Tolerance of cassava to post-emergence herbicides and determination of residues in the roots

Tolerância da mandioca a herbicidas em pós-emergência e determinação dos resíduos nas raízes

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Abstract
Assessing the tolerance of cassava to herbicides is essential for the development of weed management programs. Nevertheless, this assessment must include analyses of herbicide residues in the food so as to guarantee safety to the final consumer. Thus, this study evaluates the selectivity of cassava to different herbicides and quantifies pesticide residues in cassava roots (Manihot esculenta Crantz). The experimental design was randomized blocks with four replicates. The treatments were: control; mechanical weeding; bentazon (900 g a.i. ha⁻¹); mesotrione (120 g a.i. ha⁻¹); and imazethapyr (100 g a.i. ha⁻¹), applied in post-emergence. We used the herbicides clomazone (1080 g a.i. ha⁻¹) and fluazifop (125 g a.i. ha⁻¹) in pre-emergence and post-emergence, respectively, in all treatments. Throughout the crop cycle, we assessed the following: cassava intoxication at 10, 20, and 30 days after the application (DAA) of post-emergence herbicides; and plant height and stem diameter at 30 DAA. At the end of the crop cycle, we assessed the following: number of roots per plant; root length and diameter; root yield (kg/ha); starch content (%); and pesticide residues in cassava roots (multiresidue method). Despite its high phytotoxicity in the early development of cassava, mesotrione shows high selectivity and viability for weed control in this crop since, in general, it did not impair the other yield variables. Moreover, the study identified clomazone and sulfentrazone residues in cassava, indicating root contamination and high persistence of these herbicides in the environment.

Additional keywords: Manihot esculenta Crantz; pesticide residues; selectivity.

Resumo
A avaliação da tolerância da mandioca aos herbicidas é fundamental para o desenvolvimento de programas de manejo de plantas daninhas na cultura, porém deve ser acompanhada de estudos de análise de resíduos no alimento, a fim de garantir a segurança ao consumidor final. Diante disso, o objetivo foi avaliar a seletividade da cultura a diferentes herbicidas; e quantificar os resíduos de agrotóxicos nas raízes de mandioca (Manihot esculenta Crantz). O experimento foi conduzido em delineamento em blocos casualizados, com quatro repetições. Os tratamentos foram: testemunha; capina mecânica; bentazon (900 g i.a. ha⁻¹); mesotrione (120 g i.a. ha⁻¹) e imazethapyr (100 g i.a. ha⁻¹) aplicados em pós-emergência. Os herbicidas clomazone (1080 g i.a. ha⁻¹) e fluazifop (125 g i.a. ha⁻¹) foram utilizados em pré-emergência e pós-emergência, respectivamente, em todos os tratamentos. As variáveis avaliadas foram: intoxicação da cultura aos 10, 20 e 30 dias após a aplicação dos herbicidas pós-emergentes (DAA); estatura das plantas e diâmetro do caule, aos 30 DAA. Ao final do ciclo da cultura, foram avaliados: número de raízes por planta; comprimento e diâmetro de raízes; produtividade de raízes (kg/ha), teor de amido (%) e determinação de resíduos de agrotóxicos em raízes de mandioca (método multiresíduos). O mesotrione, apesar da elevada fitotoxicidade na fase inicial, demonstra maior seletividade e possibilidade de utilização no controle de plantas daninhas na cultura, uma vez que, em geral, não prejudicou as demais variáveis produtivas. Ainda, identificou-se a detecção de resíduos de clomazone e sulfentrazone na mandioca, indicando contaminação nas raízes e alta persistência desses herbicidas no ambiente.

Palavras-chave adicionais: Manihot esculenta Crantz; resíduos de agrotóxicos; seletividade.
Introduction

Cassava (Manihot esculenta Crantz) plays an important role in the human diet (CONAB, 2020). Its production targets mainly the manufacture of flour and starch and its fresh consumption. Brazil is the world’s fifth largest producer of cassava, with a production of 18.8 million tons in an area of 1.3 million hectares (FAOSTAT, 2017). Such figures reveal a low yield (14.4 t ha\(^{-1}\)) despite the high yield potential of the crop, which reaches values above 100 t ha\(^{-1}\) in some regions (Alves, 2007).

Among the factors concerning the low yield in the field, losses resulting from weed competition stand out. Given their rusticity context, producers usually believe that they do not need to worry about the phyto-sanitary management of crops. This is an extremely mistaken decision making since studies indicate a yield reduction of practically 100% due to weed interference (Johanns & Contiero, 2006).

