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Before or after Planting? Mycorrhizal and Bacterial Biostimulants and Extracts in Intense Strawberry (*Fragaria* × *ananassa* Duch.) Production

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Abstract: The aim of this research was to evaluate the effect of a combination of several mycorrhizal and bacterial biostimulants, applied before and after planting, on the 'Clery' strawberry's performance. Vegetative and reproductive parameters (the number of crowns per plant, root/canopy weight and dimensions, the number of fruits per plant, individual fruit weight, and fruit yield per plant) were monitored on nine harvest dates. Additionally, external and internal fruit quality (firmness, color, soluble solids content, and primary and secondary metabolites) was determined. The application of product combinations resulted in significantly improved vegetative growth, fruit dimensions, and fruit weight. Consequently, more than 30% higher yields were determined for the treated plants. A minor decrease in vitamin C (approx. 6%), total individual sugars (approx. 10%), organic acids (approx. 9%), and total phenolics (approx. 7%) was detected in the treated plants, but the differences were not uniform during the harvest. The accumulation of anthocyanins was least affected by product application, and the fruit exhibited comparable color characteristics, which is important for the consumers. The use of biostimulants in intense strawberry production is justified as the products improve the vegetative development of strawberry plants, which produce significantly more marketable fruit.

Keywords: *Fragaria* × *ananassa*; biostimulants; vegetative parameters; yield; primary metabolites; phenolics; anthocyanins

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1. Introduction

Strawberries (*Fragaria* × *ananassa*) are one of the most widely cultivated berry fruit, due to their pleasing taste, appearance, and metabolite composition [1]. Commonly, intense field strawberry production is organized in non-heated plastic greenhouses, and plants are re-planted after two or three production seasons, sometimes intermittently, with vegetables or other annual crops. A major challenge in long-term strawberry cultivation is diminishing the negative effects of soil-borne pathogens, among which fungal diseases such as *Fusarium* sp., *Phytophthora* sp., *Phytium* sp., and *Verticillium* sp. and pests such as *Agriotes* sp., *Melolontha melolontha*, *Otiorrynchus* sp., and *Pratylenchus penetrans* are the most aggressive [2]. Different measures have been proposed in their combat: for instance, fumigation with synthetic fumigants (e.g., bromomethane, chloropicrin) in combination with several fungicides is one of the most effective methods [3]. However, the use of fumigants has been banned in most countries under the Montreal Protocol, and alternative methods and products are evolving to better suit the focus of environmentally conscious production [4].

These technological measures include the use of various biostimulants, defined by the European Biostimulant Industry Council (EBIC) as "organic or natural products obtained from bioactive materials and/or microorganisms that can boost several molecular and physiological processes" [5]. Interestingly, biostimulants improve the nutrient use efficiency and quality attributes of plants, regardless of their nutrient content [6]. Positive effects are reflected in advanced vegetative growth, generative development and yield, and progressed root formation [7,8]. Biostimulants may also improve the tolerance of plants as they diminish the impact of biotic or abiotic stress [9,10] and direct antagonistic activities to phytopathogens such as *Botrytis* and *Colletotrichum* [11,12]. Biostimulants can be categorized into two basic groups: products containing live organisms (bacteria or fungi) and products containing organic (humic acids, biopolymers, seaweed extracts, etc.) or inorganic compounds (beneficial minerals) [6]. Frequently, a combination of different groups of biostimulants is applied to plants, and numerous commercial products and protocols are being tested for their efficiency.

In strawberry, the use of biostimulants is widely practiced, and products are used either as a pre-planting measure or they are supplemented during vegetation [13,14]. For example, studies report that different formulations of humic acids and minerals account for increased vegetative growth, fruit weight, fruit size, and yield [15] and tolerance to salinity stress [16] and other forms of abiotic stress [17]. Moreover, seaweed extracts in combination with silicon have been shown to advance fruit formation and yield [18], the application of *Trichoderma citrinoviride* effectively decreases the activity of soil-borne pathogenic fungi [19], and Bacillus sp. has been shown to have an antagonistic effect on *Verticillium dahlia* and *Phytophthora cactorum* [20]. Studies have also investigated combinations of several biostimulants, as in strawberry cultivation, the use of products is rarely limited to a single group. Combinations of products have been tested in connection with the growth, yield, and fruit quality of strawberry plants [21]. Experiments exploring the effect of soil pre-treatment with biostimulants in comparison with post-planting treatment of strawberries are, however, rare [22].

Strawberries are appreciated by consumers for their characteristic sweet flavor and aroma linked to the presence of many primary and secondary metabolites [23]. Strawberry fruit is an important source of vitamin C and contains essential minerals [24] and phenolic compounds [18]. The predominant groups of phenolics detected in strawberries are flavonoids (mainly color-defining anthocyanins) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids) [23,25], which are greatly affected by genetic, physiological, seasonal, and technological factors [26,27]. Studies focusing on detecting the effect of biostimulants on the individual phenolic composition of strawberry fruit are few. Roussos et al. [28] and Weber et al. [18] detected a positive correlation between treatment with *Ascophyllum nodosum* and anthocyanin accumulation. Soppelsa et al. [21] extended the study on other phenolic groups and other biostimulants, including humic acids. But research on combinations of mycorrhizal and bacterial products affecting individual phenolic compounds needs to be undertaken.

Therefore, the aim of the current study was to evaluate the effect of recommended combinations of commercial biostimulant products intended as a measure to improve the vegetative and generative parameters of strawberries, applied either before planting or during the vegetation period. Moreover, the composition of treated and non-treated fruit was evaluated in terms of primary and secondary metabolites. A detailed phenolic response was monitored during the full period of the strawberry harvest. The potential benefits of biostimulant application were discussed in terms of the plant growth, yield, and quality parameters of strawberries.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiment was performed in cooperation with a local strawberry producer near Krsko, Slovenia (latitude, 45°88′ N; longitude, 15°48′ E; altitude 151 m above sea

level), who grows strawberries according to integrated pest management. In November 2019, depleted strawberry plants were removed, and the field was tilled and left bare overwinter. In May 2020, the soil was amended with 300 kg ha^{-1} potassium sulphate and elevated beds with black polyethylene were prepared in June. Frigo strawberry plants (*Fragaria* × *ananassa* Duch.) cv. 'Clery' were planted on 26 June 2020. Plants were arranged in single rows, with 0.15 × 0.15 m spacing between them and 1.10 m spacing between the rows. The system was equipped with drip irrigation and all plants were subjected to identical agrotechnical measures: in summer and autumn 2020, flowers and runners were removed; in February 2021, dead leaves were cut back to stimulate new growth; in March 2021, tunnels were covered with plastic, and plant protection and fertilization regime were performed according to integrated production guidelines until the start of harvest.

Three treatments were established: (1) CON, control treatment with no addition of biostimulants; (2) BP, drip application of biostimulants before planting; and (3) AP, drip application of biostimulants after planting. Specifics of biostimulants' application are listed in SM Table S1.

Control plants were drip-irrigated with water only. Each treatment was repeated in five blocks and each block included ten plants. Only mature fruits were collected at individual sampling dates, and appropriate ripeness stage was ascertained according to the strawberry fruit's visual characteristics. Fruits were harvested nine (9) times from 17 May until 13 June 2021 and on each sampling date, the following parameters were monitored immediately: number of fruit per plant, individual fruit weight per plant, total fruit weight per plant, fruit diameter and height, soluble solids content, fruit firmness (digital penetrometer, TR, Turin, Italy) and fruit colorimetric analyses (color parameters *L*, *C*, h°, a^* and b^*; portable colorimeter CR-10 Chroma; Minolta, Japan). Parameter *L* presents lightness (0 – 100, 0 is black, 100 is white), *C* stands for chroma (higher value presents more intense color), h° represents color expressed in degrees (0° is red, 90° is yellow, 180° is green and 270° is blue), a^* is color from green to red (−128 to 127) and b^* is color from blue to yellow (−128 to 127). Strawberries were transported to the laboratory facility on ice, and ascorbic acid extractions were made from a portion of fresh fruit samples (5 fruit per repetition, $n = 5$). The remaining portion of strawberry samples were labelled, transferred to laboratory on ice, measured, shock frozen in liquid nitrogen, and stored at −20 °C for up to a week before primary metabolite (sugars and organic acids) and phenolic extractions. For all chemical analyses, five repetitions were carried out ($n = 5$) per treatment and sampling date; each repetition included several fruits. At the end of the trial, plants were uprooted, and the following parameters were evaluated: root weight, root length (the length of the longest root was measured), canopy weight, canopy height (the highest point of the canopy was measured), and the number of crowns.

