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## Hormesis with glyphosate depends on coffee growth stage

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### ABSTRACT

Weed management systems in almost all Brazilian coffee plantations allow herbicide spray to drift on crop plants. In order to evaluate if there is any effect of the most commonly used herbicide in coffee production, glyphosate, on coffee plants, a range of glyphosate doses were applied directly on coffee plants at two distinct plant growth stages. Although growth of both young and old plants was reduced at higher glyphosate doses, low doses caused no effects on growth characteristics of young plants and stimulated growth of older plants. Therefore, hormesis with glyphosate is dependent on coffee plant growth stage at the time of herbicide application.

**Key words:** *Coffea arabica* L., weed management, herbicide, spray drift, stimulatory effect.

### INTRODUCTION

Coffee is one of the world's most popular beverages (Fujioka and Shibamoto 2008) and the most important traded commodity in the world after oil (Naidu et al. 2008). Brazil, where coffee production is of economic as well as social importance, is the main world producer (Marana et al. 2008). Among coffee tree species planted in Brazil, *Coffea arabica* L. has the highest economic importance, producing the consumers' favorite coffee drink (Nascimento et al. 2006).

An important issue for coffee production is weed management. Brazilian growers commonly use inter-row mechanical weeding associated with intra-row chemical control. So, herbicides can reach coffee

plants both directly by accidental application and indirectly by spray drift, as in other crops such as citrus and eucalyptus where similar weed management is used (Tuffi Santos et al. 2006, Gravena et al. 2009, Machado et al. 2010).

The main herbicide used in Brazilian coffee plantations is glyphosate (*N*-(phosphonomethyl) glycine). Effects of glyphosate on plants are well understood, but its growth-promoting effects (hormesis) at very low doses have not been well studied. Researchers have reported growth-promoting effects of low glyphosate doses on different plant species (e.g., Cedergreen et al. 2007, Velini et al. 2008, Cedergreen and Olesen 2010). Hormesis can be defined as an adaptive response characterized by biphasic dose responses of generally similar

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quantitative features with respect to amplitude and range of the stimulatory response that are either directly induced or the result of compensatory biological processes following an initial disruption in homeostasis (Calabrese and Baldwin 2002), although the mechanism(s) of hormesis is poorly understood.

Some previous experiments indicate that *C. arabica* plants show some tolerance to herbicide glyphosate. This indicates that coffee plant growth cannot be reduced by glyphosate reaching the crop due to spray drift from application to weeds at doses that kill weeds. But, if accidental direct application occurs, plant growth could be reduced. We hypothesize that glyphosate hormesis in coffee plants is dependent on the growth stage at which the plant is sprayed. Thus, the objective of this research is to evaluate if there is any effect on *C. arabica* plants exposed to glyphosate at two distinctly different plant growth stages.

## MATERIALS AND METHODS

### PLANT MATERIAL AND GROWING CONDITIONS

Greenhouse experiments were carried out in Jaboticabal, SP, from October 2009 to February 2010. Five-leaf seedlings of *C. arabica* cv. Catuaí Vermelho IAC-144 were transplanted to 7-L pots filled with a substrate containing a mixture of manure and Red Latosol clay textured (1:3, v/v) with pH ( $\text{CaCl}_2$ ) = 5.1, O.M. = 20.0 g.dm<sup>-3</sup>; P (resin) = 26.0 mg.dm<sup>-3</sup>, K = 1.9, Ca = 23.0, Mg = 14.0, H+Al = 25.0, SB = 38.9, and T = 63.9 mmol<sub>c</sub>.dm<sup>-3</sup>, and V = 61%. Plants were fertilized with 3 g.pot<sup>-1</sup> of NPK (4-14-8) fertilizer just after transplanting. Water was supplied every day. Fungicides (piraclostrobin, epoxiconazol, and cooper oxichloride) and insecticide (thiamethoxan) were applied every two weeks.

