

Evaluation of leaf and root absorptions of glyphosate in the growth of coffee plants

Avaliação da absorção foliar e radicular de glifosato no crescimento de plantas de café

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ABSTRACT: Intoxication by dispersion of glyphosate droplets in coffee seedlings is common and, in addition to the problem of drift, there are reports of contamination of this herbicide to a nontarget plant via the rhizosphere. Hydroponics allows the comparison of the translocation of the glyphosate absorbed by the foliage or the roots and avoids the interaction with the soil, which could hamper the achievement of more accurate conclusions when it is absorbed by the root. Thus, the toxicity of glyphosate sublethal dosages in the initial growth of coffee plants in hydroponics was evaluated by applying four sublethal dosages in two different locations (solution and leaf). Fifty days after the application of the herbicide, the intoxication percentage and the growth of the coffee seedlings were evaluated. From the reduced dose of 115.2 g·ha⁻¹ of glyphosate, height reductions, root length; number of leaves, dry mass of leaf, roots and total, leaf area, and leaf mass ratio were observed. The first two parameters were observed only in leaf application and the others via leaf and solution. The aerial part–root ratio system had an increase in herbicide sublethal dosages when applied to leaves and the ratio of leaf area and specific leaf area increased in both applications. Sublethal dosages of glyphosate applied to young coffee plants under hydroponic conditions impair their growth, and it is more accentuated with increasing doses and when the leaves, instead of the roots, absorb the herbicide.

KEYWORDS: absorption; *Coffea arabica*; phytotoxicity; hydroponics.

RESUMO: Intoxicação por dispersão das gotas de glifosato em mudas de café são comuns, além do problema da deriva, há relatos da passagem desse herbicida para planta não alvo via rizosfera. A hidroponia possibilita comparar a translocação do glifosato absorvido pelas folhagens ou raízes e evita a interação do solo que poderia dificultar a obtenção de conclusões mais precisas quando absorvido pela raiz. Assim, a toxicidade de subdoses de glifosato no crescimento inicial de plantas de café em hidroponia foi avaliada aplicando-se quatro subdoses em dois locais distintos (solução e folha). Cinquenta dias após a aplicação do herbicida, a porcentagem de intoxicação e o crescimento das mudas de café foram avaliados. A partir da subdose de 115,2 g·ha⁻¹ de glifosato observaram-se reduções da altura; comprimento radicular; número de folhas; massa seca da folha, raiz, radicular e total; área foliar; razão de massa foliar, sendo os dois primeiros parâmetros observados somente na aplicação foliar e os demais via foliar e solução. A relação parte aérea/sistema radicular aumentaram com o incremento das subdoses do herbicida quando aplicado nas folhas e a razão de área foliar e área foliar específica aumentaram em ambas as vias de aplicação. Subdoses de glifosato aplicadas em plantas jovens de café, em condições hidropônicas, prejudicam o seu crescimento sendo mais acentuados com o aumento das doses e quando o herbicida é absorvido pelas folhas em relação à absorção radicular.

PALAVRAS-CHAVE: absorção; *Coffea arabica*; fitotoxicidade; hidroponia.

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Received on: 06/05/2018. Accepted on: 08/02/2020

INTRODUCTION

The management of weeds in crops is essential in order to achieve greater productivity (SCHRÜBBERS et al., 2014; COSTA et al., 2017). Coffee trees are sensitive to the interference of invasive plants that, even at low densities, can cause decreases in nutritional content by competing for resources in the environment which makes them scarce for coffee plants and hinders harvesting and treatment (FRANÇA et al., 2010a; CARVALHO et al., 2014). The chemical control of weeds stands out for being more efficient; however, the reduced number of selective products to coffee culture increases the application of no selective herbicides such as glyphosate (SCHRÜBBERS et al., 2014).