2.2. Extraction and Determination of Sugars and Organic Acids

Thawed strawberry fruit (5 fruit per repetition) was finely chopped and 1 g of puree was homogenized in 5 mL of bi-distilled water using an Ultra-Turrax T-25 (Ika-Labortechnik, Germany), left for 30 min at room temperature with constant stirring (Unimax 1010 shaker; Heidolph, Schwabach, Germany), centrifuged (Eppendorf Centrifuge 5810 R) at 9000× *g* for 10 min at 4 °C, and filtered through a 0.20 µm cellulose ester filter (Chromafil A-20/25; Macherey-Nagel, Düren, Germany) into vials, according to the method of Weber et al. [18]. The analyses of primary metabolites were carried out using a high-performance liquid chromatography (HPLC) (Vanquish; Thermo Scientific, Waltham, MA, USA). The injection volume was 20 µL, and the flow rate was maintained at 0.6 mL min^{-1}. Sugar separations were carried out using a Rezex RCM-monosaccharide column from Phenomenex (Ca+ 2%), operated at 65 °C (300 mm × 7.8 mm). The mobile phase was bi-distilled water, total run time was 30 min, and a refractive index (RI) detector (RI plus; RefractoMax520; Thermo Scientific, Waltham, MA, USA) was used to monitor the eluted carbohydrates, as described by Mikulic-Petkovsek et al. [29]. Analyses of organic acids were performed on the same HPLC system, equipped with a UV detector set at 210 nm, using a Rezex

ROA-organic acid (H+ (8%)) column from Phenomenex (300 mm × 7.8 mm), as described by Mikulic-Petkovsek et al. [29]. The column temperature was set to 65 °C. The elution solvent was 4 mM of sulfuric acid in bi-distilled water and the flow rate was 0.6 mL min^{-1}. The contents of sugars and organic acids were calculated with the help of a corresponding external standard and expressed as mg g^{-1} fresh weight (FW). Sugar/organic acid ratio was calculated as a sum (or ratio) of all individual sugars or organic acids.

2.3. Ascorbic Acid Extraction and Determination

Fresh strawberry fruit (5 fruit per repetition) was chopped into small pieces with a ceramic knife, and 2.5 g of tissue was immediately mixed with 5 mL of 3% metaphosphoric acid [29]. The samples were left on a shaker for 30 min, centrifuged at 9000× *g* rpm for 7 min at 4 °C (5801R; Eppendorf, Hamburg, Germany), filtered through cellulose filters (Chromafil A-20/25; MachereyNagel, Dueren, Hamburg, Germany), transferred to vials, and analyzed by HPLC (Vanquish; Thermo Scientific, Waltham, MA, USA) on a Rezex ROA-Organic acid H+ 8% column (150 mm × 7.8 mm; Phenomenex, CA, USA). The UV detector was set at 210 nm under the following analytical conditions: column temperature, 65 °C; injection volume, 20 µL; flow rate, 0.6 mL min^{-1}; and sample analysis time, 15 min. The mobile phase was 4 mM of sulfuric acid in bi-distilled water. Ascorbic acid content was determined using the calibration curves, and data were expressed in g kg^{-1} FW.

2.4. Extraction of Phenolic Compounds

Thawed strawberry fruit (5 fruit per repetition) was finely chopped, and 3 g of pulp was extracted with 6 mL of 80% methanol containing 3% formic acid in bi-distilled water. The samples were mixed by vortexing, left in a cooled ultrasonic bath (0 °C) for 1 h, and then centrifuged at 9000× *g* for 10 min at 4 °C (5810 R; Eppendorf, Hamburg, Germany). The supernatant was filtered through 0.2 mm polyamide filters (Chromafil AO-20/25; Macherey-Nagel, Düren, Germany), and put into vials prior to injection and into the HPLC system [18].

2.5. Determination of Individual Phenolic Compounds

Separation of the phenolic compounds was performed on an HPLC system (Dionex UltiMate 3000; Thermo Scientific, Waltham, MA, USA), with detection at absorbances of 280, 350, and 530 nm under the conditions described by Mikulic-Petkovsek et al. [29] with the following: flow rate: 0.6 mL min^{-1}; autosampler temperature: 10 °C; C18 column (Gemini, 150 mm × 4.6 mm, 3 µm; Phenomenex, CA, USA); column temperature: 25 °C; and injection volume: 20 µL. Mobile phase A was 3% acetonitrile and 0.1% formic acid in bi-distilled water (*v*/*v*/*v*), and mobile phase B was 3% bi-distilled water and 0.1% formic acid in acetonitrile (*v*/*v*/*v*). The gradients used were as follows: 0–15 min, 5% B; 15–20 min, 5–20% B; 20–30 min, 20–30% B; from 30–35 min, 30–90% B; 35–45 min, 90–100% B; and 45–50 min, 100–5% B.

Individual phenolic compounds were identified by comparisons of their retention times with external standards and confirmed on an ion trap mass spectrometer (LTQ XL linear; Thermo Scientific, Waltham, MA, USA) based on their mass fragmentation patterns. The injection volume of the samples was 10 µL, and the flow rate was 0.6 mL min^{-1}. The mass spectrometer was operated in negative and positive (anthocyanins) ion modes, with electrospray ionization. The capillary temperature was maintained at 250 °C, the sheath gas at 20 units, and the auxiliary gas at 8 units. The source voltage used was 4 kV, with *m*/*z* scanning from 115 to 1600. The content of phenolic compounds was quantified from corresponding external standards or similar compounds and expressed in mg 100 g^{-1} FW. Details on phenolic identification are listed in SM Table S2.

2.6. Chemicals and Products

The following standards were used for determination of sugars and organic acids: sucrose, fructose, glucose and citric, malic, fumaric, and ascorbic acid from Fluka Chemie

(Buchs, Switzerland), and shikimic acid from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Water for the mobile phase was bi-distilled and purified with a Milli-Q system (Millipore, Bedford, MA, USA). Commercial products were purchased directly from LG Italia (Lodi, Italy) and Atens (Tarragona, Spain). Sulfuric acid, formic acid, and acetonitrile were purchased from Sigma-Aldrich Chemicals. Standards used for phenolic compounds determination were as follows: caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, procyanidin B1, catechin, epicatechin, luteolin-7-*O*-glucoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, cyanidin-3-*O*-glucoside, and pelargonidin-3-*O*-glucoside (Fluka Chemie).

2.7. Statistical Analyses

The data were analyzed using the R commander i386 4.0.4. program (Manugistics, Inc., Rockville, MD, USA). Significant differences among treatments were estimated using one-way analysis of variance (ANOVA) separately for each sampling date at $p < 0.05$.

3. Results and Discussion

3.1. Strawberry Vegetative Performance

Root length (17.98–21.35 cm), root weight (42.93–59.67 g), canopy height (46.99–50.28 cm), canopy weight (286.7–347.2 g), and the number of crowns (3.2–4.3) were recorded at the end of the experiment after uprooting all the strawberry plants, and significantly higher values of most of the vegetative parameters were recorded in biostimulant treatments, with more favorable effects detected in plants that were treated after planting (Table 1).

Table 1. Root length, root weight, canopy height, canopy weight, and number of crowns of strawberry plants at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 15 plants per treatment. Different letters (a,b) indicate significant differences among treatments (LSD test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; NS, non-significant).

