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Photochemical, Anatomical, and Growth Changes in Cassava Cultivars after Application of Post-Emergent Herbicides

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Abstract: Plants develop a series of adaptive mechanisms capable of tolerating the action of herbicides; however, little is known about the physiological mechanisms developed by cassava. The purpose of this research was to evaluate the influence of post-emergence herbicides on the physiological and anatomical characteristics of two cassava cultivars subjected to six herbicide treatments. The evaluations occurred at 0, 24, 48, 72, and 168 h after herbicide application. Herbicide application induced changes in the physiological and anatomical leaf profile. These changes were observed through the thickening of the leaf blade midrib caused by the herbicides fomesafen and fenoxaprop-p-ethyl in the Campinas cultivar. On the other hand, the leaves of the Sergipana cultivar showed a reduction in the thickness of the midrib tissues. Minor effects on cassava plants were observed with the herbicide fluazifop-p-butyl.

Keywords: *Manihot esculenta* Crantz; chemical molecules; gas exchange; leaf anatomy; physiological adaptation

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1. Introduction

The global demand for food is expected to double by 2050, which implies the need to increase agricultural production to feed the world's growing population [1]. Cassava (*Manihot esculenta* Crantz) is one of the main staple foods for more than 800 million people worldwide. It has tuberous roots rich in starch and presents the potential to be cultivated in several regions of the world due to its high adaptability/resistance to adverse conditions, such as high temperatures and drought [2–8].

The slow growth of the cassava crop favors the emergence of weeds, which interfere in the cultivation, causing losses of up to 90% of commercial roots' productivity [9,10]. Initial studies on the use of post-emergent herbicides facilitate the decision-making on weed control efficiency [11].

Among the herbicides used in weed management in cassava, fomesafen is efficient at weed control by inhibiting chlorophyll synthesis and the action of the enzyme protoporphyrinogen oxidase (PPO or PROTOX) [9,12]. Additionally, fenoxaprop-p-ethyl, fluazifop-p-butyl, and clethodim are effective at inhibiting the enzyme acetyl-coenzyme-A carboxylase (ACCase).

PPO and ACCase inhibition can develop chlorosis in leaf tissues [13]. Thus, plant adaptive responses to several biotic and abiotic stresses are more accurately evidenced

in leaves as they are directly exposed to the environment, which makes leaf anatomy an important tool for understanding these processes [4,14–16].

The application of herbicides results in direct and indirect effects on the photosynthetic parameters of plants, including changes in physiological and/or phenotypic plasticity/elasticity [17–19], which are caused by hyperplasia, with reduced numbers of parenchyma cells due to the interference of cell division, causing anatomical alterations [20].

The use of herbicides in the cassava crop has been the subject of several studies [9,11,12,21], but little is known about the effect of these molecules on the physiological and anatomical characteristics of cassava during the initial growth stage.

We hypothesized that cassava cultivars could use some morpho-physiological strategies to escape the stress caused by the application of post-emergent herbicides in order to confer some advantage during crop development. To test this hypothesis, therefore, the physiological and anatomical responses of two cassava cultivars were evaluated after the application of post-emergent herbicides. These findings will be of great importance for our knowledge of herbicide selectivity and for decision making in the use of future technologies.

2. Materials and Methods

Plant material and experimental strategy: The experiment was conducted in a greenhouse at the Federal University of Alagoas, Arapiraca Campus, AL, Brazil (09°41′53.6″ S; 36°41′26.3″ W, and 264 m of altitude). Two cassava cultivars were used, Campinas and Sergipana, which are both used in industry. The two cassava cultivars have different canopy characteristics. The Campinas cultivar presents branched canopy architecture, while Sergipana has an erect canopy architecture.

The selection of the cut-stems was carried out in commercial production. The soil used as a substrate for cassava cultivation in the greenhouse was collected in this same area in the 0–20 cm-depth layer. The soil collected was air-dried and distributed in trays (10 kg tray^{-1}) of polyethylene (0.135 m^2), where cut-stems approximately 15 cm long were planted, making up four rows composed of one plant per row, and the two central rows, that is, two plants, were evaluated.

The four post-emergent action herbicides evaluated were: fomesafen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitro-benzamide); fenoxaprop-p-ethyl (Ethyl(R)-2-[4-(6-chloro-1,3-benzoxazol-2-yloxy)phe-noxy]propanoate), fluazifop-p-butyl (butyl(R)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate), and clethodim ((+/−)-2-[(E)-1-[(E)3-chloroallyloxymino]propyl]-5-[2-(ethylthio)propyl]-3-hidroxy-2-cyclohexen-1-one).

Both cassava cultivars, Campinas and Sergipana, were submitted to six with four replications treatments in a completely randomized design. The treatments consisted of: positive control (without herbicide and with weeding); negative control (without herbicide and weeding); fomesafen (250 g a.i. (active ingredient) ha^{-1}), fenoxaprop-p-ethyl (100 g a.i. ha^{-1}), fluazifop-p-butyl (187.5 g a.i. ha^{-1}), and clethodim (108 g a.i. ha^{-1}).

The herbicides were applied as recommended by the commercial product leaflet based on the concentration of the active ingredient. For the herbicides fomesafen and fenoxaprop-p-ethyl, 0.05 mL of the products were applied in 9 mL of solution; 0.02 mL of fluazifop-p-butyl was applied in 5.5 ml of solution, and 0.02 mL of clethodim was applied in 16.2 mL of solution. The applications were performed 26 days after planting (DAP), using a CO_2-pressurized sprayer with a single nozzle type Teejet XR 110.02 VP with a service pressure of 200 kPa (Herbicat, SP, Brazil). The trays were irrigated daily with a volume of 4.1 L of water, as determined by Reichardt [22].

Photosynthetic characteristics were measured daily during the first three consecutive days and on the seventh day, i.e., 0, 24, 48, 72 and 168 h after application of the herbicides, from 07:30 to 08:30 in the morning, with an initial room temperature of 27.6 °C and a final temperature of 30 °C, and presenting initial and final relative air humidity of 67.4 and 65.2%, respectively, with four readings per treatment for each cultivar.

Physiological analyses were performed with an infrared gas analyzer—IRGA (LI-6400, Photosynthesis Meter, Li-Cor Biosciences, Lincoln, Nebraska, USA). Net photosynthetic rate—*A* (μmol (CO_2) m^{-2} s^{-1}), stomatal conductance—*gs* (mmol (H_2O) m^{-2} s^{-1}), transpiration rate—*E* (mmol (H_2O) m^{-2} s^{-1}), and maximal quantum yield of photosystem II (PSII) photochemistry—*Fv/Fm* were carried out in the middle third of the first fully expanded leaf. The same leaf was used in all evaluations. For the readings of *Fv/Fm* in the dark, the leaves were previously acclimated using special paper clips to keep the leaf surface from the light.

