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Chapter 7

Procedures for Detection of Resistant Weeds Using ^{14}C-Herbicide Absorption, Translocation, and Metabolism

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Additional information is available at the end of the chapter

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Abstract

Herbicide resistance mechanisms involve altered absorption, translocation, and metabolism of herbicides (i.e., glyphosate), and this is an important component in the study of herbicide resistance mechanisms as well. ^{14}C-herbicides are used in resistant weeds studies, since they provide some advantages in comparison with chemical measures, including greater sensitivity, stepwise description of a particular element in a metabolic system, herbicide position, detection through X-ray films and/or radio image, and liquid scintillation. However, an up-to-date, organized description and standardization of research procedures and methodology on the use of radioisotopes for detection of resistant weeds, through different mechanisms of absorption, translocation, and metabolism in comparison with susceptible weeds are lacking in the literature. Techniques that use ^{14}C such as tracers are extremely useful to study the herbicides behavior in the resistant weed, since the radiometric techniques offer the possibility of accurately determining very small amounts in a relatively short time. However, mechanism of resistance to herbicides in this resistant weed population compared with the susceptible population cannot be due to differential absorption, translocation, or metabolism of herbicide in weed; so other studies are necessary to elucidate the mechanism of herbicide resistance on weed population.

Keywords: mechanism of resistance, metabolites, standard methodology, radioisotopes

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1. Introduction

Herbicides can penetrate plants through their aerial structures (leaves and stems), subterraneous (root, rhizome, stolon, and tuber), and young structures such as radicles and caulicles. The main route of penetration of the herbicides in the plant is a function of a series of intrinsic and extrinsic (environmental) factors. Absorption of herbicides by roots or leaves is influenced by the availability of the products at the sites of absorption and environmental factors (temperature, light, relative humidity, and soil moisture), which also influences the translocation of these to the site of action [1].

Among the biochemical and physiological mechanisms, the change in the absorption, translocation, or metabolism of resistant weed biotypes has been reported on several species for different herbicides. These resistance mechanisms have been studied over the last years, allowing the development and improvement of analytical techniques to diagnose this type of resistance [2]. However, an up-to-date, organized description and standardization of research procedures and methodology on the use of radioisotopes for detection of resistant weeds, through different mechanisms of absorption, translocation, and metabolism in comparison with susceptible weeds are lacking in the literature.

Radioisotopes are used on several research areas, such as for the metabolism of drugs and pesticides, environmental studies to determine biological routes and mass balance studies for organic compounds, and the ones that are most frequently used are tritium and ^{14}C. The method for using radiolabeled herbicides may be quantitative or qualitative, allowing associating the resistance to the reduced absorption and/or translocation, and/or to the accelerated metabolism in several weed species [3]. Therefore, it is important to understand concepts and measurement units of the main analytical techniques that use labeled molecules with ^{14}C to study the biochemical and physiological resistance mechanisms to herbicides, as well as for studies that evaluate the destination of these molecules on the environment. Understanding these mechanisms is fundamental for management alternatives to be planned or to improve the effectiveness of the product [4].

Considering the above, the objective of this chapter was to conduct a description of the research procedures and the methodology related for detection of resistant weeds using ^{14}C-herbicide absorption, translocation, and metabolism compared with susceptible weeds.

2. Restriction of herbicide movement in resistant weeds

As long as a plant biotype is susceptible to an herbicide, the biological activity resulting from the pulverization of the herbicide in the plant is dependant of the absorption and translocation of that herbicide in the plant.

Translocation is a desirable attribute because it allows the herbicide to reach both treated and untreated parts of the plant [5]. It is especially important when used for controlling plants that are able to regenerate themselves through structures such as bulbs, rhizomes, stolons, and tubers. If, for some reason, the herbicide fails to reach these structures due

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to restriction of movement, the plants are not going to be controlled and will therefore be resistant.

Weed Science Society of America (WSSA) defines herbicide resistance as the inheritable ability of a plant biotype to survive and reproduce following exposure to an herbicide dose that would normally be lethal to the wild type [2].

Resistance conferred by the restriction of herbicide movement mechanism is classified as non-target-site resistance (NTSR). Weeds that are resistant due to this mechanism commonly show higher foliar retention and reduced translocation, reducing the amount of herbicide that reaches the target, making it insufficient to exercise control over the weed.

