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Light Down-Conversion Technology Improves Vegetative Growth, Berry Production, and Postharvest Quality in Tunnel-Cultivated Blueberry

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Abstract

This study examined three innovative 'light-converting films' that convert green light (−23%) into red light (+8%; Red film), ultraviolet light (−80%) into blue light (+9%; Blue film), and green light (−5.7%) into red light (+4%; Pink film) but also ultraviolet light (−76%) into blue light (+5.6%; Pink film). These films were used for growing blueberry plants under cover under controlled tunnel conditions (27.3 ± 11.7 °C, 51.9 ± 21.6% RH). The use of Red film led to increases in the total plant biomass (+54.2%), and Red and Pink films enhanced the leaf thickness (+17.1% and +14.4%, respectively) as compared to the control (a transparent polyethylene film). No differences in the photosynthetic rate (P_n) were observed at the flowering stage, but a decrease (−25.9%) was observed in plants grown under the Pink film during the green fruit stage. The plants grown under Blue film boosted flower production, leading to +86.8% increase in the total yield. The Blue film improved the total phenolic content (+15.2%) in the fruit, and a +25.3% greater total antioxidant capacity was observed in fruit grown under Pink film. Freshly harvested blueberries were subjected to postharvest experiments (4 °C; in dark conditions; 90–95% RH). The results suggest the importance of Red film in enhancing plant biomass and Red and Blue films in improving fruit yield and maintaining nutraceutical postharvest quality in blueberry fruit.

Keywords: *Vaccinium corymbosum*; luminescent down-conversion films; photoselective cultivation; controlled environment horticulture; fruit anthocyanins

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1. Introduction

Blueberries (*Vaccinium* spp.) are recognized by the Food and Agriculture Organization of the United Nations as one of the healthiest foods for human consumption [1,2]. Including blueberries in the human diet alongside other fruits and vegetables has been recommended due to their high antioxidant content, with recognized health benefits [3]. Blueberries are also recognized for their organoleptic properties such as fruit color, juiciness, flavor, and texture [4,5]. Most blueberry bioactive compounds belong to the chemical class of flavonoids and phenolic acids, which may have preventive effects against heart disease, diabetes, and neurodegeneration [6–8]. Among flavonoids, anthocyanins are the main polyphenols in blueberries that are responsible for their vivid blue-purple color, being mostly synthesized in the cytoplasm and then transported into the vacuole of fruit cells [5,9].

According to Krishna et al. [8], popular species of blueberries include the wild lowbush blueberry (*Vaccinium angustifolium*), the cultivated highbush blueberry (*V. corymbosum*), and the rabbiteye blueberry (*V. virgatum*, formerly known as *V. ashei*). It is widely anticipated that blueberry fruit sales and production will naturally increase in the near future, driven by the development and selection of new cultivars based on their bioactive properties [10].

In general, blueberry plant production is typically influenced by genetic background, growing conditions, and harvest time [11]. Light is the primary environmental factor influencing the performance of photosynthesis, morphogenesis, and receptor-mediated physiological/biochemical processes [12], thereby affecting plant growth and development. A distinct effect of light was observed on blueberry varieties when subjected to artificial light or to natural sunlight, as cultivar specific responses are reported [13,14]. For instance, higher fruit weight, larger fruit diameter, higher soluble solids content (SSC), and anthocyanin accumulation were observed in 'Blueray' blueberry fruit harvested from plants grown under artificial light (0–504 μmol m^{-2} s^{-1} Photosynthetic Photon Flux Density; PPFD) when compared with fruit from plants grown under natural sunlight (0–1000 μmol m^{-2} s^{-1} PPFD). Conversely, the 'Misty' blueberry fruit had a lower weight and diameter under artificial light but accounted for a higher SSC and anthocyanin yield [13]. During critical plant phenological stages such as flowering and fruit production, moderate light irradiance is necessary for blueberry photosynthetic performance in order to avoid photosynthetic saturation due to stomatal and biochemical limitations. In addition, in a dynamic light environment, photosynthesis was further limited by a slow stomatal response to increasing light [14]. In addition to the light intensity, the light quality is also an important factor affecting blueberry plant growth and fruit quality [15–17]. For example, 100% red light (660–665) by Light-Emitting Diode (LED), thanks to the role of red-light photoreceptors named phytochromes, increased the new shoot length of blueberry cultivar 'Misty' compared to those grown under white LED light, while 100% blue light, thanks to the role of phototropins and cryptochromes, resulted in increased leaf chlorophyll and anthocyanin contents compared to white- and 100% red-light treatments [16]. These responses are mediated by specific photoreceptors: phytochromes detecting red/far-red ratios crucial for stem elongation and flowering, cryptochromes responding to blue light for chlorophyll synthesis and stomatal regulation, and phototropins governing directional growth responses [18–22]. Indeed, in literature, the roles of phytochromes are strictly correlated with light cascade pathways related to the expression of genes of the anthocyanin pathway (i.e., chalcone synthase) but also with shade avoidance syndrome, reducing chlorophyll biosynthesis [18,21,22]. Moreover, cryptochromes and phototropins are correlated to the chloroplast movement, stomatal opening, and photoperiodic flowering (cryptochrome 2) [20]. Cryptochrome 1 present in the cytosol is responsible for cotyledon expansion, anthocyanin production, and also greening of the chloroplast [20]. These light cascade response pathways influence plants at the photomorphogenic level, inducing shoot elongation under red light and the inhibition of shoot elongation under blue light [18].

