Advances in Weed Science formerly Planta Daninha

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RESEARCH ARTICLE

Peanut and sorghum are excellent phytoremediators of ^{14}C-tebuthiuron in herbicide-contaminated soil

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Abstract: **Background:** Phytoremediation is a technique used in soils contaminated with residual herbicides, such as tebuthiuron. However, the herbicide presence in the soil and plant matrices are not generally quantified.
Objective: This study aimed to select plant species to evaluate the phytoremediation of ^{14}C-tebuthiuron by showy rattlepod (*Crotalaria spectabilis*), sorghum (*Sorghum bicolor*), radish (*Raphanus sativus*), peanut (*Arachis hypogaea*), and alfalfa (*Medicago sativa*) in herbicide-contaminated soil.
Methods: The selection of the five herbicide phytoremediation plants was with the application of five rates of tebuthiuron (300, 600, 1,200, 2,400, and 4,800 g a.i. ha^{-1}) and compared to a control. Peanuts and sorghum (herbicide-tolerant plants) were sown in soil contaminated with tebuthiuron (600 g a.i. ha^{-1}) applied through a working solution containing 17.47 kBq of ^{14}C-tebuthiuron. The total of herbicide was analyzed in the soil and plant at three phenological stages.
Results: Showy rattlepod, radish, and alfalfa were sensitive to the herbicide even at the lowest application rate. Sorghum was tolerant to the herbicide up to 600 g ha^{-1} with the application of 1,200 g ha^{-1}, there was 80% injury; peanut was tolerant even at the highest rate (4,800 g ha^{-1}) with only 40% injury. Peanut and sorghum were able to phytoremediate the soil, although, peanut was more efficient in decreasing tebuthiuron contamination by 76%, while sorghum reduced at by 45% at 3rd phenological stage.
Conclusions: Thus, both plants can be recommended in succession/rotation with crops that had tebuthiuron applied from pre-emergence weed control.

Keywords: Root absorption; Rhizodegradation; Radiolabeled herbicide; Decontamination; Crop

Journal Information:
ISSN - 2675-9462
Website: http://awsjournal.org
Journal of the Brazilian Weed Science Society

How to cite: Conciani PA, Mendes KF, Sousa RN, Ribeiro AP, Pimpinato RF, Tornisielo VL. Peanut and sorghum are excellent phytoremediators of ^{14}C-tebuthiuron in herbicide-contaminated soil. Adv Weed Sci.2023;41:e020220068.
https://doi.org/10.51694/AdvWeedSci/2023;41:00002

Approved by:
Editor in Chief: Carol Ann Mallory-Smith
Associate Editor: Marcos Yannicari

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Received: September 28, 2022
Approved: November 17, 2022

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1. Introduction

The contamination of the environment by pesticides, including herbicides, is a major global concern and can be considered a major obstacle to the development of sustainable agriculture (Mendes et al., 2020). There is social and scientific appeal for the minimization of environmental impacts caused by the use of herbicides in the large areas cultivated with sugarcane and the increase of areas treated with long residual effect herbicides, such as tebuthiuron.

Tebuthiuron (1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea) is a systemic herbicide, absorbed by roots and translocated in weeds, widely used in sugarcane, with a photosystem II (PSII) inhibitors mode of action of the urea chemical group (Mendes et al., 2022). This herbicide has a high leaching potential due to its high water solubility (S_w = 2,500 mg L^{-1}) and low sorption (sorption coefficient, K_d = 1.32 and 0.85 L Kg^{-1} in clay and loamy sand soil, respectively) (Guimarães et al., 2022). A positive correlation among clay content, soil organic matter (SOM), and tebuthiuron sorption was found by Mendes et al. (2021). Also, this herbicide has a high persistence in the soil (90% degradation time, DT90 = ~385 and 334 d) (Guimarães et al., 2022). However, bacteria can effectively degrade tebuthiuron in soil (Lima et al., 2022) and seawater (Mercurio et al., 2015).

The application of herbicides with residual effects in the soil is essential for weed control in pre-emergence. Nevertheless, if the residual effect is greater than the cycle of the crop, it can cause injury in the next crop (rotation/succession), a process commonly known as carryover. On the other hand, tebuthiuron can have negative impacts on soil microorganisms (Faria et al., 2018) and water (Bernardes et al., 2014). In the sugarcane field-surrounding aquatic environment, tebuthiuron distribution ranged from 0.007 to 0.022 mg L^{-1}, leading to a high risk at in aquatic ecosystems (Qian et al., 2017), as DNA damage in exposed small fish (*Oreochromis niloticus*) can occur (Bernardes et al., 2014). Then, using strategies to remediate herbicides in contaminated soils is critical in agricultural systems.

Phytoremediation is one option to reduce the environment in soil contaminated by residual herbicides, such as tebuthiuron (Mendes et al., 2021a and 2021b).

