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Physiological and Nutritional Responses to Ozone Application in Tomato Seedling Plants

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Abstract: Research on environmentally friendly techniques for the agroindustry is growing constantly. In this sense, the supply of ozone (O_3) has been taken into consideration, especially for disinfection because of its high oxidizing power. However, there is not enough information about the application of dissolved O_3 via fertigation in crops. For that reason, in this study, two trials were carried out simultaneously to determine the consequences of O_3 application on plant growth and quality of tomato plants. The first trial aimed to assess the effects on tomato fertigated with the nutrient solution and the application of O_3. The second trial was performed to establish the ideal O_3 supply rate for tomato plants. In both experiments, we measured the biometric, physiological, and nutritional parameters of the tomato plant. The results obtained showed that the application of O_3 treatment resulted in the highest overall dry weight gain, whereas O_3 application decreased leaf proline and total soluble sugars concentrations. There was no clear effect on chlorophyll and total soluble sugars in tomato plants under O_3 application. Regarding nutritional parameters, the application of O_3 led to a higher content of P and K in tomato plants. These findings indicate that the use of dissolved O_3 via fertigation may present several advantages for tomato plants' growth and quality.

Keywords: chlorophyll; nitrogen; phosphorus; potassium; tomato; ozone

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1. Introduction

Since the 1990s, O_3 has become an important input in numerous agricultural industries for its possible applications, such as the disinfection and oxidation of organic impurities [1]. For instance, the majority of O_3 studies are related to its disinfectant power, aiming to develop environmentally safe alternatives to reduce the use of methyl bromide (CH_3Br) (banned in the European Union) for soil fumigation. Besides the disinfectant power, another advantage of the application of dissolved O_3 via fertigation is the increase in plant yield and reduction in soil pathogen pressures without detrimental environmental effects [2].

The main disadvantage of O_3 use is its high instability under certain conditions, such as pressure and temperature [3]. Nevertheless, it is important to point out that the use of O_3 is an environmental and economic sustainability practice. Therefore, it can be considered a natural compound, which decreases chemical use effectively without producing any negative byproducts [4].

For these reasons, the application of O_3 has been experimented on different crops for the treatment of soil pathogens, such as *Fusarium oxysporum*, resulting in a high mortality with a concentration of O_3 as low as 0.84 mg L^{-1}, *Phytophthora sojae* with a 100% reduction in the pathogen at a concentration of O_3 of 0.47 mg kg^{-1} of soil and nematodes, demonstrating that O_3 LD_{50} on nematodes is lower than 0.5 mg kg^{-1} of soil [3]. Treatments with O_3 have also been researched for irrigation under different growing conditions in crops, such as pepper under greenhouse conditions [5], or *Brassica rapa* L. var. Perviridis under controlled

conditions [6], showing enhanced growth compared to the control treatment without O_3. Additionally, impacts of aqueous O_3 have been tested in hydroponic tomato (*S. lycopersicum* cv Matrix F_1) yield, being applied via drip irrigation, with observed significant increases in leaf area, shoot dry matter, and also the prevention of algae persistence on the substrate surface [7].

Despite the benefits described above, researchers have addressed for many years the consequences of elevated levels of tropospheric O_3 on agricultural species, finding negative impacts on crop productivity, especially in physiological processes that are inhibited by O_3 exposure that lead to suppressed plant growth and yield [8]. In addition, the Environmental Protection Agency (EPA) established on 26 October 2015 (80 FR 65292) a recommended O_3 level of 0.070 ppm (140 μg m^{-3}) for a daily maximum 8 h concentration [9]. However, in the European Community, the threshold limits are between 180–240 μg m^{-3} (Directive 2008/50/CE), also included in Spain by the law 34/2007 of air quality [10].

Therefore, the aims of this study are to assess which are the effects on tomato growth performance in two different approaches fertigated with dissolved O_3 supply. The first scenario involves irrigation with water or fertigation with a nutrient solution, as well as the application of O_3. The second scenario determines the ideal concentration of O_3 delivery under fertigation with a nutrient solution based on changes in tomato plant biomass, physiology, and nutrition.

2. Materials and Methods

2.1. Cultivation Conditions

The University of Almeria's greenhouse (mono-tunnel) served as the site for the two experiments. With the help of HOBO SHUTTLE sensors, the weather inside the greenhouse was recorded (model U12–13, Onset Computer Corp., Bourne, MA, USA). Throughout the duration of the experiment, the average values for temperature, relative humidity, and photosynthetically active radiation (PAR) were 27.05 ± 1.4 °C, 62.89 ± 2.4 %, and 16.62 ± 1.3 MJ m^{-2} day^{-1}, respectively. For cultivation, 12 m^2 and a height of 0.80 metal tables were employed.

2.2. Plan Material

The seedlings of *S. lycopersicum* L. var. Astuto (HM Clause, Almeria, Spain) plants were acquired from a neighboring commercial nursery for use in the experimental investigations. Then, they were transferred to plastic containers (4L of volume) filled with growing medium (blond peat moss) (Kekkila Professional; Projar, Valencia, Spain). Both studies lasted 50 days (from 26 April to 15 July 2019) with a 2 plants m^{-2} planting density.

2.3. Experimental Design

Tomato seedlings were used in two different experiments performed simultaneously. The first experiment was conducted to determine the physiological and nutrient uptake differences between tomato plants irrigated with water, nutrient solution, and/or O_3. The experimental design was a split-plot bifactorial with four blocks and four plants (pots) per block and treatment (with or without fertigation and with or without supply of dissolved O_3). The second experiment examined the physiological and nutrient uptake effects of an increasing O_3 concentration in the nutrient solution on tomato plants. Four concentration levels of O_3, four blocks, and four plants (pots) per block (Table 1). The schematic view of the experimental design is depicted in Figure 1. The chemical composition of irrigation water and nutrient solution (NS) is presented in Table 2.

Table 1. Description of the different treatments performed in each experiment. (NS_0) tap water, (NS_1) treatment supplying fertigation with the nutrient solution (NS) and O_0; $O_{0.18}$; $O_{0.36}$; $O_{0.54}$: fertigation with ozone supplies with the nutrient solution at different levels: 0, 0.18, 0.36 and 0.54 mg L^{-1}; respectively.

	Treatments	O_3 Supply
Experiment 1	NS_0	O_0
	NS_0	$O_{0.54}$
	NS_1	O_0
	NS_1	$O_{0.54}$
Experiment 2	NS_1	O_0
	NS_1	$O_{0.18}$
	NS_1	$O_{0.36}$
	NS_1	$O_{0.54}$