Anatomical parameters: Four young leaves with visual symptoms of phytotoxicity were collected in each replicate 48 h after herbicide application to evaluate anatomical parameters. The phytotoxicity symptoms were identified according to EWRC (European Weed Research Council) [23]. Once collected, the leaves were immediately immersed in fixative solution FAA70% (Formol + Glacial acetic acid + Ethanol 70%) for 48 h and then transferred to 70% ethanol until the analysis. Tissue staining was done using Toluidine blue dye (1%) in 0.1 M phosphate buffer and pH 7.0, and the semi-permanent slides were assembled using a solution of water + glycerin at a proportion of 1:1 [24].

The transversal sections were made in the midrib (ADE—adaxial epidermis, ADC—adaxial collenchyma, XY—xylem, PH—phloem, ABC—abaxial collenchyma, and ABE—abaxial epidermis) and mesophyll (ADE—adaxial epidermis, PP—palisade parenchyma, SP—spongy parenchyma and ABE—abaxial epidermis) of the leaf for posterior visualization of the tissues using an optical microscope (Nova 107®—model Cx31). The photodocumentation of the anatomical images was recorded with a digital camera coupled to the microscope using a 4X digital zoom (Canon Power Short A630) and an objective lens with a magnification of ten (10X). The midrib and mesophyll tissues of the leaves were measured and analyzed using the free software Image Tool®.

Growth parameters: To obtain the growth characteristics, the plant height (PL), stem diameter (SD), number of leaves (NL), and leaf area (LA) of cassava were evaluated in the Campinas and Sergipana cultivars after the application of herbicides for weed management 33 days after planting.

Statistical analysis: Data on the physiological parameters photosynthetic rate and gas exchange rates (stomatal conductance and transpiration) and chlorophyll *a* fluorescence (*Fv/Fm*) were evaluated in a factorial scheme in a completely randomized design (treatments x times) with four replications. As the data did not fit the regression models, they were evaluated by analysis of variance (ANOVA) and compared by Tukey's test ($\alpha = 0.05$).

Anatomical parameters data were submitted to analysis of variance and the means were compared by Tukey test at 5% significance. Growth parameters were combined in a factorial scheme (treatments × cultivars) and were submitted to analysis of variance, and the means were compared by Tukey test at 5% significance using SISVAR® software [25].

3. Results

3.1. Physiological Parameters

The photosynthetic and gas exchange rates (stomatal conductance and transpiration) and chlorophyll *a* fluorescence in leaves showed changes after herbicide application in both cassava cultivars (Figure 1; Table S1). As the first parameter of differentiation among cultivars, we observed the CO_2-fixing capacity. Under positive control conditions with manual weeding, the photosynthetic rate (*A*) maintained average values that reached 15 μmol CO_2 m^{-2} s^{-1} in the Campinas cultivar, while the Sergipana cultivar presented lower values (11 μmol (CO_2) m^{-2} s^{-1}), showing that the Campinas cultivar presents a photosynthetic yield 26.4% higher when compared to the Sergipana cultivar (Figure 1A,B; Table S1).

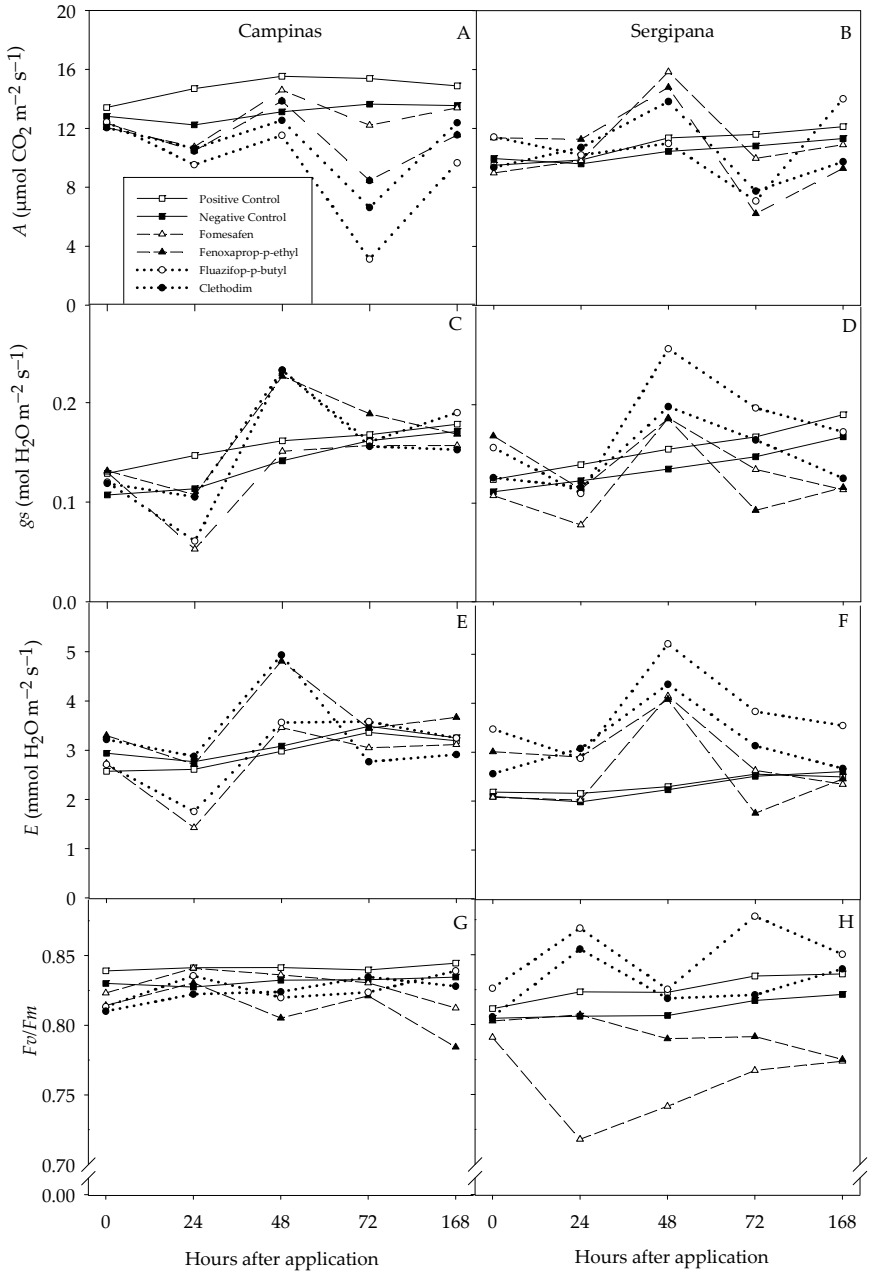


Figure 1. Net photosynthetic rate—*A* (**A**,**B**), stomatal conductance—*gs* (**C**,**D**), transpiration rate—*E* (**E**,**F**), and maximal quantum yield of PSII photochemistry—*Fv/Fm* (**G**,**H**) of two *M. esculenta* Crantz cultivars (Campinas and Sergipana) after application of chemical molecules. The data represent average values ± standard error.