Goggin et al. [6] employed ^{14}C-labeled 2,4-dichlorophenoxyacetic acid (2,4-D) to study resistance in two wild radish (*Raphanus raphanistrum* L.) biotypes from Australia. When comparing with a susceptible population, results showed that the resistance is due to an inability to translocate 2,4-D out of the treated leaf. Further investigation is necessary, but the authors suggest that the restriction of herbicide movement could be due to an alteration in the activity of a plasma membrane ABCB-type auxin transporter responsible for facilitating long-distance transport of 2,4-D.

Reduced translocation was reported as the cause of resistance to paraquat in two populations of *Hordeum leporinum* [7]. The inability to translocate paraquat out of the treated leaves was verified with the use of ^{14}C-labeled paraquat comparing the two resistant populations with a susceptible one, all from Australia.

Riar et al. [8] studied three barnyardgrass (*Echinochloa crus-galli*) biotypes from the United States with cross-resistance to imazamox, imazethapyr, penoxsulam, and bispyribac-sodium. The authors concluded that reduced translocation could contribute to imazamox and bispyribac-sodium resistance for two out of three biotypes.

Regarding glyphosate, the world's most important and widely used herbicide, NTSR has been reported as one of the most widespread type of resistance [9].

Glyphosate is a foliar applied herbicide which follows a source-to-sink pattern and kills plants through the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which are most highly expressed in the meristems and flowers of plants [10]. Since it is applied on the shoots, it must traverse the non-living structures of the leaf cuticle and the cell walls of the epidermis, apoplast, and mesophyll prior to accessing the phloem for transport to sink tissues [11]. Glyphosate's great ability to translocation in the plant reaching vital areas such as the roots and shoot meristems is one of the characteristics that makes it so important and efficient, but it also makes it highly dependent on herbicide movement.

Ferreira et al. [12] reported an increase in foliar retention in hairy fleabane (*Conyza bonariensis*) resistant to glyphosate. Reduced translocation was reported to be one of the mechanism conferring resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) [13] and perennial ryegrass (*Lolium perenne*) [14].

The mechanism of glyphosate absorption into plant cells is not well understood. There appears to be two different mechanisms of absorption. One is an active system that pumps

the herbicide into plant cells, possibly via a phosphate transporter, and operates at low concentrations. Other may be a passive mass flow system which is gradient dependent (**Figure 1**).

The exact mechanism that promotes the reduction of cellular absorption and translocation of glyphosate in resistant weeds is not clear yet. Shaner [10] described four potential mechanisms that may cause the restriction of glyphosate movement (**Figure 2**): (1) alteration in a putative phosphate transporter responsible for the active cellular absorption of glyphosate, in a way that the transporter is no longer present or no longer recognizes glyphosate, resulting in reduced absorption and translocation; (2) evolution of a new transporter that pumps glyphosate into the vacuole, thus sequestering the herbicide and preventing it from reaching either the chloroplast or the phloem; (3) evolution of a new transporter that actively pumps glyphosate out of the cell into the apoplast; or (4) evolution of a transporter at the chloroplast envelope that pumps glyphosate out of the chloroplast, preventing the herbicide from reaching its target site.

In order to study glyphosate resistance, ^{31}P nuclear magnetic resonance (NMR) spectroscopy studies were employed to track glyphosate movement and metabolism in resistant and susceptible biotypes of horseweed (*Conyza canadensis*), and the results showed that the rate of vacuole accumulation of this herbicide is faster and occurs to a greater extent in the resistant biotype rather than in the susceptible [15].

These results have been confirmed in different glyphosate-resistant *Lolium* spp. biotypes collected on three different continents [16], pointing to vacuolar glyphosate sequestration as the primary mechanism of resistance in these biotypes.

