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Article

Translocation and Root Exudation of Glyphosate by *Urochloa brizantha* and its Transport on Sugarcane and Citrus Seedlings

Translocação e Exsudação Radicular do Glyphosate por ***Urochloa brizantha*** *e seu Transporte em Mudas de Cana-de-Açúcar e Citros*

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ABSTRACT - Glyphosate is easily exuded by the roots of plants. However, there is still some lack of information in the scientific literature as to whether *Urochloa brizantha* is capable of exuding glyphosate to influence the growth of crops such as sugarcane and citrus. Thus, the objective of this research was to evaluate the translocation and root exudation of ^{14}C-glyphosate by *U. brizantha* and its transport in sugarcane and citrus. Sugarcane seedlings, varieties SP80-1842, and citrus *'Limão Rosa'* (*Citrus limonia* L. Osbeck) were cultivated with *U. brizantha* around the seedlings. Using a microsphere, ^{14}C-glyphosate was applied on the leaves of *U. brizantha*. The plants were cultured for 12 days after treatment (DAT). The radioactivity of the herbicide was evaluated by liquid scintillation spectrometer, after oxidizing the soil and parts of the plants (leaves, culms and roots) of *U. brizantha*, sugarcane and citrus. The highest amount of glyphosate was detected in the *U. brizantha* leaves, where the applications were carried out, regardless of the culture studied. Only traces of glyphosate (0.001%) were detected in soil cultivated with sugarcane. On the other hand, in citrus, *U. brizantha* exuded 9.46% of the glyphosate by the root system in the soil. The total amount of herbicide found in sugarcane and citrus seedlings was only 0.006 and 0.095%, respectively, in all parts of the plant. These concentrations are lower than those required to cause intoxication in those crops. Considering the results, it is possible to state that the translocation of glyphosate in young plants of *U. brizantha* associated with citrus was higher in relation to sugarcane, and it was not exuded by the root system of the weed with sugarcane, but presented root exudation with citrus, however, the amount did not reach what is necessary to affect the dry mass of the agricultural crops.

Keywords: behavior in the plant, radioisotope, chemical sensitivity.

*RESUMO - O glyphosate é facilmente exsudado pelas raízes das plantas. No entanto, ainda falta informação na literatura científica sobre a braquiária (**Urochloa brizantha**) é capaz de exsudar o glyphosate a ponto de influenciar o crescimento de culturas, como cana-de-açúcar e citros. Assim, o objetivo desta pesquisa foi avaliar a translocação e exsudação radicular do ^{14}C-glyphosate por **U. brizantha** e seu transporte em mudas de cana-de-açúcar e citros. Foram cultivadas mudas de cana-de-açúcar, variedade SP80-1842, e citros 'Limão Rosa' (**Citrus limonia** L. Osbeck) com a braquiária ao redor das mudas. Utilizando uma microsseringa, foi aplicado ^{14}C-glyphosate nas folhas de braquiária. As plantas foram cultivadas por 12 dias após o tratamento (DAT). A radioatividade do herbicida foi avaliada por espectrômetro de cintilação líquida, depois de oxidado o solo e as partes das*

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plantas (folhas, colmos e raízes) de braquiária, cana-de-açúcar e citros. A maior quantidade de glyphosate foi detectada nas folhas de braquiária, onde foram realizadas as aplicações, independentemente da cultura estudada. Foram detectados apenas traços do glyphosate (0,001%) no solo cultivado com cana-de-açúcar. Por outro lado, em citros, a braquiária exsudou 9,46% do glyphosate pelo sistema radicular no solo. A quantidade total do herbicida encontrada nas mudas de cana-de-açúcar e citros foi de apenas 0,006 e 0,095%, respectivamente, em todas as partes da planta - concentrações inferiores às necessárias para causar intoxicação nessas culturas. Diante dos resultados, é possível afirmar que a translocação do glyphosate em plantas jovens de ***U. brizantha*** *associadas com citros foi maior em relação à cana-de-açúcar; ele não foi exsudado pelo sistema radicular da planta daninha com cana-de-açúcar, mas apresentou exsudação radicular com citros, não sendo em quantidade necessária para afetar a massa seca das culturas agrícolas.*

Palavras-chave: comportamento na planta, radioisótopo, sensibilidade química.