### HERBICIDE AND APPLICATION CONDITIONS

A formulation of glyphosate with 48% (m/v) of active ingredient [isopropylamine salt of *N*-(phosphonomethyl)glycine] and 36% of acid

equivalent (AE) of glyphosate was used. Herbicide was applied using a CO<sub>2</sub>-pressurized sprayer with flat plan nozzles (TeeJet, 80.02, USA), pressure of 1.90 kgf.cm<sup>-2</sup>, and volume of application of 200 L.ha<sup>-1</sup>.

### TREATMENTS AND EXPERIMENTAL DESIGN

Treatments were 0, 180, 360, 720, 1,440, and 2,880 g.AE.ha<sup>-1</sup> of glyphosate, sprayed directly on coffee plants, simulating drift and accidental application conditions. Applications were made at 10 and 45 days after transplanting (DAT). Experiments were set up in a factorial scheme with two main treatments (time of glyphosate application) and six secondary treatments (glyphosate doses). A completely randomized design was used with six replicates. Each experiment was repeated at least twice.

### EVALUATION TIME AND MEASURED CHARACTERISTICS

All experiments were evaluated at 60 days after herbicide application (DAA) when plant height, stem diameter (Zaas Precision, 150 mm, Brazil), leaf number, leaf area (Li-Cor Inc., LI3000A, USA), and stem and leaf dry mass after drying for 96 h in a forced air oven at 70°C were measured.

### STATISTICAL ANALYSIS

There was no interaction between treatments and experimental repetitions detected by an ANOVA test (data not shown), so all data were pooled across experimental repetitions for further analysis and presentation. Data of plants with 10 DAT were submitted to a nonlinear regression analysis, according to the equation:

$$Y = Y_0 + A / (1 + \exp(-(X - X_0) / B))$$

where: Y describes a coffee characteristic evaluated in response to X, representing the glyphosate doses; Y<sub>0</sub> describes the bottom value while A represents the top value of the coffee characteristic; X<sub>0</sub> describes the dose reducing the coffee characteristic by 50% (I<sub>50</sub>); and B is the slope of the curve.

Data of plants with 45 DAT were submitted to nonlinear regression analysis, according to the equation:

$$Y = Y_0 + A \cdot \exp(-0.5 \cdot ((X - X_0) / B)^2)$$

where: Y describes a coffee characteristic evaluated in response to X, representing the glyphosate doses; Y<sub>0</sub> describes the bottom value while A is the difference between Y<sub>0</sub> and the maximum Y value of the coffee characteristic; X<sub>0</sub> describes the dose promoting the highest stimulation in the coffee characteristic; and B is the standard deviation on X<sub>0</sub>.

The dose reducing coffee characteristic by 50% (I<sub>50</sub>) of plants with 45 DAT was calculated based on the adjusted equations when the growth characteristics were reduced by 50% when comparing to the no-treated check.

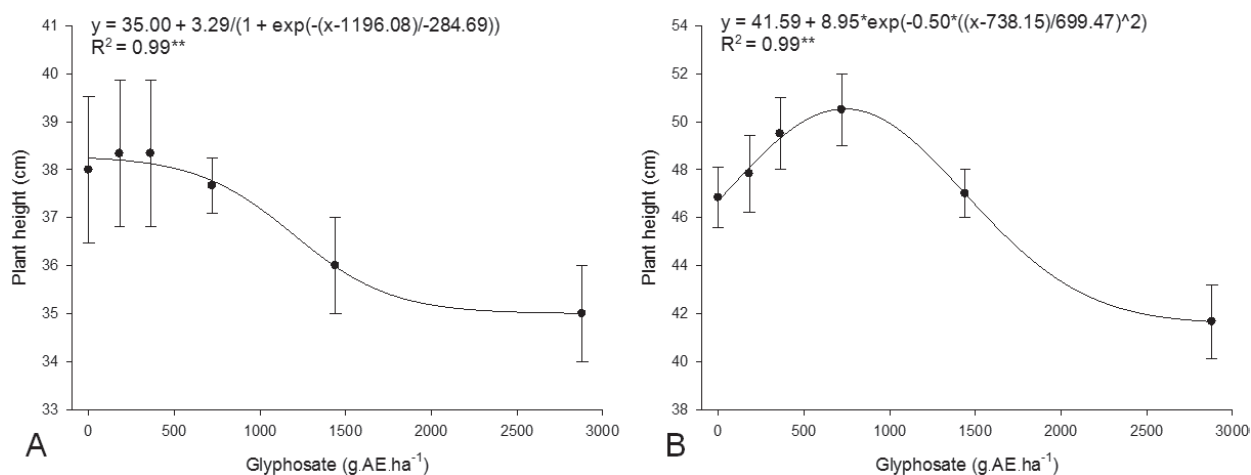
Regression analyses were performed using SigmaPlot software (Systat Software Inc., Version 10.0, USA).