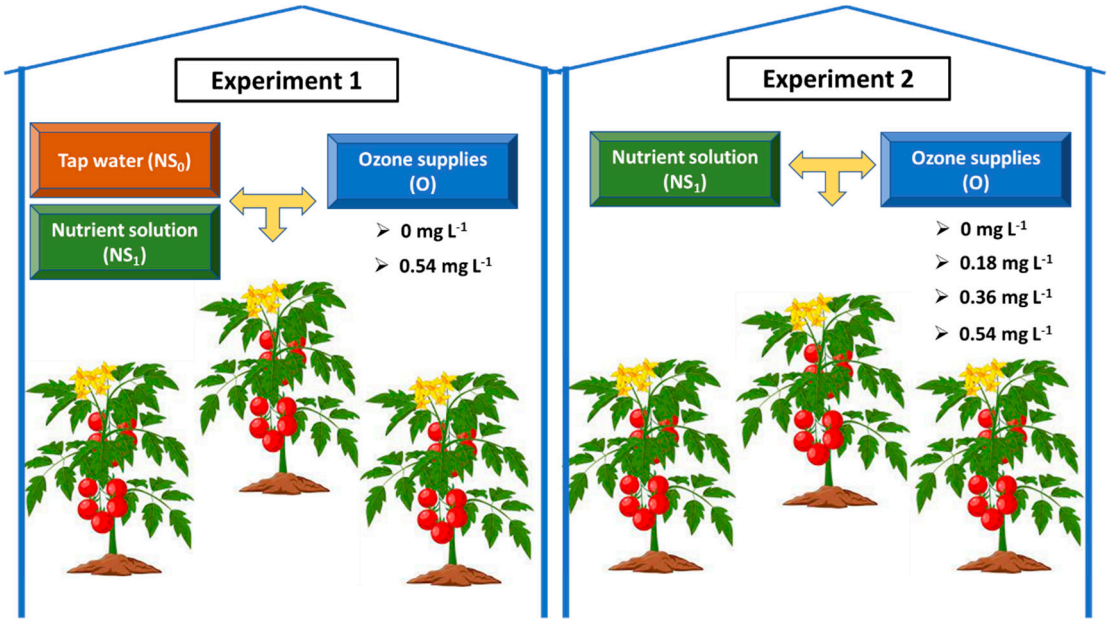

Figure 1. Schematic representation of the experimental setup.

Table 2. Chemical composition of the tap water and nutrient solution (NS) after ozone saturation with the values of electrical conductivity (EC) (dS m^{-1}) and macronutrients concentration (mmol L^{-1}).

	EC	pH	NO_3^-	HPO_4^{2-}	SO_4^{2-}	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Na^+
Water	0.9	8.1	0.0	0.0	1.1	3.5	0.0	2.0	1.4	2.6
NS	1.3	6.0	6.0	0.7	1.1	3.5	3.0	2.0	1.4	2.6

The NS used in the experiment is the same as that used by local growers for this crop, and it was obtained by mixing simple chemical fertilizers (phosphoric acid (H_3PO_4), nitric acid (HNO_3), potassium nitrate (KNO_3), and calcium nitrate ($Ca(NO_3)_2$)) into irrigation water.

The Evozon3 device was employed to produce O_3 for integration into the fertigation system (Evo Industria, Malaga, Spain). This system draws in ambient air using a centrifugal pump (3 kg cm^{-2} of pressure and 3.5 m^3 h^{-1} of flow rate), filters the oxygen with zeolite, and sends the pure oxygen to a concentrator at a flow rate of 8 L min^{-1} in order to reduce the humidity to 14%. Then, O_2 is ionized with a high voltage current of 9000 volts, releasing one of the oxygen atoms and causing this atom to bind with another O_2 molecule, generating O_3 molecules. The produced O_3 with a concentration of 10 g h^{-1} was injected into a 120 L

water tank from the network using a submersible Venturi system and a flash reactor, which promotes mixing, homogeneity, and appropriate bubble size.

In addition to measuring pH and oxidation-reduction potential (mV), the equipment probe (PHTK-160 Digital PH ORP (2 In 1), Teckoplus Ltd., Trade-mart Dr., Kowloon Bay, Hong Kong) was also implemented. After 30 min of operation, the ozonated water reaches the proper conditions to be used in the experiments, with a voltage between 850 and 1000 mV. Table 3 depicts the conversion between power redox and O_3 concentration to enable an optimum condition for the experiments based on the recommendations provided by TopOzono [11]. The different levels of ozone concentration and the maximum concentration tested in the experiment were also based on the recommendations given by the manufacturer instructions in order to avoid toxicity in crops.

Table 3. Conversion from power redox (mV) to O_3 concentration (mg L^{-1}) in the ozone machinery.

Power Redox (mV)	O_3 Concentration
0	0
225	0.18
450	0.36
675	0.54

Table 4 showed the design of the ozonated treatments and the quantities of O_3 in mg L^{-1} or mV of power redox.

Table 4. Characterization of the mixture (in %) supplied in each treatment: tap irrigation water (NS_0); fertigation with the nutrient solution (NS_1). Ozone levels: O_0: 0 mg L^{-1}, $O_{0.18}$: 0.18 mg L^{-1}, $O_{0.36.}$: 0.36 mg L^{-1}, $O_{0.54.}$: 0.54 mg L^{-1}; respectively.

		Mixing Percentage (%)		
	Treatments	**Nutrient Solution (NS_1)**	**Ozonated Water 0.8 mg L^{-1} (± 900 mV) (O)**	**Irrigation Water (NS_0)**
Experiment 1	NS_0 O_0	0	0	100
	NS_0 $O_{0.54}$	0	75	25
	NS_1 O_0	25	0	75
	NS_1 $O_{0.54}$	25	75	0
Experiment 2	NS_1 O_0	25	0	75
	NS_1 $O_{0.18}$	25	25	50
	NS_1 $O_{0.36}$	25	50	25
	NS_1 $O_{0.54}$	25	75	0

Graduate cylinders were used to manually irrigate the pots. Although each plant received 200 mL of irrigation daily, irrigation frequency was adjusted weekly to attain a drainage ratio of approximately 30%.

2.4. Biomass Assessments

At the final stage of the experiment, four plants were randomly selected from each block and treatment, and the substrate was removed from these plants with distilled water to prevent root loss. After separating plants into their roots, stems, leaves, and fruits, each of these sections was cleaned and dried with blotting paper to determine their fresh weight (FW). The corresponding dry weights of the various plant organs were then determined by oven-drying them at 60 °C for 48 h (DW). Using these values, many plant metrics were determined, including the relative root weight ratio (RWR), stem weight ratio (SWR), leaf weight ratio (LWR), and fruit weight ratio (FWR), according to the protocol reported by Garcia-Caparros et al. [12]. Total plant dry weight (TDW) was calculated using the sum of the root, stem, leaf, and fruit dry weights [13]. The computation of total leaf area using digital photographs of each plant using the computer program Idrisi Selva (Clark

Laboratories, Worcester, MA, USA) was carried out following the protocol established by Garcia-Caparros et al. [14].

2.5. Physiological Parameters

Randomly selected leaf samples (0.2 g) from four plants per block and treatment were soaked in methanol for 24 h in darkness at room temperature (RT). After removing the supernatant, pigment concentrations were measured spectrophotometrically using the Wellburn [15] equation and quantified in mg g^{-1} FW.

$$\text{Chlorophyll a} = ([(15.65 \times \text{A\_666}) - (7.34 \times \text{A\_653})] \times 15)/2 \quad (1)$$

$$\text{Chlorophyll b} = ([(27.05 \times \text{A\_653}) - (11.21 \times \text{A\_666})] \times 15)/2 \quad (2)$$

Applying the method described by Irigoyen et al. [16], the concentration of total soluble sugars and proline in the leaves of four randomly selected treatment plants was determined. The anthrone reagent was used to measure the concentration of total soluble sugars, whereas the ninhydrin reagent was employed to measure the concentration of free proline. Both parameters were analyzed colorimetrically with a spectrophotometer (model Shimadzu UV-1201, Shimadzu, Tokyo, Japan). The concentration of total soluble sugars in leaf was expressed as milligrams of glucose per gram of fresh weight (FW), while the concentration of proline in leaf was expressed as micrograms per gram of fresh weight (FW).

