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Determining the Selectivity of Herbicides and Assessing Their Effect on Plant Roots - A Case Study with Indaziflam and Glyphosate Herbicides

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Additional information is available at the end of the chapter

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Abstract

This chapter explores the general aspects of herbicide selectivity on plants, describing the various aspects of the topic, especially the action of herbicides on root crops and presenting a case study with the suggestion of a methodology to evaluate herbicide action on roots in perennial culture and thus determine their selectivity. This study was carried out under field conditions, over a period of four years, where the effect of indaziflam and glyphosate herbicides on roots of Coffee and Citrus plants was evaluated. The results demonstrate that the methodology used to assess the effect of herbicides on the roots was important to validate and qualify safe herbicide selectivity towards crops. Thus, this analysis should be indicated as a routine method for studies to assess the selectivity of herbicides to crops.

Keywords: Herbicide, roots, selectivity, coffee, citrus, indaziflam, glyphosate

1. Introduction

In the proto-Neolithic era, between 7500 and 5000 BCE, early in the development of agriculture by human communities, a psychic change is observed, resulting in an *increased awareness*, diagnosed by the many myths attached to these communities. One such myth is of the young Wunzh (Native American folktale, the Father of Indian Corn) entering adolescence, fighting and killing a divine being, burying it and fulfilling his order of "keeping the area free for plants," thus allowing the "friend" to be able to be reborn, now in the form of a beautiful corn plant, being able to grow, generating new seeds in the ears and food for the people [1].

Instinctively, man realized that the plants that lived together with the ones he was growing were harming them; therefore, since then he has sought ways to reduce the labor of controlling these plants. English agricultural pioneer Jethro Tull's book, *Horse Hoeing Husbandry* (1731), stands out among the first to indicate the use of horsepower for weed control.

In the 1950s, the relationship between cultivated plants with those that were vegetating together began to be analyzed by scientific experimental methods—observation of regular events that can be repeated and assessing assumptions—and it was found that in the implementation of an agricultural area by means of a cultivation system there are serious and significant changes in the geomorphic, edaphic, and biological subsystems, making them simpler (agroecosystem) compared with the ecosystem, this one being more complex. This transformation resulted in a drastic impairment of the system's self-regulation capacity, thus making it more unstable and susceptible to power inputs.

One of the main consequences of this transformation was the excessive increase of the populations of certain species of insects, microorganisms, nematodes, and plants in such a way as to significantly compromise crop production, which are therefore called agricultural pests and in the case of plants are named "weeds" [2].

2. Weeds [3]

Among the various definitions found in textbooks involving weeds, I highlight one that I think is the simplest and most direct: "plants that are born outside of the desired place," adding that they are always present in the agricultural ecosystems, are difficult to control, and damage agricultural crops [3].

For a long time, agricultural research has been showing that weed control in the most diverse agricultural ecosystems is essential for successful crop production. All the technological development of the crop in nutritional, phytotechnical, or improvement aspects may be compromised if weeds are not controlled.

The control of these plants is performed by combining various methods, such as preventative, cultural, weeding, and the chemical ones with the use of herbicides.

Herbicides are chemicals used to eliminate plants. They are applied in suitable doses directly on the vegetation for foliar absorption (postemergence treatment) or to the soil for absorption by tissues formed after seed germination before the emergence of the plant on the surface (preemergence treatment). They are generally used to control weeds that infest the various agricultural ecosystems, or any other ecological niches favorable to these organisms: vacant lots, edges of roads, railways beds, parking lots, and aquatic environments.

Herbicide use should be made in a technical and discerning way, always seeking to maximize its benefits of use and minimizing toxicological and environmental risks.

Its use is not without risks, among which the selectivity of herbicides stands out, particularly those applied continuously to perennial crops.

3. Selectivity of herbicides ([4] modified)

Herbicides have the selectivity characteristic when, in contact with plants of different species, they kill or slow the growth of some, while not affecting others. Therefore, in agronomic terms, a selective herbicide is the one that kills or slows the growth of weeds while others (in this case crops) are tolerant to the same treatment.

A herbicide is selective for a particular crop within certain limits, governed by complex interactions and covering factors such as the plant itself, the herbicide, and the weather.

4. The plant

Seven factors related to a particular plant influence the selectivity of herbicides: development stage (age), growth rate, morphology, physiology, and biophysical, biochemical, and genetic processes.

4.1. Development stage (age)

When the plant is young, it presents its meristems in a clear biological activity, and this influences the herbicide response. New plants are generally more susceptible to herbicides than the older ones; therefore, treatment with preemergence herbicides acts on seedlings which are germinated and not on the ones already established.

4.2. Growth rate

Probably, likewise, when the plant has an outstanding growth, this favors the reaction with the herbicide. In general, fast-growing plants are more sensitive to herbicides than those growing more slowly.

4.3. Morphology

The morphology of weeds is very important in determining the death of the plant for a given herbicide. It is differentiated in roots, in growth meristems and leaves.

4.4. Morphology of the roots

The roots are structures that may be alive or dead. They are the first structure emerging from a seed during germination. Its main purposes are to fix the plant in the soil and absorb water and minerals. Its whole, with the total of its branches, is called a root system [5].

The fine roots of the plants are a major means for removing soil resources, and their length and number are indicative of the nutrient absorption capacity [6]. The higher the roots of a plant, the greater their ability to exploit the soil and absorb available nutrients and water [7].

