GLYPHOSATE TRANSLOCATION IN HERBICIDE TOLERANT PLANTS[1]

Translocação do Glyphosate em Plantas Tolerantes ao Herbicida

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ABSTRACT - The objective of this study was to evaluate glyphosate translocation in glyphosate-tolerant weed species (*I. nil*, *T. procumbens* and *S. latifolia*) compared to glyphosate-susceptible species (*B. pilosa*). The evaluations of ^{14}C-glyphosate absorption and translocation were performed at 6, 12, 36 and 72 hours after treatment (HAT) in *I. nil* and *B. pilosa*, and only at 72 HAT in the species *T. procumbens* and *S. latifolia*. The plants were collected and fractionated into application leaf, other leaves, stems, and roots. In S. latifolia, approximately 88% of the glyphosate remained in the application leaf and a small amount was translocated to roots at 72 HAT. However, 75% of the herbicide applied on *T. procumbens* remained in the leaf that had received the treatment, with greater glyphosate translocation to the floral bud. It was concluded that the smaller amount of glyphosate observed in *S. latifolia* and *T. procumbens* may partly account for their higher tolerance to glyphosate. However, *I. nil* tolerance to glyphosate may be associated with other factors such as metabolization, root exudation or compartmentalization, because a large amount of the herbicide reached the roots of this species.

Keywords: *Ipomoea nil*, *Tridax procumbens*, *Spermacoce latifolia*, *Bidens pilosa*.

*RESUMO - Objetivou-se neste estudo avaliar a translocação de glyphosate em plantas tolerantes (**Ipomoea nil**, **Tridax procumbens** e **Spermacoce latifolia**) e suscetível (**Bidens pilosa**) a esse herbicida. As avaliações de absorção e translocação do ^{14}C-glyphosate em **I. nil** e **B. pilosa** foram efetuadas às 6, 12, 36 e 72 horas após a aplicação do herbicida (HAT), e em **T. procumbens** e **S. latifolia**, às 72 HAT. As plantas foram coletadas e fracionadas em: folha de aplicação, demais folhas, caules e raízes; em **T. procumbens** e **S. latifolia**, avaliou-se a presença do produto na inflorescência da planta. Em **S. latifolia**, aproximadamente 88% do glyphosate permaneceu na folha de aplicação, e pequena quantidade translocou para as raízes. Todavia, em **T. procumbens**, 75% do herbicida permaneceu na folha que recebeu a aplicação, observando-se maior translocação na inflorescência em relação às raízes. Conclui-se que a menor translocação do glyphosate observada em **S. latifolia** e **T. procumbens** pode ser um dos fatores responsáveis pela maior tolerância dessas espécies ao herbicida, ao passo que em **I. nil** a metabolização, a exsudação radicular ou a compartimentalização podem favorecer a tolerância, já que grande quantidade do produto atingiu as raízes da espécie.*

Palavras-chave: *Ipomoea nil*, *Tridax procumbens*, *Spermacoce latifolia*, *Bidens pilosa*.

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INTRODUCTION

Management of weeds infesting soybean-producing areas in Brazil has been demanding high expertise from the people involved with this kind of crop because the distribution and infestation are occurring sharply and causing severe losses. However, in addition to the damage that can be directly caused by weeds, another factor that has received attention is the increasing number of herbicide-tolerant species. This situation has been aggravated in many grain producing regions, particularly in the areas cultivated with glyphosate-resistant soybeans (Vargas et al., 2007).

Glyphosate stands out among the most frequently used herbicides in chemical weed control, either in no-tillage or in post-emergence in transgenic crops, (Vargas, 2007). However, this herbicide does not effectively control some tolerant weed species when it is applied alone; a mixture with other active ingredients is required. Glyphopsate has been mixed with 2,4-D, carfentrazone-ethyl, flumizin, chlorimuron-ethyl, metsulfuron-methyl or bentazon, aiming to control *Ipomoea* spp. (common morning-glory), *Commelina diffusa* e *C. benghalensis* (day flower), *Richardia brasiliensis* (Brazil pusley), *Spermacoce latifolia* (buttonweed) e *Tridax procumbens* (tridax daisy), and others (Galon et al., 2009).

