ROOT EXUDATION OF IMAZAPYR BY EUCALYPT, CULTIVATED IN SOIL[1]

Exsudação Radicular de Imazapyr por Eucalipto Cultivado em Solo

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ABSTRACT - Imazapyr has been used to control stump sprouting in stand of *Eucalyptus* plantations, where herbicide is applied to the tree trunk before cutting. The herbicide is applied exclusively on the stump to be killed, but little is known about the final fate of the molecule. Imazapyr exudation via roots of eucalypt grown in soil as the substrate was evaluated under greenhouse conditions. Different herbicide doses (0.000, 0.375, 0.750, 1.125, 1.500, and 3.000 kg ha^{-1} a.i.) were applied on the aerial parts of 8-month-old *Eucalyptus grandis* clonal seedlings, cultivated in pots with 18.0 dm^3 of soil. Forty days after this treatment, the eucalypt plants were cut and a lateral opening in the containers was made and the plants inclined 90°, with plants sensitive to herbicide presence (sorghum and cucumber) sown into the openings along the exposed soil surface. After 15-day sowing, toxicity symptoms on the shoots as well as the shoot and root system dry biomass of the bio-indicators were evaluated. The results suggest that eucalypt roots do exude imazapyr, and/or its metabolites, at concentrations high enough to cause toxicity to the bio-indicators. Toxicity effects were observed in all plants sown along the exposed soil profile of the container, with higher intensity at higher doses.

Key words: Chopper, *Eucalyptus grandis*, bioindicators, interference, exudates.

RESUMO - O imazapyr tem sido utilizado para controle de brotações na reforma de cultivos florestais com ***Eucalyptus****, por meio de sua aplicação no caule no pré-corte das árvores; dessa forma, o herbicida é aplicado exclusivamente na planta, porém há pouco conhecimento sobre o destino final da molécula. A exsudação radicular do imazapyr por eucalipto, cultivado em solo, foi avaliada em casa de vegetação, aplicando-se diferentes doses do herbicida (0,000; 0,375; 0,750; 1,125; 1,500; e 3,000 kg* ha^{-1} *i.a.) sobre a parte aérea de mudas clonais de* ***Eucalyptus grandis****, com oito meses de idade, as quais foram cultivadas em recipientes com 18,0* dm^3 *de solo. Quarenta dias após a aplicação do herbicida procedeu-se à recepa do eucalipto e abertura de uma das laterais do recipiente, a qual foi inclinada 90°, recebendo a semeadura de plantas indicadoras da presença do herbicida (sorgo e pepino) ao longo da superfície exposta. Quinze dias após a semeadura, foi feita a avaliação dos sintomas de intoxicação na parte aérea, determinando-se a biomassa seca desta e do sistema radicular das plantas indicadoras. Os resultados evidenciaram que o eucalipto apresenta exsudação radicular do imazapyr e/ou de seus metabólitos em quantidade capaz de causar toxicidade nos bioindicadores, sendo esta observada em todas as plantas ao longo do vaso, com maior intensidade sob doses maiores.*

Palavras-chave: Chopper NA, *Eucalyptus grandis*, bioindicadores, interferência, exsudados.

INTRODUCTION

One of the most common problems, after the first or second clear-cut of an eucalypt plantation submitted to coppice management, is choosing the best criteria to decide whether to reform or conduct the stand for an additional growth period (Simões et al., 1981; Lopes, 1990; Silva, 1990). An expressive production reduction is observed after the second clear-cut

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in some areas, either due to reduced sprout regeneration, or to a low coppicing productivity (Rezende et al., 1981), leading the companies to decide in favor of reforming the stand. This decision must be supported by technical data to ensure its economical and environmental feasibility (Berger et al., 1974).

If the decision is in favor of reforming the stand, it is necessary to avoid stump sprouting, which can be achieved, among other alternatives, by imazapyr application, which can be performed before harvesting, by injecting the herbicide in the trunk; after harvesting but before stump sprouting – the herbicide is applied up to 72 hours after the cut, by wetting the cambial region of the stumps (Christoffoleti et al., 1997); or to the already established coppices by foliar application. The least expensive and most effective method has been herbicide injection to the trunk prior to cutting (Respondovesk, 1999). Despite the control option, some questions have been raised in relation to the fate of the molecule in the plantation site after fulfilling its function, and to its effects on the quality of the harvested product, the environment impact and effects on the posterior plantings. When imazapyr is used prior to harvesting, no effect is observed on debarking, wood quality, or cellulose toxicity (Dantas et al., 2001). However, it is not known whether the molecule is exuded and what after-effects occur from this exudation on the posterior stand.

