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Physiological, Biochemical and Chlorophyll Fluorescence Parameters of *Physalis Peruviana* L. Seedlings Exposed to Different Short-Term Waterlogging Periods and Fusarium Wilt Infection

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Abstract: Cape gooseberry has coped with abiotic and biotic stresses such as prolonged waterlogging periods and vascular wilt in recent years. The aim of this study was to evaluate the influence of four waterlogging periods on stomatal conductance (g_s), leaf water potential (Ψ_{wf}), plant growth, leaf photosynthetic pigments, malondialdehyde (MDA) production, proline content and chlorophyll fluorescence parameters in cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph). Two-month-old ecotype "Colombia" plants were arranged in a completely randomized factorial design in eight treatments: plants without waterlogging (control), plants with waterlogging for 4, 6 and 8 d with and without Foph, respectively. The area under the disease progress curve was higher in inoculated plants subjected to 6 and 8 d of waterlogging (55.25 and 64.25) compared to inoculated plants but without waterlogging (45.25). The results also showed a lower plant growth, g_s, Ψ_{wf}, leaf photosynthetic pigments and chlorophyll fluorescence parameters (F_v/F_m, electron transport rate (ETR), Y (II) and qP) as waterlogging periods in plants with Foph increased. However, this group of plants showed a greater proline and malondialdehyde (MDA) accumulation and a higher NPQ. In conclusion, cape gooseberry shows a low acclimation to waterlogging conditions of more than 6 d in soils with Foph.

Keywords: Andean fruit species; acclimation mechanisms; plant disease

1. Introduction

Cape gooseberry (*Physalis peruviana* L.) belongs to the Solanaceae family and is native to the South American Andean region [1]. In recent years, the cape gooseberry fruit has gained economic importance due to its nutritional properties as a source of vitamins A and C [2]. In Colombia, the crop occupied 1,025 ha with a national production of 15,112 t in 2016 [3]. Also, cape gooseberry is the second most exported fruit in the country, being Colombia one of the main world producers [4].

Cape gooseberry productivity in Colombia has been significantly affected by abiotic and biotic factors in the soil such as oxygen deficiency (waterlogging) and the presence of pathogens [5,6]. *Fusarium oxysporum* f. sp. *physali* (Foph) (vascular wilt causal agent) has generated considerable reductions in yields and planted areas in the last decade, causing on certain occasions the total economic loss of this crop [7,8]. Also, Andean fruit trees (cape gooseberry, tamarillo and lulo) have been significantly affected by climate variability phenomena (ENSO), increasing the intensity and frequency of rainfall which generates prolonged waterlogging episodes [5,9,10].

The pathogen *F. oxysporum* (FO) can survive for long periods in the soil and is difficult to eliminate [11–13]. FO infection occurs through roots via direct penetration or wounds. The pathogen obstructs the plants' xylem, generating leaf water deficit and low leaf gas exchange properties (stomatal conductance, transpiration, and photosynthesis) [14–16]. As a consequence, symptoms in the infected plants include low plant growth, leaf area and dry matter accumulation, followed by vascular tissue wilting and finally plant death [15,17–21].

Soil oxygen deficiency can also affect the leaf gas exchange properties and chlorophyll fluorescence parameters (maximum quantum efficiency of photosystem II (PSII) (F_v/F_m), actual efficiency of PSII (Y (II)), photochemical quenching (qP), non-photochemical quenching (NPQ) and electron transport rate (ETR)) [22,23]. It has been reported that stomatal conductance is a physiological parameter sensitive to waterlogging conditions [24]. Flórez-Velasco et al. [9] observed a 30% reduction of stomatal conductance in lulo plants (*Solanum quitoense* cv. *Septentrionale*) subjected to short waterlogging periods. Kallestad et al. [25] also found similar effects in pecan tree (*Carya illinoinensis*) seedlings. On the other hand, chlorophyll fluorescence parameters have been widely used as indicators of the photosystem II (PSII) activity under soil oxygen deficiency conditions [26,27]. Ren et al. [23] found a reduction of 16% in the F_v/F_m and Y (II) values in two maize (*Zea mays* L.) hybrids under short waterlogging periods (6 d). Similar results were also reported by Casierra-Posada and Cutler [28] who observed that prolonged waterlogging periods (25 d) also caused lower F_v/F_m, Y (II) and qP in cabbage (*Brassica oleracea* var. *capitata*) plants.

However, few studies have determined the relationship between soil saturation duration and severity of diseases. Yanar et al. [29] observed that growth of maize (*Zea mays* L.) plants was negatively affected by increased flooding duration and presence of the pathogen *Pythium arrhenomanes*. Additionally, Kirkpatrick et al. [30] observed that *Pythium* sp. isolation frequency increased with waterlogging in soybean plants. Also, short periods of exposure to waterlogging can stimulate the germination of fusarium spores in the soil [31].

The production of malondialdehyde (MDA) and leaf photosynthetic pigments content (chlorophylls and carotenoids) can be altered by either waterlogging or FO since these compounds are biochemical markers that indicate the intensity of the stress conditions [24,32–35]. Additionally, waterlogging promotes proline content, which is also a biochemical marker that shows the plant's acclimation response. Ren et al. [23] observed a significant increase in MDA contents under waterlogging conditions in maize plants, which indicates a negative impact on cell membrane integrity. On the other hand, Fortunato et al. [36] reported a 45% increase in MDA content in two banana (*Musa AAB* Simmonds) genotypes inoculated with *F. oxysporum* f. sp. *cubense*.

Plant proline biosynthesis is stimulated by abiotic and biotic stress conditions [37,38]. Tou et al. [39] observed a greater proline accumulation in leaves of waterlogged peach plants, concluding that it was a tolerance mechanism to cope with this stress condition. Additionally, Hao et al. [40] observed an increase in proline concentrations in cucumber (*Cucumis sativus* L.) plants after FO inoculation. Regarding leaf photosynthetic pigments, reductions in chlorophyll content under waterlogging conditions have been reported in rice (*Oriza sativa* L.) [41], pigeonpeas (*Cajanus cajan* L.) [42], wheat (*Triticum aestivum* L.) [22], and jatropha (*Jatropha curcas* L.) [43]. Rajeswari et al. [44] also observed a reduction of 74% and 69% in the total chlorophyll and carotenoids values in peanut (*Arachis hypogaea* L.) plants infected with FO, respectively.

The knowledge on the impact of combined abiotic and biotic stresses is important to understand their influence on crop growth and productivity. In this sense, the effect of combined stress factors on crops cannot always be additive, because these stresses are complex and involve numerous physiological, molecular, and cellular adaptations [45,46]. Combined studies about the interaction of waterlogging and soil borne pathogens on physio-morphological and biochemical (proline, MDA, leaf photosynthetic pigments) traits are still scarce. However, available literature only shows the effect of the combination of biotic stress (pathogens) and abiotic stress which can favor the impact of

pathogenicity via increased disease levels [47]. Drought stress increased the level of MDA in plants inoculated with leaf or soil-borne pathogens. [18,48].

