Article

Ultraviolet-to-Blue Light Conversion Film Affects Both Leaf Photosynthetic Traits and Fruit Bioactive Compound Accumulation in *Fragaria × ananassa*

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Abstract: The influence of light downconversion films (red, pink and blue films) on leaf physiological features and fruit bioactive compound accumulation was studied in strawberry plants. Red, pink and blue films were able to convert light less utilised by plants into more efficient light wavebands with various possibilities depending on the film characteristics (blue film shifts UV into blue radiation; red film shifts green into red radiation, pink film shifts UV and green into blue and red radiation but to a lesser extent than red film). Indeed, by enhancing the quality of light available for photosynthesis, the utilization of these films holds the potential to improve agricultural productivity and sustainability. All of these light downconversion films resulted in higher plant fresh weight compared to a control colourless (Cnt) film, with plants grown under blue film (UV-to-blue light conversion) showing the most impressive results in terms of total leaf area (+25%), leaf thickness (+10%) and leaf mass per area (+15%). Simultaneously, during the flowering stage, plants under blue film had a higher net photosynthetic rate due to the increase in photosynthetically efficient wavelengths. Moreover, blue film resulted in the highest total phenolic (+40% and +28% than red and pink films, respectively) and flavonoid content (+54%, +84%, +70% than Cnt, red and pink films, respectively) in fruit, with specific effects on targeted phenols, i.e., quercetin, ellagic acid and its glycoside, ellagitannins, and procyanidins. In conclusion, the use of the UV-to-blue conversion light film tested herein represents an innovative solution to increase strawberry yield and promote fruit nutraceutical features, playing a pivotal role in ensuring food quality and security and sustainable agricultural practices.

Keywords: light downconversion film; light spectrum; phenolic compound; photosynthesis; shifting light film; strawberry

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1. Introduction

Balancing crop productivity with market demands is a daily challenge for most farmers. Simultaneously implementing sustainable practices and reducing energy consumption adds an extra layer of complexity. The production system is constantly evolving to align with the market in response to the high demand for fresh fruit. This evolution is facilitated by integrating new technologies to enhance fruit quality and yield [1–3]. In this context, as light is one of the main drivers that affect plant physiology, researchers have

continuously attempted to increase available light as well as to manipulate the spectral composition of light to possibly use light as an "eustress" able to modulate the accumulation of targeted bioactive compounds in fruit [4–6]. Notably, it has been observed that red and blue lights have pronounced effects on leaf photosynthetic traits when administered as monochromatic light (actually as a narrow-band spectrum) or as supplemental light in greenhouses, as reviewed by Landi et al. [4]. For instance, supplementing red light during daylight hours has been shown to enhance plant productivity in strawberries [6,7]. Red light supplementation can also influence the secondary metabolisms, leading, for example, to increased starch and sucrose levels as observed in tomatoes and higher levels of anthocyanins as detected in strawberry fruits [6,8–10]. It has long been known that supplementation with blue light commonly leads to an increase in secondary metabolite concentrations in a range of plant species, including green and red sweet basil, red lettuce, tomatoes, and strawberries [6,11,12]. However, it should be noted that high intensities of blue light supplementation (e.g., 250 μmol m^{-2} s^{-1}) could be detrimental to plant and fruit productivity, leading to a decline in physiological performance [6,13].

In horticulture, covering materials are highly used due to their versatility. The most used covering technologies enable shade adjustment by modulating light intensity or selecting specific wavebands. These techniques optimize the light environment to promote crop growth and development [14–16]. Nowadays, researchers are testing a new technology for covering films that are able to manipulate the spectral distribution of transmitted solar radiation, known as light downconversion films [17–20]. Light downconversion films, also referred as to fluorescent or light conversion films, can convert light radiation less usable by chlorophyll, such as UV (280–380 nm) and green radiation (510–575 nm), into light radiation that is more efficient to kick off photosynthesis, such as blue and red light [20]. Furthermore, the specific effects of the conversion films depend on the composition and structure of the fluorescent dye blends within the plastic polymer matrix [18].

The alteration by light downconversion films of the sunlight spectrum reaching the plant, consisting of an increase in red and blue light wavebands, has positively affected crop production without compromising fruit qualitative characteristics [21]. This effect was observed in early potato crops (+12% yield), melons, and watermelons (+10% yield) [21]. Furthermore, when subjected to a green-to-red light conversion film, 'Buttercrunch' lettuce plants had a significant increase on leaf area, leading to a 21.7% increase in total aboveground fresh weight [22]. In the case of *Solanum lycopersicum* var. Beefsteak, a light conversion film focused solely on the red light peak at 600 nm resulted in a 23% increase in light efficiency measured as crop production per unit of photosynthetically active radiation light (PAR), leading to a 10% faster vegetative growth rate and a 36% reduction in total tomato waste [23]. Furthermore, variations in light spectra can impact plant quality by influencing the accumulation of bioactive compounds [22–24]. For instance, Cozzolino et al. [24] reported increased chlorophyll and vitamin C contents in *Valerianella locusta* L. plants cultivated under blue light-diffusing films. Moreover, Shen et al. [22] reported an increase in vitamin C content in lettuce grown under a green-to-red light-converting film.

Strawberry fruits have significant economic importance in various climate zones worldwide [25,26]. In an experiment conducted by Peng et al. [27], strawberry plants grown under various light selective films differed significantly in leaf area and shoot biomass. The films that transmitted a higher proportion of red light led to the best results, including the highest anthocyanin fruit yield.

To the best of our knowledge, little information is present in the literature regarding the use of different light downconversion films on the physiological features of strawberry plants and, especially, on fruit nutraceutical properties. Hemming et al. [28] found a weak relationship between vegetative growth and fruit yield among plants grown under different light downconversion films. Only the fluorescent film in the blue spectrum showed promising effects on fruit production. Differently, a work conducted by Kang et al. [3] suggests that green-to-red spectrum downconversion films boost the electron flow, leading to an enhanced photosynthetic capacity and superior fruit nutraceutical value in strawberry.

Assuming that little and inconsistent information is available about the effects of light downconversion films on strawberry plants, the present research aimed to investigate the effects of three distinct light downconversion films (UV-to-blue, UV/green-to-blue/red, and green-to-red light shifting features) on the physiological features of strawberry leaves, as well as their impact on fruit secondary metabolites accumulation. Our hypothesis is based on the assumption that the selective enrichment with red and blue light may enhance the photosynthetic efficiency of strawberry leaves and, at the same time, stimulate the production of specific secondary metabolites in the fruit.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Commercial plantlets of *Fragaria* × *ananassa* cv. "Aromas" were cultivated under four tunnels (6 m × 4.15 m × 1.9 m) with two open ends located at the facilities of the Department of Agriculture, Food and Environment (DAFE), University of Pisa (43.7041371° N, 10.4270071° E). The transplantation took place on the 31 of August 2022; 5 plants were put in commercial substrate bags (X-BAG P30, Virgoplant Italia srl, Fombio, LO, Italy) with coco-fibre and perlite (60:40; *v/v*) under each tunnel, and the experiment lasted 3 months. The automated drip irrigation was scheduled 3 times per day with a nutrient solution containing the following nutrient concentrations: 11.0 mM NO_3^-, 0.5 mM NH_4^+, 1.6 mM $H_2PO_4^-$, 5.7 mM K^+, 5.0 mM Ca^{2+}, 1.7 mM Mg^{2+}, 2.5 mM Na^+, 2.9 mM SO_4^{2-}, 2.8 Cl^-, 18.0 mM Fe^{2+}, 19.0 mM B, 0.8 mM Cu^{2+}, 8.0 mM Zn^{2+}, 15.0 mM Mn^{2+}, and 0.5 mM MoO_4^{2-} Water chemical adjustments were carried out periodically to maintain 1.9 mS cm^{-1} for electrical conductivity (EC) and 5.5 for pH values.

A total of 60 strawberry plants were split into 3 treatments (20 plants per treatment), involving the utilization of 150 µm thick polyethylene films manufactured and provided by CASCADE SAS (Clamart, France) along with 20 plants subjected to a control colourless polyethylene film (Cnt; without the fluorescent dye formulation). The treatments were: blue film (blue; shift of UV into blue radiation), red film (red; shift of green into red radiation), light red film (pink; shift of UV and green into blue and red radiation, respectively but to a lesser extent than the red film). A spectroradiometer (SpectraPen, Photon Systems Instruments, Drásov, Czech Republic) was used to measure the light spectra distribution under each light downconversion film compared to the control film.