Results showed preliminary evidence of effective phytoremediation capacity by velvet bean *(Mucuna pruriens)*, pearl millet *(Pennisetum glaucum)*, and sunn hemp *(Crotalaria juncea)* in tebuthiuron contaminated-soil (Ferreira et al., 2021). In the highest rate of tebuthiuron (1,500 g ha^{-1}) applied, jack bean *(C. ensiformis)*, white lupin *(Lupinus albus)*, and pearl millet *(P. glaucum)* were the species with better phytoremediation effects (Pires et al., 2008). When the soil was treated with tebuthiuron at 1,000 g ha^{-1}, jack bean, followed by white lupin, and velvet bean were the species that had better phytoremediation (Pires et al., 2006). However, the phenological stages of the phytoremediation species and their association with rhizosphere microorganisms can directly interfere in the amount of herbicide degraded.

The phytoremediation technique increases in complexity, as researchers try to select species that present remediation capacity and other agronomic benefits to farmers. However, phytoremediation is sustainable and effective, less expensive and destructive when compared to other soil decontamination processes, and can be applied over large areas (Paiva, Mendes, 2021).

Through techniques of use of herbicides radiolabeled with ^{14}C, it is possible to verify the potential of phytoremediation of plant species at the laboratory condition, since this technique allows accurately determining the absorption and translocation of small amounts of herbicides in plants, such as diuron, hexazinone, sulfometuron-methyl (Teófilo et al., 2020), quinclorac, and tebuthiuron (Mendes et al., 2021a).

Many phytoremediation studies of various herbicides in contaminated soils have been carried out in several edapho-climatic conditions and with a diversity of plant species. However, there is still a lack of studies that detect herbicides by analytical techniques in the soil and plants. Thus, this study aimed to select plant species to evaluate for the phytoremediation of ^{14}C-tebuthiuron by showy rattlepod (*Crotalaria spectabilis*), sorghum (*Sorghum bicolor*), radish (*Raphanus sativus*), peanut (*Arachis hypogaea*), and alfalfa (*Medicago sativa*) in soil.

2. Material and Methods

2.1 Soil collection and preparation

To ensure that there was no previous contamination with herbicides in the soil used in this study, the soil was collected in native forests (permanent preservation area) near Piracicaba-SP, Brazil, at a minimum distance of 20 m from the edge of a road.

A preliminary cleaning was performed, eliminating leaves and pieces of plants from the soil surface and then the layer from 0 to 10 cm deep was collected. The collected soil was air-dried, homogenized, and passed through a 2 mm sieve, and the physical and chemical properties were analyzed (Table 1).

2.2 Installation of the studies

Two experiments were carried out for the development of this study. The first was to select five species of agronomic interest for phytoremediation at different levels of soil contamination by tebuthiuron. Seeds from the herbicide tolerant species that survived in the first study were collected and used in the second study, in which, the phytoremediation of sorghum and peanut to soil contaminated by tebuthiuron was determined. The studies were conducted in a glass greenhouse for one year. During this period, the averages observed for maximum, minimum, and average temperatures were 30.4, 17.2, and 23.8 °C, respectively.

2.2.1 *Plant selection for phytoremediation of tebuthiuron in contaminated soil*

The experiment was set up in a randomized block design in a factorial scheme (5x6) with three repetitions. Seeds of five plant species (showy rattlepod, sorghum, radish, peanut, and alfalfa) were grown in pots filled with soil sprayed (simulated contamination) by six rates of tebuthiuron (0x, 1/4x 1/2x, x, 2x, and 4x). in which *x* represents the maximum rate recommended for field application of the herbicide (1,200 g a.i. ha^{-1}).

Polystyrene pots with a capacity of approximately 1,500 cm^3 were used. Each pot received 800 g of dry soil and water was added to reach field capacity. Twenty-four hours after the soil reached saturation point each pot was sown with 20 seeds of each specie that then were covered by 100 g of dry soil for three repetitions.

Given the small diameter of the pot, each rate was prepared in a concentration of active ingredient per application volume equivalent to the rate of 200 L ha^{-1}. The herbicide application was used a chamber with spray nozzle TTI110.02 and the constant pressure of 279.2 kPa.

The herbicide was applied at the appearance of the first true leaf until the emergence of the second pair of leaves, every two days, visual evaluations of the injury level were performed, according to a scale of 0 to 100%, where 0 represents the absence of symptoms and 100 represents death of the plant.

After injury level evaluations, the pots were disassembled and from each plot, three plants were randomly collected. Each plant measured by plant height, and root length, and stored in paper bags. The collected plants were dried 72 h in an oven heated to 40 °C and then weighed to determine the accumulated dry matter.

For the data on percent reduction in dry matter, plant height, and root length, Tukey's test (p<0.05) was performed to verify the difference between the treatments. The level injury data were expressed as mean and standard deviation (n = 3). All figures were plotted using Sigma Plot (version 10.0 for Windows, Systat Software, Inc., Point Richmond, CA, USA).