In the Campinas cultivar, the *A* reduction started 24 h after the application of the treatments, chiefly with the herbicide fluazifop-p-butyl, and reached a maximum reduction of 79.7% in relation to the positive control 72 h after the application of the herbicides (Figure 1A; Table S1).

A rate increased significantly in the Sergipana cultivar 48 h after the application of the herbicides, exceeding the maximum recorded in the positive control, with a 39.4% increase when fomesafen was applied. After this period, there was a drastic reduction, with a minimum point recorded 72 h after application when a decrease of 46.3% was observed with the herbicide fenoxaprop-p-ethyl (Figure 1B; Table S1).

Stomatal conductance rates (*gs*) underwent significant changes in both cultivars, with a maximum increase 48 h after herbicide application (Figure 1C,D). The *gs* average

observed in the plants of the positive control treatment in the Campinas cultivar was around 0.16 mmol m^{-2} s^{-1}, while in the Sergipana cultivar these values were around 0.15 mmol m^{-2} s^{-1}.

At this point, the *gs* of the Campinas cultivar was most affected by the herbicide fomesafen and at 24 h a maximum reduction of 64% was registered, while at 48 h there was a considerable increase in the *gs* rate that was more prominent in the herbicides fenoxaprop-p-ethyl, fluazifop-p-butyl, and clethodim, which showed an increase of approximately 44%, with a subsequent reduction, keeping all treatments at levels close to the positive control.

Similar behavior was observed in the Sergipana cultivar for the *gs* rate, with a reduction of 44% occurring 24 h after application of the herbicide fomesafen when compared to the positive control. There was also a considerable increase after 48 h for the herbicide fluazifop-p-butyl, whose maximum value was 65.8% higher than the positive control, with reductions observed at 72 h remaining at a stable level (Figure 1C; Table S1).

Regarding the transpiration rates (*E*), the cultivars Campinas and Sergipana presented mean values of 2.9 and 2.3 mmol (H_2O) m^{-2} s^{-1}, respectively. It was noted that 24 h after herbicide application, transpiration rates in the Campinas cultivar suffered a marked reduction, especially when using the herbicides fomesafen and fluazifop-p-butyl, which showed reductions of 45.3 and 32.8%, respectively. However, 48 h after application, a satisfactory increase in *E* was observed for the herbicides clethodim (65.7%) and fenoxaprop-p-ethyl (61.5%) (Figure 1E; Table S1).

E rates remained practically constant in the control treatments (positive and negative) in the Sergipana cultivar, with a slight reduction 24 h after application of the molecules. However, the herbicide fluazifop-p-butyl surpassed the other treatments with a maximum point of transpiration observed at 48 h, 127% higher in relation to the positive control, and it was possible to verify that the fenoxaprop-p-ethyl molecule showed a maximum reduction of 31.2% at 72 h in relation to the positive control (Figure 1F; Table S1).

The analysis of the maximum quantum yield of PSII photochemistry (*Fv/Fm*) revealed that the average value of *Fv/Fm* observed in the positive control treatment for the Campinas cultivar was 0.84, while the Sergipana cultivar obtained a value 2.4% lower; that is, about 0.82, evidencing health in the efficiency of photosystems.

The *Fv/Fm* ratio showed less variation in the Campinas cultivar than in the Sergipana one (Figure 1G,H). This implies that 48 h after herbicide application there was a small reduction in PSII efficiency for all treatments in the Campinas cultivar, which was more prominent with the herbicide fenoxaprop-p-ethyl, showing a reduction of 4.3% in relation to the positive control.

In the last evaluation, at 168 h, it was possible to verify that the herbicide fluazi-fop-p-butyl maintained the *Fv/Fm* ratio between the rates observed for the positive control treatment, while in the fenoxaprop-p-ethyl treatment there was a reduction of 7% in relation to the positive control, making it lower than the standard PSII efficiency, which is 0.80, implying that this herbicide caused damage to photosystem II (Figure 1G; Table S1).

Regarding the Sergipana cultivar, treatments showed reductions in *Fv/Fm* immediately after 24 h, which was more evidenced with the fomesafen herbicide, with a 12.8% reduction in relation to the positive control; on the other hand, at 168 h the largest reductions were similar for the herbicides fomesafen and fenoxaprop-p-ethyl, at approximately 7.5% (Figure 1H). The average values of the maximal quantum efficiency of photosystem II (PSII) (*Fv/Fm*) were above the optimal value of 0.80 in treatments that did not show PSII dysfunction, but a reduction in *Fv/Fm* values was observed in plants exposed to fomesafen and fenoxaprop-p-ethyl (Figure 1G,H; Table S1).

3.2. Anatomical Parameters

Regarding leaf anatomical characteristics, plants exposed to herbicides, as well as those influenced by weeds during growth, underwent significant changes in all structures of the midrib and mesophyll (Table 1). On the other hand, anatomical alterations were less intense in plants exposed to the clethodim herbicide.

Table 1. Anatomical changes in leaves of *Manihot esculenta* Crantz of the cultivars Campinas and Sergipana when submitted to different chemical herbicides.

