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Oxyfertigation and Transplanting Conditions of Strawberries

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Abstract: Soilless growing systems can improve water-use efficiency, especially in closed soilless growing conditions, and determine how supplying H2O2 as an oxygen source to the rhizosphere of strawberry plants in a soilless growing system affects plant growth, fruit yield and fruit quality. Strawberry plants (Fragaria x ananassa Duch.) cv. ‘Fortuna’ were cultivated in 12 L pots filled with peat substrate, and maintained under conditions of natural light and temperature. Treated plants were supplied with hydrogen peroxide (H2O2) (H1) and control plants did not receive H2O2 (H0). In terms of the transplanting conditions, the plants were transplanted in October (T1), and either maintained in a culture chamber (T2), or refrigerated (T3), for one month, before being transplanted. A completely randomized block design with two treatment factors (transplanting conditions, and H2O2 treatment) and five replications was established. Then, we determined the fruit per plant, yield per plant (g plant−1), fruit weight (g fruit−1), fruit size (mm), SPAD values, crown number, crown diameter (mm), flower number, firmness (g cm−1), pH, total soluble solid (TSS), titratable acidity (TA) and TSS/TA. During the early crop cycle, there were not significant differences between treatment and the transplanting conditions that significantly affected the fruit weight and fruit size, although T3 produced the highest values. During the late crop cycle, the H2O2 treatment affected fruit per plant, yield per plant (g plant−1), and crown diameter, with H1 producing the highest values. Furthermore, the transplanting conditions affected yield per plant (g plant−1), old SPAD values, crown diameter, firmness, TSS, TA and TSS/TA.

Keywords: oxygen; SPAD; total soluble solid; soilless; titratable acidity

1. Introduction

Soilless growing systems are characterized by dense plants roots confined in a small volume of growth medium, and by high rates of metabolic activity, respiration and growth. As a consequence of the root confinement, the oxygen requirements in the rhizosphere are particularly high. The following factors affect the concentration of O2 in the rhizosphere: aeration capacity of the growth medium [1]; volume of the growth medium [2]; irrigation management [3]; concentration of oxygen in the irrigation water [4]; organic matter content [1]; environmental temperature [5]; salinity of the growth medium [6], and the shape and depth of the pot [7].

Hypoxic conditions in the growth medium affect growth, plant yield and the quality of horticultural crops. This may be due to alterations in physiological and biochemical processes such as water potential [8], chlorophyll content and hormonal balance caused by excess ethylene [9,10].

Hypoxia induces oxidation and the death of roots [11] because of the lowered photosynthetic rate and the transport of assimilates. The reserves are also reduced by the catabolism of carbohydrates and proteins [12]. The lack of oxygen induces anaerobic
respiration, thus increasing the concentrations of ethanol and CO$_2$ in the rhizosphere to toxic levels [9].

High root density, along with increased temperature and salinity, and low O$_2$ concentration in the irrigation water, can lead to hypoxia in soilless growing systems [13]. High temperatures in the growth medium, and the subsequent increased respiration rate of roots can also cause oxygen deficiency [3,14,15].

Oxyfertigation consists of the injection of gaseous oxygen under pressure into the irrigation water [13,16]. In physical or mechanical oxyfertigation, the system is mechanically agitated, and air is injected into the nutrient solution [13]. Chemical oxyfertigation consists of the application of peroxide-based compounds, which release oxygen during the decomposition process [17].

Hydrogen peroxide (H$_2$O$_2$) as a chemical compound is often used as an oxidizer, bleaching agent, and disinfectant. H$_2$O$_2$, as a product generated from human body or enzymatic reaction, is a valuable biomarker of inflammation [18,19], some chronic diseases [20,21], and an essential mediator in food [22], industry, medical, and environmental analysis [23,24].

It is widely accepted that H$_2$O$_2$ is one of the most prominent signaling molecules, due to its higher relative stability and mobility [25]. As a result, H$_2$O$_2$ can migrate from sites of synthesis to adjacent compartments, and even neighboring cells [26], orchestrating multiple plant physiological processes [27]. Hydrogen peroxide root pretreatment was found to induce salt tolerance in maize and wheat seedlings [28,29], drought tolerance in soybean plants [30] and heat–stress tolerance in cucumber leaves [31], mainly through the mitigation of lipid peroxidation and the regulation of the activity of antioxidant enzymes.

Under conditions of hypoxia, the use of oxyfertigation boosts the root system, enhances microbial activity, and improves mineral transformations and water use [16]. Hydrogen peroxide, as an oxygen fertilizer to improve oxygen concentration in root zone soil and soil aeration, can effectively improve the nitrogen content in crops [32]. Oxyfertigation can also compensate for the negative effects of compaction and salinity of the growth medium. It can thus improve the growth medium and plant yield.

The authors of [33] have reported that, under conditions of hypoxia, tomato flowers and fruits may fall, due to the accumulation of ethylene. Under these conditions the tomato yield was also reduced, but the fruit diameter and weight were not affected. In tomato crops, the concentration of O$_2$, which can affect the growth, yield and fruit quality, ranged from 3 to 7 mg L$^{-1}$. The minimum concentrations of O$_2$ required for oxyfertigation have been reported to be 3 mg L$^{-1}$ [34], 5.3 mg L$^{-1}$ [35], and 6.7 mg L$^{-1}$ [36].

In a soilless growing system with coir fibre as the growth medium for tomato, [33] did not find any effect on the yield at an O$_2$ concentration of 5.5 mg L$^{-1}$.