2.6. Plant Nutrient Parameters

The concentration of nutrients in plant organs was determined using ground, oven-dried samples. At 300 °C, the samples were mineralized with H_2SO_4 (96%) and H_2O_2 (P-free). The procedures of Cataldo et al. [17] and Hogue et al. [18] were assessed, respectively, for the analysis of nitrogen and phosphorous concentrations. The K^+ concentration in samples was quantified by flame spectrometry (model Jenway PFP 7, Jenway/Barloworld Scientific, Essex, UK) according to the protocol of Lachica et al. [19]. Then, using these measurements and the DW, plant nutrient content and organ distribution were determined. The partitioning was calculated by dividing the nutrient extraction in each organ by the overall nutrient uptake by the plant.

2.7. Statistical Analysis

All experiments were assessed as independent samples based on the outcomes for each plant and variable. Analysis of bifactorial and unifactorial variance (ANOVA) and Fisher's least significant difference (LSD) tests at $p < 0.05$ using Statgraphics Plus were used to determine statistically significant differences between treatment means (Statpoint Technologies Inc., Warrenton, VA, USA).

3. Results

3.1. Biomass Assessments

A significant rise in total dry weight in tomato plants was observed under nutrient solution fertigation in experiment 1. Various tendencies in biomass partitioning were observed in the various organs studied. The leaf weight ratio raised significantly with fertigation and O_3 supply, whereas the stem weight ratio did not differ significantly with fertigation and decreased significantly with O_3 supply. The root weight ratio raised with irrigation water and O_3 supply. The flower weight ratio raised with irrigation water but did not differ significantly with O_3 supply. Fruit weight ratio remained stable across all factors tested, but relative water content and leaf area decreased significantly under fertigation regardless of O_3 supply (Table 5).

Table 5. Effects of fertigation and ozone on *S. lycopersicum* seedlings' total dry weight (g) (TDW), relative leaf weight ratio (LWR), stem weight ratio (SWR), root weight ratio (RWR), flower weight ratio (FwWR), fruit weight ratio (FWR), relative water content (RWC) and leaf area (LA) (m^2). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters or an asterisk (*) denote statistically significant changes between treatments (LSD test; $p < 0.05$). ns indicates non-statistical differences between treatments.

		TDW	LWR	SWR	RWR	FwWR	FWR	RWC	LA
Fertigation treatments		*	*	ns	*	*	ns	*	*
	NS_0	8.37 ± 0.05 b	0.31 ± 0.01 b	0.55 ± 0.01	0.07 ± 0.003 a	0.04 ± 0.002 a	0.02 ± 0.005	7.20 ± 0.10 a	1.99 ± 0.04 a
	NS_1	11.19 ± 0.28 a	0.34 ± 0.01 a	0.54 ± 0.01	0.06 ± 0.002 b	0.03 ± 0.003 b	0.01 ± 0.005	6.90 ± 0.10 b	1.39 ± 0.06 b
Ozone treatments		ns	*	*	*	ns	ns	ns	ns
	O_0	9.70 ± 0.15	0.29 ± 0.02 b	0.57 ± 0.01 a	0.04 ± 0.01 b	0.03 ± 0.005	0.02 ± 0.005	7.20 ± 0.13	1.67 ± 0.04
	$O_{0.54}$	9.90 ± 0.17	0.35 ± 0.02 a	0.52 ± 0.01 b	0.07 ± 0.01 a	0.03 ± 0.005	0.01 ± 0.005	7.00 ± 0.09	1.71 ± 0.03
Interactions		ns	ns	ns	ns	ns	ns	ns	*

The leaf area of tomato plants was modified by the fertigation (NS_0 and NS_1) and O_3 (O_0 and $O_{0.54}$) treatments (Figure 2). Regarding nutrient solution, leaf area showed the highest value at $O_{0.54}$. Regarding ozone concentration, the highest values were greater in tap water (NS_0).

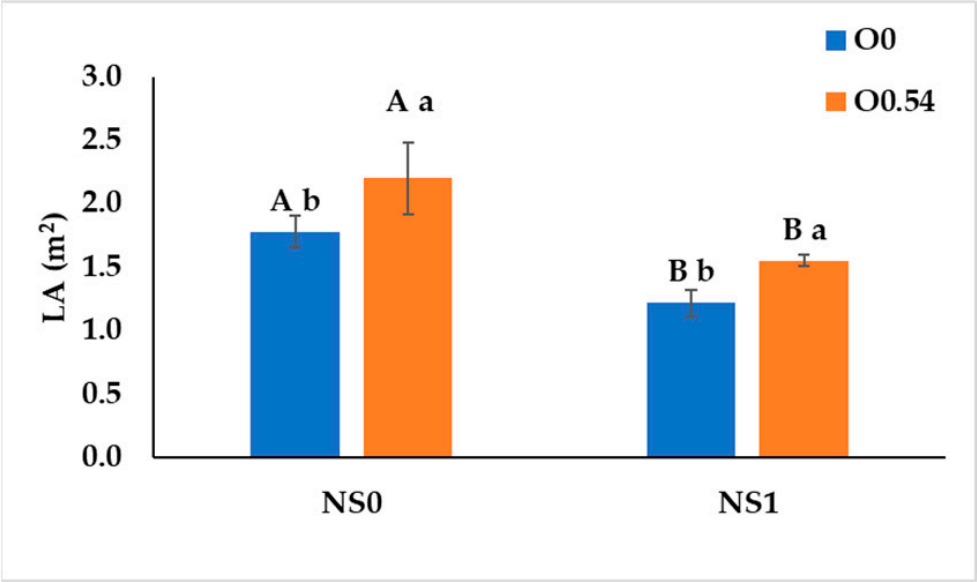

Figure 2. Interactions between fertigation treatments and O_3 supply on leaf area in tomato plants. The comparison between the ozone supplies is assessed with lowercase letters. Capital letters are used to analyze the comparability of fertigation treatments. Different letters denote significant treatment changes (LSD test, $p < 0.05$).

The $O_{0.36}$ treatment yielded the highest TDW in tomato plants in experiment 2, but, as previously stated, there were differences in biomass partitioning among treatments. For the highest dose of ozone ($O_{0.54}$), LWR and FWR showed the highest value whereas SWR showed the lowest value. Flower weight ratio (FwWR) did not show a clear tendency with increasing dosages of O_3. RWC had the highest value in tomato plants subjected to $O_{0.18}$ treatment, and LA obtained higher values in plants grown without O_3 application (Table 6).

Table 6. Effects of O_3 dosage on *S. lycopersicum* seedlings' total dry weight (g) (TDW), relative leaf weight ratio (LWR), stem weight ratio (SWR), root weight ratio (RWR), flower weight ratio (FwWR), fruit weight ratio (FWR), relative water content (RWC) and leaf area (LA) (m^2). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters denote statistically significant changes between treatments (LSD test; $p < 0.05$).

Ozone Treatments	TDW	LWR	SWR	RWR	FwWR	FWR	RWC	LA
O_0	11.18 ± 0.14 b	0.31 ± 0.01 c	0.56 ± 0.02 ab	0.03 ± 0.01 b	0.03 ± 0.004 a	0.01 ± 0.011 b	6.95 ± 0.14 b	1.55 ± 0.08 a
$O_{0.18}$	10.97 ± 0.14 b	0.32 ± 0.01 bc	0.60 ± 0.03 a	0.06 ± 0.01 a	0.03 ± 0.004 a	0.01 ± 0.012 b	7.69 ± 0.14 a	1.36 ± 0.11 ab
$O_{0.36}$	13.81 ± 0.14 a	0.35 ± 0.02 ab	0.59 ± 0.02 a	0.06 ± 0.01 a	0.02 ± 0.005 b	0.01 ± 0.013 b	6.23 ± 0.14 c	1.28 ± 0.06 b
$O_{0.54}$	11.21 ± 0.14 b	0.37 ± 0.02 a	0.52 ± 0.03 b	0.06 ± 0.01 a	0.03 ± 0.004 a	0.03 ± 0.013 a	6.84 ± 0.14 b	1.22 ± 0.07 b

3.2. Physiological Parameters

In experiment 1, compared to the control treatment, fertigation with the nutrient solution significantly improved leaf pigments, total soluble sugars, and proline concentration. Nevertheless, the increase in O_3 concentration in the fertigation did not show a similar trend between the factors assessed, since at the highest concentration (0.54 mg L^{-1}) leaf chlorophyll b and (a + b) concentration increased, leaf proline concentration decreased, while leaf chlorophyll a and total soluble sugars concentration remained without changes (Table 7).