Perennial weeds have roots that are deeper than the annual ones. Likewise, dicotyledons present roots that plunge into the ground without too many branches, deeper in relation to monocotyledons, which are hair-like and more superficial. This difference in position is significant to the susceptibility of the plant's absorption processes of herbicides by the root.

4.5. Growth meristems

In Poaceae (also called Gramineae or true grasses) and Cyperaceae, the growth meristem is situated in the base or beneath the soil surface; when the herbicide is not systemic and is applied as postemergence, these meristems are protected and can sprout again. In other species, broad leaves (dicotyledons), the growth meristems are located at the epigeal part: in the apex of the growth points and in the leaf axils, directly exposed to herbicide application; if there is death of the meristems, the plant dies.

4.5.1. Leaves

Some properties of the leaves protect the crops treated with herbicides. For example, vertical leaves hinder the attachment of the spray solution, as well as the layer and the type of waxes present in the leaves when the spray droplets to reach these kinds of leaves, that tend to bounce or wet the surfaces only on small points, thereby reducing the effect of the herbicide.

The leaf form also interferes with the selectivity of herbicides. Broadleaf trees usually have broadleaf and smooth surfaces, horizontally extending from the stem. Therefore, they easily intercept the spray droplets and these are less likely to bounce off the leaf.

Thus, when broadleaf weeds are sprayed with contact herbicides, the spray solution tends to spread as a thin film and the droplets are scattered to wet a large portion of the sheet, thus facilitating the absorption of the herbicide, unlike when the same herbicides are applied on cereals (Poaceae) or on crops of onions, when there is the rebound of drops, avoiding the absorption and effect of the herbicide, and preserving the plant.

4.6. Physiology

The plant physiology determines the mode and amount of herbicide which enter the plant (absorption) and its movement (translocation).

4.6.1. Absorption

Plants that have favorable cuticles and many large stomata favor the entry of the herbicides into the plant.

4.6.2. Translocation

After the entry of the herbicide into the plant, it may move within the plant (translocation). This movement may be downward by Liberian vessels (phloem) or ascending by timber vessels (xylem).

The movement in the conducting vessels of the plants by the herbicides is usually in one direction or another and governed by their chemical affinity: lipophilic, movement via phloem or hydrophilic, movement via the xylem, but in some cases, such as 2,4-D (2,4-Dichlorophenoxyacetic acid, weak acid), they can move in both directions.

4.7. Biochemical processes

Biochemical processes are responsible for the protection or activation of herbicides in plants.

4.7.1. Enzymatic inactivation

The biochemical reactions involve enzymes and are responsible for the activation or inactivation of the herbicides.

In this process, selectivity is expressed in the differential inactivation of the activated herbicides decreasing the enzymatic activity in a particular plant species, and another one that does not interfere with the metabolic processes of the plant, for example, in photosynthesis. This can kill certain plants and leave the others unharmed.

Biochemical reactions can inactivate the herbicides, and thus be selective, preserving the crop and killing the weeds. For example, compound 2,4-D is biochemically metabolized in plants of the Poaceae family in 2,4-DB (or 4-(2,4-dichlorophenoxy)butyric acid) and does not affect these plants.

4.8. Genetic heritage

Genetic characteristics intrinsic of plants define their morphological, physiological, and biochemical characteristics, and these, being influenced by weather conditions, determine the intensity of herbicide effect response.

Currently, science uses biotechnology tools that modify the genetic heritage of plants, obtaining genetically modified organisms (GMOs), significantly changing their physiology and turning cultivated plants, once sensitive, intolerant to a certain herbicide.

5. Herbicides

5.1. Molecular configuration

Size, shape, and chemical characteristics (molecular, acid, base) influence herbicide entry and consequently herbicide effect on the plant. Brigs [8, 9] has demonstrated that lipophilicity is related to the herbicide ability to systematically move by conducting vessels of the plant when they present $\text{Log } K_{ow}^1$ with values close to 2 that show this characteristic.

¹ Kow: octanol–water partition coefficient, measures chemical affinity of herbicides: hydrophilic (<0) and lipophilic (>0).

5.2. Toxicity

The herbicide can have two types of effect or toxicity: acute or chronic. Sharp toxicity is characterized as intense and usually quickly kills the plant; contact herbicides have this feature and this type of toxicity.

Chronic toxicity is characterized by the effect of the herbicide along time and generally has a slow action. They are herbicides that are usually applied to the soil as preemergence, have biological persistence with effect on plants, and in the case of weeds can take up to 10 weeks to eradicate them.

5.3. Concentration and formulation of herbicides

The concentration and the commercial formulation are important factors characterizing the selectivity of herbicides on plants.

The concentration of the herbicide determines whether or not it is selective for a given crop. For example, 2,4-D in low concentrations induces the increase of cell transpiration and cell division of plants, but in larger doses it reduces this process and can kill cells.

Formulation is the vehicle that, along with water, forms the spray solution application and leads the herbicide to contact the soil and plants and is preponderant to determine the selectivity of herbicides in relation to a particular species, depending on various factors: granulometry of the formulations, surfactants, adjuvants and other additives which stabilize the formulations.