Treatment	Root Length (cm)	Root Weight (g)	Canopy Height (cm)	Canopy Weight (g)	Crown Number
Control	21.35 ± 4.54	42.93 ± 14.82 b	46.99 ± 2.98 b	286.7 ± 93.8 b	3.2 ± 0.8 b
AP	20.11 ± 6.63	59.67 ± 11.29 a	50.28 ± 2.22 a	347.2 ± 47.0 a	4.3 ± 1.3 a
BP	17.98 ± 6.76	52.13 ± 13.00 ab	49.83 ± 3.35 a	301.9 ± 73.8 ab	4.3 ± 1.2 a
Significance	NS	**	**	*	*

Greater root development (higher root weight) and a significantly higher number of crowns per plant improved the nutrient and water uptake and resulted in increased aboveground vigor (canopy height and weight) of strawberry plants [30]. The number and diameter of crowns denote important positive factors affecting generative advancement of strawberry plants [31,32]. Various biostimulants have previously been reported to positively affect these vegetative parameters. Plant probiotic bacteria, including *Bacillus amyloliquefaciens*, improved plant height, canopy diameter, and root formation. Rahman et al. (2018) [33] and Madhavi et al. [30] reported superior height, root development, and number of crowns in strawberries supplemented with humic acids and a combination of beneficial microbes. Humic acids have a potent role in plant nutrient and water uptake, cell differentiation, and lateral root formation [34], which could explain the improved root and canopy parameters in AP treatment. Lombardi et al. [35] reported enhanced root length, root fresh weight, and root dry weight of plants supplemented with *various Trichoderma* strains, attributing the effect to general augmented carbon and energy metabolism as well as increased nutrient uptake and higher photosynthetic efficiency. The same group of authors linked the growth improvement in strawberry plants to amplified levels of enzymes involved in the biosynthesis of essential metabolites and precursors of structural proteins in growing fruit cells [36]. Additionally, multimicrobial inoculation with *Glomus mosseae*, *Bacillus subtilis*, and *Trichoderma harzianum* strains proved effective in promoting strawberry growth [37]. The latter was also applied to strawberries in a study by Khan et al. [38], who

speculated that enhanced growth could be attributed to secondary metabolites produced by fungi and bacteria, particularly auxins, which promote stem elongation and plant growth. Numerous mechanisms are therefore linked to the improved growth of treated strawberries by augmenting the bioavailability of essential nutrients.

3.2. Strawberry Reproductive Performance and Fruit Measurements

The number of fruits per plant, individual fruit weight, and total fruit weight per plant as well as fruit dimensions were recorded on nine sampling dates (Figure 1A–C).

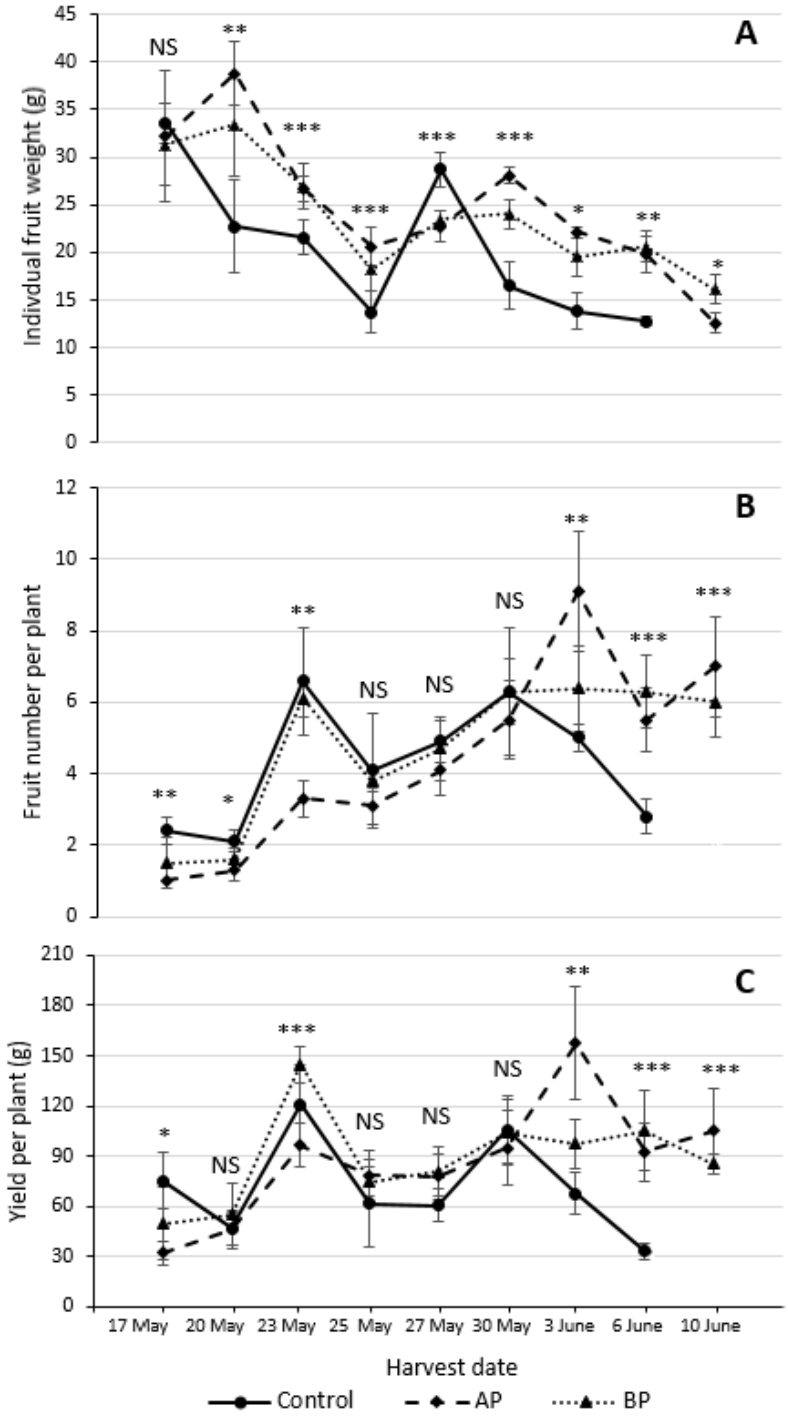

Figure 1. Individual fruit weight (**A**), fruit number per plant (**B**), and yield per plant (**C**) at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are

means ± standard errors of 15 plants per treatment at individual harvest date. Significant differences among treatments within the same harvest date were estimated using LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, non-significant; -, no data.

The dynamics of fruit development from 17 May to 10 June are typical of strawberries and correspond to time trends reported by Weber et al. [18]. In the first samplings from one to three, ripe fruits were harvested from plants dependent on the treatment, and their weight was larger compared to the fruit later in the season. This can be ascribed to a lower sink-to-source ratio at the beginning of generative development as there are fewer fruits that demand assimilates. Fruit height and width were superior in strawberries collected from treated plants in all but two samplings (Table 2).

Table 2. Strawberry fruit height and two diameters at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 15 fruit per treatment at individual harvest date. Different letters (a–c) indicate significant differences among treatments within the same harvest date (LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, non-significant; -, no data).