Glyphosate [N-(phosphonomethyl) glycine] is one of the most widely used systemic herbicides in weed control due to its broad aspect of action, low cost and low vapor pressure (KRUSE et al., 2000; GREEN, 2007; CAMPOS et al., 2013). Glyphosate inhibits the enzyme 5-enolpyruvyl-chiquimate-3-phosphate synthase (EPSP) from the metabolic pathway of shikimic acid, therefore preventing the synthesis of essential amino acids such as phenylalanine, tyrosine and tryptophan and secondary metabolites (KRUSE et al., 2000; SCHRÜBBERS et al., 2014). It also compromises metabolic processes such as carbon flow, photosynthesis (GREEN, 2007; GOMES et al., 2016) and the nutritional status of plants (FRANÇA et al., 2010a).

Despite knowing the effects of glyphosate on weeds, it is important to understand about its interference in the growth and development of nontarget plants that even with due care, cases of intoxication due to the dispersion of drops in coffee plants are common (FRANÇA et al., 2010a; CARVALHO et al., 2013). In addition to the drift problem, there are reports of the contamination of herbicide from target plants (weed) to nontarget plants (economic culture) by the rhizosphere and mycorrhizal interactions (WAGNER et al., 2003; SANTOS et al., 2008). ^{14}C -glyphosate was found in the tissues of *Eucalyptus grandis* (Myrtaceae) after the application of the herbicide in plants of *Brachiaria decumbens* (Gramineae) (SANTOS et al., 2008). However, the effects on growth and development in nontarget crops such as coffee are not known when this herbicide is absorbed by the root system of this plant. Understanding the divergences in glyphosate absorption pathways is important due to its widespread use in the management of weeds in coffee, which can improve the management in agricultural practices. The hydroponic system avoids the interaction of the herbicide with the soil, which could make it more difficult to reach conclusions that are more accurate. Therefore, it can be a method to compare the differential sensitivity of coffee plants by the glyphosate absorbed by the foliage or the roots.

This work aimed to verify the toxicity of glyphosate in coffee seedlings by two absorption routes through the hydroponic system using a nutrient solution containing the herbicide.

MATERIAL AND METHODS

Coffee seeds of 'Catuaí Vermelho IAC 99' were placed to germinate in a washed sandbox and were transferred to a tray containing $\frac{1}{4}$ of the concentration of nutrients for adaptation of the plants in the matchstick phase. Four increasing changes were made weekly until the final concentration of the solution of HOOGLAND; ARNON (1938).

Upon reaching two pairs of fully developed leaves, the seedlings were transferred to pots with 2.0 L of hydroponic solution. The pots were covered with a 1 cm thick extruded polystyrene foam (XPS) with a hole for insertion of the root in the solution and another for the entry of the hose that conducted the air.

The plants remained in the pots for one week, and then they received the sublethal dosages of glyphosate 0.0, 115.2, 230.4, and 460.8 $\text{g}\cdot\text{ha}^{-1}$ in the leaves and in the hydroponic solution corresponding to 0, respectively 8, 16 and 32% of the 1440 $\text{g}\cdot\text{ha}^{-1}$ dose of the isopropylamine salt formulation. In the application, the pots with the seedlings were lined up and each dose applied once with a CO_2 pressurized sprayer, calibrated at a constant pressure of 250 kPa, equipped with a bar, with a fan-type spray tip (TT 11002), providing the application of 200 $\text{L}\cdot\text{ha}^{-1}$ of syrup.

The design was a randomized block with a 2 by 4 factorial scheme with seven replications, the first one referring to the absorption pathway (leaf and root) and the second one to the sublethal dosages of glyphosate. The experimental unit consisted of a vase with a coffee seedling.

Fifty days after the application (DAA) of the glyphosate, the percentage of intoxication of the coffee plants by the herbicide was evaluated, using a scale from 0 to 100%, where 0 corresponds to the absence of visible symptoms and 100% to the death of the plants (FRANS, 1972). Then, the plants were collected and leaf area (ANTUNES et al., 2008), collar diameter, height, number of leaves, root length, dry mass of leaf, stem and root were evaluated. From the values obtained, the aerial part-root ratio, leaf area ratio, specific leaf area and the leaf, stem and root mass ratios were calculated according to the following formulas: aerial part-root ratio ($\text{g}\cdot\text{g}^{-1}$) = (aerial part dry mass/root dry mass); leaf area ratio ($\text{cm}^2\cdot\text{g}^{-1}$) = (leaf area/total dry mass); specific leaf area ($\text{cm}^2\cdot\text{g}^{-1}$) = (leaf area/dry leaf mass); leaf mass ratio ($\text{g}\cdot\text{g}^{-1}$) = (leaf dry mass/total dry mass); stem mass ratio ($\text{g}\cdot\text{g}^{-1}$) = (stem dry mass/total dry mass); root mass ratio ($\text{g}\cdot\text{g}^{-1}$) = (root dry mass/total dry mass).