Table 7. Effect of fertigation and ozone on pigments concentration (chlorophyll a and b (Chl a and b)) (mg g^{-1} FW), leaf total soluble sugars (TSS) (mg g^{-1} FW), and leaf proline (µg g^{-1} FW). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters or an asterisk (*) denote statistically significant changes between treatments (LSD test; $p < 0.05$). ns indicates non-statistical differences between treatments.

		Chl a	Chl b	Chla + b	TSS	Proline
Fertigation treatments		*	*	*	*	*
	NS_0	1.60 ± 0.14 b	1.24 ± 0.11 b	2.81 ± 0.12 b	26.47 ± 2.41 b	35.29 ± 2.99 b
	NS_1	7.37 ± 0.56 a	4.53 ± 0.41 a	11.69 ± 1.80 a	52.94 ± 4.54 a	203.89 ± 19.95 a
Ozone treatments		ns	*	*	ns	*
	O_0	4.42 ± 0.35	2.76 ± 0.12 b	6.81 ± 0.02 b	45.50 ± 4.79	162.52 ± 12.40 a
	$O_{0.54}$	4.53 ± 0.37	3.21 ± 0.13 a	7.69 ± 0.03 a	38.91 ± 4.81	76.66 ± 7.12 b
Interactions		ns	ns	ns	*	*

Similar results in leaf total soluble sugars concentrations were obtained in NS_0 and NS_1, being higher at $O_{0.54}$. Furthermore, the ozone concentration (O_0 and $O_{0.54}$) was always higher in NS_1 (Figure 3a). In terms of leaf proline concentration, we noted a contrary trend in NS_0 and NS_1. Under varying ozone concentrations, NS_0 had the lowest leaf proline concentration (Figure 3b).

In experiment 2, the dosage of 0.36 mg L^{-1} of O_3 showed the highest value for chlorophyll a and (a + b) concentration, whereas for chlorophyll b concentration, the highest value was reported at 0.18 mg L^{-1}. The control treatment without ozone application showed the greatest values in leaf total soluble sugars and proline concentration (Table 8).

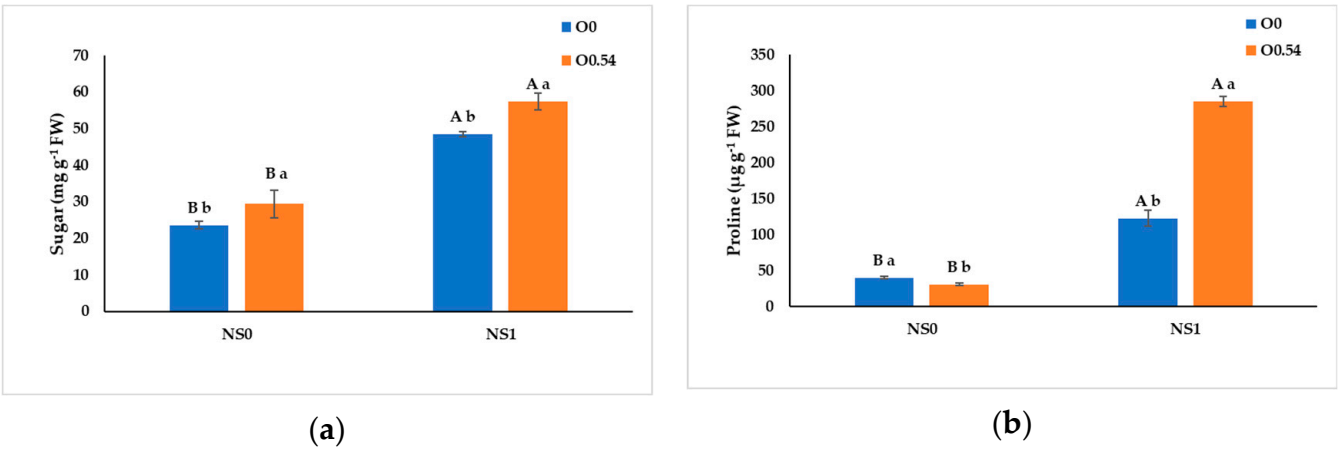

Figure 3. Interactions between fertigation treatments and O_3 supply on leaf total soluble sugars (**a**) and proline concentrations (**b**). The comparison between the ozone supplies is assessed with lowercase letters. Capital letters are used to analyze the comparability of fertigation treatments. Different letters denote significant treatment changes (LSD test, $p < 0.05$).

Table 8. Effect of O_3 dosage on leaf chlorophyll a and b (mg g^{-1} FW), TSS (mg g^{-1} FW), and proline (µg g^{-1} FW) concentration. The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters denote statistically significant changes between treatments (LSD test; $p < 0.05$).

Ozone Treatments	Chl a	Chl b	Chl a+ b	TSS	Proline
O_0	7.47 ± 0.06 b	4.36 ± 0.03 b	11.70 ± 0.17 b	57.46 ± 3.32 a	285.10 ± 20.30 a
$O_{0.18}$	7.37 ± 0.08 b	5.21 ± 0.04 a	12.50 ± 0.29 b	37.71 ± 3.21 c	102.65 ± 10.21 d
$O_{0.36}$	8.49 ± 0.08 a	4.50 ± 0.04 b	13.00 ± 0.45 a	45.77 ± 3.14 b	179.24 ± 18.50 b
$O_{0.54}$	7.28 ± 0.07 b	4.71 ± 0.05 b	12.00 ± 0.28 b	48.42 ± 3.18 b	132.68 ± 11.21 c

3.3. Nutritional Parameters

In experiment 1, the highest levels of nitrogen, phosphorous, and potassium in leaves of tomato plants were reached with fertigation with NS, regardless of O_3 application, except in K, where an increase was observed with O_3 application (Table 9).

Table 9. Effect of fertigation and ozone on nitrogen, phosphorous, and potassium leaf concentration (mg g^{-1} DW). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters or an asterisk (*) denote statistically significant changes between treatments (LSD test; $p < 0.05$). ns indicates non-statistical differences between treatments.

		N	P	K
Fertigation treatments		*	*	*
	NS_0	47.32 ± 4.22 b	2.29 ± 0.24 b	15.34 ± 1.14 b
	NS_1	78.36 ± 7.96 a	2.89 ± 0.16 a	24.49 ± 2.16 a
Ozone treatments		ns	ns	*
	O_0	65.70 ± 16.04	2.55 ± 0.15	16.54 ± 1.25 b
	$O_{0.54}$	59.97 ± 19.40	2.43 ± 0.17	23.29 ± 2.47 a
Interactions		ns	*	ns

The concentration of P in leaves was greatest in NS_0 and NS_1 when no ozone was present (O_0). Concerning ozone treatments, there were no significant statistical variations in P concentration between the presence and absence of ozone supply (Figure 4).