In recent years, small fruit production in plastic tunnels has gained popularity, as they help to reduce external input and extend the growing season of specialty crops such as blueberries [23]. As blueberries have specific growing requirements under covered tunnels that provide uniformity in growth and shield against environmental factors [8]. Protected cultivation systems offer precise environmental control but introduce challenges in light transmission and spectrum modification. Passive spectrum conversion technologies represent a potentially economical approach to light quality management [24].

In tunnel houses, the coverings can also be an instrument to modify incident sunlight quality perceived by plants and fruit, inducing changes in plant physiological responses and fruit quality [14,23,25]. In this context, photoluminescent spectrum-converting films are a newly developed technology able to increase blue and red light reaching the plant. The light down-conversion technology, patented by Light Cascade® (LC®; Clamart, France), allows for the absorbance of UV (300–390 nm) and green wavelengths (520–565 nm), re-emitting into blue (400–500 nm) and red (600–700 nm) wavelengths, respectively [26]. In particular, the Red film converts green light (−23%) into red light (+8%), the Blue film converts ultraviolet light (−80%) into blue light (+9%), and the Pink film converts green light (−5.7%) into red light (+4%) but also ultraviolet light (−76%) into blue light (+5.6%; Pink film). Recent research has demonstrated the advantages of light spectrum modulation using LC® films in improving crop production and fruit quality. Light down-conversion efficiency, measured as the ratio of converted photons to absorbed photons, typically ranges from 60 to 90% depending on the phosphor or quantum dot material used [21]. Energy losses through the Stokes shift must be considered when evaluating the net photosynthetic benefit of spectrum conversion technologies. The agents in agricultural polymer films include fluorescent dyes and rare-earth complexes, which convert sunlight into specific wavelengths that promote plant growth and photochemical processes [21]. Unlike light selective films, light conversion films are more effective in modulating light intensity, as they convert specific spectrum ranges to longer wavelengths instead of merely absorbing light, as is the case with selective films [22]. Strawberry plants grown under the LC® Blue film had 10% increased leaf thickness, 15% higher leaf mass area (LMA), and enhanced photosynthesis as compared to the standard control film [24]. Additionally, stimulation of flowers production in raspberries under the Pink LC® film (+19%) led to a 16% increase in marketable fruit yield [26]. Converting UV to red light is equally important and has shown positive effects on the chlorophyll fluorescence parameters in both strawberries and eggplant [27]. Despite physiological changes in strawberry var. ‘Albion’ under red-colored film—such as reduced photosynthetic rates and unchanged chlorophyll content—fruit productivity remained unaffected [28].