On the field, non-destructive analyses were focused on leaf physiological traits through gas exchanges and chlorophyll *a* (chl *a*) fluorescence measurements. Leaf physiological analysis started after 25 days of plant acclimation under the agricultural films (T1; Figure 1); thereafter, the same analyses were carried out around every 20 days for a total of 3 times (T2, T3 and T4; Figure 1). Ambient daily minimum and maximum temperatures during the trial period are reported in Figure 1.

Strawberry fruits were harvested at the commercial stage, ensuring that fruit from different treatments had visually reached the desired ripening level of 75–90% maximum redness. Fruit harvest started at the end of October and was prolonged until the end of November. For biochemical investigations on the fruit, fruit samples were stored at −80 °C using liquid nitrogen and then cryogenically ground for homogenous replicates.

2.2. Plant and Leaf Morphological Parameters

At the end of the experiment, five plants were randomly selected from each treatment for morphological measurements [plant fresh and dry weight, leaf thickness, leaf area and leaf mass per area (LMA)]. Plant fresh weight (n = 5) was obtained by weighing the whole aerial part of the plant composed by leaf, stem, and collar. Then, the aerial part samples were put in a ventilated oven (Memmert GmbH Co., KG Universal Oven UN30, Schwabach, Germany) at 80 °C on the first day, followed by 105 °C on the second day until a constant weight was reached for the determination of the dry weight. Leaf thickness and foliar area measurements (n = 20) (4 leaves for each biological replicate) were performed using High Precision Digital Thickness Gauge and ImageJ software (version 1.52t). LMA was

evaluated utilising one leaf per biological replicate (n = 5), calculated as the ratio of leaf dry weight to leaf area, and expressed as g m^{-2}.

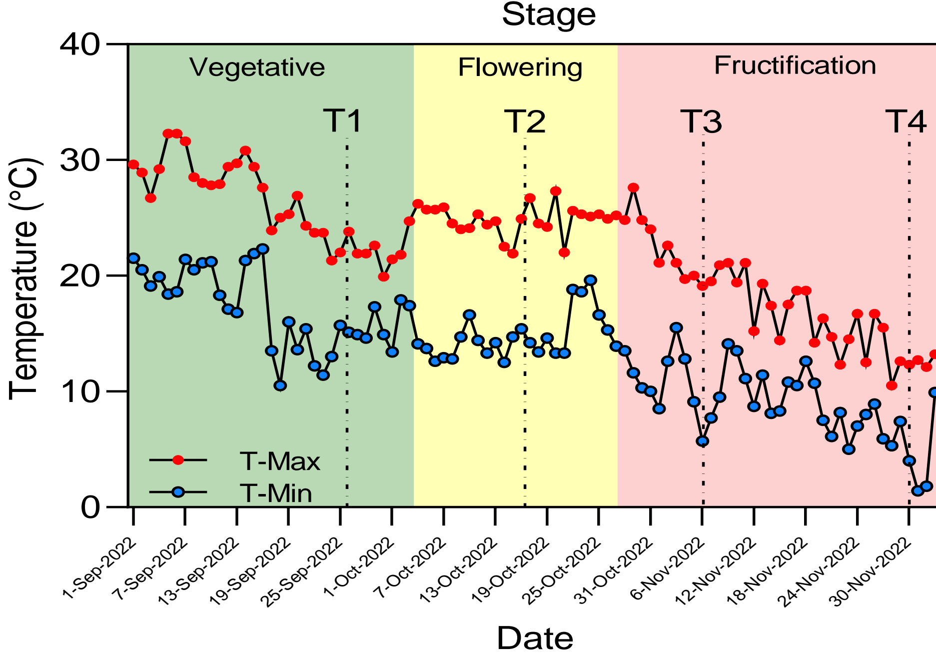

Figure 1. Ambient daily minimum and maximum temperatures during the trial period with corresponding dates of non-destructive data analysis times (T1: vegetative stage; T2: flowering stage; T3: fruit stage; T4: fruit stage).

2.3. Gas Exchange and Chl a Fluorescence Analysis

Gas exchange measurements (n = 10) were performed using a portable infrared gas analyser LI-6800 system (Li-Cor, Lincoln, NE, USA) on randomly selected fully expanded leaves from 11:00 a.m. to 1:00 p.m. at a light intensity of 1500 µmol photons m^{-2} s^{-1}. Using the CO_2 mixer, the CO_2 concentration inside the leaf chamber was set at 400 µmol mol^{-1}, and the flow rate was 500 µmol s^{-1}. Once the steady state was reached, the following parameters were recorded: net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and apparent carboxylation efficiency (P_n/C_i).

Chl *a* fluorescence parameters were measured (n = 6) on 20 min dark-adapted leaves using a portable fluorometer (Plant Efficiency Analyzer–Handy PEA, Hansatech Ltd., Norfolk, UK). Samples were flashed for 1 s with a saturated (2700 µmol m^{-2} s^{-1}) red light emitting diodes (LED) light pulse (650 nm). Plant photosynthetic performance was assessed using the maximum quantum efficiency of Photosystem II (PSII), i.e., F_v/F_m, where F_v represents the difference between the maximal (F_m) and minimal (F_0) fluorescence of a dark-adapted sample, F_m is the maximum Chl *a* fluorescence recorded after a saturating light pulse, and F_0 is the basal fluorescence before a saturation pulse, extrapolated from the line of best fit determined through the initial data point recorded at the onset of illumination.

2.4. Phenolic Extraction for Spectrophotometric Assay

Homogenous fruit samples (stored at −80 °C) of about 0.1 g fresh material were combined with 1 mL of a solution of methanol diluted to 80% (*v/v*). The homogenates were sonicated at 4 °C with a sonicator (Digital ultrasonic Cleaner, DU-45, Argo Lab, Modena, Italy) for 30 min and then centrifuged at 4 °C with a laboratory centrifuge (MPW 260R, MWP Med. instruments, Warsaw, Poland) at 10,000 rpm for 15 min. The obtained extracts were stored at −20 °C until the analysis.

2.5. Total Phenolic Content (TPC)

The procedure presented by Dewanto et al. [29] for TPC was followed with some reagent volume modifications. Basically, 62.5 µL extract sample was mixed with 62.5 µL Folin–Ciocalteu reagent and 250 µL distilled water and kept standing for 6 min at room temperature. Then, 625 mL of Na_2CO_3 7% (*w/v*) aqueous solution was added and the mixtures were incubated for 90 min in the dark at room temperature. The absorbance at 760 nm was measured with a spectrophotometer (Ultrospec 2100 Pro, GE Healthcare Ltd., Chalfont, Buckinghamshire, UK) against a blank solution (free of fruit sample). All of the obtained results (n = 5) were compared with a gallic acid standard curve (y = 0.002x + 0.0008; R^2 = 0.9934) and were expressed as mg gallic acid equivalents per g fresh weight (FW; mg GAE g^{-1} FW).

2.6. Total Flavonoid Content (TFC)

TFC was determined following the procedure described by Silva et al. [30]. An aliquot (100 µL) of fruit extract, 30 µL of $NaNO_2$ 5% (*w/v*) aqueous solution and 400 µL distilled water were mixed. After 5 min, 30 µL of $AlCl_3$ 10% (*w/v*) aqueous solution was added for the aluminium–flavonoid complexes formation. After 6 min, 200 µL of NaOH 1 M and 240 µL of distilled water were added to the mixture. The absorbance at 510 nm was spectrophotometrically measured against a blank solution for each extract replicate (which contained all of the reagents except the $AlCl_3$ 10% (*w/v*) aqueous solution, which was replaced with distilled water). All of the obtained results (n = 5) were compared with a catechin standard curve (y = 0.0005x + 0.0465; R^2 = 0.9956) and were expressed as mg catechin equivalents per g FW (mg CAE g^{-1} FW).