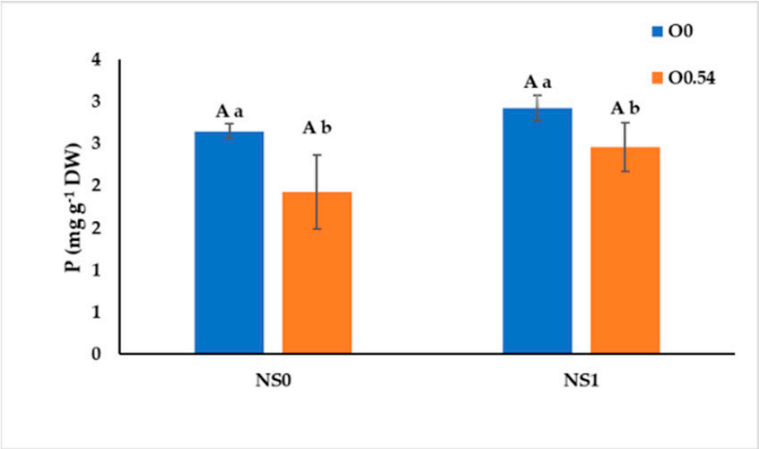

Figure 4. Interactions between fertigation treatments and O_3 supply on P concentration. The comparison between the ozone supplies is assessed with lowercase letters. Capital letters are used to analyze the comparability of fertigation treatments. Different letters denote significant treatment changes (LSD test, $p < 0.05$).

In experiment 2, the rise in O_3 concentration caused significant increases in P and K concentrations in leaves. Meanwhile, leaf nitrogen concentration did not demonstrate significant treatment variations (Table 10).

Table 10. Effect of O_3 dosage on nitrogen, phosphorous, and potassium leaf concentration (mg g^{-1} DW). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters denote statistically significant changes between treatments (LSD test; $p < 0.05$).

Ozone Treatments	N	P	K
O_0	78.61 ± 7.10 a	2.46 ± 0.08 b	20.96 ± 1.32 c
$O_{0.18}$	80.26 ± 7.63 a	2.73 ± 0.12 ab	25.38 ± 0.79 b
$O_{0.36}$	73.64 ± 6.92 a	2.99 ± 0.11 a	28.03 ± 0.96 a
$O_{0.54}$	78.36 ± 7.96 a	2.93 ± 0.08 a	30.11 ± 1.23 a

3.3.1. Nitrogen Extraction and Partitioning

The total nitrogen absorbed by plants (TNE) rose regardless of O_3 supply in the first trial. Under fertigation, the root nitrogen ratio (RNR) dropped, but it increased with the supply of O_3 for the organs assessed for partitioning. The stem nitrogen ratio (SNR) rose after fertigation but not with the addition of O_3. Leaf nitrogen ratio (LNR) only increased with O_3 application, regardless of fertigation. Flower nitrogen ratio (FwNR) only increased with irrigation water, independent of O_3 application. Fruit nitrogen ratio (FNR) was unaffected by the two evaluated parameters (Table 11). In the different parameters studied, no interactions were found between fertigation treatments or ozone treatments.

In experiment 2, the total nitrogen extracted by tomato plants presented no significant differences between O_3 concentration tested, as well as FNR. The significantly higher value of RNR was achieved at $O_{0.36}$ treatment, whereas for SNR it was achieved without O_3 application (O_0). LNR values increased when O_3 concentration increased, the lowest value being the one without O_3 application (O_0). Tomato plants treated with $O_{0.18}$ treatment exhibited the highest FwNR (Table 12).

Table 11. Effect of fertigation and ozone on total nitrogen extracted by plant (TNE) (mg plant^{-1}) and their distribution in root (RNR), stem (SNR), leaf (LNR), flower (FwNR), and fruit (FNR) (dimensionless). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters or an asterisk (*) denote statistically significant changes between treatments (LSD test; $p < 0.05$). ns indicates non-statistical differences between treatments.

		TNE	RNR	SNR	LNR	FwNR	FNR
Fertigation treatments		*	*	*	ns	*	ns
	NS_0	358.34 ± 44.94 b	0.08 ± 0.01 a	0.49 ± 0.01 b	0.35 ± 0.04	0.04 ± 0.001 a	0.02 ± 0.006
	NS_1	750.13 ± 90.70 a	0.03 ± 0.01 b	0.53 ± 0.01 a	0.41 ± 0.04	0.03 ± 0.002 b	0.01 ± 0.006
Ozone treatments		ns	*	*	*	ns	ns
	O_0	582.59 ± 53.45	0.04 ± 0.01 b	0.55 ± 0.03 a	0.34 ± 0.02 b	0.04 ± 0.002	0.02 ± 0.006
	$O_{0.54}$	525.87 ± 54.86	0.07 ± 0.01 a	0.47 ± 0.02 b	0.41 ± 0.03 a	0.04 ± 0.002	0.01 ± 0.006
Interactions		ns	ns	ns	ns	ns	ns

Table 12. Effect of O_3 dosage on total nitrogen extracted by plant (TNE) (mg plant^{-1}) and their distribution in root (RNR), stem (SNR), leaf (LNR), flower (FwNR), and fruit (FNR) (dimensionless). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters denote statistically significant changes between treatments (LSD test; $p < 0.05$).

Ozone Treatments	TNE	RNR	SNR	LNR	FwNR	FNR
O_0	772.92 ± 73.09 a	0.03 ± 0.00 b	0.56 ± 0.04 a	0.36 ± 0.04 b	0.04 ± 0.01 b	0.01 ± 0.005 a
$O_{0.18}$	693.18 ± 64.26 a	0.03 ± 0.01 b	0.46 ± 0.05 b	0.41 ± 0.02 ab	0.08 ± 0.01 a	0.01 ± 0.004 a
$O_{0.36}$	722.75 ± 61.70 a	0.05 ± 0.002 a	0.46 ± 0.04 b	0.45 ± 0.03 a	0.04 ± 0.01 b	0.01 ± 0.003 a
$O_{0.54}$	727.33 ± 75.61 a	0.03 ± 0.01 b	0.48 ± 0.03 b	0.45 ± 0.02 a	0.04 ± 0.01 b	0.01 ± 0.005 a

3.3.2. Phosphorus Extraction and Partitioning

In experiment 1, total phosphorus extracted from plants (TPE) increased regardless of the use of O_3 during fertigation. Regarding biomass partitioning, there were variations across treatments. Root phosphorus ratio (RPR) showed an increase in tomato plants under fertigation and O_3 application. Steam phosphorus ratio (SPR), compared to RPR, exhibited a contrary tendency with lower values with the application of O_3 and without significant differences with fertigation. Leaf phosphorus ratio (LPR) decreased only in tomato plants with O_3 application. Flower phosphorus ratio (FwPR) increased under irrigation with water and the application of O_3. Fruit phosphorus ratio (FPR) did not present significant differences for both treatments (Table 13).

Table 13. Effect of fertigation and ozone on total phosphorus extracted by plant (TPE) (mg plant^{-1}) and their distribution in root (RPR), stem (SPR), leaf (LPR), flower (FwPR), and fruit (FPR) (dimensionless). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters or an asterisk (*) denote statistically significant changes between treatments (LSD test; $p < 0.05$). ns indicates non-statistical differences between treatments.

