Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v36i3.17035

The absorption and translocation of imazaquin in green manures

Flávia Garcia Florido[1], Patrícia Andrea Monquero[1*], Ana Carolina Ribeiro Dias[2] and Valdemar Luiz Tornisielo[3]

*[1]Centro de Ciências Agrárias, Universidade Federal de São Carlos, Rod. Anhanguera, km 174, Cx. Postal 153, Araras, São Paulo, Brazil. [2]Departamento de Produção Vegetal, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil. [3]Laboratório de Ecotoxicologia, Centro de Energia Nuclear na Agricultura, Piracicaba, São Paulo, Brazil. *Author for correspondence. E-mail: pamonque@hotmail.com*

ABSTRACT. Green manure species that are tolerant to the herbicide imazaquin can be used in crop rotation schemes that aim to reduce herbicide carryover to sensitive plants such as sunflower or corn. Three different doses of imazaquin (0, 0.15 and 0.28 kg ha^{-1}) were applied during the pre-emergence growth stage to *Dolichos lablab, Cajanus cajan, Canavalia ensiformis, Crotalaria juncea, C. breviflora, C. spectabilis, Mucuna deeringiana, M. cinerea, M. aterrima, Lupinus albus, Helianthus annuus, Pennisetum glaucum, Avena strigosa* and *Raphanus sativus*, and the results were evaluated in a greenhouse. *C. ensiformis* and *M. cinerea* were selected from these species for being the most tolerant, and they were then evaluated for absorption and translocation of ^{14}C-imazaquin in two different growth stages: the cotyledonary stage and the emergence of the first pair of true leaves. *M. cinerea* individuals showed the best potential for translocating imazaquin to the shoot when compared to *C. ensiformes*, which accumulated the herbicide mostly in its roots. These plants had a higher ability to accumulate herbicide during their most advanced stage of development, which demonstrates their potential for use in areas that have residual imazaquin.

Keywords: herbicide, residual, tolerant species.

A absorção e translocação de imazaquin em adubos verdes

RESUMO. Espécies de adubos verdes tolerantes ao herbicida imazaquim podem ser utilizadas em esquemas de rotação de culturas visando diminuir o carryover deste herbicida em plantas sensíveis como o girassol ou milho. Foram avaliadas, em casa-de-vegetação, três doses do herbicida imazaquim (0; 0,15 e 0,28 kg ha^{-1}), aplicado em pré-emergência de *Dolichos lablab, Cajanus cajan, Canavalis ensiformis, Crotalaria juncea, C. breviflora, C. spectabilis, Mucuna deeringiana, M. cinerea, M. aterrima, Lupinus albus, Helianthus annuus, Pennisetum glaucum, Avena strigosa* and *Raphanus sativus*. Entre estas espécies, *C. ensiformis e M. cinerea* foram selecionadas como as mais tolerantes e avaliadas em seguida, para determinar a absorção e translocação de ^{14}C-imazaquim em dois estádios fenológicos distintos: estádio cotiledonar e no primeiro par de folhas verdadeiras. A espécie *M. cinerea* apresentou maior potencial para translocar o imazaquim para a parte aérea em relação à *C. ensiformes*, a qual acumulou o herbicida preferencialmente nas raízes. As plantas apresentaram maior potencial para acumularem o herbicida no estádio de desenvolvimento mais avançado, o que demonstra potencial dessas espécies para uso em áreas com residual de imazaquim.

Palavras-chave: herbicida, residual, espécies tolerantes.

Introduction

Agrochemicals are classified according to the target organisms they are designed to control (e.g., insects, weeds or fungi). Of all the target organisms, weeds cause by far the greatest economic loss as a consequence of their interference in crop production. It is therefore not surprising that herbicides are the most common class of agrochemicals that are used in Brazil (48% of total expenditures), outstripping insecticides (30%) and fungicides (21%) (PINHEIRO et al., 2011).

Predicting the movement and fate of herbicides in soils is an important step in limiting their environmental impacts (CARABIAS-MARTINEZ et al., 2000). Herbicides may be sorbed by mineral and organic colloids and, depending on their binding energy, they may become unavailable to the plants (via bond residue fractions) they are intended to kill and can also become unavailable for biodegradation or desorption in soil solution, causing them to be transported or absorbed by plants (HORNSBY et al., 1995).

Herbicide persistence in soil exerts a strong influence on the control of weeds, can cause damage to succession crops and can lead to environmental contamination risks. This persistence varies with the chemical structure of the molecule, the type of soil to which it is applied and the climatic conditions,

such as the soil humidity, which in turn affects absorption, leaching and microbial/chemical decomposition (SILVA et al., 1999).

Imazaquin is an imidazolinone herbicide that is widely used for broadleaf and grassy weed control in soybeans and warm-season turf grasses (SEIFERT et al., 2001). The absorption of imazaquin herbicide, also known as 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]quinoline-3-carboxylic acid, occurs through both the roots and the leaves, while translocation occurs through the phloem and xylem, accumulating in the meristems of plants where it produces necrosis (SHANER, 2003). This molecule acts by inhibiting the acetolactate synthase enzyme (ALS), resulting in a blockage of the synthesis of the amino acids valine, leucine and isoleucine. The phytotoxic effect of imidazolinone is caused by deficiency of these amino acids, leading to a decrease in DNA and protein synthesis, which adversely affects cellular division and photosynthate translocation to growing points. These processes cause a reduction in plant growth as well as an elongation of leaves and chlorosis between leaf ribs (TAN et al., 2005).

Imazaquin is an ionizable organic molecule that contains both an ionizable carboxyl group with a pKa of 3.8 and a basic quinoline ring with a dissociation constant of 2.0. At the most common pH range for tropical soils (pH 4.0-6.0), imazaquin predominantly behaves as an organic anion (NOVO et al., 1997), causing low sorption by soil colloids, which are also negatively charged at this pH range. However, organic matter can react with polyvalent cations to form chelates or ionic bridges with acid herbicides, which decreases the pH effect (AICHELE; PENNER, 2005). Several factors, such as speciation, soil solution and sorbent surface pH, charge, ionic strength, and solution composition, must be considered due to the herbicide's amphoteric nature for the user to successfully predict soil sorption (REGITANO et al., 1997, 2001; ROCHA et al., 2003).

