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Response of Jabuticaba [*Plinia cauliflora (Mart) Kausel*] seedlings to herbicides

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Abstract

Jabuticaba (*Plinia sp.)* is one of the most important fruit species in the Brazilian flora; it is appreciated for fresh consumption or products produced from its fruits. The establishing of orchards requires the determination of management methods that favor the development and productivity of this species. The objective of this work was to evaluate the response of jabuticaba seedlings to application of herbicides and determine possible phytotoxicity. The experiment was carried out at the Federal Technological University of Paraná, in Dois Vizinhos, PR, Brazil. A randomized block experimental design with four replications was used, with 5 plants per experimental unit. The treatments consisted of applications of pre-emergence herbicides (Dual Gold® and Spider®) and post-emergence herbicides (2,4-D, Ally®, Enlist®, Primatop®, Fusilade®, Imazetapyr, Flex®, Plenum®, and Volcane®). The applied herbicide rates were equivalent to 50% of their commercial recommendation. A spray volume equivalent to 250 L ha^{-1} was applied, using water as diluent. The following variables were evaluated 45 days after applications: stem diameter at 1 cm above ground (cm); plant height (cm); numbers of necrotic, chlorotic, shriveled, and healthy leaves; number and length of shoots (cm); survival rate (%); and chlorophyll contents (*a*, *b*, and total). The application of the herbicides Ally®, Enlist®, and Flex® resulted in less harmful effects on jabuticaba seedlings. The lowest survival rates were found in plants in the treatments with the herbicides Dual Gold®, Volcane®, and Primatop®.

Keywords: chemical control, pre-emergence, post-emergence, selectivity

Introduction

Jabuticaba is among the most promising native trees for the fruit market due to its plasticity, as it can adapt to different edaphoclimatic conditions, which enables its cultivation in subtropical and tropical regions; it tolerates low temperatures and short-term frosts, without severe damage to plants, ensuring its survival (Kinupp et al., 2011).

In recent years, awareness of the benefits of fruit consumption for human health has increased the number of research studies (Rosa et al., 2022), focused mainly on elucidating the bioactive components in fruits that provide nutraceutical gains. In this sense, the scientific community has paid attention to the fruit biodiversity in Brazil, and studies have found that compounds in jabuticaba peels provide countless benefits to human health (Fidelis et al., 2021).

These characteristics, combined with the search for a better quality of life and the consumption of health foods, create a demand for increased availability of jabuticaba fruits in the market. However, there are few commercial jabuticaba orchards in Brazil because these fruits are mainly obtained through the extraction of natural resources, which may not meet the demands of fresh, pharmaceutical, and processed fruit markets due to the lack of an organized production chain.

Considering this potential market and the need for fruit supply, establishing commercial orchards while following technical recommendations for growing jabuticaba trees is important and still requires further studies.

Weed management in orchards is one of the main fundamental practices, mainly regarding the use of pre- and post-transplantation of seedlings. Weeds compete with plants of the crop of interest for water, light, nutrients, and space, and can serve as hosts for diseases

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and pests (Vasconcelos & Lima, 2012).

Weed competition can delay plant development during orchard establishment and hinder the initial production. Thus, control methods should include preventive, cultural, mechanical, biological, physical, and chemical methods, or their combinations, considering the weeds present in the area, terrain slope, and operational costs (Souza, 2021).

Chemical control is one of the most common methods for managing weeds, using herbicides that eliminate or inhibit the development of weeds of interest (Maciel, 2014), as their application is practical, requires little labor, and is efficient in weed control.

Guerra (2014) emphasized the importance of understanding herbicides dynamics in orchards, including their selectivity and effectiveness under different edaphoclimatic conditions. Improper application of some herbicides, such as 2,4-D, can cause stress to plants and stomatal closure after application, which affects their photosynthetic capacity (Rubert, 2021).

