tested, which corresponded to 0.0, 8.0 and 32.0%, respectively, of the recommended commercial dose for weed control (1.440 g acid equivalent of glyphosate ha^{-1}). Each experimental parcel consisted of a pot containing one plant. At 120 days after transplantation, when the coffee plants had approximately 21 pairs of leaves and six plagiotropic branches, the glyphosate was applied in a way that it would not reach the upper third of the coffee plants; a backpack sprayer pressurized with CO_2 and calibrated for a constant pressure of 250 kPa was used for the application. The sprayer was equipped with a bar and two flat fan tips (TT-110.02) spaced 0.50 m from each other, which provided a volume of 200 L ha^{-1}. During the application, the air temperature (25.3°C ± 1), relative air humidity (80% ± 3) and wind velocity (2 km h^{-1}) were measured. After the application of glyphosate, the plants remained outside of the greenhouse for 24 hours, with the leaves protected from irrigation or rainwater to avoid the removal of the product.

At 15 and 45 days after the application (DAA), the stomatal conductance of water vapor (*gs* – mol m^{-1} s^{-1}) and transpiration rate (*E* – mol H_2O m^{-2} s^{-1}) were measured, calculating the water use efficiency (*WUE* – mol CO_2 mol H_2O^{-1}) with the values of CO_2 fixed by photosynthesis and the water transpired. The physiological assessments were performed using the last and fourth completely expanded leaves (counting from the base of the plant) using an infrared gas analyzer (IRGA) (model LCA PRO, Analytical Development Co. [ADC] Ltd, Hoddesdon, UK).

The data were subjected to a variance analysis using the F test ($p \leq 0.05$). The interaction was then unfolded and subjected to Tukey's test at 5% probability, resulting in separate comparisons among the three cultivars and three dosages at 15 and 45 DAA; a comparison among the leaf positions was not explored because this was not an objective of this study.

Results and discussion

An interaction between the cultivars and subdoses of glyphosate was found for the assessed variables, depending on the evaluated leaf and time of evaluation. Regardless of the significance or non-significance, the results of each assessment were unfolded and are presented in a standardized manner.

The measurements for the fourth leaf at 15 days after the application (DAA) of glyphosate demonstrated that there was a difference among the cultivars in relation to the means for the stomatal conductance (*gs*) and transpiration rate (*E*). The means of both parameters for Catucaí Amarelo showed inferior values to Topázio. This last cultivar also showed a lower water use efficiency (*WUE*) when glyphosate was applied at 460.8 g ha^{-1}. A decrease in the mean for *gs* with an increase in the subdose was observed. *E* and *WUE* showed lower means only with the highest concentration of herbicide. However, the subdose did not affect the *gs*, *E* and *WUE* values of the Acaiá cultivar (Table 1).

Table 1. Stomatal conductance of water vapor (*gs*), transpiration rate (*E*) and water use efficiency (*WUE*), as measured in the fourth completely expanded leaf of three coffee cultivars (*Coffea arabica*) at 15 days after the application of glyphosate. Viçosa, Minas Gerais State, 2009.

Treatments	*gs* - mol m^{-1} s^{-1}			
	----------Doses - g ha^{-1}----------			Mean
Cultivars	0	115.2	460.8	
Acaiá	0.29 bA	0.26 aA	0.25 aA	0.27 ab
Catucaí Amarelo	0.36 bA	0.25 aB	0.08 bC	0.23 b
Topázio	0.49 aA	0.30 aB	0.15 bC	0.32 a
Mean	0.38 A	0.26 B	0.16 C	CV(%) = 20.26
Cultivars	*E* - H_2O mol m^{-2} s^{-1}			
Acaiá	4.19 aAB	4.28 aA	3.66 aB	4.05 a
Catucaí Amarelo	3.69 abA	3.67 bA	2.10 bB	3.15 b
Topázio	3.38 bA	3.50 bA	3.36 aA	3.41 b
Mean	3.76 A	3.82 A	3.04 B	CV(%) = 13.50
Cultivars	*WUE* - mol de CO_2 mol de H_2O^{-1}			
Acaiá	2.40 aA	2.23 aA	2.23 aA	2.29 a
Catucaí Amarelo	2.61 aA	2.33 aA	1.74 bB	2.23 a
Topázio	2.38 aAB	2.49 aA	2.14 aB	2.34 a
Mean	2.46 A	2.35 A	2.04 B	CV(%) = 15.78

The means followed by the same lowercase letter in a column and uppercase letter in a row do not differ from each other by the Tukey test at a 5% probability.

