*horticulturae*

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# Effects of Different Irrigation Rates on Remontant Strawberry Cultivars Grown in Soil

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**Abstract:** The present study assessed the responses, in terms of vegetative, productive, qualitative, and nutritional features, of plants and berries of three remontant strawberry cultivars cultivated in soil and irrigated using three irrigation regimes: standard irrigation regime (W100), 20% (W80) less irrigation than the standard irrigation, and 40% (W60) less irrigation than the standard irrigation. The tested plants were "Albion", "San Andreas", and "Monterey", which were cultivated in the east coast area of Marche, Italy. Specifically, the study examined the response of the genotype to irrigation deficit, highlighting the performance of the "Monterey" cultivar, which showed improvement in terms of fruit firmness, folate content, and antioxidant capacity at the W80 irrigation regime without a significant yield reduction. In all the cultivars, when irrigation was reduced by up to 20% of the standard irrigation regime (W100), there were no significant losses of yield or reduction in the fruits' sensorial quality or antioxidant activity. The results showed that the standard irrigation regime (W100) commonly adopted by the farmers in the Marche area uses more water than necessary. With more accurate water management, it will be possible to save almost 226 $m^3$ of water per hectare per cultivation cycle.

**Keywords:** strawberry; water stress; remontant; sensorial quality; nutritional compounds; soil



**Citation:** Marcellini, M.; Raffaelli, D.; Mazzoni, L.; Pergolotti, V.; Balducci, F.; Armas Diaz, Y.; Mezzetti, B.; Capocasa, F. Effects of Different Irrigation Rates on Remontant Strawberry Cultivars Grown in Soil. *Horticulturae* **2023**, *9*, 1026. https://doi.org/10.3390/horticulturae9091026

Academic Editor: Toktam Taghavi

Received: 11 July 2023
Revised: 30 August 2023
Accepted: 5 September 2023
Published: 11 September 2023



## 1. Introduction

In recent decades, the strawberry (*Fragaria* × *ananassa* Duch.) has garnered much interest because of its various features [1]. Consumers appreciate this fruit because of the color, shape, taste, and nutritional properties of the berry [2–9]. However, the current changes in the climate, leading to extreme weather conditions, for example, drought [10], have increased the stress on the plant, leading to uncertainty in the strawberry market. According to the FAO 2021 [11], the annual worldwide water withdrawal from natural water bodies was about 4250 $km^3$, where agriculture used 71.7% of the total water consumed. By 2050, the human population is estimated to reach nine billion; to feed this population, agriculture will have to cope with several challenges [12]. About 60% extra food will be needed, implying an even higher water consumption [13]. Recently, the Sustainable Development Goal (SDG) 6.4.2 [11] was created to evaluate a country's water stress level. It considers the ratio of the total freshwater withdrawn by all major sectors to the difference between the total renewable freshwater resources and the environmental water requirements, multiplied by 100 [14]. Between 2015 and 2018, Italy achieved 30% of S.D.G. 6.4.2, demonstrating a low level of water stress [15]. The S.D.G. of 2022 is not available yet. Nevertheless, in the January–June semester, with +0.76 °C, Italy faced the warmest period ever recorded [16]. In this scenario, in agriculture, the level of water consumption must be maintained within certain limits, avoiding water abuse and groundwater contamination [17,18]. Increased

temperature negatively influences the plants' growth and development [19,20], leading to stunted reproductive organs because of lowered carbon assimilation. Many studies have highlighted the importance of selecting the optimal performing genotype to save water in strawberry cultivation [21]. New breeding programs should select new cultivars with reduced water need [22]. Furthermore, appropriate studies on cultivar, environment, and cultivation system interactions should be developed to define the proper irrigation regimes for more sustainable cultivation protocols and better fruit quality. Regarding strawberries, particular attention is now being paid to the development of cultivation systems that can promote out-of-season production using remontant cultivars able to fruit in different climatic conditions without the need for the winter season and in different growing conditions. Therefore, there is a need to identify appropriate cultivation conditions for remontant strawberry cultivars. In this work, we tested the response of three strawberry remontant cultivars, grown in open-field conditions and adopting reduced irrigation regimes, with the aim to develop the most sustainable and quality production practice.

## 2. Materials and Methods

### *2.1. Plant Materials*

This one-year-cycle experiment was set in the experimental farm of the regional extension service (Agenzia Servizi al Settore Agroalimentare delle Marche, ASSAM) located in Petritoli, Italy (43°03′10′′, 13°41′20′′). We used remontant strawberry cultivars grown in open fields for a single production. On 24 April 2019, frigo plants were planted in soil and covered by a plastic tunnel and the fruits were collected in the summer of 2019. The studied cultivars were frigo plants "Albion" (A+), "San Andreas" (A++), and "Monterey" (A+), three remontant cultivars well known for their consistent productivity during the season ("Albion"); earliness, rusticity, and quality fruit ("San Andreas"); and yield, quality, and resistance to diseases ("Monterey") [23].

### *2.2. Experimental Design and Irrigation Scheduling*

The plants were planted in double rows. The plants in each row were 30 cm apart and the rows were 35 cm apart, resulting in a density of 5.5 plants $m^{-2}$. The plants were grown in non-fumigated, chalky, and high-pH soil, as described in Table 1. The fertigation program, controlled by a Dosatron® D8R (Dosatron SAS, Tresses, FR), involved the distribution of N (120 unit $ha^{-1}$), P (100 unit $ha^{-1}$), and K (150 unit $ha^{-1}$) during the cultivation cycle with daily treatment. Each line had two dripline hoses Toro® Acqua-Traxx with a 1.1 L $hour^{-1}$ flow rate. For the cultivation, we followed the standard integrated pest management (IPM) (Directive128/2009). Before the start of the irrigation treatment, all plants received the same amount of water (1378 $m^3$ $ha^{-1}$) to ensure good establishment of the plant. We started the experimental irrigation at the flowering stage (stage 6 BBCH) and ended it on the last harvest date (stage 8 BBCH). Three irrigation treatments (W) were applied: W100 (control) with an irrigation rate suggested by the Marche Region Directive 786 on 10 July 2017 [24], corresponding to 1183 $m^3$ $ha^{-1}$, and W80 and W60, with 20% and 40% less water used, respectively, corresponding to 957 $m^3$ $ha^{-1}$ and 665 $m^3$ $ha^{-1}$ of total water used for irrigation by the end of the experiment. The soil humidity was monitored by six tensiometers, two per treatment, and placed at a 15 cm depth, approximately the root exploration area. The moisture probes, Watermark®, were characterized by a datalogger that took daily measurements (Figure S1). The temperature over the experimental period was monitored through the ASSAM weather station (Figure S2). The split-plot design of the experimental field consisted of three main blocks, differentiated by three different water supply levels, repeated for "San Andreas", "Albion", and "Monterey" cultivars. Each cultivar represented a sub-block and was composed of three replicates, called "plots", consisting of 8 plants each, for a total of 27 plots and 216 plants (3 blocks × 3 cultivars × 3 replicates) as shown in Figure S3.