Chemical weed control is a fast, effective, and interesting cost-benefit alternative. Notwithstanding, there are few registered herbicides for cassava, most of which are pre-emergence herbicides (AGROFIT, 2020). The most common agricultural system for cassava is family farming, which reduces the interest and investment of large multinational companies to register and develop molecules for use in this crop.

The search for selective herbicides capable of eliminating weeds without causing phytotoxic effects to the crop is fundamental to expand management options for the farmer and to increase its yield potential. However, this should not be the only concern of the professionals in the area.

Selectivity studies on cassava do not evaluate possible pesticide residues in the food after application (Agostinetto et al., 2002; Biffe et al., 2010; Silva et al., 2012). This determination is extremely important to assess the risks arising from the use of pesticides regarding contaminated food and organisms. It provides useful information to assist in the development of prevention programs, risk control, and regulation of foreign trade, ensuring safety to the population.

Therefore, this study evaluates the selectivity of cassava to different herbicides with the following mechanisms of action (Senseman, 2007): bentazon (photosystem II inhibitor), mesotrione (carotenoid inhibitor), and imazethapyr (acetolactate synthase inhibitor). The study also quantifies pesticide residues in the roots at the end of the crop cycle.

Materials and Methods

We conducted the experiment in an agricultural area (29° 03’ 34.9” S, 49° 36’ 19.8” W, and approximate altitude of 14 meters) in Sombrio city, Santa Catarina State, Brazil, from September 2018 to July 2019. The soil in the area is a Quartzarenic Neosol with sandy texture (EMBRAPA, 2013), and the previous crop was tobacco (Nicotiana tabacum L.). The relief is flat and, according to the Köppen classification (Ometto, 1981), the climate of the region is Cwa type, humid mesothermal subtropical.

The soil had the following chemical characteristics: pH (CaCl\(_2\)) = 5.2; SMP index = 6.7; phosphorus = 466 mg dm\(^{-3}\); potassium = 159 mg dm\(^{-3}\); calcium = 1.8 cmol\(_d\) dm\(^{-3}\); magnesium = 1.2 cmol\(_d\) dm\(^{-3}\); aluminum = 0.1 cmol\(_d\) dm\(^{-3}\); and soil organic matter = 2.1%. Soil analysis showed no need for base fertilization and liming. However, we performed cover fertilization at 45 days after planting, using 20 kg N ha\(^{-1}\) (COFS, 2016).

The experimental design was randomized blocks with four replicates. The treatments were: control; mechanical weeding (weeeded the plants with the aid of a hoe until the last herbicide application, in January 2019); and bentazon (900 grams of active ingredient per hectare), mesotrione (120 g a.i. ha\(^{-1}\)) and imazethapyr (100 g a.i. ha\(^{-1}\)) applied in post-emergence. We chose these herbicides and doses from their effectiveness in controlling broadleaf weeds. Two post-emergence applications took place throughout the cycle, from which we used mean phytotoxicity values for statistical analysis.

We used the herbicides clomazone (1080 g a.i. ha\(^{-1}\)) and fluazifop (125 g a.i. ha\(^{-1}\)) in pre-emergence and post-emergence, respectively, in all treatments, considering it to be a standard management due to the frequent use of producers in the region.

The plots were 2.4 m long and 3.6 m wide, with four rows. For the evaluations, we considered the two central rows as the working area, discarding a plant at each end. Thus, each plot presented six useful plants in the evaluations.

Planting took place in October. For that, we used 21 cm long (Viana et al., 2002) stem cuttings of the cultivar Sangão (SCS-253). We planted the stem cuttings horizontally in 5 cm deep furrows, at a spacing of 0.60 m. At about 30 days before planting, we cleared the cultural remains of the previous crop, desiccating the entire area with the herbicide glyphosate (1440 g a.i. ha\(^{-1}\)).

At 10, 20, and 30 days after the application (DAA) of post-emergence herbicides, we assessed crop intoxication visually using a percentage scale ranging from 0 (zero) to 100 (one hundred), where 0 implies the absence of any injuries and 100 corresponds to plant death. At 30 DAA, we assessed first branch height (m) and stem diameter (cm; with the aid of a digital caliper).

At 10 months after planting, we harvested the plants and assessed the following variables: number of roots per plant; root length and diameter (m); root yield (kg ha\(^{-1}\)); and starch content (%), with the aid of a hydrostatic balance (Grossman & Freitas, 1950, adapted by Oliveira et al., 2011). For the latter, we used the following equations: DW = 15.75 + 0.0564x, where DW is the dry weight (%) and “x” is the weight of 3 kg of fresh cassava in water; Starch content = DW - 4.65.