The means for *E* and *gs* of each cultivar were similar for the control (0.0 g ha^{-1}), whereas differences were observed among the cultivars when glyphosate was applied.

The *gs* value was reduced with an increase in the glyphosate subdose. Any change caused by abiotic stress can be demonstrated as stomatal sensitivity in many cultivars of *C. arabica* and *C. robusta* (DAMATTA; RAMALHO, 2006). Such conditions stimulate stomatal closure and increase stomatal resistance, such as mechanisms of defense against dehydration, thus the plant reduces the loss of water by transpiration (LARCHER, 2004). However, reduced transpiration in plants cannot be considered beneficial because this factor can be directly correlated to carbon assimilation and, consequently, to plant growth. Although the data showed a *gs* decrease of 58% with the application of 460.8 g ha^{-1} glyphosate (Table 1), other authors noted reductions of 29% in the photosynthetic rate in the same leaf and on the same day (CARVALHO et al., 2013), with 32% showing visual intoxication at 10 DAA (FRANÇA et al., 2010a).

Although water is abundant in the world, less than 1% is water that is fresh and suitable for human consumption and use in agriculture, such that the use of weed control methods that do not negatively affect the water use efficiency of crops should be emphasized, along with use of the best cultivars for efficient water use.

The means of the *gs* parameter for the last leaf differed for the cultivars at 15 DAA. This difference was noted in cultivars that had not received glyphosate, whereas there was no difference in the cultivars treated with glyphosate. The *gs* of each cultivar decreased with the herbicide application, but no change was noted for the *E* and *WUE* means. Only the subdose of 115.2 g ha^{-1} decreased the *WUE* of Topázio and increased the *WUE* of Catucai Amarelo, though the mean between the two cultivars was not affected (Table 2).

Table 2. Stomatal conductance of water vapor (*gs*), transpiration rate (*E*) and water use efficiency (*WUE*), as measured in the last completely expanded leaf of three coffee cultivars (*Coffea arabica*) at 15 days after the application of glyphosate. Viçosa, Minas Gerais State, 2009.

Treatments	*gs* - mol m^{-1} s^{-1}			
	----------Doses - g ha^{-1}----------			Mean
Cultivars	0	115.2	460.8	
Acaiá	0.33 bA	0.24 aA	0.22 aA	0.26 b
Catucaí Amarelo	0.74 abA	0.75 aA	0.21 aA	0.57 ab
Topázio	1.36 aA	0.44 aA	0.23 aA	0.68 a
Mean	0.81 A	0.48 AB	0.21 B	CV(%) = 85.04
Cultivars	*E* - H_2O mol m^{-2} s^{-1}			
Acaiá	3.57 aA	3.62 aA	3.60 aA	3.60 a
Catucaí Amarelo	3.86 aA	3.88 aA	3.37 aA	3.71 a
Topázio	3.84 aA	3.66 aA	3.48 aA	3.66 a
Mean	3.76 A	3.72 A	3.48 A	CV(%) = 21.58
Cultivars	*WUE* - mol de CO_2 mol de H_2O^{-1}			
Acaiá	2.14 abA	2.13 aA	2.13 aA	2.13 a
Catucaí Amarelo	1.54 bB	2.70 aA	1.99 aAB	2.08 a
Topázio	2.54 aA	1.16 bB	2.20 aA	1.96 a
Mean	2.07 A	2.00 A	2.10 A	CV(%) = 25.16

The means followed by the same lowercase letter in a column and uppercase letter in a row do not differ from each other by the Tukey test at a 5% probability.