The imidazolinone group has a high water solubility and high persistence in the environment, which is predicated on an imazaquin half-life that varies from 16 weeks (AICHELE; PENNER, 2005) to 210 days (VIDAL, 2002). On the one hand, this half-life is good because the herbicide can provide residual weed control during the entire life cycle of the soybean, but on the other hand, it may become a risk to succession crops such as winter maize, cotton, sunflower and brassica crops (ARTUZI; CONTIERO, 2006; DAN et al., 2011; RODRIGUES; ALMEIDA, 2011; SEIFERT et al., 2001; YODER et al., 2000) or lead to environmental contamination. In the U.S., sixteen active ingredients of herbicides belonging to the sulfonylureas, sulfonamides and imidazolinones were found in samples that were collected from surface and groundwater (BATTAGLIN et al., 2000).

One possible method to assuage the effects of herbicide residues is phytoremediation. This technique aims to decontaminate soil and water by using plants as cleansing agents (NEWMAN et al., 1998). The cleansing action may occur by direct assimilation of the contaminating agents and subsequent accumulation of non-toxic metabolites in the vegetable tissue, such as structural components, or by the stimulation of microbial activity by the plant (SCRAMIN et al., 2001).

The use of phytoremediation is based on the natural or purposefully developed ability that some species exhibit to specific types of compounds or action mechanisms. Phytoremediation is commonly performed with agricultural species and weeds that are tolerant to certain herbicides (PIRES et al., 2003). Plant selection is related to the fact that organic compounds may be translocated by plants to other tissues within the transpiration stream, from which the compounds could be volatilized (GENT et al., 2007). They may also suffer partial or complete degradation or be transformed into less toxic compounds, especially less phytotoxic compounds, when combined and/or connected to plant tissue (via compartmentalization) (ACCIOLY; SIQUEIRA, 2000; SCRAMIN et al., 2001).

A plants' capacity to metabolize pesticides into compounds that are non-toxic (or less toxic) is the principle behind phytodegradation. Another possible strategy is phytostimulation in which plants stimulate microbial activity, promoting the release of root exudates that degrade the compound in the soil, which in some situations, determines the rhizosphere's capacity to engage in the bioremediation of toxic compounds (PIRES et al., 2003).

In Brazil, phytoremediation has been shown to have promise in the decontamination of soils that are contaminated with various herbicides such as tebuthiuron (PIRES et al., 2005), trifloxysulfuron-sodium (SANTOS et al., 2004) and picloram (CARMO et al., 2008). This method has been advantageous when the levels of compound in the soil are high and conditions favor herbicide leaching.

Thus, it is important to select plant species that have phytoremediation potential. It is also important to understand the physiological mechanisms that are responsible for these characteristics in order to create and plan new crop protection strategies, in

addition to perfecting already existing treatments. Therefore, this work aims to select species of green manures that are tolerant to imazaquin and to determine the physiological bases for their different responses.

Material and methods

Selection of imazaquin-tolerant plants

This experiment was installed and conducted in a greenhouse. The imazaquin tolerance of 14 species of plants, among which were leguminous, cruciferous, gramineous and composed plants, was evaluated. The evaluated species were *Dolichos lablab, Cajanus cajan, Canavalis ensiformis, Crotalaria juncea, C. breviflora, C. spectabilis, Mucuna deeringiana, M. cinerea, M. aterrima, Lupinus albus, Helianthus annuus, Pennisetum glaucum, Avena strigosa* and *Raphanus sativus*. Samples of a soil classified as Hapludox (EMBRAPA, 1999) without a history of herbicide use were taken from a depth of 0-20 cm for the plantings. Physical and chemical analyses were performed in the Soil Chemistry and Fertility Laboratory at UFSCar [Federal University of São Carlos] (Table 1).

The experiments were set up in randomized blocks with three repetitions. These blocks were arranged in three arrays of 14 x 3 (factorial), which contained 14 species of green manures and three different herbicide doses. The experimental unit was a polyethylene vase that was lined with a plastic bag to avoid the outflow of herbicide, and the vase contained 20 $dm^3$of soil.

Imazaquin herbicide (at concentrations of 0, 0.15 and 0.28 kg i.a ha^{-1}) was applied during the plant pre-emergence stage with a CO_2-pressurized backpack sprayer that had a sprayer lance with two Teejet AI-110.02 fan-type nozzles and a spray volume of 200 L ha^{-1}.

Seeding was performed the day after herbicide application. The vases were then irrigated frequently using a fixed conventional sprinkler system to maintain the soil's humidity at approximately 80% of field capacity. Visual intoxication that was caused by the herbicide was evaluated at 15, 30 and 45 days after seeding (DAS). Grades from 0 to 100 were given according to the intoxication symptoms demonstrated by the plant shoots, where 0 represents an absence of symptoms and 100 represents the death of the plant. At 46 DAS, the dry matter masses of the shoots and roots were also determined. The shoots and roots were placed in a heater with forced air circulation (60 ± 2ºC) for 72 hours to achieve drying.

An analysis of weight averages and variances was performed and compared with the Tukey test at a 5% probability.

Evaluation of the absorption and translocation of imazaquin

This experiment was performed at the Ecotoxicology Laboratory of CENA/USP [*Nuclear Energy in Agriculture Centre/University of São Paulo*]. Based on the previous study, the green manure species *Canavalis ensiformis* (jack bean) and *Mucuna cinerea* (grey mucuna) were selected for their high tolerance to imazaquin herbicide.

Samples of Hapludox were extracted from the 0-10 cm layer (Table 1) from an area without a history of herbicide use and were used as the substrate for the planting of these two species. The soil was dried and passed through a 2-mm sifter to remove part of the organic matter and to increase the soil's homogeneity before planting.