Table 1 - Soil physical and chemical properties

Chemical properties											
pH	**P**	**S**	**K**	**Ca**	**Mg**	**Al**	**H+Al**	**OM**	**BS**	**CEC**	**SB**
$CaCL_2$	mg cm^{-3}		mmol$_c$ dm^{-3}					g dm^{-3}	mmol$_c$ dm^{-3}		%
6.6	10	6	3.3	74	18	<1	20	69	95.5	116	83

Physical properties			
Sand	**Silt**	**Clay**	**Textural class**
%	%	%	clay
40.6	15.0	44.4	

P: phosphorous, S: sulfur, K: potassium, Ca: calcium, Mg: magnesium, Al: aluminum. H+Al: potential acidity, OM: organic matter, BS: base sum, CEC, cationic exchange capacity, SB: saturation base

Source: Soil Laboratory, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil

2.2.2 *Phytoremediation of ^{14}C-Tebuthiuron in contaminated soil*

Pots with a capacity of 2,800 cm^3, filled with 900 g of air-dried soil, were used. Water was added to the trays of the pots until it reached the soil surface by capillarity action. After 24 h, each pot received either 5 sorghum or 5 peanut seeds (species selected in the first study) and a layer of 100 g of dry soil was deposited over the seeds.

The study was a randomized block design with a factorial scheme (2x3), with two plant species (sorghum and peanut) in three phenological stages of development, with three repetitions. In addition, a control treatment (no herbicide application) was added for comparison of the other treatments.

Given the small surface area of the pots and the handling of a radioactive product, the herbicide application was performed by adding 100 g of treaded dry soil. A working solution containing 17.47 kBq of ^{14}C-tebuthiuron (^{14}C-UL, specific activity = 3.01 MBq mg^{-1}, radiochemical purity > 98%) and technical product (non-radiolabeled) was prepared to reach 600 g a.i. ha^{-1} of herbicide rate recommended in the field.

Whenever necessary, the re-establishment of the moisture in the pots occurred through the deposition of water in the trays to maintain soil moisture close to field capacity and avoid herbicide leaching.

When the plants reached the desired three stages of phenological development [one pair of true leaves formed (1st phenological stage), 1st pair of branches (2nd) and beginning of flowering (3rd) for peanut; and 3rd leaf (1st phenological stage), 5th leaf (2nd), and fully extended (3rd) flag leaf for sorghum]. The plants were removed from the pots and placed on plastic trays. A KCl (0.02 mol L^{-1}) solution was used to wash the roots.

The trays were left to stand for 24 h for sedimentation of the solution. The soil solution was collected to quantify the herbicide remaining and centrifuged (Hitachi CF16RXII, Hitachi Koki Co., Ltd., Indaiatuba, SP, Brazil) in Teflon flasks at 4,000 rpm for 15 min to remove the soil suspension. The sediment soil was returned to the trays and dried in an oven for 72 h at 40 °C. The dried soil was crumbled, homogenized, and ground. Three samples of 0.2 g each were burned in a biological oxidizer (R.J. Harvey Instrument Corporation OX500, Tappan, NY, USA) at 900 °C in porcelain dishes by 3 min to determine the radioactivity amount of ^{14}C-herbicide mineralized to $^{14}CO_2$. The ^{14}C-tebuthiuron concentration was determined using a Liquid Scintillation Spectrometry (LSS) for 15 min with a Tri-Carb 2910 TR LSS counter (PerkinElmer, Waltham, MA, USA).

After centrifugation to separate the soil, the solution from the pots was diluted with distillated water. Three aliquots (10 mL each) of this solution were taken from each pot. To each sample 10 mL of the scintillator solution (Insta-gel plus) was added, and then measured in LSS.

The phytoremediation was evaluated by absorption and translocation of ^{14}C-tebuthiuron. This study was qualitatively analyzed by autoradiography and quantitatively by combustion of plant tissues. Plants were washed, pressed, and dried in a forced circulation oven at 45 °C for 120 h. One triplicate in each plant was used according to Mendes et al. (2017) and Cruz et al. (2021), on phosphorus film plates for 120 h and analyzed in a radio-scanner (PerkinElmer® Storage Phosphor System, model Cyclone Plus, Shelton, WA, USA).

After drying, the plants were removed and divided into leaves, roots, stems, and cotyledon (when this was still present) to quantify the radioactivity in each part of the plant.

The samples were burned in a biological oxidizer, with three repetitions for each part of the plant. The $^{14}CO_2$ released in the combustion was collected in a flask containing scintillator solution plus monoethanolamine and methanol. The radioactivity contained in this flask was determined in a LSS.

Radioactivity present in all parts of the plants was considered as translocation because the initial herbicide application was in the soil. The data were expressed as mean (n=3).

3. Results and Discussion

3.1 Plant selection for phytoremediation of tebuthiuron in contaminated soil

At the rates of 300 and 600 g ha^{-1} of tebuthiuron, there was a small initial incidence of injury level (<8%) in sorghum compared to the control treatment (no herbicide). However, after 10 days after emergence (DAE) there was a tendency in decreased injury symptoms and at 20 DAE the injury did not differ from the control treatment (Figure 1).