We identified samples from each experimental plot and sent them to the Laboratory for Analysis of Pesticide Residues (TECPAR - Curitiba/PR) (with the exception of the bentazon and imazethapyr treatments,
due to insufficient material) for the verification of possible pesticide residues in the cassava roots. These samples consisted of 0.5 kg of fresh cassava, which we stored after harvest in a refrigerator (temperature between 4-5 °C) until transport. During transport, according to the guidelines of the company, the samples remained in a thermal box with ice. The laboratory used liquid chromatography for the determination of pesticide residues, with detection by sequential mass spectrometry - LC-MS/MS multiresidue method.

We submitted the data to analysis of variance (p≤0.05). In the case of statistical significance, we compared the means using the Tukey test (p≤0.05), except for the evaluation of pesticide residues, which consisted of descriptive analysis.

### Table 1 – Phytotoxicity (%) resulting from bentazon (900 g a.i. ha\(^{-1}\)); mesotrione (120 g a.i. ha\(^{-1}\)) and imazethapyr (100 g a.i. ha\(^{-1}\)) herbicides; besides the control and weeding, to the cassava culture, evaluated at 10, 20 and 30 days after the application of the treatments (DAA).

| Treatment | 10 DAA | 20 DAA | 30 DAA |
|-----------|--------|--------|--------|
| Control   | 0.00 c\(^1\) | 0.00 b | 0.00 c |
| Weeding   | 0.00 c | 0.00 b | 0.00 c |
| Bentazon  | 13.75 b | 35.00 a | 75.00 b |
| Mesotrione| 33.75 a | 45.00 a | 80.00 ab |
| Imazethapyr | 5.00 c | 38.75 a | 82.50 a |
| CV (%)    | 28.83  | 23.53  | 6.07   |

\(^1\)Means followed by the same letters in the columns do not differ from each other by the Tukey test at 5% probability.

Silva et al. (2012) indicate the selectivity of cassava to post-emergence application of the herbicides mesotrione, bentazon, and trifloxysulfuron-sodium (the latter belonging to the group of ALS inhibitors, the same mechanism of action of imazethapyr). The authors found intoxication values below 7%; contrasting with the results of the present study. A hypothesis is that, in the present study, the higher dosage and the climatic stress conditions resulting from the drought period that the crop went through decreased its tolerance to the herbicides. This suggests that these factors directly influence plant behavior in response to the treatment for weed control.

Weed interference affected stem diameter and mesotrione treatments showed similar behavior. The results of the analysis of pesticide residues led to an estimate of human exposure to pesticides in

### Results and Discussion

Regarding phytotoxicity, at 10 DAA, imazethapyr caused less injury than the herbicides bentazon and mesotrione (Table 1). This result is possibly due to the greater systemic action of imazethapyr, where the symptoms become more apparent after weeks of application. This proved to be true after analyzing the last evaluation of this variable (30 DAA), when the herbicide, which belongs to the group of acetolactate synthase (ALS) inhibitors, showed high phytotoxicity values. These values were similar to those of the other herbicides, indicating that the products had a high phytotoxic effect.

| Treatment | Stem diameter (cm) | Height (m) |
|-----------|--------------------|------------|
| Control   | 2.18 c\(^1\)       | 0.32 a     |
| Weeding   | 7.69 a             | 0.35 a     |
| Bentazon  | 3.57 bc            | 0.05 b     |
| Mesotrione| 4.50 b             | 0.24 a     |
| Imazethapyr | 3.04 bc       | 0.06 b     |
| CV (%)    | 24.48              | 26.24      |

\(^1\)Means followed by the same letters in the columns do not differ from each other by the Tukey test at 5% probability.
fresh food, which Table 4 shows along with the maximum residue limits (MRL) established by the National Health Surveillance Agency (ANVISA, 2015). The main herbicide in this analysis was clomazone, a carotenoid inhibitor present in all pre-emergence treatments of this experiment. Its maximum allowed limit is 0.05 mg kg\(^{-1}\), and the control treatment showed higher values.