Two vases with 1.5 dm^3 of soil were used for each species and phenological stage under study. The soil's density was calculated (1083 g mL^{-1}) with the purpose of estimating the total weight that a vase could support (1270 g). Thus, the necessary weight to fill a vase to a depth of 2-cm of superficial soil (307 g) was calculated. The procedure consisted of contaminating the first soil layer with radiolabeled herbicide and then positioning the seeds between the cold soil layer (that is, the soil without radiolabeled product) and the radiolabeled layer for germination.

The 2-cm vase soil layer was ground prior to the application of ^{14}C-imazaquin solution to ensure homogeneous herbicide application. The herbicide was not homogenized within all the soil inside the vase because the results obtained during the pre-experimentation phase on imazaquin leaching revealed that the herbicide attaches itself strongly to the superficial layer of the soil, and the objective was to evaluate the capacity and behavior of the species that were chosen to tolerate the herbicide under field conditions.

Table 1. Physical and chemical characteristics of the soil that was used in the experiment.

Sample	pH $CaCl_2$	MO	P	K	Ca	Mg	H + Al	SB	CTC	V%	Clay	Silt	Sand
		g dm^{-3}	mg dm^{-3}	----------mmol$_c$ dm^{-3}----------						%	---------g kg^{-1}---------		
0-10	6.2	36	14	2.4	29	13	0	44.4	68.4	64.9	560	240	200

Twenty seeds per species were planted (10 seeds per vase). One phenological stage was evaluated per vase (stage 1: cotyledonary leaves and stage 2: first pair of true leaves).

Preparation of radiolabeled imazaquin solution

The herbicide product that was used for experimental purposes was commercial imazaquin (Scepter technical) (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid), from the Cyanamid brand (purity = 98.26%). Its respective radioactive isotope (^{14}C-imazaquin, labeled carboxylic group, specific activity = 0.80 MBq mg^{-1}; radiochemical purity = 98%) was used as the radiolabeled product (Figure 1).

For the labeled spray, a solution containing 18,933.73 dpm μL^{-1} of ^{14}C-imazaquin was prepared in which the labeled imazaquin was diluted in a solution containing unlabeled imazaquin (technical product) in a manner that ensured that the final herbicide concentration was equal to the commercial dose of 161 g ha^{-1}, with a total spray volume of 1 L ha^{-1}.

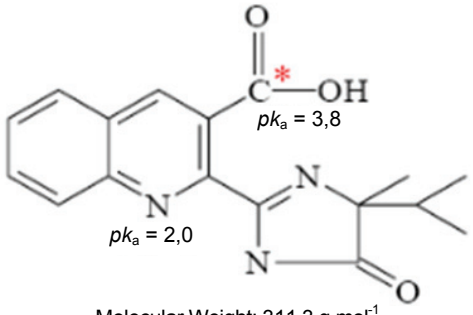


Figure 1. Imazaquin Molecule. *Radioactively labeled carbon (^{14}C).

Experimental assembly

This experiment was conducted in a randomized block design with ten replications *Canavalis ensiformis* and *Mucuna cinerea* were planted. Sifted dry soil was added to each vase, and its surface was leveled and cleared of possible fragments that could direct the behavior of the herbicide in the soil in a preferential manner.

The green manure seeds underwent a process of mechanical chiseling to break their dormancy. All of the seeds were immersed in 10% sodium hypochlorite for 10 minutes to avoid the proliferation of fungi and bacteria.

Each species' seeds were placed on the 'cold' soil (without radiation), and then the radiolabeled layer was added. The humidity level was reestablished during this first phase with the addition of water to the vase dishes until the water reached the surface of the soil. The vases were then positioned on a tray to avoid possible radioactive contamination.

To quantify the amount of remaining herbicide, the water and soil that remained after the vase disassembly process were analyzed. The soil was dried at room temperature and the solution of exceeding soil was centrifuged at 4,000 rpm for 15 minutes to remove the suspended soil. The vials were then dried in a heater at 40ºC, and the centrifuged solution was added to the dry soil. After being ground, four replicates of 0.2 g of soil each were examined in a biological oxidizer to quantify the remaining herbicide.

The supernatants from the centrifugation step were stored in vials of 500 mL each and filtered with the aid of a vacuum pump and common filter paper to remove the organic matter. The solution that resulted from the disassembly of the vases (SDV) was analyzed by examining 3 aliquots of 0.1 mL each in 10 mL of scintillation solution that were then quantified in a liquid scintillation spectrometer. After these evaluations, the SDV density was 1 g mL^{-1}. The filter paper and the filtered solution were dried in a heater at 40ºC, and after drying, the organic matter was incorporated into the soil that was being analyzed.

The absorption and translocation of imazaquin were qualitatively studied by autoradiography and quantitatively evaluated through the combustion of plant tissue. The plants were washed, pressed and dried in a heater with forced air circulation at 45ºC for 72 hours. Three specimens from each growth stage were autoradiographed using Crafts and Yamaguchi's protocol (1964) and analyzed in a autoradiography detector.

After drying, the plants were taken out and divided into groups of leaves, roots, stems and cotyledons (whenever present) with the objective of quantifying the radioactivity in each group. The combustion was performed with a biological oxidizer, with six repetitions for each part of the plant. The radioactivity present in all parts of the plants was considered to have been translocated. An average of six repetitions was calculated, and the radioactivity of each plant part was compared to the total radioactivity absorbed by the plant to calculate the translocation.

Results and discussion

Selection of imazaquin-tolerant plants

At 15 days after seeding (DAS), the species that were more sensitive to imazaquin were clearly observable after they received a dose of 0.28 kg ha^{-1}.