2.7. Total Ascorbic Acid Content

Total ascorbic acid content was measured following the procedure described by Kampfenkel et al. [31]. An amount (0.2 g) of homogenous fresh fruit sample powder stored at −80 °C was homogenized with 6% (*w/v*) trichloroacetic acetic acid (TCA) aqueous solution and centrifuged at 10,000 rpm for 10 min at 4 °C. From each replicate supernatant, 50 µL of extract was combined with 50 µL of 10 mM (*w/v*) dithiothreitol and 100 µL of 0.2 M (*w/v*) phosphate buffer Na-P (pH 7.4) and then the mixture was placed in a water bath at 42 °C. After 15 min incubation, 50 µL 0.5% (*w/v*) *N*-ethylmaleimide, 250 µL 10% (*w/v*) TCA, 200 µL 42% (*w/v*) H_3PO_4, 200 µL 4% (*w/v*) 2,2′-dipyridil and 100 µL 3% (*w/v*) $FeCl_3$ were added to the mixture, which was incubated again at 42 °C for 40 min. The blank solution was prepared by replacing the extract with 6% TCA (*w/v*). The adsorption of the final solution was measured spectrophotometrically at 525 nm and all of the obtained results (n = 5) were expressed as mg ascorbic acid per g FW (mg ASA g^{-1} FW) using an ascorbic acid standard calibration curve (y = 0.0147x − 0.0042; R^2 = 0.9954).

2.8. Total Anthocyanin Content (TAC)

TAC was determined following the differential pH method [32]. For the sample extraction, an amount (0.2 g) of fresh fruit sample (stored at −80 °C) was homogenized with 1% (*v/v*) acidified methanol and centrifuged at 10,000 rpm for 15 min at 4 °C. For the TAC determination, 100 µL of fruit extract was diluted in 900 µL of either 0.4 M (*w/v*) sodium acetate buffer (pH 4.5) or 0.025 M (*w/v*) potassium chloride (pH 1), depending on the pH to test. After 15 min at room temperature, the absorbance at 530 and 700 nm was spectrophotometrically registered for both pH dilutions and the final absorbance (A_f), and TAC was calculated as follows:

$$A_f = (A_{530} - A_{700})_{pH1.0} - (A_{530} - A_{700})_{pH4.5}$$

$$TA = \frac{Af \times MW\ Pg3glu \times Df}{\mathcal{E} \times 1 \times Vext \times Wsample}$$

where *MW* is the molecular weight of pelargonidin 3-*O*-glucoside, *Df* is the dilution factor, 1 is the path length (in cm), ε is the molar extinction coefficient (in L mol^{-1} cm^{-1}), *Vext* is the volume extract and *Wsample* is the weight of the sample (in g).

All obtained results (n = 5) were expressed as mg pelargonidin 3-*O*-glucoside equivalent on g FW (mg Pg-3-*O*-glu g^{-1} FW).

2.9. Antioxidant Activity (AA) Assays

The AA was measured using both the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [33] and the 2,29-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test [34].

For the DPPH assay, after the preparation of the methanolic solution of 3.12 $\times$ 10^{-5} M DPPH (*w/v*), 10 µL of fruit extract was mixed with 990 µL of DPPH methanolic solution. The absorbance was spectrophotometrically determined at 515 nm against a blank solution (free of extract).

For the ABTS assay, a buffer solution (5 mM *w/v*) was prepared from Na_2PO_4 and $Na_2H_2PO_4$ to reach pH = 7.4. Firstly, 0.0192 g of 7 mM (*w/v*) ABTS and 0.0034 g of 2.5 mM (*w/v*) $K_2S_2O_8$ solution were solubilized in 5 mL of buffer solution and the mixture was stored in a dark environment. For the analysis, 50 µL of fruit extract was mixed with 950 µL of ABTS solution and the kinetics of the reaction were spectrophotometrically followed for 90 s at 734 nm.

The obtained results (n = 5) from both antioxidant activity assays were expressed as mg Trolox equivalents per g FW (mg TE g^{-1} FW).

2.10. Chemical Fingerprint by UHPLC-DAD-HR-ESI-MS Analysis

Each strawberry fruit sample was subjected to ultrasound-assisted extraction utilizing an ethanol solution 80% (*v/v*) for the extraction of phenolic compounds (solid:liquid ratio of 1:3 g mL^{-1}) and a mixture of 2% HCl in methanol (solid:liquid ratio of 1:6 g mL^{-1}) for anthocyanin recovery. The defrosted strawberry samples (1.5 g each) were placed in the corresponding amount of solvent and treated with ultrasound at 20 °C for 15 min. After centrifugation for 5 min at 3500 rpm, the supernatants were directly analysed in triplicate using ultra-high performance liquid chromatography (UHPLC) coupled to a diode array detector (DAD) and a high-resolution mass spectrometer (HR-MS).

The LC-MS system employed a Vanquish Flex Binary pump coupled with DAD and a HR-MS Q Exactive Plus Orbitrap-based FT-MS with an electrospray ionization (ESI) source and Xcalibur 4.1 software (Thermo Fisher Scientific Inc., Bremen, Germany). A volume of 2 µL of each phenolic extract and of 5 µL of anthocyanin extract were injected into the LC-MS system. All of the analyses were performed on a C-18 Kinetex® Biphenyl column (100 $\times$ 2.1 mm, 2.6 µm particle size) provided with a Security Guard™ Ultra Cartridge (Phenomenex, Bologna, Italy), eluting with a mixture of HCOOH in H_2O 0.1% (*v/v*) for solvent A and HCOOH in methanol 0.1% (*v/v*) for solvent B at a flow rate of 0.5 mL min^{-1}, using a splitting system of 1:1 to MS and DAD. For phenols, a linear gradient was applied, increasing from 5 to 80% of solvent B over a period of 32 min. In the case of anthocyanins, the solvent gradient was 10 to 35% of solvent B in 5 min. The column and autosampler temperatures were maintained at 35 and 4 °C, respectively. Data from the DAD were registered within the range of 200–600 nm. Selected channels at 254, 280, and 325 nm were utilized according to the characteristic absorbances of phenolic compounds, while for anthocyanins, the absorbance was targeted at 515 nm. The ESI interface was used in negative ion mode to analyse phenols and in positive ion mode for anthocyanins. The HR-MS spectra were acquired in a *m/z* scan range of 170–1200 for phenols and *m/z* 250–1200 for anthocyanins, operating in full (resolution 70,000 and maximum injection time of 220 ms) and data dependent-MS/MS (resolution 17,500 and maximum injection time of 60 ms). The ionization parameters were optimized as reported in Cioni et al. [35].

2.11. Statistical Analysis

After checking the normality of distribution (Shapiro–Wilk test, 95% confidence interval), one-way ANOVA was performed on the plant morphological and fruit nutraceutical compounds data using the type of film as the factor of variation. A two-way ANOVA was carried out on the physiological data (gas exchange and chlorophyll fluorescence parameters) using the type of film and time as factors of variation. Significant differences among treatments were determined by the LSD Fisher *post hoc* test ($p \leq 0.05$). GraphPad software (v.10, GraphPad, La Jolla, CA, USA) was used for the statistical analysis.

3. Results

3.1. Light-Converting Properties of Polyethene Films

The blue film significantly increased the intensity of the blue light spectrum, which falls in the range of 420–480 nm, by a noticeable 8.4% when compared to the Cnt film (Figure 2). On the other hand, the red film primarily boosted the red light spectrum, encompassing wavelengths from 600–700 nm, showing an increase of 12.6% in comparison to the Cnt film. Interestingly, the pink film demonstrated enhancements in both the red and blue light spectra, registering increases of 4 and 5%, respectively, over the Cnt film. However, despite these differences in specific spectra, it is important to note that the PAR, which spans from 400–700 nm, remained consistent across all of the treatments, hovering around approximately 1200 μmol $m^{-2}s^{-1}$ on a full sunny day. Furthermore, through a comparison with PAR natural sunlight, all of the films exhibited an irradiance that fell short of the sun's irradiance, which measured at around 1500 μmol $m^{-2}s^{-1}$ (Figure 2).