5.4. Root system

Plant roots are used for fixation in the soil and uptake of water and ions. They are also the site of synthesis of various compounds, for example, hormones and substances of allelopathic effects, and are also used as storage organs.

Morphologically, we highlight the meristems of the roots of higher plants, which at their apex are wrapped by a hoodlike structure (calyptra) whose cells produce mucilage to protect the meristematic cells from mechanical damage, and the cell elongation zone, which turn into differentiation areas and of root hairs. Theoretically, plants as a whole are capable of absorbing water, but the leaves and the stem are covered by the cuticle, which, depending on its thickness, prevents significant water absorption compared with the roots, for these have no cuticle and have absorbent hairs that increase much of the total area for absorption of water and may reach very high values; for example, a single rye plant may have roots with an absorbing area of 400 m².

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Soil is composed of several fractions: sand, silt, clay, and organic matter, plus air and water. Clay and organic matter are constituents of the colloidal fraction of the soil, having charges that adsorb on the surface: ion attraction, anion–cation, molecules, hydrogen bonds, or van der Waals forces (or interactions). Herbicides are examples of molecules adsorbed by the soil.

On condition with higher soil moisture, there is competition for the adsorption site in the colloids by water, leading to the release of herbicide molecules, moving them to the soil solution and making them available for absorption by the roots.

5.5. Assessment of selectivity of herbicides on the roots

Soil is an opaque and solid environment of high density that creates resistance to root growth and thus makes it difficult to observe and assess root development "in loco" [10]. This shows that to analyze the performance of herbicides on the roots and assess their selectivity, the researcher is constantly working "in the dark" [11].

In fact, roots grow naturally in the soil porous volume, distributing in this volume according to non-uniform directions (anisotropy), dictated by the tropisms of each type of roots (e.g. ortho-geotropism in primary axes), and by the endogenous branching patterns [11].

Therefore, the root systems are complex branched structures that vary in space and time [11], and this has direct implications on the methodological aspects of the studies. Moreover, soil has its own patterns of spatial variability, expressed as different gradients of physical and chemical properties, which, superimposed to the endogenous variability of the roots, strengthen the anisotropy of the root system [11].

Generally, the assessment of the selectivity of the herbicides on the roots is done indirectly by assessing the epigeal parts of the plant, assuming that the mass of the roots is similar. For Hooker et al. [12], this comparison is not valid because significant variations may occur, both spatial and temporal, between the epigeal and hypogeal (roots) parts.

Thus, assays to qualify the selectivity of herbicides, when the object of study are the roots, are complex, all have advantages and limitations [11].

5.6. Methods of herbicide effect assessment

This method is based on separation by washing and/or sieving of the soil to distinguish the roots; depending on the plant stage of development, the methodology is different. For example, assessing the selectivity of herbicides acting on seedlings can be a direct and comprehensive approach to the root system, carefully removing this one to assess the mass, length, and visual harm. However, for annual crops established for many years in the full production stage, the assessment of the root system has to be carried out by an appropriate sampling of the roots,

and thus estimate the depth of rooting, biomass, and complex root demographics measurements in cycling studies of roots.

The measurement of these growth and development parameters of the root system is very difficult due to the difficulty in obtaining reliable data to assess the null hypothesis (H^0) among treatments. In general, the test tends to present a type II error,² mainly if, to carry out the assessments of the root system, the plant is removed by means of direct uprooting of the trees or shrubs. In this case, they are exposed only to assess the primary roots, for the remaining, secondary and mostly root hairs, remain attached to the ground and are not considered in the assessment.

In this case, an adequate sampling of the roots is advisable to assess the root system [11, 12, 13, 14].

In this regard, appropriate methodologies for adequate root system assessments are studied by means of roots sampling methods and strategies under field conditions by direct and indirect sampling, by means of destructive techniques and extraction of soil root.

Among these techniques are the use of cylindrical auger (volumetric ring), digging the roots system, and opening trenches.

The major problem in using this method, including selectivity studies, is the minimum number of samples. This should be determined by the statistical criteria used, thus making it possible to detect statistical effects by the analysis of variance of the data that must be representative. For each test, a prior sampling and analysis of the data is suggested, using the coefficient of variation $\leq 30\%$ as an indication of the minimum number of samples sufficient to obtain reliable results.

Another factor which complicates the use of the destructive method is the necessary time and labor. For example, in soil removal of a volume of 1.0 m^3 , with the soil with a real density of 1.2 g.cm^3 , 1.2 tons of soil is removed.

Another way is to use trenches with a lattice framework on the soil profile wall for roots direct counting, assessing their thickness and thus estimating the root system of the plants.

Another way of assessing the roots is by using images: rhizotron, a system that uses glass to observe the roots; with the placement of this one in the subsoil, to observe the roots *in vivo*.

Another way of assessing the roots is by using images: rhizotron, a system that uses glass to observe the roots, with the placement of this one in the subsoil, to observe the roots *in vivo*, not interfering in the shoot, which is exposed to ambient conditions.

Due to the lack of resistance on the glass surface, the roots have a tendency to grow on its surface. Thus, this method is more suitable for phenological studies of the roots and not for analyzing the selectivity of the herbicides on them [15].