The sensitive species were *R. sativus* (with 66.67% of phytotoxicity), *P. glaucum* (73.33%) and *C. juncea* (46.67%), and the more tolerant ones were *C. spectabilis* (0%) and *C. cajan* (13.33%) (Table 2). At 30 DAS, the more tolerant species were *C. cajan* with 0 to 31.67% phytotoxicity at the highest and lower doses, respectively, and *C. ensiformis* with 10% phytotoxicity for both doses (Table 2). Usually the basis for imidazolinone selectivity results from a difference in the nature or rate of herbicide metabolism or through a version of acetolactate synthase (ALS) that is insensitive to inhibition by the herbicide (NEWHOUSE et al., 1992). At 45 DAS, there was a visual observation of green manure responses, with the species *C. ensiformis, M. aterrima, C. cajan* and *M. cinerea* showing the greatest herbicide tolerance. Seedlings of *R. sativus*, *P. glaucum* and *H. annuus* were the most susceptible, especially at the highest product dose. Imazaquin acts by inhibiting the ALS, which is essential for the synthesis of the amino acids valine, leucine and isoleucine. ALS-inhibiting herbicides are widely used because of their low dose rate, sound environmental properties, low mammalian toxicity, wide crop selectivity and high efficacy (TAN et al., 2005).

C. ensiformis, *C. breviflora* and *M. cinerea* did not present significant shoot biomass differences between the different herbicide doses. However, a higher accumulation of biomass in *C. ensiformis* (10.57 g in the highest dose), *M. cinerea* (6.30 g in the highest dose) and *M. aterrima* (9.64 g in the highest dose) was observed. It was verified that plants with the most significant root production were *C. ensiformis* and *M. cinerea* (Table 3). Although the imidazolinones are weed-selective in some crops such as peanut (*Arachis hypogaea*) and soybean (*Glycine max*), severe injury is normally observed when applied to other crops such as melon (*Cucumis melo*), cucumber (*Cucumis sativus*), sunflower (*Helianthus annuus*) and mustard (*Brassica* sp.) (THOMPSON et al., 2005). Imazaquin carryover was most pronounced after pre-plant incorporation in soils with higher organic matter and clay content (SMITH et al., 2005).

Table 2. Percentage of phytotoxicity from imazaquin herbicide in green manure species at 15, 30 and 45 days after seeding (DAS).

Species	Number of days after seeding (DAS)								
	15			30			45		
	Imazaquin doses kg i.a ha^{-1}								
	0.15	0.28	0	0.15	0.28	0	0.15	0.28	0
D. lablab	6.67 cB	30.00 cdeA	0.00 aC	0.00 dB	36.67cdA	0.00 aB	16.67 cdB	56.67 cdA	0.00 aC
C. cajan	11.67 cA	13.33 efgA	0.00 aB	0.00 dB	31.67 dA	0.00 aB	13.33 cdAB	16.67 fA	0.00 aB
C. ensiformis	0.00 cB	20.00 defA	0.00 aB	10.00 dA	10.00 eA	0.00 aB	0.00 dA	13.33 fA	0.00 aA
C. juncea	6.67 cB	46.67 bA	0.00 aB	28.33 cA	40.00 cdA	0.00 aB	46.67 bB	63.33 cA	0.00 aC
C. breviflora	13.33 cA	16.67 efgA	0.00 aB	28.33cA	40.00 cdA	0.00 aB	0.00 dB	26.67 efA	0.00 aB
C. spectabilis	46.67 bB	73.33 aA	0.00 aC	43.33 bcA	50.00 cA	0.00 aB	33.33 caA	40.00 deA	0.00 aB
M. deeringiana	13.33 cA	16.67 efgA	0.00 aB	6.67 dB	80.0 bA	0.00 aB	13.33 cdB	73.33 bcA	0.00 aC
M. cinerea	65.00 aB	28.33 cdeA	0.00 aC	0.00 dB	38.33 cdA	0.00 aB	0.00 dA	13.33 fA	0.00 aA
M. aterrima	36.67 bA	40.00 bcA	0.00 aB	0.00 dB	40.00 cdA	0.00 aB	0.00 dB	20.00 efA	0.00 aB
L. albus	6.67 cA	0.00 gA	0.00 aA	10.00 dB	93.33 abA	0.00 aB	3.33 dB	90.00 abA	0.00 aB
H. annus	13.33 cA	10.00 fgAB	0.00 aB	55.00 bB	90.00 abA	0.00 aC	83.33 aA	93.67 abA	0.00 aB
P. glaucum	1.67 cB	28.33 cdeA	0.00 aB	56.67 bB	90.00 abA	0.00 aC	93.33 aA	96.67 aA	0.00 aB
A. strigosa	10.0 cAB	16.67 efgA	0.00 aB	38.33 cB	86.67 abA	0.00 aC	40.00 bB	90.00 abA	0.00 aC
R. sativus	66.67 aB	81.67aA	0.00 aC	96.67 aA	100.00 aA	0.00 aB	96.67 aA	100.0 aA	0.00 aB
C.V (%)	35.96			20.89			26.35		

Equal lower-case letters in the column and capital letters in the lines do not differ significantly from each other according to the Tukey test at a 5% probability within each evaluation.

Table 3. Biomass (g) of the shoots and roots of green manure species at 46 days after seeding (DAS).

Species	Imazaquin doses kg i.a ha^{-1}					
	0.15	0.28	0	0.15	0.28	0
	Biomass of the shoot (g)			Biomass of the roots (g)		
D. lablab	3.70 cdB	2.32 bB	6.10 bcA	1.57 abA	0.80 bcB	1.60 bcA
C. cajan	1.07 defA	0.93 bB	2.20 deA	0.79 cdB	0.64 cdB	1.45 bcA
C. ensiformis	10.57 aA	9.24 aA	10.96 aA	1.68 abB	2.10 aAB	2.42 aA
C. juncea	0.92 defB	0.72 bB	3.63 cdA	0.55 cdeB	0.48 deB	1.82 abA
C. breviflora	0.29 fA	0.15 bA	0.37 eA	0.28 deA	0.18 efgB	0.17 hB
C. spectabilis	0.73 defA	0.26 bB	1.20 deA	0.20 eA	0.34 efA	0.36 ghA
M. deeringiana	3.71 bB	1.59 bC	6.27 bcA	0.61 cdeA	0.49 deAB	0.49 fghA
M. cinerea	6.30 bcA	6.87 aA	6.33 bcA	1.74 aA	1.41 abAB	1.08 cdeA
M. aterrima	9.64 aA	2.92 bB	7.89 bA	1.06 aA	1.36 abA	1.03 cdeA
L. albus	8.93 abB	2.10 bC	13.69 aA	1.70 aA	1.20 bB	1.75 abA
H. annus	0.25 fB	0.19 bB	2.39 deA	0.15 eB	0.06 fgB	1.41 bcA
P. glaucum	0.18 fB	0.06 cA	0.41 eA	0.97 cA	0.29 efB	0.84 efgA
A. strigosa	0.27 fB	0.20 bA	1.50 deA	1.55abA	0.67 cdB	1.68 bcA
R. sativus	0.62 efB	0.15 bB	5.54 bcA	0.08 eB	0.01 gB	1.06 cdeA
C.V (%)	30.51			24.40		