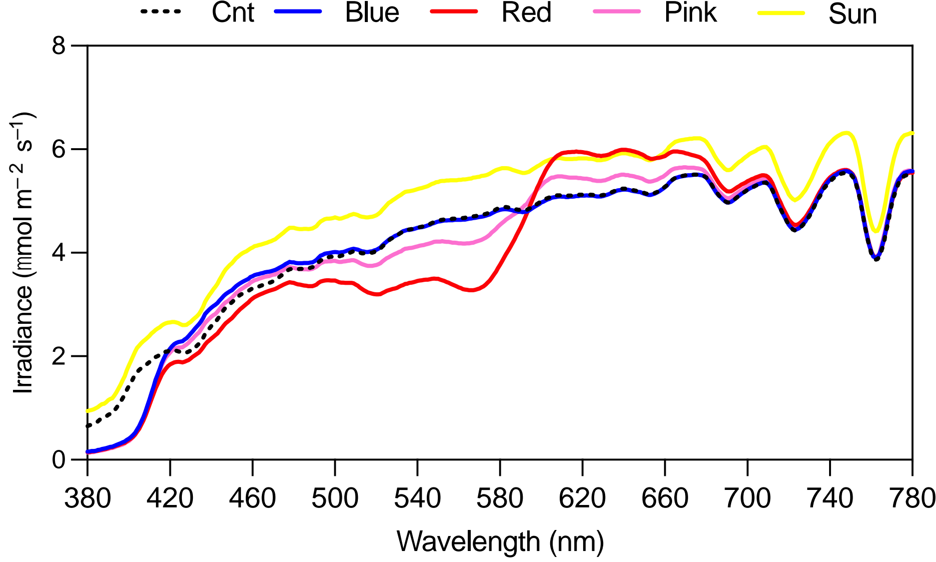

Figure 2. Effect of control (Cnt), blue, red, and pink light downconversion films used in the experiment on the transmitted wavelengths of sunlight inside the tunnel.

3.2. Plant Morphological Parameters

The plants cultivated under the light downconversion films experienced a notable increase in both fresh and dry weights, showing an uplift of +40 and +34%, respectively, when compared to those grown under the Cnt film, as evidenced by the data presented in Table 1. Strawberry plants under blue and red films showed a greater leaf area (+25 and 31%, respectively) than those under Cnt film (Table 1). Delving deeper into the values, the blue film exhibited particularly impressive results. The plants grown under the blue film had higher values of leaf thickness and LMA (10 and 15%, respectively) when compared to the plants grown under the Cnt film.

Table 1. Plant fresh and dry weight, leaf area, leaf thickness, and leaf mass area (LMA) of *Fragaria × ananassa* plants grown under control (Cnt; no fluorescence), blue, red, and pink light downconversion films.

Variable [1]	Cnt	Blue	Red	Pink
Plant fresh weight (g plant^{-1})	71.98 ± 23.17b	100.10 ± 11.99a	105.60 ± 6.04a	96.67 ± 8.61a
Plant dry weight (g plant^{-1})	16.50 ± 4.43b	22.08 ± 2.52a	21.97 ± 2.62a	22.30 ± 3.02a
Leaf area (cm^2)	50.87 ± 10.69b	63.40 ± 13.19a	66.52 ± 11.93a	53.00 ± 9.810b
Leaf thickness (µm)	247.2 ± 26.8b	271.7 ± 35.80a	233.5 ± 23.00b	242.9 ± 29.70b
LMA (g m^{-2})	70.09 ± 3.19b	80.56 ± 5.06a	63.39 ± 5.74b	69.98 ± 10.39b

[1] Each value is the mean ± SD of 5 replicates for plant fresh, dry weight and leaf mass area measurements, mean ± SD of 20 replicates for leaf morphological parameters (leaf area and leaf thickness). Means were subjected to one-way ANOVA with type of film as the source of variations. Means with different letters are significantly different at $p \leq 0.05$ for Fisher's least significant difference *post hoc* test.

3.3. Gas Exchange and Chl a Fluorescence Analysis

The analysis of gas exchanges during different phenological stages indicated that plants under the blue film had superior net CO_2 assimilation performances than Cnt, with an average increase of 16% throughout all stages. The blue film promoted an enhancement of P_n, closely followed by the red film, which reported a 15% average increase. The pink film demonstrated a comparable P_n improvement (+16%; Figure 3a) only during the final two stages (T3 and T4).

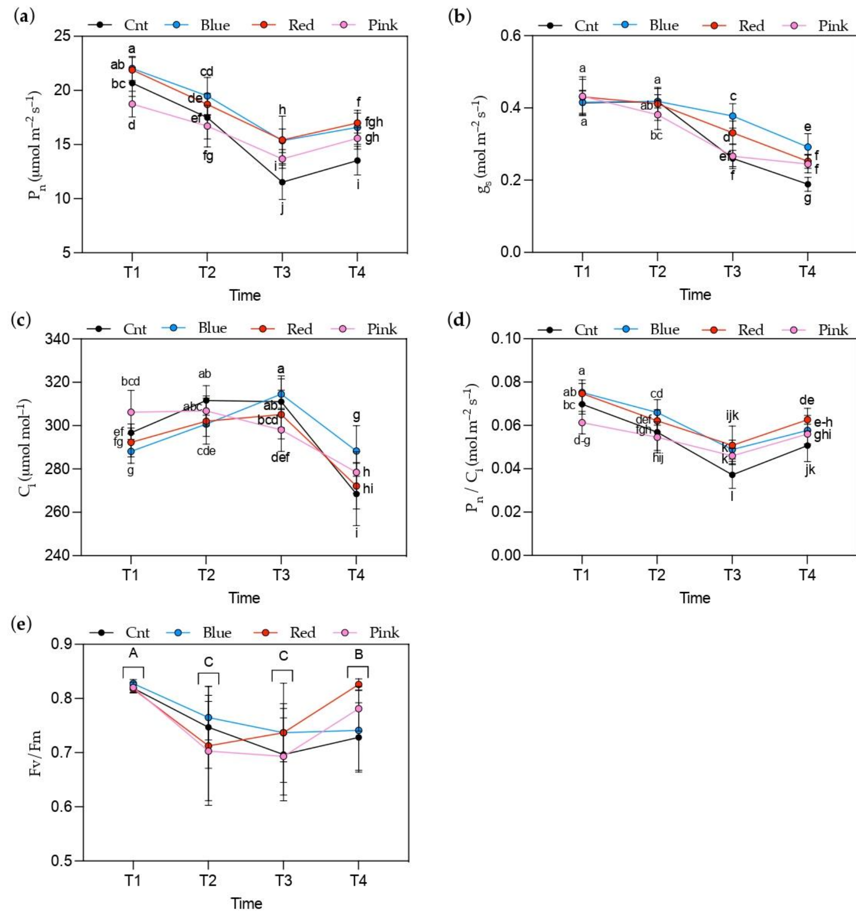

Figure 3. Net photosynthesis (P_n; (**a**)), stomatal conductance (g_s; (**b**)), intercellular CO_2 concentration (C_i; (**c**)), apparent carboxylation efficiency (P_n/C_i; (**d**)) and photosystem II maximum photochemical efficiency (F_v/F_m; (**e**)) of *Fragaria × ananassa* plants grown under control (Cnt; no fluorescence), blue,

red, and pink light downconversion films, measured at vegetative (T1), flowering (T2), fructification (T3 and T4) stages. Means were subjected to two-way ANOVA with the type of film and sampling time as the source of variations. Means with different letters are significantly different at $p \leq 0.05$ with Fisher's least significant difference *post hoc* test. When the F ratio of the interaction between the variability factors was not significant, the letters indicate statistically significant differences between means over time.

During T3, all treatments exhibited the lowest P_n values, but the differences reported above were maintained. The g_s declined in accordance with the plant phenological stage. From T2 onwards, the highest g_s value was observed in plants under the blue film (Figure 3b). At T3, plants under the blue and red films displayed statistically significant differences compared to those under the Cnt film, with improvements of 45 and 27%, respectively (Figure 3b). At T4, all of the light downconversion films showed higher g_s values than the Cnt film (+54, +34 and +29% for blue, red and pink compared to the Cnt film, respectively; Figure 3b). At T1, the highest C_i values were observed in plants under the pink film, showing a 3% increase compared to the Cnt, whereas the blue film led to a 3% reduction (Figure 3c). At T2, both the red and blue films showed approximately 3% lower C_i values compared to the Cnt. However, by T4, the blue film led to the highest C_i values, with a 7% increase relative to the Cnt. The P_n/C_i patterns mirrored the P_n trends. Throughout the experiment, plants under the blue and red films consistently recorded higher P_n/C_i values than those under the Cnt film, averaging increases of 15 and 16%, respectively (Figure 3d). The lowest P_n/C_i values were observed across all treatments at T3. Starting from T3 to T4, plants grown under the pink film again showed higher values of P_n/C_i than the Cnt plants (+16% on average).