Herbicides approved for weed management in orchards include Ametryn, Atrazine, Diquat, Diuron, Glufosinate, Glyphosate, Linuron, Metam, Oryzalin, Paraquat, and Simazine (Vargas & Roman, 2003). Although several herbicides are available in the market, with different active ingredients and modes of action, there are currently no approved herbicides for jabuticaba orchards, and there is little information on possible plant phytotoxicity after application due to drift.

In this context, the objective of this work was to evaluated the response of jabuticaba seedlings [*Plinia cauliflora* (Mart) Kausel], suitable for planting in the field, to herbicides, regarding phytotoxicity and growth after application.

Material and Methods

The experiment was carried out at the Fruit Seedling Production Nursery of the Unit of Teaching and Research at the Universidade Tecnológica Federal do Paraná (UTFPR), in Dois Vizinhos, state of Paraná, Brazil.

Two-year-old jabuticaba trees grown from seeds were used; they were kept in 3-liter pots with a substrate composed of a mixture of a Oxisol (Latossolo Vermelho Distroferrico humico; Santos et al., 2018) and sand (3:1 v v^{-1}).

Before implementing the experiment, the plants were kept under a 50% shade screen and received daily irrigation with two shifts (early morning and late afternoon) until reaching the field capacity.

The treatments consisted of application of eleven herbicides and a control (water) (**Table 1**). The applied herbicide rates were equivalent to 50% of their commercial recommendation, aiming for better herbicide selectivity. A spray volume equivalent to 250 L ha^{-1} was applied, using water as diluent. Pre-emergence herbicides (Dual Gold® and Spider®) and post-emergence herbicides (2,4-D, Ally®, Enlist®, Primatop®, Fusilade®, Imazetapyr, Flex®, Plenum®, and Volcane®) were used.

Table 1 - Treatments and herbicides and rates applied to jabuticaba (*Plinia* sp.) seedlings.

Treatment	Herbicide	Active ingredient	Applied rate*
T1	Control		Water
T2	Spider®	Diclosulam	0.119 g
T3	Ally®	Metsulfurom-metílico	0.2 g
T4	Enlist®	2,4-D	0.05 mL
T5	Plenum®	Fluroxipir and Picloram	2.5 mL
T6	Dual Gold®	S-Metolacloro	7.5 mL
T7	Volcane®	MSMA	9 mL
T8	2,4-D	2,4-D	7.5 mL
T9	Fusilade®	Fluazifope-p-butílico	2.5 mL
T10	Imazetapyr	Imazetapir	2.5 mL
T11	Primatop®	Atrazine e Simazine	30 mL
T12	Flex®	Fomesafem	5 mL

*Rate equivalent to 50% of the herbicide commercial recommendation

The weather conditions at the time of application of the treatments were: relative air humidity of 70%, air temperature of 25 °C, and no wind. The herbicides were applied using a knapsack sprayer, with a pressure gauge and a spray boom with four nozzles (TT110.02 fan tips) spaced 0.5 m apart, operating at a pressure of 250 kPa (SANTOS et al., 2005).

After applying the herbicides, the seedlings were placed on benches in a greenhouse, with air temperature of 25°C and air humidity of 80%; irrigation was carried out manually directly on the substrate, using 2 liters of water per seedling per day.

The experiment was carried out in a randomized block design with four replications, using 12 treatments and 5 plants per experimental unit.

Stem diameter at 1 cm above ground (mm), plant height (cm), total number of leaves, and number and length of primary shoots (cm) were evaluated before applying the herbicides.

Stem diameter at 1 cm above ground (cm) and plant height (cm) were evaluated 45 days after application (DAA), as well as percentages of necrotic, chlorotic, shriveled, and healthy leaves; number and length of primary shoots (cm); survival rate (%); and chlorophyll contents (a, b and total). The differences between the results obtained before the herbicide applications and at 45 DAA were considered as increases.

Plant survival rate was determined by the percentage of live plants in relation to the total number of plants in each replication. Chlorophyll contents (*a*, *b*, and total) were assessed using a chlorophyll meter (ClorofiLOG CFL 1030, Falker), with readings taken on healthy leaves.