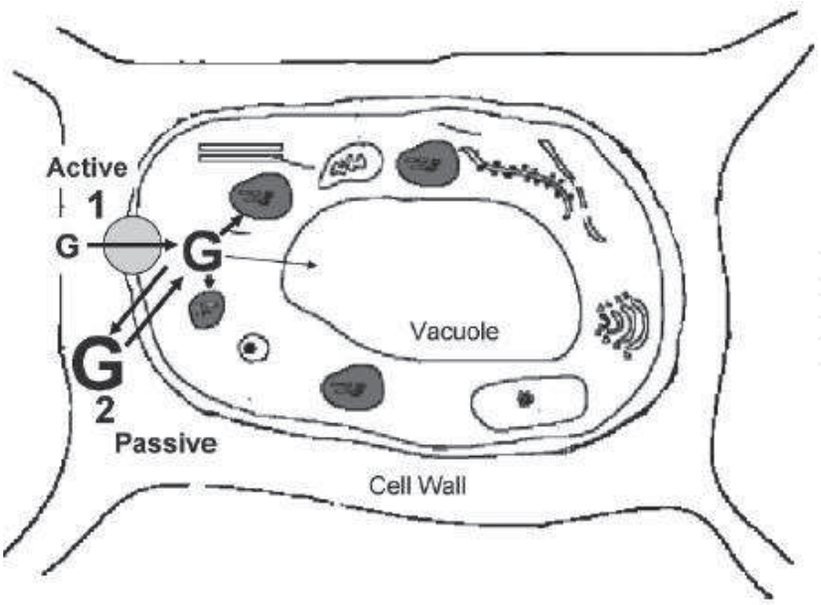


Figure 1. Proposed mechanisms of glyphosate absorption into plant cells. G, glyphosate (the size of the letter indicates relative size of glyphosate pool). (1) Active absorption of glyphosate into cell. (2) Passive diffusion of glyphosate into the cell. Arrows indicate direction of movement of glyphosate pools into and out of the cell, chloroplast, and vacuole. Source: Shaner [10].

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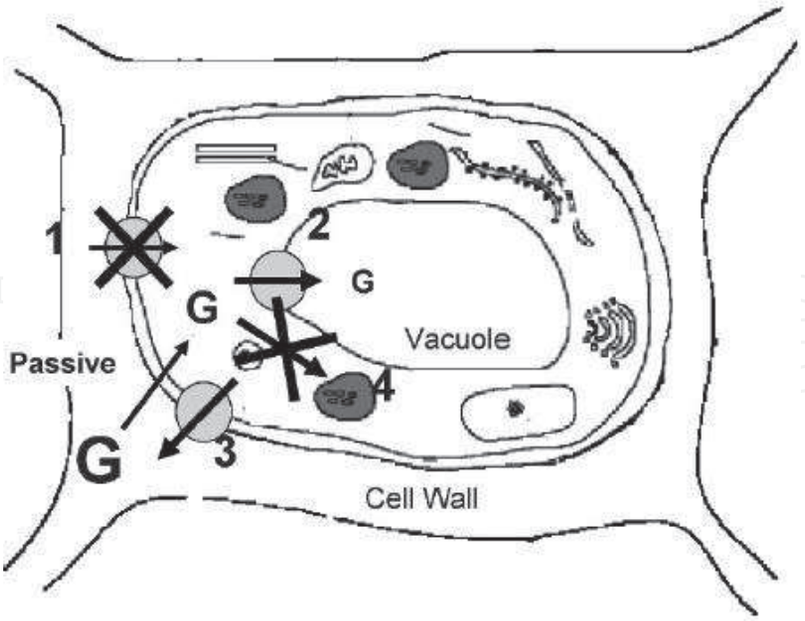


Figure 2. Potential mechanisms for reduced glyphosate cellular absorption in glyphosate-resistant (GR) biotypes. (1) Inhibition of active absorption by a modification of active transporter. (2) An active transporter that pumps glyphosate into the vacuole. (3) An active transporter that pumps glyphosate from the cell into the apoplast. (4) Inhibition of glyphosate absorption into the chloroplast by a transporter that pumps it out of the chloroplast. G, glyphosate. Source: Shaner [10].

3. Preparation of resistant weed samples by oxidizer

According IRSN [17], the ^{14}C contained in the resistant weed (test portion) is transformed to $^{14}CO_2$ from which a sample is prepared for measurement by liquid scintillation spectrometry (LSS), and combustion by oxidizer (**Figure 3**) is main method used.