		TPE	RPR	SPR	LPR	FwPR	FPR
Fertigation treatments		*	*	ns	ns	*	ns
	NS_0	14.47 ± 0.14 b	0.06 ± 0.01 b	0.49 ± 0.01	0.40 ± 0.01	0.08 ± 0.01 a	0.02 ± 0.006
	NS_1	26.76 ± 0.16 a	0.09 ± 0.01 a	0.48 ± 0.01	0.40 ± 0.01	0.04 ± 0.01 b	0.01 ± 0.005
Ozone treatments		ns	*	*	*	*	ns
	O_0	20.51 ± 0.15	0.06 ± 0.004 b	0.51 ± 0.01 a	0.42 ± 0.01 a	0.03 ± 0.01 b	0.02 ± 0.005
	$O_{0.54}$	20.71 ± 0.17	0.07 ± 0.003 a	0.46 ± 0.01 b	0.38 ± 0.01 b	0.06 ± 0.01 a	0.01 ± 0.006
Interactions		*	*	*	*	*	ns

Looking at the interaction of TPE with fertilizer treatments and O_3 supplies, the highest values were found in O_0 in both fertigation treatments. Regarding ozone treatments, the highest values were found in NS_1. In RPR, there were differences in NS_o showing the highest value at $O_{0.54}$. Regarding ozone treatments, there were only differences in O_0 being higher in NS_1. In SPR, regarding fertigation treatments, in NS_o, the highest value was O_0 whereas in NS_1, the trend was the opposite. In the case of ozone supplies, there were different trends; where in O_0 the greatest value was in NS_0, in the other case, the trend was the contrary. In LPR, there were only differences in NS_1 being higher at O_0, whereas considering ozone treatments, there were differences in O_0 being higher at NS_1. In FwPR, the fertigation treatments showed the same trend as in RPR, but when considering ozone treatments, the highest values were noted in NS_o (Figure 5).

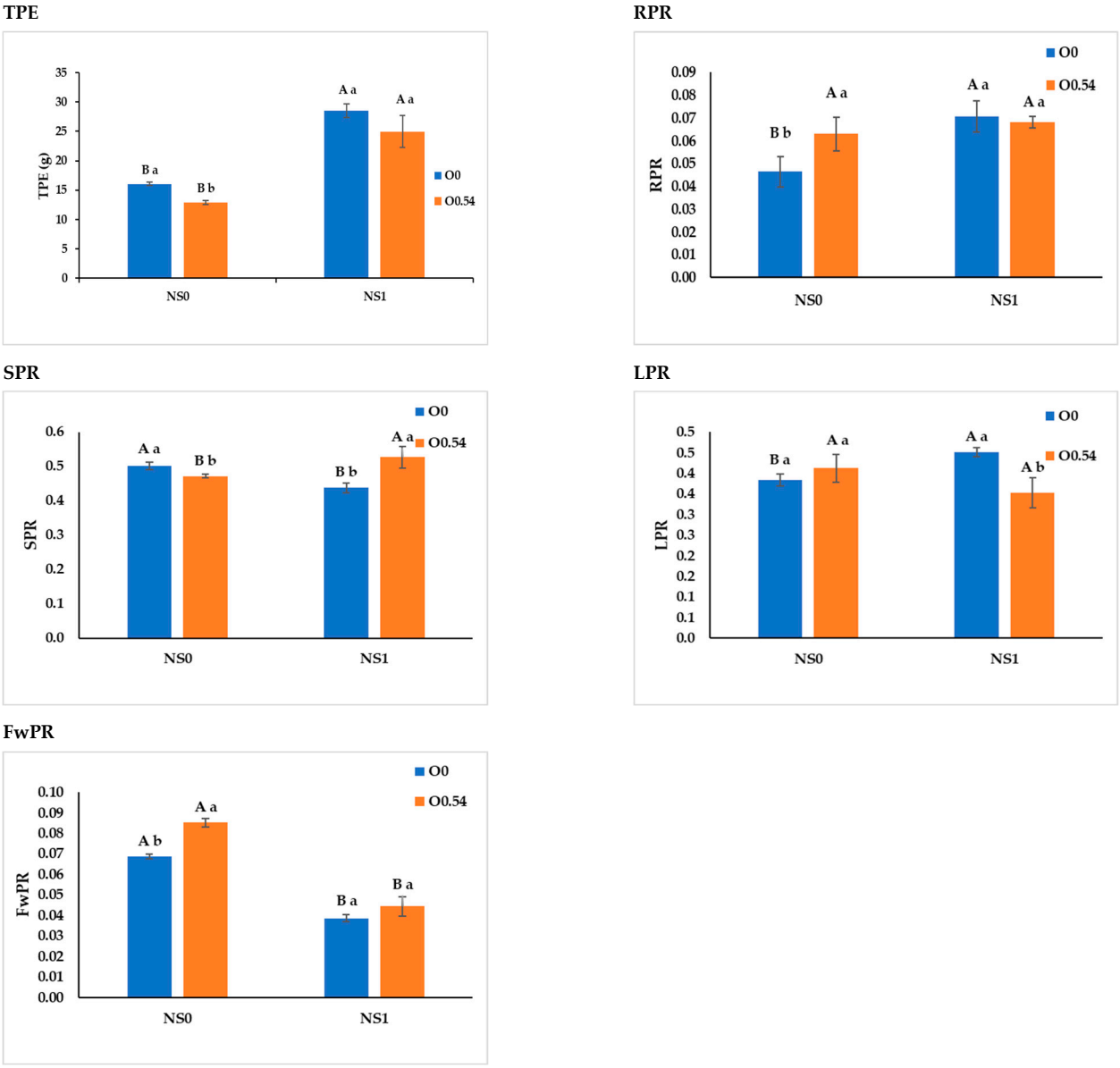

Figure 5. Interactions between fertigation treatments and O_3 supply on total phosphorus extracted (TPE) from plants, RPR, SPR, LPR, and FwPR. The comparison between the ozone supplies is assessed with lowercase letters. Capital letters are used to analyze the comparability of fertigation treatments. Different letters denote significant treatment changes (LSD test, $p < 0.05$).

In the second experiment, tomato plants produced under the $O_{0.36}$ treatment had the greatest TPE value, whilst the $O_{0.18}$ treatment had the highest SPR value. The ratio of leaf phosphorus was substantially greater with $O_{0.54}$ and declined with decreasing O_3 levels. There were no significant changes between the treatments with or without O_3 concentrations for any of the other variables (Table 14).

Table 14. Effect of O_3 dosage on total phosphorus extracted by plant (TPE) (mg plant^{-1}) and their distribution in root (RPR), stem (SPR), leaf (LPR), flower (FwPR), and fruit (FPR) (dimensionless). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters denote statistically significant changes between treatments (LSD test; $p < 0.05$).

Ozone Treatments	TPE	RPR	SPR	LPR	FwPR	FPR
O_0	24.99 ± 0.12 c	0.07 ± 0.01 a	0.53 ± 0.01 ab	0.35 ± 0.02 c	0.04 ± 0.01 a	0.01 ± 0.002 a
$O_{0.18}$	28.83 ± 0.17 b	0.07 ± 0.01 a	0.57 ± 0.03 a	0.34 ± 0.02 c	0.04 ± 0.01 a	0.01 ± 0.003 a
$O_{0.36}$	34.77 ± 0.14 a	0.07 ± 0.01 a	0.48 ± 0.02 bc	0.41 ± 0.01 b	0.03 ± 0.01 a	0.01 ± 0.003 a
$O_{0.54}$	28.53 ± 0.19 b	0.07 ± 0.01 a	0.44 ± 0.01 c	0.45 ± 0.02 a	0.04 ± 0.01 a	0.01 ± 0.003 a

3.3.3. Potassium Extraction and Partitioning

In experiment 1, the total potassium obtained from plants (TKE) was enhanced under fertigation conditions and in the absence of O_3 treatment. Regardless of O_3 applications, the root potassium ratio (RKR) rose as a result of fertigation in relation to organ partitioning. Regardless of fertigation, the stem potassium ratio (SKR) only dropped with the application of O_3 and the leaf potassium ratio (LKR) only rose with the application of O_3. Flower potassium ratio (FwKR) dropped with fertigation but remained unchanged with O_3 treatment, while fruit potassium ratio (FKR) was unaffected by fertigation or O_3 application (Table 15).