A study performed by Pires et al. (2003a), showed the effects of different rates of tebuthiuron in multiple species, in which, the authors also found a lower injury (25%) in pearl millet (*Pennisetum glaucum)* treated with 500 g ha^{-1} of tebuthiuron at 60 days after application (DAA).

At rates of 1,200, 2,400, and 4,800 g ha^{-1} of tebuthiuron, the initial symptoms of injury in sorghum increased over time, reaching 80% with 1200 g ha^{-1} and values above 95% in rates of 2,400 and 4,800 g ha^{-1} (Figure 1). These results corroborate those of Pires et al. (2003a) in which pearl millet plants had an injury level of 76% compared to the control when treated with 1000 g ha^{-1} of tebuthiuron and 100% with the rate of 2,000 g ha^{-1} at 60 DAA.

Even at the lowest rate of tebuthiuron (300 g ha^{-1}), all showy rattlepod plants died at 12 DAE (Figure 2), the same can be observed for alfalfa (Figure 3) and radish (Figure 4), respectively, at 8 and 10 DAE. At 60 DAA, showy rattlepod died, and had 83% injury to radish treated with a rate of 500 g ha^{-1} (Pirest et al., 2003a). Corroborating this data, in another study, the injury levels of 95% at 15 DAA and

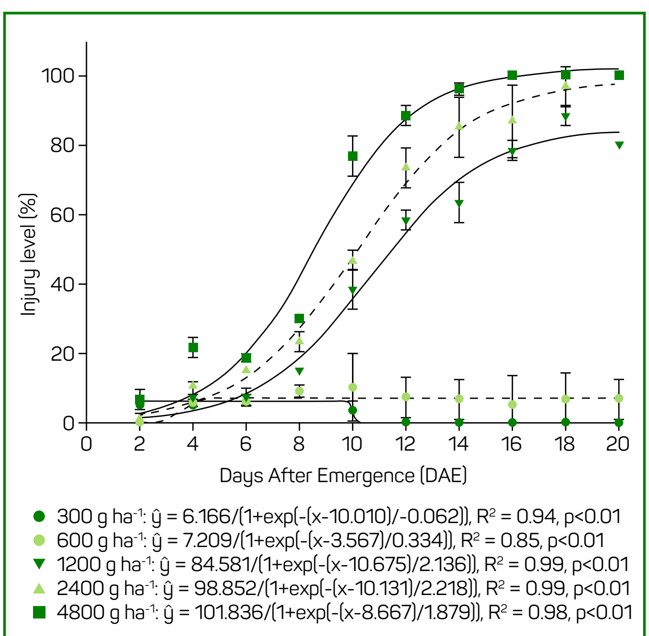

Figure 1 - Injury level in sorghum (*Sorghum bicolor*) caused by the application of five rates of tebuthiuron with evaluations at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after emergence (DAE). Vertical bars represent standard deviations (± SD) of means (*n* = 3)

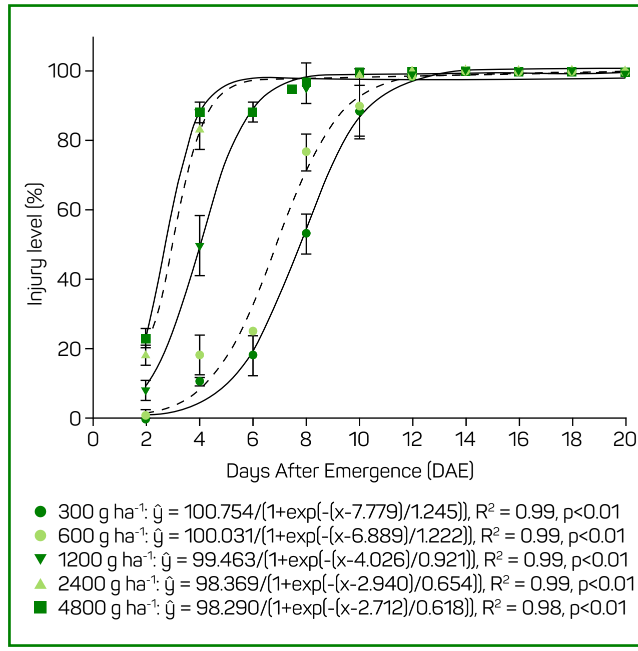

Figure 2 - Injury level in showy rattlepod (*Crotalaria spectabilis*) caused by the application of five rates of tebuthiuron with evaluations at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after emergence (DAE). Vertical bars represent standard deviations (± SD) of means (*n* = 3)

