IMAZAPYR ROOT EXUDATION FROM EUCALYPT SEEDLINGS CULTIVATED IN NUTRITIVE SOLUTION[1]

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ABSTRACT – Imazapyr has been used in Brazilian eucalypt cultivation for the maintenance of clearings and coppicing control in areas undergoing stand reform. However, inquiries have been made as to the final fate of the molecule. Imazapyr root exudation in eucalypt plants was evaluated through a bioassay under greenhouse conditions, by applying different herbicide doses (0.000, 0.375, 0.750, 1.125, and 1.500 kg ha^{-1} a.i.) on *Eucalyptus grandis* seedlings derived from vegetative propagation, hydroponically cultivated in 2.500 ml vases. Forty-day-old seedlings of the same clone were used as bioindicators, transplanted to the vases two days after herbicide application. After a period of 13 days of coexistence, the sprayed plants were removed and discarded; ten days later, the visual symptoms of toxicity were evaluated and the total dry biomass (aerial part and roots) of the bioindicators were determined. The lowest herbicide dose (0.375 kg ha^{-1} a.i.) affected the total biomass and growth, being most evident in the aerial part, with larger I_{50} for root dry biomass. The *E. grandis* seedlings exuded imazapyr, and/or its metabolites, in concentrations capable of affecting the growth of plants of the same species.

Keywords: *Eucalyptus grandis*, root exudates and herbicide.

EXSUDAÇÃO RADICULAR DE IMAZAPYR POR MUDAS DE EUCALIPTO CULTIVADAS EM SOLUÇÃO NUTRITIVA

RESUMO – Na eucaliptocultura brasileira, vem-se utilizando o imazapyr para manutenção de aceiros e erradicação de cepas e brotações em áreas de reforma dos povoamentos. Entretanto, têm sido levantadas indagações quanto ao destino final da molécula. A exsudação radicular de imazapyr em plantas de eucalipto foi avaliada por meio de bioensaios em casa de vegetação, aplicando-se diferentes doses do herbicida (0,000; 0,375; 0,750; 1,125; e 1,500 kg ha^{-1} i.a.) sobre mudas de ***Eucalyptus grandis****, provenientes de propagação vegetativa e cultivadas em sistema hidropônico, em vasos de 2.500 mL. Como bioindicador, empregaram-se mudas do mesmo clone com 40 dias de idade, as quais foram transplantadas para os vasos dois dias após a aplicação do herbicida. Depois de um período de 13 dias de convivência, retiraram-se as plantas que receberam a aplicação, descartando-as; 10 dias após, foram avaliados os sintomas visuais de toxicidade e determinadas as biomassas secas de parte aérea e raízes das mudas do bioindicador. A menor dose do herbicida (0,375 kg ha^{-1} i.a.) afetou o crescimento e a produção de biomassa total, sendo mais pronunciado na parte aérea, com maior valor de I_{50} na biomassa seca de raiz. As mudas de* ***E. grandis*** *exsudam o imazapyr e, ou, seus metabólitos, em concentrações capazes de afetar o crescimento de plantas da mesma espécie.*

Palavras-chave: ***Eucalyptus grandis****, exsudados radiculares e herbicida.*

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1. INTRODUCTION

The forest sector plays an essential role in the Brazilian economy, since contributes to foreign exchange earnings as well as the creation of direct and indirect jobs, on grounds of: the high competitiveness of the sector on the international market, investments aiming at productivity increase, forest marketing (FSC and ISO 1400), and the incessant search for sustainable programs, with a more holistic view by entrepreneurs.

When eucalypt is chosen for the stand reform, one of the factors responsible for the increase in productivity is the use of herbicides for coppice control, providing a highly efficient control of suckers and a small physical impact on the soil (compaction), since no tractors are needed to perform the application. Glyphosate and imazapyr are the most frequently used herbicides for this purpose, with the latter offering greater flexibility to be used in the period between application and tree clearcutting (RESPONDOVESK, 1999).

Imazapyr is injected into trees with an insertion of about 2.5 cm at breast height – 1.30 m. The dose must be determined according to the species, hybrid or clone used, due to the possibility of different responses to the herbicide (SILVA et al., 2004). Imazapyr controls coppicing efficiently (DANTAS et al., 2001), because it acts on the plant's growing points, inhibiting regrowth (RODRIGUES and ALMEIDA, 1998). The aspects of great concern are the doses used and the final destination of the molecule; imazapyr might not remain restricted to eucalypt, but it can be metabolized to non-toxic compounds and/or be exuded into the soil.