Besides the assessment of roots being done in two ways—directly in the soil profile or by means of washed roots—there are also methods that analyze digital images, a progress in the

² This occurs when one accepts the H_0 null hypothesis as true when this one is false, due to a β probability.

techniques for studying the root system. In practice, in the herbicide selectivity studies, when the roots are assessed, this technique is interesting to check its effect on seedlings in trials conducted in a greenhouse or phytotrons, where the root system can be entirely removed in orchards or coffee plantations with large and deep root systems. It is necessary to build trenches to obtain many images and repetitions, thus obtaining data on which the statistics by the F (5%) test has the power to find significant differences.

Thus, this chapter describes an original research, in order to contribute to the research aimed to assess the selectivity of herbicides on perennial crops, having as a parameter the assessment of the effect of these on the root system.

6. Case study

Determining the selectivity of indaziflam and glyphosate herbicides, assessing their effect on the roots when applied for four years in a row in Coffee and Citrus crops [16, 17, 18].

Herbicides assessed:

Indaziflam: ($C^{16}H^{20}FN^5$), name IUPAC: N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1fluoroethyl]-1,3,5-triazine-2,4-diamine, belongs to the group of alkylazinas and has the following chemical characteristics: vapor pressure (25°) 5.1×10^{-10} mm Hg, $\text{Log } K^{ow} (pH^7) = 2.8$; solubility ($pH^{6.8}$) and 2.8 mg.L^{-1} and dissociation constant (pK_a) = 3.5. Its mode of action is the inhibition of cellulose biosynthesis and is suitable for preemergence applications for a broad spectrum of weed control, monocotyledons and dicotyledons by means of the the cell wall biosynthesis inhibition, acting on the growth of the meristematic cells and affecting the germination of sensitive weed seeds.

Glyphosate: ($C^{16}H^{20}FN^5$), name IUPAC: N- (phosphonomethyl) glycine, belongs to the group of substituted Glycine and has the following chemical characteristics: vapor pressure (45°) 2.45×10^{-8} mm Hg, $\text{Log } K^{ow} = -3.22$ to -2.76 ; solubility (pH^7) $15,700 \text{ mg.L}^{-1}$ and the dissociation constant (pK_a) = 2.6; 5.6; 10.3. Its mode of action is the inhibition of the enzyme enol-pyruvyl-shikimate-phosphate synthase (EPSPS), applied as postemergence for controlling a broad spectrum of weeds, monocotyledons and dicotyledons by inhibiting the biosynthesis of amino acids, such as phenylalanine, tyrosine and tryptophan.

6.1. Treatments

In crops with more than five years in the full production stage, tests were installed in the crops of Coffee cv. Catuaí Vermelho and Citrus cv. Valência (Table 1). The study commenced in December 2008.

The tests were performed on soil of medium texture with 28 g/dm^3 organic matter.

	Treatments	Dose		
		Grams – a.i. ha ⁻¹ (1)	Liters – pc ha ⁻¹ (2)	n (3)
1	Weeded control	--	--	--
2	Indaziflam	75	0.15	1
3	Indaziflam	100	0.20	1
4	Indaziflam	150	0.30	1
5	Glyphosate	960	2.00	3

1. AI: active ingredient
2. CP: commercial product
3. Number of annual applications

Table 1. Treatments applied to the crops of Coffee and Citrus

6.2. Periods of application

Herbicide applications were done in the following times: indaziflam, only one time at the beginning of the rainy season (spring); glyphosate in three seasons: spring, summer, and fall.

6.3. Unit and experimental design

In the Coffee crop, the plots consisted of two rows, containing in total 16 plants, measuring 8 m x 7 m and in the Citrus test, 4 x 16 m covering four plants. Experimental design, randomized block design with four replications.

Each year and during the next four years, the same treatments were repeated on the same plots, and these treatments and their frequencies are described in Table 1.

6.4. Assessments of the roots

In the winter of the following year, after the first treatment application with indaziflam and the series of three glyphosate applications, before the new series of applications, the effect of the treatments on the root system of the Citrus and Coffee crops was assessed in each plot.

For opening the trenches, the methodology cited in Ref. [19] was used, in a modified manner. For each plot, the excavation of the trenches was in the longitudinal direction of the crop planting row, close to the root collar of the plant (10–15 cm), measuring 1.20 x 2.00 x 1.20 m (width, length, and depth).

After this procedure, the profile surface was adequately prepared following the methodology of the lattice framework for the assessment of the roots: cutting the exposed roots in the profile, profile scarification for new exposure of roots, profile painting with white paint, and washing with water for a highlighted exposure of the painted white roots, where a framework was placed for the assessments, number, and percentage of roots (Photos 1–5).

Photo captions by Flavio Martins Garcia Blanco.



Figure 1. Backhoe for construction of the trenches.



Figure 2. Exposure of roots.



Figure 3. Fixation of the picture and profile painting.



Figure 4. Washing profile, soil removal highlighting the colorful roots of white.