Similar O$_2$ thresholds have been reported for different plants: 2.7 mg L$^{-1}$ for cucumber (Cucumis sativus) [36], and 2 mg L$^{-1}$ for pepper (Capsicum annuum) [37]. In a soilless growing system with perlite as the growth medium, pepper did not observe any effect on fertirrigation parameters, height of the plant, biomass or productivity due to O$_2$ concentration (ranging from 4.2 mg O$_2$ L$^{-1}$ to 21.8 mg O$_2$ L$^{-1}$) [13]. On the other hand, also regarding pepper, other authors have reported that yield increased in response to the application of chemical oxifertirrigation in the nutrient solution [13]. For zucchini (Cucurbita pepo), soybean (Glycine max) and cotton (Gossypium hirsutum) plants grown in a clay soil in which hypoxia occurred, some authors obtained an increase in the total biomass and yield by applying H$_2$O$_2$ in fertigation [38].

H$_2$O$_2$ has been associated with increasing photosynthetic capacity, reducing lipid membrane stability and enhancing thermo-tolerance of plants during the heat-stress period [39,40]. For example, exogenously applied H$_2$O$_2$ has been found to activate a defensive system of barley and maize [28]. Another report suggested that the provision of H$_2$O$_2$ could increase the crop yield in a soilless growing system, with coir fibre used as the growth medium for strawberry plants [41].
The aim of the present study was to evaluate the effects of different transplanting conditions, and how supplying H$_2$O$_2$ as an oxygen source to the rhizosphere of strawberry plants in a soilless growing system would affect plant growth, fruit yield and fruit quality.

2. Materials and Methods

2.1. Experimental Site and Cropping System

The experiment was conducted in methacrylate tunnel-type greenhouses in the Rábida Campus, Huelva University (37°12’ latitude, 6°55’ longitude and 24 m above sea level), Huelva (SW Spain), under conditions of natural light and temperature between October 2016 and June 2017. Huelva was first area of strawberry production in the European Union and is the largest export of fresh strawberry fruit in the world. Huelva growers harvested approximately 6355 ha, with an average yield of 54,940 kg ha$^{-1}$, in the 2016–2017 season, with a return of about €454 million and a crop production of 349,143 ton [42]. The cropping season in the area is divided into two different periods: a cold, early crop season (low temperatures and high relative humidity) between January and March (early production), and a warmer, late crop season (high temperatures and low relative humidity) between April and June (late production) [43].

Strawberry plants, (Fragaria × ananassa Duch.) cv. ‘Fortuna’, were cultivated in a soilless growing system in 12 L pots filled with peat substrate (Gramoflor GmbH and Co. KG, Vechta, Germany), held under conditions of natural light and temperature, and watered with a drip irrigation system (1 drip per plant with a flow of 2.3 L h$^{-1}$).

2.2. Experimental Design

A completely randomized block design with two factors (2 H$_2$O$_2$ treatments × 3 transplanting conditions) and 5 replications was established. Treated plants were supplied with H$_2$O$_2$ (H1) and control plants did not receive H$_2$O$_2$ (H0). The plants were transplanted under different conditions: T1 plants were transplanted in October; T2 plants were maintained in the culture chamber for one month before being transplanted; T3 plants were maintained under refrigeration (4 °C) for one month before being transplanted. The nutrient solution consisted of (mg L$^{-1}$): N 271, P 702, K 586, Mg 207, S 414, Fe 8, Mn 4, Cu 0.3, Zn 0.8, B 0.7 and Mo 0.3, in accordance with standard crop-cultivation practices [43]. The pH and EC of the drainage were monitored weekly. The pH in water was measured by pHTestr® 30 (Oakton®, Vernon Hills, IL, USA), with a previous calibration with buffer solution pH = 7, and the EC were measured by ECStrr® 11+ (Envco®, Australia, New Zealand). Analyses of the drainage water revealed an average EC of approximately 0.47 mS m$^{-1}$ and a pH of 7.02 (Figure 1).

![Figure 1. pH and CE on drained solution A = H0 (without H$_2$O$_2$) B = H1 (with H$_2$O$_2$) on ‘Fortuna’ strawberry plants, recorded during the crop cycle in 2016/2017.](image-url)

The H$_2$O$_2$ was injected directly into each plant rhizosphere once a week, with a calibrated syringe. For this purpose, 100 mL of 35% H$_2$O$_2$ was dissolved in 5 L of distilled water, and
11 mL (2.56 mmol H$_2$O$_2$) of this solution was supplied to each plant. The O$_2$ concentration was determined using an Oxymeter (OXI 330i) fitted with a sensor (CellOx 325, Crison®, Edmonton, Alberta, Canada). The O$_2$ concentration (mg L$^{-1}$) in the drained solution was measured weekly during the crop cycle, in treatments H1 and H0. The temperature and the relative humidity (data not shown) were recorded every 60 min during the crop growing period, with a temperature and humidity data logger (Watchdog A-Series Logger-A150, Spectrum Technologies, Inc., Aurora, IL, USA).

2.3. Laboratory Measurements

Strawberry fruits were graded for size and external colour, and damaged fruits were discarded.