Campinas							
Treatments	*Midrib						
	ADE [µm]	ADC [µm]	XY [µm2]	PH [µm2]	ABC [µm]	ABE [µm]	Total [µm]
Positive Control	20.75 a	162.22 a	1022.72 d	624.71 a	88.20 a	17.68 ab	1545.46 d
Negative Control	15.97 b	162.16 a	976.57 d	611.04 a	85.93 a	13.52 c	1479.46 d
Fomesafen	12.68 b	104.82 c	2272.26 a	233.90 d	44.19 c	14.24 c	3072.90 a
Fenoxaprop-p-ethyl	12.08 b	90.30 d	2247.61 a	225.30 d	52.99 b	13.00 c	3027.02 a
Fluazifop-p-butyl	21.43 a	153.39 b	1185.15 c	380.75 c	85.75 a	18.21 a	1844.50 c
Clethodim	21.18 a	153.56 b	1332.07 b	513.79 b	49.48 bc	15.15 bc	2085.22 b

Treatments	*Mesophyll				
	ADE [µm]	PP [µm]	SP [µm]	ABE [µm]	Total [µm]
Positive Control	19.07 a	82.37 a	56.45 a	16.99 b	174.87 a
Negative Control	13.50 c	80.25 a	45.88 b	17.75 b	157.38 b
Fomesafen	11.74 c	53.23 b	36.17 b	16.88 b	118.01 c
Fenoxaprop-p-ethyl	13.56 bc	54.12 b	43.48 b	18.62 b	129.79 c
Fluazifop-p-butyl	16.96 a	79.12 a	61.93 a	26.99 a	185.00 a
Clethodim	16.62 ab	83.98 a	61.17 a	17.59 b	179.36 a

Sergipana							
Treatments	*Midrib						
	ADE [µm]	ADC [µm]	XY [µm2]	PH [µm2]	ABC [µm]	ABE [µm]	Total [µm]
Positive Control	19.00 ab	149.11 a	2457.36 b	656.54 c	68.99 c	16.90 ab	3367.90 b
Negative Control	15.72 c	138.38 a	2005.13 c	642.52 c	73.38 bc	14.50 bc	2889.65 c
Fomesafen	15.31 c	148.84 a	897.74 e	947.51 b	44.11 d	14.82 bc	2017.91 d
Fenoxaprop-p-ethyl	17.33 bc	98.42 c	1044.35 e	980.33 a	48.10 d	11.30 d	2250.26 d
Fluazifop-p-butyl	21.71 a	141.17 a	1623.04 d	356.12 d	85.66 a	14.30 c	2241.99 d
Clethodim	19.49 ab	116.91 b	2891.85 a	653.10 c	80.73 ab	17.42 a	3779.52 a

Treatments	*Mesophyll				
	ADE [µm]	PP [µm]	SP [µm]	ABE [µm]	Total [µm]
Positive Control	18.86 a	91.23 a	78.25 a	21.54 abc	209.88 a
Negative Control	17.07 ab	86.85 a	74.76 a	16.56 c	195.25 a
Fomesafen	15.66 b	56.50 b	50.00 bc	22.50 ab	144.66 c
Fenoxaprop-p-ethyl	14.50 b	52.00 b	43.25 c	25.37 a	135.12 c
Fluazifop-p-butyl	14.60 b	86.44 a	56.98 b	18.07 bc	176.09 b
Clethodim	19.59 a	86.50 a	73.45 a	19.78 bc	199.32 a

Means followed by the same lowercase letter in the column do not differ by the Tukey test at 5% significance. Legend: CV—coefficient of variation; * Legend: ADE—adaxial epidermis; ADC—adaxial collenchyma; XY—xylem; PH—phloem; ABC—abaxial collenchyma; ABE—abaxial epidermis; PP—palisade parenchyma; SP—spongy parenchyma; Total—total thickness.

On the surface of the adaxial epidermis (ADE), in the midrib region of the Campinas cultivar, the greatest reduction in tissue thickness was evident when cassava plants were exposed to the herbicide fenoxaprop-p-ethyl, with a reduction of 41.8% when compared to the positive control treatment (Table 1). In the mesophyll region, the ADE reduction was significantly higher with the herbicide fomesafen, reaching 38.4% (Table 1, Figure 2E).

In the ADE of the Sergipana cultivar, the largest reductions in the midrib region were caused by the herbicides fomesafen (19.4%) and fenoxaprop-p-ethyl (8.8%), which did not differ statistically from the negative control (17.3%), while the highest values were observed for the herbicides fluazifop-p-butyl and clethodim, which did not differ statistically from the positive control (Table 1, Figure 2M–O,S–U). Regarding the mesophyll region, a reduction of 23% was observed when the herbicide fenoxaprop-p-ethyl was used in relation to the positive control, while clethodim showed an increase of 3.9% (Table 1, Figure 2P,R,X).

Reductions in the cell layer thicknesses of the adaxial collenchyma tissue (ADE) were observed. In relation to the structures of the control plants of the Campinas cultivar, there was a reduction in the thickness of the ADC, whose values decreased by 44.3% after the application of fenoxaprop-p-ethyl. Regarding the Sergipana cultivar, the reduction in the thickness of this structure was observed only in the leaves of plants submitted to treatment with fenoxaprop-p-ethyl (a 34% reduction was observed) (Table 1).

Regarding the analysis of the cell layers of the abaxial collenchyma (ABC), it was possible to observe different responses among the two cassava cultivars studied. The largest reductions were observed after the application of the herbicide fomesafen (49.9%) in the Campinas cultivar (Table 1; Figure 2B), followed by fenoxaprop-p-ethyl (39.9%) and clethodim (43.9%) (Table 1). The opposite behavior was observed in the Sergipana cultivar, with the herbicides fluazifop-p-butyl and clethodim causing an increase in the ABC tissue thickness of 24 and 17%, respectively (Table 1; Figure 2T,U).

The area of xylem vessels (XY) in the leaves of the Campinas cultivar increased considerably when exposed to herbicides, with the largest areas of vessels found in leaves treated with the herbicides fomesafen and fenoxaprop-p-ethyl, whose increases were about 122.2 and 119.8%, respectively (Table 1; Figure 2B,C). Contrary to what was observed in the Campinas cultivar, the area of the XY vessels decreased in the Sergipana cultivar, with the largest reductions occurring when the leaves were exposed to the same herbicides, fomesafen and fenoxaprope-p-ethyl, which showed reductions of 63.5 and 57, 5%, respectively (Table 1; Figure 2N,O).

Regarding the phloem vessels (PH) present in the midrib of the leaf, they also showed some sensitivity to the action of herbicides. In the Campinas cultivar, the largest reductions were observed with the herbicides fomesafen and fenoxaprop-p-ethyl, with reductions of 62.6 and 63.9% of the vessel area, respectively (Table 1; Figure 2B,C). Reinforcing the differential response pattern of the two cassava cultivars studied to the application of herbicides, a substantial increase in the area of the leaf phloem vessels of the Sergipana cultivar was observed, which was verified when it was treated with the herbicides fomesafen and fenoxaprop-p-ethyl, whose increases reached 44.3 and 49.3%, respectively (Table 1; Figure 2N,O).