Morphological changes in leaves were evaluated in fully expanded leaves without visible injuries. Leaf samples were collected, placed between two expanded polystyrene plates, and cut; the sections were placed in petri dishes filled with distilled water and, then, sodium hypochlorite was added; subsequently, they were rinsed with distilled water and subjected to three drops of methylene blue dye, which is absorbed and accumulated in the cytoplasm, facilitating the visualization of structures, following a methodology adapted from Kraus &Arduin (1997).

Slides were mounted for each treatment, using an electron microscope; the anatomical structures of the leaves were observed to identify potential effects and injuries caused by the herbicides.

The data were subjected to the Lilliefors normality test, and the means of survival rate, number of necrotic, chlorotic, shriveled, and healthy leaves, number and length of primary shoots, and chlorophyll *a*, *b*, and total contents were transformed using the square root of x + 1.

The transformed or non-transformed means of the variables were subjected to analysis of variance (ANOVA) and the Duncan's multiple range test (α = 0.05), using the software Genes.

Results and Discussion

The analysis of variance showed significant effects of the treatments on almost all the variables analyzed, except for the percentages of chlorotic and necrotic leaves and stem diameter and plant height (**Tables 2** and **3**, respectively).

Regarding the survival rate of jabuticaba seedlings, the highest means were found for the control and the application of Ally®, Enlist®, Plenum®, Flex®, and Imazataphyr. The lowest means were found for seedlings in the treatments with Dual Gold®, Volcane®, and Primatop® (Table 2).

The highest mortality rates found for application of Dual Gold® (pre-emergence), Volcane® (post-emergence), and Primatop® (post-emergence) are probably due to their active ingredients and the concentrations used in the study, which hindered the seedlings' survival.

Some herbicides can affect non-target organisms (Peixoto et al., 2010), as is the case of S-methachlor present in Dual Gold®, which causes inhibition of cell division and, consequently, directly inhibits shoot and root growth (Markwell et al., 2019). Experiments with tomato applying the active ingredients S-metolachlor and atrazine showed plant death 14 days after application (Alves, 2020).

The application of the herbicide Volcane® resulted in the lowest percentage of healthy leaves, presenting the highest incidence of shriveled leaves (Table 2). Shrinking causes damage to the photosynthetic mechanism, limiting sugar production and energy for plant metabolism, restricting plant growth and development, which may lead to plant death.

Active ingredients such as 2,4-D and Picloran, which are found in the composition of products like Plenum®, 2,4-D, and Enlist®, have already been reported as compounds that cause wrinkling, depending on the application rate used. Zandoná et al. (2021) reported these symptoms in pecan trees when products containing these compounds were applied, presenting similar results to those found in the present work.

The anatomical analysis showed an intense activity of the herbicide Volcane® due to its presence in the phloem (Figure 1 B), with a transport route in the plant similar to sugars.

According to Wolf (1977), chlorophyll biosynthesis, as well as the development of chloroplasts, can be inhibited by herbicides, which affect structures of this organelle, causing loss of pigmentation; thus, it affects photosynthesis and leads to plant death.

Loss of pigmentation is shown by the results of chlorophyll contents (*a*, *b*, and total) (Table 3). The damage caused to photosynthesis by some herbicides was shown by the results of total chlorophyll content after application of Dual Gold®, Volcane®, Primatop®, and Fusilade®, which resulted in the lowest means. These four herbicides also resulted in the lowest chlorophyll *a* and *b* contents (Table 3), followed by Spider® and Imazataphyr.

Similar results were found in a study evaluating pecan trees, in which the application of atrazine (active ingredient of the product Primatop®) caused chlorosis followed by necrosis in leaves (Zandoná et al., 2021)

Despite Imazataphyr was among herbicides that resulted in the lowest mean chlorophyll *b* content, it was one of those that resulted in the highest total chlorophyll contents, which may be associated with its mode of action, inhibiting synthesis of AHAS and ALS, which cause chlorosis. The lack of homogeneity in leaf pigmentation may have led to accumulation of pigments in some leaf regions and, consequently, the evaluation overestimated total chlorophyll contents.