**Table 1.** Soil feature of the Agenzia Servizi Settore Agroalimentare delle Marche (ASSAM) experimental field.

| Soil Parameter | Unit | Results | Method |
|---|---|---|---|
| pH | | 8.14 | [25] |
| Sand | g $Kg^{-1}$ | 304 | [26] |
| Silt | g $Kg^{-1}$ | 399 | [26] |
| Clay | g $Kg^{-1}$ | 297 | [26] |
| Active limestone | g $Kg^{-1}$ | 61 | [26] |
| Total limestone | g $Kg^{-1}$ | 174 | [26] |
| Assimilable P | g $Kg^{-1}$ | 3.7 | [27] |
| Exchangeable Na | g $Kg^{-1}$ | 15 | D.M. 13/09/99 GU SO n.248 del 21/10/1999 III.2, XIII.2.6 |
| Cation exchange capacity | mEQ 100 $g^{-1}$ | 21.9 | D.M. 13/09/99 GU SO n.248 del 21/10/1999 III.2 |
| Assimilable iron | g $Kg^{-1}$ | 9.7 | [28] |
| Assimilable M n | g $Kg^{-1}$ | 4.1 | [29] |
| Assimilable Z n | g $Kg^{-1}$ | 0.52 | [29] |
| Assimilable C u | g $Kg^{-1}$ | 2.7 | [29] |
| Boron soluble | | 0.1 | [30] |
| C/N | | 7.7 | |
| Organic matter | g $Kg^{-1}$ | 11.9 | D.M. 13/09/99 GU SO n.248 del 21/10/1999-VII.3. VII.3.6 |
| Total N | g $Kg^{-1}$ | 0.90 | [31] |
| Mg/K | | 2.7 | |
| Exchangeable Mn | mg $Kg^{-1}$ | 155 | [29] |
| Exchangeable K | mg $Kg^{-1}$ | 410 | [32] |

### *2.3. Plant Growth and Vegetative Parameters*

The leaf number and plant height were recorded three times during the season. The measurements were taken on 7 July 2019, 7 August 2019, and 7 September 2019. One measurement date (7 August 2019) was applied for the number of crowns, inflorescences number, leaf length, and leaf width.

### *2.4. Fruit Production*

Strawberries were harvested on 11 dates: 2 July, 9 July, 15 July, 22 July, 29 July, 5 August, 12 August, 19 August, 26 August, 2 September, and 9 September. To evaluate the ripening stage, we used the methods described by Capocasa et al. [33] and a precocity index (IP), which represents the average number of weighted days needed to collect the whole production of a cultivar from 1 January. The other parameters were the average fruit weight (AFW), the total yield, and the marketable production (fruits $\geq$ 22 mm and not rotted or deformed).

### *2.5. Fruit Quality*

For each harvest date, 10 fully ripe strawberry fruits were collected from each plot. Fruits for the qualitative analyses, both organoleptic and nutritional, were selected from the first, second, and third main pickings. We collected the fruits from six plants at the center of each plot and pooled together the fruits deriving from the three replicates of each

cultivar. The collected strawberries were fully ripe, without any visible injuries, and of a homogenous size.

#### 2.5.1. Fruit Organoleptic Quality

Ripe fruits were analyzed for color, firmness, total soluble solids, and titratable acidity in accordance with Marcellini et al. [34]. For each thesis (genotype/treatment), at each harvest, we selected 10 fruits to evaluate the chroma, also known as color saturation (Minolta Chromameter CR 400, Konica Minolta, Tokyo, Japan) and firmness (Penetrometer 327, Effegì, Ravenna, Italy). To evaluate the external color of fresh fruits, the CR-400 was used, measuring two points on opposite sides of each fruit using CIELAB values (L*, a*, b). The chroma was evaluated from a and b values. The genotype and the ripeness stage influenced the chroma value. Next, we perforated the same fruits using the penetrometer, through a 6 mm star probe. Until the total soluble solids (TSS) and titratable acidity (TA) evaluation, the samples were frozen at −18 °C. A soluble solids measurement was performed using a digital refractometer (PR-101$\alpha$ ATAGO, Tokyo, Japan) for TSS and acid–base titration was carried out for TA. The TA was calculated as mEQ of NaOH per 100 g of fresh weight (FW) as follows: on 10 g of strawberry juice as the base, we added 10 g of distilled water and a few droplets of bromothymol blue (pH indicator) with a 0.1 N NaOH solution. The final acidity content was expressed as described by the following formula [35]:

$$\% \text{ acid} \left( \frac{\text{wt}}{\text{wt}} \right) = \frac{\text{N} \times \text{V} \times \text{Eq. wt.}}{\text{W} \times 1000} \times 100$$

where

N = normality of the titrant, NaOH (mEQ/mL)
V = volume of the titrant (mL)
Eq. wt. = equivalent weight of the predominant acid (mg/mEQ)
W = mass of the sample (g)
1000 = factor relating milligrams to grams (mg/g) (1/10 = 100/1000).