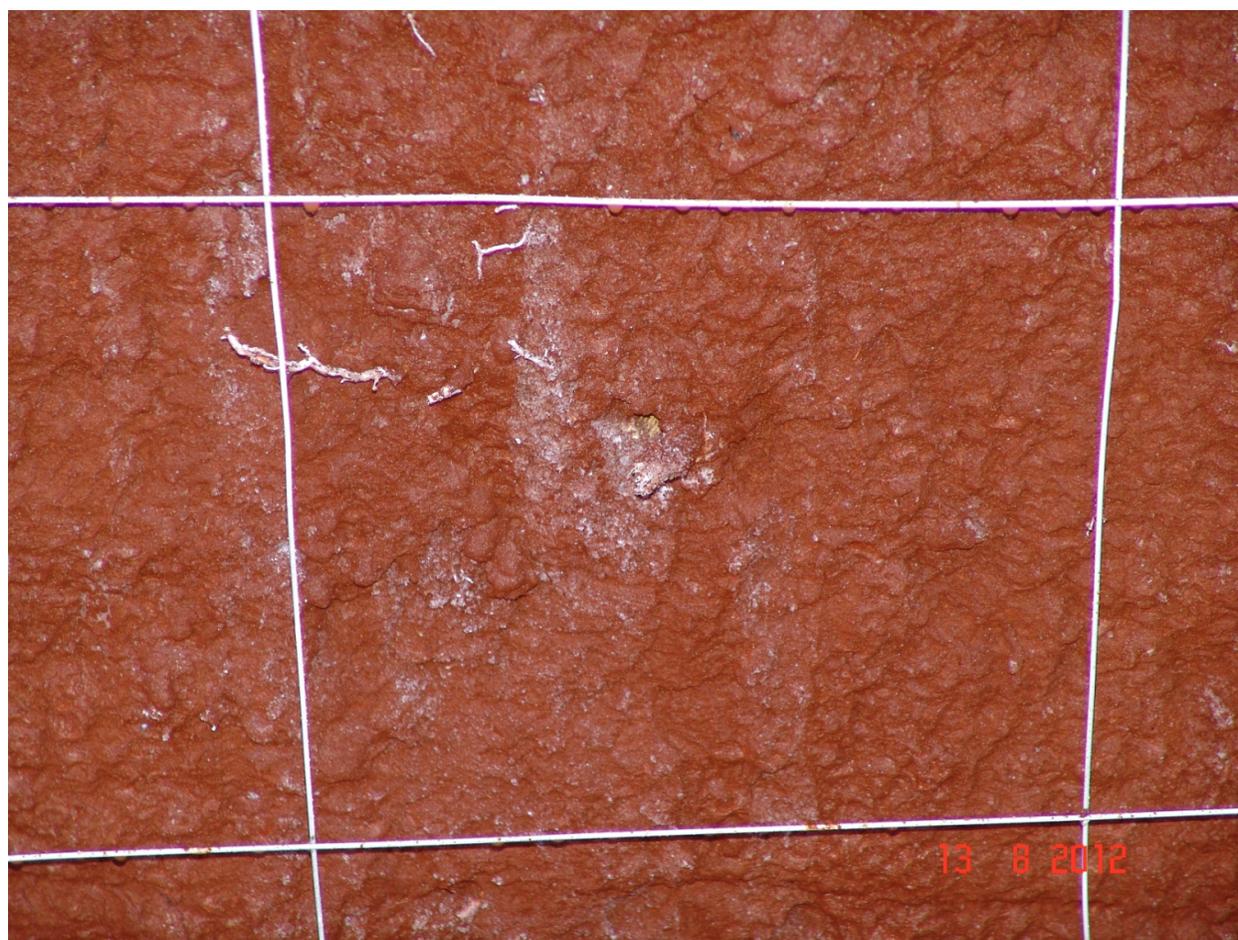


Figure 5. Roots highlighted facilitating reviews.

The wood frame 1 x 1 m (1 m²) had 16 subdivisions, grids of 0.25 x 0.25 m (0.0625 m²), and it was fixed in the soil profile after a preparation which outlined the roots by the white coloring. Each grid was assessed by counting the number of roots and visually estimating the percentage of area occupied by these grids.

Data were assessed at three levels: (1) spanning all the profile (1 m²), (2) upper position of the profile (0–50 cm), and (3) lower position (50–100 cm), each of 0.5 m². It was thus possible to determine whether there was any phytotoxic action of herbicides in the development of the roots due to the root position in relation to the soil profile.

6.5. Statistical assessment

Data were subjected to analysis of variance, indicating the coefficient of variation. When the analysis of variance was significant at 5% probability, the *t*-test (5%) means were performed, individually comparing the null hypothesis between the means of the treatments with herbicides and the means of control weeded treatment.

For the standardization of data, these were transformed in $\sqrt{x+1}$ and arcsine $\sqrt{x/100}$ for the count and percentage of the roots, respectively.

7. Results

The results are shown in tables, separated into crops.

7.1. Citrus crop

Tables 2–9 describe the analyses of the annual assessments in the Citrus cv. Valência crops. It can be seen that the tables were separated by characteristics of counting the number of roots, Tables 2, 4, 6, and 8, and their respective cover was estimated and expressed in cover percentage, Tables 3, 5, 7, and 9, showing the assessment in the 0–100-cm overall shape profile and the two subdivisions, 0–50 cm and 50–100 cm.

	Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer		
			0–100 cm 1 m ²	0–50 cm 0.5 m ²	50–100 cm 0.5 m ²
1	Weeded control	--	16.42	11.10	12.14
2	Indaziflam	75	15.79	12.19	10.07
3	Indaziflam	100	16.55	12.58	10.73
4	Indaziflam	150	15.39	10.07	11.61
5	Glyphosate	3 x 960	15.84	10.69	11.57
	<i>F</i>		0.17 ns ⁽¹⁾	1.71 ns	0.57 ns
	CV %		14.4	14.0	19.3

1. Non-significant.

Table 2. Number of roots due to the treatments: first year. Data were transformed into $\sqrt{x + 1}$. Average of four replications

	Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile		
			0–100 cm 1 m ²	0–50 cm 0.5 m ²	50–100 cm 0.5 m ²
1	Weeded control	--	0.18	0.21	0.15
2	Indaziflam	75	0.18	0.21	0.14
3	Indaziflam	100	0.19	0.24	0.13
4	Indaziflam	150	0.17	0.19	0.14
5	Glyphosate	3 x 960	0.16	0.18	0.13
	<i>F</i>		0.82 ns ⁽²⁾	0.89 ns	0.56 ns
	CV %		17.4	24.5	19.1

1. Non-significant.

Table 3. Percentage of roots cover due to the treatments: first year. Data transformed in $\arcsin \sqrt{x / 100}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer			
		0–100 cm 1 m ²	0–50 cm 0.5 m ²	50–100 cm 0.5 m ²	
1	Weeded control	--	10.15	7.32	6.98
2	Indaziflam	75	9.29	7.07	5.90
3	Indaziflam	100	9.97	7.44	6.70
4	Indaziflam	150	9.54	6.96	6.57
5	Glyphosate	3 x 960	9.19	7.02	5.98
	<i>F</i>		0.36 ns ⁽¹⁾	0.12 ns	0.47 ns
	CV %		9.6	7.1	21.1

1. Non-significant.

Table 4. Number of roots due to the treatments: second year. Data were transformed into $\sqrt{x + 1}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile			
		0–100 cm 1 m ²	0–50 cm 0.5 m ²	50–100 cm 0.5 m ²	
1	Weeded control	--	0.20	0.25	0.14
2	Indaziflam	75	0.19	0.24	0.12
3	Indaziflam	100	0.18	0.23	0.12
4	Indaziflam	150	0.20	0.25	0.12
5	Glyphosate	3 x 960	0.18	0.23	0.12
	<i>F</i>		0.49 ns ⁽²⁾	0.74 ns	0.73 ns
	CV %		14.5	16.6	20.8

1. Non-significant.

Table 5. Root cover percentage: second year. Data transformed in $\arcsin \sqrt{x / 100}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer			
		0–100 cm 1 m ²	0–50 cm 0.5 m ²	50–100 cm 0.5 m ²	
1	Weeded control	--	14.32	12.04	12.04
2	Indaziflam	75	12.96	10.22	10.22
3	Indaziflam	100	11.53	9.26	9.26
4	Indaziflam	150	13.68	10.45	10.45
5	Glyphosate	3 x 960	11.80	9.87	9.87
	<i>F</i>		2.49 ns ⁽¹⁾	3.05 ns	1.05 ns
	CV %		11.7	11.9	27.7

1. Non-significant.

Table 6. Number of roots due to the treatments: third year. Data were transformed into $\sqrt{x + 1}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the Profile			
		0–100 cm	0–50 cm	50–100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	--	0.19	0.24	0.12
2	Indaziflam	75	0.20	0.24	0.13
3	Indaziflam	100	0.18	0.22	0.12
4	Indaziflam	150	0.19	0.22	0.14
5	Glyphosate	3 x 960	0.18	0.22	0.12
	<i>F</i>		0.40 ns ⁽²⁾	0.28 ns	0.55 ns
	CV %		13.6	16.3	18.6

1. Non-significant.

Table 7. Root cover percentage: third year. Data transformed in $\sqrt{x/100}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer			
		0–100 cm	0–50 cm	50–100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	--	11.23	8.97	6.57
2	Indaziflam	75	11.56	8.82	7.49
3	Indaziflam	100	11.97	9.34	7.50
4	Indaziflam	150	12.23	8.46	8.82
5	Glyphosate	3 x 960	12.37	8.43	9.10
	<i>F</i>		2.49 ns ⁽¹⁾	3.05 ns	1.05 ns
	CV %		11.7	11.9	27.7

1. Non-significant.

Table 8. Number of roots due to the treatments: fourth year. Data were transformed into $\sqrt{x+1}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile			
		0-100 cm	0-50 cm	50-100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	--	0.23	0.28	0.15
2	Indaziflam	75	0.22	0.28	0.14
3	Indaziflam	100	0.22	0.27	0.15
4	Indaziflam	150	0.23	0.28	0.15
5	Glyphosate	3 x 960	0.24	0.31	0.15
	<i>F</i>		0.78 ns ⁽²⁾	0.74 ns	0.22 ns
	CV %		8.7	11.0	17.3

1. Non-significant.

Table 9. Root cover percentage: fourth year. Data transformed in $\sqrt{x/100}$. Average of four replications

In all samples, analyses of variance of the treatments were performed, calculating the value of *F*, indicating its significance and its coefficient of variation.

Covering all depth ranges, the assessments of the parameters, score, and percentage of root cover, the values of the coefficient of variation had a range of 9.6% to 27.7% and 8.7% to 24.6%, respectively. These values are compatible for testing using this methodology, collaborating with the indication that technically the conduct of the tests was adequate. It is also observed that for the probability level (5%), used in the methodology to determine the significance of the analyses of variance, the value of *F* was always non-significant (ns).