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] is a water-soluble herbicide, and it penetrates the cuticles of the leaves of the plant, where it is simplistically translocated through the phloem to the apical meristems and, also, through the apoplast (Shaner, 2009). The translocation of glyphosate in plants follows the source-sink route of photoassimilates and it is translocated to fast-growing sprouts and root tissues (Bromilow et al., 1993). Glyphosate shows little or no metabolism in most plants, and it is easily exudated by the roots of the plants, being eventually released into the rhizosphere, probably through a diffusion process, together with sugars, amino acids and other low molecular weight compounds (Kremer et al., 2005). Exudation through the root is also considered one of the possible mechanisms involved in the environmental destination of herbicides, mas but relatively few data is available in the literature on the exudation of herbicides through the roots of plants (Dinelli et al., 2007).

When glyphosate gets in touch with the soil during spraying or when it is exudated by weeds, it may be strongly adsorbed to the soil particles (Al-Rajab et al., 2008; Gómez Ortiz et al., 2017), degraded by the microorganisms on the soil (Rueppel et al., 1977; Yu et al., 2015) or absorbed by the roots of adjacent plants, mainly those very close to the root of weeds treated with the herbicide (Tuffi Santos et al., 2005, 2008).

Considering this scenario, sugarcane and citrus crops are currently still frequently infested by brachiaria (*Urochloa brizantha*), and such infestation was already common in the past, as reported by Carmona (1995), in which *Urochloa* spp. Prevailed between the rows in citrus orchards. However, such weeds stand out due to their high competitive ability in relation to sugarcane. Galon et al. (2011) reported that the infestation by *U. brizantha* interfered in the morphological components of sugarcane, with a reduction in the mean productivity of stems of up to approximately 60 t ha^{-1}. Thus, among the herbicides used on sugarcane and citrus cultures, glyphosate stands out due to its effective control on a large number of weed species, since it is a non-selective herbicide.

Concomitantly, glyphosate applied at the doses recommended by the manufacturer caused an accumulation of d” 0.3 mM of the herbicide on root tissue of susceptible plants (Honegger et al., 1986). It is important to point out that glyphosate may interfere in the photosynthesis of sugarcane, since it reduces the chlorophyll and carotenoid contents of the plant, and, consequently, it reduces its productivity (Meschede et al., 2011). On the other hand, in citrus seedlings, glyphosate show low toxicity (Gravena et al., 2009, 2012).

Further information is still necessary in the scientific literature as to whether brachiaria (*U. brizantha*) is able to exudate glyphosate to the point of influencing the growth of crops such as sugarcane and citrus. Therefore, it is necessary to evaluate whether the contamination of the main crop by glyphosate residues may occur by the absorption of the herbicide on the soil as root exudates coming from weeds controlled by glyphosate. Thus, the objective of this research was to evaluate the translocation and root exudation of ^{14}C-glyphosate by *U. brizantha* and its behavior in sugarcane and citrus seedlings.

MATERIAL AND METHODS

The experiments were conducted in a greenhouse using 10 L vases, where sugarcane seedlings from variety SP80-1842 were grown, as well as citrus *'Limão Rosa'* (*Citrus limonia*) seedlings from the Areão farm (520 m of altitude, 22°41'43"S and 47°38'29"W), Piracicaba, SP, Brazil. The latter is considered in this study as a non-target plant. The vases were duly labeled, identified and filled with Red Nitosol soil and washed sand, at a proportion of 10:1, to make it easier for the roots of the plants to be removed during the root system evaluations. The soil was sieved using a 2 mm mesh and then homogenized and analyzed. Its physical-chemical characteristics are shown in Table 1.

Table 1 **-** Physical-chemical properties of the soil (0-10 cm of depth) used in this study

pH ($CaCl_2$)	P (mg dm^{-3})	K	Ca	Mg	H+Al	BS	CEC
		(mmol$_c$ dm^{-3})					
5.4	7	4.4	65	48	32	117.4	149.8

V	OC	Sand	Silt	Clay	Texture class
(%)					
78	2.2	47.1	12.6	40.3	Sandy clay

BS = base saturation; CEC = cation exchange capacity; V = base saturation levels and OC = organic carbon. Source: Soil Science Department – ESALQ/USP, Piracicaba, SP, Brazil.