#### 2.5.2. Fruit Nutritional Quality

We stored the strawberries and harvested for the analysis of nutritional compounds in plastic bags at −18 °C in laboratory freezers until the day of the extraction. For the extraction, we followed the method described by Mezzetti et al. [36]. In short, from each bag, we chose five strawberries and cut each fruit into four pieces: for the analysis, we used only half of the fruit, the part derived from opposite faces of the fruit, so as to avoid any bias connected to the influence of sunlight during cultivation. The strawberry pieces were chopped and weighed: 10 g was designated for the methanolic extract suitable for detecting phenolic acids, polyphenols, anthocyanins, and antioxidant capacity; 1 g for extracting vitamin C; and 2 g for extracting folates. After the extraction, the fruit samples were analyzed by two methods: HPLC, to detect ascorbic acid, folates, and phenolic acids, as well as spectrophotometry, to evaluate polyphenols, anthocyanins, and antioxidant capacity.

#### 2.5.3. HPLC

Both ascorbic acid content and folate content were quantified as described by Mezzetti et al. [28]. For vitamin C, we added 4 mL of the extraction buffer made of Milli-Q water, 5% meta-phosphoric acid, and 1 mM ethylenediaminetetraacetic acid to 1 g of strawberry sample, homogenized the mixture using an Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark), and sonicated the same for 5 min. After centrifuging the samples (2500 rpm at 4 °C, for 10 min), we filtered them through a 0.22 µm nylon syringe filter. The samples with the vitamin C extracted were analyzed in the HPLC system, specifically, a Jasco PU-2089 Plus controller (Jasco Inc., Easton, MD, USA) and a Jasco UV-2070 Plus ultraviolet (UV) (Jasco Inc., Easton, MD, USA) detector set at an absorbance of 260 nm. The HPLC column used was an Ascentis Express C18 150 × 4.6 mm (Supelco, Bellefonte, PA, USA), protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard

column (Phenomenex, Torrance, CA, USA). The calibration curve was prepared by the standard concentration of the vitamin. Finally, the unit of measurement was mg Vit C per 100 g of fruit weight (FW) from three replications per sample.

For folate extraction, we added 2 g of frozen strawberries to 8 mL of the extraction buffer (0.1 M phosphate buffer containing 1.0% of L(+)-ascorbic acid ($w/v$) and 0.1% 2,3-dimercapto-1-propanol ($v/v$) at pH 6.5, freshly prepared) and homogenized the mixture using an Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark) at a high speed. The falcon tube containing the sample was immersed in a water bath at 100 °C for 10 min and then rapidly cooled at −18 °C. Next, to deconjugate polyglutamylation folates, we added 150 µL of folate conjugase from the hog kidney to each sample and incubated the mixture in a shaking oven at 37 °C for 3 h. We placed the tube in a thermal bath at 100 °C for 5 min and then rapidly cooled the mixture at −18 °C and centrifuged it at 4000 rpm at 4 °C for 20 min. The supernatant was collected in a 25 mL falcon tube. Then, the pellet was reprocessed with the same extraction buffer, warmed in a water bath, cooled, and centrifuged. We added the supernatant to the extracted sample and filled the falcon tube with the extraction buffer so that the volume became 25 mL. We filtered the samples (0.45 µm syringe filter) as described by Iniesta et al. [37] and Jastrebova et al. [38] with some modifications. The filtrates were purified through solid-phase extraction on anion-exchange Isolute cartridges. Finally, we carried out the HPLC analysis in accordance with Strålsjö et al. [39] with some modifications. The analytical column was a Luna C18, 250 × 4.6 mm, 5 µm (Phenomenex, Torrance, CA, USA), protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA). A fluorescence detector (FLD) FP-2020 Plus (Jasco, Easton, MD, USA) set at wavelengths of 290 nm excitation and 360 nm emission and an autosampler AS-4050 (Jasco, Easton, MD, USA) were also used. The results were expressed as µg of folate per 100 g of FW. The results were obtained with three replications ± standard deviation.

For phenolic acid analysis, the procedure adopted was in accordance with Frederick et al. [40]. The HPLC setup consisted of a Jasco PU-2089 plus controller, a Jasco UV-2070 plus ultraviolet detector, and a Jasco AS-4050 autosampler, all from Jasco (Easton, MD, USA). The chromatographic column employed was an Aqua Luna C18, with dimensions of 250 mm × 4.6 mm, manufactured by Phenomenex (Torrance, CA, USA). This column was safeguarded by a Phenomenex 4.0 mm × 3.0 mm C18 ODS guard column. The separation process involved a gradient program with two mobile phases: A (containing 2% acetic acid) and B (composed of acetic acid, acetonitrile, and water in a ratio of 1:50:49). The gradient initiated with 55% A and 45% B for 50 min, followed by a 10-min phase of 100% B. Subsequently, it was reduced to 10% B until the analysis concluded. To quantify and recognize only phenolic acids, the UV/VIS detector was set to 320 nm. Three standard solutions were prepared from the following pure phenolic acids: chlorogenic acid, caffeic acid, and ellagic acid. For caffeic and chlorogenic acids, ethanol ($C_2H_6O$) was used as a solvent. For ellagic acid, sodium hydroxide (NaOH, 1 M) was used as a solvent. The results were expressed as mg of phenolic acids per 100 g of fresh fruit.

#### 2.5.4. Spectrophotometry

We measured the anthocyanin content (ACY) using the pH differential shift method [41]. Each sample was diluted at a ratio of 1:10 with two solutions: potassium chloride (pH 1.00) and sodium acetate (pH 4.50). Then, the absorbance for both solutions was measured at 500 and 700 nm. The data were expressed as mg pelargonidin-3-glucoside (molar extinction coefficient 15,600 L $mol^{-1}$ $cm^{-1}$; molecular weight 433.2 g $mol^{-1}$) per kg of FW. The total antioxidant capacity (TAC) was measured through the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay [42,43]. ABTS is a colorless substance that turns into the colored monocationic radical form when exposed to an oxidative agent. The extent of decolorization of the monocationic radical form is a function of the antioxidants present in the strawberries and was calculated relative to the reactivity of Trolox, a water-soluble vitamin E analog. Antioxidant activity is expressed as mg Trolox equivalent per kg of FW and

the results were expressed as the mean of six replications ± standard deviation. The total polyphenol content (TPH) was measured using the Folin–Ciocalteu reagent method [44]. Briefly, we filled a glass test tube with 7.0 mL of water. To this, we first added 1 mL of the diluted sample (1:20) and then added 500 mL of the 2 N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA). The solution was vortexed and allowed to react for 3 min. Then, we added 1.5 mL of a 20% sodium carbonate solution. The contents of the tube were mixed again and the tube was stored in the dark for 60 min. After this, we measured the sample absorbance at 760 nm. The data were expressed as mg gallic acid per kg of FW. The results were obtained as the mean of six replications ± standard deviation.