Linder et al. (1964) verified that some plants are capable of exuding substances applied to stem or leaves into the soil. There are little studies on root exudation mechanisms, but the biological importance of exudation must be taken into consideration. According to Smith (1976), compound exudation through plant roots can modify the nutrient pool and other compounds in the soil, possibly giving rise to considerable alterations in the system; besides, the liberation of these substances can be an inhibiting factor for the development of neighboring plants. Nicosulfuron, for example, when applied to Johnsongrass (*Sorghum halepense* (L.) Pers.), can be exuded in its original form and/or in toxic metabolites by the root system of weeds, and it can be taken up by corn plants. It may also modify the allelochemichal composition of Johnsongrass, producing a phytotoxicity effect through Johnsongrass residues on the crop (GUBBIGA et al., 1996).

Several organic substances can be released by means of root exudates from herbaceous and woody species, in soil or in solution, such as: carbohydrates, amino acids, nucleotides, flavonoids, enzymes, and organic acids (BÖNER, 1960; SILVA et al., 2001). It has been reported that some herbicides can also be exuded through the root systems of weeds and agricultural crops. Coupland and Caseley (1979) verified root exudation of glyphosate to couchgrass (*Agropyron repens* (L.) Beuv.) after accumulation of the herbicide in roots and rhizome nodes of the plant. Fites et al. (1964) confirmed accumulation in the plant structure, with posterior exudation, in jimsonweed (*Datura stramonium*), six weeks after foliar application of 2,4-D.

The aim of this work was to evaluate exudation of imazapyr from *Eucalyptus grandis* plants into nutritive solution, since herbicide exudation by eucalypt still lacks research.

2. MATERIALS AND METHODS

The assay was carried out in a hydroponic system under greenhouse conditions, using plastic vases containing 2,500 ml nutritive solution, pH adjusted to 5.50 ± 0.10 with NaOH or HCl every other day. The vases received silver coating on the outside and a double plastic layer, the first black and the other transparent, on the inside. The vase lids were perforated with two 14-mm holes to receive the eucalypt seedlings, and another 2-mm hole to insert an air tube, which was linked to a compressor with constant airflow, into the solution.

The three-month-old *E. grandis* seedlings, 15-to-20-cm high, were propagated vegetatively, and cultivated in 55-cm^3 plastic tubes, in the substrates carbonized rice chaff (46%), vermiculite (46%) and B horizon soil (8%). Homogeneously high plants were used as transplants, after careful washing of root systems. They were immediately transferred, one plant per vase, to a nutritive Clark solution (CLARK, 1975), and kept there during 40 days (phase of adaptation to solution) to grow new leaves and roots. The nutritive solution was weekly replaced; with the last change on the day of imazapyr foliar application. The herbicide was applied by a carbon dioxide pressurized backpack sprayer regulated to deliver 200 L ha^{-1} at a constant pressure of 30 lb pol^{-2}, after the vase surfaces have been protected with a double clingfilm layer to avoid contaminating the nutritive solution.

The treatments consisted of a control (without herbicide), and four imazapyr doses (1.5, 3.0, 4.5, and 6.0 L ha^{-1} Arsenal NA), corresponding to 0.375, 0.750, 1.125, and 1.500 kg ha^{-1} a.i., respectively.

Two days after the applications (DAT), one 6-9 cm high, 40-day-old seedling per vase of the same clone was transplanted to the solution as bioindicator of the herbicide in the solution. During 13 days, the seedling treated with imazapyr and the bioindicator seedling grew side by side in the vase. The treated seedling was thereafter removed and the bioindicator grew alone in the solution for ten more days. Phytotoxicity was thereupon evaluated according to the symptomatic scale proposed by the European Weed Research Council (EWRC, 1964), which attributed grades from 1 to 9, representing a toxicity of 0, 1-15, 16-30, 31-45, 46-60, 61-75, 76-90, 91-99, and 100%, respectively, where 0 (zero) is absence of symptoms and 100% is tip plant death.

For determining root dry biomass, roots were separated from the aerial part, wrapped separately in paper bags, and placed in a stove with air circulation, at 72 ± 1 °C, until they reached a constant weight after approximately 72 hours.