## RESULTS

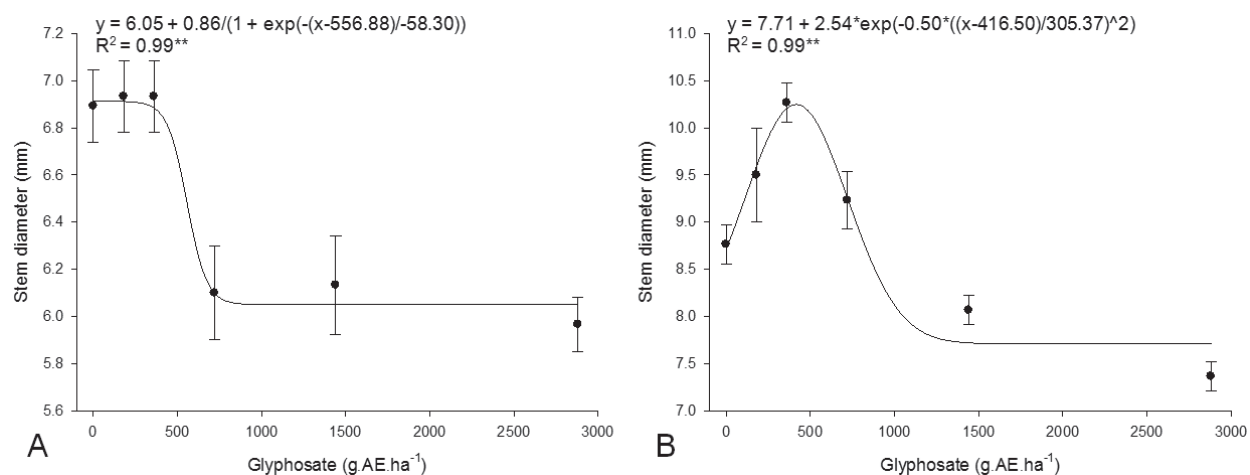
Coffee plants exposed to the highest glyphosate dose at 10 and 45 DAT showed reduction of all growth characteristics. In 10-DAT plants, plant height, stem diameter, leaf number, leaf area,

stem dry mass, leaf dry mass, and above ground dry mass were reduced by 9, 12, 30, 53, 31, 50, and 44%, respectively, whereas, in 45 DAT-plants, those same characteristics were reduced by 11, 12, 18, 42, 36, 44, and 40%, respectively (Figures 1-5).