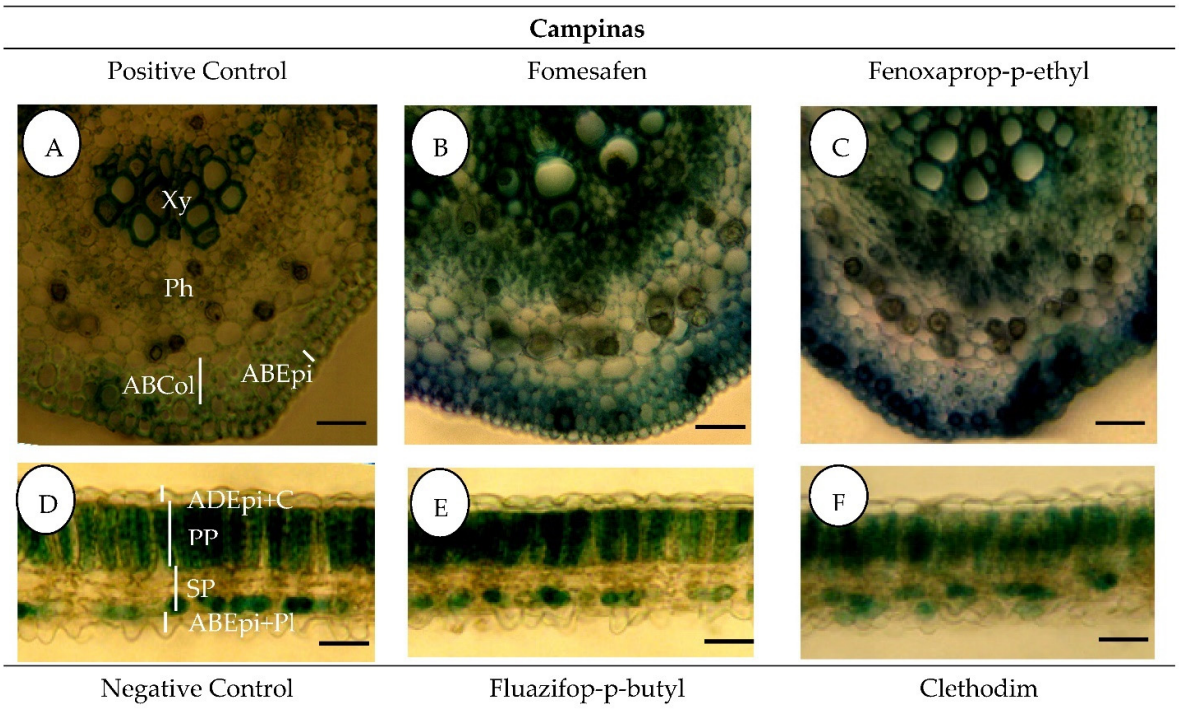
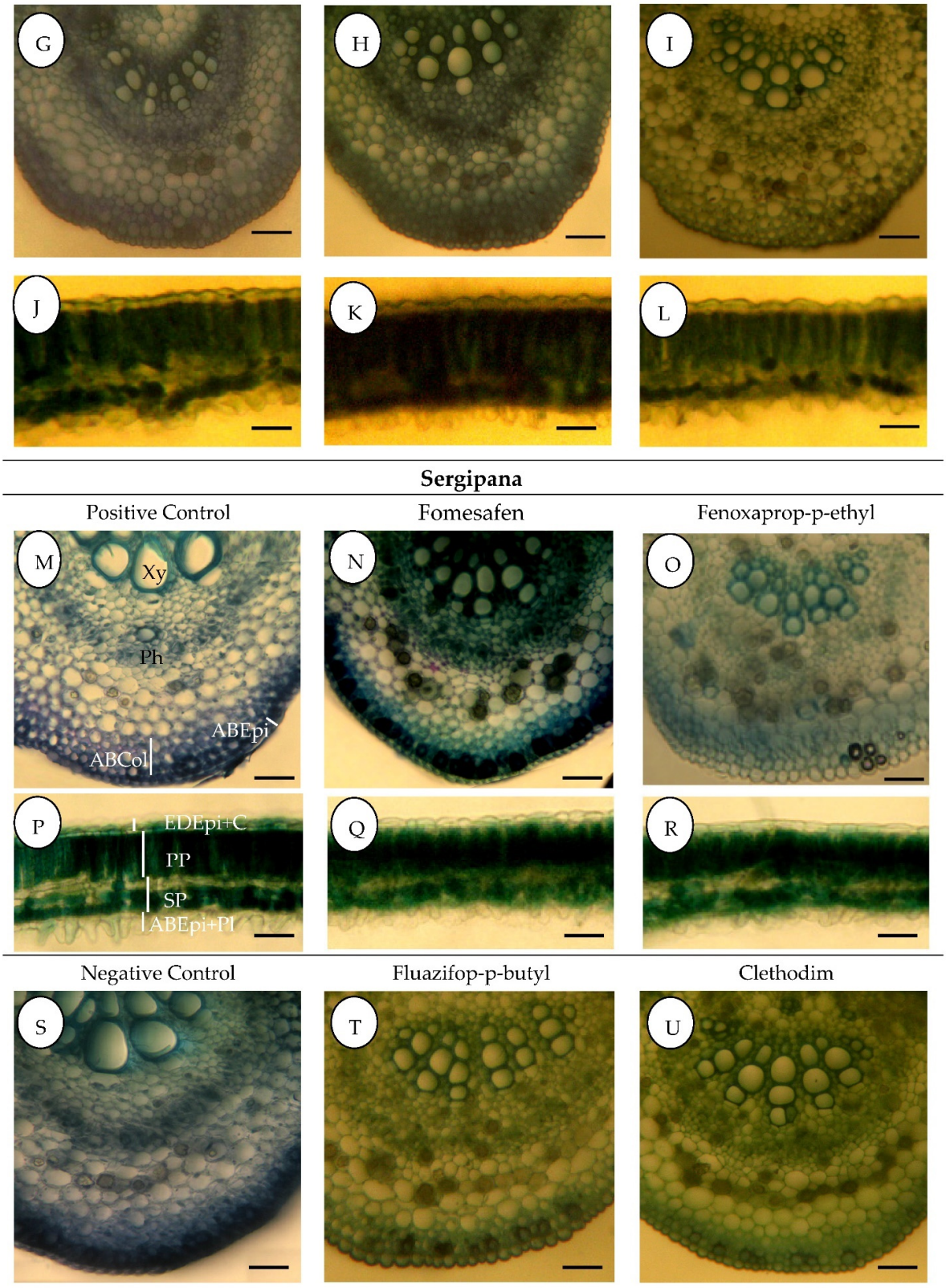