Despite extensive research on LED lighting effects in berries, the specific impacts of passive spectrum conversion on the intricate relationships between plant development, fruit biochemistry, and postharvest stability remain unexplored in *V. corymbosum* production systems. Indeed, photoluminescent spectrum-converting films exploit radiation already present in the solar spectrum (without supplementation as in the use of LED lights) and without the selection of specific wavelengths as in photoselective netting because they are able to convert light wavebands not useful for the photosynthetic process of plants but naturally present in the solar radiation into wavebands of photosynthetically useful radiation. From a postharvest perspective, fruit appearance, flavor, and texture stability during storage are major quality characteristics that influence consumer satisfaction and the retailers’ needs [11]. According to Gorzelany et al. [3], fresh blueberry fruit has a shelf life of 10 to 18 days when stored at the recommended temperature and humidity, but the fruit’s nutraceutical quality can potentially change during the storage. Xie et al. [10] analyzed fruit phenolic compounds and antioxidant activities of four rabbiteye blueberry cultivars at five ripening stages and observed that the total phenolic content, total flavonoid concentration, and antioxidant activity of all the cultivars increased nonlinearly with ripening. The preharvest application of light modulation is thoroughly documented in the existing literature, yet its impact on postharvest quality remains largely underexplored [11,29]. Fresh blueberries are particularly vulnerable to decay during storage, and specific doses of light application may help to mitigate postharvest changes in essential fruit quality parameters, including reduced weight loss, enhanced firmness, and consistent skin color.

Overall, the optimal organoleptic characteristics of blueberries are typically observed after six weeks of storage [11].

To date, researchers have extensively explored the nutraceutical value of blueberry fruit's changes during the berries' ripening [10,20,29,30]. However, little information is available in literature about the modulatory effects of preharvest light conditions on the nutraceutical profile and antioxidant activity during fruit storage [10]. The aim of the present research was, therefore, to evaluate the effects of three innovative LC® down-conversion films on blueberry plant growth and development, fruit production, and quality in a field experiment. Additionally, it assessed the shelf life and the nutraceutical quality of fruit from plants grown under the LC® photoluminescent spectrum-converting films during cold storage.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The study was carried out during the 2024 growing season from March to July in the experimental site of the Department of Agriculture, Food and Environment (DAFE) at the University of Pisa (43.7041371° N, 10.4270071° E). The selected 2-year-old blueberry plants (*V. corymbosum* L.) of the 'Duke' variety were purchased at vegetative bud burst stage from Vivai Veimaro (Cossato, Biella, Italy). The plants were grown in a commercial peat and selected for experiments at the woody-stem stage. The transplantation took place in March 2024 in 12-L pots filled with a commercial ready mixture of acidic substrate with a blond peat base (Professional TYP 5, Vialca srl, Uzzano, Pistoia, Italy; pH 4.5; electrical conductivity EC = 0.35 dS m^{-1}). In addition, during the transplantation, the pot substrate was mixed with slow-release granulated fertilizer for acidic plants, KB osmocote (Pet&Green Srl, Perugia, Italy), at a dose of 30 g $plant^{-1}$, and the acidity corrector sulfosprint®, composed of 87% sulfur (Delta, Verona, Italy). The substrate pH and EC were measured once a week and refreshed when the solution exceeded 10% of the initial values. The pots were covered at the stem base with pine bark to help protect the superficial rhizosphere moisture. The plants were regularly irrigated with tap water, using drip irrigation and adapting the irrigation time and frequency to the plant phenological stage. The pH of the tap water was adjusted to 5.0–5.5 with diluted sulfuric acid. The EC was 0.81 dS m^{-1}. Both the pH and EC of the irrigating water were checked once a week.

During the trial period (n = 100 days), the relative humidity and the average temperature were monitored every 30 min, using a data logger (Tinytag Ultra 2—TGU-4500, Gemini Data Loggers Ltd., Chichester, UK) installed under one of the four films, in the middle of the tunnel, reporting the following data: 27.3 ± 11.7 °C average temperature and 51.9 ± 21.6% average relative humidity. The temperature and relative humidity were not affected by the covered film since the tunnels were open and in equilibrium with the environmental temperature and relative humidity.

The 40 newly transplanted blueberry plants (10 per treatment) were positioned in 4 tunnels with two open ends (6 m × 4.15 m × 1.9 m; L × W × H) in a hoop structure shape. Under each tunnel, the plants were positioned in two rows with an interspacing of 3 m and intra-space of 30 cm between the pots. The experimental setup and details of the tunnels are reported in Figure 1.

Three types of polyethylene photoluminescent spectrum-converting films designed by LC® were used to cover the tunnels. All films were 150 µm thick and had the following characteristics:

- Blue film (Blue; shift of UV (300 nm–390 nm) to blue light radiation (420 nm–495 nm);
- Red film (Red; shift of green (520 nm–560 nm) to red light radiation (600 nm–700 nm);

- Pink film (Light Red; shift of UV (300 nm–390 nm) and green (520 nm–560 nm) to blue (420 nm–495 nm) and red light radiation (600 nm–700nm), respectively, but with a lesser extent than Red film).