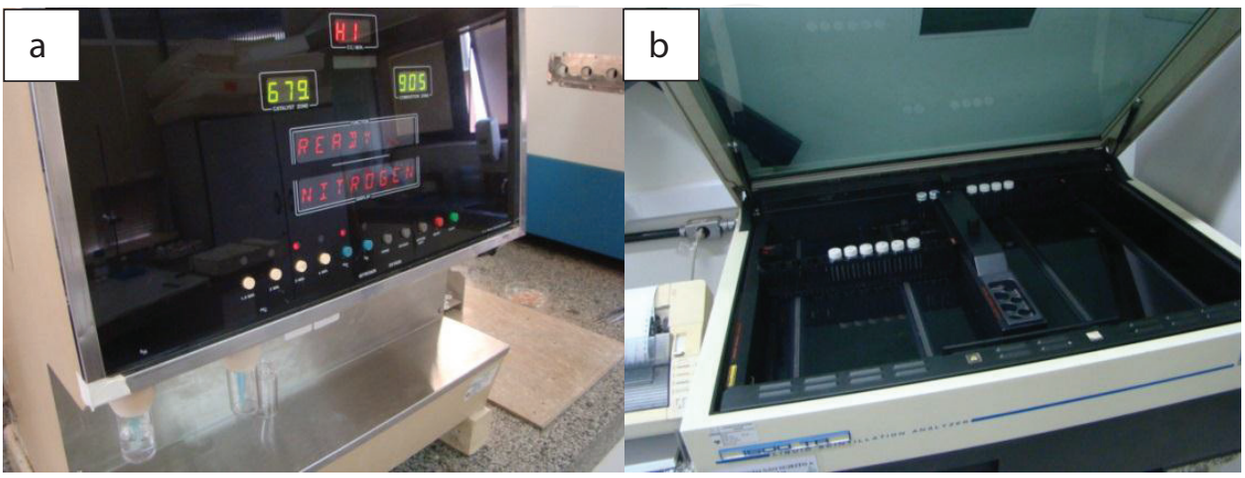


Figure 3. Oxidizer OX500 (R.J. Harvey Instrument Corporation) (a) and liquid scintillation equipment, Tri-Carb 2910 TR LSA counter (PerkinElmer) (b) from the Laboratory of Ecotoxicology of CENA/USP.

Resistant weed samples are not readily soluble on scintillation cocktails. Due to this reason, such samples go through biological combustion on oxidizer. The combustion of the sample creates an atmosphere that is rich in hydrogen, which is oxidized by the water, while the entire carbon content is oxidized by the carbon dioxide containing ^{14}C ($^{14}CO_2$). Evolved $^{14}CO_2$ is trapped in a 2 M NaOH solution and subsequently mixed in an adequate scintillating cocktail for ß counting on a LSS [18].

Coughtrey et al. [19] described a wet oxidation technique using potassium dichromate and concentrated sulfuric and phosphoric acid, which can be done in a modified filter flask. This technique can accommodate up to 0.3 g of dry resistant weed. Recovery of ^{14}C is consistent between batches, with an average recovery of 97.2% over 15 standards. These authors reported that technique described does not involve large capital expenditure and is relatively rapid.

The expression of the resistant weed sample's activity in becquerel (Bq) of ^{14}C per kg of carbon also requires measuring its elementary carbon content, generally by gas chromatography. According Nandula and Vencil [20], the commonly accepted unit of measurement of radioactivity is the Bq, derived from the International System of Units. It is defined as follows:

$$1 \text{ becquerel } (\text{Bq}) = 1 \text{ disintegration/s } (\text{dps}) = 60 \text{ disintegrations/min } (\text{dpm}) \quad (1)$$

A description of the research procedures and the methodology related for detection of resistant weeds using ^{14}C-herbicide absorption, translocation, and metabolism compared with susceptible weeds will be described below, based on Nandula and Vencil [20] and Mendes et al. [21].

4. Herbicide absorption and translocation in resistant weeds

Studies on the absorption and translocation of herbicides in plants are usually conducted to evaluate the behavior of a new herbicide on a certain plant species, comparing two or more herbicides, specific formulations, additives, or the effect of environmental standards. The growing problem regarding the resistance of weeds to herbicides promoted the studies on the absorption, translocation, and metabolism of herbicides as the methodology to elucidate the resistance mechanisms [20]. So these procedures need to be better explained to researchers, as will be described in this chapter.

The studies on the absorption of herbicides use a destructive sampling of treated plants on several post-treatment periods, which allows the characterization of the absorption standard on the plant, considering the planning and adequate statistical analyses [22].

On the adequate phenological stage for each species, susceptible and resistant weeds must be adequately identified by treatment. The leaves that have been predetermined to receive the radiolabeled herbicide must be covered with plastic film, aluminum paper, or small paper envelopes. Then, the "cold" herbicide is applied to the plants (without the radioisotope) at the dose recommended by the manufacturer, as a solution with adjuvant (when indicated) and water, followed by the immediate removal of the protective plastic film of the applied leaf.

The radiolabeled herbicide solution must be prepared on a solution containing its commercial formulation at the recommended dose for the considered phenological stage. After applying

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the "cold" product, its radiolabeled version is applied. It is important for the radiolabeled herbicide to be applied with at least 170 Bq of specific activity, in the case of studies with most of the annual weeds [20].