The chlorophyll *a* and *b* contents found in the present study, with lower means after application of

Table 2 - Survival rate (%), chlorotic, shriveled, necrotic, and healthy leaves (%), and total number of leaves in jabuticaba seedlings after application of 11 herbicides and in the control treatment

Treatment	Survival rate (%)	Chlorotic leaves (%)	Wrinkled leaves (%)	Necrotic leaves (%)	Healthy leaves (%)	Total leaves
Control	80.0 a	6.4 ns	6.31 de	8.38 ns	78.78 a	95.04 a
Spider®	45.0 bcd	5.6	6.67 bcde	18.75	68.99 a	47.94 b
Ally®	80.0 a	5.01	6.06 cde	9.38	79.55 a	61.83 ab
Enlist®	85.0 a	6.74	2.56 e	8.74	81.97 a	43.86 b
Plenum®	75.0 ab	2.64	10.88 bcde	14.33	72.16 a	52.41 b
Dual Gold®	5.02 e	5.14	20.49 bcd	11.58	62.79 a	50.15 b
Volcane®	15.0 de	0.14	74.85 a	5.84	19.18 b	32.21 b
2,4 D	45.0 bcd	3.43	16.96 bcde	5.91	73.12 a	43.27 b
Fusilade®	40.0 cd	4.75	24.92 b	7.63	62.7 a	59.63 ab
Imazataphyr	60.0 abc	2.93	12.84 bcde	11.18	73.05 a	40.17 b
Primatop®	20.0 de	2.38	22.71 bc	4.91	70.01 a	37.24 b
Flex®	60.0 abc	2.71	12.69 bcde	9.08	75.52 a	52.72 b
CV(%)	25.87	41.33	35.62	30.08	19.89	21.85

CV = coefficient of variation. Means followed by different letters in the columns are significantly different from each other by the Duncan's test (a = 0.05). ns = not significant by the F test.

Table 3 - Increases in diameter and height (cm) and chlorophyll contents (*a*, *b*, and total) in jabuticaba seedlings after application of 11 herbicides and in the control treatment

Treatment	Increase in diameter	Increase in height	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
Control	0.88 ns	47.0 ns	22.95 ab*	5.83 ab	28.78 a
Spider®	0.73	39.35	14.63 abc	6.07 ab	20.70 a
Ally®	0.73	42.7	17.28 ab	9.48 a	26.77 a
Enlist®	0.95	45.78	17.58 ab	4.78 abc	22.36 a
Plenum®	0.83	42.43	18.34 ab	3.39 bcd	21.73 a
Dual Gold®	0.7	40.0	2.46 c	0.19 e	2.65 b
Volcane®	0.9	47.0	5.83 bc	2.87 bcde	8.70 ab
2,4 D	0.73	45.95	24.85 ab	3.98 bcd	28.83 a
Fusilade®	0.83	46.28	8.78 abc	1.41 cde	10.18 ab
Imazataphyr	0.78	47.45	31.88 a	2.55 bcde	34.43 a
Primatop®	0.7	33.73	10.61 abc	0.73 de	11.35 ab
Flex®	0.73	50.43	29.48 a	5.96 ab	35.44 a
CV (%)	6.77	19.13	30.64	21.39	29.55

CV = coefficient of variation. Means followed by different letters in the columns are significantly different from each other by the Duncan's test (a = 0.05). ns = not significant by the F test.

the herbicides Dual Gold®, Volcane®, Fusilade®, and Primatop®, may have affected the survival rate (below 40%), reflecting the potential damage caused by the inhibition of photosynthetic activity.

Giraldeli et al. (2018) evaluated sugarcane plantations seven days after application of the herbicide Volcane® and found chlorotic spots on leaves that quickly evolved to necrosis. Durigan et al. (2004) evaluated the application of different herbicides to sugarcane plantations and found necrotic spots on the edges of leaves.