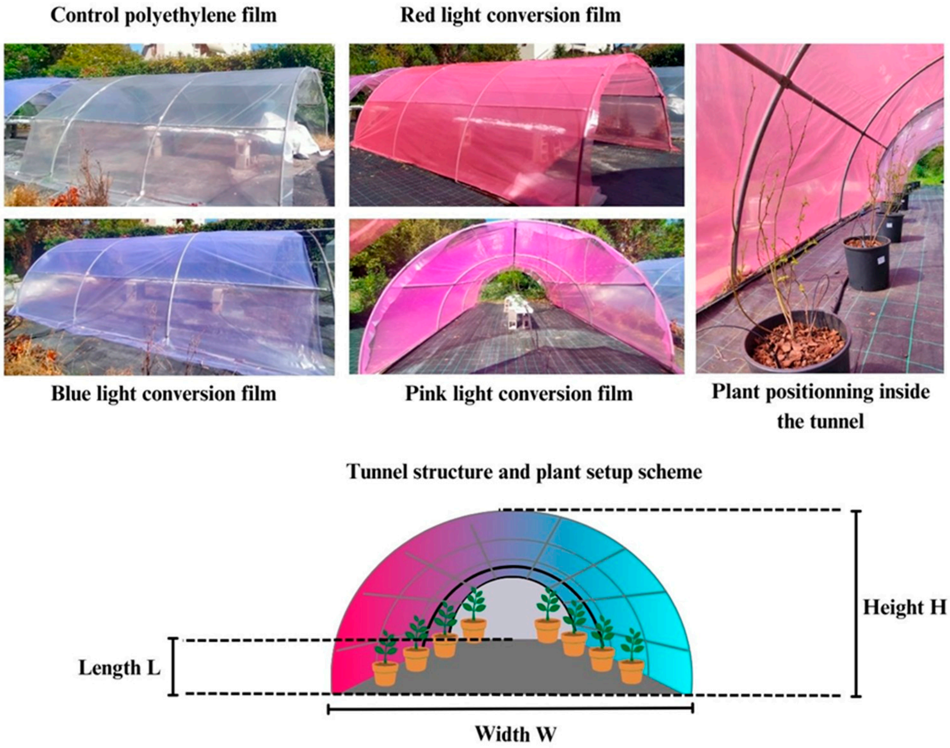

Figure 1. The experimental setup and details of the tunnels.

As a control (Cnt), a fourth tunnel was covered with a transparent polyethylene film, which simulated the optical properties of the LC® conversion films adopted by Light Cascade before the incorporation of the fluorescent chromophore, which allows for light conversion. The light spectrum of each photoluminescent spectrum-converting film (Supplementary Materials, Figure S1) was measured by the spectroradiometer (Ocean HR Series: HR2 Spectrometer, Ocean Optics, FL, USA) to identify the sunlight spectrum modulation, as reported by El Horri et al. [25]. The differential light transmittance relative to the control indicated spectral conversion, as shown in Supplementary Figure S2. In average, the Blue and Pink films allowed for an increase of 8.9% and 5.6%, respectively, in blue light. The Red and Pink films boosted red light by 7.9% and 3.9%, respectively. In terms of absorption, the ultraviolet light range was reduced in all three light conversion films, with an average of 77%. In contrast, green light was absorbed exclusively by the Red and Pink films by 23% and 5.7%, respectively. In comparison to sunlight, the control allows for a decrease of 64% of abundant UV light and a decrease of an average of 15.39% of the blue, green, and red lights.

Light intensity within the tunnel structure perceived by plants was hovering around approximately 1020 μmol m^{-2} s^{-1} on a full sunny day. The experimental trial lasted in total 108 days after transplanting (DAT), and the plants' development timeline is detailed in the Supplementary Materials, Figure S3.