Some plants have the capacity to exude, through their roots, exogenous compounds applied to their leaves and/or stem surface (Linder et al., 1964). Coupland and Caseley (1979) observed that couch grass (*Agropyron repens*) exuded glyphosate through the roots after the herbicide had accumulated in the roots and rhizomes. Reid and Hurtt (1970) verified root exudation of picloram and 2,4,5-trichlorophenoxyacetic acid when they were applied in sublethal doses on the leaves of two wood species, red maple (*Acer rubrum*) and green ash (*Fraxinus pennsylvanica* Marsh.) with a greater picloram exudation being observed in the latter species.

According to Zambolim (1990), the germination of symbiont endo and ectomycorrhizal fungi in the rhizosphere region of eucalypt primary roots is subject to the influence of root exudates, which can be beneficial or not, depending on the characteristic of the exuded substance and the involved microorganism. Matsumoto et al. (1979) found that a substance exuded by maize roots (*Zea mays*) can interfere with the growth of non-symbiotic nitrogen fixing microorganisms in the rhizosphere region.

Root exudation of organic substances can be affected by a variety of factors, such as soil water stress, temperature, light intensity, plant age and species, mineral nutrition, soil microorganisms, degree of anaerobiosis, and application of chemicals (Hale et al., 1971). These factors, together with the physical-chemical properties of the herbicides, determine the exudation potential, as well as the interference degree of root exudates with plants adjacent to those previously treated with herbicide.

Root tip, or the region immediately below it, can be considered the most important site of exudation (Pearson and Parkinson, 1961), although other root parts can also exude organic compounds (Bowen, 1968; Rovira, 1969). Therefore, the toxicity caused by the herbicide exudation and/or its metabolites depends on biotic and abiotic factors, and not only on the molecule presence in the soil.

Furthermore, an exudate may be insufficient to deleteriously interfere with adjacent plants, because of the exuded quantity, the toxicological characteristics of the exudate, its reactions with soil constituents, or due to plant tolerance to the exuded compound. Rodrigues et al. (1982), for instance, did not verify interference of glyphosate exuded through wheat roots (after spraying using doses of 1.1, 3.4, and 6.7 kg ha^{-1}) in the height and dry biomass of soybean plants (*Glycine max*).

This study aimed to evaluate root exudation of imazapyr in the soil by eucalypt plants (*Eucalyptus grandis*) and its effect on the growth of bio-indicators.

MATERIALS AND METHODS

Eucalypt clonal seedlings (*Eucalyptus grandis*), cultivated in plastic tubes of 55 cm^3

with carbonized rice chaff and vermiculite as substrate, were used for the experiment under greenhouse conditions. At the age of three months they were transplanted to aluminum vases, perforated at the bottom, and filled with 18.0 dm^3 of soil (Table 1).

Before filling the containers, the soil was homogeneously blended with 3.05 kg m^{-3} of lime and 5.0 kg m^{-3} of 4-14-8 fertilizer, equivalent to 54.09 and 90.0 g/container of lime and fertilizer, respectively.

The containers were maintained outside the greenhouse until the application of imazapyr (doses of 0.000, 0.375, 0.750, 1.125, 1.500, and 3.000 kg ha^{-1} a.i.) to the eucalypt seedlings, which were 1.60 m high and presented a well-developed root system (Figure 1a). Five months after seedling out-planting, herbicide application was carried out with a carbon dioxide pressurized backpack sprayer, adjusted to deliver 200 L ha^{-1} at a constant pressure of 30 lb pol^{-2}. The soil surface in the containers was covered with a double layer of cling film, in order to avoid contamination by the herbicide molecule. Crown overlapping was prevented to avoid misapplying the herbicide. Subsequently, frequent irrigation maintained the soil moisture near field capacity.