The radiolabeled product is applied using a micro-syringe, by applying a 1 μL droplet (the total radiolabeled product applied depends on the molecule and the radioactivity of the radiolabeled molecule), on the leaf blade of the upper part of the expanded leaf of each plant (**Figure 4**). The choice for the leaf on which the application will occur depends on the studied species. Each plant (or part of the plant) must be collected according to the pre-established times for each situation. However, it is suggested that at least six collection times are used, in addition to time zero (immediately after the application), and that the untreated plants are included as control. For each collection, the treated leaf from each plant must be rinsed with the adequate solvent. The concentration (v v^{-1}) of the solvent must be established on preliminary tests with the studied molecule. Then, the radioactivity during the rinsing must be quantified by LSS in order to determine the non-absorbed radioactivity. The leaf absorption is calculated by the difference between the applied and the non-absorbed radioactivity. The plants must be dried with an absorbing paper, pressed, and dried on an air circulation oven at 70°C for 48 h.

In the preparation of the absorption studies, we must select resistant and susceptible weeds of the same age and/or growth stage. According Nandula and Vencill [20] to plot, the figure is necessary use at least six time points in addition to a 0 time point of tissue harvest, as illustrated in **Figure 5**. However, under conditions of limited resources, it is better to increase the number of time points and reduce the number of replications ($n \geq 2$). Include non-treated weeds as a blank or control is very important for research. Then, the steps to evaluate the translocation are conducted.



Figure 4. Application of ^{14}C-glyphosate with a micro-syringe on glyphosate tolerant *Spermacoce verticillata* leaves at the Laboratory of Ecotoxicology of CENA/USP.

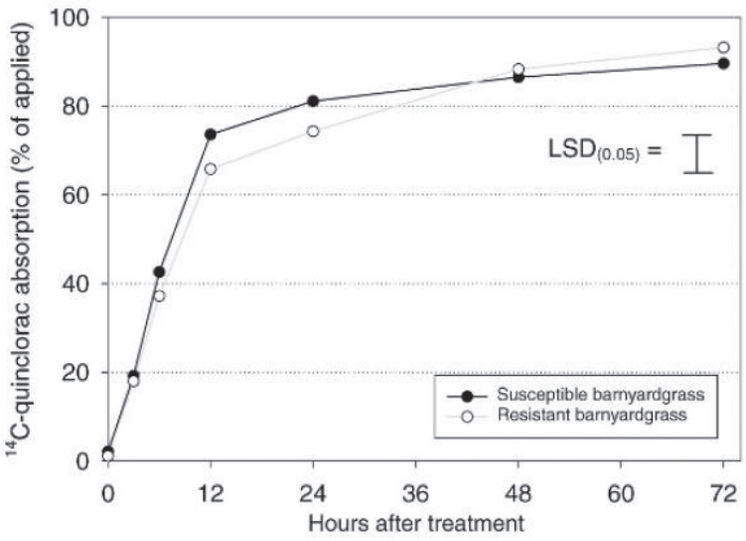


Figure 5. Absorption of ^{14}C-quinclorac by propanil- and quinclorac-resistant and susceptible barnyardgrass (*Echinochloa crus-galli*) biotypes over time. No differences were detected between biotypes at any time. LSD (0.05) bar to make comparisons between biotypes at a particular time interval. Source: Lovelace et al. [23].

Usually, the translocation studies are conducted right after the absorption studies, although they demand more work and time. Differently from the absorption, which occurs within hours after the treatment, the translocation of herbicides may take up to days after the treatment. Due to this reason, in order to evaluate the translocation, the previous knowledge must be considered in order to determine the times after the treatment in which this variable should be evaluated.

The biological combustion is the most used procedure to quantify the translocation of herbicides on plants. However, care must be taken when stating that the detection of the radioactivity on other parts of the plant, outside the treated leaf, means that the herbicide is on its parental form. It might have been converted into a non-phytotoxic metabolite. In order to state this, one must investigate the potential for the herbicide to have been metabolized by the studied weed, through the information available in the literature.

To study the movement of herbicides on weeds, the qualitative techniques involving autoradiography or phosphorus blade images have been used for over 50 years [20]. While the biological combustion offers a quantitative estimation of the herbicide on the treated weed, autoradiography (**Figure 6**), or the phosphorus blade image provides a qualitative measurement of the movement of the herbicide on the weed, in addition to the location where it occurs.

For the exposition of the treated and untreated plants, the use of phosphorus blade images is safer in comparison to the use of autoradiography, since it does not require handling chemical compounds that are harmful to the health. Despite more expensive, the technique is also quicker. A single day of exposition of a plant on a phosphorus blade resulted on images with superior quality than the exposition for 3 weeks with the X-ray film [24].