2.2. Plant Biomass, Macro-Morphological Measurements, and Fruit Yield

During the experimental period, plants subjected to different treatments were monitored to follow the main phenological phases. At the flowering stage, the flower number per plant (B; n = 5) was assessed, and at the harvest time, the fruit number per plant (A;

n = 5) and single fruit weight (n = 5); the total fruit production (n = 4) was determined as the sum of fruit weights per plant. The fruit set rate (n = 5) was calculated as:

$$\text{Fruit set rate (\%)} = (\text{A}/\text{B}) \times 100$$

At the end of the experiment, the number of leafy secondary branches (new shoots), was recorded and measured in length and diameter (n = 3) using a digital caliper. The shoot diameter (mm) was measured at the middle portion of the shoots. At the full flowering stage, the number of flowers per plant was counted (n = 5), whereas the rest of the parameters, such as the total leaf weight per plant (kg plant^{-1}; n = 3), total plant biomass consisting of leaves and shoots (kg plant^{-1}; n = 3), leaf area (cm^2; n = 10), and leaf thickness (µm; n = 10), were measured. Fully expanded leaves were subjected to morphological measures. Leaf thickness was recorded using a high precision digital thickness gauge (Shenzhen Yidier Technology, Shenzhen, China) by measuring the leaf's middle part and avoiding the mid-ribs; the leaf area was obtained by first scanning single leaves (mature leaves) and then using ImageJ software (version 1.52t, Bethesda, MD, USA) for leaf area measurement.

Fully expanded leaves (n = 4) from the control and treated plants were sampled to measure the dry matter percentage (DM). Fresh vegetative material was first weighted with the tare weight and then placed at 105 °C until a constant weight was reached in a ventilated oven (Memmert GmbH Co., KG Universal Oven UN30, Schwabach, Germany). The DM percentage was calculated using the following equation:

$$\text{DM (\%)} = (\text{DW}/\text{fresh weight}) \times 100$$

2.3. Micro-Morphological Measurements of Leaf: Leaf Anatomical Observations

To assess the micro-morphological characteristics of leaves, anatomical observations were carried out to evaluate the influence of LC® films on leaf tissue. After 83 DAT, the expanded mature leaf samples with an approximate age of 53 days were taken in the morning at 11:00 a.m. from the upper shoot layer of the plants. For each treatment, two-centimeter leaf segments from the median leaf surface (the leaf part targeted for leaf gas exchange analysis) were fixed in 1:1:8 *v*/*v* Formalin-Aceto-Alcohol (FAA) solution containing 10% formaldehyde, 5% glacial acetic acid, and 45% ethyl alcohol. Then, the samples were dehydrated in graded ethanol series and embedded in histoplast following the standard paraffin procedures [31]. Toluidine blue (0.01% *v*/*v*) was used to stain the leaf transverse sections (≈12 µm) obtained using a microtome (Shandon AS325 Manual Rotary Microtome, Model 0325R, Thermo Fisher Scientific, Cheshire, UK). Leaf sections were analyzed under an optical microscope (Carl Zeiss Axio Imager 2, Oberkochen, Germany).

Anatomical characteristics were determined from the digitized images by the measurements of leaf components' thickness (upper epidermis, palisade parenchyma, spongy parenchyma, and lower epidermis). Sections (n = 5), obtained from three leaf samples, were treated as units and were analyzed using ZEISS ZEN 3.7 software (associated with the optical microscope). The thickness of each component in the leaf sections (A) was measured, and the percentage of each component was then calculated as:

$$\text{Leaf component thickness (\%)} = (\text{A}/\text{total leaf thickness}) \times 100$$

The sum of all leaf component thickness was also calculated (n = 5) to obtain leaf thickness data from an anatomical point of view.

2.4. Gas Exchange and Pigment Analyses

Non-destructive measurements were taken on fully grown leaves during the flowering and shoot growth stage (t1: 35 DAT) and during the green fruit stage (t2: 55 DAT). Gas exchange was carried out on the fully developed leaves. Net photosynthesis (P_n), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were assessed from 11:00 a.m. to 1:00 p.m. on randomly chosen leaves (n = 4) using a LI-6400 portable infrared gas analyzer (Li-Cor, Lincoln, NE, USA) under sunlight. A CO_2 mixer was utilized to provide a CO_2 concentration of 400 µmol mol^{-1} with a flow rate of 500 µmol s^{-1}.

Pigment measurements were conducted using the Dualex® scientific sensor (Force-A, Orsay, France) for each treatment, with 40 measurements taken on the upper leaf side at various sampling times while avoiding the mid-ribs. The device can detect chlorophyll content (Chl) in the leaf epidermis based on the fluorescence emitted by Chl molecules when excited by the light source provided by the instrument clip.

2.5. Postharvest Storage: Fruit Shape, Weight, Organoleptic Properties, and Nutraceutical Quality

To examine the effect of the preharvest use of photoluminescent spectrum-converting films on blueberry fruit production, harvesting was carried out one week after the skin reached a blue-purple color. Non-destructive measurements of the fruit shape (length and diameter) were taken. Since the length was found to be similar among harvested fruit from different treatments, the fruit was graded into four classes on the basis of the fruit diameter (Table 1).