The data were submitted to analysis of variance, and the variables were compared by regression analysis, with the choice

of models based on their significance, biological phenomenon, and determination coefficient.

RESULTS AND DISCUSSION

The glyphosate caused intoxication in coffee plants, with increasing values proportional to the increase of sub-lethal dosages and greater intensity when applied to the leaves, reaching

70 and 50%, in 50 DAA via leaf and solution respectively (Fig. 1a). The symptoms observed were narrowing of the leaf blade and internerval chlorosis in new leaves, similar to those reported by other authors (FRANÇA et al., 2010b; SILVA et al., 2016). Internerval chlorosis and narrowing of the leaf blade can occur due to Mg and Zn deficiencies respectively (BOTT et al., 2008; FRANÇA et al., 2010a). Glyphosate is a phosphonic acid that acts as a chelator, blocking the absorption of minerals by the plant with high specificity for the micronutrients Mn, Fe, Zn and Cu (COUTINHO; MAZO,

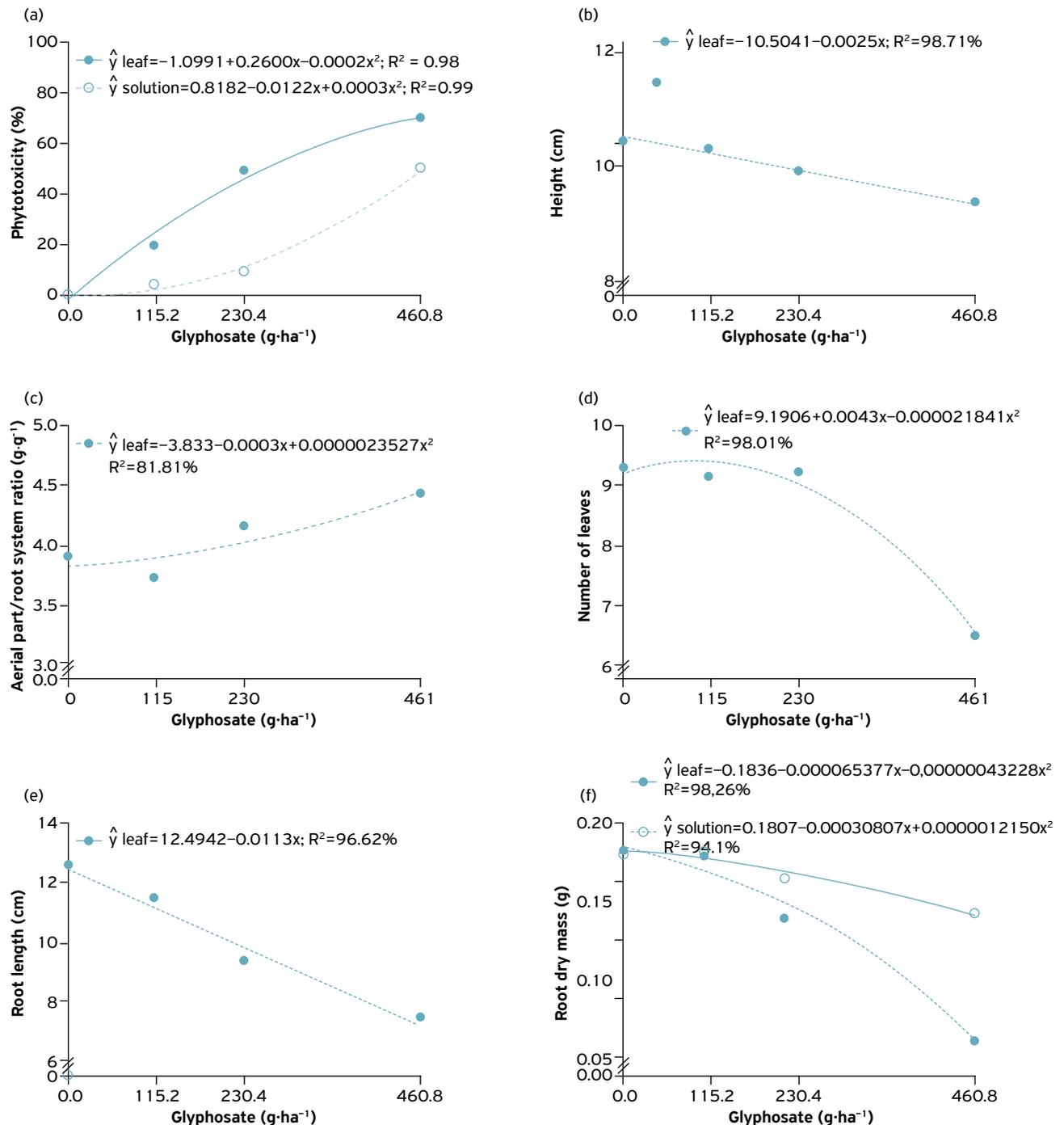


Figure 1. Phytotoxicity (a); height (b); aerial part/root ratio (c); number of leaves (d); root length (e); root dry mass (f) of young coffee plants submitted to different doses of glyphosate, 50 days after application.

2005). Another possible cause of leaf lesion is the accumulation of aminomethylphosphonic acid (AMPA), which is the first metabolite of glyphosate (DING et al., 2011). The lesser effect when applied to the solution may have occurred due to the dilution of the herbicide when applied in the solution, reducing the absorbed concentration.

The stem diameter and stem mass ratio of coffee plants were not altered in 50 DAA with sublethal dosages of glyphosate regardless of the route of application (solution or leaf). A similar result was observed for stem diameter in young coffee plants grown in pots with sublethal dosages of this herbicide (FRANÇA et al., 2010b).