The eucalypt plants were cut 40 DAT (days after treatment application), 20 cm above the soil surface, leaving some viable buds for posterior evaluation of sprouts in the treated plants. Thereafter, the container was cut up laterally and inclined 90°, exposing the soil. On this surface, pre-germinated seeds of sorghum and cucumber were sown at a depth

Table 1 - Chemical-physical characteristics of the soil used in the bioassay

Chemical analysis						
pH	P	K	Ca	Mg	Al	H+Al
($CaCl_2$)	(mg dm^{-3})		($cmol_c$ dm^{-3})			
4.2	1.4	23.0	0.54	0.17	1.3	4.11

Physical analysis	
(dag kg^{-1})	
Sand	36.4
Silt	5.3
Clay	58.3
Soil textural class: clayish	

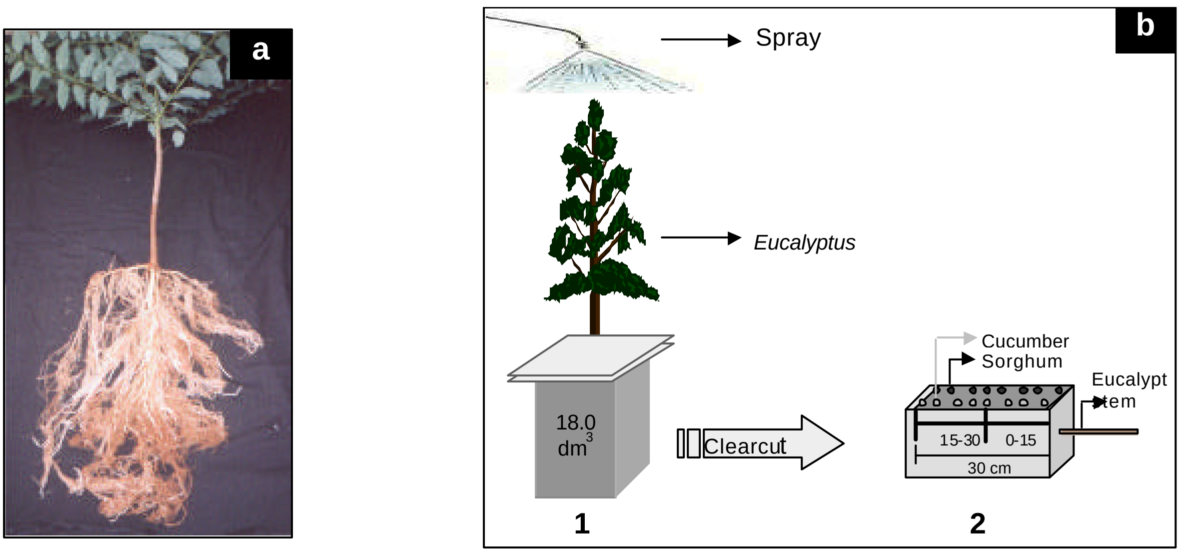

Figure 1 - **a**) Visual aspect of the root system distribution of a eucalypt plant, eight months of age, at the moment of the foliar imazapyr doses application; **b**) Experimental sequence: **1** – foliar imazapyr application on *E. grandis* plants, eight months of age; and **2** – removal of one container side and its inclination of 90° and sowing of pre-germinated sorghum and cucumber on the exposed soil surface, 40 days after treatment application.

of one cm all along the container profile (Figure 1b), which was subdivided in sections of 0-15 and 15-30 cm from the surface, where the clear-cut had been performed. The toxicity symptoms in the aerial part as well as the aerial part and root system dry biomass of the bio - indicators were evaluated after 15 days, with grades of 0-100 being attributed, according to the intensity of the toxicity symptoms, where 0% (zero) expresses the absence of visible symptoms in the plants and 100% signifies plant death. After separating the aerial part from the roots, the latter were wrapped separately in paper bags, put in an oven with air circulation at 72 ± 1 ºC, until they reached constant weight (after approximately 72 hours), to determine the dry matter.

The treatments were laid out as completely randomized with a 2 x 6 x 2 factorial design: two species (sorghum and cucumber), six treatments (herbicide doses), and two depths (0-15 and 15-30) with the latter corresponding to the sub-plots. The analyses of variance (ANOVA) were carried out using the statistical software package Saeg (SAEG, 1993), after verifying its presuppositions (Demetrio, 1978). The logistic model was adopted to evaluate the response of the dose, and the SigmaPlot software (SIGMAPLOT, 1997) to estimate the model regression parameters.