It must be emphasized that some aspects may favor the selection of herbicide-tolerant weed biotypes. For example, there are characteristics associated with the weeds themselves, the herbicides and, above all, farmers' cultural practices (Winkler et al., 2003). The management methods adopted by farmers can significantly increase the seed bank of tolerant species in the soil. This occurs when only glyphosate is used in the control, thus making this herbicide a selector of tolerant species and, to a lesser extent, resistant ones (Christoffoleti & López-Ovejero, 2003).

When applied to plants with intense photosynthetic activity, glyphosate inhibits the synthesis of aromatic chains aminoacids phenylalanine, tyrosine and thryptophan. Immediately after its absorption, glyphosate is promptly translocated, along with photoassimilates, from the application points – normally the leaves – to distant drains (Rodrigues & Almeida, 2011). In sensitive plants, this herbicide inhibits the activity of the plastidic enzyme 5-enolpiruvilshikimate-3-phosphate syntase (EPSPs), which is responsible for the reaction that condenses shikimate-5-phosphate and phosphoenolpiruvate in 5-enolpiruvilshikimate-3-phosphate (EPSP) and inorganic phosphate (Pi) in the pre-chorismate phase of the shikimate pathway (Shaner & Bridges, 2004).

When glyphosate is applied post-emergence, its efficacy is dependent of processes associated with the retention of the molecule on the leaf surface, foliar penetration, translocation inside the plant to the site of action and inhibition of the target-enzyme - in this case, EPSPs (Kirkwood & Mckay, 1994). A plant can be considered susceptible if it dies when submitted to the recommended herbicide dose. On the other hand, tolerance is an innate characteristic of the species (Vargas et al., 2007). In this case, the plants are able to survive and reproduce after being sprayed with a herbicide dose normally recommended for other species (Vargas et al., 2007).

I. nil has a high and widespread occurrence; it is a significant weed that infests a great number of both perennial and annual crops, and many of the species of this genus are glyphosate-tolerant. It has a longer cycle than annual crops, which can hamper harvesting because its branches get tangled up in crop plants and in harvesting equipment (Kissmann & Groth, 1999). Some other species of glyphosate-tolerant weeds have gained importance in Brazil, especially *T. procumbens* and *S. latifolia* (Galon et al., 2009). These species have very high occurrence, particularly in the midwestern, mortheastern, northern and southeastern regions, where they infest annual crops, orchards and coffee plantations.

B. pilosa is distributed in all regions of Brazil, and it infests most of the annual and perennial crops in winter and summer conditions (Kissmann & Groth, 1999). In many places in Brazil, this weed has become resistant to acetolactate synthase inhibitor (ALS) herbicides (Christoffoleti & López-Ovejero, 2003).

Control of both *T. procumbens* and *S. latifolia* in glyphosate-resistant transgenic soybean fields is ineffective because of the low efficacy of the herbicide on these species. In most cases, mixtures with other herbicides are required, as previously discussed (Vargas et al., 2007; Eubank et al., 2008; Galon et al., 2009). However, even if glyphosate is mixed with other herbicides, certain aspects must be taken into account: current stage of weed development, climatic conditions, soil characteristics and, especially, the application technology at the time of control. If these aspects are not taken into account, there can be an ineffective control and an increase in production costs.

It should also be considered that glyphosate is the most used broad-spectrum herbicide both in no-tillage system and in selective weed control in resistant crops. As a result, knowledge has been gained on the reasons why infestation by tolerant species has rapidly expanded in crops where this herbicide is the main control tool (Vargas et al., 2007). In this way, studies must be implemented in order to clarify the tolerance mechanisms of weed species to glyphosate and, thus, more accurately determine management strategies associated with the chemical control method based on this active ingredient.