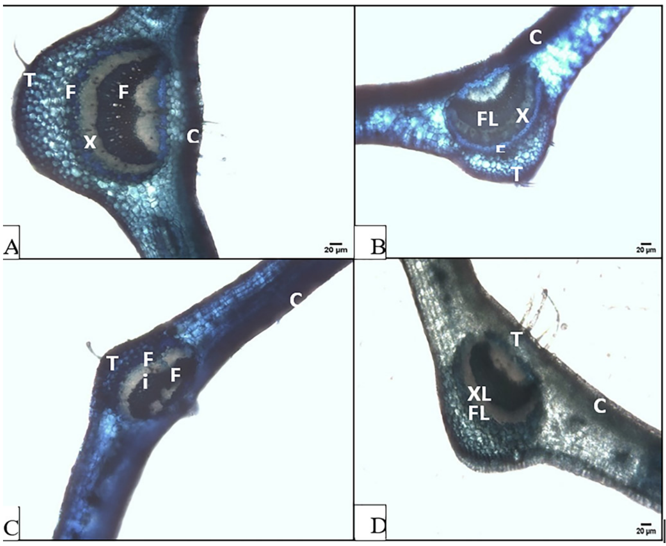
The application of the herbicides to the jabuticaba seedlings did not affect the plant growth in height and stem diameter, whose means were 43.34 cm and 0.79 mm, respectively (Table 3). The lack of significant effect of herbicides on these variables may be connected to the relatively short period of evaluation (45 days), which was not enough to express differences in stem diameter and plant height.

Tiburcio et al. (2012) emphasized that weed management in crops for which there is a limited number of approved products for chemical control should be conducted with caution, as the products used may result in injuries and productivity losses due to drift.

The visual damage observed on the leaves (**Figure 1**) indicated phytotoxicity due to the action of the herbicides, which resulted in changes in the leaf internal structures (Figures 1B, C and D) compared to the use of water (Figure 1A).

According to Foresti et al. (2015), incorrect applications of herbicides cause drift, which can damage fruit trees with potential occurrence of chlorosis e necrosis in the leaves. In eucalyptus, another species from the family Myrtaceae, this toxicity is more severe, especially for young plants (Santos Junior et al., 2015).

Furthermore, the plants exhibited druses (Figure 1), which are common in species of the family Myrtaceae; they are produced by cellular secondary metabolism and act in the defense mechanism of the plant. Structures such as trichomes and oil channels, whose function is



xl = xylem; ct = cuticle; fl = phloem; tr = trichomes.

Figure 1 - Image of a histological slide of a jabuticaba (Plinia sp.) leaf sprayed with water (A) and with the herbicides Dual Gold® (B) Volcane® (C), and Primatop® (D).

excretion, were also found. Plasmolysis of the epidermal tissue occurred in practically all the evaluated plants, indicating that the resulting injuries were severe.

The production of such structures is probably for assisting in the elimination of the herbicide molecule, as described by Carvalho (2013) and Fiuza et al. (2008).

Decreases and increases in leaf cuticle were also observed in the histological sections, which may be related to the stress caused by the application of herbicide (Figures 1B, C and D). However, further studies should be carried out to confirm this hypothesis, as there is a lack of information to assess this change.

Procópio et al. (2003) observed that trichomes intercept herbicide drops, preventing them from being absorbed by the plant's epidermis, regardless of the size and amount of this structure, characterizing them as a defense structure of the plant.

The analysis of leaf blades from jabuticaba (*Plinia* sp.) seedlings subjected to the application of herbicides showed anatomical data with some changes, which can be used in comparative studies and standardization assessments.

Morphoanatomical changes in leaves of fruit species can be caused by toxicity due to drift of herbicides (Costa et al., 2009).

Conclusion

The application of the herbicides Ally®, Enlist®, and Flex® resulted in less harmful effects on jabuticaba seedlings. The lowest survival rates were found for seedlings in treatments with the application of Dual Gold®, Volcane®s, and Primatop®. Further studies are necessary to determine ideal rates that can be recommended for jabuticaba orchards without hindering plant growth, development, and production, as this study represents preliminary research for the species.

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