Ripe fruits from the plants in each treatment were harvested throughout the experimental period. Fruit per plant and the total marketable fruit yield (sum of early and late production) between 1 January and 31 May was recorded weekly, as grams per plant and fruit weight (g fruit$^{-1}$), during the crop cycle. The early and late marketable fruit yields were respectively recorded between 1 January and 31 March, in grams per plant. Fruit size (mm) was determined by the maximum diameter of the equatorial section. To evaluate the effects of the treatments/conditions, during the early and late crop cycle we determined the chlorophyll content (SPAD values), crown number, crown diameter (mm) and flowers number. Rapid, non-destructive measurements of leaf chlorophyll content were obtained with a chlorophyll meter (SPAD-502, Minolta Camera Co., Ltd., Osaka, Japan). Thus, the chlorophyll content of young leaves on the same plant was recorded weekly, at random times during the crop cycle, by obtaining SPAD readings with the meter. Measuring the young leaves with SPAD guaranteed that we never measured the same leaf.

The fruit quality was also evaluated by means of firmness (g cm$^{-1}$), pH, total soluble solid (TSS), titratable acidity (TA) and TSS/TA ratio. The firmness was evaluated in a sub-sample of 3–4 fruits from each treatment using a portable penetrometer, and the results were expressed in g cm$^{-2}$. The TSS was determined using an automatic temperature-compensated PR101 digital refractometer (Atago Pallette PR101; Atago Co., Itabashi-Ku, Tokyo, Japan). TA was measured in each treatment by titrating 10 g of the pulp, plus 10 mL of H$_2$O with 0.1 mol L$^{-1}$ NaOH, up to a pH of 8.1, and it was expressed as g of citric acid per 100 g$^{-1}$ fresh weight.

2.4. Statistical Analysis

Data were analysed by two-way ANOVA with SPSS software version 27.0 (SPSS, IBM, Chicago, IL, USA), and when the F value was significant, means were separated by Tukey’s multiple range test. Significance was accepted with $p < 0.05$. The variables analyzed by ANOVA were fruit per plant, yield, fruit weight, fruit size, SPAD values, crown numbers, crown diameter, flowers, firmness, pH, TSS, TA, TSS/TA ratio, O$_2$, pH and CE on drained solution.

3. Results and Discussion

The results suggested that the H$_2$O$_2$ treatment affected fruit per plant and yield per plant (g plant$^{-1}$), crown number, crown diameter and TA (%); on the other hand, the transplanting conditions affected yield per plant (g plant$^{-1}$), fruit weight (g fruit$^{-1}$), fruit size (mm), old and new SPAD, crown number, crown diameter (mm), firmness (g cm$^{-1}$), TSS (mg kg$^{-1}$), TA (%) and TSS/TA ratio.

During the early crop cycle there were not significant differences between treatments (Table 1). However, during the late crop cycle, there were significant differences between treatments and fruit per plant, with treatment H1 producing a significant higher fruit per plant (3.95) than the control treatment H0 (2.97) (Table 2). Similarly, other authors suggested that the application of H$_2$O$_2$ in a soilless growing system with coir fibre as the growth medium for the strawberries increased the number of strawberry fruits per plant [41].
**Table 1.** Combined effects of hydrogen peroxide and transplanting conditions on yield per plant and growth parameters (mean ± standard deviation) recorded during the early crop cycle (before week 16) in 2016/2017 on ‘Fortuna’ strawberry plants.

| Experimental Factor | Fruit per Plant (Numbers) | Yield per Plant (g plant⁻¹) | Fruit Weight (g fruit⁻¹) | Fruit Size (mm) | Old SPAD | New SPAD | Crown Diameter (mm) | Flowers (Numbers) |
|---------------------|---------------------------|----------------------------|--------------------------|-----------------|----------|----------|---------------------|------------------|
| **Transplanting conditions (T)¹** | | | | | | | | |
| T1 (control)        | 2.77 ± 1.85               | 59.46 ± 37.39              | 22.49 ± 7.36 b           | 33.14 ± 4.62 b  | 52.85 ± 4.27 a | 38.48 ± 5.00 | 1.85 ± 0.90 b        | 34.50 ± 15.06 b   |
| T2                  | 2.58 ± 1.28               | 58.18 ± 29.74              | 23.28 ± 8.24 b           | 33.30 ± 4.68 b  | 52.13 ± 3.75 ab| 38.83 ± 5.64 | 1.84 ± 0.83 b        | 34.59 ± 13.64 b   |
| T3                  | 2.55 ± 1.97               | 65.54 ± 40.45              | 28.08 ± 10.48 a          | 36.51 ± 4.75 a  | 50.41 ± 6.56 b | 37.32 ± 4.91 | 2.45 ± 1.01 a        | 41.04 ± 15.87 a   |
| Significance        | ns                         | ns                        | **                       | ns              | **       | ns       | ns                  | ns               |
| H₀ (without H₂O₂)   | 2.40 ± 1.48               | 57.40 ± 33.94              | 25.23 ± 9.96             | 34.28 ± 5.21    | 51.95 ± 6.14 | 38.30 ± 5.40 | 1.90 ± 0.91 b        | 35.78 ± 14.56 a   |
| H₁ (with H₂O₂)      | 2.88 ± 1.93               | 65.52 ± 38.22              | 24.34 ± 8.33             | 34.59 ± 4.60    | 51.64 ± 3.79 | 38.13 ± 5.06 | 2.18 ± 0.98 a        | 37.59 ± 15.68 a   |
| Interaction T × H  | ns                         | ns                        | ns                       | ns              | ns       | ns       | ns                  | ns               |

¹ Transplanting conditions: T1 plants were transplanted at the usual planting time (October); T2 plants were maintained in the culture chamber for one month before transplanting; T3 plants were maintained in cold storage for 1 month at T = 4 °C before transplanting. Different letters indicate significant differences in mean values (Tukey’s test, p < 0.05). * Significant at p < 0.05. ** Significant at p < 0.01. ns: effect of treatment not significant.