Climate change models predict longer flooding periods with favorable environments for biotic stresses associated with phytopathogens [49,50]. Studies on the effect of interactions between abiotic (waterlogging) and biotic (soil-borne vascular pathogens) stresses have shown an increase in the severity of the disease under waterlogging periods. For example, Sanogo et al. [51] observed a 140% increase in vascular wilt severity caused by *Verticillium dahliae* in waterlogged chili pepper (*Capsicum annuum* L.) plants compared to non-waterlogged plants. Flooding and soil-borne diseases can cause severe crop losses of tomato during the hot and wet summer months in the tropics [52]. Villarreal-Navarrete et al. [6] also observed a greater progress of vascular wilt caused by FO in cape gooseberry under waterlogging conditions. Furthermore, these authors reported a reduction in dry matter accumulation, root length, leaf area, plant height and dry matter distribution under the same conditions mentioned above.

Increases in the intensity and frequency of rainfall are projected in Colombia for the coming years [53,54]. Consequently, studies on the response of Andean fruit trees acclimation to waterlogging scenarios have gained importance in recent years [5,9,10]. However, research on the interaction between the frequency and intensity of waterlogging periods and the incidence and severity of FO in cape gooseberry is still scarce [6]. We hypothesized that waterlogging conditions may favor the progress of the disease and condition growth and development in cape gooseberry plants. For this reason, the objective of this study was to evaluate different waterlogging periods on physiological, biochemical and chlorophyll fluorescence parameters in cape gooseberry plants infected with Foph.

2. Materials and Methods

2.1. Growth Conditions and Inoculation of the Pathogen

An experiment was carried out from November 2016 to March 2017 under greenhouse conditions at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia's Bogotá campus (4°35′56″ N, 74°04′51″ W). The environmental growth conditions during the experiment were: 25/20 °C day/night temperature, 60%–80% relative humidity and a natural photoperiod of 12 h (photosynthetically active radiation was 1500 μmol m^{-2} s^{-1} at noon). Two-month-old cape gooseberry ecotype "Colombia" seedlings purchased from a local nursery were used. Seedlings were indexed following the methodology described by [55] to discard the infection with Foph. Then, seedlings were subjected to an acclimation period for 15 days. After the acclimation period ended, the cape gooseberry seedlings were transplanted in 2 L plastic pots, containing a soil-based substrate and rice husk (3:1 v/v) with and without Foph inoculum. Before substrate inoculation, the methodology described by [56] was used to confirm the absence of Foph in the soil used for the mixture.

The inoculation with Foph was carried out by incorporating the pathogen's propagules at a concentration of 1×10^6 microconidia mL^{-1} at the time of substrate preparation prior to transplantation [4]. One hundred mL of an Foph suspension in sterile distilled water were added for every 1 kg of substrate used (soil + rice husk). The Map5 strain (Laboratorio de Microbiología Agrícola, Agrosavia, Colombia) was used as source of inoculum, which was cultured in 250 mL liquid medium of potato dextrose broth in constant agitation in an orbital incubator-shaker (Lab-Line, Melrose Park, IL, USA) at 125 rpm for 8 d at room temperature (28 °C) under dark conditions [57].

The seedlings were irrigated daily with 50 mL of a nutrient solution prepared with a complete liquid fertilizer (Nutriponic®, Walco SA, Colombia) at a dose of 5 mL L^{-1} H_2O from the transplant to the beginning of the waterlogging periods. The concentration of the nutrient solution was as follows: 2.08 mM Ca $(NO_3)_2 \cdot 4H_2O$, 1.99 mM $MgSO_4 \cdot 7$ H_2O, 2.00 mM $NH_4H_2PO_4$, 10.09 mM KNO_3, 46.26 nM H_3BO_3, 0.45 nM $Na_2MoO_4 \cdot 2H_2O$, 0.32 nM $CuSO_4 \cdot 5H_2O$, 9.19 nM $MnCl_2 \cdot 4H_2O$, 0.76 nM $ZnSO_4 \cdot 7H_2O$, and 19.75 nM $FeSO_4 \cdot H_2O$. The volume of water used was obtained by daily quantification of the plant evapotranspiration needs using the gravimetric technique described by [58].

2.2. Waterlogging Treatments

At inoculation time, two groups of 100 plants each [inoculated (substrate with Foph) and non-inoculated (substrate without Foph)] were obtained. Then, each group was divided into groups of 25 plants, which were subjected to a different waterlogging period (0, 4, 6 and 8 d) at 5 d after inoculation (DAI), obtaining eight groups of treatments: plants without waterlogging (control), inoculated plants without waterlogging, inoculated plants with waterlogging for 4, 6 and 8 d, and non-inoculated plants with waterlogging for 4, 6 and 8 d. Subsequently, the seedlings had a recovery period until the end of the trial (53 DAI).

The imposition of the waterlogging periods was performed by placing the plastic pots inside 120 L plastic boxes, which were filled with 60 L to guarantee approximately 5 cm water level over the pot´s soil surface. At the end of each waterlogging period, the pots were removed from the plastic boxes and drained until reaching the substrate's moisture field capacity. After waterlogging, the seedlings were watered during the recovery period according to the evapotranspiration requirements of each of the treatments until the end of the experiment. Additionally, the different groups of waterlogging treatments were arranged in a completely randomized design where each treatment was repeated five times. Therefore, a total of five plants were used per treatment at each sampling point. Finally, the experiment lasted 70 days.

2.3. Analysis of Vascular Wilt Severity

At the beginning of the waterlogging periods (5 DAI), the severity of vascular wilt was determined visually every three days until the end of the experiment (53 DAI), following the scale described by Moreno [59]: 0) asymptomatic plants; 1) slight hyponasty and mild chlorosis of the lower third of the plant; 2) hyponasty in between 30%–50% of the leaves and moderate chlorosis in mature leaves; 3) hyponasty between 60%–80% of the leaves and moderate chlorosis in the middle third; 4) hyponasty in all the leaves of the plant, severe chlorosis and defoliation and 5) wilting, severe defoliation and/or dead plant. Subsequently, the disease severity index was determined using Equation (1) described by Townsend and Heuberger [60]:

$$\text{Severity index } (\%) = \left(\sum (nv)/V\right) \quad (1)$$

where n is the level of infection according to the scale, v is the number of plants present in each level and V is the total number of plants evaluated.

Finally, the intensity of the disease in each treatment was estimated by calculating the area under the disease progress curve (AUDPC) by the trapezoidal integration method [61,62] using Equation (2):

$$\text{AUDPC} = \left\{\sum_{i=1}^{n-1} [(y_i + y_{i+1})/2] * (t_{i+1} - t_i)\right\} \quad (2)$$

where n is the number of evaluations, y_i and y_{i+1} are the values of the severity scale that were presented at each time of evaluation and $(t_{i+1} - t_i)$ is the time interval between evaluations. Finally, Foph presence or absence in plants of the different treatments was confirmed by planting segments taken from the base of the plant in potato dextrose agar (PDA) medium and incubating them at 25 °C at each evaluation time (13, 33, and 53 DAI) [55].

2.4. Stomatal Conductance and Leaf Water Potential

Stomatal conductance (g_s) was estimated using a portable porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA) with a range of 0 to 1000 mmol m^{-2} s^{-1} and a sample chamber aperture of 6.35 mm. Measurements were taken by clipping the sensor of the porometer onto the third fully expanded leaf of the middle portion of the canopy. g_s measurements were taken from two leaves per plot and the two readings were averaged. g_s was measured in completely sunny days between 0900 and 1100 hours.