This demonstrates that the methodology was not able to find significant differences among treatments by the assessed parameters, thus indicating that the treatments with the herbicides did not affect root development, therefore characterizing them as selective for growing Citrus cv. Valência.

The same format for reporting the results in the assessments in the coffee crop are described below.

7.2. Coffee crop

Following the same form of presentation of the previous crop, Tables 10–17 describe the analyses of the annual assessments on the crop Coffee cv. Catuaí Vermelho. The tables were also separated according to the parameters assessed, score of the number of roots, Tables 10, 12, 14, and 16, and their percentage of cover, Tables 11, 13, 15, and 17, all showing the assessment of the overall shape profile and in two subdivisions 0–50 cm and 50–100 cm. For each year, analyses of variance of the treatments were performed, calculating the value of *F*, indicating its significance, and also the coefficient of variation for each analysis.

	Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer		
			0–100 cm 1 m ²	0–50 cm 0.5 m ²	50–100 cm 0.5 m ²
1	Weeded control	--	14.18	11.51	8.33
2	Indaziflam	75	13.63	11.45	7.42
3	Indaziflam	100	14.47	11.82	8.36
4	Indaziflam	150	12.65	9.66	8.16
5	Glyphosate	3 x 960	12.04	9.42 ^{*(2)}	7.52
	<i>F</i>		1.46 ns ⁽¹⁾	3.04 ^{*(3)}	0.33 ns
	CV %		12.6	10.7	19.5

1. Non-significant.
2. Significant in relation to the weeded control by the $t_{(5\%)}$ test of means.
3. Significant by the analysis of variance, $F_{(5\%)}$ test.

Table 10. Number of roots due to the treatments: first year. Data were transformed into $\sqrt{x + 1}$. Average of four replications

	Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile		
			0–100 cm	0–50 cm	50–100 cm
			1 m ²	0.5 m ²	0.5 m ²
1	Weeded control	--	0.14	0.16	0.12
2	Indaziflam	75	0.12	0.14	0.08
3	Indaziflam	100	0.15	0.18	0.11
4	Indaziflam	150	0.11	0.12	0.10
5	Glyphosate	3 x 960	0.12	0.14	0.10
	<i>F</i>		1.70 ns ⁽¹⁾	1.70 ns	3.00 ns
	CV %		20.29	25.2	19.16

1. Non-significant.

Table 11. Percentage of roots cover due to the treatments: first year. Data transformed in $\arcsin \sqrt{x/100}$. Average of four replications

	Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer		
			0–100 cm	0–50 cm	50–100 cm
			1 m ²	0.5 m ²	0.5 m ²
1	Weeded control		10.05	8.87	4.76
2	Indaziflam	75	11.26 ⁽²⁾	9.61	5.86
3	Indaziflam	100	11.74 [*]	10.14 [*]	5.96
4	Indaziflam	150	10.67	9.33	5.26
5	Glyphosate	3 x 960	10.05	8.23	5.68
	<i>F</i>		3.77 ⁽³⁾	6.09 ⁽³⁾	0.74 ns ⁽¹⁾
	CV %		7.7	16.1	20.8

1. Non-significant.

2. Significant in relation to the weeded control by the $t_{(5\%)}$ test of means.

3. Significant by the analysis of variance, $F_{(5\%)}$ test.

Table 12. Number of roots due to the treatments: second year. Data were transformed into $\sqrt{x+1}$. Average of four replications

	Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile		
			0–100 cm	0–50 cm	50–100 cm
			1 m ²	0.5 m ²	0.5 m ²
1	Weeded control		0.22	0.30	0.08
2	Indaziflam	75	0.19	0.25	0.09
3	Indaziflam	100	0.21	0.28	0.11
4	Indaziflam	150	0.20	0.27	0.10
5	Glyphosate	3 x 960	0.18 ⁽²⁾	0.24	0.09
	<i>F</i>		2.94 ⁽³⁾	2.39 ns ⁽¹⁾	1.59 ns
	CV %		14.5	12.3	21.2

1. Non-significant.

2. Significant in relation to the weeded control by the $t_{(5\%)}$ test of means.

3. Significant by the analysis of variance, $F_{(5\%)}$ test.

Table 13. Root cover percentage: second year. Data transformed in $\arcsin \sqrt{x/100}$ Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer			
		0–100 cm	0–50 cm	50–100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	15.48	13.02	8.39	
2	Indaziflam	75	16.35	13.49	9.16
3	Indaziflam	100	16.57	13.97	8.91
4	Indaziflam	150	16.32	13.59	9.00
5	Glyphosate	3 x 960	13.61	11.69	6.98
	<i>F</i>	1.51 ns ⁽¹⁾	1.02 ns	1.58 ns	
	CV %	15.6	13.3	16.1	

1. Non-significant.

Table 14. Number of roots due to the treatments: third year. Data were transformed into $\sqrt{x + 1}$. Average of four replications

Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile			
		0–100 cm	0–50 cm	50–100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	0.15	0.19	0.11	
2	Indaziflam	75	0.17	0.21	0.12
3	Indaziflam	100	0.21 ^{*(2)}	0.26 ^{*(2)}	0.13
4	Indaziflam	150	0.19	0.23 ^{*(2)}	0.13
5	Glyphosate	3 x 960	0.13	0.16 ^{*(2)}	0.10
	<i>F</i>	12.56 ^{*(3)}	10.50 ^{*(3)}	4.54 ^{*(3)}	
	CV %	9.3	11.9	5.5	

1. Non-significant.

2. Significant in relation to the weeded control by the $t_{(5\%)}$ test of means.

3. Significant by the analysis of variance, $F_{(5\%)}$ test.

Table 15. Root cover percentage: third year. Data transformed in $\arcsin \sqrt{x / 100}$ Average of four replications.