### *2.6. Statistical Analysis*

The results are presented as the values ± standard deviation and were subjected to a two-way analysis of variance (ANOVA) at confidence levels of 95% and 99%. Significant differences were calculated according to Fisher's LSD test and differences at $p < 0.05$ were significant. Principal component analysis (PCA) was also used to evaluate the levels of association among the nutritional parameters. Statistical analyses were performed using Statistica 7 software (StatSoft, TIBCO Software, Palo Alto, CA, USA).

## 3. Results and Discussion

### *3.1. Vegetative Parameters*

Considering the vegetative parameters, in "Albion" and "San Andreas" there was an increase in the branch crown number at high and moderate water supply (W100 and W80, respectively), while in "Monterey", there was a significant decrease in this parameter at a slight water shortage (W80). On severe water reduction (W60), the number of branches per plant was reduced in comparison with what was detected in plants grown at W100, like the results provided by Gehrmann [45], Awang et al. [46], and Marcellini et al. [34], underlining the negative influence of the salinity arising from the water shortage on the vegetative apparatus of the strawberry plant. The number of inflorescences in the plants was quite low, even at full irrigation. In addition, in this case, "Monterey" showed a significant sensitivity to water shortage at W60 compared to W100 (Table 2). As already described [47,48], water shortages tended to influence the vegetative structure of the plants, indicated by the lower plant height and leaf number, possibly leading to a reduction in photosynthesis [49]. Therefore, when the water supply is low, the plant's habit is more compact. For all treatments, "S. Andreas" was the most vigorous, with the highest value in terms of the plant height and the number and size of leaves, followed by "Monterey" and "Albion". "Monterey" had fewer branch crowns and inflorescences at reduced irrigation and reduced plant height at W80. Generally, "Albion" was smaller in height than other cultivars, with a particularly negative influence at W60 (Table 2). The leaf number was also significantly reduced at W60. The different treatments did not influence the leaf size in any of the cultivars (Table 2). The development of the plants in terms of plant height and leaf number was monitored between July and September 2019 (Figure 1). We noted that for the three cultivars examined, the leaf number increased, particularly at W100 and W80, while at W60 during the summer season, this parameter remained constant. Contrarily, the plant height tended to decrease slightly for all treatments.

**Table 2.** Effects of water availability on branch crown number, the number of inflorescences, leaf length, and leaf width in different strawberry cultivars.

| | | **Cultivar** | |
|---|---|---|---|
| Number of branch crowns | Albion | Monterey | S. Andreas |
| W100 | 2.3 ± 0.8 cd | 2.6 ± 0.9 bc | 3.2 ± 0.8 a |
| W80 | 2.6 ± 1.0 b | 2.2 ± 0.8 d | 3.1 ± 1.0 a |
| W60 | 2.3 ± 0.7 cd | 2.3 ± 0.8 cd | 2.7 ± 0.7 b |
| Number of inflorescences | | | |
| W100 | 2.2 ± 2.4 bc | 2.7 ± 3.0 a | 2.0 ± 3.2 cd |
| W80 | 2.5 ± 2.0 ab | 2.5 ± 2.6 ab | 1.8 ± 2.7 d |
| W60 | 2.0 ± 2.7 cd | 2.3 ± 2.3 bc | 2.0 ± 3.4 cd |
| Leaf length (cm) | | | |
| W100 | 7.3 ± 1.1 bc | 7.0 ± 1.1 cd | 7.9 ± 1.2 a |
| W80 | 7.3 ± 1 bc | 6.8 ± 1.1 d | 7.9 ± 1.3 a |
| W60 | 7.4 ± 1.0 b | 7.0 ± 1.0 cd | 7.8 ± 0.9 a |
| Leaf width (cm) | | | |
| W100 | 6.7 ± 0.8 bc | 6.7 ± 1.1 bc | 7.2 ± 1.0 a |
| W80 | 6.9 ± 1.3 ab | 6.5 ± 0.9 c | 7.2 ± 0.8 a |
| W60 | 6.8 ± 0.8 bc | 6.7 ± 1.0 bc | 7.0 ± 1.0 ab |

Note: Values with the same lowercase letter for the same parameter were not statistically different in Fisher's LSD test ($p < 0.05$). Values are expressed as the means of one year (2019) ± standard deviation.