Leaf water potential (Ψ_{wf}) was also estimated in the same leaves used to determine g_s with a Scholander pressure chamber (PMS Instruments, Albany, OR, USA). Measurements were taken midday on a sunny day with clear sky. The time between chamber pressurization and leaf excision was as brief as possible, generally less than 15 s. The leaf petiole was cut with a sharp razor from the shoot and placed in the chamber. The chamber was then sealed and gradually and slowly pressurized with nitrogen gas. The pressure increased forcing sap out of the xylem system, which is observed with a 15× magnifying glass at the cut end of the leaf petiole. The measurements of g_s and Ψ_{wf} were recorded at 13, 33 and 53 DAI.

2.5. Chlorophyll Fluorescence Parameters

The same leaves used to determine g_s were also taken for the measurement of chlorophyll *a* fluorescence parameters using a modulated fluorometer (MINI-PAM, Walz, Effeltrich, Germany). Before taking the measurements, the leaves were adapted to darkness using clips for 20 minutes. After adaptation in the dark, the maximum efficiency of the photosystem (PSII) (Fv/Fm), electron transport rate, actual efficiency of PSII (Y(II)), photochemical quenching (qP), and non-photochemical quenching (NPQ) were estimated. Then, the leaves received a pulse of actinic light of up to 2,600 μmol m^{-2} s^{-1} on the surface of the leaf to obtain the fluorescence parameters. These measurements were also taken at 13, 33 and 53 DAI.

2.6. Diameter of the Stem and Leaf Area

The leaves of each plant per treatment were collected and photographed with a digital camera (D3300, Nikon, Thailand) and saved in TIFF (Tagged Image File Format) format. The leaf area was measured from the digital images using a Java image processing program (Image J; National Institute of Mental Health, Bethesda, MD, USA). The fresh and dry weight (FW and DW, respectively) of the same leaves were measured gravimetrically. The diameter of the stem was also determined as a growth measure. These variables were also evaluated at 13, 33 and 53 DAI.

2.7. Leaf Photosynthetic Pigments

The equations described by [63] were used to estimate leaf chlorophylls and carotenoids content. Thirty mg of leaf tissue sample from the middle part of the canopy were collected and homogenized in 3 mL of 80% acetone (v/v). Then, the samples were centrifuged (Model 420101, Becton Dickinson Primary Care Diagnostics, MD, USA) at 5000 rpm for 10 minutes to remove particles. The supernatant was diluted to a final volume of 6 ml by adding acetone [64]. Chlorophyll content was determined at 663 and 646 nm, and carotenoids were determined at 470 nm using a spectrophotometer (Spectronic BioMate 3 UV-vis Thermo, Madison, WI, USA).

2.8. Proline and Malondialdehyde Content

Proline content was estimated for all treatments using the method described by [65]. Approximately 300 mg of the same leaves collected for the determination of photosynthetic pigments were homogenized in liquid nitrogen and stored for further analysis. Then, 10 mL of a 3% aqueous sulfosalicylic acid solution were added to the stored samples and filtered through Whatman paper (No. 2). Two mL of this filtrate were reacted with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid. The mixture was placed in a water bath at 90 °C for 1 h. The reaction was stopped by incubation in ice. The resulting solution was dissolved in 4 mL of toluene by shaking the test tubes vigorously using a vortex shaker and the absorbance readings were determined at 520 nm with the same spectrophotometer used in the quantification of photosynthetic pigments (Spectronic BioMate 3 ultraviolet-visible (UV-Vis), Thermo, Madison, WI, USA). Proline content was calculated using the fresh weight of the sample with a standard calibration curve (Equation (3)).

$$\frac{\text{µmol Proline}}{\text{fresh vegetal material}} = \frac{\left[\frac{\left(\frac{\mu g\ Proline}{mL} \times mL\ Toluene\right)}{\frac{115.5\ \mu g}{\mu mol}}\right]}{\left[\frac{g\ sample}{5}\right]} \quad (3)$$

The thiobarbituric acid (TBA) method described by [66] was used to estimate membrane lipid peroxidation (MDA). Approximately 300 mg of plant material were homogenized in liquid nitrogen. Samples were centrifuged at 5000 rpm, and then their absorbances were estimated at 440, 532 and 600 nm with the spectrophotometer. Finally, an extinction coefficient (157 $M \cdot mL^{-1}$) was used to obtain the MDA concentration. Proline, leaf photosynthetic pigments and MDA measurements were performed at 13 and 53 DAI.

2.9. Experimental Design and Data Analysis

Data were analyzed by a factorial arrangement where the main factor was plant inoculation (with and without Foph) and the second factor was the four different waterlogging periods (0, 4, 6 and 8 days). Each treatment group consisted of 5 plants. An analysis of variance (ANOVA) was performed and when significant differences ($p \leq 0.05$) were found, and a Tukey post hoc test was used for the comparison of means. The percentage values were transformed using the arcsine function. Data were analyzed using the Statistix v 9.0 software (Analytical Software, Tallahassee, FL, USA), and SigmaPlot (Systat Software, San Jose, CA, USA) was used for the figures.

3. Results and Discussion

The analysis of variance that shows the effect of the waterlogging treatments, *F. oxysporum* f. sp. *physali* (Foph) inoculation and their interaction on the leaf photosynthetic pigments (chlorophyll *a*, *b*, and total content and carotenoids), malondialdehyde and proline content at 53 DAI is summarized in Table 1. The analysis of variance that shows the effect between *Fusarium oxysporum* f. sp. *physali* (Foph), waterlogging periods, and sampling time on stomatal conductance (g_s), leaf water potential (Ψ_{wf},), stem diameter, leaf area (LA), foliar dry weight (FDW), the maximum efficiency of the photosystem (PSII) (F_v/F_m), actual efficiency of PSII (Y(II)), photochemical quenching (qP), non-photochemical quenching (NPQ) and electron transport rate (ETR) of cape gooseberry plants is summarized in Table 2.

Table 1. Summary of the analysis of variance of the effect of four waterlogging (W) periods on the leaf photosynthetic pigments (chlorophyll *a*, *b*, and total content and carotenoids), malondialdehyde (MDA) and proline of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at 53 DAI.

	Abbr.	**Source Variation**		
		Foph	**Waterlogging**	**Foph × Waterlogging**
Chlorophyll *a*	Chl *a*	***[z]	***	***
Chlorophyll *b*	Chl *b*	***	***	**
Total chlorophyll	Chl total	***	***	***
Carotenoids	Cx + c	***	***	***
Malondialdehyde	MDA	***	***	*
Proline		***	***	***

[z] *, **, and *** significantly different at the 0.05, 0.01 and 0.001 probability levels, respectively.

3.1. Vascular Wilt Severity

The area under the disease progress curve (AUDPC) and the disease severity index at the end of the trial (53 DAI) are shown in Table 2. All inoculated plants showed the characteristic symptoms of vascular wilt; additionally, the presence of FO was confirmed by isolation in PDA from affected material (data not shown). Plants without pathogen inoculation did not show any disease symptoms

and pathogen isolation in PDA was also negative for all cases. Significant differences were observed in the AUDPC ($p < 0.001$) and the disease severity index ($p < 0.05$). Plants subjected to 8 d of waterlogging showed the highest AUDPC values. Then, plants subjected to an intermediate waterlogging (6 d), while plants with waterlogging for 4 d and plants that were only inoculated with the pathogen showed no differences between them. On the other hand, the highest levels of vascular wilt severity were also found in 8 d of waterlogging, being statistically different from treatments of 4 and 6 d and plants inoculated with Foph under field capacity conditions (Table 3).