Therefore, in order to study the translocation, the plants treated as on the absorption study must be exposed on phosphorus blade for 72 h, in order to scan the image for qualitative

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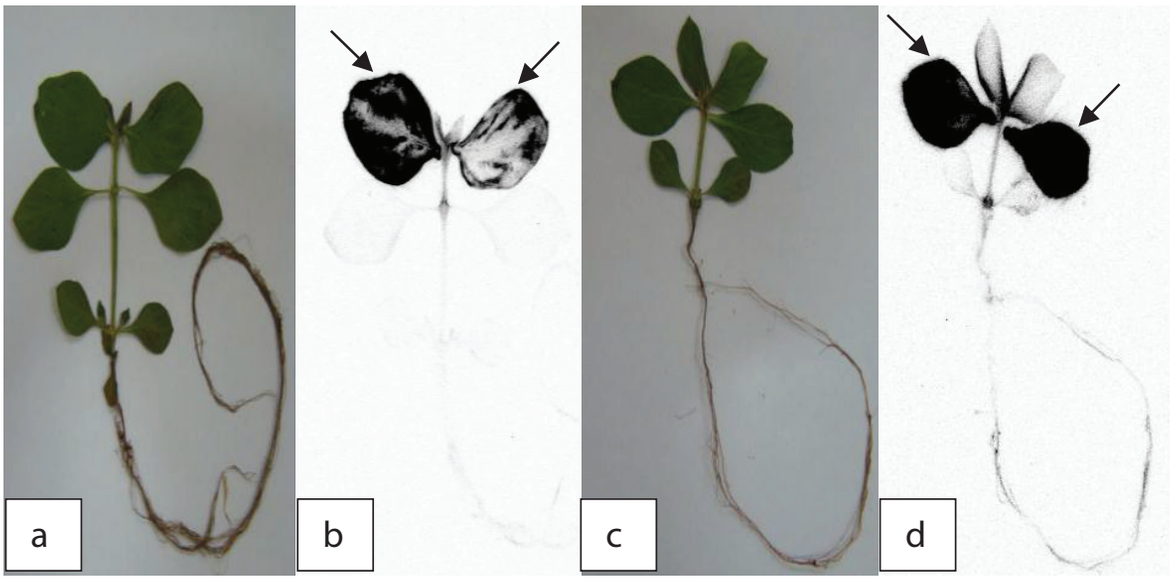


Figure 6. Autoradiography of glyphosate tolerant (a and b) and susceptible (c and d) *Richardia brasiliensis* with application of the leaf of ^{14}C-glyphosate at 48 HAT (hours after the treatment). Photograph weed to the right (a and c) and autoradiography of the weed translocation to the left (b and d) at the Laboratory of Ecotoxicology of CENA/USP. Arrows indicate the sites of application.

analysis. The usual procedure to quantify the translocation of herbicides on plants is the biological combustion, in which dry samples of each part of the plant (both the treated leaf and the part above and below it, as well as the roots) are oxidized by the presence of O_2, and the resulting CO_2 is captured on a special solvent. Then, the radioactivity must be measured on the scintillation counter.

The quantitative analysis of the translocation may also be conducted through the volume analysis, offered by the software provided together with the image scanner, as of its purchase. The volume is the total signal intensity of the radioactivity within defined limits of the image. The translocation is then expressed as the rate between the percentage of signal intensity on the applied zone, as well as above and below it, and the total signal intensity on a defined image containing ^{14}C [25].

5. Herbicide metabolism in resistant weeds

The use of radiolabeled herbicides to investigate whether the herbicide is being metabolized in the resistant weed is an efficient method, and it is the most indicated method to diagnose the resistance related to other phenomena that are not related to the change on the action site of the herbicide [26]. The analytical method aiming at studying the metabolism of herbicides in plants comprehends three fundamental steps: preparation of the plants and application of treatments; extraction and separation; and identification of the herbicide and its metabolites, if any.

The steps to conduct the study on the metabolism of herbicides in plants are described as follows. The preparation of plants and application of the treatments must be conducted as

described for the absorption and translocation study. In case the fresh samples of plants are not adequate for processing after the collection, the ideal is to store them at −20°C to assure the stability of the active substances and metabolites. The techniques employed on studies on the metabolism of herbicides in plants are thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC), depending on the herbicide molecule.