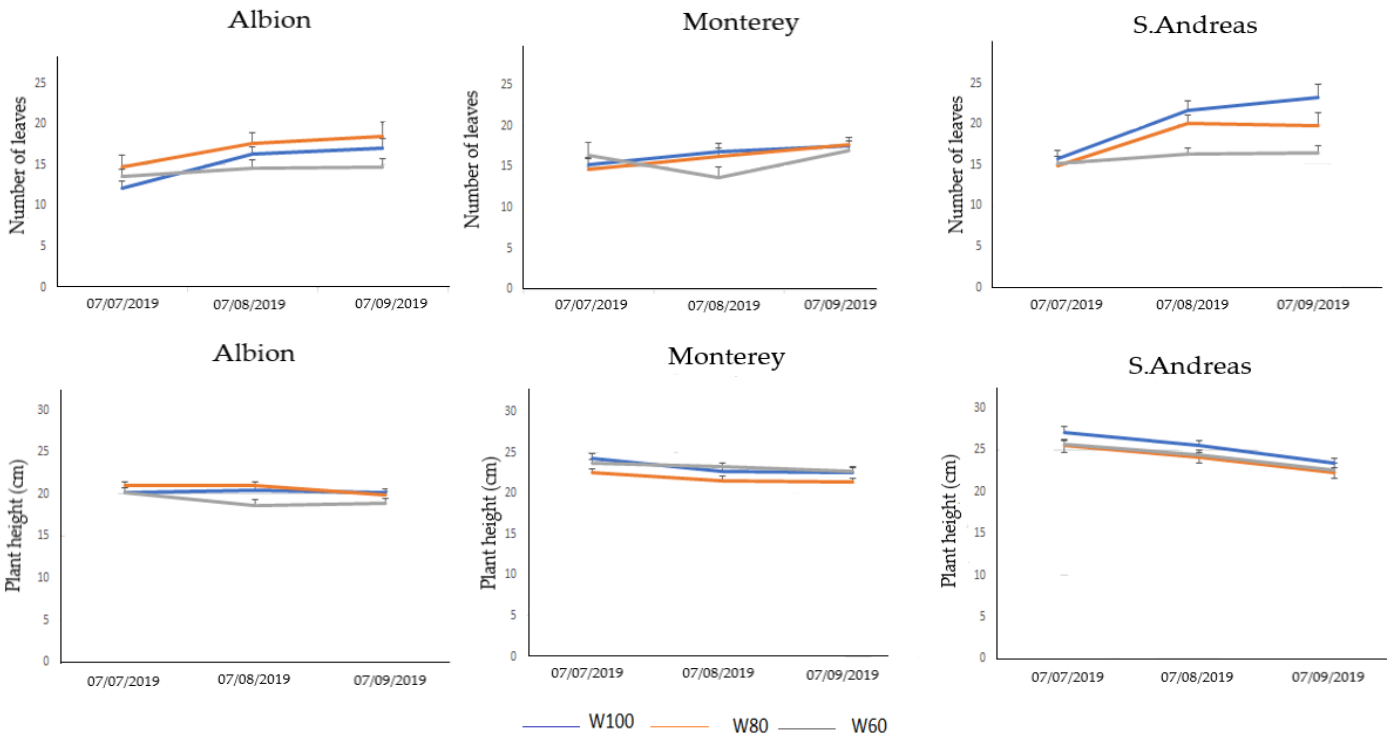


**Figure 1.** The number of leaves and plant height during water reduction (from July to September 2019).

### *3.2. Productive Parameters*

Generally, reduced water restitution led to the fruits maturing earlier than usual. In fact, at full irrigation (W100), the three cultivars ripened at the same time. However, from

W100 to W60, the fruits matured 6 days earlier for "Albion", 10 days earlier for "Monterey", and 12 days earlier for "San Andreas" (Table 3). On average, the difference in the harvest time was 5 days earlier for W80 and 9 days earlier for W60 with respect to the control treatment (W100). The average fruit weight appeared to be independent of the difference in the amount of water restitution. The W80 treatment negatively influenced the AFW (even when compared to the W60 treatment), though the difference was not statistically significant. Concerning commercial production, "Albion" appeared to be the most sensitive cultivar, with a reduction in 37% in the commercial yield (statistical difference), followed by "San Andreas" (−34%) and "Monterey" (−21%), but without a significant difference (Table 3). For total production too, a similar trend was detected. Compared to W100, in "Albion", the total production was reduced by about 60 g; in "Monterey", the total production was reduced by about 54 g; and in "S. Andreas", the total production was reduced by about 55 g. Among the cultivars, "Monterey" showed the highest commercial and total production for all treatments. As already known from the literature [50–54], proper irrigation treatment is a prerequisite for exploiting the yield potential of a cultivar. In fact, the differences in the production among cultivars in the same water condition demonstrate that efficient water use is genotype-dependent [47,48].

**Table 3.** Effects of water availability on precocity index and average fruit weight in different strawberry cultivars.

| | **Cultivar** | | |
|---|---|---|---|
| Precocity index (days) | Albion | Monterey | S. Andreas |
| W100 | 214.2 ± 2.4 ab | 214.6 ± 3.4 ab | 215.1 ± 6.3 a |
| W80 | 209.4 ± 1.4 abcd | 209.7 ± 0.1 abc | 211.1 ± 4.1 ab |
| W60 | 208.6 ± 4.5 bcd | 204.4 ± 2.7 cd | 203.6 ± 2.8 d |
| Average fruit weight (g) | | | |
| W100 | 11.0 ± 0.1 cd | 12.2 ± 1.8 abc | 13.4 ± 0.8 a |
| W80 | 10.5 ± 0.5 d | 10.5 ± 0.9 d | 11.6 ± 1.4 bcd |
| W60 | 11.1 ± 0.4 cd | 12.1 ± 1.2 abcd | 12.9 ± 0.5 ab |
| Commercial production (g/plant) | | | |
| W100 | 162.9 ± 24.8 ab | 181.2 ± 42.3 a | 122.1 ± 11.4 bcd |
| W80 | 122.6 ± 26.2 bcd | 174.0 ± 44.2 a | 79.2 ± 26.5 d |
| W60 | 103.1 ± 15.9 cd | 142.3 ± 17.1 abc | 80.9 ± 22.0 d |
| Total production (g/plant) | | | |
| W100 | 204.8 ± 26.1 ab | 241.8 ± 32.4 a | 159.4 ± 14.9 bc |
| W80 | 164.5 ± 26.0 bc | 206.3 ± 50.8 ab | 107.5 ± 26.8 d |
| W60 | 144.7 ± 31.7 cd | 187.9 ± 15.0 bc | 104.5 ± 19.2 d |

Note: Values with the same lowercase letter for the same parameter were not statistically different in Fisher's LSD test ($p < 0.05$). Values are expressed as the means of one year (2019) ± standard deviation.

### *3.3. Qualitative Parameters*

In fruits, there is a positive relationship between reduced irrigation and the increased content of soluble solids [49–51]. In our experiment, the sugar content of the fruits did not differ from that shown in previous works, highlighting a positive correlation between less water restitution and fruits' °Brix content (Table 4). The cultivars "Monterey" and "Albion" stood out for the high sugar content in their fruits, which increased, respectively, by 1.2 °Brix and 1 °Brix at W60 with respect to W100. In all the treatments, the fruits of "S. Andreas" showed a lower sugar content in comparison with the other cultivars; however, they also showed a significant increase in the content of soluble solids with a lower irrigation supply. At the lower water restitution, the fruits of stressed plants accumulated higher SSC,