The experimental arrangement was a completely randomized design with five treatments, consisting of five herbicide doses carried out in four replications. Data were analyzed by analysis of variance (ANOVA) after verifying the presuppositions (DEMÉTRIO, 1978). Following ANOVA, the regression analysis was carried out, using the logistic model to evaluate the dose-response for total dry biomass. The statistical software Saeg was run for analysis of variance and SigmaPlot for parameter estimation in the regression model.

The logistic model – equation 1 (SOUZA et al., 2000) – has three parameters, where **a** is denominated "saturation level", corresponding to the bioindicator response at lower dose; $\mathbf{x_o}$ is the inflection point of the curve, which corresponds to the I_{50} value; and **b** describes the slope of the curve around I_{50}.

$$y = a/[1+(x/x_o)^b] \quad \text{(eq. 1)}$$

3. RESULTS AND DISCUSSION

Figure 1 shows that the logistic function gave excellent adjustment to dose-response to imazapyr ($R^2 > 0.90$) for the evaluated characteristics; which was also observed by Souza et al. (2000) when they used this equation to study imazapyr dose-response in tomato plants.

The eucalypt seedling transplants used as bioindicators presented an average dry biomass of the aerial part (DBAP) of 0.118 g/plant (data estimated from other clones at the same growth stage). As biomass has low variability, and considering the average value of DBAP at the moment of the transplant as reference, there was an approximately six-fold DBAP increase compared to the control (0.6824 g/plant) while the bioindicator remained in the solution. *E. grandis* was able to exude a sufficient quantity of imazapyr and/or its metabolites at the lowest dose (0.375 kg ha^{-1} a.i.), resulting in toxicity to the bioindicator seedlings (40 days old) transplanted after the application (Figure 2). This can be observed by the smaller DBAP increases based on the I_{50} value (0.2083 kg ha^{-1} a.i.), which was 0.3412 g/plant under this dose and 0.2239 g/plant under the highest applied dose (1.500 kg ha^{-1} a.i.).

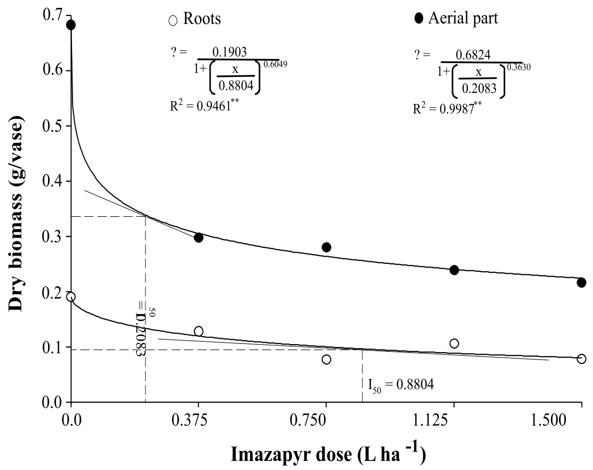

Figure 1 – Dry biomasses of aerial part (●) and roots (○) of eucalypt seedlings used as bioindicator and cultivated in nutritive solution containing root exudates from eucalypt plants subjected to foliar application of imazapyr, at 23 days after transplanting. I_{50} = dose that causes 50% decrease in dry biomass compared to control.

***Figura 1** – Biomassas secas da parte aérea (●) e das raízes (○) de mudas de eucalipto utilizadas como bioindicador e cultivadas em solução nutritiva contendo exsudados radiculares de plantas de eucalipto submetidas à aplicação foliar de imazapyr aos 23 dias após o transplante. I_{50} = dose que causa 50% de redução na biomassa em relação ao controle.*

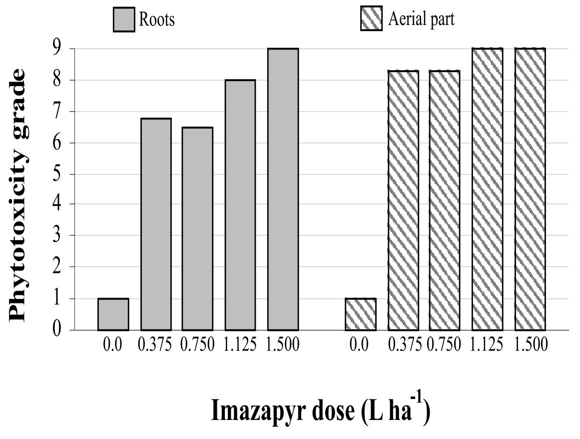

Figure 2 – Average of visual grades of toxicity in aerial part (□) and roots (▩) of eucalypt seedlings used as bioindicator and cultivated in nutritive solution containing root exudates from eucalypt plants subjected to foliar application of imazapyr, at 23 days after transplanting.