There are at least six general mechanisms that can explain weed tolerance to herbicides and influence the mechanism of action of these compounds: lower absorption or translocation, higher metabolization of the herbicide to less phytotoxic substances, compartmentalization of the intact herbicide molecules, lack of affinity of the herbicide with the specific site of action, and super-production of the target enzyme (Galon et al., 2009).

The present study aimed to evaluate glyphosate translocation in weed species which are tolerant and susceptible to this herbicide.

MATERIAL AND METHODS

The experiment was installed in a greenhouse from September to December, 2008, in a randomized complete block design with four replicates. Glyphosate-tolerant (*Ipomoea nil, Tridax procumbens* and *S. latifolia*) and glyphosate-susceptible (*Bidens pilosa*) weed species were cultivated in pots containing 500 mL of substrate composed of soil (with previous fertility correction) and sand in a 3:1(v:v) ratio.

At 45 days after weed emergence, a mixture of ^{14}C-glyphosate and commercial glyphosate was applied, with the final herbicide concentration being equivalent to a dose of 1.440 g ha^{-1}. Treatments were applied at the center of the adaxial face of the leaf on the third knot of the evaluated plants. A microsyringe was used to apply 10 μL of the spray volume with a specific activity of 1.400 Bq, approximately.

Absorption and translocation of ^{14}C-glyphosate were evaluated in the species *I. nil* and *B. pilosa* at intervals of 6, 12, 36 and 72 hours after product application (HAT) but only at 72 HAT in the species *T. procumbens* and *S. latifolia* because of their low germination. For this reason, unlike what had been done for the other species, it was not possible to obtain enough experimental units to apply all the treatments. At the end of each exposure period, the plants were collected and fractionated into application leaf, other leaves, stems and roots. For *S. latifolia*, the presence of the product in the inflorescence was also assessed.

The application leaf was washed with 9.0 mL of methanol at the time each treatment was collected for evaluation in order to extract the product not absorbed. All plant material was dried in a forced-air circulation oven at 65 ± 1 °C for 72 hours to obtain dry matter. Then, the material was ground to provide a particle size equivalent to 200 mesh. Next, 100 mg samples of dry matter for each plant component (application leaf, stem, other leaves, roots and flower bud of *T. procumbens*) were placed into 20 mL vials for counting. Samples were mixed with 1 mL Triton-X-100 for 14 seconds in a shaker; 5 mL of scintillation cocktail was then added, and the samples were homogenized again. The prepared vials were placed in a Beckman 6.500 liquid scintillation spectrometer with automatic quench correction. The methodology used to determine glyphosate translocation in the tested weed species followed the methodologies proposed by Monquero et al. (2004) and Ferreira et al. (2006).

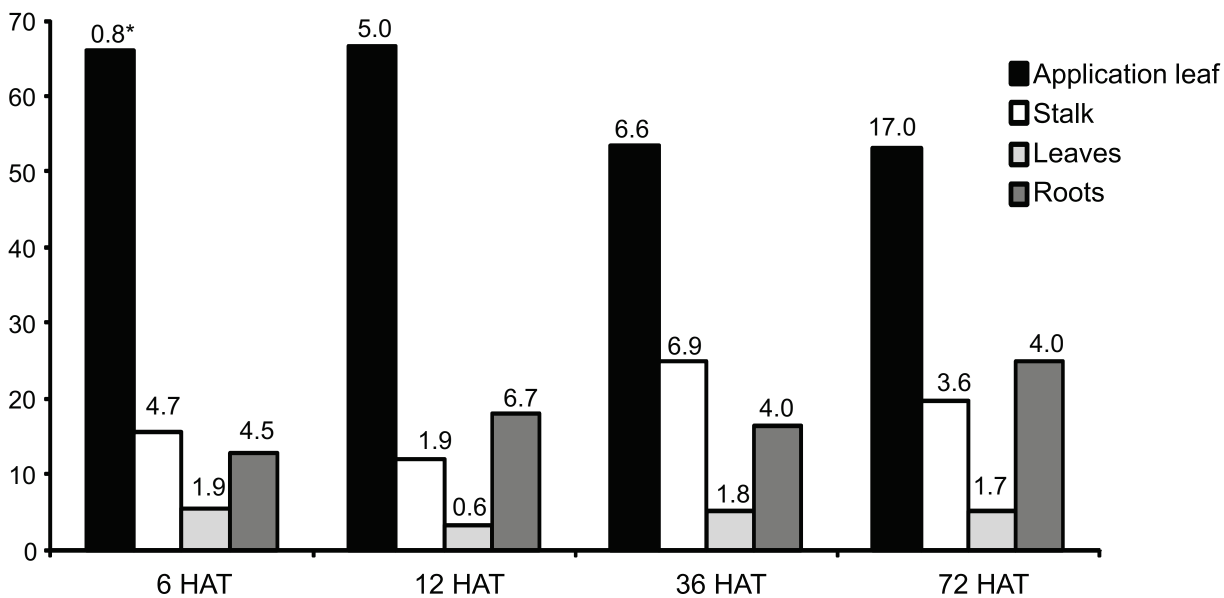
The values of ^{14}C-glyphosate radiation in plants were converted to a percentage of the total glyphosate distributed in the treated leaf, stem, other leaves, roots and flower bud. The results were shown in graphs, with the respective means and standard error.