probably in terms of higher concentrations of fructose and glucose [47]. Regarding acidity, the common tendency among the fruits was the lack of a correlation between the water administrated and the acid content. In each irrigation treatment, the fruits of "Albion" had the highest acidity, followed by those of "Monterey" and "S. Andreas", with similar values. The fruit firmness increased when irrigation was reduced by 20% (Table 4). These results are different from those previously obtained by Krüger et al. [48], where the fruit firmness decreased in a reduced water regime. In the present study, when grown in an optimal condition of irrigation (W100), the cultivars showed an appreciable fruit firmness: "Monterey", 422.1 g; "Albion", 371.0 g; and "S. Andreas", 338.9 g. However, when "S. Andreas" and "Albion" were treated at W60, the fruit firmness increased by 20 g and 25 g, respectively. "Monterey" demonstrated a constancy in terms of fruit firmness. At full irrigation (W100), the chroma value was higher in the fruits of "S. Andreas": they seemed brighter than the other tested cultivars (Table 4). "Albion" and "Monterey" were pretty much the same. The brightness of the fruits of "Albion" and "Monterey" increased in higher-water-stress conditions in contrast to those of "S. Andreas". Our findings were partially in accordance with those of other studies [45,46] but in contrast to the results of the study by Adak et al. [47], where water stress did not affect the fruit color parameters.

**Table 4.** Effects of water availability on sugar content, titratable acidity, firmness, and chroma in different strawberry cultivars.

| | | **Cultivar** | |
|---|---|---|---|
| Sugar content (°Brix) | Albion | Monterey | S. Andreas |
| W100 | 14.1 ± 1.2 c | 14.5 ± 0.8 bc | 11.2 ± 0.7 e |
| W80 | 14.6 ± 1.2 bc | 15.2 ± 1.1 ab | 11.4 ± 0.8 e |
| W60 | 15.1 ± 1.3 ab | 15.7 ± 1.4 a | 12.3 ± 0.8 d |
| Titratable acidity (mEQ of NaOH/100 g of fruit weight) | | | |
| W100 | 14.9 ± 0.8 a | 13.2 ± 1.1 b | 13.5 ± 1.3 b |
| W80 | 14.5 ± 1.7 a | 13.3 ± 1 b | 13.2 ± 1.0 b |
| W60 | 14.5 ± 1.3 a | 13.3 ± 1.2 b | 13.5 ± 1.0 b |
| Firmness (g) | | | |
| W100 | 371.0 ± 94.2 c | 422.1 ± 105.7 a | 338.9 ± 78.2 d |
| W80 | 391.9 ± 88.9 b | 419.7 ± 110.3 a | 361.4 ± 100.1 c |
| W60 | 395.6 ± 91.4 b | 420.3 ± 121.5 a | 359.1 ± 105.5 c |
| Chroma | | | |
| W100 | 43.7 ± 5.5 cd | 43.2 ± 6.5 d | 45.6 ± 5.7 a |
| W80 | 44.4 ± 5.0 bc | 44.2 ± 5.8 bc | 44.8 ± 5.9 ab |
| W60 | 45.4 ± 5.3 a | 44.4 ± 5.9 bc | 44.5 ± 5.9 bc |

Note: Values with the same lowercase letter for the same parameter were not statistically different in Fisher's LSD test ($p < 0.05$). Values are expressed as the means of one year (2019) ± standard deviation.

### *3.4. Nutritional Parameters*

The fruit content of ascorbic acid seemed to depend on the interaction between the plant genotype and water restitution to the plants (Table 5). Briefly, reduced water content negatively affected vitamin C accumulation in "Monterey" fruits (26.63 mg/100 g of FW at W100 compared to 22.18 mg/100 g of FW at W60). However, the fruits of "Albion" and "San Andreas" did not show any significant variation between treatment at W100 and at W60, even though they performed worse at W80. On average, "Monterey" performed as the best cultivar in terms of fruit vitamin C content.

Concerning the vitamin B9 fruit content, in all the tested genotypes, reduced water restitution tended to stimulate fruit production with increased folate content (Table 5). "Monterey" plants produced fruits with a significantly higher folate content at W60 thesis than at W100. On average, "Monterey" fruits expressed the highest concentration of folates compared to the other cultivars. "Albion" fruits did not exhibit significant differences among the thesis and "San Andreas" accumulated the highest concentration of vitamins at W60.

In the fruits, the accumulation of total phenolic compounds was not significantly influenced by water regimes or by their interaction with genotype (Table 5), as was also described by Martínez-Ferri et al. [53]. The highest accumulation was detected in fruits from "San Andreas" plants at W60, with 422.50 mg GA/100 g of FW, and the lowest accumulation was detected in fruits harvested from "Albion" plants at W80, with 351.38 mg GA/100 g. Differently from these cultivars, "S. Andreas" plants produced fruits with a lower accumulation of TPH at W100 than at W80 and W60. The same response in terms of fruit anthocyanins content was also detected in plants treated with reduced water regimes, where no significant differences were detected among treatments. Among the cultivars, the fruits of "San Andreas" had the highest amount of fruit anthocyanins (Table 5).

The antioxidant capacity of strawberry fruits harvested from plants treated with reduced water regimes was higher than that of fruits harvested at 100% water restitution (Table 5). Even though this trend was confirmed in each cultivar, only "S. Andreas" presented significant differences, with the fruits harvested at W60 treatment presenting a significantly higher value of TAC (599.23 mg Trolox eq/100 g of FW) than those harvested at W100 (506.66 mg Trolox eq/100 g of FW). The cultivar-dependent effect for this important fruit trait has already been described by Cardeñosa et al. who showed that the "Primoris" cultivar exhibits higher fruit antioxidant capacity under higher saline conditions because of the plant's response to abiotic stress [54].

Concerning the phenolic acid content, different water regimes did not significantly influence the amount of these compounds in strawberry fruits, while, again, the genotype was a determinant factor. In fact, fruits of "Monterey" had the highest content of phenolic acids, presenting the highest amount at the W60 trial. In this case, both "Monterey" and "S. Andreas" displayed a similar trend, showing increasing fruit concentrations of phenolic acids with decreasing water supply. Furthermore, the fruits of "S. Andreas" showed a significant difference in terms of the phenolic acid content detected at W60 (30.52 mg/100 g of FW) and that detected at W100 (27.37 mg/100 g of FW) (Figure 2). Among phenolic acids, strawberry fruits showed the highest concentration of ellagic acid, followed by chlorogenic acid; the low quantity of caffeic acid did not seem to be influenced by reduced water supplies.