Treatment	Harvest Date	Fruit Height (mm)	Fruit Diameter 1 (mm)	Fruit Diameter 2 (mm)
Control	17 May	51.6 ± 3.9	43.1 ± 4.0	36.6 ± 2.5
AP		47.8 ± 10.9	42.6 ± 1.47	38.5 ± 1.5
BP		49.2 ± 5.6	42.8 ± 5.2	36.4 ± 2.5
Significance		NS	NS	NS
Control	20 May	46.5 ± 4.0 b	36.4 ± 4.5 b	32.9 ± 2.1 b
AP		51.9 ± 4.8 a	46.1 ± 5.0 a	35.6 ± 2.2 a
BP		51.4 ± 3.8 a	42.9 ± 4.4 a	37.3 ± 2.2 a
Significance		**	***	***
Control	23 May	46.5 ± 4.1 b	35.3 ± 2.6 b	33.6 ± 2.3 b
AP		51.8 ± 4.3 a	37.8 ± 3.3 a	35.5 ± 3.3 a
BP		50.2 ± 3.4 a	37.9 ± 3.7 a	35.6 ± 1.4 a
Significance		**	*	*
Control	25 May	37.2 ± 2.7 b	27.9 ± 2.1 b	26.8 ± 2.1 c
AP		43.6 ± 3.1 a	33.2 ± 2.1 a	32.6 ± 2.0 a
BP		41.5 ± 3.0 a	32.3 ± 4.6 a	30.2 ± 1.8 b
Significance		***	***	***
Control	27 May	47.5 ± 2.9	41.4 ± 4.3 a	36.6 ± 2.5 a
AP		46.3 ± 2.7	35.6 ± 2.7 b	32.9 ± 1.8 b
BP		46.5 ± 3.4	36.4 ± 2.4 b	34.1 ± 2.1 b
Significance		NS	***	***
Control	30 May	38.2 ± 2.9 c	31.5 ± 2.2 b	33.0 ± 2.3 b
AP		46.6 ± 2.3 a	39.0 ± 2.9 a	35.3 ± 2.5 a
BP		43.9 ± 4.6 b	37.3 ± 3.7 a	34.0 ± 2.8 ab
Significance		***	***	*
Control	3 June	34.9 ± 2.6 b	30.7 ± 3.1 b	29.0 ± 2.9 c
AP		42.6 ± 4.0 a	35.6 ± 2.4 a	34.2 ± 2.3 a
BP		42.5 ± 2.8 a	34.7 ± 2.3 a	32.2 ± 2.7 b
Significance		***	***	***
Control	6 June	32.0 ± 3.2 b	30.4 ± 2.5 b	27.9 ± 2.4 b
AP		37.4 ± 2.7 a	35.5 ± 1.9 a	33.5 ± 1.6 a
BP		38.2 ± 2.5 a	36.1 ± 2.2 a	34.0 ± 2.0 a
Significance		***	***	***
Control	10 June	-	-	-
AP		31.5 ± 4.6 b	30.3 ± 2.5 b	28.0 ± 2.4 b
BP		34.8 ± 3.3 a	33.1 ± 4.3 a	30.3 ± 3.7 a
Significance		*	*	*

Correspondingly, the individual fruit weight was significantly higher in both biostimulant treatments compared to the control in all but two samplings (Figure 1A), even if the number of fruits was significantly higher than in the control treatment, which suggests superior plant conditions during the harvest season. This agrees with a study by Mikiciuk et al. [39], who applied several mycorrhizal and bacterial products to strawberry plants and measured 31 to 70% greater fruit weight of treated strawberries. Other authors have reported that the application of *Trichoderma* stimulates strawberry plant's physiological processes, specifically nutrient uptake, protein metabolism, and carbon/energy metabolism, resulting in more fruit, larger fruit, and improved crop quality [35]. In the present study, the combined yield per plant was also recorded and was significantly higher in the BP and AP treatments (33% and 30% more marketable fruits were collected cumulatively during the season, respectively) compared to the control plants. Our results agree with Shafir et al. [40] and Khan et al. [38], who supplemented strawberry plants with *Trichoderma* and biofertilizers and recorded 21% to 45% yield increases, as well as Mikiciuk et al. [39], who detected 26 to 38% yield increases in strawberries inoculated with *Funneliformis mosseae* and other mycorrhizal and bacterial products. It has been reported that *Trichoderma* sp. not only improves the nutrient use efficiency, especially nitrogen, but it also solubilizes Mn^{4+}, Fe^{3+}, and Cu^{2+}. Consequently, the availability of nutrients stimulates plants to increase the number of fruits and their weight [38,41].

Previous authors have endorsed the use of bacteria and fungi at the beginning of the strawberry growing cycle by root dipping or foliar application [33,42], but research on the effect of pre-planting applications of products is rare. Interestingly, the treated plants in our experiment produced significantly more fruit in the last three samplings compared to the control plants (Figure 1B), resulting in substantial yield increases late in the season (Figure 1C). The lasting positive effects of mycorrhizal fungi and bacteria suggest that inoculating the root system of strawberry plants as a pre-planting measure or early in the season is beneficial even in the following production season.

In addition to fruit dimensions, fruit color also determines the strawberry's external quality, which is valued by consumers. Differences in color parameters were non-significant on most harvest dates, indicating similar visual characteristics of strawberry fruit at all treatments (Table 3). Color characteristics were comparable to previous reports on the 'Clery' strawberry [43,44]. Similarly, the strawberry fruit's firmness did not differ among treatments on any harvest date (Table 4), which suggests equal fruit transport and storage potentials, as fruit firmness considerably affects these logistic traits [45].

Table 3. Strawberry fruit color parameters at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 15 fruit per treatment at individual harvest date. Different letters (a,b) indicate significant differences among treatments within the same harvest date (LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; NS, not significant; -, no data).

Treatment	Harvest Date	L *	C	h°	a *	b *
Control	17 May	36.0 ± 2.8	47.8 ± 4.1	33.4 ± 2.8	39.8 ± 2.5	26.4 ± 4.0
AP		36.5 ± 1.3	50.3 ± 3.5	34.7 ± 1.3	41.4 ± 2.9	28.7 ± 2.3
BP		36.6 ± 1.8	49.4 ± 2.7	33.7 ± 2.3	41.0 ± 1.9	27.4 ± 2.7
Significance		NS	NS	NS	NS	NS
Control	20 May	35.7 ± 2.0	48.3 ± 3.5	34.0 ± 1.7	40.0 ± 2.2	27.0 ± 3.0
AP		35.9 ± 2.3	48.6 ± 3.5	34.7 ± 1.5	40.0 ± 2.5	27.7 ± 2.8
BP		37.3 ± 1.9	50.2 ± 3.1	34.9 ± 2.2	41.1 ± 2.0	28.7 ± 3.0
Significance		NS	NS	NS	NS	NS
Control	23 May	35.0 ± 1.6	46.3 ± 3.7	33.2 ± 2.1 ab	38.7 ± 2.8	25.3 ± 2.6
AP		36.0 ± 1.5	47.9 ± 2.7	33.5 ± 1.4 a	39.9 ± 1.9	26.5 ± 2.3
BP		34.6 ± 1.9	46.7 ± 3.5	32.2 ± 2.1 b	39.5 ± 2.3	25.0 ± 3.2
Significance		NS	NS	*	NS	NS

Table 3. *Cont.*

Treatment	Harvest Date	L *	C	h°	a *	b *
Control	25 May	34.8 ± 4.1	47.3 ± 4.8	35.6 ± 1.5 a	38.4 ± 3.7	27.5 ± 3.3
AP		35.6 ± 2.3	45.6 ± 4.5	33.6 ± 1.7 b	38.5 ± 3.0	25.9 ± 3.3
BP		36.2 ± 2.1	47.3 ± 3.3	34.1 ± 2.2 b	39.1 ± 2.0	26.6 ± 3.0
Significance		NS	NS	*	NS	NS
Control	27 May	34.0 ± 2.1	44.6 ± 3.3	32.1 ± 2.6	37.7 ± 2.2	23.7 ± 3.2
AP		35.0 ± 1.3	46.4 ± 2.8	33.5 ± 1.9	38.6 ± 1.9	25.7 ± 2.7
BP		34.8 ± 2.0	45.6 ± 3.3	32.6 ± 2.1	38.4 ± 2.1	24.7 ± 3.1
Significance		NS	NS	NS	NS	NS
Control	30 May	33.0 ± 2.7	41.7 ± 5.2 b	32.1 ± 2.7 b	35.9 ± 3.9 ab	22.3 ± 4.3 b
AP		34.5 ± 2.0	45.0 ± 3.8 a	33.2 ± 1.8 ab	37.6 ± 2.6 a	24.7 ± 3.1 ab
BP		34.4 ± 1.2	44.4 ± 3.1 ab	34.2 ± 1.2 a	34.6 ± 4.2 b	25.3 ± 2.2 a
Significance		NS	*	*	*	*
Control	3 June	32.7 ± 1.2	43.2 ± 2.3 a	32.0 ± 1.9	36.6 ± 2.2 a	22.9 ± 1.5 ab
AP		32.1 ± 2.3	42.1 ± 3.9 a	33.0 ± 1.1	35.3 ± 3.1 a	23.4 ± 2.9 a
BP		31.7 ± 2.3	39.0 ± 5.3 b	32.7 ± 1.9	32.7 ± 4.3 b	21.1 ± 3.3 b
Significance		NS	*	NS	**	*
Control	6 June	34.7 ± 1.8	45.2 ± 3.5	33.7 ± 1.3	37.6 ± 2.6	25.2 ± 2.6 ab
AP		33.5 ± 2.2	43.2 ± 4.1	32.6 ± 1.9	36.3 ± 3.0	23.3 ± 3.0 b
BP		34.5 ± 2.0	46.0 ± 4.1	33.7 ± 1.6	38.2 ± 2.8	25.7 ± 3.4 a
Significance		NS	NS	NS	NS	*
Control	10 June	-	-	-	-	-
AP		33.5 ± 1.6 b	43.1 ± 2.9 b	32.0 ± 1.2 b	36.5 ± 2.2 b	22.2 ± 4.1 b
BP		35.9 ± 2.2 a	46.2 ± 3.6 a	33.7 ± 1.8 a	38.4 ± 2.5 a	25.7 ± 3.0 a
Significance		**	*	**	*	*