RESULTS AND DISCUSSION

The results show that in *Ipomoea nil*, 6 hours after herbicide application (HAT), approximately 66% of the product remained in the application leaf and about 16, 13 and 5% were distributed in the stem, roots and leaves, respectively (Figure 1). In this glyphosate-tolerant species, glyphosate movement can be considered fast, because 17% of the xenobiotic present in the plant was found in the roots after six hours. Ferreira et al. (2006), when researching glyphosate resistant and susceptible ryegrass biotypes, found that the herbicide was already present in the roots two hours after application. Monquero et al. (2004) studied glyphosate movement through the phloem in tolerant weeds. They found that this herbicide follows the same route of photosynthesis products (sugars), occurring from the photosynthetically active leaves towards the plant parts that use these sugars for growth, maintenance and metabolism, or storage for future use, i.e. roots, tubers, rhizomes, young leaves and meristematic zones.

In the evaluations made at 12 HAT, glyphosate concentration in the application leaf of *I. nil* was similar to the one observed at 6 HAT and higher in the roots, with a small reduction in the stem and leaves (Figure 1). At 36 HAT, however, glyphosate concentration in the application leaf was reduced to approximately 53%, and it was higher in the stem: around 25% (Figure 1C). In roots, the value of glyphosate concentration was 16.34%, similar to the one found in the 12 HAT evaluation. In the application leaf, only 5% was found in this evaluation (Figure 1).

At 72 HAT, an increase by around 24.8% was observed in glyphosate concentration in the roots, while in the stem, this value decreased from 25% at 36 HAT to approximately 19% at 72 HAT (Figure 1). This variation in the concentration of the product present in the leaves and stem as a function of time may be associated with a retranslocation of the herbicide to other parts of the plant (Santos et al., 2005). In both the application leaf and the other leaves, there was little variation in



* Standard deviation of treatment means.

Figure 1 - Percentage of ^{14}C-glyphosate in *Ipomoea nil* at 6, 12, 36 and 72 HAT (hours after herbicide application).

the concentration of the product compared to the 36 HAT evaluation (Figure 1). Ferreira et al. (2006) investigated resistant and susceptible ryegrass biotypes, and they verified that in the resistant biotype at 64 HAT, 81.64% of the total glyphosate absorbed was still retained in the leaf where ^{14}C-glyphosate had been applied, while there was 54.66% retention in the susceptible biotype.