The seedlings were planted at a depth of approximately 5 cm. After 40 days from when the sugarcane seedlings were planted and 75 days from the citrus seedling transplantation, approximately 30 seeds of the target plant brachiaria (*U. brizantha*) – were sown around the seedlings, at a distance of approximately 9 cm from the stem of the non-target seedlings. The vases in which brachiaria was planted were irrigated by capillarity using vase plates, in order for the soil not to be compacted and to avoid the leaching of nutrients.

The brachiaria seeds were covered with 1 cm of soil with the same initial composition, so that the vase would not be moved, in order to avoid damages to the root system of the plants. During the growth of the brachiaria plants, thinning was conducted, keeping only the six most vigorous plants with three leaves each. Before applying the herbicide, stakes were used on these brachiaria plants, preventing their leaves from touching one another or from touching the leaves of the cultures.

In order to prepare the work solution of 1,100 µL, 40 µL of radiomarked glyphosate were used with ^{14}C on the phosphonomethyl radical. The specific radioactivity of the product was 165.11 Bq μL^{-1}. Since the radiomarked product is acid, before it was used, it was aminated by adding monoisopropylamine (MIPA), after which, the pH of the solution was around 4.5. In order to reach the recommended dose for the field (2,440 g a.e. ha^{-1}), the solution was completed with a commercial formulation of the glyphosate herbicide RoundUp® (Monsanto Company, Marysville, OH, USA).

Using a Hamilton microsyringe and a PB600-1 repeating dispenser (Figure 1), which releases 1 µL drops at a time, 15 µL of the solution were applied on each brachiaria leaf, at a total of 45 µL per plant (three leaves) or 270 µL per vase, when the brachiaria seedlings were approximately 30 cm high, corresponding to 30 days after sowing. A total radioactivity of 7.294 KBq was applied to each plant, totaling 43.767 KBq per vase (six plants). The product was applied with a microsyringe, to avoid any type of deviation to other plants or even to the soil.

After applying glyphosate, the vases stayed in the laboratory where the same radiological protection procedures were used for drying and total penetration of the herbicide solution on the brachiaria leaves; then, they were once again carefully taken to the greenhouse. Then, the plants were grown for 12 days after treatment (DAT). The soil, roots and leaves of brachiaria and

Figure 1 - Application of ^{14}C-glyphosate in *Urochloa brizantha* leaves using a Hamilton microsyringe and a PB600-1 repetitive dispenser, and distribution of sugarcane (A) and citrus (B) seedlings.

of the other crops, in addition to the sugarcane stem, were carefully separated with the help of scissors; when necessary, running water was used to remove the excess of soil on the roots; then they were placed on a tray to dry at room air.

Representative samples of the soil were collected for further analysis of glyphosate. The samples were stored at an ultrafreezer at -85 °C up to lyophilization. The vegetable material and the soil were dried using a freeze dryer at a vacuum pressure of approximately 0.064 mBar and temperature of approximately -51 °C. The root samples were collected on Petri dishes for lyophilization; the leaves were lyophilized in paper bags, and the soil, in aluminum vessels. After the lyophilization, the samples were stored in the freezer up to the time of the analysis. After the material had dried, the samples were weighted, in order to quantify the dry mass.

The soil was ground using a mechanical mill (Marconi MA330, Piracicaba, SP, Brazil), for further oxidation. The samples of the brachiaria leaves were chopped and manually homogenized. The sugarcane samples (leaves, stems and roots) and citrus samples (leaves and roots) were ground using a blender.

All the samples (0.2 g each) in triplicates were subjected to combustion in a biological oxidizer (OX500, RJ Harvey Instrument Corporation, Tappan, NY, USA) at 900 °C. The detection of glyphosate was conducted by capturing the $^{14}C\text{-}CO_2$ released during oxidation. The efficiency of the oxidizer was calculated before combustion of the samples, in order to correct any recovery error.

After each sample had been burned, a new flask was used (only 10 mL of scintillation solution), to verify the absence of radioactivity of the sample (control). The radioactivity of glyphosate was measured for five minutes in a liquid scintillation spectrometer, using a Tri-Carb 2910 TR counter (LSA Perkin Elmer, Waltham, MA, USA). The radioactivity mass balance of glyphosate was calculated.