To investigate whether one or more nutritional compounds are linked together in their determination, we analyzed the data obtained for the three cultivars using principal component analysis (PCA) (Figure 3). The two main factors reported on the graph justify the 60% of the variability registered in this study. It is interesting to note that the vectors TAC and TPH fall close to each other, indicating a strong relationship between the amount of total phenolics and the antioxidant capacity of fruits. This result confirms the finding that the phenolic compounds are mainly responsible for the TAC of strawberries. Furthermore, the vector phenolic acids is placed in the third quadrant and this was also expected, given the antioxidant capacity exerted by this class of compounds. What is surprising is that the vector folates is in this quadrant, even though these compounds are not strong antioxidants. The vectors ACY and Vit C are placed in opposite quadrants (second and fourth, respectively), indicating that high amounts of one of them in the strawberry fruits of this study corresponded to low amounts of the other and vice versa. However, they are both good antioxidant compounds and they are equidistant from the TAC vector.

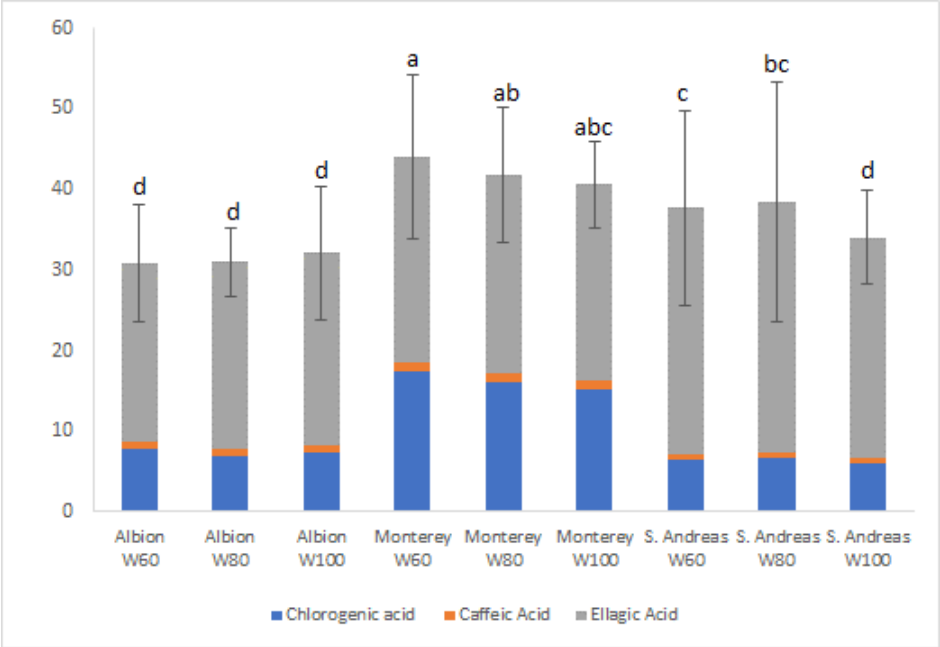


**Figure 2.** Phenolic acid content (mg 100 $g^{-1}$ of FW) based on different water supplies. Values are expressed as the mean total phenolic content of one year (2019) ± standard deviation. Different letters indicate significant differences (Fisher's LSD test; $p < 0.05$).

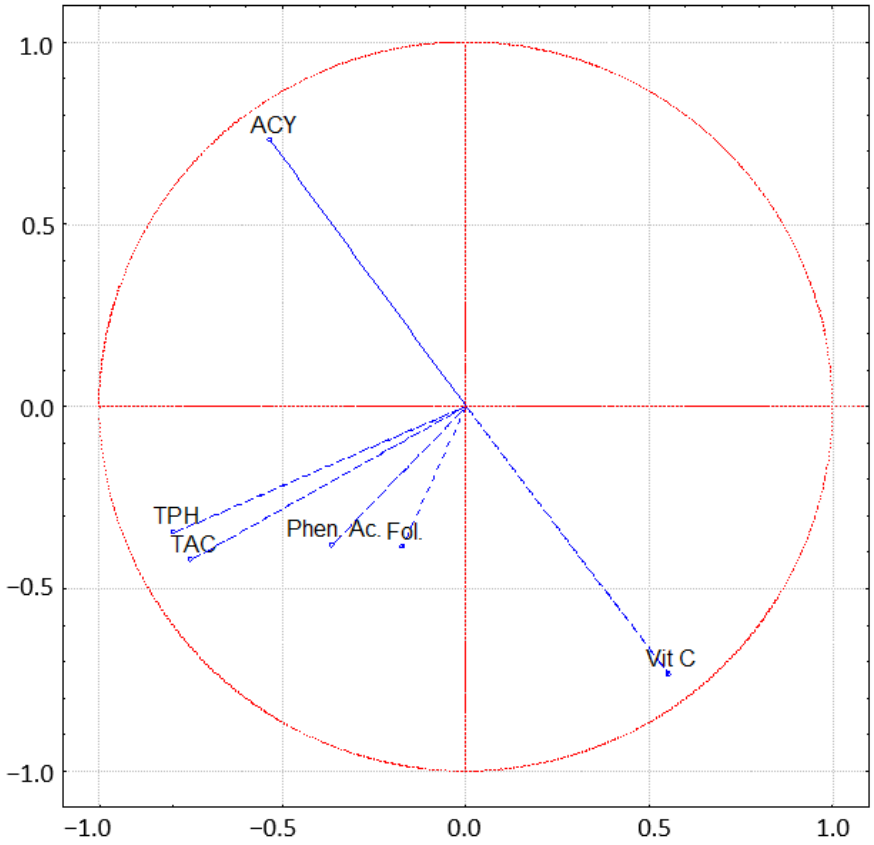


**Figure 3.** Principal components analysis (PCA). Factor 1: 32.50% and Factor 2: 27.68%. The abbreviations referred to ACY: anthocyanins; TPH: total phenolics; TAC: total antioxidant capacity; Phen.A.: phenolic acids; Fol: folates; Vit C: vitamin C.