Table 15. Effect of fertigation and ozone on total potassium extracted by plant (TKE) (mg plant^{-1}) and their distribution in root (RKR), stem (SKR), leaf (LKR), flower (FwKR), and fruit (FKR) (dimensionless). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters or an asterisk (*) denote statistically significant changes between treatments (LSD test; $p < 0.05$). ns indicates non-statistical differences between treatments.

		TKE	RKR	SKR	LKR	FwKR	FKR
Fertigation treatments		*	*	ns	ns	*	ns
	NS_0	184.64 ± 12.14 b	0.02 ± 0.005 b	0.68 ± 0.03	0.22 ± 0.03	0.04 ± 0.005 a	0.01 ± 0.002
	NS_1	423.44 ± 30.16 a	0.04 ± 0.005 a	0.73 ± 0.04	0.21 ± 0.03	0.02 ± 0.005 b	0.01 ± 0.002
Ozone treatments		*	ns	*	*	ns	ns
	O_0	332.10 ± 15.15 a	0.03 ± 0.01	0.75 ± 0.03 a	0.14 ± 0.02 b	0.03 ± 0.01	0.02 ± 0.006
	$O_{0.54}$	275.97 ± 18.17 b	0.03 ± 0.01	0.66 ± 0.04 b	0.28 ± 0.03 a	0.03 ± 0.01	0.01 ± 0.006
Interactions		*	*	ns	ns	*	ns

In TKE, RKR, and FwKR, there were significant interactions between fertigation treatments and O_3 supplies (Figure 6). Those interactions in TKE and RKR appeared because under NS_0 there were no differences with or without O_3 supplies. Comparing fertigation treatments, TKE was higher in O_0 whereas in RKR showed an opposite trend. Regarding FwKE, there were no changes between fertigation treatments for FwKE, and O_0 values were the greatest.

In experiment 2, the total K extracted from plants (TKE) was significantly greater in tomato plants grown under $O_{0.36}$, whereas the value was lowest in the control treatment. Nonetheless, the greatest RKR and SKR values were observed in the control treatment (0 mg L^{-1} of O_3). LKR increased under increasing O_3 concentrations, being the highest

value reached in the $O_{0.54}$ treatment. In FwPR and FPR, there were no statistically significant differences between treatments with rising O_3 concentrations (Table 16).

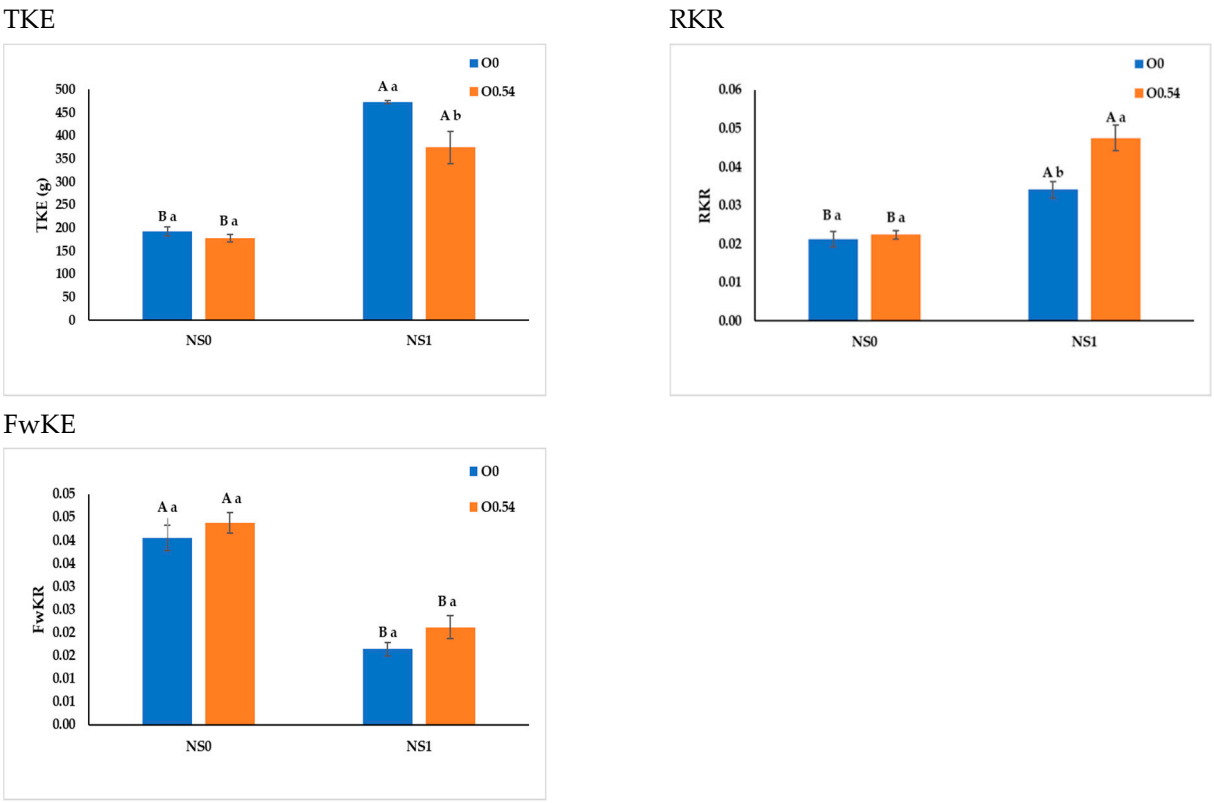

Figure 6. Interactions between fertigation treatments and ozone supply on total potassium extracted (TKE) from plants, RKR, and FwPR. The comparison between the ozone supplies is assessed with lowercase letters. Capital letters are used to analyze the comparability of fertigation treatments. Different letters denote significant treatment changes (LSD test, $p < 0.05$).

Table 16. Effect of O_3 dosage on total potassium extracted by plant (TKE) (mg plant^{-1}) and their distribution in root (RKR), stem (SKR), leaf (LKR), flower (FwKR), and fruit (FKR) (dimensionless). The data recorded are the means ± standard deviation of four samples per treatment at the end of the experimental period. In each column, different letters denote statistically significant changes between treatments (LSD test; $p < 0.05$).

	TKE	RKR	SKR	LKR	FwKR	FKR
O_0	374.37 ± 28.11 c	0.06 ± 0.01 a	0.78 ± 0.03 a	0.14 ± 0.03 c	0.02 ± 0.01 a	0.01 ± 0.005 a
$O_{0.18}$	434.83 ± 37.59 b	0.03 ± 0.01 b	0.74 ± 0.02 ab	0.21 ± 0.02 b	0.02 ± 0.01 a	0.01 ± 0.004 a
$O_{0.36}$	661.84 ± 50.27 a	0.03 ± 0.01 b	0.80 ± 0.03 a	0.15 ± 0.02 c	0.01 ± 0.01 a	0.01 ± 0.004 a
$O_{0.54}$	472.50 ± 40.14 b	0.03 ± 0.01 b	0.68 ± 0.01 b	0.27 ± 0.03 a	0.02 ± 0.01 a	0.01 ± 0.005 a

4. Discussion

The results obtained in this trial reported that the fertigation with the nutrient solution led to increased total plant dry weight in tomato. These outcomes are consistent with previous findings reported by other researchers who have stated that fertigation with the correct application of fertilizers enhanced the plant growth and yield in different horticultural crops [20,21]. Regarding biomass partitioning, fertigation with the nutrient solution increased leaf weight ratio to the detriment of the root and flower weight ratios. Nevertheless, the increase in LWR did not show a positive relationship with the relative water content and leaf area, since these parameters declined under fertigation with the nutrient solution. These results may indicate that the fertigation with the nutrient solution contributed to the

plant's compactness, reducing the leaf area and, in consequence, reducing the water status. The reduction in RWC and leaf area can be also ascribed to the higher value of electrical conductivity (EC) of the nutrient solution supply as reported by different authors related to salinity conditions [22] and the consequent restriction of water uptake [23]. Similarly, Parra-Terraza et al. [24] noted that there was an increase in the osmotic potential under increasing nutrient concentration in the nutrient solution resulting in an impairment of water uptake.