Both experiments with brachiaria were conducted using independent randomized designs, prepared with each crop, sugarcane and citrus, with four replicates, in addition to control, which did not receive the herbicide application, in order to compare the radioactivity calculations found in the plants; thus, it was possible to verify in which part of the soil-plants system a greater concentration of the herbicide occurred. With the purpose of evaluating the translocation of glyphosate in the crop separately, a treatment with monocultures was not evaluated (brachiaria, citrus and eucalypt). All the data from the experiments was subjected to variance analysis. When significant, Tukey's test ($p<0.05$) was conducted to compare the treatment means, in order to verify the translocation and root exudation of ^{14}C-glyphosate by the plants. The figures were plotted using the Sigma Plot program (version 10.0 for Windows, Systat Software Inc., Point Richmond, CA, USA).

RESULTS AND DISCUSSION

The mass balance of ^{14}C-glyphosate applied in Association with sugarcane and citrus seedlings was, on average, 85.08 and 80.86%, respectively, which is the ratio of the sum of the radioactivity of the herbicide found by biological oxidation (soil, root, stem and leaves of brachiaria and other crops) and the total radioactivity applied to the individual plant. According to Nandula and Vencill (2015), a mass balance equal to or higher than 80% is adequate to detect the translocation process of the herbicide on the plant using radioisotopes.

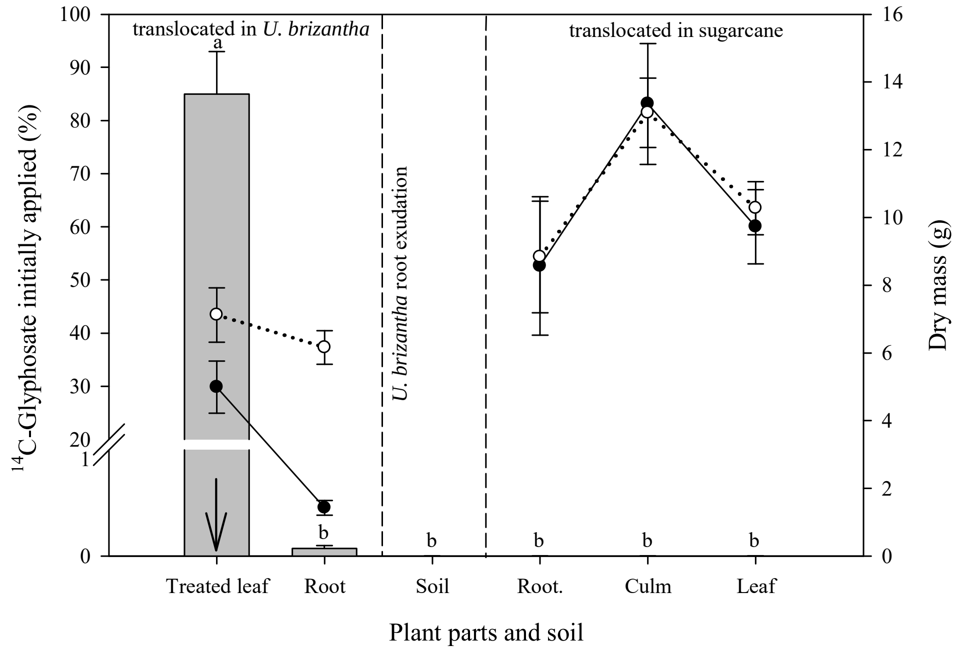
The radioactivity of glyphosate found on the soil and on the parts of the brachiaria, sugarcane and citrus plants were from each other (F = 451.03 and 622.45; $p<0.01$, respectively) (Figures 2 and 3, respectively). The translocation and root exudation of glyphosate by brachiaria and its transportation in sugarcane and citrus seedlings may be seen on Figures 2 and 3, respectively.

The highest amount of glyphosate (~85 and 69%) was detected on the treated leaves of brachiaria, with associated to sugarcane and citrus, respectively (Figures 2 and 3, respectively). Therefore, it may be assumed that the translocation of glyphosate in brachiaria was relatively lower when associated to sugarcane; however, for brachiaria in associated to citrus seedlings, this behavior of glyphosate was more intense, showing a differentiated translocation of the herbicide in the same species. This fact may be justified by the small difference on the dry mass of the shoot and root of brachiaria with sugarcane (~5 and 1.5 g, respectively), in comparison to citrus (~6 and 2 g, respectively), after the application of glyphosate (Figures 2 and 3, respectively). The differentiation on the development of the main crops (sugarcane and citrus) may have directly affected the growth of. It is important to point out that the herbicides that showed greater translocation on the plant, such as glyphosate, are absorbed and translocated with greater efficiency by plants in initial development stages (Roman et al., 2007), such as the stage in which the brachiaria plants evaluated in this study were.