Equal lower-case letters in the column and capital letters in the lines do not differ significantly from each other according to the Tukey test at a 5% probability.

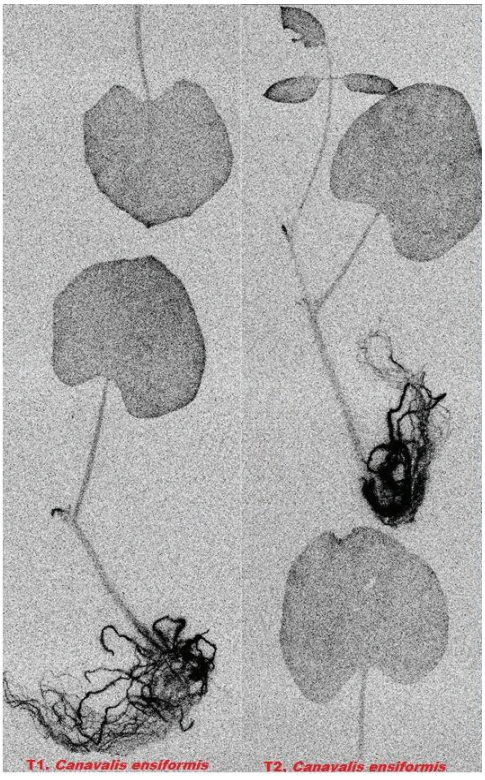
Following these data, *M. cinerea* and *C. ensiformis* were selected for further imazaquin absorption and translocation study, as a result of both visual observations and the production of dry mass by shoots and roots in these species.

Absorption and translocation

Canavalis ensiformis

Autoradiography was performed at time 1-T1 (first pair of cotyledonary leaves) and at time 2-T2 (first pair of true leaves) in the species that are shown in Figure 2. One can observe that a higher quantity of radiation is concentrated in the roots. This sequestration resulted from the direct contact between the plants and the radiolabeled herbicide because the imazaquin is absorbed through the roots (SHANER, 2003). Because the entire plant is completely visible in the picture, one can infer that the herbicide that was absorbed by the roots was translocated through all the plant parts because the figure only reveals the locations with observable radiation. A darker outline was also observed in all of the leaves. This indicates a higher quantity of herbicide in these areas, and one possible hypothesis is that the imazaquin is being translocated through the xylem and phloem and is accumulating in the growth points, where there is higher acetolactate synthase enzyme activity (TROXLER et al., 2007).

The values in Table 4 show how the herbicide is translocated inside the plant. At both times, a higher concentration of herbicide was observed in the roots (49.90% and 54.38% in T1 and T2, respectively), which demonstrated that despite the translocation, most of the imazaquin remained in the root system. This same behavior was observed when the weeds *Ipomoea lacunosa* and *I. hederifolia* were used in which the largest percentage of applied ^{14}C imazaquin was found in the roots of the plants (RISLEY; OLIVER, 1992). However, when the plants presented their first pair of true leaves, the percentage of herbicide in the leaves increased to 36.06%. The increased herbicide that was found in the leaf over the course of the plant's life cycle proves that herbicide translocation to the shoot tends to increase in plants during the more developed phenological stages. These results showed an increase in the accumulation of radiation per plant of approximately 2.7 times, or the amount that was absorbed in the cotyledonary leaf stage almost tripled by the true leaf stage. Askew and Wilcut (2002) reported that cotton (*Gossypium hirsutum*), jimsonweed (*Datura stramonium*), peanut (*Arachis hypogaea*), and sicklepod (*Senna obtusifolia*) rapidly absorbed ^{14}C trifloxysulfuron (another ALS inhibitor) between 0 and 4 hours after treatments, but total absorption after 72 hours varied by species between 30 and 70%. By comparison, tobacco absorbed 40% of the applied ^{14}C-imazaquin at 8 days after treatment and translocated 22% (WALLS et al., 1993). It is important to note that *C. ensiformis* does not have cotyledons when the first pair of true leaves is present.



Caption: T1. Jack Bean | T2. Jack bean.

Figure 2. Autoradiography indicating the absorption and translocation of imazaquin herbicide in *C. ensiformis* at time 1-T1 (first pair of cotyledonary leaves) and time 2-T2 (first pair of true leaves).

Out of all the applied radiation, *C. ensiformis* plants absorbed 2.57% and 7.91% during the cotyledonary leaf and first pair of true leaves stages, respectively (Table 5). Thus, one can observe that the plant continued to absorb imazaquin during its growth, increasing absorption as a consequence of the accumulation of dry matter. When observing individual absorption data, an absorption of 0.30% in the cotyledonary stage and 0.98% during the first pair of leaves stage was observed. Marcacci et al. (2006) showed that the high biomass production of these plants is of crucial importance for the progress of phytoremediation. These results provide evidence for the importance of increasing the growth of green manures in areas with herbicide residue, which would increase the potential removal of these products from the soil through the actions of these plants.

Table 4. Translocation of imazaquin herbicide inside *C. ensiformis* during different phenological stages.