Table 2. Summary of the analysis of variance between *Fusarium oxysporum* f. sp. *physali* (Foph), waterlogging (W) periods, and sampling time on the physiological variables of cape gooseberry plants.

Source Variation	df	g_s	Ψ_{wf}	SD	LA	FDW	F_v/F_m	Y(II)	qP	NPQ	ETR
Foph	1	<0.001	<0.001	0.0011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Waterlogging (W)	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Foph*W	3	<0.001	0.0221	0.3399	<0.001	<0.01	<0.001	<0.001	0.2914	0.408	<0.001
Time	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time*Foph	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0051	<0.001	<0.001	<0.001
Time*W	6	<0.001	<0.001	0.0163	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time*W*Foph	6	<0.001	0.3034	<0.05	<0.001	<0.05	<0.001	<0.01	<0.01	0.149	<0.01
C.V. (%) [1]		8.78	18.53	12.7	8.42	10.11	3.69	13.1	9.56	7.2	10.98

gs, stomatal conductance; Ψwf, leaf water potential; SD, stem diameter; LA, leaf area; FDW, foliar dry weight; Fv/Fm, the maximum efficiency of the photosystem (PSII), Y(II), actual efficiency of PSII; qP, photochemical quenching; NPQ, non-photochemical quenching; ETR, electron transport rate. [1] C.V.: coefficient of variation.

Kumar et al. [67] stated that environmental factors played an important role in the incidence of diseases and also reported that the development of FO was favored by warm temperatures and dry periods in the soil. However, other authors mentioned that soil pathogens such as *Phytophthora* and *Pythium* are favored in waterlogged soil [68,69]. The present study showed similar findings in which medium (6 d) and prolonged (8 d) waterlogging periods caused a greater vascular wilt severity generated by Foph in cape gooseberry seedlings. Moslemi et al. [70] also found that FO severity was higher under 4 d of waterlogging in pyrethrum (*Tanacetum cinerariifolium*) plants. These authors stated that the greater severity in waterlogged soils could be due to the fact that hypoxia conditions weaken the root favoring infection and colonization of the pathogen.

Table 3. Effect of four waterlogging periods on the area under the disease progress curve (AUDPC) and the disease severity index of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at 53 DAI.

Treatment	AUDPC	Disease Index
F. oxysporum	45.25 c [1]	2.5 b
4 days + Foph	41.25 c	2.75 ab
6 days + Foph	55.25 b	3.75 ab
8 days + Foph	64.25 a	4.25 a
Significance	*** [2]	**
CV (%) [3]	8.29	26.23

[1] Values within one column followed by different letters are significantly different to $p \leq 0.05$ according to Tukey Test. [2] **, *** significant to $p \leq 0.01$ or $p \leq 0.001$. [3] C.V.: coefficient of variation.

3.2. Stomatal Conductance (g_s) and Leaf Water Potential (Ψ_{wf})

Differences were found between the triple interaction (waterlogging, Foph inoculation and sampling point) on g_s ($p < 0.001$) (Table 2). At 13 DAI, control plants (without waterlogging and with or without pathogen inoculation) had the highest g_s (~410 mmol m^{-2} s^{-1}). Then, a progressive g_s decrease was observed with the increase of the waterlogging periods in both inoculation conditions. The lowest g_s (~210 mmol m^{-2} s^{-1}) was obtained in cape gooseberry plants with waterlogging for 8 d (with or without pathogen inoculation). At 33 DAI, g_s continued being higher in the non-inoculated

control plants and without soil oxygen deficiency. Subsequently, a significant drop in this variable was observed due to the pathogen infection in non-waterlogged plants. Finally, g_s progressively decreased as waterlogging periods increased (4, 6 and 8 d) in both inoculation conditions. A greater influence of the pathogen on this variable was observed at 4 d of waterlogging. At 53 DAI, similar negative trends occurred with the increase of waterlogging periods in plants without inoculation. Foph presence also caused a reduction, with a greater effect on plants without waterlogging (~84.27 mmol m^{-2} s^{-1}) Figure 1).

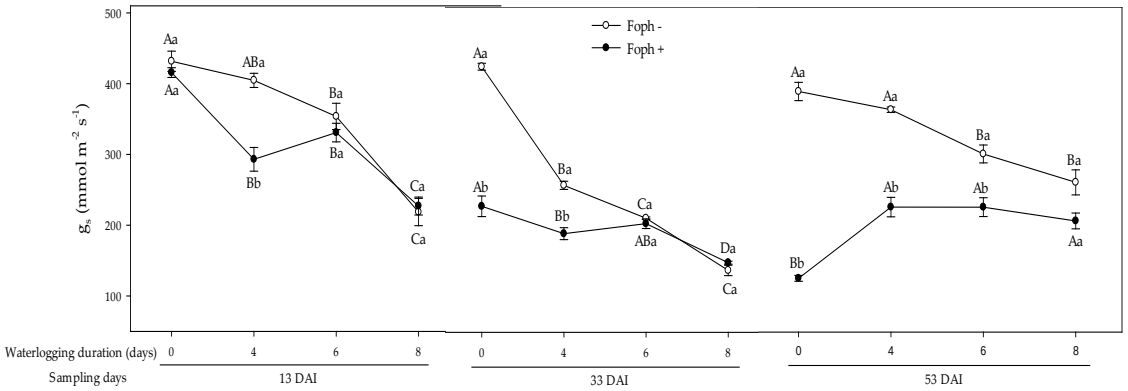

Figure 1. Effect of four waterlogging periods on stomatal conductance (g_s) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at three different sampling points (13, 33, and 53 days after inoculation (DAI)). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

Significant differences ($p < 0.05$) between waterlogging periods and Foph presence on Ψ_{wf} were only obtained at 53 DAI (Table 2). In general, the lowest Ψ_{wf} values were registered in plants inoculated with Foph regardless of the waterlogging period. On the other hand, Ψ_{wf} values were statistically different among the waterlogging periods in plants without inoculation, observing a progressive drop as the waterlogging period increased (Figure 2).

One of the initial leaf responses to the waterlogging condition is a lower g_s to avoid water loss as a result of a hypoxia condition in the soil [71,72]. In the present study, g_s was negatively affected (reduction between 30% and 40%) mainly at 6 and 8 d of waterlogging. Similar results were observed by Olmo-Vega et al. [73] where intermediate waterlogging periods (6 d) reduced g_s around 60% in three pomegranate (*Punica granatum* L.) genotypes. Herrera [74] also suggested that species sensitive to waterlogging show an initial reduction in g_s accompanied by a decrease in Ψ_{wf}.

The above was observed in the present study in waterlogged and non-inoculated cape gooseberry plants. Castonguay et al. [75] observed that alfalfa (*Medicago sativa* L.) plants showed a reduction in Ψ_{wf} of approximately 44% under a waterlogging period from 2 to 6 d. Striker and Colmer [76] mentioned that lower g_s and Ψ_{wf} f are due to a low hydraulic conductivity of the root. FO produces toxins such as fusaric acid that can also influence the stem hydraulic conductance, affecting Ψ_{wf} negatively [18,77]. In the current study, Foph presence in both waterlogging situations caused a more negative Ψ_{wf}. Dong et al. [78] also observed lower Ψ_{wf} in banana (*Musa* AAA) plants infected with *F. oxysporum* f. sp. *cubense* and stated that this reduction could have been due to a blockage of xylem elements by the structures of the pathogen.