Santos et al. (2007, 2008) stated that young *U. brizantha* plants, when they had approximately 10 cm of height and when the plants had four to five tillers with approximately 20 cm of height, are easily controlled with glyphosate between 15 and 60 days after the application. The height of the brachiaria plants and the application period of the herbicide for this study are similar to those used by these authors, thus, an effective control of the weed by the translocation of glyphosate in the plant was possible. However, for citrus plants, a reduction of the dry mass of the shoot and root of brachiaria of approximately two and three times, respectively, was observed in relation to the control treatment, which received no glyphosate application (Figure 3); the same occurred for sugarcane, with a reduction of approximately 1.5 and 4.5 times, respectively, in comparison to the control (Figure 2).

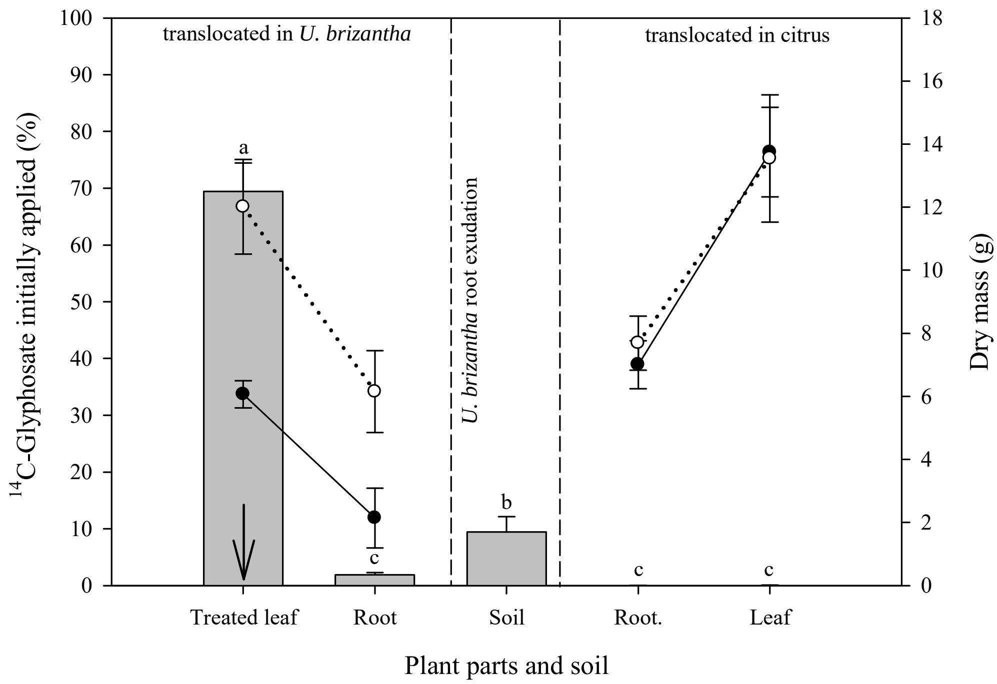
Only 0.08 and 1.89% of the glyphosate translocated by brachiaria went to the roots of the weed, when associated to sugarcane and citrus, respectively, at 12 days after the application (Figures 2 and 3, respectively). In another *Urochloa* species, such as *U. ramosa*, only 10.8% of the applied radioactivity was translocated from the treated leaf to the rest of the plant (including the root) when glyphosate was applied in isolation 72 hours after treatment (Burke et al., 2007). These results show the small amount of the herbicide that reaches the root system of brachiaria.