The opening and closing of stomata depends on such factors as solar radiation, mesophilic CO_2 concentration, relative moisture (air vapor pressure deficit), water potential and other factors of less importance, including the wind, growth components, and endogenous rhythms of each species (LARCHER, 2004). The stomatal conductance varies with the frequency, size, form and diameter of the stomata, in addition to environmental factors and leaf ontogeny. Many studies have suggested that the change in stomatal conductance is related to stomatal development (ENGLAND; ATTIWILL, 2011).

The regulation mechanism of stomatal conductance did not result in corresponding alterations in transpiration because *E* did not decrease according to the lower values of *gs*. The control of transpiration can be complex, and the difference in the water potential between the leaf and atmosphere may influence transpiration, as this gradient strongly affects the transpiration rate (TAIZ; ZEIGER, 2009).

The *gs*, *E* and *WUE* parameters of the cultivars were different, as were those for the cultivars that were not subjected to glyphosate, on the fourth leaf at 45 DAA. It was noted that the differences in the means are due to the characteristics of each cultivar. However, the herbicide application did not reduce the mean for all of the cultivars, but interfered only with *gs* and *E* of Topázio cultivar (Table 3). This behavior, regardless of the differences between the observed means at 45 DAA, may be explained by duration of the glyphosate action. The herbicide is rapidly absorbed and translocated in the plant and has a maximum effect of control between 15 and 25 DAA (SILVA; SILVA, 2007); thus, after this time, plants that had not died can recover.

Table 3. Stomatal conductance of water vapor (*gs*), transpiration rate (*E*) and water use efficiency (*WUE*), as measured in the fourth completely expanded leaf of three coffee cultivars (*Coffea arabica*) at 45 days after the application of glyphosate. Viçosa, Minas Gerais State, 2009.

Treatments	*gs* - mol m^{-1} s^{-1}			
	----------Doses - g ha-1----------			Mean
Cultivars	0	115.2	460.8	
Acaiá	0.12 bA	0.13 aA	0.12 aA	0.12 b
Catucaí Amarelo	0.14 bA	0.13 aA	0.12 aA	0.13 b
Topázio	0.20 aA	0.15 aB	0.14 aB	0.16 a
Mean	0.15 A	0.13 A	0.13 A	CV(%) = 23.86
Cultivars	*E* - H_2O mol m^{-2} s^{-1}			
Acaiá	1.70 bA	1.79 aA	1.73 bA	1.74 b
Catucaí Amarelo	1.18 cA	1.20 bA	1.27 cA	1.22 c
Topázio	2.29 aA	1.85 aB	2.16 aA	2.10 a
Mean	1.73 A	1.61 A	1.72 A	CV(%) = 8.84
Cultivars	*WUE* - mol de CO_2 mol de H_2O^{-1}			
Acaiá	3.90 aA	3.15 aA	3.50 aA	3.51 ab
Catucaí Amarelo	4.50 aA	4.60 aA	4.25 aA	4.45 a
Topázio	2.04 bA	3.67 aA	2.66 aA	2.79 b
Mean	3.48 A	3.81 A	3.47 A	CV(%) = 32.22

The means followed by the same lowercase letter in a column and uppercase letter in a row do not differ from each other by the Tukey test at a 5% probability.

In fact, to assimilate the same amount of CO_2, the amount of water transpired by the Topázio cultivar was approximately 64% higher than the spent by the Catucaí Amarelo cultivar. In contrast, Topázio showed a higher *gs* compared to the other cultivars.

Plants that fix a higher volume of CO_2 than transpired water have a higher water use efficiency. According to these data, Catucaí Amarelo showed a higher water use efficiency, which may be explained by the lower transpiration rate in this cultivar.

The Acaiá cultivar showed an increase of 67% in *E* in relation to the other cultivars when the last leaf was evaluated at 45 DAA. The same cultivar had approximately 58% more transpiration than the other cultivars when not treated with the herbicide. The Topázio cultivar achieved a lower *E* at all of the subdoses, whereas the *E* of Acaiá decreased with herbicide application (Table 4).