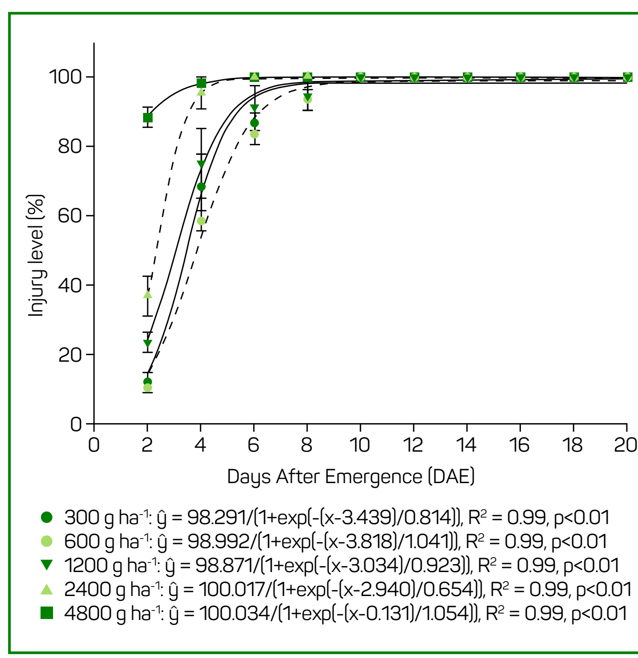

Figure 3 - Injury level in alfalfa (*Medicago sativa*) caused by the application of five rates of tebuthiuron with evaluations at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after emergence (DAE). Vertical bars represent standard deviations (± SD) of means (*n* = 3)

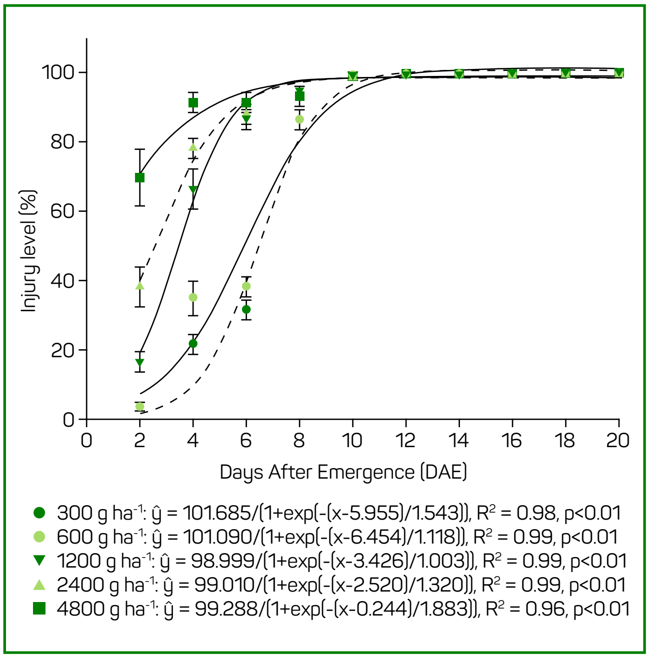

Figure 4 - Injury level in radish (*Raphanus sativus*) caused by the application of five rates of tebuthiuron with evaluations at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after emergence (DAE). Vertical bars represent standard deviations (± SD) of means (*n* = 3)

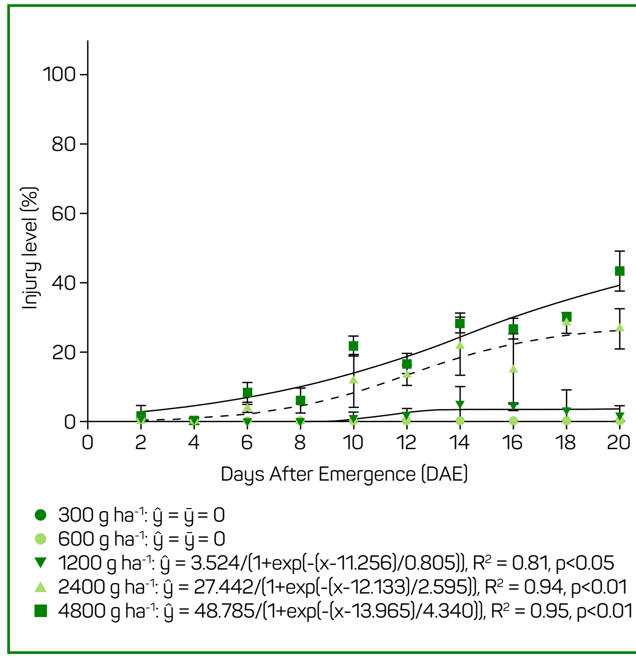

Figure 5 - Injury level in peanut (*Arachis hypogaea*) caused by the application of five rates of tebuthiuron with evaluations at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after emergence (DAE). Vertical bars represent standard deviations (± SD) of means (*n* = 3)

100% (plant death) at 30 DAA were reported in radish with 500 g ha^{-1} of this herbicide (Pires et al., 2003b).

In peanuts, with 300 and 600 g ha^{-1} of tebuthiuron, there were no symptoms of injury, however, in the rate of 1,200 g ha^{-1}, slight symptoms (<5%) were observed between 10 to 20 DAE (Figure 5). In the two highest rates (2,400 and 4,800 g ha^{-1}) of the herbicide, there was a constant increase in peanut injury over time. At 20 DAE there were injury levels of 30 and 40%, respectively. These results are in agreement with Fernandes et al. (2012), who reported injury of 24% at 28 DAA in bean with a rate application of 800 g ha^{-1}. Over time, there was a decrease in symptoms, with 18% injury at 56 DAA.