Tomato plants grown at 0.36 mg L^{-1} of O_3 showed the greatest value of TDW. Leaf weight ratio increased under increasing O_3 concentration. The degradation of O_3 and the consequent enhancement of root respiration may have led to a better growth and plant development, resulting in higher leaf weight ratio [25]. Identical outcomes have been recorded in other horticultural crops, such as sweet pepper, melon, and cucumber [26], as well as in tomato [27]. The other biomass partitioning parameters and relative water content did not exhibit a clear tendency under increasing O_3 concentrations. This fact can be ascribed to the levels of O_3 concentration supplied which could not induce a clear trend in these parameters studied. On the other hand, the reduction in leaf area under increasing O_3 concentrations can be related to the toxic role of ozone in some crops [28].

The supply of the nutrient solution enhanced the concentration of pigments, leaf TSS, and proline concentration. The highest concentration of TSS in our experiment can be related to the increase in photoassimilates linked to higher rates of photosynthetic activity ascribed to higher pigment concentration in leaves [29].

In the case of proline, the higher values can be ascribed to the higher value of the EC of the nutrient solution as already noted in other crops, such as strawberry [30] and tomato [31]. The rise in ozone concentration also increased chlorophyll b and (a + b) concentration but reduced the leaf proline concentration. The enhancement of pigments concentration with increasing ozone reported in our experiment was consistent with those obtained by Sloan and Engelke [32] in bentgrass irrigated with ozonated water. Similarly, the supply of increasing ozone concentrations (from 0 to 2 mg L^{-1}) to the nutrient solution increased pigments concentrations in tomato plants as reported by Tahamolkonan et al. [33]. Nevertheless, the decline in leaf proline concentration under rising O_3 was consistent with the results obtained by Colunje et al. [34] in pepper grown under similar experimental conditions.

Similarly to TDW, tomato plants grown at 0.36 mg L^{-1} of O_3 showed the greatest concentration of chlorophyll a and (a + b), highlighting in this way the relationship in our experiment between the photosynthetic performance and the plant development and growth. The clear reduction in leaf TSS and proline concentration under increasing O_3 concentration can be associated with the oxidative damage caused by the application of increasing ozone O_3 concentrations [35].

As it was expected, leaf N, P, and K concentrations rose in tomato plants fertigated with the nutrient solution. This fact relies on the higher availability of nutrients and consequently the higher nutrient uptake rate as already reported by several researchers [36,37]. Regarding ozone treatments, only leaf K concentration demonstrated a clear increase. Similar findings were reported by Diaz-Lopez et al. [38], who observed that tomato plants grown under increasing ozone concentrations in the nutrient solution exhibited an increase in leaf K concentration and showed that ozone has a beneficial influence on nutrient availability.

Under increasing concentrations of O_3, tomato plants increased leaf P and K concentration. These results may suggest the possible activation of several nutrient transporters and the consequential nutrient uptake increase by tomato plants under increasing O_3 concentration as reported Xu et al. [39] with the following nitrogen transporters: SlAMT1-1 (ammonium nitrogen absorption-related gene) and SlNRT2.3 (nitrate nitrogen absorption-related gene).

Regarding N extraction, fertigation with the nutrient solution increased the total nitrogen uptake by the plant with a higher allocation of N in the stem to the detriment of the root and flowers. Similar findings were reported by Colunje et al. [34] in pepper

seedlings fertigated with nutrient solution, where there was a higher accumulation of N in the stems.

On the same hand, Xu et al. [39] noted that increasing concentrations of ozone water irrigation (from 1 to 4 mg L^{-1}) enhanced the content of nitrogen in leaves of tomato plants compared to the control treatment without ozone application. Ozone treatments did not affect the total nitrogen extracted by the plant but modify the allocation of N between organs, resulting in a rise in the root and leaf in a decrease in the shoot. These outcomes agree with those reported by Martinez-Sanchez and Aguayo [40] in watermelon under ozonized water conditions. Under increasing ozone concentration, the total nitrogen extracted by the plant was not affected but there was a higher accumulation of N in the leaf. Similar results were noted by Martínez-Sánchez and Aguayo [5] in pepper seedlings grown under increasing O_3 concentration.

Similar to nitrogen extraction, the total phosphorous extracted by tomato plants fertigated with the nutrient solution was greater exhibiting a higher accumulation of P at root level. Different results have been noted by Colunje et al. [34] in a trial with pepper plants fertigated with nutrients, since in this case there was a clear accumulation of P in the stems and leaves. Ozone treatments have no impact on the total P extracted by the plant but modified the allocation of P between organs, resulting in an increase in root and flowers to the detriment of the shoot and leaf. No variations in P extraction in cucumber plants treated with ozonated water has also been reported by Najarian et al. [41]. There was no clear relationship between TPE and rising O_3 concentrations and, in the nutrient partitioning, there was only a clear positive relationship between leaf phosphorus ratio and O_3 concentrations. Similar tendencies have been documented by Colunje et al. [34] in pepper seedlings fertigated with nutrient solution.

The fertigation with the nutrient solution increased the total amount of potassium taken by the plant, resulting in a greater accumulation at the root level. This fact can be due to the higher availability of potassium and the activation of potassium transporters under increasing nutrient concentrations [42]. Although ozone treatments reduced the TKE, there was a higher accumulation of K in the leaf to the detriment of the shoot. These improvements in translocation were not obtained in pepper when ozonated fertigation was used [34]. In our experiment, no clear trends were reported in K extraction and partitioning in tomato plants grown under increasing O_3 concentrations. Similar results were obtained by Martinez-Sanchez and Aguayo [40] in watermelon under ozonized water conditions (750 mV).

5. Conclusions

Tomato plants fertigated with nutrient solution showed a rise in total plant dry weight and pigments concentration. Even though these plants had a greater leaf weight ratio, the values of RWC and LA fell when they were fertigated with nutritional solution. Under increasing O_3 concentrations, tomato plants were characterized by a decrease in the leaf area and an increase in the total dry weight and pigment concentrations, being maximum at a dose of 0.36 mg L^{-1}. The fertigation with the nutrient solution increased N, P, and K leaf concentration as well as the total extraction of these nutrients by tomato plants. Nevertheless, the partitioning of these nutrients between organs showed a specific trend of allocation and accumulation depending on the nutrient studied. Ozone treatments only increased leaf K concentration, did not change the total nitrogen and phosphorus extracted by plants, but decreased the total potassium extracted. In tomato plants growing under increasing O_3 concentrations, leaf P and K increased but the total extraction of N, P, and K by plants and the partitioning between organs did not show a clear trend. In conclusion, it is vital to note that investigating the effects of increasing O_3 concentrations on other horticultural species would necessitate additional research.

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