Table 4. Stomatal conductance of water vapor (*gs*), transpiration rate (*E*) and water use efficiency (*WUE*), as measured in the last completely expanded leaf of three coffee cultivars (*Coffea arabica*) at 45 days after the application of glyphosate. Viçosa, Minas Gerais State, 2009.

Treatments	*gs* - mol m^{-1} s^{-1}			
	----------Doses - g ha-1----------			Mean
Cultivars	0	115.2	460.8	
Acaiá	0.19 aA	0.23 aA	0.18 aA	0.19 a
Catucaí Amarelo	0.12 aA	0.13 aA	0.15 aA	0.13 a
Topázio	0.29 aA	0.12 aA	0.12 aA	0.17 a
Mean	0.20 A	0.16 A	0.15 A	CV(%) = 70.71
Cultivars	*E* - H_2O mol m^{-2} s^{-1}			
Acaiá	2.68 aA	2.07 aB	1.88 aB	2.21 a
Catucaí Amarelo	1.64 bA	1.61 bA	1.57 aA	1.61 b
Topázio	1.46 bAB	1.57 bA	1.09 bB	1.37 b
Mean	1.93 A	1.75 AB	1.51 B	CV(%) = 15.56
Cultivars	*WUE* - mol de CO_2 mol de H_2O^{-1}			
Acaiá	2.61 bA	3.17 aA	3.33 bA	3.04 b
Catucaí Amarelo	3.78 abA	3.22 aA	3.87 abA	3.62 b
Topázio	4.46 aA	4.07 aA	4.70 aA	4.41 a
Mean	3.61 A	3.49 A	3.97 A	CV(%) = 21.94

The means followed by the same lowercase letter in a column and uppercase letter in a row do not differ from each other by the Tukey test at a 5% probability.

In many species, such as coffee, the stomatal conductance is highly correlated with the photosynthetic rate, and the stomatal conductance has a strong sensitivity to different conditions (DAMATTA; RAMALHO, 2006). However, differences were observed among the cultivars only at 45 DAA, and the determination of the most efficient cultivar in water use depended of the leaf sampled. In the Topázio cultivar, a lower water use efficiency in the fourth leaf and the highest efficiency in the last leaf were observed.

The translocation of glyphosate to the site of action follows the same route of photosynthesis products, that is, from the photosynthetically active leaves (sources) toward the organs that use the photoassimilate (sinks) (WANAMARTA; PENNER, 1989; ZABLOTOWICZ; REDDY, 2007). Thus, the observed effects on the last fully expanded leaf or the leaves that were being formed during the application (as sinks) were expected due to the translocation of glyphosate.

The *gs* and *WUE* did not differ after glyphosate application, independently of the subdose applied, at 45 DAA in the last leaf. However, *E* showed a reduction of approximately 22% with the application of 460.8 g ha^{-1}, less than *E* without the application of herbicide (Figure 4). After absorption, glyphosate is actively translocated to growing tissues where it starts inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) in the shikimate pathway, which is responsible for the biosynthesis of many compounds in plants (ZOBIOLE et al., 2010). Therefore, the effects observed after 45 days in the young tissues is related to the translocation of the herbicide to target sites.

When subjected to the application of glyphosate up to 460.8 g ha^{-1}, the cultivars show effects with regard to the amount of carbon fixed per volume water lost to the atmosphere. Although damage was transient and only observed in leaves that received direct application of the product, differences in the ratio of CO_2 assimilated to H_2O transpired were only observed at 15 DAA and on the fourth leaf, where the product was sprayed. Thus, glyphosate may affect the resistance of coffee to water stress, as glyphosate-resistant soybean plants show less resistance to drought (ZOBIOLE et al., 2010).

Conclusion

The differences among the cultivars treated with glyphosate were related to the intrinsic properties of each cultivar because the differences between the cultivars were similar with or without the application of glyphosate. However, the *WUE* of Acaiá cultivar was more tolerant to glyphosate, as it was not affected by the glyphosate application. Glyphosate has negative effects on the water use efficiency of plants, with transitory damage that can result in irreversible damage to a crop under drought conditions. Thus, care should always be taken to avoid the herbicide drift.

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