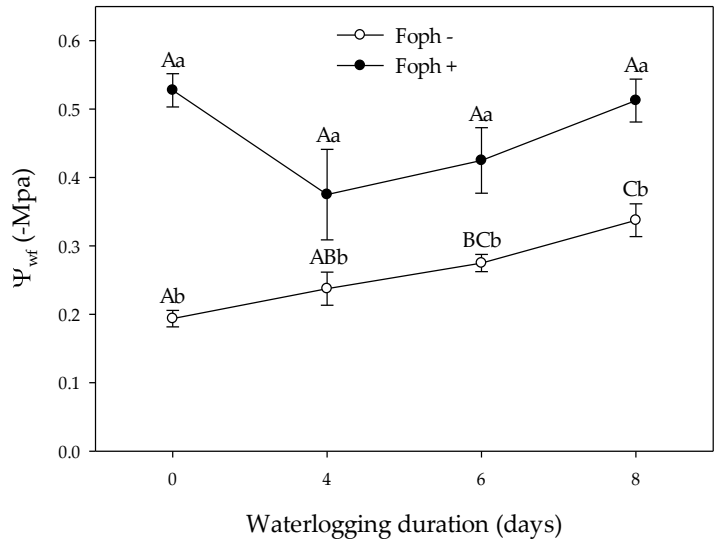

Figure 2. Effect of four waterlogging periods on leaf water potential (Ψ_{wf}) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at 53 days after inoculation (DAI). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

3.3. Growth Response

Growth parameters (foliar dry weight (FDW), leaf area (LA) and stem diameter) of cape gooseberry plants showed differences ($p < 0.05$, <0.001, and <0.05, respectively) in the interaction between the waterlogging periods, Foph presence and time (Table 2). From the second and third sampling points (33 and 53 DAI), plants without Foph presence, in general, showed higher FDW values compared to inoculated plants. In both inoculation conditions, the FDW decreased as the waterlogging periods increased, with the lowest values with waterlogging for 8 d at the above-mentioned sampling days (Figure 3).

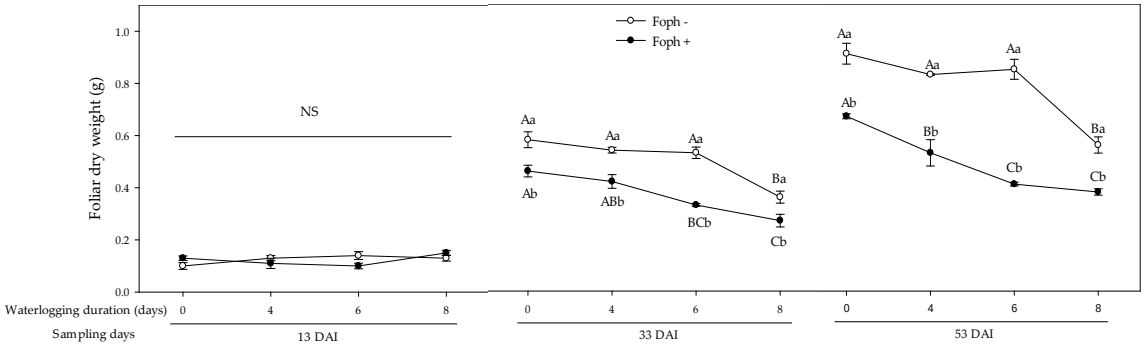

Figure 3. Effect of four waterlogging periods on foliar dry weight (FDW) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at three different sampling points (13, 33, and 53 days after inoculation (DAI)). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

Significant differences were not observed on LA and stem diameter between inoculation and waterlogging periods at 13 DAI. At 33 and 53 DAI, a progressive LA reduction was observed with

the increase of the waterlogging periods in plants with both inoculation conditions. Likewise, the inoculated plants showed a lower LA through the experiment. In addition, the lowest LA values were obtained in plants with waterlogging for 8 d and with Foph (Figure 4A). Regarding stem diameter, similar trends were observed between waterlogging periods and Foph inoculation at 33 DAI, in which an increase in stem diameter was observed as waterlogging periods increased in both inoculation conditions. However, contrasting trends were obtained on this variable at 53 DAI. Stem diameter was favored in plants subjected to different episodes of waterlogging in the absence of the pathogen. On the other hand, stem diameter increased when plants with Foph were subjected to 4 d of waterlogging; then, longer waterlogging periods (6 and 8 d) caused a drop in this variable (Figure 4B).

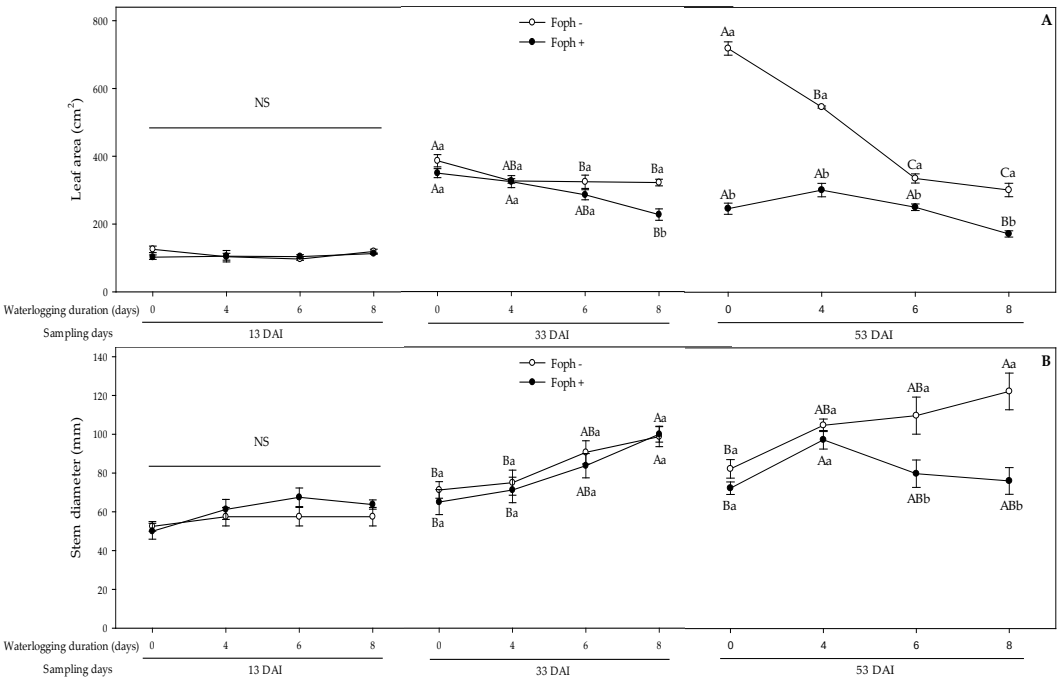

Figure 4. Effect of four waterlogging periods on leaf area (LA) (**A**) and stem diameter (**B**) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at three different sampling points (13, 33, and 53 days after inoculation (DAI)). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

The combination of abiotic and biotic stresses has an impact on plant growth [46]. Therefore, the interaction between waterlogging and the presence of Foph caused a lower plant growth (LA, FDW and stem diameter) in this study, which was mainly observed at 53 DAI. Villarreal-Navarrete et al. [6] also observed a decrease of 57.3% in the values of the stem diameter and 42.1% in LA values in cape gooseberry plants with 6 d of waterlogging and inoculated with FO compared to control plants. Suzuki et al. [79] reported that the interaction between abiotic and biotic (pathogen) stresses can also generate alterations in physiological traits such as photosynthetic activity (photosystems efficiency or rubisco enzyme activity), hormonal signaling or nutritional status. On the other hand, the present work also showed that the stem diameter of cape gooseberry plants was favored mainly by the increase in the duration of waterlogging in non-inoculated plants. Flórez-Velasco et al. [9] also observed a greater stem diameter in lulo plants with a period of 4 d of waterlogging and mentioned that an increase in the thickness of the stem may be due to the formation of aerenchyma that facilitates the transport of oxygen from the stems to the roots under hypoxia or anoxia conditions. On the other hand, a decrease in stem diameter was observed from a waterlogging period of 6 d in inoculated plants. The reduction

of stem diameter in this group of plants was related with the plant infection since the fungal hyphae cause the reduction of the diameter of the xylem vascular elements. This causes a higher resistance to water flow which results in leaf vascular wilt due to water deficiency [77].