For the extraction, the adequate system of solvents for the studies herbicide must be known. The treated leaf must be rinsed with non-polar solvent (usually ethanol or methanol). Then, the plant must be dried with an absorbing paper, immediately frozen in liquid nitrogen and stored at −80°C up to its use. The plant tissue must be macerated in crucibles that must be previously cooled with N_2, and homogenized with the specific cold solvent at a concentration of 80% (v v^{-1}). A stainless steel homogenizer may also be used. The solution must be centrifuged; the supernatant decanted; and the residue must go through re-extraction with the chosen cold solvent at 80%, followed by extraction with the same cold solvent at 50% (v v^{-1}). The supernatants must be mixed, and the radioactivity must be determined by LSS, in order to know the mass balance, which is expressed as the rate between the radioactivity applied at the beginning of the experiment and the total radioactivity measured (originated from rinsing all parts of the plant). The mass balance may be also referred to as the radioactivity recovery percentage. Approximately 7 mL of the supernatant must be evaporated, resuspended in 300 mL of the solvent at 50%, and centrifuged. The final sample may be analyzed by any previously described technique, usually TLC or HPLC, with the respective solvent system [4].

6. Results of differential absorption, translocation, and metabolism of herbicides in resistant and susceptible weeds

Several researchers have studied herbicides behavior in weeds in order to find resistance mechanics through the differential of ^{14}C-herbicide absorption, translocation, and metabolism, according to **Table 1**. These results suggest that reduced translocation and accelerated metabolism of herbicide plays a major role in herbicide resistance in resistant biotypes of weed. Likewise, differences in absorption may contribute to the differential sensitivity of herbicide resistant and susceptible weed populations.

Overall, herbicide absorption is similarly compared with resistant and susceptible biotypes, but the difference in herbicide translocation is notorious in most studies reported (**Table 1**). Although differential translocation can be observed between resistant and susceptible weeds, it is unclear whether this difference is a cause of herbicide resistance or an effect of some other physiological process [23].

Herbicide metabolism studies are not always researched together with herbicide absorption and translocation studies, because the increased herbicide metabolism in resistant biotypes compared with susceptible transforms this product on metabolites without herbicidal action (**Table 1**). Among herbicides reported, glyphosate is more studied. Studies on glyphosate metabolism, expression, and sensitivity of target enzyme EPSPS synthase

Herbicide	Weed	Biotype	Absorption	Translocation	Metabolism	Reference
Clopyralid	*Centaurea solstitialis*	Resistant	=	=	↑	Valenzuela et al. [27]
		Susceptible	=	=	↓	
Quinclorac	*Echinochloa crus-galli*	Resistant	=	↓	NA	Lovelace et al. [23]
		Susceptible	=	↑	NA	
Glyphosate	*Lolium multiflorum*	Resistant	=	↓	NA	Pérez et al. [28]
		Susceptible	=	↑	NA	
Glyphosate	*Conyza canadensis*	Resistant	=	↓	=	Feng et al. [29]
		Susceptible	=	↑	=	
Glyphosate	*Conyza canadensis*	Resistant	=	↓	NA	Koger and Reddy [30]
		Susceptible	=	↑	NA	
Glyphosate	*Lolium rigidum*	Resistant	=	=	=	Feng et al. [31]
		Susceptible	=	=	=	
Glyphosate	*Lolium rigidum*	Resistant	=	↓	NA	Yeboah et al. [32]
		Susceptible	=	↑	NA	
MSMA	*Xanthium strumarium*	Resistant	=	=	NA	Keese and Camper [33]
		Susceptible	=	=	NA	
Chlorsulfuron	*Kochia scoparia*	Resistant	=	=	=	Saari et al. [34]
		Susceptible	=	=	=	
2,4-D	*Glechoma hederacea*	Tolerant	↓	=	NA	Kohler et al. [35]
		Susceptible	↑	=	NA	
2,4-D	*Raphanus raphanistrum*	Resistant	=	↓	=	Goggin et al. [6]
		Susceptible	=	↑	=	

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Herbicide	Weed	Biotype	Absorption	Translocation	Metabolism	Reference
Paraquat	*Crassocephalum crepidioides*	Resistant	=	↓	=	Ismail et al. [36]
		Susceptible	=	↑	=	
Paraquat	*Hordeum leporinum*	Resistant	=	↓	NA	Preston et al. [7]
		Susceptible	=	↑	NA	
Bispyribac sodium	*Echinochloa crus-galli*	Resistant	=	↓	NA	Riar et al. [8]
		Susceptible	=	↑	NA	
Imazamox	*Echinochloa crus-galli*	Resistant	=	↓	NA	Riar et al. [8]
		Susceptible	=	↑	NA	
Penoxsulam	*Echinochloa crus-galli*	Resistant	=	=	NA	Riar et al. [8]
		Susceptible	=	=	NA	
Propoxycarbazone Sodium	*Bromus tectorum*	Resistant	=	=	↑	Park et al. [37]
		Susceptible	=	=	↓	

(=) % absorbed of the total applied radioactivity, % translocate of the total absorbed, and amount of metabolites formed were similar in resistant and susceptible weeds. (↓) values were lower in this biotype. (↑) values were higher in this biotype. NA: non-available.