The reduction in dry matter, plant height, and roots of five plants obtained after the application of different rates of tebuthiuron are shown in Table 2. With the increase of tebuthiuron rates, all plants had decreased dry matter and reduced plant height and roots length, when compared to control (Table 2).

Alfalfa and radish showed 100% of death due to their high sensitivity to the herbicide. Showy rattlepod showed a reduction of 90, 77, and 97% in dry matter, plant height, and root length, respectively, even at the lowest rate (300 g ha^{-1}). This result did not differ from radish and alfalfa in any of the variables evaluated for any of the rates (Table 2). Similar results were observed in showy rattlepod (Pires et al., 2003a) and in radish (Pires et al., 2003b), which showed 100% mortality when applied to a rate of 500 g ha^{-1}.

It was observed that sorghum showed 62 and 72% reduction in dry matter, 19 and 28% in plant height, and 18 and 25% in root length at rates of 300 and 600 g ha^{-1} of tebuthiuron, respectively, regardless of the rates (Table 2). Another study produced similar results, in which 44% reduction of dry matter of sorghum at 50 DAA of sulfentrazone (500 g ha^{-1}), was observed with a protoporphyrinogen oxidase (PPO) inhibitor (Belo et al., 2011).

Peanuts showed a 19% reduction in the dry matter at 300 g ha^{-1} and 36% at 600 g ha^{-1}, which did not differ from the other rates. In the variables above and root length, there were no differences between the evaluated rates in peanuts (Table 2).

3.2 Phytoremediation of ^{14}C-Tebuthiuron in Contaminated Soil

There was a lower concentration of ^{14}C-tebuthiuron (~4%) in the water from the pots containing peanut compared to those with sorghum. In addition, there was a higher percentage of the herbicide recovered (~8%) in the 1st phenological stage (one pair of true leaves formed for peanut and 3rd leaf for sorghum). of the plant compared to the others (Table 3), showing the reduction in the amount of herbicide available for plant absorption in the soil solution.

Peanut had a higher percentage of ^{14}C-tebuthiuron than sorghum in all phenological stages, indicating greater efficiency in herbicide absorption (Table 3). Although, there

Table 2 - Percent reduction in dry matter, plant height, and root length compared to the control treatment of five plant species cultivated in soil with five rates of tebuthiuron at 20 days after emergence (DAE)

Plant	Rate (g ha^{-1})				
	Dry matter				
	300	600	1200	2400	4800
Sorghum	62.31 bA	71.92 bA	81.64 bB	100.0 bC	100.0 bC
Showy rattlepod	90.30 cA	100.0 cA	100.0 cA	100.0 bA	100.0 bA
Alfalfa	100.0 cA	100.0 cA	100.0 cA	100.0 bA	100.0 bA
Radish	100.0 cA	100.0 cA	100.0 cA	100.0 bA	100.0 bA
Peanut	19.38 aA	35.93 ABC	43.40 aC	29.73 aB	32.10 aB
	Plant height				
	300	600	1200	2400	4800
Sorghum	18.89 aA	27.56 aA	57.06 bB	100.0 bA	100.0 bA
Showy rattlepod	76.97 bA	100.0 bA	100.0 cA	100.0 bA	100.0 bA
Alfalfa	100.0 bA	100.0 bA	100.0 cA	100.0 bA	100.0 bA
Radish	100.0 bA	100.0 bA	100.0 cA	100.0 bA	100.0 bA
Peanut	20.12 aA	27.03 aA	17.88 aA	19.51 aA	14.83 aA
	Root length				
	300	600	1200	2400	4800
Sorghum	17.85 aAB	24.75 aB	8.52 aA	100.0 bC	100.0 bC
Showy rattlepod	97.35 bA	100.0 bA	100.0 bA	100.0 bA	100.0 bA
Alfalfa	100.0 bA	100.0 bA	100.0 bA	100.0 bA	100.0 bA
Radish	100.0 bA	100.0 bA	100.0 bA	100.0 bA	100.0 bA
Peanut	7.11 aA	10.29 aA	6.37 aA	10.29 aA	3.92 aA

Means followed by the same lowercase letter in the column and capital in the row do not differ by Tukey's test (p<0.05)

was a considerable variation in the percentage of herbicide recovered, there was no difference in the concentration of ^{14}C-tebuthiuron found in the analyzed plants throughout the phenological stages, showing that there was herbicide absorption by the plants.

Thus, it can be affirmed that the root absorption of the ^{14}C-tebuthiuron present in the soil and the translocation of this herbicide for the two species occurred continuously throughout the phenological stages (Figure 6).

Over the evaluation time, there was a decrease in the sharpness of the ^{14}C in the autoradiography The decreases is due to the percentage of radioactivity recovered from the herbicide in the plants remained constant, while the concentration of radioactivity per gram of dry matter decreased as the plants grew (Figure 6). This confirmed that there was no accumulation of the herbicide molecule in the plant. Possibly, the plants metabolized the herbicide, as found by Johnsen (1992) in Utah junipers trees (*Juniperus osteosperma*). Loblolly pine (*Pinus taeda*) and eastern redcedar (*Juniperus virginiana*) can prevent the accumulation of tebuthiuron in the leaves within 24 h. Demethylation was determined to be the primary detoxification mechanism of this herbicide by eastern redcedar, loblolly pine, and bur oak (*Quercus macrocarpa*) (McNeil et al., 1984).