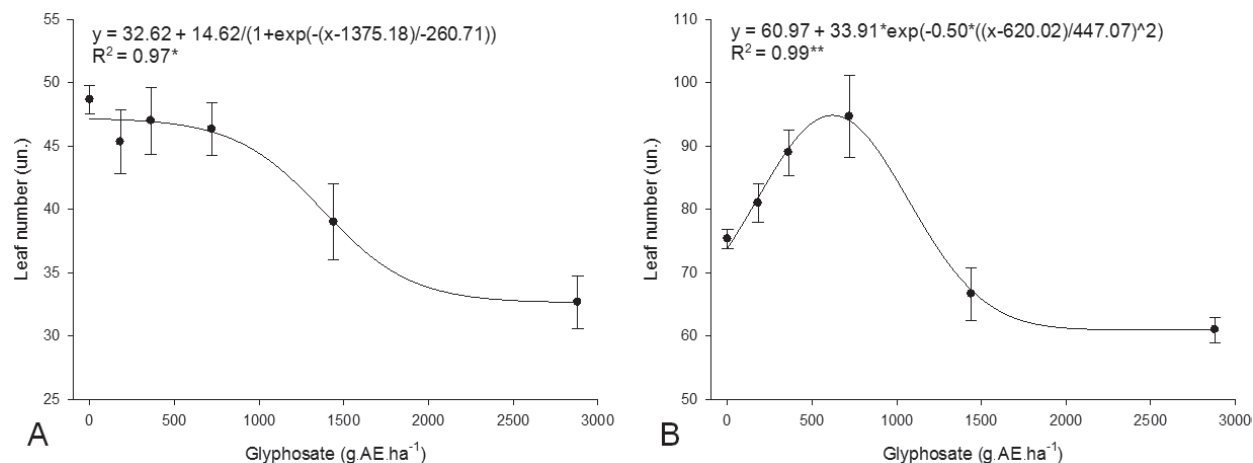
When applied at 10 DAT, the only effect of glyphosate on plant height was inhibition at the two highest doses (Figure 1A). Similar results were observed on stem diameter (Figure 2A), leaf number (Figure 3A), leaf area (Figure 4A), stem dry mass (Figure 5A), leaf dry mass (Figure 5C), and above ground dry mass (Figure 5E) of coffee plants that were exposed to glyphosate application at the same growth stage. Stem diameter was more sensitive to glyphosate than the other growth parameters. On the other hand, stimulatory effects of low doses of glyphosate were measured on all coffee plant growth characteristics after exposure to glyphosate at 45 DAT. Maximum stimulatory effects of 21%, 18%, 28%, 39%, 24%, 31%, and 27% were observed on plant height, stem diameter, leaf number, leaf area, stem dry mass, leaf dry mass, and above ground dry mass when coffee plants were exposed to 738, 416, 620, 536, 488, 530, and 513 g.AE.ha<sup>-1</sup>, respectively (Figures 1B, 2B, 3B, 4B, 5B, 5D, and 5F, respectively), characterizing a hormetic effect.



**Figure 1** - Coffee plant height at 60 days after being exposed to glyphosate applied on plants with 10 (A) and 45 (B) days after transplanting. Jaboticabal, 2009-10. Vertical bars indicate standard error of the mean, \*\*significant at  $p < 0.01$ .



**Figure 2** - Coffee stem diameter at 60 days after being exposed to glyphosate applied on plants with 10 (**A**) and 45 (**B**) days after transplanting. Jaboticabal, 2009-10. Vertical bars indicate standard error of the mean, \*\*significant at  $p < 0.01$ .