The increase in glyphosate doses linearly reduced the height of the coffee plants by 0.0025 cm for each gram of the herbicide applied via the leaf, but with no effect when applied to the solution (Fig. 1b). This reduction is possibly related to the fact that glyphosate inhibits the synthesis of auxin, a growth hormone, by inhibiting the tryptophan precursor (CHANG et al., 2016; GREEN, 2007). The lower growth induced by this herbicide may have the consequence of reducing the concentrations of micronutrients in the leaves (FRANÇA et al., 2010a). Different results were observed in peach trees [(*Prunus persica* (Rosaceae))] (SANTOS et al., 2006) and Varjão [*Parkia multijuga* (Leguminosae – Mimosoideae)] (YAMASHITA et al., 2006) in which sublethal dosages of glyphosate did not provide growth suspension, indicating greater sensitivity for young coffee seedlings. Differential tolerance between species to agrochemicals can occur due to differences in applied doses, absorption and translocation to the target site, mechanisms that reduce the absorption of drops such as trichomes, thick layer of wax and overlapping leaves (REDDY et al., 2008; SILVA et al., 2016).

Stimulation of vegetative growth in low doses of toxic substances, known as hormone, was observed for glyphosate (BRITO et al., 2017) characterized by the greater thickness of the spongy parenchyma (SILVA et al., 2016). However, this effect was not observed in the coffee seedlings in any of the absorption routes, regardless of the sublethal dosages applied. The hormone effect is dependent on the plant's growth stage at the time of application, which can lead to different results (CARVALHO et al., 2013; CEDERGREEN, 2008).