**Table 2.** Combined effects of hydrogen peroxide and transplanting conditions on yield per plant and growth parameters (mean ± standard deviation) recorded during the late crop cycle (between week 16 and 22) in 2016/2017 on ‘Fortuna’ strawberry plants.

| Experimental Factor | Fruit per Plant (Numbers) | Yield per Plant (g plant⁻¹) | Fruit Weight (g fruit⁻¹) | Fruit Size (mm) | Old SPAD | New SPAD | Crown Diameter (mm) | Flowers (Numbers) |
|---------------------|---------------------------|----------------------------|--------------------------|-----------------|----------|----------|---------------------|------------------|
| **Transplanting conditions (T)¹** | | | | | | | | |
| T1 (control)        | 3.33 ± 1.97               | 47.70 ± 30.49 ab           | 15.19 ± 3.81             | 33.02 ± 3.97    | 49.93 ± 4.48 | 36.06 ± 2.51 | 2.77 ± 1.16         | 49.55 ± 9.25 b    |
| T2                  | 3.00 ± 2.22               | 42.24 ± 31.50 b            | 14.33 ± 4.15             | 31.52 ± 4.20    | 48.93 ± 4.48 | 37.13 ± 4.40 | 2.79 ± 1.26         | 53.45 ± 8.22 b    |
| T3                  | 4.03 ± 2.11               | 62.06 ± 36.38 a            | 16.70 ± 4.91             | 32.80 ± 4.05    | 49.08 ± 4.08 | 36.66 ± 3.46 | 3.44 ± 1.12         | 60.50 ± 5.95 a    |
| Significance        | ns                         | ns                        | ns                       | ns              | ns       | ns       | ns                  | ns               |
| H₀ (without H₂O₂)   | 2.97 ± 1.55 b             | 42.48 ± 26.84 b            | 15.66 ± 4.33             | 32.31 ± 3.84    | 49.43 ± 5.20 | 36.60 ± 3.14 | 2.79 ± 1.26         | 51.29 ± 10.68 b   |
| H₁ (with H₂O₂)      | 3.95 ± 2.53 a             | 58.98 ± 37.99 a            | 15.08 ± 4.27             | 32.62 ± 4.35    | 49.20 ± 3.74 | 36.62 ± 3.91 | 3.18 ± 1.18         | 57.10 ± 6.18 a    |
| Interaction T × H  | ns                         | ns                        | ns                       | ns              | ns       | ns       | ns                  | ns               |

¹ Transplanting conditions: T1 plants were transplanted at the usual planting time (October); T2 plants were maintained in the culture chamber for one month before transplanting; T3 plants were maintained in cold storage for 1 month at T = 4 °C before transplanting. Different letters indicate significant differences in mean values (Tukey’s test, p < 0.05). * Significant at p < 0.05. ** Significant at p < 0.01. ns: effect of treatment not significant.
During the late crop cycle there were significant differences between treatments and yield per plant (g plant$^{-1}$), with treatment H1 producing a significantly higher yield (58.98 g plant$^{-1}$) than the control treatment H0 (42.48 g plant$^{-1}$). Furthermore, the transplanting conditions also significantly affected the yield per plant, with T3 producing the highest value (62.06 g plant$^{-1}$) (Table 2). The findings were consistent with the suggestion of other authors that said the application of H$_2$O$_2$ in a soilless growing system with coir fibre as the growth medium for strawberry could increase the crop yield [41]. Another study suggested that significant increases in marketable yield were found for a commercial greenhouse cucumber crop grown on rockwool slabs with oxyfertigation [44]. Another report suggested that significant increases in marketable yield were found for a spring melon grown on rockwool slabs with chemical oxygen enrichment [17]. On the other hand, no marketable yield differences were observed by other authors for a watermelon crop grown on perlite grow-bags [3].

During the early crop cycle the transplanting conditions also significantly affected the fruit weight and fruit size, with T3 producing the highest values (28.08 g fruit$^{-1}$ and 36.51 mm, respectively) (Table 1). However, during the late crop cycle the fruit weight and fruit size were lower than during the early crop cycle. The reduction in fruit weight at the end of the late crop cycle was related to an increase in temperature. According to another study, temperatures above 30 $^\circ$C may cause a reduction in the size and weight of strawberry fruits [45]. Another report suggested that the yield of strawberry cultivars under different temperature conditions, the number of inflorescences, the number of fruits and the average fruit weight, were all significantly lower when the day/night temperature ratio was 30/25 $^\circ$C, compared with 23/18 $^\circ$C [46].

The results indicated that during the early crop cycle, crown number was affected by treatment, with treatment H1 producing a significantly higher crown number (2.18) than the control treatment H0 (1.90). Furthermore, the transplanting conditions also significantly affected the old SPAD, crown number and crown diameter. The treatment T3 showed the highest values of crown number (2.45) and crown diameter (41.04 mm) (Table 1).