3.4. Biochemical Tests on Leaves (Malondialdehyde Content, Photosynthetic Pigments and Proline)

Significant differences in the interaction between waterlogging periods and inoculation on leaf chlorophyll a ($p < 0.001$), chlorophyll b ($p < 0.05$), total chlorophyll ($p < 0.001$), carotenoids ($p < 0.001$), MDA ($p < 0.05$) and proline ($p < 0.001$) were found only at 53 DAI (Table 1). In general, leaf photosynthetic pigments content (chlorophyll a, b, total and carotenoids) was lower in plants inoculated with Foph and/or subjected to 4, 6 and 8 d of waterlogging compared to plants without any stressful condition (Figure 5A–D). However, MDA contents increased under both stress conditions, with higher values observed in plants with Foph and with waterlogging for 8 d (~14 μmol g^{-1} FW) (Figure 6A). On the other hand, proline accumulation was mainly favored with the increase of the waterlogging periods, whereas proline synthesis was not stimulated by Foph presence (Figure 6B).

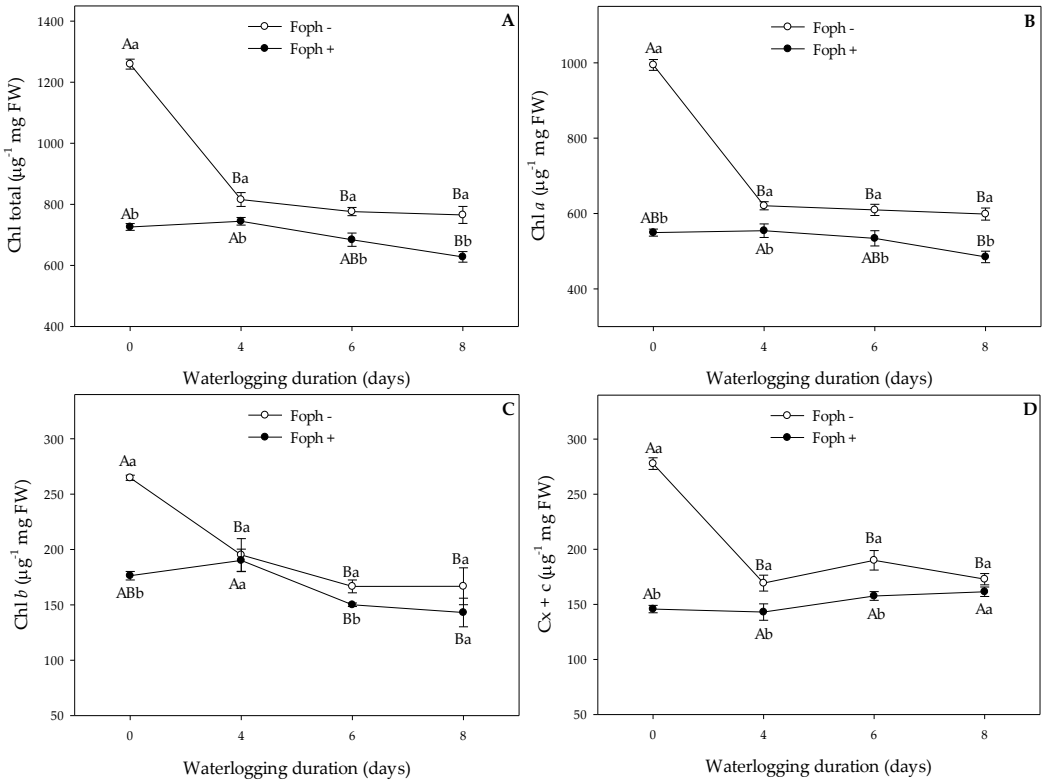

Figure 5. Effect of four waterlogging periods on chlorophyll total (Chl total). (**A**), chlorophyll *a* (Chl *a*) (**B**), chlorophyll *b* (Chl *b*) (**C**) and carotenoids (Cx+c) (**D**) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at 53 days after inoculation (DAI). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

It has been widely reported that leaf photosynthetic pigments content (chlorophylls a, b and carotenoids) and biochemical markers such as proline and MDA may indicate plant susceptibility or tolerance to abiotic or biotic stress conditions [80–82]. In the present study, leaf photosynthetic pigments were negatively affected by waterlogging and/or Foph. Alwathnani and Perveen [83] also found that tomato plants (*Solanum lycopersicum* L.) infected with *F. oxysporum f. sp. lycopersici* showed an 80% decrease in leaf chlorophyll content. Leaf chlorophyll degradation was also observed in Andean

fruit trees under conditions of soil oxygen deficit [5,9,10]. A low leaf chlorophyll content under both factors studied may be due to the fact that FO has the capacity to produce fusaric acid (pathogenicity factor), which alters the plant's metabolism and favors leaf photosynthetic pigments degradation [11]. Also, waterlogging periods can cause an increase in chlorophyllase activity (chlorophyll degradation) as well as in the ethylene synthesis, which generates leaf senescence [24,42].

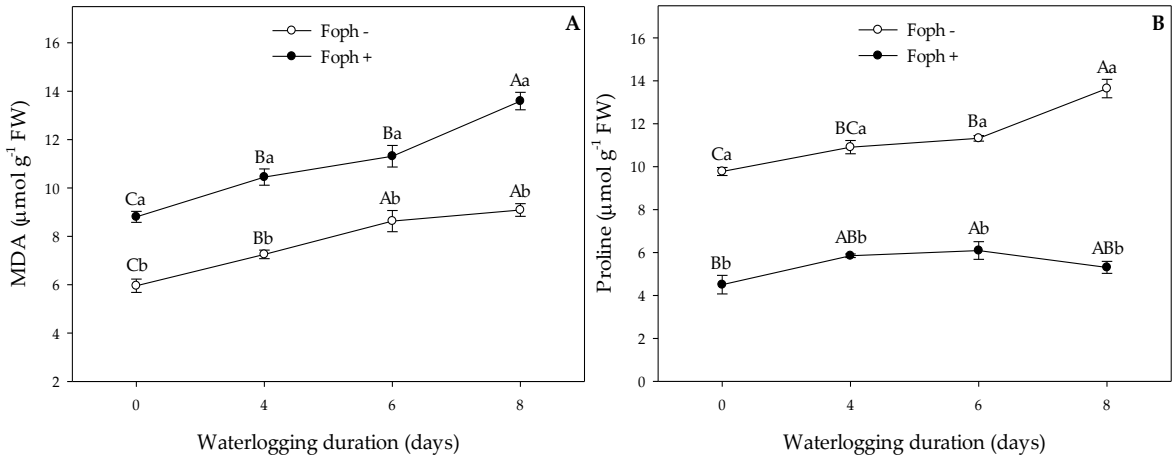

Figure 6. Effect of four waterlogging periods on malondialdehyde (MDA) production. (**A**) and proline content (**B**) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at 53 days after inoculation (DAI). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