The aerial part–root ratio was affected in a quadratic way by the doses of glyphosate (Fig. 1c). Leaf absorption at a dose of 460.8 g·ha⁻¹, observed an increase of 13.86% compared to the control, but with no effect when applied to the solution regardless of the dose. A different result was observed for barley [(*Hordeum vulgare* (Poaceae))] in hydroponics with an increase of 10 to 30% in the root-aerial part ratio (CEDERGREEN, 2008). The decrease in the root length along with the increase in the aerial part indicates a greater investment of photoassimilates for the leaves rather than to the root system (CARVALHO et al., 2014; FRANÇA et al., 2010a; SANTOS et al., 2008). This result suggests that coffee roots may be more affected

after glyphosate drift and the distribution of resources in one organ may have priority over another depending on the species.

The number of leaves was influenced by the sublethal dosages of glyphosate with the lowest value in the treatment in which the herbicide was applied to the aerial part, reaching the highest point with 98.44 g·ha⁻¹ of the isopropylamine salt formulation with reduction from the increment of the doses (Fig. 1d). However, reduction in the number of leaves was not observed for the cultivars Catucaí Amarelo, Oeiras and Topázio (FRANÇA et al., 2010b) showing distinct susceptibility among coffee cultivars to sublethal dosages of glyphosate. The smaller number of leaves observed may be related to the lower growth of the plant and the interference in the allocation of resources. Leaf reduction reduces the possibility of maintaining fruit production due to the lower photosynthetic rate and lower carbohydrate fixation (DAMATTA, 2004).

The length and root dry mass of coffee reduced when the glyphosate dosage was increased, with effect on the route of application for the last parameter, where a greater reduction was observed when the herbicide was applied on the leaves (Fig. 1e and f). This is due to the fact that this herbicide is systemic and translocates through the path of photoassimilates from the leaves to the other parts, affecting the growth points such as root meristems (KRUSE et al., 2000). The growth of young plant roots was expanding and presented high cell division; thus, amino acids, hormones or enzymes that participate in these processes may have been inhibited with increased doses of the herbicide due to their translocation to the growth zone (CARVALHO et al., 2014). The presence of glyphosate in the *Brachiaria* roots was observed by darkening them (SANTOS et al., 2008) and the damage to the cotton roots, in hydroponics, included inhibition of development, deposition of a thick layer of dead cells and cellular exudates (PLINE et al., 2002). If the root system of soil coffee seedlings is affected by glyphosate in a similar way to plants growing in hydroponic environment, the interruption of growth induced by this herbicide at the root can lead to a more severe water stress or a slower development of seedlings.

The dry leaf, stem and total variables had the lowest values when the herbicide was applied to the leaves (Fig. 2a, b and c). Corn plants, in hydroponic solution, were more sensitive to glyphosate in the aerial part than in the root with 12 and 32% of herbicide accumulated in the apex of this plant when applied via solution and leaf, respectively (ALISTER et al., 2005). There was an effect of the interaction between the variables dry mass of leaves (Fig. 2a), stem (Fig. 2b), roots (Fig. 1e) and total (Fig. 2c) and the increase in glyphosate doses reduced the mass of the coffee plants. Reductions of these parameters were also observed in physic nut plants, *Jatropha curcas* L. (Euphorbiaceae), treated with sublethal dosages of this herbicide (COSTA et al., 2009). Physiological stress caused shortly after application of glyphosate may have reduced dry mass, as it showed symptoms of injuries, which intensified when