**Table 3** - Number of roots per plant (NRP), root length (RL), root diameter (RD), yield (kg/ha) and starch content (%) resulting from bentazon (900 g a.i. ha\(^{-1}\)); mesotrione (120 g a.i. ha\(^{-1}\)) and imazethapyr (100 g a.i. ha\(^{-1}\)) herbicides; besides the control and weeding, to the cassava crop, evaluated at 275 days after planting.

| Treatment   | NRP   | RL (m) | RD (m) | Yield (kg ha\(^{-1}\)) | Starch (%) |
|-------------|-------|--------|--------|-------------------------|------------|
| Control     | 1.78 ab\(^1\) | 0.09 ab | 0.09 bc | 157.69 b                | 6.03 ab    |
| Weeding     | 3.31 a  | 0.17 a  | 0.29 a  | 1039.23 a               | 11.11 a    |
| Bentazon    | 0.00 b  | 0.00 b  | 0.00 c  | 0.00 b                  | 0.00 b     |
| Mesotrione  | 3.16 a  | 0.16 a  | 0.23 ab | 651.85 ab               | 11.84 a    |
| Imazethapyr | 0.00 b  | 0.00 b  | 0.00 c  | 0.00 b                  | 0.00 b     |
| CV (%)      | 46.24  | 32.12   | 20.59   | 46.44                   | 23.34      |

\(^1\) Means followed by the same letters in the columns do not differ from each other by the Tukey test at 5% probability.

Studies identify risks from the use of clomazone, pointing to the capacity of this herbicide to induce oxidative stress and inhibit erythrocytes in humans (Santi et al., 2011). Moreover, it has a toxic effect on fish such as Prochilodus lineatus (Valenciennes, 1837), leading to hematological and biochemical changes in the species (Pereira et al., 2013); and on microbial populations, decreasing the abundance of microorganisms and nitrogen-fixing bacteria (Du et al., 2018).

The physicochemical characteristics of the herbicide are also important since they indicate high solubility (S = 1100 mg L\(^{-1}\)) and moderate affinity with organic matter (Koc = 300 mL g\(^{-1}\)) (Sensenman, 2007). Along with these factors, the soil conditions in the experimental region (low pH and organic matter) enhance herbicide leaching (Pereira et al., 2017). This leads to possible problems of groundwater contamination and exposure risks for humans.

The second most important herbicide in the analysis was sulfentrazone, which is a protoporphyrinogen oxidase (Protox) inhibitor (Sensenman, 2007). Its concentration was 0.02 mg kg\(^{-1}\) (Table 4). Interestingly, the present experiment did not include this herbicide. Its detection was possibly due to the history of the experimental area since the previous crop was tobacco and its management included the use of this product. In addition, this herbicide has a long half-life (time in which the compound decreases by 50%) in the soil, reaching values of 302 days (Sensenman, 2007). This certainly contributes to the persistence of the herbicide in the environment, increasing the risk of contamination to the subsequent crop (cassava).

Studies also report possible problems arising from the use of sulfentrazone, with a negative effect on the neurological development of rats exposed to this herbicide (Castro et al., 2007). Furthermore, sulfentrazone is an endocrine disruptor (Ahmad et al., 2017), that is, it interferes with the synthesis, release, transport, binding, action, or elimination of natural hormones in the body, which are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (Crisp et al., 1998). There is a particular concern about endocrine disrupting pesticides as they are metabolism-resistant and bioaccumulate through the food chain. They can concentrate in body fats and move to the developing offspring and/or newborns through breast milk (Bila & Dezotti, 2007).

**Table 4** – Pesticide residues in cassava roots resulting from the treatment with mesotrione herbicide (120 g a.i. ha\(^{-1}\)); beyond the control and weeding.

| Parameter \(^1\) | MRL\(^2\) (mg kg\(^{-1}\)) | Control | Treatments |
|------------------|--------------------------|---------|------------|
|                  |                          |         | Weeding    | Mesotrione |
| Bifentrin\(^*\)  | 2                        | < LQ    | *          |            |
| Clomazone        | 0.05                     | 0.06    | 0.02       | 0.04       |
| Metolac\(^*\)    | Not authorized           | < LQ    | *          | < LQ       |
| Fluazifope-p-butilico | 0.02      | < LQ    | *          | *          |
| Sulfentrazone    | Not authorized           | < LQ    | *          | 0.02       |
| Chlorproprop    | Not authorized           | < LQ    | *          | < LQ       |

\(^1\) The other active ingredients researched were not detected in the analyzed material; \(^2\) MRL - Maximum residue level recommended by the Brazilian Health Regulatory Agency – ANVISA; <LQ - detected at a concentration lower than the limit of quantification; * Pesticide not identified in sampling.

The herbicide can also affect the symbiosis between rhizobia and legumes, reducing amino acids and nitrate concentration in xylem sap. This leads to less production and less amount of photos assimilates in nodules, which impairs nitrogen assimilation and fixation in soybean plants (Arruda et al., 2001).

**Conclusions**

Despite its high phytotoxicity in the early development of cassava, mesotrione shows high selectivity and viability for weed control in this crop since, in general, it did not impair the other yield variables.
Moreover, the study identified clomazone and sulfentrazone residues in cassava, indicating root contamination and high persistence of these herbicides in the environment.

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