Strawberry soluble solids content (°Brix) differed among treatments, and the fruit of the control treatment was generally characterized by the highest values (Table 4).

Table 4. Strawberry fruit firmness, total soluble solids, and sugar/organic acid ratio at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 15 fruit per treatment at individual harvest date. Different letters (a,b) indicate significant differences among treatments within the same harvest date (LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant; -, no data).

Treatment	Harvest Date	Fruit Firmness (kg cm^{-1})	Total Soluble Solids (°Brix)	Sugar/Organic Acid
Control	17 May	0.87 ± 0.31	8.28 ± 0.94	4.11 ± 0.41 a
AP		1.06 ± 0.24	7.68 ± 0.34	3.43 ± 0.33 b
BP		0.89 ± 0.34	7.85 ± 1.27	4.08 ± 0.25 a
Significance		NS	NS	*
Control	20 May	0.84 ± 0.49	7.80 ± 0.47 a	3.74 ± 0.55
AP		0.68 ± 0.26	7.25 ± 0.39 b	3.47 ± 0.31
BP		0.79 ± 0.33	7.80 ± 0.43 a	3.74 ± 0.24
Significance		NS	**	NS
Control	23 May	0.91 ± 0.41	8.22 ± 0.84 a	4.29 ± 0.46
AP		0.73 ± 0.51	7.53 ± 0.54 b	4.21 ± 0.86
BP		0.70 ± 0.33	7.67 ± 0.41 b	4.16 ± 0.53
Significance		NS	*	NS
Control	25 May	1.12 ± 0.26	7.96 ± 0.78 a	3.06 ± 0.41 b
AP		1.19 ± 0.34	6.72 ± 1.26 b	4.08 ± 0.79 a
BP		1.07 ± 0.18	7.48 ± 0.70 a	4.05 ± 0.52 a
Significance		NS	**	*

Table 4. *Cont.*

Treatment	Harvest Date	Fruit Firmness (kg cm^{-1})	Total Soluble Solids (°Brix)	Sugar/Organic Acid
Control	27 May	0.76 ± 0.41	8.89 ± 0.68 a	4.26 ± 0.83 a
AP		0.83 ± 0.37	7.09 ± 0.63 b	3.30 ± 0.24 b
BP		0.73 ± 0.32	7.44 ± 0.64 b	3.63 ± 0.80 ab
Significance		NS	***	*
Control	30 May	1.25 ± 0.32 a	8.65 ± 0.97 a	3.44 ± 0.29
AP		1.26 ± 0.21 b	7.58 ± 0.88 b	3.79 ± 0.56
BP		1.28 ± 0.33 a	8.23 ± 1.01 ab	3.41 ± 0.32
Significance		NS	*	NS
Control	3 June	0.48 ± 0.31	9.33 ± 1.27 a	3.69 ± 0.63 ab
AP		0.54 ± 0.23	7.31 ± 1.41 b	3.34 ± 0.41 b
BP		0.51 ± 0.27	7.59 ± 0.81 b	4.19 ± 0.37 a
Significance		NS	***	*
Control	6 June	1.00 ± 0.30	8.26 ± 1.28	3.29 ± 0.60
AP		1.07 ± 0.27	7.58 ± 1.07	3.36 ± 0.54
BP		1.05 ± 0.24	7.77 ± 0.86	3.12 ± 0.21
Significance		NS	NS	NS
Control	10 June	-	-	-
AP		0.76 ± 0.33	7.97 ± 1.19	3.62 ± 0.70
BP		0.74 ± 0.31	7.68 ± 0.62	3.19 ± 0.29
Significance		NS	NS	NS

This can potentially be linked to larger fruits from biostimulants treatments and dilution effects due to the increased water content of fruit. Previously reported data on the 'Clery' strawberry are in accordance with the values determined in our study [46]. Moreover, the seasonal trends in these parameters are similar to those determined by Weber et al. [18], who ascribed differences to the altered water content, sink/source ratio, and higher temperature in later harvests.

3.3. The Content of Sugars, Organic Acids, and Sugar/Acid Ratio of Strawberry Fruits

The content of individual and total sugars (38.01–61.34 g kg^{-1}) and individual and total organic acids (11.06–15.33 g kg^{-1}) and their ratios were monitored on all nine harvest dates (Tables 5 and 6).

Table 5. Total and individual sugar contents of strawberry fruit at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 5 replicates per treatment at individual harvest date. Different letters (a–c) indicate significant differences among treatments within the same harvest date (LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant; -, no data).

Treatment	Harvest Date	Total Sugar Content (g kg^{-1})	Sucrose (g kg^{-1})	Glucose (g kg^{-1})	Fructose (g kg^{-1})
Control	17 May	52.92 ± 3.11	11.06 ± 1.80	19.47 ± 1.39 a	22.39 ± 1.04 a
AP		49.22 ± 3.47	13.47 ± 1.66	16.58 ± 1.12 b	19.17 ± 1.29 b
BP		53.04 ± 5.06	12.14 ± 2.73	18.99 ± 1.72 a	21.92 ± 1.49 a
Significance		NS	NS	*	**
Control	20 May	50.09 ± 3.81 a	11.56 ± 1.17	17.90 ± 1.49 a	20.63 ± 1.50 a
AP		43.04 ± 2.42 b	9.76 ± 0.82	15.17 ± 0.89 b	18.11 ± 1.02 b
BP		45.57 ± 3.98 ab	10.82 ± 1.84	15.89 ± 1.01 b	18.86 ± 1.14 b
Significance		*	NS	**	*