Table 1. Absorption, translocation, and metabolism of ^{14}C-herbicides in resistant and susceptible weeds.

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are necessary to elucidate the mechanism of glyphosate resistance in weed population [28]. However, Feng et al. [29] suggested that glyphosate resistance is likely due to altered cellular distribution that impaired phloem loading and plastidic import of glyphosate resulting in reduced overall translocation as well as inhibition of EPSPS. Taken together, these results suggest that metabolic deactivation is not a likely mechanism for glyphosate resistance in weeds.

7. Radiation safety orientation

The purpose of radiation safety orientation is to protect researchers, employees, students, and the general public from overexposure to radiation. In that matter, it will be necessary to comply with regulations, laws, and guidelines regarding the safe use of radioactive material, such as ^{14}C-herbicide.

It is mandatory that personal involved with the handling of radioactive material must attend to a training of radiological protection (RP), given by professionals certified by the regulatory agencies of each country.

The training should aim to achieve the clear and convincing transfer of the knowledge and recommendations on the subject. The main objective is to avoid deterministic health effects and to reduce the probability of stochastic health effects of ionizing radiation. For annual limits of exposure to ionizing radiation check the annals of the International Commission on Radiological Protection (ICRP) [26].

When handling a radiolabeled ^{14}C-herbicide, the orientation for individual protection is to wear a Personal Protective Equipment (PPE), which consists of: laboratory coat exclusive for radiolabeled material handling, disposable plastic gloves, and protective goggles.

For general protection, the use of the international symbol of radioactive material is mandatory in every room or equipment where radiolabeled material is handled or stored, and only authorized personal should be allowed.

It is mandatory to have a radiation detector (usually Geiger-Müller) that must be turned on when handling radiolabeled material and the surface where it will be handled should be covered with an impermeable plastic film in order to prevent equipment contamination.

8. Radioactive waste management

The use of ^{14}C-herbicide generates some waste that can be in the form of liquid scintillation vials, refuse, and biological waste. The volumes of the waste generated in research activities using ^{14}C-herbicide are much smaller than those generated by reactor and fuel reprocessing operations; however, it still needs to be managed if the activity is superior of a certain threshold. This threshold will depend solely on which state of matter the waste is presented.

In Brazil, the *Comissão Nacional de Energia Nuclear* (CNEN) determines that if the solid waste activity is above 1×10^4 kBq/kg (Norm CNEN-NN-8.01, 2014) [38], it must be stored on a flask specific for radioactive solid waste storage with the international radioactive symbol. If the activity is below the same value, it can be discarded as common waste.

The liquid waste generated by the utilization of ^{14}C-herbicide is usually in the form of scintillation solution, and since the organic solvent used in the scintillation solution is not only toxic but also water insoluble, all the radiolabeled scintillation solution must be considered radioactive waste.

Every radioactive waste must be identified with all the information about the radionuclide, including: activity, volume, physical and chemical properties.

9. Conclusion

Absorption, translocation, and metabolism of herbicides are dependent upon active ingredient form and sensitivity of the target weed species. There is the need of further disclosure within the scientific community connected to the study of weeds regarding the use of ^{14}C-herbicides on absorption, translocation, and metabolism studies in resistant and susceptible weed, mainly in the Brazilian conditions. In this chapter, a step-by-step methodology was suggested in order to meet this need, including the radiation safety orientation and management of resulting radioactive waste from the studies conducted in the laboratory. Techniques that use ^{14}C such as tracers are extremely useful to study the herbicides behavior in the resistant weed, since the radiometric techniques offer the possibility of accurately determining very small amounts in a relatively short time. However, mechanism of resistance to herbicides in this resistant weed population compared with the susceptible population cannot be due to differential absorption, translocation, or metabolism of herbicide in weed; so other studies are necessary to elucidate the mechanism of herbicide resistance on weed population.

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