At the beginning of the experiment, during the vegetative stage (T1), strawberry leaves under all films showed the highest F_v/F_m (Figure 3e) when compared with the other growth stages (flowering and fructification). During the flowering (T2) and the fructification (T3) stages, leaves from all treatments showed a significant decrease of F_v/F_m, with a subsequent increase at the end of the fructification stage, independent of the treatment.

3.4. Fruit Nutraceutical Features

The fruit from plants grown under the blue film showed the highest content in TPC (+38% than the Cnt fruit; Figure 4a), even though no significant differences were reported between fruits harvested under blue and Cnt film. Moreover, fruits from plants grown under Cnt film yielded similar TPC to those grown under the red and pink films.

The TFC was significantly higher in fruits under the blue film (+127% than the Cnt fruit) than in those under the Cnt film or the rest of the light downconversion films (Figure 4b). Fruits under the red film had lower TFC in comparison with those under the Cnt film but similar to those harvested under the pink film. For TAC, the pink film fruits exhibited the lowest value in comparison to the rest of the films (−43% compared to the Cnt fruit; Figure 4c). However, the light downconversion films did not positively affect the total ascorbic acid content of strawberry fruit (−36, −21 and −36% for fruit grown under blue, red, and pink films, respectively, compared with the Cnt fruit; Figure 4d). No significant differences were observed in the antioxidant activity between the DPPH and ABTS assays (Figure 4e,f).

The chemical composition of strawberry fruit subjected to light downconversion films was elucidated through the application of the highly sensitive UHPLC-DAD-HR-ESI-MS technique. The LC-MS obtained chromatograms for phenolic and anthocyanin profiles are reported in Figures 5 and 6, respectively.

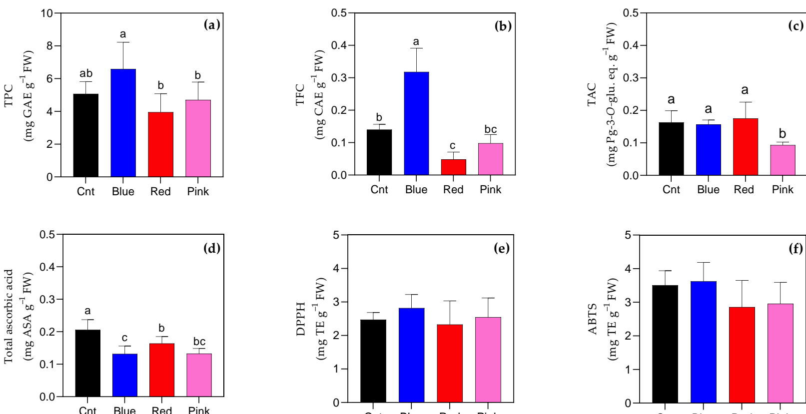

Figure 4. Total phenolic (**a**), flavonoid (**b**), anthocyanin (**c**), ascorbic acid (**d**) content and antioxidant activity detected with DPPH (**e**) and ABTS (**f**) assays of *Fragaria* × *ananassa* fruit from plants grown under control (Cnt; no fluorescence), blue, red and pink light downconversion films. Means were subjected to one-way ANOVA with the type of film as the source of variation. Means (n = 5 ± SD) with different letters are significantly different at $p \leq 0.05$ for Fisher's least significant difference *post hoc* test.

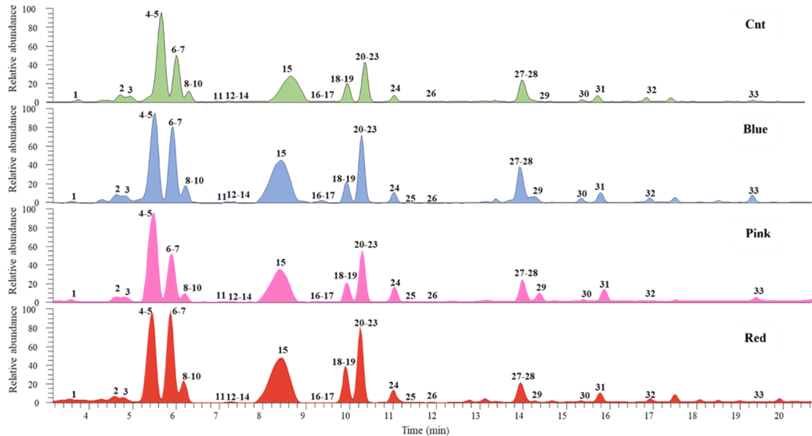

Figure 5. Phenolic UHPLC-DAD-HR-ESI-MS profile recorded in negative ion mode of *Fragaria* × *ananassa* fruit from plants grown under control (Cnt; no fluorescence), blue, red and pink light downconversion films. Peak data are shown in Table 2.

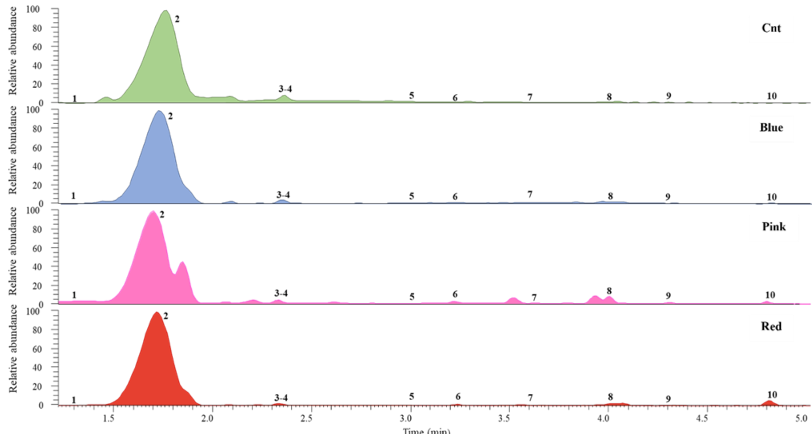

Figure 6. Anthocyanin UHPLC-DAD-HR-ESI-MS profile recorded in positive ion mode of *Fragaria* × *ananassa* fruit from plants grown under control (Cnt; no fluorescence), blue, red and pink light downconversion films. Peak data are shown in Table 3.

Table 2. Retention time (t_R) and mass spectral data of phenols tentatively identified in *Fragaria × ananassa* fruit from plants grown under control (Cnt; no fluorescence), blue, red and pink light downconversion films.