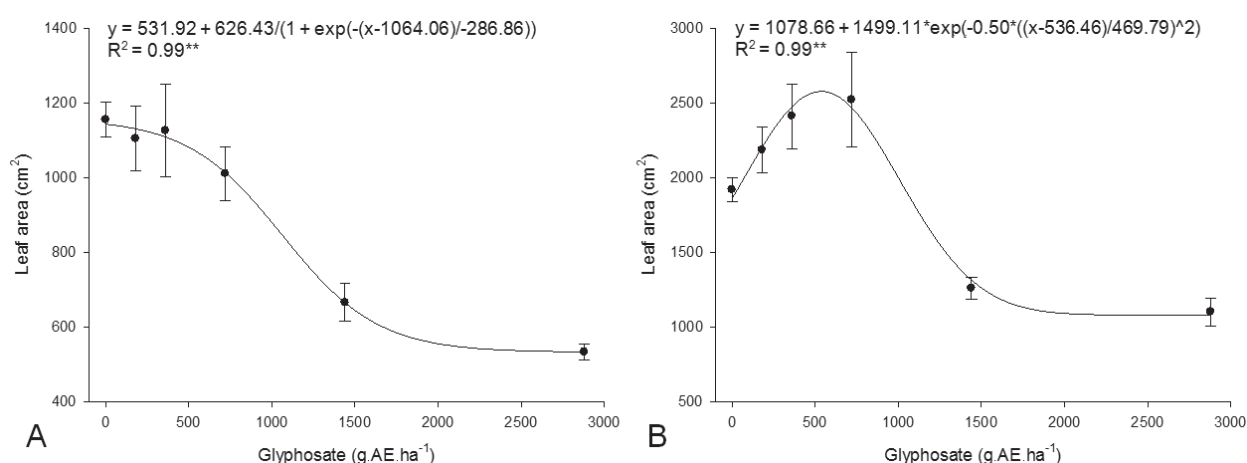


**Figure 3** - Coffee number of leaves at 60 days after being exposed to glyphosate applied on plants with 10 (**A**) and 45 (**B**) days after transplanting. Jaboticabal, 2009-10. Vertical bars indicate standard error of the mean, \*, \*\*significant at  $p < 0.05$  and  $p < 0.01$ , respectively.

### DISCUSSION

We assume that reductions in plant height, stem diameter, leaf number, leaf area, stem dry mass, leaf dry mass, and above ground dry mass observed in coffee plants treated with high doses of glyphosate at 10 and 45 DAT occurred due to inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (EC 2.5.1.19) of the shikimate pathway. Inhibition of EPSPS results in reduction in synthesis of EPSP and metabolic products of EPSP, such as the aromatic acids phenylalanine,

tyrosine, and tryptophan that are required for protein synthesis (Siehl 1997, Herrman and Weaver 1999), as well as indoleacetic acid, lignin, and many secondary compounds required for plant defence and other aspects of chemical ecology (Lydon and Duke 1989). Deregulation of the shikimate pathway also causes shortages of compounds necessary for carbon fixation (Siehl 1997), a process that is quickly inhibited by glyphosate (Servaites et al. 1987). These metabolic changes cause plant growth reduction and even plant death when the