The potential of showy rattlepod, jack bean, velvet bean, and white lupin, in the soil phytoremediation treated with ^{14}C-quinclorac and ^{14}C-tebuthiuron was studied, and all species were reported to have the potential to remedy soils contaminated with both herbicides (Mendes et al., 2021a). The same authors also observed that the translocation of ^{14}C-tebuthiuron was greater in the old leaves than roots and young leaves, also the translocation of ^{14}C-quinclorac (synthetic auxin) was higher in the young leaves compared to the old leaves, roots, and cotyledons. The same ^{14}C-tebuthiuron behavior in was observed in our study.

There was a reduction in the percentage of ^{14}C-tebuthiuron recovered in the soil over time for both plants, showing that peanuts and sorghum

Table 3 - Amount of ^{14}C-tebuthiuron (%) found in the different matrices about the initially rate applied (600 g ha^{-1}) in peanut (*Arachis hypogaea*) and sorghum (*Sorghum bicolor*) in three phenological stages

^{14}C-tebuthiuron (%) - Supernatant water of the pots				
Plant	Phenological stage			Mean
	1st	2nd	3rd	
Peanut	6.74	3.12	2.09	3.98 b
Sorghum	8.70	6.55	4.50	6.58 a
Mean	7.72 a	4.84 b	3.29 b	
CV%	36.78			
^{14}C-tebuthiuron (%) - Plant				
Plant	Phenological stage			Mean
	1st	2nd	3rd	
Peanut	0.83	1.16	0.56	0.85 a
Sorghum	0.06	0.30	0.39	0.25 b
Mean	0.44 a	0.73 a	0.48 a	
CV%	39.58			
^{14}C-tebuthiuron (%) - Soil				
Plant	Phenological stage			Mean
	1st	2nd	3rd	
Peanut	68.77	42.64	24.20	45.20 b
Sorghum	98.42	85.56	55.51	79.83 a
Mean	83.60 a	64.10 b	39.85 c	
CV%	12.60			
^{14}C-tebuthiuron (%) – Total (Supernatant water + Plant + Soil)				
Plant	Phenological stage			Mean
	1st	2nd	3rd	
Peanut	76.35	46.94	26.86	50.05 b
Sorghum	107.20	92.42	60.41	86.67 a
Mean	91.77 a	69.68 b	43.63 c	
CV%	12.31			

One pair of true leaves formed (1st phenological stage), 1st pair of branches (2nd) and beginning of flowering (3rd) for peanut; and 3rd leaf (1st phenological stage), 5th leaf (2nd), and fully extended (3rd) flag leaf for sorghum. Means followed by the same lowercase letter in the column and capital in the row do not differ by Tukey's test (p<0.05)

were efficient in the phytoextraction of tebuthiuron, phytodegradation (metabolization) by the plant, and/or rhizodegradation by the microorganism's activity in the roots. Seedlings of winged elm (*Ulmus alata*), bur oak, black walnut (*Juglans nigra*), eastern redcedar, and loblolly pine also had root absorption and leaf accumulation of ^{14}C-tebuthiuron (McNeil et al., 1984). Less than 5.5% of the recovered activity of ^{14}C-tebuthiuron for species, [Japanese brome (*Bromus japonicas*) and corn (*Zea mays*)] was above ground, less than 3% in the roots, and less than 1.5% was in the nutrient solution (Steinert, Stritzke, 1977). Generally, tebuthiuron and its metabolites are found in plants, however, in low concentration (Johnsen, Morton, 1991).

In this study, peanut at all phenological stages was more efficient in decreasing the amount of ^{14}C-tebuthiuron with ~69, 43, and 24% in stages 1st, 2nd, and 3rd. In sorghum was recovered 98, 86, and 55% of the herbicide initially applied, respectively (Table 3). Peanut and sorghum were able to phytoremediate, however, peanut was more efficient in reducing contamination of tebuthiuron (76%) from the soil than sorghum (45%) at the 3rd phenological stage.

Native microorganisms from sugarcane's rhizosphere had a synergistic bacterial pool able to produce 90 mg CO_2 day^{-1} upon the target tebuthiuron at 5 mmol g^{-1} (Lima et al., 2022). Thus, these authors clearly showed the microbial degradation of tebuthiuron in the rhizosphere soil.

It is evident that further studies are needed with these species over the whole life cycle and to establish a comparison of the effect with uncultivated soils. In order to demonstrate the maximum potential for herbicide phytoextraction/phytodegradation by these species.