Figura 2 – *Médias das notas visuais de toxicidade na parte aérea (□) e nas raízes (▩) de mudas de eucalipto utilizadas como bioindicador e cultivadas em solução nutritiva contendo exsudados radiculares de plantas de eucalipto submetidas à aplicação foliar de imazapyr aos 23 dias após o transplante.*

When the plants were transferred to the nutritive solution, the ones in the same growth stage of the bioindicator presented dry root biomass (DRB) of 0.068 g/plant. In conclusion, under the highest herbicide dose used (1.500 L ha^{-1}), the quantity of root exudates in solution was able to cause an reduction of 58% in the biomass accumulation, compared to the biomass produced in the absence of the herbicide (0.1903 g/ plant) (Figure 1).

The dose related to 50% response, referred to I_{50} doses in this paper, is the most common dose used to measure the plant sensitivity to herbicide (STREIBIG and GREEN, 1994). The I_{50} value for the DRB was 0.8804 kg ha^{-1} a.i., in other words, when the commercial dose is applied to control already established coppices (0.750 kg ha^{-1} a.i.), the quantity of imazapyr exudates in the solution was not sufficient to cause a 50% in inhibition of the root growth of bioindicator seedlings.

A shallow dose-response curve around the I_{50} value was obtained for the DRB compared to the DBAP, since the straight tangent line to the curve forms a smaller angle with the axis of the herbicide doses at this value, indicating that the increases in concentration of the herbicide applied to *E. grandis* plants resulted in exudate concentrations in the solution which led to a higher toxicity in the aerial part of the bioindicator seedlings (Figure 2), because of the drastic reductions in biomass of aerial part and height, as it shows figures 1 and 3, respectively.

The presence of root exudation containing the imazapyr molecule caused root death (characterized by its darkening), height and total biomass reduction, due to the action of the herbicide in the plant growing points (meristems) causing inhibition of ALS (acetolactate synthase) activity, which involves inhibition of the branched-chain amino acid biosynthesis (leucine, isoleucine and valine) (CHIPMAN et al., 1998). Consequently, protein production and enzyme biosynthesis which are dependent on these amino acids are stopped, affecting DNA synthesis and cellular growth, besides reducing assimilate translocation (LIEBL and BRIDGES, 2000).

Figure 3 – Visual aspect of eucalypt seedling development used as bioindicator cultivated in nutritive solution containing root exudates from eucalypt plants subjected to foliar application of imazapyr, at 23 days after transplanting.

Figura 3 – *Aspecto visual do crescimento de mudas de eucalipto utilizadas como bioindicador e cultivadas em solução nutritiva contendo exsudados radiculares de plantas de eucalipto submetidas à aplicação foliar de imazapyr aos 23 dias após o transplante.*

The conclusion that can be drawn from the results is that when imazapyr is applied to tree trunks for coppicing control in commercial eucalypt plantations, it is likely the occurrence of exudation of herbicide and/or its possible metabolites, which can cause a detrimental effect on the growth of eucalypt cultivated between rows of the previous crop (reform). This may be caused by the greater persistence of the molecule in the soil (LIEBL and BRIDGES, 2000); the weak linkages with constituents, therefore strongly influenced by the pH variation (PUSINO et al., 1997); as well as by the reverse leaching, in other words, by the capillary mobility of the molecule (FIRMINO, 2001).

4. CONCLUSIONS

Under the experimental conditions, the results indicate that *E. grandis* seedlings presented root exudation of imazapyr and/or its metabolites when the herbicide was applied to the aerial part, and the quantity of exuded toxic composts can affect the growth of eucalypt seedlings used as bioindicator, even with the lowest dose (0.375 kg ha^{-1} a.i.); besides, the toxicity of root exudates is more intense in the aerial part of the eucalypt plant used as bioindicator than in the root system. However, areas with increased quantity of organic matter (MCDOWELL et al., 1997) and humic acid (LEONE et al., 2002), allied to low pH values (PUSINO et al., 1997), high temperature and precipitation (MCDOWELL et al., 1997) have greater adsorption of imazapyr in the soil, and consequently fewer detrimental effects on eucalypt seedlings cultivated between the rows of the previous crop

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