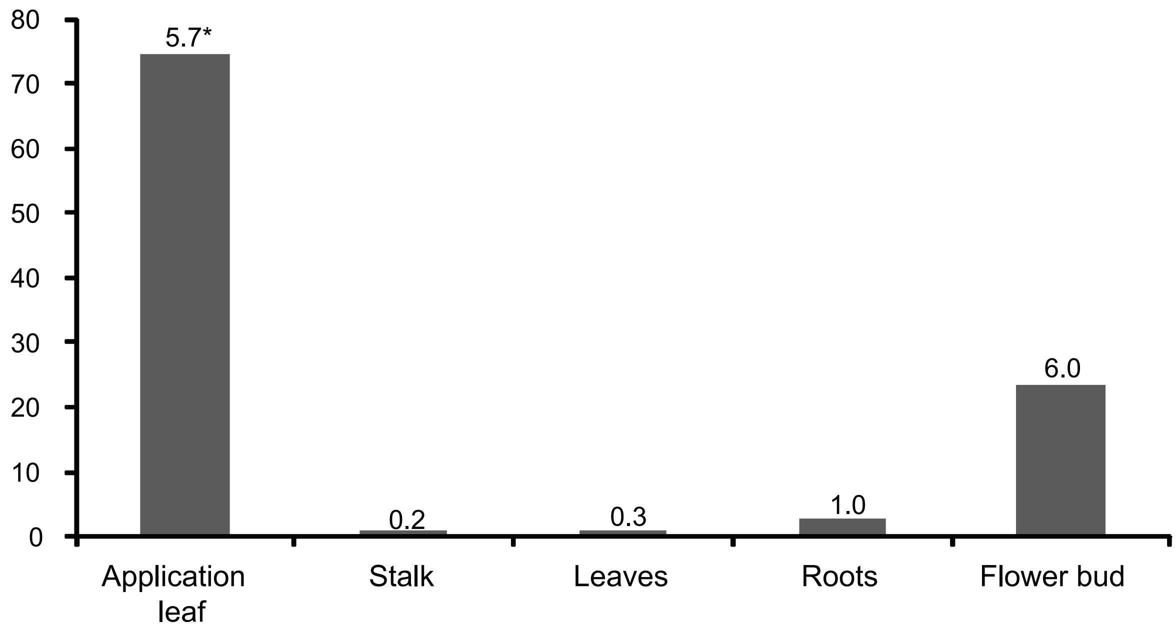
High glyphosate retention was observed in the application leaf of *I. nil* for all evaluated time intervals. This can be attributed to the mechanism of action of the herbicide, which interrupts the carbon cycle in the chloroplast, causing a reduction in the synthesis of carbohydrates, decreasing their transport to drains and, subsequently, decreasing glyphosate transport. This herbicide is transported through the plasma membrane by a protein carrier. The phosphate carriers, which are contained in the plasmalema, are able to bind to glyphosate molecules and transport them to the cytoplasm (Mcallister & Haberlie, 1985). Glyphosate movement is associated with carbohydrates, which govern the distribution of the herbicide in the plant, as a greater accumulation of that herbicide occurs in the shoot tips and roots (McAllister & Haberlie, 1985).

In general, *I. nil* proves to be effective in the translocation and distribution of glyphosate to all parts of the plant, and variations occur in the herbicide's distribution throughout the measured time intervals. It could be seen at 72 HAT that the higher concentration of herbicide translocated from the application leaf is firstly directed to the roots and, secondly, to the stem (Figure 1). This greater product translocation in this species discards differential translocation as a glyphosate tolerance mechanism of *I. nil*. The mechanism in this species is possibly associated with other ones, such as metabolization, root exudation or compartmentalization of the herbicide. However, Monquero et al. (2004), when investigating glyphosate-tolerant *I. grandifolia,* found lower herbicide translocation in the plant, and concluded that this is due to greater tolerance of this species to the product.

For *Tridax procumbens* at the 72 HAT evaluation, a high concentration of glyphosate was observed in the application leaf, corresponding to approximately 75% of the total glyphosate in the plant (Figure 2). At 45 days after emergence, when the herbicide was applied, this species was at full reproductive stage; it produced inflorescences which were evaluated. There was a high translocation to the inflorescence at 72 HAT, with about 24% of the product present in these organs. In this species, a small amount of the herbicide was detected in the leaves, stem and roots (1.1, 0.8 and 2.7%, respectively). Glyphosate translocation to all parts of the plant is an essential requirement for its control, thus avoiding the occurrence of regrowth and interference in the crops. However, when weeds known to be glyphosate-tolerant occur in crops, producers mix glyphosate with other herbicides (2,4-D, metsulfuron-methyl, carfentrazone-ethyl, bentazon, among others) in the spray tank in most situations. This raises production costs and causes more contamination of humans and the environment.