Figure 2. *Cont.*



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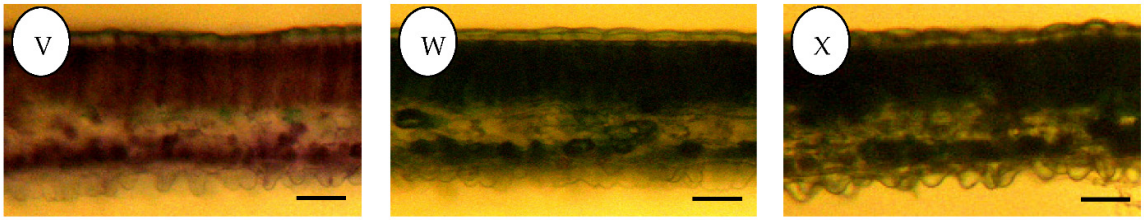

Figure 2. Leaf transversal sections of the *Manihot esculenta* Crantz, Campinas cultivar, central region of the midrib (**A**–**C**,**G**–**I**) and mesophyll (**D**–**F**,**J**–**L**), and Sergipana cultivar, central region of the midrib (**M**–**O**,**S**–**U**) and mesophyll (**P**–**R**,**V**–**X**). Legend: ADE—adaxial epidermis (µm), ABE—abaxial epidermis (µm), XY—xylem (µm^2), PH—phloem (µm^2), ABC—abaxial collenchyma (µm), PP—palisade parenchyma (µm), SP—spongy parenchyma (µm), CU—cuticle (µm), PL—papilla (µm); Bars—50 µm.

The herbicides fomesafen and fenoxaprop-p-ethyl reduced the palisade parenchyma (PP) by 35% and 34% in the Campinas cultivar and 38% and 43% in the Sergipana cultivar, respectively (Table 1; Figure 2E,F,Q,R).

The spongy parenchyma (SP), located close to the ABE mesophyll, showed a reduction in thickness when exposed to the herbicides fomesafen and fenoxaprop-p-ethyl in the Campinas cultivar, whose reductions covered approximately 36 and 23% of the leaf tissue, respectively, and did not differ statistically from the negative control (Table 1; Figure 2E–J). In relation to the same tissue, when studied in the Sergipana cultivar the largest reductions were observed when the herbicides fomesafen, fenoxaprop-p-ethyl, and fluazifop-p-butyl were used, with reductions of 36, 45, and 27%, respectively (Table 1; Figure 2Q–V).

Regarding the abaxial epidermis (ABE) of the midrib, it underwent thickness changes even without coming into direct contact with the herbicides. This variation was observed in the Campinas cultivar with respective reductions of 19.4, 26.5, and 14.3% when exposed to the herbicides fomesafen, fenoxaprop-p-ethyl, and clethodim, when compared to the positive control (Table 1; Figure 2B,C,I). Regarding the ABE mesophyll, the herbicides showed no thickness variation, except when the leaves were exposed to the herbicide fluazifop-p-butyl, which increased the thickness by 59% (Table 1; Figure 2K).

The analysis of the ABE midrib of the Sergipana cultivar leaves showed that the largest reduction was caused by the herbicide fenoxaprop-p-ethyl, with 33% (Table 1; Figure 2O). However, for the thickness of the leaf mesophyll ABE, the molecules did not differ from the positive control treatment (Table 1; Figure 2P,Q,R,V–X).

In general, the increase in the midrib region total thickness of the leaves of the Campinas cultivar was mainly influenced by the increase in the area of the XY vessels when the leaves were exposed to the herbicides fomesafen and fenoxaprop-p-ethyl, whose values were 99 and 96% higher, respectively. However, in the mesophyll region, negative effects were observed, with tissue reductions of 32.5 and 26% when plants were subjected to fomesafen and fenoxaprop-p-ethyl treatments, which did not differ from the negative control (Table 1).

The total thickness of the midrib in the Sergipana cultivar in response to herbicide application resulted in tissue reductions caused mainly by the XY vessels, whose largest reductions were observed in the treatments with the herbicides fomesafen, fenoxaprop-p-ethyl, and fluazifop-p-butyl, with values of 40, 33, and 33.4%, respectively, in addition to a considerable increase of 12% with clethodim. In the mesophyll region, there was a reduction in parenchyma tissues by 31 and 36% when the herbicides fomesafen and fenoxaprop-p-ethyl were applied, respectively (Table 1).

3.3. Growth Parameters

Weeds and application of post-emergent herbicides significantly affected the initial growth of cassava cultivars in the variables analyzed (Table 2). Phytosociological analyses of weed species that appeared during cassava cultivation were cataloged (Table S2).

Table 2. Plant height (PL), stem diameter (SD), number of leaves (NL), and leaf area (LA) of cassava in the cultivars Campinas and Sergipana after the application of herbicides in weed management 33 days after the planting.

	Cultivars							
	Campinas	Sergipana	Campinas	Sergipana	Campinas	Sergipana	Campinas	Sergipana
Treatments	PL (cm)		SD (mm)		NL		LA (cm^2)	
Positive Control	13.00 a A	7.67 a B	5.77 a A	3.17 a B	21.33 a A	21.00 a A	1463.61 a A	1039.23 a B
Negative Control	9.00 b A	6.00 ab B	3.23 c A	3.60 a A	8.00 c B	10.67 bc A	316.81 de A	314.03 c A
Fomesafen	9.17 b A	5.00 b B	4.47 b A	3.53 a B	8.33 c A	7.67 c A	235.94 e A	108.56 d B
Fenoxaprop-p-ethyl	9.50 b A	6.67 ab B	3.23 c B	4.17 a A	16.00 b A	11.67 b B	360.97 cd A	353.14 c A
Fluazifop-p-butyl	11.67 a A	7.66 a B	4.90 ab A	3.23 a B	18.67 ab B	21.67 a A	1094.97 b A	835.55 b B
Clethodim	8.33 b A	6.67 ab B	4.23 bc A	4.13 a A	10.50 c A	11.00 b A	428.61 c A	394.63 c A

Averages followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other by Tukey's test at 5% probability.

The herbicide fluazifop-p-butyl was the one that presented the best averages in the evaluated parameters, being statistically equivalent to the positive control. This was possible due to the greater weed control ability of this treatment. The lowest weed control was observed when plants submitted to the herbicide clethodim were analyzed, which showed reduced efficiency made possible by the greater dominance of the plant species (Tables 2 and S2).