N. [1]	t_R	[M-H]⁻	Product Ions	Error ppm	Formula	Compound	Peak Area × 10⁶			
							Cnt	Blue	Red	Pink
						Phenols				
2	4.6	299.0779	137.02	+2.21	$C_{13}H_{16}O_8$	Hydroxybenzoic acid hexoside	74.16 ± 13.14ab	78.92 ± 13.71a	49.98 ± 16.17b	72.12 ± 9.44ab
12	7.2	447.1506 ([M+HCOO]⁻)	401.14, 269.10, 161.04, 113.01, 101.02	−0.44	$C_{18}H_{26}O_{10}$	Icariside F2	48.17 ± 12.20	55.72 ± 14.15	50.59 ± 10.09	39.41 ± 20.70
23	11.0	317.1030	271.06, 164.01, 137.02	+0.18	$C_{17}H_{18}O_6$	Hydroxybenzoic acid derivative	57.30 ± 5.06b	86.83 ± 17.51a	73.93 ± 10.10ab	94.80 ± 14.89a
						Flavonoids				
14	8.4	431.0982	269.04	+0.39	$C_{21}H_{20}O_{10}$	Apigenin glucoside	744.32 ± 185.72	764.70 ± 20.33	745.19 ± 131.46	562.73 ± 92.57
19	10.2	449.1089	287.04, 269.04, 243.06	−0.09	$C_{21}H_{22}O_{11}$	Dihydrokaempferol glucoside	340.26 ± 102.53	364.15 ± 15.29	364.61 ± 80.15	274.11 ± 55.75
21	10.5	595.1692	448.82, 287.06, 269.04	+0.60	$C_{27}H_{32}O_{15}$	Dihydrokaempferol rhamnosylglucoside	21.80 ± 5.75	21.24 ± 3.61	18.56 ± 4.52	14.36 ± 2.23
24	11.4	463.0519	300.99	+0.19	$C_{21}H_{18}O_{13}$	Quercetin 3-*O*-glucoside	18.99 ± 6.68b	28.86 ± 6.23a	14.29 ± 2.03b	14.24 ± 2.70b
28	14.4	477.0673	301.03	+2.34	$C_{21}H_{20}O_{11}$	Quercetin 3-*O*-glucuronide	32.43 ± 4.26	47.49 ± 9.13	34.75 ± 11.90	44.38 ± 16.47
29	15.4	447.0932	285.04	−0.20	$C_{21}H_{18}O_{12}$	Kaempferol 3-*O*-glucoside	45.07 ± 19.12	33.53 ± 10.49	26.30 ± 1.29	28.47 ± 2.60
30	15.9	461.0725	285.04	−0.11	$C_{23}H_{22}O_{12}$	Kaempferol 3-*O*-glucuronide	80.63 ± 15.60	76.93 ± 8.92	60.86 ± 15.86	68.08 ± 9.98
31	16.7	489.1038	327.05, 285.04, 284.03, 255.03	−0.10	$C_{24}H_{22}O_{14}$	Kaempferol acetyl hexoside	9.86 ± 1.41	10.02 ± 3.17	7.56 ± 1.24	7.31 ± 1.77
32	16.8	533.0937	285.04	+0.04	$C_{30}H_{26}O_{13}$	Kaempferol *O*-malonyl-O-hexoside	21.60 ± 3.22ab	23.63 ± 3.73a	18.00 ± 3.31ab	16.18 ± 3.19b
33	19.4	593.1301	447.09, 285.04	+0.07	$C_{21}H_{20}O_{10}$	Kaempferol 3-*O*-(6-*O*-p-coumaroylglucoside)	44.66 ± 29.45	34.53 ± 28.03	24.12 ± 8.70	42.62 ± 12.96
						Ellagitannins				
1	3.5	783.0695	300.99	+1.08	$C_{34}H_{24}O_{22}$	Pedunculagin	12.85 ± 4.02b	19.55 ± 2.41a	10.15 ± 1.53b	12.74 ± 3.06b
7	6.0	633.0735	633.07, 300.99	+0.25	$C_{27}H_{22}O_{18}$	Strictinin	24.85 ± 8.03b	38.14 ± 9.41a	20.39 ± 4.30b	21.54 ± 4.09b
15	9.3	467.0359 ([M-H]²⁻)	633.07, 391.03, 300.99	+0.96	$C_{41}H_{28}O_{26}$	Galloyl-diHHDP-glucose (casuarictin)	51.16 ± 2.81ab	64.79 ± 28.00a	35.29 ± 8.77b	30.38 ± 7.98b
22	10.6	466.0264 ([M-H]²⁻)	466.03, 300.99	−0.51	$C_{41}H_{26}O_{26}$	Castalgin	16.66 ± 3.22b	31.20 ± 12.03a	15.92 ± 1.74b	18.14 ± 1.49b
25	11.9	934.0717 ([M-H]²⁻)	1567.14, 935.07, 783.06, 633.07, 300.99	−0.07	$C_{82}H_{54}O_{52}$	Digalloyl-tetraHHDP-diglucose/Sanguin H-6 isomer	0.31 ± 0.16b	28.02 ± 3.43a	2.64 ± 0.88b	27.89 ± 10.99b
						Ellagic acid conjugates				
26	13.9	447.0566	300.99, 299.99	−0.67	$C_{20}H_{16}O_{12}$	Ellagic acid deoxyhexose	163.72 ± 43.63b	263.61 ± 21.79a	149.52 ± 1.18b	154.12 ± 8.41b
27	14.0	300.9988	257.00	−0.63	$C_{14}H_6O_8$	Ellagic acid	83.07 ± 16.34b	126.32 ± 12.16a	76.39 ± 6.47b	80.92 ± 11.86b

Table 2. *Cont.*

N. [1]	t_R	[M-H]$^-$	Product Ions	Error ppm	Formula	Compound	Peak Area × 10^6			
							Cnt	**Blue**	**Red**	**Pink**
Cinnamic acid conjugates										
5	5.9	325.0929	187.04, 163.04, 145.03	+0.03	$C_{15}H_{18}O_8$	p-Coumaric acid glucoside (isomer I)	364.40 ± 56.53ab	454.59 ± 46.69a	381.59 ± 83.21ab	325.10 ± 65.62b
10	6.3	325.0929	187.04, 163.04, 145.03	+0.03	$C_{15}H_{18}O_8$	p-Coumaric acid glucoside (isomer II)	93.3 ± 24.90ab	130.52 ± 11.49a	126.00 ± 22.09a	73.18 ± 27.26b
17	9.9	355.1034 ([M+HCOO]$^-$)	309.10, 207.05, 147.04	+0.38	$C_{15}H_{18}O_7$	Cinnamoyl glucose	140.63 ± 10.31	109.04 ± 85.33	142.97 ± 37.28	123.94 ± 21.05
18	10.1	487.1456 ([M+HCOO]$^-$)	441.14, 147.04	+0.18	$C_{21}H_{28}O_{13}$	Cinnamoyl xylosylglucose	14.92 ± 0.47a	14.50 ± 2.68a	13.38 ± 3.56a	8.81 ± 0.61b
Catechin and proanthocyanidins										
3	4.8	577.1353	451.10, 425.09, 289.07, 125.02	+0.26	$C_{30}H_{26}O_{12}$	Procyanidin dimer (isomer I)	97.31 ± 18.82ab	122.85 ± 10.79a	90.41 ± 11.29b	123.75 ± 16.62a
4	5.3	577.1353	451.10, 425.09, 289.07, 125.02	+0.26	$C_{30}H_{26}O_{12}$	Procyanidin dimer (isomer II)	90.55 ± 28.42ab	123.04 ± 2.72a	64.29 ± 1.29b	67.35 ± 21.07b
5	5.5	289.0718	289.07, 245.08, 109.03	+0.14	$C_{15}H_{14}O_6$	Catechin	414.32 ± 327.94	649.95 ± 58.15	526.28 ± 18.90	618.72 ± 118.12
8	6.2	561.1408	435.11, 289.07, 271.06	+1.00	$C_{30}H_{26}O_{11}$	Propelargonidin dimer (isomer I)	12.27 ± 2.03b	16.59 ± 2.94a	14.43 ± 0.33ab	13.58 ± 2.05ab
9	6.3	865.1998	577.14, 407.08, 289.07, 125.02	+0.30	$C_{45}H_{38}O_{18}$	Procyanidin trimer	34.23 ± 10.83ab	46.01 ± 2.43a	25.44 ± 3.48ab	22.13 ± 12.73b
11	6.9	561.1408	435.11, 289.07, 271.06	+1.00	$C_{30}H_{26}O_{11}$	Propelargonidin dimer (isomer II)	15.04 ± 2.26b	20.68 ± 1.60a	14.33 ± 1.34b	16.78 ± 2.86ab
12	7.2	720.1595 ([M-H]$^{2-}$)	863.17, 575.11, 407.08, 289.07, 125.02	+0.67	$C_{75}H_{62}O_{30}$	Procyanidin pentamer	11.60 ± 1.42b	27.64 ± 2.36a	12.31 ± 1.33b	15.71 ± 3.48b
13	7.4	576.1276 ([M-H]$^{2-}$)	407.08, 289.07, 125.02	+0.31	$C_{60}H_{50}O_{24}$	Procyanidin tetramer	4.19 ± 0.70b	8.09 ± 0.83a	3.65 ± 0.78b	6.28 ± 2.74ab
16	9.4	865.1998	577.14, 407.08, 289.07, 125.02	+0.30	$C_{45}H_{38}O_{18}$	Proanthocyanidin C1 (catechin trimer)	22.16 ± 3.15	25.78 ± 2.21	19.68 ± 4.09	21.62 ± 4.52
20	10.3	864.1915 ([M-H]$^{2-}$)	407.08, 289.07, 125.02	+0.91	$C_{90}H_{74}O_{36}$	Procyanidin hexamer	2.47 ± 0.44c	11.37 ± 1.99a	1.93 ± 0.34c	6.13 ± 1.79b

[1] Means (n = 3 ± SD) with letters are significantly different after one-way ANOVA followed by Fisher's LSD *post hoc* test (p = 0.05) considering the type of film as the source of variability.