Stages	Accumulated radiation (dpm)					Translocation (%)			
	Total	Leaf	Stem	Root	Cotyledons	Leaf	Stem	Root	Cotyledons
Cotyledonary leaves	7647.36	1914.23	927.34	3816.05	989.74	25.03	12.13	49.90	12.94
First pair of true leaves	20383.8	7350.17	1949.89	11083.76	-	36.06	9.57	54.38	-

Table 5. Recovery of the radioactivity of imazaquin in tests performed in different phenological stages of *C. ensiformis*.

Stage	Radioactivity recovered with the solution used in the disassembly of the vases (%)	Radioactivity recovered in the soil (%)	Number of plants in each vase	Radioactivity recovered in the plants (%)	Total of Recovered Radioactivity (%)
Cotyledonary leaves	5.58	88.20	8	2.37	96.16
First pair of true leaves	7.18	69.57	8	7.91	84.67

The total radioactivity that was recovered during the second time period was 7.91%, more than three times that of the cotyledonary leaf stage (2.37%). Pester et al. (2001) reported that the recovery of applied ^{14}C-imazamox (another ALS inhibitor) ranged from 80 to 94% for jointed goatgrass and feral rye. This difference can be explained by his method in which herbicide was applied to the leaves.

Mucuna cinerea

The autoradiography results from the imazaquin herbicide treatment of M. cinerea during the first pair of cotyledonary leaves stage (T1) and the first pair of true leaves stage (T2) are shown in Figure 3. We can observe that the radiation is homogeneously distributed throughout the plant when compared to *C. ensiformes*.

Caption: T1. Grey Mucuna | T2. Grey Mucuna.

Figure 3. Autoradiography indicating the absorption and translocation of imazaquin herbicide in the *M. cinerea* at time 1 (first pair of cotyledonary leaves) and time 2 (first pair of true leaves).

During the cotyledonary leaf stage, it was observed that translocation was higher to the leaves (31.14%), surpassing the values observed for the roots (29.34%). When the plants presented their first pair of true leaves, an increase in the leaf herbicide concentration (44.54%) and a reduction in the root values (28.45%) and in the cotyledons (18.26%) were observed (Table 6). There was an increase of almost 2000 dpm of total accumulated radiation per plant as the cycle of the cotyledonary stage (5528.93 dpm) advanced toward the first pair of true leaves stage (7289.28 dpm). These results agree with those of Salihu et al. (1998), who showed that the translocation of absorbed radioactivity from roots to shoots increased with time. Once absorbed, herbicide translocation can be affected by many factors, including plant growth stage, photosynthetic rate, phloem mobility, sink strength, and environment (PESTER et al., 2001).

In tobacco plants 40% of the ^{14}C imazaquin that was applied during postemergence was absorbed, 54% remained in the water extract on the leaf surface and 6% stayed in the epicuticular wax layer, and the translocation of herbicide from the treated leaves to the roots was very low (4-5%). By contrast, the application of herbicide to the soil resulted in retention of 40 to 53% of the radiolabeled products in the roots and a translocation of 47 to 60% to the shoots (WALLS et al., 1993).

These results are important because they show that this plant has the potential to accumulate imazaquin in the leaves, which is an advantage because it is easier to remove the shoot of a plant from a location that has residual herbicide than to remove the roots (LEAL et al., 2008).

From the total applied radiation, the *M. cinerea* plants absorbed 1.72% during the first pair of cotyledonary leaves stage and 2.55% in the first pair of true leaves stage (Table 7). In an experiment performed with another ALS-inhibiting herbicide called nicosulfuron, *Elytrigia repens* plants absorbed more ^{14}C-nicosulfuron when the plants presented one leaf than when they had five leaves; however, their translocation rates were similar, regardless of the weed's phenological stage (BRUCE et al., 1996). Differences in imidazolinone absorption and translocation by different plant species have been previously reported (BUKUN et al., 2012; HEKMAT et al., 2008), and have been mainly attributed to different metabolic rates (SHANER; MALLIPUDI, 1991).

Table 6. Translocation of imazaquin herbicide in *M. cinerea* during different phenological stages.

Stage	Accumulated radiation (dpm)					Translocation (%)			
	Total	Leaf	Stem	Root	Cotyledons	Leaf	Stem	Root	Cotyledons
Cotyledonary leaves	5528.93	1721.56	520.03	1622.22	1665.11	31.14	9.41	29.34	30.12
First pair of true leaves	7289.28	3246.42	637.63	2074.08	1331.15	44.54	8.75	28.45	18.26

Table 7. Recovery of radioactivity from imazaquin tests that were performed during different phenological stages of *M. cinerea*.

Stage	Radioactivity recovered with the solution used in the disassembly of the vases (%)	Radioactivity recovered in the soil (%)	Number of plants in each vase	Radioactivity recovered in the plants (%)	Total of Recovered Radioactivity (%)
Cotyledonary leaves	18.12	85.26	8	1.72	100.00
First pair of true leaves	8.48	91.13	8	2.55	100.00

Future studies with more plant growth times could be conducted to build a mathematical model of absorption, translocation and metabolism. It is important to reach the maximum practical potential of *M. cinerea* and *C. ensiformes* for phytoremediation strategies.

Conclusion

The species *M. cinerea* and *C. ensiformis* had the highest herbicide tolerance and were therefore selected for the study of imazaquin absorption and translocation. Considering the absorption and translocation of imazaquin from the cotyledonary stage to the first pair of true leaves stage, *M. cinerea* showed a higher potential for translocating imazaquin to the shoot of the plant when compared to *C. ensiformes*, which accumulated the herbicide mainly in the roots. The plants presented a higher potential for accumulating herbicide during the more advanced development stage, which shows greater potential for their use in areas with imazaquin herbicide residue.

References

ACCIOLY, A. M. A.; SIQUEIRA, J. O. Contaminação química e biorremediação do solo. In: NOVAIS, R. F.; ALVAREZ, V. V. H.; SCHAEFER, C. E. G. R. (Ed.). **Tópicos em ciência do solo**. Viçosa: SBCS, 2000. v. 1, p. 299-352.