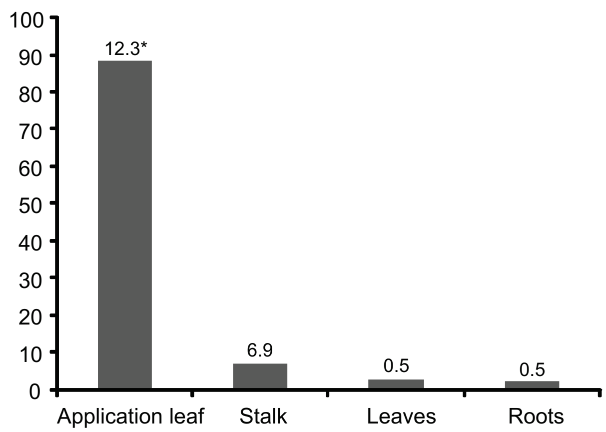
For *S. latifolia*, results show that approximately 89% of the glyphosate remained in the application leaf at 72 HAT, and only 2% of the herbicide reached the roots (Figure 3). The reduced glyphosate movement in this species may be associated with increased product tolerance; this fact may be associated with the stage of plant development, differences in morphology (leaf blade area and shape, angle or orientation of the leaves in relation to the jet spraying) and leaf anatomy (presence of stomata in the adaxial surface, presence of hair, cuticle layer thickness and composition) as well as with differences in absorption, translocation, and compartmentalization in the herbicide metabolism (Westwood et al. 1997; Santos et al., 2002). Among all the factors mentioned above, according to Monquero et al. (2004), interference occurs in the herbicide absorption by tolerant plants because glyphosate is highly hydrophilic and chemical composition of epicuticular waxes is typically hydrophobic.

In this study, *Bidens pilosa* was used as a glyphosate susceptible standard weed. It was verified that at 6 HAT, around 86% of the applied herbicide remained in the application leaf, while approximately 5, 6, and 3% translocated



* Standard deviation of treatment means.

Figure 2 - Percentage of ^{14}C-glyphosate in *Tridax procumbens* at 72 HAT (hours after herbicide application).



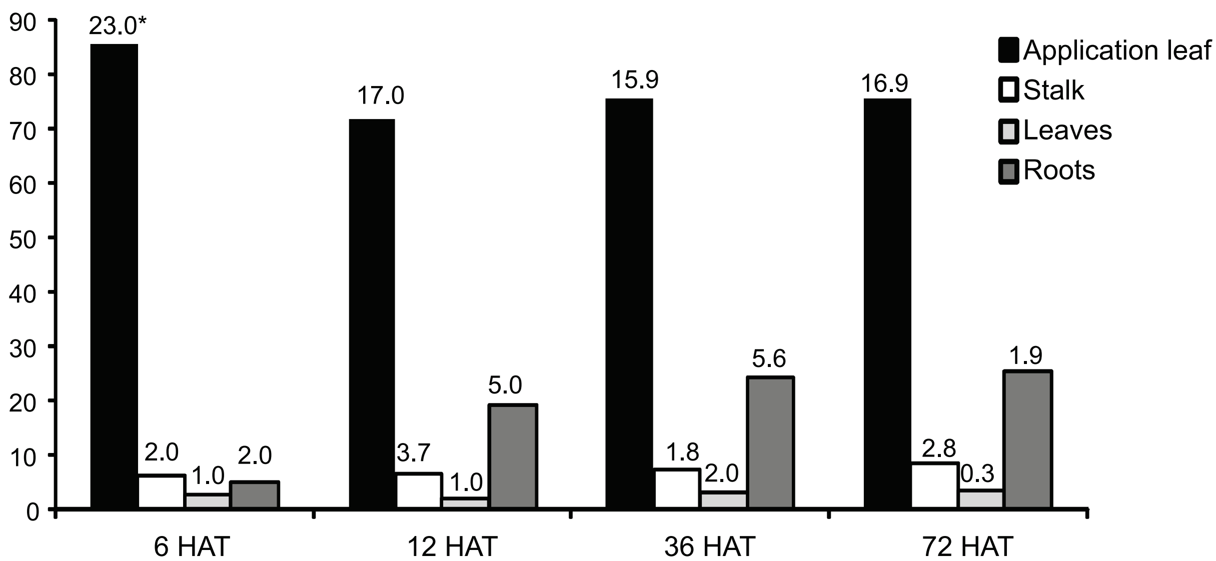
* Standard deviation of treatment means.