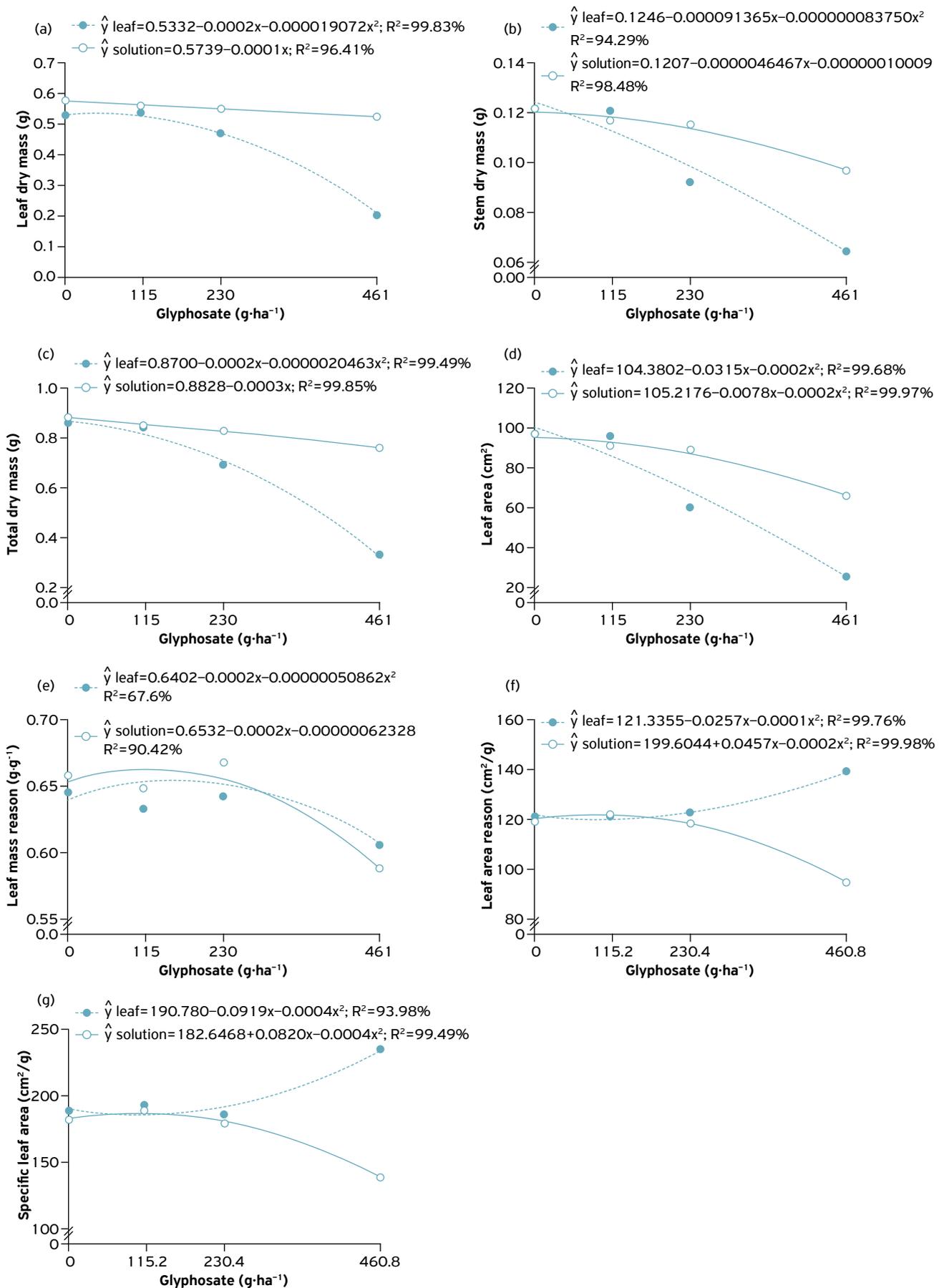


Figure 2. Leaf dry mass (a), stem dry mass (b), total dry mass (c); leaf area (d); leaf mass ratio (e); reason of leaf area (f) and specific leaf area (g) of young coffee plants submitted to different doses of glyphosate, at 50 days after application.

doses were increased. Glyphosate can interfere with carbon absorption, reducing the accumulation of dry matter and the deregulation of the shikimate pathway, causing a shortage of compounds necessary for carbon fixation, a process that is quickly inhibited (SERVAITES et al., 1987).

The leaf area was reduced in a quadratic way by the doses of glyphosate (Fig. 2d). In the dose of 460.8 g·ha⁻¹, the lowest values were observed, with 44.77 cm² in the leaf and 72.11 cm² in the solution, when compared to the control with an average leaf area of 104.49 cm². A similar result was observed for the coffee cultivars Topázio, Catucaí and Oeiras (FRANÇA et al., 2010b). This reduction may have been caused by the injuries caused by the herbicide, showing that sublethal dosages cause intoxication of the plants, reflecting less development of the leaves. Reduction of leaf area and number of leaves can decrease the photosynthetic rate and the accumulation of carbohydrates, impairing plant growth (GEIGER; BESTMAN, 1990; DAMATTA, 2004).