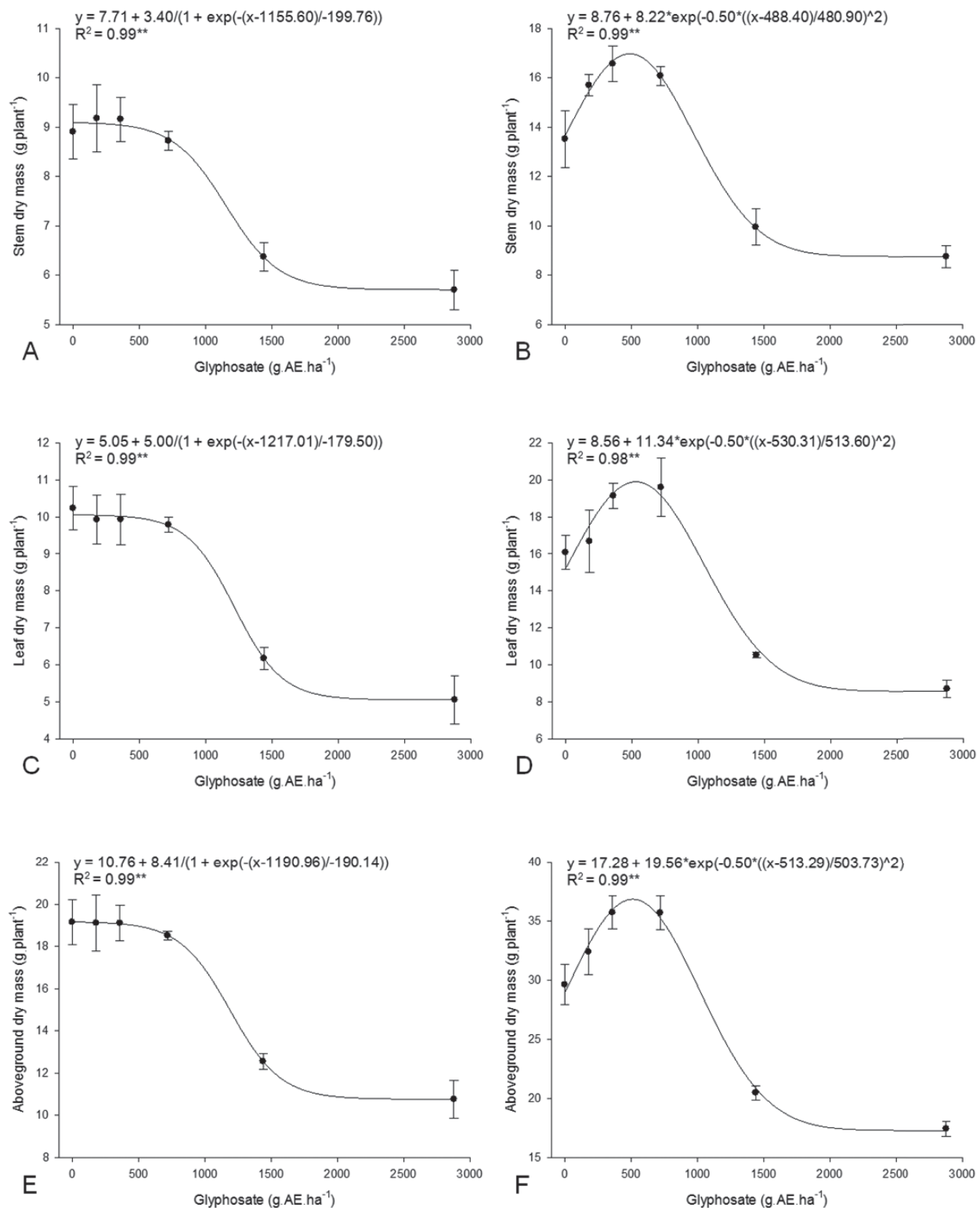
**Figure 4** - Coffee leaf area at 60 days after being exposed to glyphosate applied on plants with 10 (A) and 45 (B) days after transplanting. Jaboticabal, 2009-10. Vertical bars indicate standard error of the mean, \*\*significant at  $p < 0.01$ .

herbicide is applied to glyphosate-sensitive plants at a susceptible growth stage. Coffee plants at both growth stages of this study tolerated lower glyphosate doses and, when applied at 45 DAT, grew even more rapidly after exposure to low glyphosate doses.

The hormetic effect of glyphosate has also been observed in the growth of *Abutilon theophrasti* Medik., *Commelina benghalensis* L., *Echinochloa crus-galli* (L.) P.Beauv., *Eucalyptus grandis* Hill Ex. Maiden, *Glycine max* (L.) Merr., *Hordeum vulgare* L., *Pinus caribea* L., *Stellaria media* (L.) Vill./Cyr., *Tripleurospermum inodorum* (L.) Sch. Bip., *Saccharum* spp., and *Zea mays* L. plants (Schabenberger et al. 1999, Wagner et al. 2003, Cedergreen et al. 2007, Velini et al. 2008, Silva et al. 2009, Cedergreen and Olesen 2010, Bott et al. 2011).

The mechanism of the stimulatory effect is not well understood, and few studies have suggested a molecular mechanism of hormesis in plants (as reviewed by Cedergreen et al. 2007). Both Velini et al. (2008) and Cedergreen and Olesen (2010) indicate that the hormetic effect is in some way related to partial inhibition of EPSPS at low glyphosate doses. The absence of any growth effect of glyphosate at any dose on glyphosate-resistant soybean with an insensitive EPSPS supports this hypothesis (Velini