The logistic model – equation 1 (Streibig, 1988; Souza et al., 2000) – has three parameters: **a** is the-“saturation level”, corresponding to the bio-indicator response at zero dose; $\mathbf{x_0}$ is the inflection point of the curve, which corresponds to the value of I_{50}; and **b** is the slope of the curve around I_{50}.

$$y = \frac{a}{1+\left(\frac{x}{x_0}\right)^{b}} \qquad \text{(eq. 1)}$$

RESULTS AND DISCUSSION

Figures 2, 3 and 4 show the results of toxicity as well as the root system and shoots dry biomass of the plants used as indicators of eucalypt root exudates after the application of different imazapyr doses.

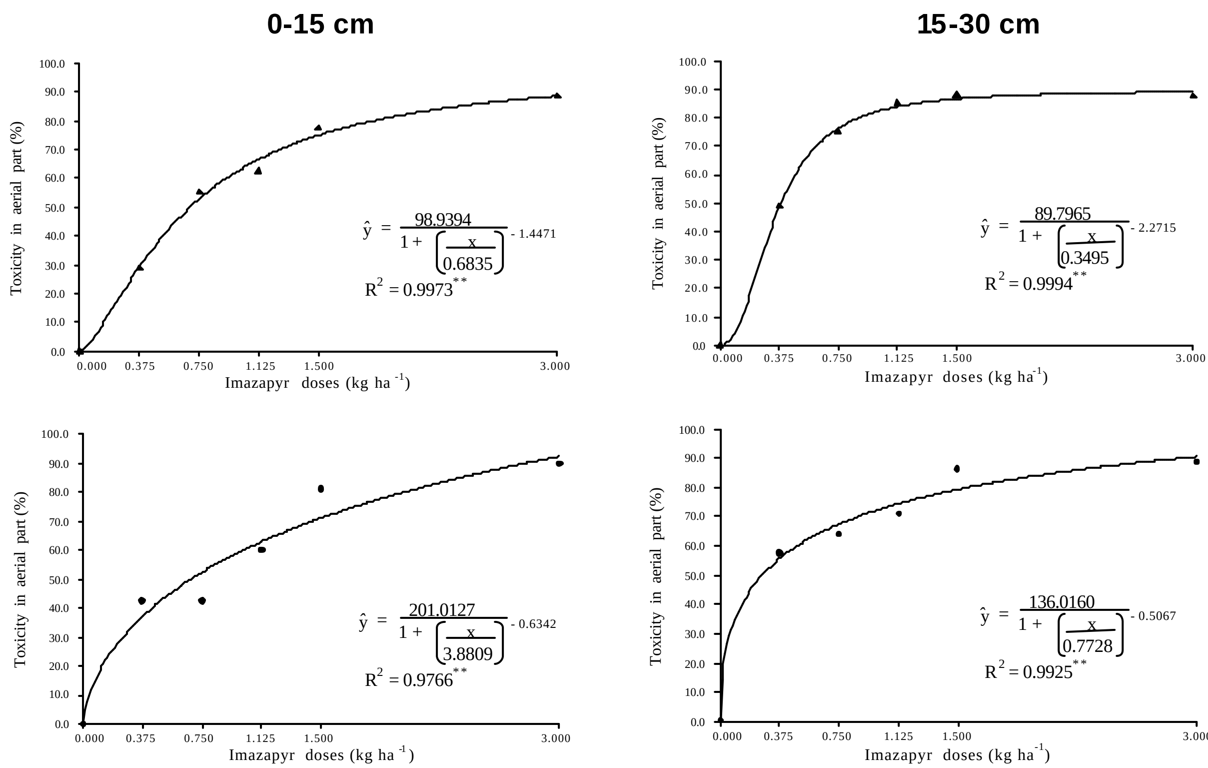

Figure 2 - Percent of toxicity of the shoot of sorghum (▲) and cucumber (●) plants 15 days after sowing, as affected by foliar application of imazapyr doses.