These results that show the low transportation of the applied glyphosate at a dose of 2.440 g a.e. ha^{-1} on the leaves of the plant may be justified by the short initial days (only 12 DAT)



Black (●) and white (o) circles represent the dry mass of plants from foliar-treated *U. brizantha* with glyphosate and control (without herbicide). The vertical bars associated with each column or circle represent the standard deviation (SD) of each mean value (n = 4). Means followed by the same letter in each top column do not differ by Tukey test (p<0.05). LSD, least significance difference = 7.49 and CV, coefficient of variation = 23.04%. The arrow represents the plant part that was treated with herbicide initially.

Figure 2 **-** Translocation and root exudation of glyphosate by *Urochloa brizantha* and its transport process on sugarcane seedlings at 12 days after treatment (DAT).



Black (●) and white (o) circles represent the dry mass of plants from foliar-treated *U. brizantha* with glyphosate and control (without herbicide). The vertical bars associated with each column or circle represent the standard deviation (SD) of each mean value (n = 4). Means followed by the same letter in each top column do not differ by Tukey test (p<0.05). LSD, least significance difference 5.42 and CV, coefficient of variation = 14.88%. The arrow represents the plant part that was treated with herbicide initially.

Figure 3 **-** Translocation and root exudation of glyphosate by *Urochloa brizantha* and its transport process on citrus seedlings at 12 days after treatment (DAT).

in which the parts of the brachiaria plants were evaluated. Corroborating this data, Santos et al. (2008) reported that, with the application of the highest dose of glyphosate (2.880 g a.e. ha^{-1}), it was possible to observe a maximum control of 74.78% for *U. brizantha* at 15 DAT, and the total control (100%) was only observed at 30 and 60 DAT. However, it would be necessary to evaluate the transportation of glyphosate for more days, in order to better understand the translocated amount of the herbicide to the root system of the weed.

It is also important to point out that this result related to the translocation of glyphosate may not be due to the compartmentalization or metabolization, since the metabolism of this herbicide in different crops showed a slow process, and since it is considered a non-selective herbicide; also, few or no metabolites of glyphosate were reported in several weeds (Duke, 2011). This author also stated that plants that are able to quickly metabolize the herbicide must have some degree of natural resistance to it. The fact that several crops are resistant to herbicides due to their quick metabolism collaborates with this. However, further data is still needed in the scientific literature related to the glyphosate behavior in *U. brizantha* plants, in order to better understand the transportation of the herbicide.

Considered the results presented for the behavior of glyphosate in Young brachiaria plants in association with sugarcane, it was already expected that the root exudation of the herbicide would not occur, due to the small amount of the herbicide found on the root system of the weed. Therefore, the data was confirmed; and only traces of glyphosate (0.001%) were detected on the soil where both species, - brachiaria and sugarcane – were grown (Figure 2). On the other hand, brachiaria exuded 9.46% of glyphosate by the root system on the soil, when associated to citrus (Figure 3).

Furthermore, the exudation of glyphosate by the root system of brachiaria grown with citrus may influence the microbial activity of the soil. Kremer et al. (2005) showed that the glyphosate detected on the exudates stimulated the growth of fungi on the soybean rhizosphere, possibly providing a selective source of C and N combined with the high levels of soluble carbohydrates and amino acids from the degradation of the herbicide. However, the increase of the fungal population that developed with the glyphosate treatment may adversely affect the growth of the plants and the biological processes on the soil and rhizosphere and, consequently, the agricultural productivity.

Corroborating the data, the total amount of glyphosate found on sugarcane and citrus seedlings was only 0.006 and 0.095%, respectively, in all parts of the plant (root, stem and leaves), at lower concentrations than those necessary to cause intoxication on the crops and reduce their dry mass, as presented on Figures 2 and 3, respectively. However, it is important to point out that each crop presents a different behavior when it comes to the application of glyphosate; thus, some measures must be taken when applying the herbicide, and alternative weed control methods may be chosen, avoiding the contamination of the non-target crop and possible productivity reductions.

The data on this research support the data found by Tuffi Santos et al. (2005, 2008), since the low exudation of glyphosate by brachiaria (*U. decumbens*), combined to the low absorption of the herbicide by the roots of eucalypt, is not enough to cause damages to the crop.

Thus, it is possible to conclude that the translocation of glyphosate in young *U. brizantha* plants associated with citrus was higher in relation to the associated with sugarcane. It was not exudated by the root system of the weed with sugarcane, but, with citrus, the root exudation occurred, however, not in a sufficient amount to affect the dry mass of the agricultural crops.

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