**Table 5.** Effects of water availability on vitamin C, folates, total phenolics, anthocyanins, and total antioxidant capacity concentration in different strawberry cultivars.

| | | **Cultivar** | |
|---|---|---|---|
| Vitamin C (mg 100 $g^{-1}$ of FW) | Albion | Monterey | S. Andreas |
| W100 | 23.89 ± 0.81 c | 26.63 ± 0.05 a | 17.76 ± 0.03 f |
| W80 | 23.38 ± 0.10 d | 25.23 ± 0.01 b | 16.90 ± 0.25 g |
| W60 | 23.72 ± 0.01 cd | 22.18 ± 0.06 e | 18.08 ± 0.12f |
| Folates (µg 100 $g^{-1}$ of FW) | | | |
| W100 | 27.21 ± 0.74 de | 28.87 ± 0.32 cd | 29.94 bc ± 0.73 bc |
| W80 | 28.51 ± 1.17 cde | 31.50 ± 0.67 ab | 27.02 ± 2.38 e |
| W60 | 28.47 ± 0.65 cde | 33.12 ± 0.19 a | 30.78 ± 0.65 b |
| TPH (mgGA 100 $g^{-1}$ of FW) | | | |
| W100 | 367.80 ± 27 ab | 367.10 ± 3.58 ab | 349.81 ± 22.69 b |
| W80 | 351.38 ± 14.67 b | 358.72 ± 1.46 b | 393.11 ± 31.77 a |
| W60 | 359.38 ± 7.64 b | 391.15 ± 9.94 a | 422.50 ± 33.81 a |
| ACY (mg PEL-3- GLU 100 $g^{-1}$ of FW) | | | |
| W100 | 35.49 ± 4.68 b | 36.24 ± 0.62 b | 44.27 ± 3.93 a |
| W80 | 33.58 ± 2.52 b | 33.82 ± 0.25 b | 42.50 ± 5.50 a |
| W60 | 32.25 ± 1.32 b | 34.06 ± 1.72 b | 44.63± 5.86 a |
| TAC (mg TroloxEq 100 $g^{-1}$ of FW) | | | |
| W100 | 509.13 ± 19.93 bc | 552.06 ± 8.98 abc | 506.66 ± 31.91 c |
| W80 | 539.86 ± 49.26 bc | 559.79 ± 18.45 abc | 557.57 ± 37.87 abc |
| W60 | 535.92 ± 36.63 bc | 561.27 ± 42.33 ab | 599.23 ± 13.12 a |

Note: Values with the same lowercase letter for the same parameter were not statistically different in Fisher's LSD test ($p < 0.05$). Values are expressed as the means of one year (2019) ± standard deviation.

## 4. Conclusions

In conclusion, the present study analyzed the responses of three different remontant cultivars of strawberry in water stress conditions. Although the experiment takes into account only one-year cultivation cycle, the results obtained are interesting and confirmed the need of future trials regarding the optimization of the irrigation management. In our experiment, even though the production was not as we expected, independently by the treatment, these cultivars showed satisfying results for all the evaluated parameters when irrigation was reduced by 20%. A further reduction of up to 40% in water amount led to a significant decline in the plants' vegetative and productive parameters. If the amount of water administrated is reduced by 20%, about 226 $m^3$ of water per hectare per cultivation cycle can be saved. A water shortage increased the fruits' sugar content, firmness, IP, folate content, total phenolic content, and total antioxidant capacity and the content of some phenolic acids. In addition, the genotype had a consistent impact on the plant's performance. Independent of the treatment, "S. Andreas" exhibited the highest fruit weight and the most balanced taste, with 14.6 °Brix and 14.5 mEQ of NaOH 100 $g^{-1}$ of FW. Nevertheless, our results suggest that "Monterey" is the most preferable remontant cultivar among the studied genotypes. In fact, for all the treatments, "Monterey" exhibited an appreciable firmness, around 420 g, indicating that this plant's fruits are most suitable for a longer shelf life on the market. "Monterey" also performed well in terms of commercial production (g/plant), showing a reduction in only about 4% at W80 compared to the production at W100. For this cultivar, these results are appreciable, especially when considering that "Monterey" achieved good values in terms of the contents of vitamin C

(26.63 mg 100 $g^{-1}$ of FW at W100 and 25.23 mg 100 $g^{-1}$ of FW at W80), folate (28.87 µg 100 $g^{-1}$ of FW at W100 and 31.50 µg 100 $g^{-1}$ of FW at W80), TAC (552.06 mg Trolox eq 100 $g^{-1}$ of FW at W100 and 559.79 Trolox eq 100 $g^{-1}$ of FW at W80), and phenolic acids (chlorogenic acid, caffeic acid, and ellagic acid were up to 40 mg 100 $g^{-1}$ of FW for all treatments). This type of experiment is essential for identifying the cultivars most suitable for a specific environment. Nevertheless, to avoid climatic and environmental influences, a "closed" experimental site, with controlled cultivation conditions, is strongly suggested. Furthermore, nowadays, a specific protocol for water management is mandatory to ensure greater economic and environmental sustainability for high-quality strawberry production for consumers.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9091026/s1, Figure S1: Soil water potential calculated by the tensiometers; Figure S2: Temperature from July 2019 until October 2019 in the ASSAM experimental field; Figure S3: Experimental design of the trial.

**Author Contributions:** Conceptualization, B.M. and F.C.; methodology, M.M. and D.R.; software, M.M., D.R. and F.C.; validation, L.M., B.M. and F.C.; formal analysis, V.P., F.B., Y.A.D., D.R. and M.M.; investigation, M.M., D.R. and F.C.; resources, B.M. and F.C.; data curation, M.M., D.R. and F.C.; writing—original draft preparation,—D.R.; writing—review and editing, L.M. and B.M.; visualization, M.M. and D.R.; supervision, F.C. and B.M.; project administration, F.C.; funding acquisition, F.C. and B.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by European Union's Horizon 2020 research and innovation program, grant number no. 679303 (GOODBERRY project).

**Data Availability Statement:** Publicly available datasets were analyzed in this study. This data can be found here: https://hdl.handle.net/11566/274632, accessed on 1 March 2023.

**Conflicts of Interest:** The authors declare no conflict of interest.

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