Table 3. Retention time (t_R) and mass spectral data of anthocyanins tentatively identified in *Fragaria* × *ananassa* fruit from plants grown under control (Cnt; no fluorescence), blue, red and pink light downconversion films.

N. [1]	t_R	[M-H]$^-$	Product Ions	Error ppm	Formula	Compound	Peak Area ×10^6			
							Cnt	Blue	Red	Pink
1	1.4	579.1496	433.11, 271.06	−0.17	$C_{30}H_{27}O_{12}$	Pelargonidin *p*-coumaroylhexoside (isomer I)	30.32 ± 7.98a	20.91 ± 5.01ab	15.30 ± 0.80b	14.87 ± 3.85b
2	1.7	433.1130	271.06	+0.18	$C_{21}H_{21}O_{10}$	Pelargonidin 3-*O*-glucoside	1641.74 ± 187.24a	971.00 ± 106.53bc	1241.09 ± 183.71b	684.79 ± 170.04c
3	2.3	519.1133	271.06	−0.04	$C_{24}H_{23}O_{13}$	Pelargonidin malonylglucoside	2.07 ± 0.40a	1.36 ± 0.54a	1.34 ± 0.39ab	0.52 ± 0.42b
4	2.6	579.1496	433.11, 271.06	−0.17	$C_{30}H_{27}O_{12}$	Pelargonidin *p*-coumaroylhexoside (isomer II)	10.49 ± 6.00	6.56 ± 1.35	4.52 ± 0.26	5.90 ± 1.89
5	3.0	533.1292	271.06	+0.43	$C_{25}H_{25}O_{13}$	Pelargonidin derivative	2.18 ± 0.1a	1.58 ± 0.65ab	1.55 ± 0.64ab	0.90 ± 0.68b
6	3.2	449.1077	287.05	−0.31	$C_{21}H_{21}O_{11}$	Cyanidin 3-*O*-glucoside	12.30 ± 0.95a	8.83 ± 1.98ab	10.00 ± 2.92ab	7.85 ± 1.17b
7	3.6	535.1083	287.05	+0.13	$C_{24}H_{23}O_{14}$	Cyanidin malonylglucoside	3.71 ± 0.44a	2.70 ± 0.61ab	2.53 ± 0.83b	1.60 ± 0.56b
8	4.0	477.1026	287.05	−0.31	$C_{22}H_{21}O_{12}$	Cyanidin derivative	16.56 ± 2.48a	12.00 ± 1.62ab	10.82 ± 4.03b	13.81 ± 2.00ab
9	4.3	549.1241	287.05	+0.40	$C_{25}H_{25}O_{14}$	Cyanidin derivative	5.59 ± 1.35	5.80 ± 0.76	4.62 ± 0.72	4.71 ± 1.17
10	4.8	595.1444	287.05	−0.37	$C_{30}H_{27}O_{13}$	Cyanidin *p*-coumaroylhexoside	6.99 ± 2.48	8.12 ± 3.85	15.75 ± 8.82	6.56 ± 2.12

[1] Means (n = 3 ± SD) with letters are significantly different after one-way ANOVA followed by Fisher's LSD *post hoc* test (p = 0.05) considering the type of film as the source of variability.

All phenolic and anthocyanin compounds were tentatively identified by comparison of their elution order, molecular formula, and both full and fragmentation mass spectra (Tables 2 and 3) with data reported in the literature [6,35–38]. A mass error < 5 ppm was considered for compound annotation. All analysed strawberry samples exhibited a highly comparable composition, showing a chemical fingerprint that was almost superimposable. In total, 43 phenolic compounds were attributed as constituents of strawberry fruit, according to previously reported studies. The identified substances belong to different classes, such as simple phenols (hydroxybenzoic acid derivatives and icariside F2), cinnamic acid derivatives (coumaric and cinnamic acid glycosides), flavonoids (mainly flavonols as kaempferol and quercetin glycosides), anthocyanins (pelargonidin and cyanidin glycosides), ellagic acid and its glycoside, ellagitannins, and catechin together with proanthocyanidins (in the form of dimers, trimers, tetramers, and larger molecules). Among the anthocyanins, pelargonidin hexoside was the most represented compound in all of the samples. Although the presence of strawberry metabolites was confirmed in all studied samples by LC-MS metabolomic analysis, variation in their amount was observed among fruit from plants under different light downconversion films. The change in the concentration of each metabolite throughout the treatments was estimated by relative quantification integrating the peak areas in the chromatograms obtained by LC-MS experiments (Tables 2 and 3). Based on the results, blue, red, and pink downconversion films did not show benefit on anthocyanins production compared to the Cnt film. Indeed, a slight but significant decrease in their level was observed (Table 3), particularly in fruit from plants exposed to the pink film (isomer I of pelargonidin *p*-coumaroylhexoside, pelargonidin 3-*O*-glucoside, pelargonidin malonylglucoside, pelargonidin derivative, cyanidin 3-*O*-glucoside, and cyanidin malonylglucoside) and exposed to the red film (isomer I of pelargonidin *p*-coumaroylhexoside, pelargonidin 3-*O*-glucoside, cyanidin malonylglucoside and a cyanidine derivative). On the contrary, fruit from plants grown under the blue film showed a slight but significant increase in the content of almost all of the other phenolic compounds (hydroxybenzoic acid derivative, quercetin 3-*O*-glucoside, pedunculagin, strictinin, castalgin, digalloyl-tetraHHDP-diglucose, ellagic acid and ellagic acid deoxyhexose, propelargonidin dimer, procyanidin pentamer, tetramer and hexamer) compared to fruit from plants under the Cnt film, even though the use of light downconversion films did not seem to dramatically promote any class of metabolites or, inside each group, any individual phenolic molecules. Anyway, the compounds wholly influenced were found to be ellagic acid conjugates and ellagic tannins. In the plants subjected to red or pink films, no significant differences were observed for most of the phenolic metabolites content when compared with those found in the Cnt fruit, except for cinnamoyl xylosylglucose, the content of which was significantly lower in fruit from plants under the pink film when compared with the Cnt fruit. Interestingly, the proportion of the main metabolites in the strawberry samples was not altered by the application of the light downconversion films.

4. Discussion

4.1. Strawberry Plant Growth Was Boosted by Light Downconversion Films, Especially by the UV-to-Blue Shifting One

Light plays a pivotal role in modulating plant developmental processes, strongly influencing photomorphogenesis and photosynthesis. This modulation arises from a complex network of photoreceptors such as cryptochromes for blue light and phytochromes (Pfr) for the red/far-red (R:FR) light ratio, acting as primary molecular channels, translating light quality to photomorphogenesis signals as reviewed by Landi et al. [4]. There is a growing interest in the cost-effective manipulation of the solar spectrum transmitted by plastic film by enhancing the blue and red light, which is able to enhance the ability of plants to use the film converted light as a "eustress" to induce an accumulation of bioactive compounds in fruit [7]. Indeed, recent advances have introduced polyethylene films with solar spectrum downconversion technology for tunnel crop applications. This innovative technology employs fluorescent agents (dyes, organic and inorganic rare-earth complexes)

that are adept at converting less photosynthetically active wavelengths, such as green or UV radiation, to more effective radiation, such as red and blue [3,20,21,23].

In our experiment, strawberry plants exhibited enhanced vegetative growth (both aerial biomass and leaf area) with all of the light downconversion films tested herein. Several studies have assessed the effect of downconversion films that promote red light on plant biomass and nutraceutical characteristics [22,23,39,40]. For instance, Li et al. [39] demonstrated that light downconversion film, by shifting green–yellow light wavebands into orange–red light wavebands, significantly enhanced the crop yield of leafy vegetables, especially in haze-prone regions like North China, by addressing weak light challenges and boosting photosynthetically active radiation. Similarly, Shen et al. [22] achieved promising results in enhancing lettuce yield by converting green light into more active red light. Other reports include those of Hebert et al. [23] and Parrish et al. [40], who utilized quantum dots in greenhouse films to shift UV/blue photons to red emissions to modify the sunlight spectrum, resulting in improved plant growth parameters in tomato (+9% of vine growth) and lettuce plants (+11% of fresh biomass and +13% leaf area).