During the late crop cycle, the results showed that there were significant differences between treatment and crown diameter (mm), with treatment H1 producing a significantly higher crown diameter (57.10 mm) than the control treatment H0 (51.29 mm). Furthermore, the transplanting conditions also significantly affected the crown diameter, with T3 producing the highest value (60.50 mm) (Table 2). Low temperature lowered the cell elongation [47]. Another report suggested that low temperature increased shoot and root length, and this increased shoot and root growth might be due to higher cell expansion in primed seeds of maize [48].

Our results indicated that flower number was not affected by treatment and that the flower number did not differ in relation to the transplanting conditions (Tables 1 and 2). Finally, the H$_2$O$_2$ treatment did not affect the firmness, pH, TA and TSS/TA ratio during the early crop cycle (Table 3). Another report suggested that in a greenhouse hydroponic tomato crop, there was no effect of hydrogen peroxide treatment on the fruit quality [49]. Significant differences in TSS and TA were observed during the early crop cycle in relation to transplanting conditions (Table 3).

During the late crop cycle, the firmness, TSS (mg kg$^{-1}$) and TA (%) were affected by transplanting conditions (Table 4). However, the pH did not differ in relation to the transplanting conditions. The transplanting conditions also affected the TSS/TA ratio, and the highest values were observed in T3 plants (13.82) (Table 4).

Figure 2 shows values for pH and electrical conductivity (EC) of the nutrient solution measured during the early and late crop cycle. The maximum values were recorded during the late crop cycle. The highest value of EC (0.54 mS m$^{-1}$) was obtained in the month of April. For strawberry soilless cultivation, EC values of around 0.32 mS m$^{-1}$ at the beginning of the bioassay were indicated to obtain high yield and fruit quality [50]. With respect to pH values, these tended to decrease during the late crop cycle. The lowest values were registered in May (6.6), with pH values of around 6.7 (Figure 1).
Table 3. The combined effects of hydrogen peroxide and transplanting conditions on fruit quality parameters (mean ± standard deviation) recorded during the early crop cycle (between week 1 and week 15) in 2016/2017 on ‘Fortuna’ strawberry plants.

| Experimental Factor      | Firmness (g cm\(^{-2}\)) | pH | Total Soluble Solids (TSS) (mg kg\(^{-1}\)) | Titratable Acidity (TA) (%) | Total Soluble Solids TA \(^{-1}\) |
|-------------------------|-------------------------|----|----------------------------------|------------------------|------------------|
| Transplanting conditions (T) \(^1\) |                        |    |                                  |                        |                  |
| T1 (control)            | 282.13 ± 33.21          | 3.44 ± 0.28 | 6.11 ± 1.15                      | 0.54 ± 0.08 b         | 11.18 ± 2.78     |
| T2                      | 290.69 ± 35.98          | 3.34 ± 0.25 | 6.07 ± 1.20                      | 0.59 ± 0.10 b         | 10.38 ± 2.81     |
| T3                      | 294.74 ± 30.78          | 3.34 ± 0.44 | 6.58 ± 1.48                      | 0.73 ± 0.09 a         | 11.69 ± 2.76     |
| Significance             | ns                      | ns  | ns                               | ns                     |                  |
| Hydrogen peroxide (H\(_2\)O\(_2\)) |                        |    |                                  |                        |                  |
| H0 (without H\(_2\)O\(_2\)) | 289.66 ± 34.85          | 3.36 ± 0.39 | 6.24 ± 1.43 b                   | 0.69 ± 0.08           | 10.79 ± 2.86     |
| H1 (with H\(_2\)O\(_2\))  | 289.61 ± 32.22          | 3.38 ± 0.27 | 6.31 ± 1.17 a                   | 0.56 ± 0.09           | 11.38 ± 2.76     |
| Significance             | ns                      | ns  | ns                               | ns                     |                  |
| Interaction T × H        | ns                      | ns  | ns                               | ns                     |                  |

\(^1\) Transplanting conditions: T1 plants were transplanted at the usual planting time (October); T2 plants were maintained in the culture chamber for one month before transplanting; T3 plants were maintained in cold storage for 1 month at T = 4 °C before transplanting. Different letters indicate significant differences in mean values (Tukey’s test, \(p < 0.05\)). * Significant at \(p < 0.05\). ns: effect of treatment not significant.

Table 4. The combined effects of hydrogen peroxide and transplanting conditions on fruit quality parameters (mean ± standard deviation) recorded during the late crop cycle (between week 16 and week 22) in 2016/2017 on ‘Fortuna’ strawberry plants.

| Experimental Factor      | Firmness (g cm\(^{-2}\)) | pH | Total Soluble Solids (TSS) (mg kg\(^{-1}\)) | Titratable Acidity (TA) (%) | Total Soluble Solids TA \(^{-1}\) |
|-------------------------|-------------------------|----|----------------------------------|------------------------|------------------|
| Transplanting conditions (T) \(^1\) |                        |    |                                  |                        |                  |
| T1 (control)            | 304.11 ± 47.30 a        | 3.19 ± 0.15 | 6.33 ± 1.51 ab                  | 0.57 ± 0.07 b         | 12.63 ± 1.93 ab  |
| T2                      | 297.86 ± 49.14 ab       | 3.16 ± 0.31 | 6.78 ± 1.70 ab                  | 0.62 ± 0.06 a         | 10.94 ± 3.51 b   |
| T3                      | 272.63 ± 55.58 b        | 3.18 ± 0.12 | 5.79 ± 1.43 b                   | 0.49 ± 0.07 c         | 13.82 ± 2.26 a   |
| Significance             | *                      | ns  | *                               | ns                     |                  |
| Hydrogen peroxide (H\(_2\)O\(_2\)) |                        |    |                                  |                        |                  |
| H0 (without H\(_2\)O\(_2\)) | 294.26 ± 49.65          | 3.22 ± 0.26 | 6.33 ± 1.65                     | 0.59 ± 0.09 a         | 12.60 ± 2.65     |
| H1 (with H\(_2\)O\(_2\))  | 291.19 ± 54.26          | 3.14 ± 0.12 | 6.23 ± 1.36                     | 0.55 ± 0.06 b         | 12.03 ± 3.06     |
| Significance             | ns                      | ns  | ns                               | ns                     |                  |
| Interaction T × H        | *                      | ns  | *                               | ns                     |                  |