AICHELE, J. M.; PENNER, D. Adsorption, desorption, and degradation of imidazolinones in soil. **Weed Technology**, v. 19, n. 1, p. 154-159, 2005.

ARTUZI, J. P.; CONTIERO, R. L. Herbicidas aplicados na soja e produtividade do milho em sucessão. **Pesquisa Agropecuária Brasileira**, v. 41, n. 7, p. 1119-1123, 2006.

ASKEW, S. D.; WILCUT, J. W. Absorption, translocation, and metabolism of foliar-applied CGA 362622 in cotton (*Gossypium hirsutum*), peanut (*Arachis hypogea*), and selected weeds. **Weed Science**, v. 50, n. 5, p. 293-298, 2002.

BATTAGLIN, W. A.; FURLONG, E. T.; BURHARDT, M. R.; PETER, C. J. Ocurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. **Science of the Total Environment**, v. 248, n. 2-3, p. 123-133, 2000.

BUKUN, B.; NISSEN, S. J.; SHANER, D. L.; VASSOS, J. D. Imazamox absorption, translocation, and metabolism in red lentil and dry bean. **Weed Science**, v. 60, n. 3, p. 350-354, 2012.

BRUCE, L. A.; CAREY, B.; PENNER, D.; KELLS, J. J. Effect of growth stage and environment on foliar absorption, translocation, metabolism, and activity of nicosulfuron in quackgrass (*Elytrigia repens*). **Weed Science**, v. 44, n. 3, p. 447-454, 1996.

CARABIAS-MARTÍNEZ, R.; RODRÍGUEZ-GONZALO, E.; FERNÁNDEZ-LAESPADA, M. E.; SÁNCHEZ-SAN ROMÁN, F. J. Evaluation of surface- and ground-water pollution due to herbicides in agricultural areas of Zamora and Salamanca (Spain). **Journal of Chromatography**, v. 869, n. 2, p. 471-480, 2000.

CARMO, M. L.; PROCOPIO, S. O.; PIRES, F. R.; CARGNELUTTI FILHO, A.; BRAZ, G. B. P.; SILVA, W. F. P.; BARROSO, A. L. L.; SILVA, G. P.; CARMO, E. L.; BRAZ, A. J. B. P.; ASSIS, R. L. Influência do período de cultivo de *Panicum maximum* (cultivar Tanzânia) na fitorremediação de solo contaminado com picloram. **Planta Daninha**, v. 26, n. 2, p. 315-322, 2008.

CRAFTS, A. S.; YAMAGUCHI, S. The autoradiography of plant materials. **California Agricultural Experiment Station Manual**, v. 35, n. 1, p. 143, 1964.

DAN, H. A.; DAN, L. G. M.; BARROSO, A. L. L.; PROCÓPIO, S. O.; OLIVEIRA JR., R. S.; ASSIS, R. L.; SILVA, A. G.; FELDKIRCHER, C. Atividade residual de herbicidas pré-emergentes aplicados na cultura da soja sobre o milheto cultivado em sucessão. **Planta Daninha**, v. 29, n. 2, p. 437-445, 2011.

EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de Pesquisa de Solos. **Sistema brasileiro de classificação de solos**. Rio de Janeiro: Embrapa, 1999.

GENT, M. P. N.; WHITE, J. C.; PARRISH, Z. D.; ISLEYEN, M.; EITZER, B. D.; MATTINA, M. I. Uptake and translocation of *p,p*-dichlorodiphenyldichloroethylene supplied in hydroponics solution to *cucurbita.*

Environmental Toxicology and Chemistry, v. 26, n. 12, p. 2467-2475, 2007.

HEKMAT, S.; SOLTANI, N.; SHORSPHERE, C.; SIKKENA, P. H. Effect of imazamox plus bentazon on dry bean. **Crop Protection**, v. 27, n. 12, p. 1491-1494, 2008.

HORNSBY, A. G.; WAUCHOUPE, R. D.; HERNER, A. E. **Pesticide properties in the environment**. New York: Springer-Verlag, 1995.

LEAL, I. G.; ACCIOLY, A. M. A.; NASCIMENTO, C. W. A.; FREIRE, M. B. G. S.; MONTENEGRO, A. A. A.; FERREIRA, F. L. Fitorremediação de solo salino sódico por *atriplex nummularia* e gesso de jazida. **Revista Brasileira de Ciências do Solo**, v. 32, n. 3, p. 1065-1072, 2008.

MARCACCI, S.; RAVETON, M.; RAVANEL, P.; SCHWITZGUÉBEL, J. P. Conjugation of atrazine in vetiver (*Chrysopogon zizanioides* Nash) grown in hydroponics. **Environmental and Experimental Botany**, v. 56, n. 205, p. 205-215, 2006.

NEWHOUSE, K. E.; SMITH, W. A.; STARRETT, M. A.; SCHAEFER, T. J.; SINGH, B. K. Tolarence to imidazolinone herbicides in wheat. **Plant Physiologist**, v. 100, n. 2, p. 882-886, 1992.

NEWMAN, L. A.; DOTY, S. L.; GERY, K. L.; HEILMAN, P. E.; MUIZNIEKS, I.; SHANG, T. Q.; SIEMIENIEC, S. T.; STRAND, S. E.; WANG, X.; WILSON, A. M.; GORDON, M. P. Phytoremediation of organic contaminants: a review of phytoremediation research at the University of Washington. **Journal of Soil Contamination**, v. 7, n. 4, p. 531-542, 1998.

NOVO, M. C.; CRUZ, L. S. P.; PEREIRA, J. C. V. N.; TREMOCOLDI, W. A.; IGUE, T. Persistência de imazaquim em Latossolo Roxo cultivado com soja. **Planta Daninha**, v. 15, n. 1, p. 30-38, 1997.

PESTER, T. A.; NISSEN, S. J.; WESTRA, P. Absorption, translocation and metabolism of imazamox in jointed goatgrass and feral rye. **Weed Science**, v. 49, n. 5, p. 607-612, 2001.