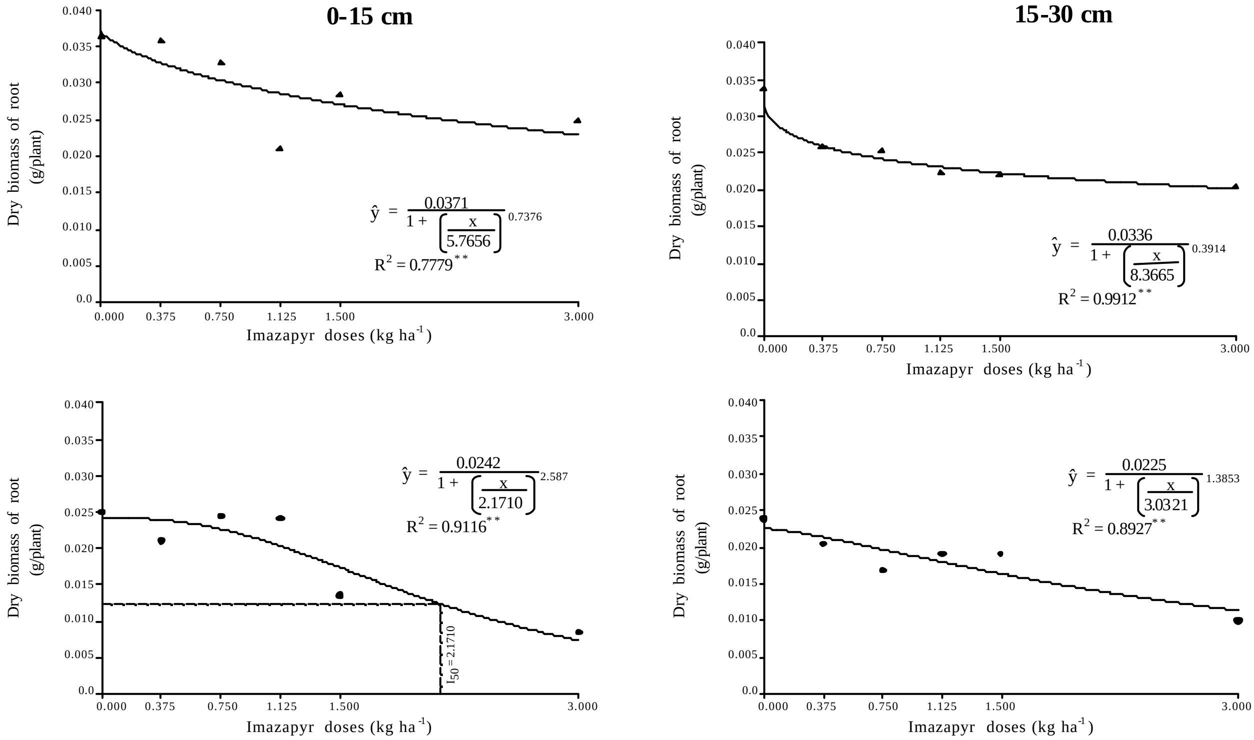

Figure 3 - Dry biomass of root of the sorghum (▲) and cucumber (●) plants 15 days after sowing, as affected by foliar application of imazapyr doses.