In peanut and sorghum, there was a decrease in the total radioactivity recovered over the evaluation time, confirming the degradation of the herbicide in the soil, which it was probably mineralized to ^{14}C-CO_2 (Table 3). Under higher tebuthiuron concentrations (>1,000 g ha^{-1}), the mean CO_2 evolution rate was higher in the rhizospheric soil of jack bean, followed by velvet bean, and Georgia velvet bean (Pires et al., 2005b). Accordingly, when tebuthiuron was applied at 1,000 g ha^{-1}, jack bean showed the best phytoremediation results (Pires et al., 2005a).

4. Conclusions

Alfalfa, radish, and showy rattlepod were sensitive to the tebuthiuron presence in the soil, not showing potential for phytoremediation of soil with residues of this herbicide. Conversely, sorghum and peanut were tolerant to tebuthiuron. Peanut was able to phytoremediate 31% more herbicide from soil than sorghum at the 3rd phenological stage (even though the life cycle of the plants was not completed). Thus, both plants can be recommended in succession/rotation with crops that have the tebuthiuron applied for pre-emergence weed control. This study is an important contribution to new insights into herbicide physiology.

Author's contributions

All authors read and agreed to the published version of the manuscript. PAC, KFM, and VLT:

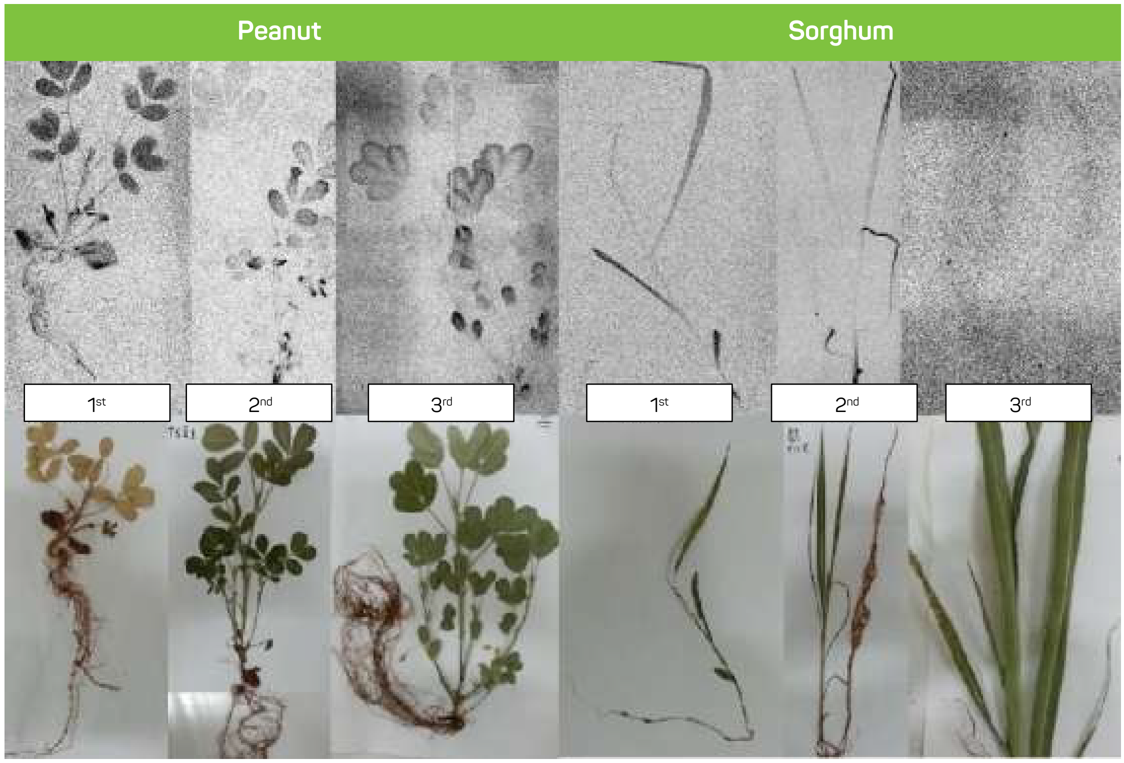

Figure 6 - Visualization of phosphorescence images of autoradiography (top) and digital images (bottom) of peanut (*Arachis hypogaea*) and sorghum (*Sorghum bicolor*) with ^{14}C-tebuthiuron application (600 g ha^{-1}) in three phenological stages. One pair of true leaves formed (1st phenological stage), 1st pair of branches (2nd) and beginning of flowering (3rd) for peanut; and 3rd leaf (1st phenological stage), 5th leaf (2nd), and fully extended (3rd) flag leaf for sorghum. The darker the gray, the greater the radioactivity emitted by ^{14}C

conceptualization of the manuscript and development of the methodology. PAC, and RFP: data collection and curation. PAC, and KFM: data analysis. PAC, RNS, and APR: data interpretation. VLT: funding acquisition and resources. PAC, and VLT: project administration. VLT: supervision. PAC, KFM, RNS, APR, and RFP: writing the original draft of the manuscript. PAC, KFM, RNS, APR, and VLT: writing, review, and editing.

Acknowledgments

The first author thanks *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for granting the scholarship.

Funding

This research received no external funding.

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