Similar growth for PL was observed in plants treated with fluazifop-p-butyl herbicide, which did not differ from the positive control in either cultivar. Regarding the NL in the Campinas cultivar, a reduction of 12.5% was observed with fluazifop-p-butyl. In the Sergipana cultivar, the fluazifop-p-butyl treatment showed superiority over other treatments, not differing from the control treatment. For the LA of cassava plants, the fluazifop-p-butyl treatment showed differences of 25.2 and 19.6% in relation to the weeded control in the cultivars Campinas and Sergipana, respectively (Table 2).

4. Discussion

There were physiological and anatomical differences among the two cassava cultivars after herbicide application. The results presented in this research show that the reduction in phloem vessels area (PH) in the Campinas cultivar and xylem vessels area (XY) in the Sergipana cultivar were necessary to maintain the pressure exerted by the plant during the translocation flow of substances, which is reflected in the reduction in photosynthetic rates and gas exchange and can therefore be considered characteristics of structural adaptation due to the use of herbicides in commercial cassava plantations (Table 1; Figures 1 and 2). There is a high correlation between the physiological variables studied and the anatomical aspects of cassava [26] so that under normal conditions, without stress, the responses are more satisfactory.

In particular, cassava plants showed significant reductions in photosynthetic rates for both cultivars, which reflects changes in plant growth (Figure 1). When exposed to herbicides, physiological mechanisms change, such as reductions in photosynthetic rates or even death of herbicide-treated plants, caused by limitations of the enzymatic activity of Rubisco and several other proteins related to photosynthesis [21,27]. Similar reductions were also observed in two biotypes of *Conyza bonariensis*, one resistant and the other sensitive, in which strong phytotoxic symptoms were observed seven days after application in the sensitive biotype, showing cellular damage that caused complete inhibition of CO_2 assimilation, while in the resistant biotype these effects were practically imperceptible [28].

Previous studies with the use of the herbicide fomesafen in the cassava crop showed that this plant can present symptoms such as intoxication, necrosis, and leaf twisting as herbicide doses increase with changes in the *A*, *gs*, and *E* rates [29]. On the other hand, there are also studies with *M. esculenta* in which no changes were observed in *gs* when plants were submitted to the herbicides fomesafen and fluazifop-p-butyl [12]. These differences found for *gs* among the two cassava cultivars, Campinas and Sergipana, may be due to the differential expression of genes as a resistance factor to the applied herbicides [21].

Changes in *E* rates were observed for both cultivars under study, with an increase in rates at 48 h in plants that received treatment and, later, normalization from the 5th day after herbicide application. In line with these results, Silva et al. [12] observed an increase in *E* when using the herbicides fluazifop-p-butyl and fomesafen in cultivars of *M. esculenta*, verifying that these molecules significantly interfere in the *E* of leaves of susceptible cultivars, causing considerable losses in the photosynthetic metabolism since the leaves continue to transpire while they do not perform photosynthesis due to damages caused in the photosystems, evidencing the chlorosis of the leaves as observed after the application of the herbicide fomesafen.

Under normal conditions, high *E* rates cause higher dry matter production in cassava plants that are in full growth of their shoot and root system [12,29]. However, the application of some herbicides generates more severe reductions in photosynthetic rates and, consequently, reductions in mass gain, causing retardation by the chlorotic action of the molecules.

It is important to say that the herbicide fomesafen acts as an inhibitor of the enzyme protoporphyrinogen oxidase (PPO), whose chemical group is diphenyl ether, which acts on the inhibition of chlorophyll synthesis, interfering in the photosynthetic process. Without the normal functioning of chloroplasts, photosynthesis decreases, causing the accumulation of free radicals due to the malfunction of the chlorophyll molecules, directly affecting photosynthesis and indirectly affecting stomatal conductance and transpiration [9].

Fenoxaprop-p-ethyl, fluazifop-p-butyl, and clethodim belong to the chemical group aryloxyphenoxypropionate that acts as a barrier and inhibits CO_2 fixation, lipid synthesis, and the action of the enzyme acetyl-coenzyme-A carboxylase (ACCase). They act on the cytosol, preventing the plants from producing important fatty acids that constitute the cell membranes. Hence, they present indirect action in photosynthesis, conductance, and transpiration [13]. The ACCase inhibition presents fast chlorosis in foliar tissues, with systemic action on plant tissues and roots. However, it is important to emphasize that plants recover the chlorotic effect as new leaves appear [12,29].

The enzymatic sensitivity of cassava to the metabolization of fomesafen is related to the tolerance presented by some *M. esculenta* cultivars when submitted to herbicides, which, when in contact with the site of action of the enzymes, cause alterations in the photosynthetic process [9,21]. The results obtained by Silva et al. [9] and Silva et al. [12], report that the herbicide fluazifop-p-butyl responded promisingly in the control of weeds since it acts selectively to the *M. esculenta* crop. These reports are contrary to the one obtained in this work, in which the action of the herbicide fenoxaprop-p-ethyl as an ACCase inhibitor is evidenced.

The application of the herbicides caused changes in the *Fv/Fm* rates, especially when the plants were treated with the herbicides fomesafen and fenoxaprop-p-ethyl (Figure 1G,H). Chlorophyll fluorescence values, under normal conditions, were around 0.80 in plants without stress conditions [12]. A reduction in this parameter indicates that the PSII were damaged [30].

The photosynthetic apparatus of both Campinas and Sergipana cultivars was more affected by the herbicides fomesafen and fenoxaprop-p-ethyl; in contrast, lower damage was observed for the quantum efficiency of the PSII of the Campinas cultivar, which requires a longer period of time to present *Fv/Fm* damage and is less intense (Figure 1G,H). Significant changes in the *Fv/Fm* rates after application of *N*-(phosphonomethyl)glycine

in the *Saccharum officinarum* crop were also observed by Silva et al. [31], which showed reductions in the maximum efficiency of the photosystem.

Cassava plants are demanding in high radiation, so the luminosity is important in chlorophyll synthesis, enzymes, and all processes involving the photosynthetic yield of the plants [32]. Herbicide application interferes in the synthesis of chlorophyll and modifies the plant metabolism by reducing the chlorophyll molecule efficiency, which causes the inhibition of the biosynthesis of carotenoids in the plants and, consequently, leaf chlorosis generated by the photooxidation of the chlorophyll molecules [33].

Regarding the leaf blade total thickness reduction in the presence of herbicides (Table 1), this can be attributed to the reduced size of the cells in both palisade and spongy parenchyma tissues. The development of smaller cells can be attributed to a form of adaptation to the stress caused by the action of herbicides, such as fomesafen and fenoxaprop-p-ethyl (Table 1).