\(^1\) Transplanting conditions: T1 plants were transplanted at the usual planting time (October); T2 plants were maintained in the culture chamber for one month before transplanting; T3 plants were maintained in cold storage for 1 month at T = 4 °C before transplanting. Different letters indicate significant differences in mean values (Tukey’s test, \(p < 0.05\)). * Significant at \(p < 0.05\). ** Significant at \(v < 0.01\). ns: effect of treatment not significant.
crop cycle. The highest O₂ concentration was recorded during December (9.89) mg L⁻¹ and the lowest O₂ concentration was recorded during April (5.99) mg L⁻¹ (Figure 2).

The highest temperatures were recorded during the late crop cycle, and the highest values were recorded during December (29 °C). The faster rate of ripening related to the high temperatures. Another study also reported the relationship between high temperatures and the rate of ripening [43], and similar effects on fruit production have previously been reported for strawberry plants [51]. Moreover, temperature and day length may play a key role in fruit production [52]. The O₂ concentration in the drained solution was lower at the end than at the beginning of the crop cycle. Therefore, the decrease in O₂ concentration during the late crop cycle may be due to the higher temperatures at this time. High temperatures are also known to contribute to soil–oxygen deficiencies [14,15]. Another study reported that in a soilless growing system, with rock wool used as the growth medium for melon (Cucumis melo), the O₂ concentration decreased from 4–5 mg L⁻¹, at the beginning of the crop cycle, to 2 mg L⁻¹ at the end of the crop cycle [53]. Increased temperature can lead to hypoxic conditions in soilless growing systems [13]. In the present study, the O₂ concentration tended to decrease at the end of the crop cycle. This may have been due to an increase in the respiration rate in the plant rhizosphere, caused by the higher temperatures at this time. Supply of H₂O₂ at the end of the crop cycle could compensate for the decrease in O₂ concentration registered at this time.

4. Conclusions

The data presented here indicate that the application of H₂O₂ (Treatment H1) during the late crop cycle in a soilless growing system, with peat as the growth medium for strawberries, affects the strawberry yield and growth parameters. However, supplying the strawberry plants with H₂O₂ (treatment H1) did not affect the fruit quality.

The transplanting conditions (T3) affected strawberry yield and growth parameters during the early and late crop cycle. However, only fruit quality was affected during the late crop cycle. The study findings suggest that the supply of H₂O₂ may be a successful means of increasing the concentration of O₂ during the late crop cycle, thus increasing the total strawberry yield in soilless growing systems in the study area. Similarly, oxyfer- tigation proved a good method of improving the yield and growth of strawberry crops in the soilless growing system, providing economic and environmental benefits. These results represent an important contribution for the improvement of soilless growing-system use in strawberry cultivation. Further research should be carried out with different cultivars to confirm the influence of H₂O₂ treatment on strawberry plants. Considering the crucial role of H₂O₂ treatment in crops from a broad range of abiotic stresses, we hypothesized that high-temperature-induced damage to strawberry plants can be minimized by applying H₂O₂.

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Agronomy 2021, 30. Ishibashi, Y.; Yamaguchi, H.; Yuasa, T.; Iwaya-Inoue, M.; Arima, S.; Zheng, S.H. Hydrogen peroxide spraying alleviates drought

5. Bonachela, S.; Acuña, R.; Casas, J. Environmental factors and management practices controlling oxygen dynamics in agricultural irrigation ponds in a semiarid Mediterranean region: Implications for pond agricultural functions. Water Res. 2007, 41, 1225–1234. [CrossRef]

6. Barret-Lennard, E. The interaction between water logging and salinity in higher plants: Causes, consequences and implications. Plant Soil 2003, 253, 35–54. [CrossRef]

7. Urrestarazu, M. Bases y Sistemas de los Cultivos Sin Suelo. In Tratado Internacional de Cultivo Sin Suelo; Urrestarazu, M., Ed.; Mundi Prensa: Almería, Spain, 2004; pp. 3–48.