Several factors could contribute to the enhanced development of plants exposed to films that convert different light wavelengths into red light. According to our results, some studies have reported increased photosynthetic rates in various plants, including strawberries, pepper, and lettuce, when grown under light downconversion films that shift blue or green wavelengths into red and far-red regions [3,41,42]. However, the pink, red and blue films used in the present experiment did not influence the activity of PSII since its photochemical efficiency in light downconversion was not statistically different from those of plants under Cnt film at each plant stage according to the findings of Khramov et al. [42]. Therefore, the observed variations in carbon assimilation rates are likely attributable to other intrinsic factors that influence photosynthesis. In our experiment using pink and red films, the observed increased plant biomass could not just be related to the higher percentage of available red light for photosynthesis. It is conceivable that a higher R:FR light ratio increased the active form of Pfr due to the greater quantity of red light emitted by these two films compared to the Cnt film. Elevated Pfr levels can lead to increased CO_2 assimilation rates, promoting plant growth and resilience [42,43] and explaining the less pronounced decrease in gas exchange parameters such as P_n and P_n/C_i rates during colder periods at both fruiting stages.

Unfortunately, there is a lack of research into the impacts of UV-to-blue light conversion films on plant physiology and development, with only a few reports [28,44]. In these cases, it was observed that blue film can exert a stimulating growth effect on crop species. For example, Hemming et al. [28] reported an increase in fruit production by 11% in strawberry plants grown under UV-to-blue light downconversion film, while Guerrero-Gonzalez et al. [44] reported increased growth rate indices of about 125% in seedlings of Ipomea compared to controls. In our experiment, the increase in the blue light fluence under the blue film improved plant photosynthetic processes, as underscored by the very elevated photosynthetic rate values with respect to controls in all of the analysed plant stages. This increase in photosynthetic performances resulted in increased LMA since more C was potentially available for the vegetative structures with respect to other films [45]. Notably, a higher blue light fraction can induce the development of sun-adapted leaves with high LMA, thickness and photosynthetic capacity [46,47]. The elevated photosynthetic rates in plants grown under the blue film were attributed to both leaf biochemistry, as indicated by higher P_n/C_i values, and enhanced stomatal conductance, which remained higher compared to the Cnt film at certain plant stages (T3 and T4). Indeed, it is worth noting that Pfr can also respond in plants subjected to blue light wavelengths, even though in these plants, the conversion to Pfr is less effective than in plants subjected to red light [4].

Additionally, in cucumber seedling leaves grown under pure blue light, Su et al. [48] observed an increase in the Rubisco biosynthesis and related gene expression that, together with a higher stomatal conductance, improved the photosynthetic process compared to other monochromatic lights. The higher stomatal conductance exhibited by plants grown

under the blue film (compared to the others) was more evident during the fruiting stage, coinciding with a significant temperature drop. This phenomenon was directly linked to the properties of the additional blue light in stimulating stomatal opening, mediated by the blue light absorbing carotenoid zeaxanthin [4].

Although research on plant physiological responses to blue film remains limited, blue light exposure has shown remarkable effects on plant physiology, including increased photosynthetic efficiency and positive influences on leaf morphology. These findings emphasize the significance of light spectrum manipulation as a promising approach to optimizing plant growth and resilience across different agricultural contexts. Our observations are in agreement with the work of Hemming et al. [28], where fluorescent pigments in films emitting blue spectrum fluorescence have potential to positively affect the growth and development of strawberry plants.

4.2. UV-to-Blue Downconversion Film Enhances the Accumulation of Polyphenols in Strawberry Fruit

The current literature lacks any information concerning the accumulation of secondary metabolites in fruit from crops cultivated under the influence of light downconversion films. This conspicuous absence of data underscores the need for clarification and investigation in this specific domain. To clarify how light downconversion films affect the accumulation of secondary metabolites in fruit, findings related to the use of LED monochromatic lights, an aspect that has been extensively studied [4,49,50], will be considered to discuss our results.

Anthocyanins are of pivotal importance in strawberries because, besides their powerful antioxidant prerogatives [51], they are responsible for the bright fruit colour, thereby driving consumer selection of the fruit [52]. It is reported in the literature that short and high-energetic wavelengths, such as UV and blue, are the most efficient for stimulating the increase in fruit anthocyanin contents [53,54]. However, there is also evidence that longer wavelengths, such as red, can enhance the intensity of fruit redness [6,53]. Indeed, in a previous work with monochromatic supplemental LED lighting, red light has proven to be the most efficient in improving the accumulation of anthocyanins in strawberry fruit [6]. The possible discrepancies between our results and others from studies using LED monochromatic light can probably be attributed to the different growing conditions and then to the different light quality and quantity.

In the present study, blue film induced a higher TPC and TFC in strawberry fruit than Cnt, red and pink films. Indeed, blue film was the most promising light downconversion film for increasing the phenolic compounds in strawberry fruit when compared with Cnt film, with a specific effect on hydroxycinnamic acids (hydroxybenzoic acid derivative), flavonoids (quercetin 3-*O*-glucoside), and most of the ellagitannins, ellagic acid conjugates and condensed tannins identified in the present experiment. In particular, the contents of quercetin 3-*O*-glucoside and ellagic acid conjugates increased by more than 50% in fruit grown under blue film compared to Cnt fruit.

Ellagic acid and quercetin 3-*O*-glucoside are powerful antioxidant molecules with many biological effects, including antioxidant, anti-inflammatory, antidiabetic, cardioprotective, and neuroprotective effects [55,56], so their increase in fruit grown under the blue film is interesting for human health. To the best of our knowledge, no research has been conducted on the effect of blue light on ellagic acid content. However, it was reported for *Triticum aestivum* [57], *Camellia sinensis* [58] and *Pisum sativum* [59] that there was an increase in gallic acid content, a precursor of ellagic acid, in plants grown under blue light. Furthermore, the observed increase in flavonoids, and especially quercetin 3-*O*-glucoside, can be due to the ability of the blue light waveband to increase the activity of shikimate dehydrogenase and the transcript levels of genes responsible for phenylalanine ammonia-lyase (PAL) and flavonoid-3′-hydroxylase (F3′H) [60,61].

While blue light enrichment has been shown to positively impact the accumulation of TPC and TFC by stimulating the phenylpropanoid metabolism, it is important to note that the use of all light downconversion films led to reduced levels of ascorbic acid (vitamin C)

in fruit. Despite the decrease in ascorbic acid, the antioxidant activity of the strawberries grown under all of the light downconversion films remained comparable to that of Cnt fruit, suggesting the compensatory effect of other ROS-scavenging molecules differentially stimulated by downconversion films. Ascorbic acid plays a key role in plants, acting mainly as an antioxidant in the response and adaptation to environmental stressors such as UV radiation [62]. Previous studies have shown that exposure to UV radiation promoted the increase in ascorbic acid content in vegetative and fruit tissues [63–65]. Conversely, UV depletion resulted in reduced levels of ascorbic acid [66]. Therefore, we suggest that the observed reductions in the ascorbic acid could be due to the higher limitation of UV light transmission (Figure 2) to plants grown under light downconversion films compared to Cnt.

5. Conclusions

Light downconversion films are promising for improving protected cultivation. The common theme throughout this study is the potential to modify light conditions to optimize plant photosynthesis and growth and, at the same time, to enhance the ability of plants to use the converted light as a "eustress" to induce an accumulation of antioxidant and healthy compounds in fruit. In this regard, plants developed under a blue film (UV-to-blue light conversion) showed the highest vegetative growth, with an enhanced leaf area, thickness and mass area and an elevated net photosynthetic rate associated with higher apparent carboxylation efficiency values. At the same time, the use of the blue film induced an increase in total phenolic and flavonoid contents in fruit and promoted the stimulation of targeted health-related polyphenols. Therefore, the use of a pre-harvest blue film resulted in an efficient solution to enhance the nutraceutical properties of fruit from tunnel crops, maintaining their productivity and physiological plant status. These innovative downconversion films, which combine traditional agricultural practices with cutting-edge technologies, might play a vital role in ensuring food quality and security and sustainable agricultural practices. Further research might focus on a deeper investigation into the organoleptic quality induced by the use of these films to assess consumer appreciation of the horticultural products grown under these conditions.

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