This plasticity of the foliar tissues was found by Doupis et al. [15] when working with water stress and UV-B radiation in *Vitis vinifera*. They reported an increase in the leaf tissue density, which is associated with the smaller intercellular space, as well as a higher density of mesophyll, proteins, and chlorophylls per unit of leaf area. Reduction mesophyll thickness was also reported by Figueiredo et al. [34] after plants were submitted to chemical stress.

The reduction in leaf thickness is conducive to plants that undergo some type of stress, reducing photosynthetic activity (Figure 1) and thus causing lower proportions in the carbon allocation to the leaves [15]. It is known that the increase in the thickness of the leaf mesophyll epidermis is essential to the photosynthetic process since it influences the chloroplast efficiency to reflect a greater amount of solar radiation and to avoid transpiration [35].

For the responses of the midrib and mesophyll regions of the ADE to the application of herbicides, similar effects to those observed in this study were also observed in other works [36–38]. The ADE differentiation occurs as a form of structural adaptation of the leaf tissues in response to the duration of the stress severity, as well as to the stage of development in which the crop is found [35,39,40].

Regarding the tissue thickness of the adaxial and abaxial collenchyma, the alteration of these tissues after the application of herbicides was noticeable, as well as the difference in responses among the two cultivars. In this study, the Sergipana cultivar was more stable in relation to the tissue thickness when the herbicides were applied; in other words, the non-differentiation of the tissue measured in the positive control treatment was superior to that of the Campinas cultivar (Table 1). This fact can be explained by the considerable increase in the XY vessels, with which the ADC and ABC tissues presented adequacy, acting mainly as mechanical support to the new vessel structure (Figure 2).

Collenchyma tissues are also responsible for conferring resistance to vessel tissues in response to stress since they act as a support barrier due to the pressure exerted by the xylem and phloem vessels [41]. A reduction in the collenchyma tissues has also been reported by Costa et al. [36] when using different herbicides for weed control.

Regarding the area of the vessels, the fact that calls attention to these morpho-physiological changes in the XY and PH vessels is that there is an inverse relation among them; that is, when an increase in the XY vessels area is observed there is a concomitant reduction in the PH midrib vessels area in cassava leaves.

The reduction in the PH vessels area in the Campinas cultivar can be explained by the dispersion of the molecules in the leaves facilitated by the foliar architecture of the cultivar, which allows the plasticity verified in the area of the XY vessels, which probably occurred to supply the nutritional needs occasioned by the reduction in photoassimilates as an effect of leaf chlorosis and consequently caused damage to the photosystems (Figure 2).

A lower proportion of PH vessels is a characteristic that may be related to the behavior of the cultivar in relation to the stress as a way to supply the organs that function as a drain favored by the pressure caused by the translocation of photoassimilates from the

leaves, and may also occur as a form of adaptation to the stress exerted by the herbicide in which the plants end up modifying their structures by increasing their XY vessels and plasmolysis of the parenchyma tissues, which are responsible for maintaining the structure of the tracheids during the transpiration process [39,42].

On the other hand, according to Bianchini and Corso [20] the reduction in the XY vessels area in the Sergipana cultivar evidenced by the foliar hypertrophy/hyperplasia and caused by the reduction in the collenchyma tissues and XY vessels (Figure 2) is a way for the plant to adapt to the adverse conditions, thus allowing for an increase in the area of the PH vessels as a way to supply the plant in the formation of new tissues responsible for growth.

Considering the anatomical data obtained, it can be inferred that plants of *M. esculenta* present particular adjustments that allow for the maintenance of a functional state during chemical stress. This adjustment leads the plants to reduce the area of the vessels, indicating tissue adjustment; that is, the plant has a greater capacity to expand or contract its tissues, maintaining the same internal pressure. This factor essential to maintain the processes of cell division and plant growth [40,43,44].

This proportional difference among the XY and PH vessels in response to the use of herbicides has also been evidenced in other studies [20,35,37,38,45,46]. These authors observed greater increases in the vessels, which in consequence provided a higher potential for the translocation of photo-assimilates, promoting better root development, which is the main commercial interest of the product.

Among the treatments studied, only the herbicides fomesafen and fenoxaprop-p-ethyl showed a small decrease in the thickness of the PP and SP tissues but with a higher intensity for SP in the Sergipana cultivar. The maintenance in space presented in the PP tissue can confer a high photosynthetic capacity to the plant due to the larger intercellular spaces capable of providing better conditions for chloroplast allocation. This may confer greater adaptive capacities to species or adaptive plasticity under stress conditions of high luminosity, drought, and/or herbicide-related injury, as has already been observed in other studies with *M. esculenta* Crantz [26,35,40]. Several studies have also reported anatomical changes in PP and SP tissues of other species subjected to the application of herbicides [37,38,46].

The presence of weeds, mainly observed in treatment without weeding, interfered with the initial growth of cassava, leading to the quantification of the lowest values in the biometric parameters of cassava. The growth of cassava plants was also hampered by the visual symptoms of phytotoxicity that was promoted by the herbicides fomesafen and fenoxaprope-p-ethyl, which was reflected in the reduction in plant growth in the initial stages.

5. Conclusions

The application of herbicides to cassava cultivars can undoubtedly affect the photosynthetic process and anatomically alter leaf structure and the growth of the plants. Campinas and Sergipana cultivars responded differently to the application of herbicides, especially when exposed to fomesafen and fenoxaprop-p-ethyl. The Sergipana cultivar was more sensitive to the action of the herbicides since it presented greater changes in the foliar structures based on the physiological responses.

The herbicides fomesafen and fenoxaprop-p-ethyl did not present selectivity for the cassava crop. The herbicide fluazifop-p-butyl presented the best physiological, anatomical, and growth responses, as well as selectivity to the crop, similar to the positive control.

This is an innovative work that addresses the interaction among physiology and morphology in the initial stages of growth of cassava plants after contact with herbicides. The study shows beneficial or unhealthy events that cover morpho-physiological aspects and growth.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12070950/s1, Table S1: Net photosynthetic rate—A, stomatal conductance—gs, transpiration rate—E and maximal quantum yield of PSII photochemistry—Fv/Fm of two M. esculenta Crantz cultivars (Campinas and Sergipana) after application of chemical molecules. Table S2: Relative frequency (Rf). relative density (Rd). relative dominance (RDo) and importance value index (IVI) of weed species in the culture of M. esculenta Crantz.

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