8. Drew, M. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1997, 48, 223–250. [CrossRef]

9. Irving, L.; Sheng, Y.; Woolley, D.; Matthew, C. Physiological effects of waterlogging on two lucerne varieties grown under glasshouse conditions. J. Agron. Crop Sci. 2007, 193, 345–356. [CrossRef]

10. Horchani, F.; Galluscì, P.; Baudet, P.; Cabasson, C.; Maucourt, M.; Rolin, D.; Aschi-Smiti, S.; Raymond, P. Prolonged root hypoxia induces ammonium accumulation and decreases the nutritional quality of tomato fruits. J. Plant Physiol. 2008, 165, 1352–1359. [CrossRef]

11. Horchani, F.; Khayati, H.; Raymond, P.; Brouquisse, R.; Aschi-Smiti, S. Contrasted effects of prolonged root hypoxia on tomato root and fruit (Solanum lycopersicum) metabolism. J. Agron. Crop Sci. 2009, 195, 313–318. [CrossRef]

12. Devaux, C.; Baudet, P.; Joubert, J.; Dieuaide-Noubhani, M.; Just, D.; Chevalier, C. Physiological, biochemical and molecular analysis of sugar-starvation responses in tomato roots. J. Exp. Bot. 2003, 54, 1143–1151. [CrossRef]

13. Marfà, O.; Cáceres, R.; Guri, S. Oxyfertilization: A new technique for soilless culture under Mediterranean conditions. Acta Hortic. 2005, 697, 65–72. [CrossRef]

14. Asplund, P.T.; Curtis, W.R. Intrinsic oxygen use kinetics of transformed plant root culture. Biotechnol. Prog. 2001, 17, 481–489. [CrossRef]

15. Ityel, E.; Ben-Gal, A.; Silberbush, M.; Lazarovitch, N. Increased root zone oxygen by a capillary barrier is beneficial to bell pepper irrigated with brackish water in an arid region. Agric. Water Manag. 2014, 31, 108–114. [CrossRef]

16. Bhattarai, S.; Su, N.; Midmore, D. Oxygation unlocks yield potentials of crops in oxygen-limited soil environments. Adv. Agron. 2005, 88, 313–377.

17. Urrestarazu, M.; Mazuela, P. Effect of slow-release oxygen supply by fertigation on horticultural crops under soilless culture. Sci. Hortic. 2005, 106, 484–490. [CrossRef]

18. Halliwell, B.; Clemente, M.V.; Long, L.H. Hydrogen peroxide in the human body. FEBS Lett. 2000, 486, 10–13. [CrossRef]

19. Long, L.H.; Evans, P.J.; Halliwell, B. Hydrogen peroxide in human urine: Implications for antioxidant defense and redox regulation. Biochem. Biophys. Res. Commun. 1999, 262, 605–609. [CrossRef] [PubMed]

20. Yorek, M.A. The role of oxidative stress in diabetic vascular and neural disease. Free Radic. Res. 2003, 37, 471–480. [CrossRef] [PubMed]

21. Fanari, F.M. Hydrogen peroxide: Watery fuel for change in vascular biology. Arter. Thromb. Vasc. Biol. 2006, 26, 1931–1933. [CrossRef]

22. Marks, N.E.; Grandison, A.S.; Lewis, M.J. Use of hydrogen peroxide detection strips to determine the extent of pasteurization in whole milk. Int. J. Dairy Technol. 2001, 54, 20–22. [CrossRef]

23. Notsu, H.; Tatsuma, T.; Fujishima, A. Tyrosinase-modified boron-doped diamond electrodes for the determination of phenol derivatives. J. Electroanal. Chem. 2002, 523, 86–92. [CrossRef]

24. Nikolelis, D.P.; Krull, U.; Wang, J.; Mascini, M. Biosensors for direct monitoring of environmental pollutants in field. Klueser Acad. Publ. 1998, 38, 220–226.

25. Moller, I.M.; Jensen, P.E.; Hansson, A. Oxidative Modifications to Cellular Components in Plants. Annu. Rev. Plant. Physiol. 2007, 58, 459–481. [CrossRef]

26. Bienert, G.P.; Schjoerring, J.K.; Jahn, T.P. Membrane transport of hydrogen peroxide. Biochim. Biophys. Acta-BiOMEMBR. 2006, 1758, 994–1003. [CrossRef]

27. Neill, S.; Deskman, R.; Hancock, J. Hydrogen peroxide signalling. Curr. Opin. Plant Biol. 2002, 5, 388–395. [CrossRef]

28. De Azevedo Neto, A.D.; Prisco, J.T.; Enas-Filho, J.; Medeiros, J.V.R.; Gomes-Filho, E. Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. J. Plant Physiol. 2005, 162, 1114–1122. [CrossRef]

29. Wahid, A.; Perveen, M.; Gelani, S.; Basra, S.M.A. Pretreatment of seed with H2O2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. J. Plant Physiol. 2007, 164, 283–294. [CrossRef]

30. Ishibashi, Y.; Yamaguchi, H.; Yuasa, T.; Iwaya-Inoue, M.; Arima, S.; Zheng, S.H. Hydrogen peroxide spraying alleviates drought stress in soybean plants. J. Plant Physiol. 2011, 168, 1562–1567. [CrossRef]
31. Gao, Y.; Guo, Y.K.; Lin, S.H.; Fang, Y.Y.; Bai, J.G. Hydrogen peroxide pretreatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat-stressed cucumber leaves. *Sci. Hortic.* 2010, 126, 20–26. [CrossRef]

32. Yuan, L.; Wenguan, N.; Jingwei, W.; Jian, X.; Mingzhi, Z.; Kangyong, L. Review on advances of airjection irrigation. *Int. J Agric. Biol. Eng.* 2016, 9, 1–10.