Figure 3 - Percentage of ^{14}C-glyphosate in *Spermacoce latifolia* at 72 HAT (hours after herbicide application).

to the roots, stem and leaves, respectively (Figure 4). At 12, 36 and 72 HAT, the product was observed to move from the application leaf to the other parts of the plant, with values ranging from 72 to approximately 76%. Glyphosate was preferably translocated to the roots, where almost 19, 24 and 26% of the herbicide was found by the evaluations made at 12, 36 and 72 HAT (Figure 4), respectively.

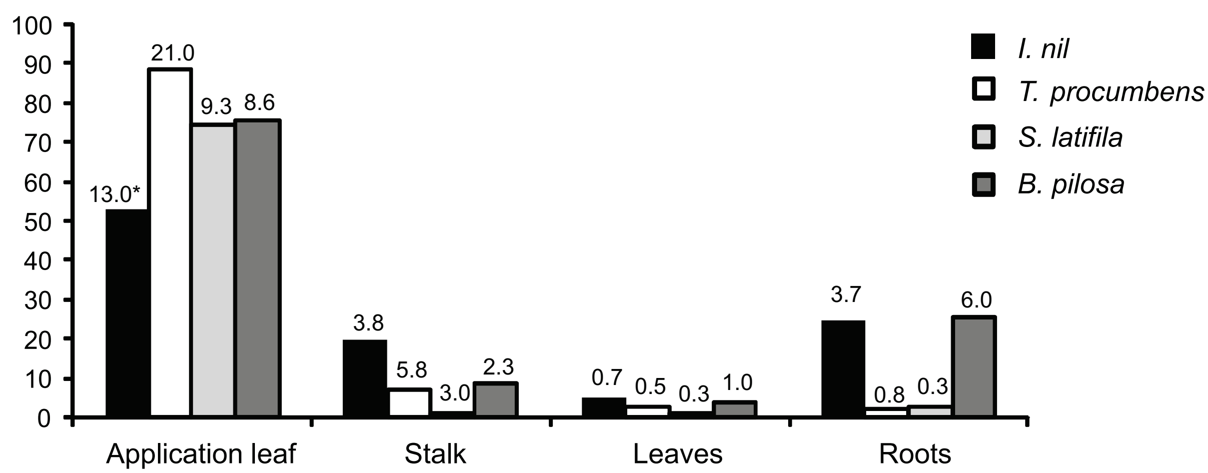
Moreover, a very low concentration of the herbicide was observed in the stem and leaves at 12 HAT (Figure 4). Similarly, Mcallister & Haberlie (1985) and Monquero et al. (2004) observed that glyphosate absorption increased over time after herbicide application when sprayed on susceptible species *Amaranthus hybridus*, *Sorghum halepense* and *Elytrigia repenses*. These results suggest that much lower concentrations than the one applied in the field are sufficient to promote death of glyphosate susceptible species, because the occurrence of rainfall, even four hours after application, would not prevent control efficacy (Costa et al., 2005).