Treatments	g a.i. ha ⁻¹	Parameters: number of roots in the profile layer			
		0–100 cm	0–50 cm	50–100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	16.58	14.54	8.03	
2	Indaziflam	75	16.10	13.73	8.27
3	Indaziflam	100	15.66	13.74	7.49
4	Indaziflam	150	16.01	14.05	7.72
5	Glyphosate	3 x 960	14.36	12.42	7.17
	<i>F</i>	0.92 ns ⁽¹⁾	1.23 ns	0.23 ns	
	CV %	12.6	10.3	23.1	

1. Non-significant.

Table 16. Number of roots due to the treatments: fourth year. Data were transformed into $\sqrt{x + 1}$ Averages of four replicates.

Treatments	g a.i. ha ⁻¹	Parameters: percentage of cover in the profile			
		0–100 cm	0–50 cm	50–100 cm	
		1 m ²	0.5 m ²	0.5 m ²	
1	Weeded control	0.19	0.24	0.13	
2	Indaziflam	75	0.19	0.23	0.15
3	Indaziflam	100	0.20	0.26	0.13
4	Indaziflam	150	0.19	0.23	0.13
5	Glyphosate	3 x 960	0.16	0.19	0.11
	<i>F</i>	2.10 ns ⁽²⁾	2.01 ns	1.90 ns	
	CV %	12.8	14.3	16.0	

1. Non-significant.

Table 17. Root cover percentage: fourth year. Data transformed in $\arcsin \sqrt{x/100}$. Average of four replications.

Regarding the coefficient of variation, in all the depth ranges the assessments of the parameters, scores, and percentage of cover had a range of 7.7% to 23.1% and 5.5% to 25.2%, respectively. These ranges were compatible for testing using this methodology, indicating that these were technically well conducted.

Analyzing the tables, it is observed that the probability level (5%) used as a threshold to determine the significance of the analysis of variance, the *F* value, was significant in some cases, thus demonstrating that the methodology was able to find differences.

This occurred in the statistical analyses indicated in the tables and their parameters: Table 10: first year, number of roots; Tables 12 and 13: second year, number of roots and percentage of cover; and Table 15: third year, percentage of cover.

In these tables, testing of the *t* (5%) means comparing the weeded control treatments with the treatments using herbicides (null hypothesis) was performed. It was observed in Table 16 in the assessment of the parameter percentage of roots cover in the soil profile in the 50–100-cm layer that the null hypothesis was accepted in all comparisons between each treatment with herbicides against the weeded control. Therefore, even if the *F* value is significant, because of the research objective, that is, to assess the effect of herbicides compared to the weeded control, the null hypothesis was accepted in all comparisons.

This situation did not occur in the other analyses where *F* was significant since the comparisons among treatments with herbicides and weeded control were significant by the rejection of the null hypothesis.

The treatment with herbicide glyphosate was the one with the largest number of rejected null hypotheses when compared with the weeded control treatment. In the first year, the number of roots in the upper layer was significantly lower (Table 10). This also occurred for the percentage of root cover that was lower in the second year throughout the soil profile assessed 0–100 cm (Table 13) and in the third year in the most superficial layer of the profile, 0–50 cm (Table 15).

This can be explained by two factors: first, possible drift that hit the lower leaves of the coffee crop. Due to the downward movement of glyphosate by means of phloem vessels, it was transported to the roots, damaging them. The second factor is the exudation of this herbicide by the roots of weeds that received its application. Therefore, the coffee roots could absorb the herbicide expressing harm [18, 19, 20, 21, 22, 23].

The indaziflam herbicide also differs from the control in the parameter percentage of roots cover in the third year of assessment of the roots (Table 15), in treatments 100 g a.i. ha⁻¹, in all of the assessed profile range (0–100 cm) and also in the upper layer (0–50 cm). The same was found for this range of the profile for treatment 150 g a.i. ha⁻¹ (Table 15). Only the parameter percentage of cover for this herbicide, regardless of the dose, was rejected by the null hypothesis and always with higher values than those obtained in the weeded control.

Research carried out by Blanco [24] has determined that indaziflam had long persistence in soil under conditions similar to the ones in the testing described herein. Thus, it can be inferred that indaziflam remaining in the soil solution can act on the secondary metabolism of the coffee roots and interfere in their secondary growth, which would explain their thickening.

In the fourth year of the test, assessing the number and percentage of roots, it was found that the analyses of variance were not able to find differences among treatments [25].

Thus, in the test scenario it can be concluded that:

- The methodology to determine the selectivity of herbicides by assessing the roots by means of trench and grid framework was appropriate.
- Herbicide glyphosate applied for four years, in three annual applications, can affect the development of crops of Coffee cv. Catuaí Vermelho.
- Herbicide indaziflam applied once for four years in crops of Coffee cv. Catuaí Vermelho and Citrus cv. Valência is selective for these crops.

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