33. Horchani, F.; Aloui, A.; Brouquisse, R.; Aschi-Smiti, S. Physiological responses of tomato plants (*Solanum lycopersicum*) as affected by root hypoxia. *J. Agron. Crop Sci.* 2008, 194, 297–303. [CrossRef]

34. Gislerod, H.; Adams, P. Diurnal variations in the oxygen content and acid requirement of recirculating nutrient solutions and in the uptake of water and potassium by cucumber and tomato plants. *Sci. Hortic.* 1983, 21, 311–321. [CrossRef]

35. Zheng, Y.; Wang, L.; Dixon, M. An upper limit for elevated root zone dissolved oxygen concentration for tomato. *Sci. Hortic.* 2007, 113, 162–165. [CrossRef]

36. Gislerod, H.; Adams, P. Diurnal variations in the oxygen content and acid requirement of recirculating nutrient solutions and in the uptake of water and potassium by cucumber and tomato plants. *Sci. Hortic.* 1983, 21, 311–321. [CrossRef]

37. Ehret, D.; Edwards, D.; Helmer, T.; Lin, W.; Jones, G.; Dorais, M. Effects of oxygen-enriched nutrient solution on greenhouse cucumber and pepper production. *Sci. Hortic.* 2010, 125, 602–607. [CrossRef]

38. Bhattachar, S.; Huber, S.; Midmore, D. Aerated subsurface irrigation water gives growth and yield benefits to zucchini, vegetable soybean and cotton in heavy clay soils. *Ann. Appl. Biol.* 2004, 144, 285–298. [CrossRef]

39. Larkinadle, J.; Huang, B. Thermostolerance and antioxidant systems in Agrostis stolonifera: Involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. *J. Plant Physiol.* 2004, 161, 405–413. [CrossRef]

40. Fahad, S.; Hussain, S.; Saud, S.; Khan, F.; Hassan, S.; Nasim, W.; Arif, M.; Wang, F.; Huang, J. Exogenously applied plant growth regulators affect heat stressed rice pollens. *J. Agron. Crop Sci.* 2016, 202, 139–150. [CrossRef]

41. Orihuela, D.L.; Hernández, J.C.; Flores, F.; Tornos, P. La oxifertirragación y su efecto sobre algunos parámetros productivos en fresa (*Fragaria x ananassa* D.). *Comunicaciones SECH* 2012, 20, 161–165.

42. MAPA. Ministry of Agriculture, Fisheries and Food (Spain). 2017. Available online: [https://www.mapa.gob.es/estadistica/pags/anuario/2018/CAPITULOSPDF/CAPITULO07/pdfc07_6.30.2.pdf](https://www.mapa.gob.es/estadistica/pags/anuario/2018/CAPITULOSPDF/CAPITULO07/pdfc07_6.30.2.pdf) (accessed on 30 November 2021).

43. Palencia, P.; Martinez, F.; Medina, J.J.; Medina, J.L. Strawberry yield efficiency and its correlation with temperature and solar radiation. *Hortic. Bras.* 2013, 31, 93–99. [CrossRef]

44. Marfà, O.; Guri, S. Efectos de la aplicación de oxígeno en el agua de riego en cultivos sin suelo. *Agrícola Vergel* 2001, 239, 593–596.

45. Ledesma, N.A.; Nakata, M.; Sugiyama, N. Effect of high temperature stress on the reproductive growth of strawberry cvs. “Nyoho” and “Toyonoka”. *Sci. Hortic.* 2008, 116, 186–193. [CrossRef]

46. De Miranda, F.R.; da Silva, V.B.; dos Santos, F.S.R.; Guimaraes, A.; da Silva, C.F.B. Production of strawberry cultivars in closed hydroponic systems and coconut fibre substrate. *Cienc. Agronômica* 2014, 45, 833–841. [CrossRef]

47. Ben-Haj-Salah, H.; Tardieu, F. Temperature affects expansion rate of maize leaves without change in distribution of cell length. *Plant Physiol.* 1995, 109, 861–870. [CrossRef][PubMed]

48. Ahmad, I.; Basra, M.S.A.; Hussain, S.; Hussain, S.A.; Rehman, H.U.; Rehman, A.; Ali, A. Priming with ascorbic acid, salicylic acid and hydrogen peroxide improves seedling growth of spring maize at suboptimal temperature. *J. Environ. Agric. Sci.* 2015, 3, 14–22.

49. Soto-Bravo, F. Oxifertirrigación química mediante riego en tomate hidropónico cultivado en invernadero. *Agron. Mesoam.* 2015, 26, 227–289. [CrossRef]

50. Martínez, F.; Castillo, S.; Borrero, C.; Pérez, S.; Palencia, P.; Avilés, M. Effect of different soilless growing systems on the biological properties of growth media in strawberry. *Sci. Hortic.* 2013, 150, 59–64. [CrossRef]

51. Caruso, G.; Villari, G.; Melchionna, G.; Contic, S. Effects of cultural cycles and nutrient solutions on plant growth, yield and fruit quality of alpine strawberry (*Fragaria vesca* L.) grown in hydroponics. *Sci. Hortic.* 2011, 129, 479–485. [CrossRef]

52. Adams, P. Nutritional Control in Hydroponics. In *Hydroponic Production of Vegetables and Ornamentals*; Savvas, D., Passam, H.C., Eds.; Embassy Publications: Athens, Greece, 2002; pp. 211–261.

53. Bonachela, S.; Acuña, R.A.; Magan, J.J.; Malfa, O. Oxygen Enrichment of Nutrient Solution of Substrate-Grown Vegetable Crops under Mediterranean Greenhouse Conditions: Oxygen Content Dynamics and Crop Response. *Span. J. Agric. Res.* 2010, 8, 1231–1241. [CrossRef]