A high MDA accumulation indicates a greater damage in the cell membrane stability due to stressful conditions [84]. Higher MDA contents have been reported under waterlogging conditions in tomato or FO presence in pigeonpea, mung (*Vigna radiata*) and black bean (*Vigna mungo*) plants [33,35]. On the other hand, proline is a molecule that confers plant tolerance under different stress conditions; an increase of this amino acid can suggest a plant acclimation mechanism [81]. In this study, proline synthesis was only favored by soil oxygen deficit (Figure 6B). Pérez-Jiménez et al. [85] also observed a greater proline accumulation (>60%) in four sweet cherry (*Prunus avium* L. Batsch) genotypes with waterlogging for 7 d compared to genotypes without waterlogging. In this regard, a higher proline content in plants under waterlogging conditions may help to maintain a normal plant osmoregulation and/or act as a compatible solute to adjust the osmotic potential in the cytoplasm [84,86]. However, proline content did not show any variations in Foph inoculated plants under different waterlogging periods in this study. Sun et al. [18] did not observe any variations in proline production in cucumber (*Cucumis sativus* L.) plants inoculated with *F. oxysporum* f. sp. *cucumerinum*. Additionally, these authors stated that FO presence affects plant nitrogen metabolism, conditioning the synthesis of this molecule.

3.5. Chlorophyll Fluorescence Parameters

Table 2 shows that differences between waterlogging, Foph inoculation, and sampling time were obtained on Fv/Fm ($p < 0.001$), Y(II) ($p < 0.01$), ETR ($p < 0.01$) and qP ($p < 0.01$).

Differences were only obtained between inoculation treatments at 13 DAI. Nevertheless, a lower Fv/Fm ratio was recorded in cape gooseberry plants only inoculated with Foph at 33 DAI. At the end of the experiment (53 DAI), Fv/Fm ratio showed a progressive decrease in relation to the higher soil oxygen deficit period. Also, Foph caused a greater affectation of this ratio in plants under waterlogging for 8 d compared with inoculated plants under 4 and 6 d of waterlogging, respectively (Figure 7).

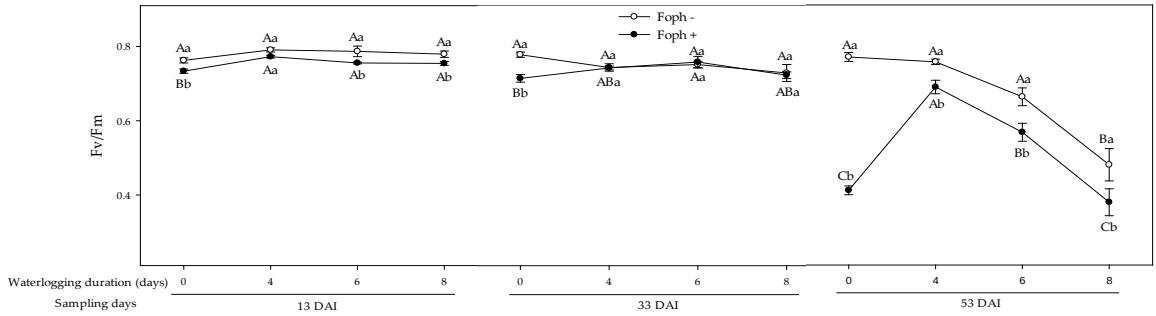

Figure 7. Effect of four waterlogging periods on maximum efficiency of the photosystem (PSII) (F_v/F_m) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at three different sampling points (13, 33, and 53 days after inoculation (DAI)). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

In general, differences between interaction factors were not recorded in Y(II) and ETR at 13 DAI. At 33 DAI, Y(II) and ETR values decreased as the waterlogging periods evaluated increased in non-inoculated plants. Foph inoculation caused a drop of approximately 45% in these variables, mainly at 0 and 4 d of waterlogging. At 53 DAI, similar trends were maintained, in which Y(II) and ETR values decreased when waterlogging periods (4, 6 and 8 d) increased in plants without Foph inoculation. On the other hand, Foph presence also influenced Y (II) and ETR values, observing a similar reduction (>50%) in inoculated plants in the different waterlogging periods (Figure 8A,B).

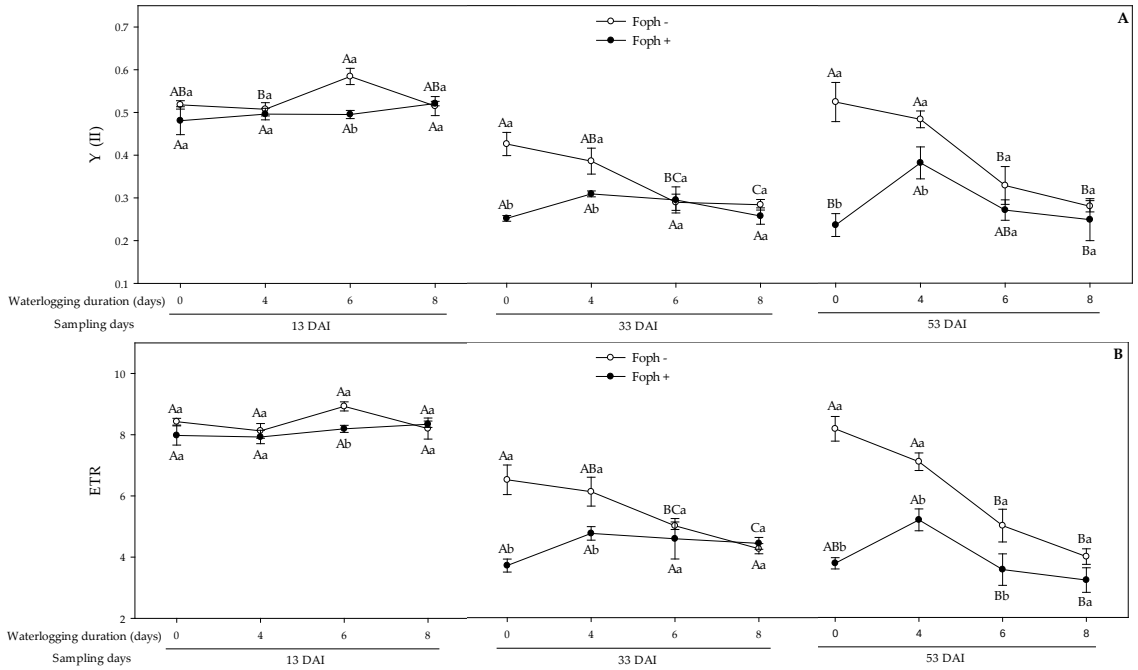

Figure 8. Effect of four waterlogging periods on actual efficiency of PSII (Y(II)) (**A**) and electron transport rate (ETR) (**B**) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at three different sampling points (13, 33, and 53 days after inoculation (DAI)). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

The qP and NPQ did not show any significant differences between factors interaction (waterlogging periods and Foph inoculation) in the first sampling point (13 DAI). However, qP started to show differences between the evaluated factors separately at 33 DAI, where plants subjected to the longest waterlogging periods (6 and 8 d) had the lowest value (Figure 9A).