et al. 2008). Low doses of glyphosate are used to enhance sucrose accumulation in sugarcane (Dusky et al. 1986, Su et al. 1992, McDonald et al. 2000). More carbon is partitioned into sucrose when lignification is inhibited (Liu et al. 1997). So, since lignin synthesis is dependent on the shikimic acid pathway, low doses of glyphosate may inhibit lignification sufficiently to allow plant to partition more carbon into sucrose (Velini et al. 2008). Another possible explanation for sugar accumulation at low glyphosate doses might be that glyphosate at low doses preferentially inhibits metabolic activity of meristematic tissues, thereby slowing translocation of sugar to such metabolic sinks. Glyphosate is preferentially translocated to metabolic sinks, in which it is accumulated (reviewed by Duke 1988). Thus, sugar synthesis might not be inhibited in plant leaf tissues with glyphosate concentrations too low to have an adverse effect, while concentrations of glyphosate in meristematic tissues of the same plant might be high enough to stop growth, thereby no longer importing sugar. Perhaps both mechanisms could be involved in glyphosate-enhanced sugar accumulation in sugarcane; however, the latter explanation would not explain glyphosate-caused hormesis in the case of overall growth stimulation. Nevertheless, if lignin synthesis is preferentially



**Figure 5** - Coffee stem, leaf and above ground dry mass at 60 days after being exposed to glyphosate applied on plants with 10 (A, C and E) and 45 (B, D and F) days after transplanting. Jaboticabal, 2009-10. Vertical bars indicate standard error of the mean,  $^{**}$ significant at  $p < 0.01$ .



inhibited at low glyphosate doses, cell walls would remain elastic for a longer period, allowing for greater cell expansion, and, thus, enhanced growth. However this mechanism would not explain stimulatory effects on total dry weight.

Other studies have shown that plant growth stimulation by glyphosate is a result of resource allocation to the whole plant (Cedergreen 2008). To achieve the biomass increase, plants must either increase photosynthetic rates or decrease respiration rates in response to the low glyphosate doses (Cedergreen and Olesen 2010). From a theoretical viewpoint, it is unlikely that respiration rates decrease, as addition of xenobiotics usually induces detoxification processes which are energy demanding (Cole 1994, Purrington and Bergelson 1999). So, an increase in photosynthesis rates (by increasing light harvesting and/or efficiency of carbon fixation) could explain the plant growth stimulation (Cedergreen and Olesen 2010).

A single specific physiological mechanism of hormesis has not been found. The finding that glyphosate does not produce hormesis in glyphosate-resistant crops indicates strongly that the mechanism requires inhibition of EPSPS (Velini et al. 2008). This suggests that the mechanism of hormesis is dependent on its effects on the shikimate pathway. Since glyphosate is the only phytotoxin known to inhibit the shikimate pathway, the mechanism of hormesis for glyphosate appears to be unique. Whatever the mechanism, our study shows that the hormetic effect can be strongly dependent on the plant growth stage. We could observe the hormetic effect only in plants exposed to glyphosate at 45 DAT, while exposure of younger plants (10 DAT) did not show this growth stimulation. We can only speculate on the age dependency for glyphosate-associated hormesis that we have observed.

Younger plants are almost always more susceptible to herbicides than older plants of the same species. In our study, this is apparent in  $I_{50}$  values mainly for shoot plant height and stem diameter,

resulting in a factor of tolerance greater than 1.5 (Table I). At the time of glyphosate application, the youngest plants (10 DAT) had just six leaf pairs and no stem ramifications, whereas the 45 DAT-plants had formed a greater leaf biomass with stem ramifications when the herbicide was applied. Another difference is that the younger plants were sprayed with herbicide exposure only a short period after transplanting, so that they had less time to establish their roots in the substrate. The older plants may have had greater capacity to detoxify glyphosate and less absorption of the herbicide through plants surfaces. They had greater biomass to dilute the herbicide. Furthermore, the ratio of more sensitive meristematic tissues to mature tissues would be smaller in older than younger plants, so that the concentration reaching the meristem would probably be less. There are no data with coffee to support any of these mechanisms, but reduced absorption in older plants has been described in literature with other species. Younger plants usually have a thinner epicuticular wax layer on the leaf epidermis than older ones, although this does not always translate to greater herbicide absorption by younger leaves (e.g., Viougeas et al. 1995). Epicuticular wax layer composition and thickness influences glyphosate penetration through the leaf tissue (Michitte et al. 2007, Nandula et al.