The results indicate that in the four species evaluated at 72 HAT, glyphosate concentration remained in a higher proportion in the application leaf. However, a higher herbicide translocation rate was detected in *B. pilosa* and *I. nil* compared with *S. latifolia* and *T. procumbens* (Figure 5). Higher glyphosate translocation in *I. nil* (herbicide tolerant), specially for the roots, indicates that the tolerance mechanism of this species is not associated with translocation. Another common tolerance mechanism is the metabolization of the herbicide to aminomethylphosphonic acid (AMPA). The main glyphosate degradation pathway in tolerant plants is the AMPA pathway, which leads to formation of the aminomethyl phosphonic acid and glyoxylate from glyphosate. Once formed, the AMPA is cleaved into methylamine and inorganic phosphate as the products of final degradation. The glyphosate degradation pathway in higher plants is not well understood. Some studies have suggested that glyphosate metabolism does not occur (Mallik et al., 1989), while others show that the chemical transformation of this herbicide can occur (Monquero et al., 2004; Ferreira et al., 2006). The presence or absence of the ^{14}C-isotope in the AMPA, a glyphosate degradation metabolite, depends on which functional group was marked on the glyphosate molecule. Normally, the molecule present in the phosphonomethyl functional group is the



* Standard deviation of treatment means.

Figure 4 **-** Percentage of ^{14}C-glyphosate in *Bidens pilosa* at 6, 12 and 36 and 72 HAT (hours after herbicide application).



* Standard deviation of treatment means.

Figure 5 **-** Percentage of ^{14}C-glyphosate, distributed among the organs of *Ipomoea nil*, *Tridax procumbens*, *Spermacoce latifolia*, *Bidens pilosa* at 72 HAT, compared to the total glyphosate applied.

one marked, resulting in the presence of this molecule in the AMPA (Bonfleur et al., 2011). In this study, in the tolerant species, glyphosate may have been degraded to a non-toxic substance by the AMPA pathway, thus preventing control of these weeds.

It was observed that glyphosate translocation was very low in *T. procumbens*, i.e., approximately 80% of the herbicide in the plant was found in the application leaf at 72 HAT. In this case, it cannot be stated that the retention of the product in the application leaf is due to the tolerance of this species, considering that at the time of assessment the plants were at reproductive stage. *S. latifolia*, which is glyphosate-tolerant, showed different translocation pattern to the one seen in *B. pilosa*, a species which is extremely susceptible to the herbicide. By comparing the movement of the product in the two species, it is observed that in *B. pilosa* there was a higher translocation to the roots, while in *S. latifolia* translocation to the roots was minimal (Figure 5). In fact, it is difficult to relate glyphosate translocation in *S. latifolia* to the tolerance of the species to this herbicide because during evaluations the plants were at full bloom stage. It is known that the reproductive structures are the major sink of photosynthates in the plant and that many herbicides can be translocated along those pathways (Monquero et al., 2004).

At 10 HAT, signs of injury were observed in the leaves of *T. procumbens* and *S. latifolia* at the sites where glyphosate was applied. These injuries can also be considered one of the reasons for the smaller product translocation from the application leaf to other plant organs.

The glyphosate-tolerant species are distributed from the north to the south of Brazil, and they cause many losses to agriculture, such as yield loss due to competition. In addition, they are hosts to pests and diseases which reduce the quality and quantity of the harvested product. It should also be noted that many farmers tend to use higher doses of the herbicide than those recommended to control tolerant species. This often causes severe poisoning of the crops, reduces production and causes major impacts to humans and the environment. For these reasons, further research using analysis techniques and more accurate identification is required to verify whether these effects actually occur as a result of a differential metabolism, particularly to glyphosate, which is one of the most commonly used herbicides in agriculture. This way, possible alternatives can be sought to control glyphosate-tolerant weeds.

According to the results, it can be inferred that the species *S. latifolia* showed lower glyphosate translocation, and this could be one of the mechanisms that provide greater tolerance to this herbicide. *I. nil* showed efficient glyphosate translocation, exceeding that of *B. pilosa*, a herbicide-susceptible species. The tolerance mechanism in this case may associated with metabolization or exudation of the product, because a large amount of the herbicide reached the roots in this species.

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