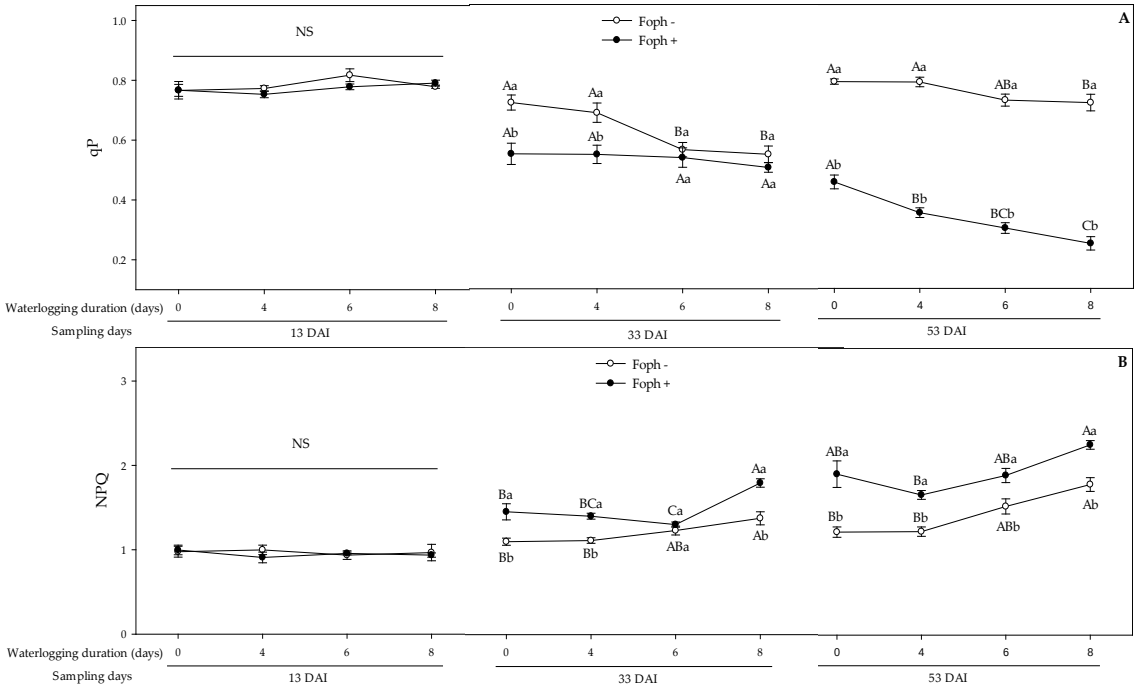

Figure 9. Effect of four waterlogging periods on photochemical quenching (qP) (**A**) and non-photochemical quenching (NPQ) (**B**) of cape gooseberry plants infected with *Fusarium oxysporum* f. sp. *physali* (Foph) at three different sampling points (13, 33, and 53 days after inoculation (DAI)). The data represent the mean of five plants ± standard error per treatment (n = 5). Capital letters refer to differences between waterlogging periods under each inoculation condition. Lower case letters refer to differences between inoculation treatments within the same waterlogging period. The same letters indicate that means are not statistically significantly different at $p \leq 0.05$.

Furthermore, Foph inoculation factor showed that plants with pathogen presence registered the lowest qP values. A significant interaction at 53 DAI was observed between waterlogging and inoculation in which plants without Foph under the different waterlogging periods recorded the highest qP values. Additionally, Foph presence caused a drastic fall in this variable, which was accentuated by the different waterlogging times (Figure 9A). Regarding NPQ, opposite results were found, where this variable was higher in plants with Foph and subjected to the four different waterlogging periods evaluated at both 33 and 53 DAI (Figure 9B).

The measurement of chlorophyll *a* fluorescence is a rapid and non-destructive technique that allows estimating the plant's tolerance or acclimation level to abiotic and biotic stress conditions [87,88]. Parameters such as Fv/Fm, qP, Y(II), ETR and NPQ have been applied as plant acclimation indicators to stressful conditions [84,87,89]. In this sense, Fv/Fm ratio is a sensitive indicator of the environmental effects on the plant acclimation [22]. In the present study, Fv/Fm was affected both by the waterlogging periods and the pathogen presence. Zhang et al. [90] also found a decrease in Fv/Fm values in ninebark (*Physocarpus amurensis* Maxim.) plants with a waterlogging period of 16 d. On the other hand, the inoculation with *F. oxysporum* f. sp. *cubense* and f. sp. *ciceri* also caused a decrease in Fv/Fm in banana (*Musa* AAA) and chickpea (*Cicer arietinum* L.) plants, respectively [91,92].

The qP is a parameter that is used to estimate PSII saturation level under stress conditions [93]. A lower qP has been reported under waterlogging conditions in avocado (*Persea americana* Mill.) plants inoculated with *Phytophthora cinnamomi* [94]. Similar results were also reported in this study,

in which cape gooseberry plants inoculated with Foph under the different waterlogging periods showed a decrease in qP. Lower Fv/Fm and qP under stress conditions (waterlogging and Foph) may be due to the fact that these factors cause leaf photosynthetic pigments degradation, a high ethylene and reactive oxygen species (ROS) production which affect the biochemical function of chloroplasts [24,91,92]. Likewise, NPQ is an important plant photoprotective mechanism to abiotic and biotic stress conditions [95]. In this study, high NPQ values were mainly observed in cape gooseberry plants inoculated with Foph and subjected to waterlogging periods. Zhou et al. [96] also observed a higher NPQ in sweat weed (*Kosteletzkya virginica* L.) plants with waterlogging for 35 d. High NPQ values have also been reported in tomato plants after inoculation with FO [97,98]. A higher NPQ indicates a dissipation of excess energy in the PSII in the form of heat as a plant protection mechanism to stress [84,99].

4. Conclusions

In summary, this study revealed that cape gooseberry plants are susceptible to waterlogging periods of over 6 d, which favor the severity of vascular wilt caused by Foph. The interaction between waterlogging and the pathogen caused important physiological disorders such as a decrease in stomatal conductance, leaf water potential, leaf area, proline and fluorescence parameters of chlorophyll *a* (F_v/F_m, ETR, Y(II) and qP). In addition, longer waterlogging periods caused stem diameter reduction in inoculated plants. NPQ and malondialdehyde (MDA) can also be considered as physiological and biochemical markers to quantify plant susceptibility to combined stress, since higher NPQ and MDA indicate that the plant can be seriously compromised. The results obtained suggest that cape gooseberry plants ecotype "Colombia" have a low acclimation to events where waterlogging periods of more than 6 d can be expected in soils with the presence of the pathogen *F. oxysporum* f. sp. *physali*. Additionally, the use of chlorophyll fluorescence parameters can be considered a reliable tool for understanding cape gooseberry acclimation mechanisms to abiotic and biotic stress conditions.

Author Contributions: C.C.C.-A. conducted the experiments, performed the analyses, collected the data and wrote the manuscript. S.G.-C. Supervision, review and edited the manuscript. H.R.-D. conceived and designed the experiments, read and edited the manuscript. All authors approved the final manuscript.

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