The sublethal dosages of glyphosate reduced the leaf mass ratio (LMR), which lowest values were obtained at the dose of 460.8 g·ha⁻¹ (Fig. 2e), being 0.60 g·g⁻¹, when the herbicide was applied to the leaves of the plants and 0.59 g·g⁻¹ when sprayed in solution. Which can be compared to the average of 0.65 g·g⁻¹ in treatments that did not receive the herbicide. Lower LMR values indicate low allocation of assimilates to the leaves (FALQUETO et al., 2009), suggesting that sublethal dosages of glyphosate compromise the allocation of photoassimilates in the leaves of young coffee plants, causing intoxication and reflecting in less accumulation of dry matter in the leaves. Glyphosate can reduce CO₂ fixation, resulting in less carbohydrates exported to the source (GEIGER; BESTMAN, 1990; GEIGER et al., 1999).

The leaf area ratio (LAR) and the specific leaf area (SLA) decreased when glyphosate was absorbed via the root, and presented a quadratic increase when it was absorbed through the air. Which proves it to be more sensitive to interference by the leaf with the increase in the dose of 230.4 to 460.8 g·ha⁻¹. There were increases of 49.29 and 16.89 (cm²·g⁻¹) for SLA

and LAR, respectively (Fig. 2f and g). These two variables correlate with photosynthetic capacity and the source-drain ratio. The increase in SLA may be correlated with lower leaf mass, this is due to leaf weight being a factor for SLA, being inversely proportional to this variable (CANCELLIER et al., 2010). Weight reduction, in addition to the lower carbohydrate accumulation already mentioned, may occur due to injuries, chlorosis and foliar narrowing caused by the herbicide. The decrease in the photosynthetic rate may occur due to the accumulation of shikimic acid caused by the inhibition of its route by glyphosate, which may represent a strong carbon drain in the Calvin cycle, due to the deviation of erythrose-4-phosphate from this route (SHIEH et al., 1991).

Glyphosate was less active in coffee seedlings when captured by the roots and may be related to the absorption path and, when applied to the leaves, it follows the symplast path (GEIGER et al., 1999; BROMILOW; CHAMBERLAIN, 2000) and the root movement is mainly by the apoplast and, eventually, the herbicide enters the symplast (ALISTER et al., 2005). The formation of the cation-glyphosate complex reduces the activity of this herbicide (HALL et al., 2000) and this complex may not be formed in the symplast because the cations create complexes with organic compounds (TAIZ; ZEIGER, 2002), so the activity of the glyphosate by this route may be greater. This may explain the lower toxicity of this herbicide when absorbed by the roots. Another hypothesis is that dilution of glyphosate in solution decreases the availability for absorption, which is different from when it was applied to the leaves.

CONCLUSIONS

Sublethal dosages of glyphosate applied to young coffee plants under hydroponic conditions impair their growth with market effects when doses were increasing and the herbicide was absorbed by the leaves, rather than root absorption.

ACKNOWLEDGEMENTS: The authors thank the Universidade Federal dos Vales do Jequitinhonha e Mucuri and Fundação de Amparo à Pesquisa do Estado de Minas for granting the resources to carry out this study.

FUNDING: Not applicable.

CONFLICTS OF INTEREST: All authors declare that they have no conflict of interest.

ETHICAL APPROVAL: Not applicable.

AVAILABILITY OF DATA AND MATERIAL: All data generated or analyzed during this study are included in this published article.

AUTHORS' CONTRIBUTIONS: Conceptualization: Silva, I.M.; Barbosa, E.A. Methodology: Matos, C.C. Supervision: França, A.C.; Silva, E.B. Writing - original draft: Silva, I.M.; Barbosa, E.A. Writing - review & editing: Silva, I.M.; Barbosa, E.A.

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