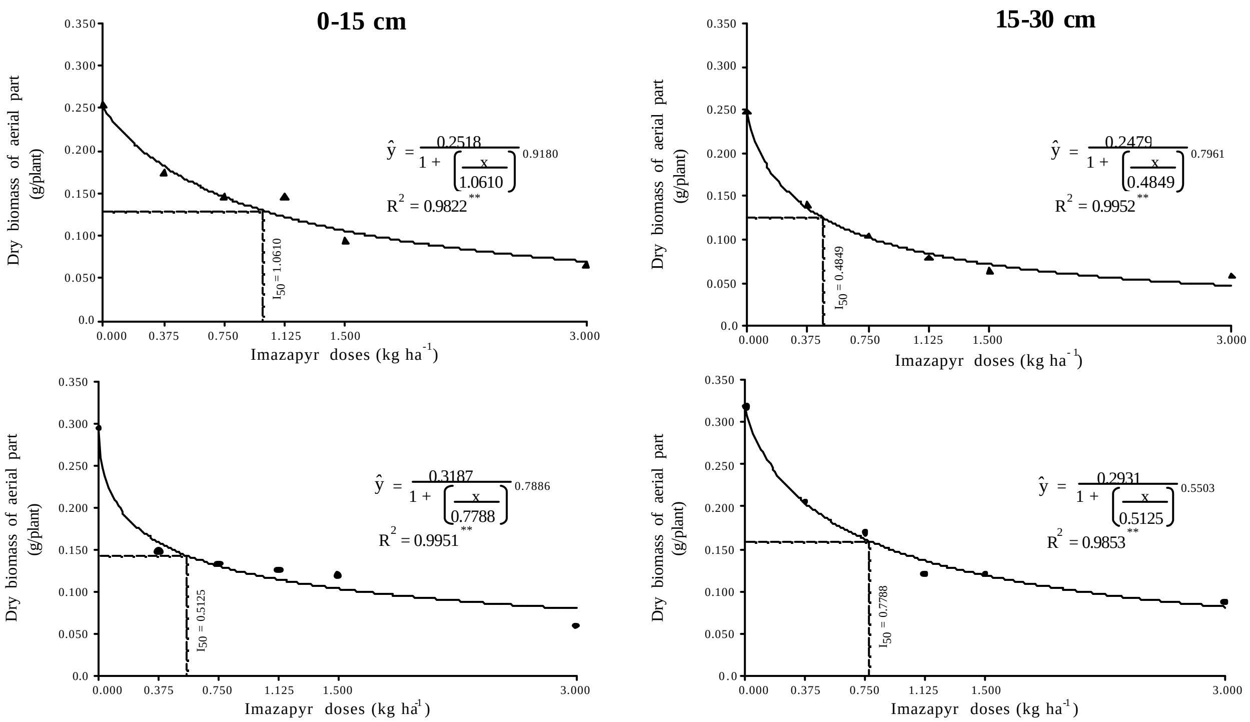

Figure 4 - Dry biomass of aerial part of the sorghum (▲) and cucumber (●) plants 15 days after sowing, as affected by foliar application of imazapyr doses.

The toxicity in the aerial part (Figure 2) was equivalent for both bio-indicators from the container surface to a depth of 15 cm, but the cucumber plants were less tolerant in the 15-30 cm layer, with the percent of visual toxicity symptoms above 50% at the dose of 0.375 kg ha^{-1} a.i. At higher herbicide doses applied to the eucalypt plants, the bio-indicators showed more severe symptoms of toxicity in the aerial part, indicating a greater root exudation of imazapyr, and/or its metabolites in the soil. This fact is due to the high sensitivity of the plants to the root exudates (Figure 5).

Based on sorghum and cucumber plant roots dry biomass (Figure 3), it was concluded that the cucumber plants were more sensitive to the exudates in the soil in the 0-15 cm layer of the container, obtaining an I_{50} value of 2.1710 kg ha^{-1} a.i. This was not verified for sorghum, since it was not possible to determine I_{50} for the range of doses used for this plant. In the studied interval (0.000 to 3.000 kg ha^{-1} a.i.), there was less interference with the dry biomass of sorghum roots than with cucumber, since it was not possible to determine I_{50} for the grass by the equation obtained.

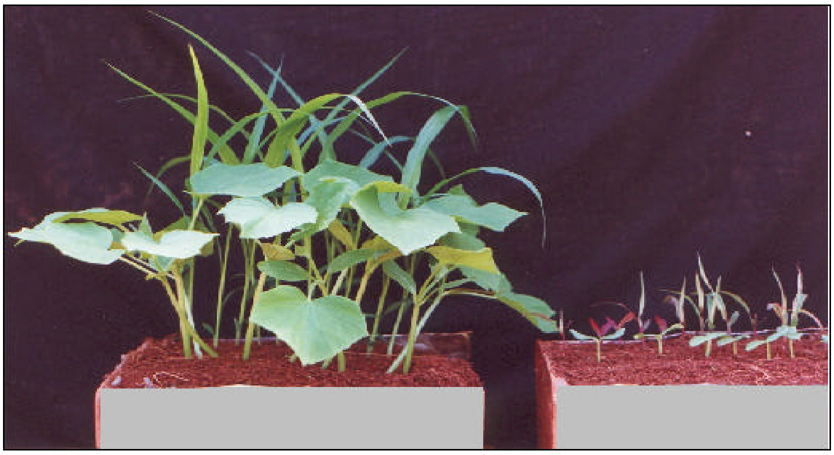

Figure 5 - Visual aspect of the bio-indicators (sorghum and cucumber) 15 days after planting, cultivated in the soil along the lateral surface of containers with root exudates of eucalypt plants, 40 d after foliar application of imazapyr. On the left, a treatment without herbicide and, on the right, the highest applied dose (3.000 kg ha^{-1} a.i.), 15 d after cutting the eucalypt plant.