Table 5. *Cont.*

Treatment	Harvest Date	Total Sugar Content (g kg^{-1})	Sucrose (g kg^{-1})	Glucose (g kg^{-1})	Fructose (g kg^{-1})
Control	23 May	50.91 ± 3.93 a	11.37 ± 1.87	18.14 ± 1.12 a	21.40 ± 1.19 a
AP		44.39 ± 5.43 b	10.77 ± 2.13	15.35 ± 1.73 b	18.27 ± 1.96 b
BP		46.02 ± 1.87 ab	11.52 ± 1.57	15.76 ± 0.32 b	18.74 ± 0.36 b
Significance		*	NS	**	**
Control	25 May	45.34 ± 3.05	10.19 ± 1.45	15.97 ± 0.84	19.17 ± 0.88
AP		45.54 ± 5.51	11.96 ± 1.85	15.26 ± 1.77	18.33 ± 1.99
BP		47.95 ± 6.53	12.79 ± 2.49	15.97 ± 2.07	19.19 ± 2.33
Significance		NS	NS	NS	NS
Control	27 May	61.34 ± 6.73 a	17.61 ± 2.51 a	20.13 ± 2.22 a	23.61 ± 2.37 a
AP		44.55 ± 3.89 b	9.90 ± 1.77 c	15.85 ± 1.46 b	18.80 ± 1.68 b
BP		48.17 ± 7.64 b	13.50 ± 3.26 b	15.85 ± 2.15 b	18.82 ± 2.44 b
Significance		**	**	**	**
Control	30 May	50.43 ± 4.25	12.26 ± 2.76	17.33 ± 0.95	20.83 ± 1.08
AP		51.84 ± 11.89	14.21 ± 6.69	17.27 ± 2.72	20.36 ± 2.94
BP		44.92 ± 3.59	11.11 ± 1.80	15.50 ± 1.22	18.31 ± 1.36
Significance		NS	NS	NS	NS
Control	3 June	49.52 ± 4.78 a	12.03 ± 3.12	17.14 ± 1.01 a	20.35 ± 0.95 a
AP		41.22 ± 3.38 b	9.83 ± 2.05	14.22 ± 1.11 b	17.16 ± 1.19 b
BP		48.69 ± 3.16 a	12.78 ± 3.37	16.50 ± 0.62 a	19.41 ± 0.73 a
Significance		***	NS	***	***
Control	6 June	45.52 ± 6.28	11.46 ± 2.65 a	15.41 ± 1.88	18.65 ± 1.90
AP		40.01 ± 3.58	8.73 ± 1.11 b	14.11 ± 1.43	17.17 ± 1.52
BP		41.86 ± 1.87	11.29 ± 1.51 ab	13.77 ± 1.10	16.80 ± 1.32
Significance		NS	*	NS	NS
Control	10 June	-	-	-	-
AP		43.58 ± 2.37 a	12.84 ± 1.62	14.01 ± 0.42 a	16.72 ± 0.46
BP		38.01 ± 2.76 b	10.21 ± 3.42	12.28 ± 0.52 b	15.52 ± 1.58
Significance		**	NS	***	NS

Fructose and glucose were the prevalent sugars in strawberry fruit (detected in quantities of 15.52–23.61 g kg^{-1} and 12.28–20-13 g kg^{-1}, respectively), followed by sucrose (8.73–14.21 g kg^{-1}), and citric (7.76–10.10 g kg^{-1}) and malic acids (2.60–5.06 g kg^{-1}) were the major organic acids, which is in accordance with previous studies [44,47].

Ascorbic acid was also quantified at levels of approx. 0.4 g kg^{-1}, which is similar to the results gathered in a study by Garzoli et al. [43]. Seasonal variations in the levels of primary metabolites can be linked to the number of fruits, fruit dimensions, and fruit weight, as reported in a previous study by Weber et al. [18]. Significant differences in the content of total sugars were detected between the control and treated fruits on four harvest dates, which can be explained by the higher number of fruits on the treated plants (Table 5). Considering the entire harvest period, a 10% decrease in total sugars was determined in the pre-harvest application of biostimulants, and an 11% decrease was detected in the post-harvest application. The distribution of organic acids was not as uniform as for sugars. The content of total organic acids was generally higher in the control fruits, but the difference was less significant (Table 6), leading to 10% lower levels of total organic acids in the pre-harvest treatment and 8% lower levels in the post-harvest treatment. The differences in the composition of primary metabolites are minor and predictable. as a higher sink-to-source ratio was characteristic of treated plants. Ascorbic acid accumulation was significantly higher in the control strawberries at three harvest dates, but the levels of this metabolite were comparable at other dates (only 7% and 5% reductions were detected in the pre-harvest and post-harvest treated plants). The sugar/organic acid ratio determines the fruit's sensory value [29], and a comparison among treatments revealed a time-dependent and treatment-

dependent trend in this characteristic. The control strawberries were characterized by a higher ratio at the beginning of harvest, and an improved sugar/organic acid ratio was determined in the treated fruit at later dates (Table 4). Other authors have also indicated a time-dependent trend in this parameter, which has been linked to the fruit number, temperature, water, and nutrient availability, as well as light [23,48]. Lombardi et al. [35] experimented with different *Trichoderma* strains and concluded that the effect on ascorbic acid accumulation was not uniform. Most strains had a negative effect on the content of this organic acid, but one boosted its formation.

3.4. Phenolic Profile of Strawberry Fruits

Strawberries contained phenolic compounds classified into six groups: flavan-3-ols (procyanidin dimer > procyanidin trimer > epicatehin > catechin), flavonols (apigenin rhamnoside > quercetin-3-*O*-glucuronide > kaempferol-3-*O*-β-glucuronide > kaempferol-3-*O*-rutinoside > kaempferol-malonylglucoside > kaempferol-3-*O*-hexoside > quercetin-3-*O*-galactoside > quercetin-3-*O*-hexoside), hydroxybenzoic acids (ellagic acid deoxyhexoside > ellagic acid derivative), hydroxycinnamic acids (ferulic acid hexoside derivative > p-coumaric hexoside > glucocaffeic acid > cinnamic acid-3-*O*-hexoside > caffeic acid derivative), and anthocyanins (pelargonidin-3-*O*-β-glucoside > cyanidin-3-*O*-β-glucoside). Chromatograms of identified phenolics at 280 nm, 350 nm, and 530 nm are supplemented in SM Figures S1–S3. Flavan-3-ols constituted the largest share of all the identified phenolic compounds in strawberry fruits (335.7–580.9 mg kg^{-1}), followed by anthocyanins (308.3–582.6 mg kg^{-1}) and other compounds detected in significantly lower quantities (Table 7). Strawberries contained from 769.7 to 1235.0 mg kg^{-1} total detected phenolic compounds, which is consistent with previous reports [18,23].

The phenolic profile of the strawberry fruit is in accordance with the research of Garzoli et al. (2020) [43], Weber et al. [18,44], and Simkova et al. [23]. Comparable seasonal variations in phenolic compounds have been reported in strawberries in a study by Paparozzi et al. [1] and Simkova et al. [23]. Both groups of authors have reported that strawberries exposed to longer periods of sunshine later in the season are richer in health-promoting compounds. This is most evident in the group of anthocyanins, which are directly linked to the visual characteristics of fruit tissues and are synthesized during the season and fruit maturation [23,49]. Linear relationships between the concentration of total anthocyanins as well as the prevalent pelargonidin-3-*O*-β-glucoside and color parameters L* and H° have been reported by Yoshida et al. [50] during the fruit development of different strawberry cultivars.

Significantly higher levels of flavan-3-ols, hydroxycinnamic acids, and flavonols were detected in the control fruits compared to the treated strawberries on the first five harvest dates, but the differences in the total detected phenolics were minor during the entire harvest period (8% in the pre-harvest and 6% in the post-harvest treatment) (Table 7).

These groups of secondary metabolites play an important role as defense phenolics, and their production is triggered by different external stressors [51]. It can be assumed that biostimulators alleviate selective negative effects on plants, which decrease the synthesis of specific phenolics. Similar findings were reported by Weber et al. [18].

On the other hand, the accumulation of anthocyanins (especially the major anthocyanin pelargonidin-3-*O*-β-glucoside) was not as uniform in control vs. treated fruit. In the first harvest, the control fruit contained more anthocyanins, but later in the season, both pre-planting and post-planting biostimulant treatments exhibited a positive effect on the anthocyanin content. Similarly, the total anthocyanin content (TAC) was not significantly affected by humic acid applications in a study by Soppelsa et al. (2019) [21]. And the content of individual anthocyanins was shown to be independent of inoculation treatment with different *Bacillus* strains in a study of Morais et al. [52]. Lombardi et al. (2020a) [36] stated that a strain-dependent and time-dependent pattern of phenolic accumulation can be detected in strawberries treated with *Trichoderma*, and that decreased levels of the prevailing pelargonidin-3-*O*-β-glucoside can be characteristic for some strains [37].