**TABLE I**  
**Dose reducing the growth characteristics by 50% ( $I_{50}$ ) and the factor of tolerance (FT) of coffee plants exposed to glyphosate at 10 and 45 days after transplanting. Jaboticabal, 2009-10.**

Characteristics	$I_{50}$ (g.AE.ha <sup>-1</sup> )		FT
	10 DAT	45 DAT	
Plant height	1,196	1,845	1.54
Stem diameter	557	968	1.74
Number of leaves	1,375	1,434	1.04
Leaf area	1,064	1,307	1.23
Stem dry mass	1,156	1,237	1.07
Leaf dry mass	1,217	1,335	1.10
Aboveground dry mass	1,191	1,298	1.09



2008). Since glyphosate is a hydrophilic herbicide, its penetration through thinner epicuticular wax layers may be increased in younger plants.

Because of these factors, more glyphosate is probably reaching EPSPS in 10 DAT-plants than in 45-DAT plants. On the other hand, glyphosate application at high doses can increase its penetration efficiency due to the high differences in herbicide concentrations between the leaf surface and leaf tissues, since diffusion is the main process of glyphosate transport through the plant cuticle (Stahlman and Phillips 1979, Caseley and Coupland 1985). This might explain the similar reduction in plant growth of younger and older coffee plants exposed at higher glyphosate doses.

Only a few studies on effects of glyphosate on coffee plants have been published. Hormesis with glyphosate was not observed in coffee plant growth in a range of doses from 57.6 to 460.8 g.AE.ha<sup>-1</sup>, but a significant reduction in plant height, leaf area, stem dry mass, leaf dry mass, and root density occurred (França et al. 2010a), and reductions in leaf nutrient content were observed (França et al. 2010b). Field observations indicated that effects of glyphosate can persist until harvesting, reducing coffee bean yield (Nelson 2008). Comparing results with others is difficult, because different dose-responses are expected, depending on growth conditions (Belz and Cedergreen 2010) and plant growth stage (Velini et al. 2008). For these reasons, predicting beneficial or stimulatory effects of sub-lethal glyphosate doses under agricultural conditions is very imprecise and it is not likely to be useful in agricultural production (Belz et al. 2011).

The findings presented here show that coffee plants cv. Catuaí Vermelho IAC-144 are tolerant to sub-lethal doses of glyphosate and that low doses of glyphosate can even stimulate growth of the plants if exposure is at the right growth stage. So, reported deleterious effects of glyphosate observed in the field that are attributed to the glyphosate drift may be due to other factors. Further research is needed to evaluate the effects of low doses of glyphosate on

plants and to understand the mechanisms involved. Such work is fundamental to improve our knowledge of glyphosate dynamics in production systems and to have precise information about the effects of glyphosate on non-target plants (Velini et al. 2008).

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#### RESUMO

Os sistemas de manejo de plantas daninhas em quase todos os pomares de café do Brasil permitem que o herbicida pulverizado sofra deriva sobre a cultura. Com objetivo de avaliar se há algum efeito do glyphosate, herbicida mais comumente utilizado em pomares de café, sobre as plantas de café, uma ampla faixa de doses do herbicida foi aplicada diretamente sobre plantas de café em dois distintos estádios de crescimento da planta. Embora o crescimento de ambas as plantas novas e velhas tenha sido reduzido em doses mais altas de glyphosate, baixas doses não causaram efeitos sobre características de crescimento de plantas novas e estimularam o crescimento das mais velhas. Portanto, o efeito hormético do glyphosate é dependente do estágio de crescimento da planta de café no momento da aplicação do herbicida.

**Palavras-chave:** *Coffea arabica* L., manejo de plantas daninhas, herbicida, deriva simulada, efeito estimulante.

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