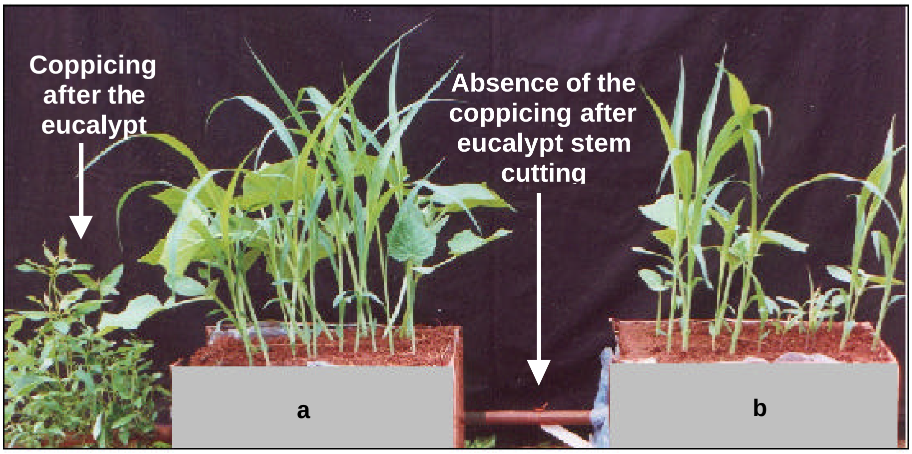

Figure 6 - Visual aspect of bio-indicators and coppice growth in the absence of imazapyr application to eucalypt plants (**a**) and visual symptoms of the herbicide toxicity in the bio-indicators and absence of coppices after cutting the eucalypt plant which had received a foliar application of 0.375 kg ha^{-1} a.i. of imazapyr (**b**).

In relation to the dose-effect response of the dry aerial part biomass (Figure 4), the I_{50} values were similar among cucumber plants at the depths 0-15 (x_o = 0.7788) and 15-30 cm (x_o = 0.5125). In the 15-30 cm layer, in relation to sorghum plants, I_{50} was approximately twice as low as in the upper layer (0.485 and 1.061 kg ha^{-1} a.i., respectively). In other words, sorghum was more sensitive to imazapyr, and/or its metabolites at this depth.

Although the cucumber plants presented a higher sensitivity, as shown by their I_{50} values in the 0-15 cm layer, there was no statistical difference between the bio-indicator species. Nevertheless, in terms of the exudate visual symptoms, sorghum was more symptomatic, since it presented chlorosis along the leaf limbs and between the nervures even at very low concentrations, as long as these are characteristic symptoms of the herbicides belonging to the chemical group imidazolinones.

The applied doses were sufficient to cause coppicing inhibition of eucalypt after clear-cut, due to the influence of the herbicide on plant growth points, probably inhibiting the protein synthesis and interfering with the DNA biosynthesis, and consequently, with cellular division, thus interrupting plant growth (Liebl and Bridges, 2000). Even under the lowest dosage (0.375 kg ha^{-1} a.i.), no regrowth was observed, while the plants not treated with imazapyr presented vigorous coppicing (Figure 6).

The results led to the conclusion that eucalypt exuded imazapyr, and/or its metabolites, via root system, since the toxic effects of the root exudates were observed in the whole soil area comprised by the eucalypt root system. With the increasing imazapyr doses, smaller increases of the total dry biomass of cucumber and sorghum plants were observed, since the latter presented more characteristic toxicity symptoms of imazapyr exudates than the former.

Therefore, it can be inferred that, when imazapyr is applied to tree trunks for coppice control in commercial eucalypt plantations, the likelihhod of exudation of imazapyr, and/or of its possible metabolites, must be taken into consideration, with the possibility of aftermath deleterious interference with the growth of eucalypt planted in between rows during the previous tillage. However, further research under field conditions is required.

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