Table 6. Total and individual organic acid contents of strawberry fruit at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 5 replicates per treatment at individual harvest date. Different letters (a,b) indicate significant differences among treatments within the same harvest date (LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant; -, no data).

Treatment	Harvest Date	Total Content of Detected Organic Acids (g kg^{-1})	Citric Acid (g kg^{-1})	Malic Acid (g kg^{-1})	Fumaric Acid (g kg^{-1})	Shikimic Acid (g kg^{-1})	Ascorbic Acid (g kg^{-1})
Control	17 May	13.41 ± 1.61 ab	8.86 ± 1.08 ab	4.09 ± 0.50	0.009 ± 0.001	0.035 ± 0.004	0.42 ± 0.02 a
AP		14.70 ± 0.40 a	9.77 ± 0.17 a	4.52 ± 0.26	0.01 ± 0.001	0.037 ± 0.002	0.36 ± 0.01 b
BP		13.37 ± 0.57 b	8.74 ± 0.48 b	4.20 ± 0.22	0.009 ± 0.002	0.035 ± 0.002	0.38 ± 0.04 ab
Significance		*	*	NS	NS	NS	*
Control	20 May	13.91 ± 1.47 a	9.23 ± 0.81	4.25 ± 0.65 a	0.008 ± 0.000 a	0.036 ± 0.003 a	0.38 ± 0.03
AP		12.81 ± 0.65 ab	8.83 ± 0.59	3.55 ± 0.25 b	0.009 ± 0.001 ab	0.030 ± 0.003 b	0.39 ± 0.03
BP		12.53 ± 0.58 b	8.55 ± 0.37	3.58 ± 0.19 b	0.008 ± 0.000 b	0.030 ± 0.003 b	0.36 ± 0.04
Significance		*	NS	*	*	**	NS
Control	23 May	12.31 ± 0.90	8.08 ± 0.67	3.80 ± 0.20 a	0.009 ± 0.001 a	0.034 ± 0.003 a	0.38 ± 0.03
AP		11.06 ± 1.35	8.07 ± 0.54	2.60 ± 1.31 b	0.008 ± 0.001 b	0.029 ± 0.003 b	0.35 ± 0.03
BP		11.54 ± 1.25	7.76 ± 1.00	3.37 ± 0.25 ab	0.008 ± 0.000 ab	0.030 ± 0.002 b	0.37 ± 0.03
Significance		NS	NS	*	*	*	NS
Control	25 May	15.33 ± 1.25 a	9.84 ± 0.75 a	5.06 ± 0.51 a	0.010 ± 0.001 a	0.040 ± 0.004 a	0.38 ± 0.02
AP		11.80 ± 2.18 b	8.23 ± 1.26 b	3.15 ± 1.19 b	0.008 ± 0.001 b	0.027 ± 0.003 b	0.38 ± 0.07
BP		12.26 ± 0.87 b	8.55 ± 1.10 ab	3.28 ± 0.93 b	0.008 ± 0.001 b	0.029 ± 0.003 b	0.40 ± 0.04
Significance		**	*	*	**	***	NS
Control	27 May	15.06 ± 1.49	10.10 ± 1.01	4.48 ± 0.56	0.009 ± 0.001	0.037 ± 0.004	0.43 ± 0.04 a
AP		13.88 ± 0.41	8.80 ± 0.31	4.65 ± 0.36	0.009 ± 0.002	0.032 ± 0.003	0.39 ± 0.02 ab
BP		13.97 ± 2.31	9.25 ± 1.72	4.29 ± 0.60	0.009 ± 0.001	0.037 ± 0.005	0.38 ± 0.03 b
Significance		NS	NS	NS	NS	NS	*
Control	30 May	15.12 ± 1.08	10.10 ± 1.13	4.56 ± 0.27	0.009 ± 0.001	0.032 ± 0.003	0.42 ± 0.04
AP		14.00 ± 1.24	9.14 ± 0.56	4.41 ± 0.77	0.009 ± 0.002	0.034 ± 0.003	0.41 ± 0.04
BP		13.62 ± 1.50	9.13 ± 1.23	4.06 ± 0.35	0.009 ± 0.001	0.031 ± 0.003	0.39 ± 0.05
Significance		NS	NS	NS	NS	NS	NS
Control	3 June	14.08 ± 1.94	9.02 ± 1.33	4.55 ± 0.80	0.009 ± 0.001	0.034 ± 0.004 a	0.46 ± 0.04 a
AP		12.84 ± 1.48	8.35 ± 0.91	4.05 ± 0.56	0.009 ± 0.001	0.030 ± 0.004 ab	0.41 ± 0.04 b
BP		12.08 ± 0.89	7.63 ± 0.47	4.00 ± 0.43	0.006 ± 0.003	0.029 ± 0.003 b	0.41 ± 0.03 b
Significance		NS	NS	NS	NS	*	*

Table 6. *Cont.*

Treatment	Harvest Date	Total Content of Detected Organic Acids (g kg^{-1})	Citric Acid (g kg^{-1})	Malic Acid (g kg^{-1})	Fumaric Acid (g kg^{-1})	Shikimic Acid (g kg^{-1})	Ascorbic Acid (g kg^{-1})
Control	6 June	14.44 ± 1.49 a	9.91 ± 1.19 a	4.03 ± 0.25	0.008 ± 0.001	0.029 ± 0.004 a	0.47 ± 0.06
AP		12.52 ± 1.30 b	8.35 ± 1.01 b	3.67 ± 0.26	0.008 ± 0.001	0.025 ± 0.003 b	0.47 ± 0.04
BP		13.87 ± 0.95 ab	9.54 ± 0.79 ab	3.88 ± 0.23	0.007 ± 0.001	0.026 ± 0.002 ab	0.42 ± 0.04
Significance		*	*	NS	NS	*	NS
Control	10 June	-	-	-	-	-	-
AP		12.73 ± 2.01	7.99 ± 1.50	4.32 ± 0.50	0.007 ± 0.001	0.027 ± 0.004	0.39 ± 0.08
BP		12.42 ± 1.86	8.07 ± 1.35	3.93 ± 0.57	0.006 ± 0.001	0.025 ± 0.004	0.38 ± 0.03
Significance		NS	NS	NS	NS	NS	NS

Table 7. The content of phenolic groups in strawberry fruit at individual treatments (control, AP: after planting, and BP: before planting) and harvest dates. Data are means ± standard errors of 5 replicates per treatment at individual harvest date. Different letters (a–c) indicate significant differences between treatments within the same harvest date (LSD and *t*-test at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant;-, no data).

Treatment	Harvest Date	Total Content of Detected Phenolic Compounds (mg kg^{-1})	Hydroxycinnamic Acids (mg kg^{-1})	Hydroxybenzoic Acids (mg kg^{-1})	Flavan-3-ols (mg kg^{-1})	Flavonols (mg kg^{-1})	Anthocyanins (mg kg^{-1})
Control	17 May	952.9 ± 42.5 a	8.60 ± 0.53 a	12.87 ± 1.20	467.0 ± 21.1 a	59.34 ± 4.90 a	405.1 ± 31.5 a
AP		769.9 ± 45.8 b	7.30 ± 0.85 b	13.32 ± 1.47	387.8 ± 24.8 b	52.94 ± 4.11 b	308.3 ± 42.0 b
BP		824.0 ± 36.2 b	7.38 ± 0.57 b	12.88 ± 0.57	414.2 ± 19.7 b	47.81 ± 2.19 b	341.5 ± 29.7 b
Significance		***	*	NS	***	**	**
Control	20 May	837.3 ± 19.7 a	7.64 ± 0.61 a	14.35 ± 1.52	408.1 ± 22.4 a	52.93 ± 2.75 a	354.3 ± 26.3 b
AP		782.4 ± 37.8 b	6.27 ± 0.65 b	12.80 ± 1.11	335.7 ± 16.5 c	20.96 ± 2.24 c	406.7 ± 28.3 a
BP		769.7 ± 41.8 b	6.95 ± 0.88 ab	13.59 ± 1.17	368.8 ± 26.1 b	43.60 ± 3.58 b	336.8 ± 30.2 b
Significance		*	*	NS	***	***	**
Control	23 May	989.5 ± 62.0 a	8.63 ± 0.40 a	14.86 ± 2.06	491.8 ± 29.8 a	68.19 ± 7.07 a	406.0 ± 35.3
AP		783.1 ± 34.0 c	6.55 ± 0.76 b	14.00 ± 1.68	348.2 ± 28.4 c	36.29 ± 4.23 c	378.1 ± 28.5
BP		865.5 ± 37.3 b	7.36 ± 0.79 b	13.66 ± 1.34	410.3 ± 27.1 b	45.54 ± 4.48 b	388.7 ± 27.0
Significance		***	**	NS	***	***	NS