PINHEIRO, A.; MORAES, J. C. S.; SILVA, M. R. Pesticidas no perfil de solos em áreas de plantação de cebolas em Ituporanga, SC. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 15, n. 5, p. 533-538, 2011.

PIRES, F. R.; SOUZA, C. M.; SILVA, A. A.; PROCÓPIO, S. O.; FERREIRA, L. R. Fitorremediação de solos contaminados com herbicidas. **Planta Daninha**, v. 21, n. 2, p. 335-341, 2003.

PIRES, F. R.; SOUZA, C. M.; SILVA, A. A.; CECON, P. R.; PROCÓPIO, S. O.; SANTOS, J. B.; FERRREIRA, L. R. Fitorremediação de solos contaminados com tebuthiuron utilizando-se espécies cultivadas para adubação verde. **Planta Daninha**, v. 23, n. 4, p. 711-717, 2005.

REGITANO, J. B.; ALLEONI, L. R. F.; TORNISIELO, V. L. Atributos de solos tropicais e a sorção de imazaquin. **Scientia Agricola**, v. 58, n. 4, p. 801-807, 2001.

REGITANO, J. B.; BISCHOFF, M.; LEE, L. S.; REICHERT, J. M.; TURCO, R. F. Retention of imazaquin in soil. **Environmental Toxicology and Chemistry**, v. 16, n. 3, p. 397-404, 1997.

RISLEY, M. A.; OLIVER, L. R. Absorption, translocation, and metabolism of imazaquin in Pitted (*Ipomoea lacunosa*) and entireleaf (*Ipomoea hederacea* var. integriuscula) Morningglory. **Weed Science**, v. 40, n. 4, p. 503-506, 1992.

ROCHA, W. S. D.; ALLEONI, L. R. F.; REGITANO, J. B. Energia livre da sorção de imazaquin em solos ácricos. **Revista Brasileira de Ciência do Solo**, v. 27, n. 2, p. 239-246, 2003.

RODRIGUES, B. N.; ALMEIDA, F. L. S. **Guia de herbicidas**. Londrina: Edição dos Autores, 2011.

SALIHU, S.; HATZIOS, K. K.; DERR, J. F. Comparative uptake, translocation, and metabolism of root-applied isoxaben in Ajuga (*Ajuga reptans*) and two ornamental. **Pesticide Biochemistry and Physiology**, v. 60, n. 2, p. 119-131, 1998.

SANTOS, J. B.; PROCÓPIO, S. O.; SILVA, A. A.; PIRES, F. R.; RIBEIRO JR., J. I.; SANTOS, E. A.; FERREIRA, L. R. Fitorremediação do herbicida trifloxysulfuron sodium. **Planta Daninha**, v. 22, n. 2, p. 323-330, 2004.

SCRAMIN, S.; SKORUPA, L. A.; MELO, I. S. Utilização de plantas na remediação de solos contaminados por herbicidas – levantamento da flora existente em áreas de cultivo de cana-de-açúcar. In: MELO, J. S. (Ed.). **Biodegradação**. Jaguariúna: Embrapa Meio Ambiente, 2001. p. 369-371.

SEIFERT, S.; SHAW, D. R.; KINGERY, W. L.; SNIPIS, C. E.; WESLEY, R. A. Imazaquin mobility and persistence in a sharkey clay soil as influenced by tillage systems. **Weed Science**, v. 49, n. 2, p. 571-577, 2001.

SHANER, D. L. Imidazolinone herbicides. In: PLUMMER, D.; RAGSDALE, N. (Ed.). **Encyclopedia of agrochemicals**. Hoboke: John Wiley and Sons, 2003. p. 769-784.

SHANER, D. L.; MALLIPUDI, N. M. Mechanisms of selectivity of the imidazolinones. In: SHANER, D. L.; O'CONNER, S. L. (Ed.). **The imidazolinone herbicides**. Boca Raton: CRC Press, 1991. p. 91-102.

SILVA, A, A.; OLIVEIRA, J. R. R. S.; COSTA, E. R.; FERREIRA, L. R. Efeito residual no solo dos herbicidas imazamox e imazethapyr para as culturas de milho e sorgo. **Planta Daninha**, v. 17, n. 3, p. 345-354, 1999.

SMITH, M. C.; SHAW, D. R.; MILLER, D. K. In field bioassay to investigate the persistence of imazaquin and pyriothiobac. **Weed Science**, v. 53, n. 1, p. 121-129, 2005.

TAN, S.; EVANS, R. R.; DAHMER, M. L.; SINGH, B. K.; SHANER, D. L. Imidazolinone tolerant crops: history, current status and future. **Pest Management Science**, v. 61, n. 3, p. 246-257, 2005.

THOMPSON, A. M.; ROSALES-ROBLES, E.; CHANDLER, J. M.; NESTER, P. R.; JINGLE, C. H. Crop tolarence and weed management systems in imidazolinone tolerant corn. **Weed Technology**, v. 19, n. 4, p. 1037-1044, 2005.

TROXLER, S. C.; FISHER, L. R.; SMITH, D. W.; WILCUT, J. W. Absorption, translocation, and

metabolism of foliar applied trifloxysulfuron in tobacco. **Weed Technology**, v. 21, n. 2, p. 421-425, 2007.

VIDAL, R. A. **Ação dos herbicidas**: absorção, translocação e metabolização. Porto alegre: Evangraf, 2002.

WALLS, F. R.; CORBIN, F. T.; COLLINS, W. K.; WORSHAM, A. D.; BRADLEY, J. R. Imazaquin absorption, translocation, and metabolism in flue-cured tobacco. **Weed Technology**, v. 7, n. 2, p. 370-375, 1993.

YODER, R. N.; HUSKIN, M. A.; KENNARD, L. M.; ZABIK, J. Aerobic metabolismo of diclosulam on U.S. and South American soils. **Journal of Agricultural and Food Chemistry**, v. 48, n. 1, p. 4335-4340, 2000.

Received on May 2, 2012.

Accepted on October 3, 2012.