Table 7. *Cont.*

Treatment	Harvest Date	Total Content of Detected Phenolic Compounds (mg kg^{-1})	Hydroxycinnamic Acids (mg kg^{-1})	Hydroxybenzoic Acids (mg kg^{-1})	Flavan-3-ols (mg kg^{-1})	Flavonols (mg kg^{-1})	Anthocyanins (mg kg^{-1})
Control	25 May	1091 ± 53.4 a	9.60 ± 1.28 a	20.32 ± 1.31 a	501.1 ± 19.4 a	70.11 ± 7.39 a	489.4 ± 33.9 a
AP		831.6 ± 46.1 b	7.66 ± 0.78 b	15.73 ± 1.51 b	349.5 ± 29.3 c	49.18 ± 4.87 b	409.5 ± 25.1 b
BP		887.9 ± 35.1 b	8.57 ± 0.45 ab	16.77 ± 1.68 b	393.6 ± 21.8 b	53.36 ± 2.90 b	415.7 ± 26.7 b
Significance		***	*	**	***	***	**
Control	27 May	896.7 ± 49.8	7.28 ± 0.72 b	15.38 ± 1.36 b	454.7 ± 27.0 a	67.53 ± 7.67 a	351.8 ± 32.3 b
AP		926.5 ± 33.1	8.40 ± 0.67 a	20.77 ± 2.36 a	359.9 ± 33.8 b	46.69 ± 2.91 b	490.7 ± 18.1 a
BP		914.3 ± 55.1	8.18 ± 0.63 ab	19.23 ± 1.63 a	380.6 ± 30.2 b	47.94 ± 4.29 b	458.4 ± 35.1 a
Significance		NS	*	**	***	***	***
Control	30 May	1235 ± 49.0 a	9.93 ± 0.61 a	22.32 ± 2.12 a	580.9 ± 30.9 a	52.23 ± 3.46 a	570.0 ± 32.0 a
AP		1074 ± 42.2 b	9.18 ± 0.65 ab	19.93 ± 1.16 b	494.9 ± 36.0 b	50.14 ± 5.71 ab	500.0 ± 34.7 b
BP		985.5 ± 40.2 c	8.86 ± 0.72 b	21.30 ± 1.78 ab	430.3 ± 24.8 c	44.30 ± 4.05 b	480.8 ± 30.0 b
Significance		***	*	*	***	*	**
Control	3 June	1008 ± 55.9 b	8.09 ± 0.64 b	18.25 ± 0.88 a	467.8 ± 36.2 ab	42.01 ± 3.01 b	472.1 ± 33.3 b
AP		1171 ± 82.1 a	10.00 ± 0.62 a	20.12 ± 1.71 a	514.1 ± 36.0 a	44.57 ± 5.76 b	582.6 ± 41.7 a
BP		947.3 ± 82.4 b	6.96 ± 0.57 c	15.19 ± 1.98 b	431.4 ± 44.5 b	52.91 ± 5.29 a	440.9 ± 37.1 b
Significance		**	***	**	*	*	***
Control	6 June	1057 ± 59.8 b	8.30 ± 1.13	17.44 ± 1.31 b	515.8 ± 33.3	46.51 ± 6.40 b	469.7 ± 29.0 c
AP		1143 ± 69.0 ab	9.13 ± 1.29	20.78 ± 1.74 a	508.4 ± 32.2	44.95 ± 4.23 b	559.3 ± 37.9 a
BP		1151 ± 61.9 a	9.47 ± 1.02	18.75 ± 1.06 b	551.7 ± 39.0	59.16 ± 3.21 a	512.4 ± 20.8 b
Significance		*	NS	**	NS	***	**
Control	10 June	-	-	-	-	-	-
AP		1089 ± 60.6 a	9.53 ± 0.85 a	19.47 ± 1.30 a	460.3 ± 41.4 a	45.52 ± 2.41 a	554.1 ± 32.3 a
BP		874.4 ± 65.1 b	7.13 ± 0.55 b	16.64 ± 1.32 b	381.8 ± 22.7 b	29.96 ± 1.73 b	438.9 ± 33.5 b
Significance		***	***	**	**	***	***

4. Conclusions

The application of widespread biostimulant products before and after planting exhibited improved the vegetative and reproductive traits of the 'Clery' strawberry. A significantly higher number of crowns per plant, root/leaf weight and spread, the number of fruits per plant, individual fruit weight, and fruit yield per plant were detected during the entire harvest. On the other hand, the fruit's compositional characteristics were slightly affected by biostimulant application. A decrease in the soluble solids content, vitamin C, total individual sugars, and organic acids was detected in the treated plants. This can be ascribed to higher production of fruit on treated plants and a greater source-to-sink ratio compared to the control plants. A similar response was also detected in the case of most phenolic groups. Significantly higher levels of flavan-3-ols, hydroxycinnamic acids, and flavonols were detected in the control fruits compared to treated strawberries on several samplings, but the differences in total phenolics were minor during the entire harvest period. Coincidentally, the composition of phenolics in strawberries is mostly disregarded by average consumers. Most often, the decision on strawberry purchase is based on the visual characteristics of the fruit. Among those, the strawberry's fruit color, size, and shape are the most important factors, and all these traits were similar in the fruit of treated plants. Interestingly, the accumulation of anthocyanins was least affected by product application among phenolic groups, and fruit exhibited comparable color characteristics. The use of biostimulants product combinations before planting or several times after planting in intense strawberry production is justified as the products significantly improve the vigor and vegetative growth of strawberry plants, which produce over 30% more marketable fruit and prolong the harvest period and income for the producers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070769/s1, Figure S1: HPLC-MS (280 nm) chromatogram of individual phenolic compounds. **1**, glucocaffeic acid; **2**, procyanidin dimer; **3**, procyanidin dimer; **4**, procyanidin dimer; **5**, procyanidin trimer; **6**, catechin; **7**, *p*-coumaric hexoside; **8**, apigenin rhamnoside; **9**, epicatechin; **10**, procyanidin dimer; Figure S2: HPLC-MS (350 nm) chromatogram of individual phenolic compounds. **1**, caffeic acid derivative; **2**, ferulic acid hexoside derivative; **3**, *p*-coumaric hexoside, ellagic acid derivative; **4**, ellagic acid deoxyhexoside; **5**, ellagic acid deoxyhexoside; **6**, cinnamic acid-3-*O*-hexoside; **7**, quercetin-3-*O*-glucuronide; **8**, quercetin-3-*O*-hexoside, kaempferol-3-*O*-rutinoside; **9**, kaempferol-3-*O*-hexoside; **10**, kaempferol-3-*O*-β-glucuronide; **11**, kaempferol-malonylglucoside; Figure S3: HPLC-MS (530 nm) chromatogram of individual phenolic compounds. **1**, cyanidin-3-*O*-glucoside; **2**, pelargonidin-3-*O*-glucoside; **3**, pelargonidin-3-*O*-glucoside; **4**, pelargonidin-3-*O*-glucoside; **5**, pelargonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside; **6**, pelargonidin-3-*O*-glucoside; Table S1: Specifics of biostimulant applications in 2020 and 2021 on 'Clery' strawberries (CON control; BP before planting; AP after plating); Table S2: Standards of phenolic compounds, used for identification and quantification of individual phenolic compounds, together with their retention times and analyses conditions.

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