Table 1. Blueberry fruit classification classes postharvest based on fruit diameter.

Class Number	**1**	**2**	**3**	**4**
Fruit diameter (cm)	2.1–2.4	1.7–2.0	1.3–1.6	1–1.2

For each fruit harvest time equivalent to the three fruit production peaks (n = 3), the fruit number per class was counted, and the percentage was determined using the following equation:

$$\text{Fruit class percentage (\%)} = (\text{fruit number per class}/\text{total number of fruits per harvest time}) \times 100$$

Blueberries harvested during the second fruit harvest time were moved to the laboratory in cold conditions within 2 h after the harvest. The total harvested fruits were separated into two sub-groups for each treatment; one group was frozen in liquid nitrogen, labeled as T0 samples, and stored at −80 °C for further biochemical analyses.

The other group was used for the cold storage experiment. Briefly, 50 homogenous fruits (class 3, Table 1) were stored in commercial PET boxes (14.3 × 9.6 × 2.5 cm) purchased from Imballagi360 (Urbania, Pesaro-Urbino, Italy). The samples were randomly split into four boxes (technical replicate) and subjected to refrigeration in a cooling cell (4 °C; in dark conditions; 90–95% HR). A data logger 'EL-USB-1-LCD' (Lascar electronics, Whiteparish, UK) was placed closed to fruit boxes to record any changes in storing humidity and temperature. The postharvest experiment timeline is described in the Supplementary Materials, Figure S4.

2.5.1. Postharvest Fruit Weight

The average weight of blueberry fruit was measured at T0 and during cold storage at specific intervals T1 (6 days after harvest; DAH), T2 (14 DAH), and T3 (20 DAH). The weight of each box (n = 3) was measured before fruit sampling, and the average fruit weight per box was determined using the following equation:

Fruit weight at each postharvest time (g) = (Total fruit weight per box)/(Total fruit number per box)

2.5.2. Fruit Dry Matter, Soluble Solids Content, and Titratable Acidity

At T0 and at each postharvest time (T1, T2, and T3), the fruit was homogenized, sampled, and stored at −20 °C for fruit dry matter (DM) determination (n = 3), soluble solids content (SSC) (n = 6), and titratable acidity (TA) (n = 3) analysis.

At the end of each postharvest time, fruit samples (n = 3 per treatment) were used for the DM percentage measurement, as described above for leaves. TA was measured using 1.5 g of frozen fruit puree mixed with 30 mL of distilled water and analyzed with 0.1 M NaOH until reaching pH 8.1 with a pH meter (XS Instruments, Modena, Italy). Recorded values were expressed as g citric acid per 100 g^{-1} of fresh weight (FW). SSC was assessed using a digital refractometer (ATC, Polsinelli srl, San Giuliano Terme, Italy) and reported in °Brix.

2.5.3. Fruit Nutraceutical Quality

For biochemical analyses, sampled fruit from each postharvest time was used for total phenol, total anthocyanin, and antioxidant activity investigations. Technical replicates were considered as each fruit box. For a representative fruit sample of T0, T1, T2, and T3 postharvest times, around 10 blueberries were cryogenically homogenized using liquid nitrogen to create uniform samples and ultimately stored at −80 °C until analysis.

Extraction of Total Phenolic Content (TPC)

About 0.1 g of frozen sample were mixed with an 80% (*v*/*v*) methanolic solution to extract the phenolic compounds. The homogenates were put into an ultrasonic bath (Digital ultrasonic Cleaner, DU-45, Argo Lab, Modena, Italy) at a temperature of 4 °C for 30 min and then centrifuged in a cooled centrifuge (MPW 260R, MWP Med. instruments, Warsaw, Poland) at 10,000× *g* for 10 min. The supernatant represented the extract for assaying the total phenolic content and antioxidant activity.

TPC Assay

Dewanto et al.'s [32] protocol was used for TPC measurement, with minor modifications. Briefly, 62.5 µL of fruit extract, 62.5 µL of Folin–Ciocalteu reagent, along with 250 µL of distilled water were mixed and incubated at room temperature for 6 min. After introducing 625 µL of a 7% (*w*/*v*) Na_2CO_3 water solution, the mixture was incubated at room temperature for 90 min in the dark. Using a spectrophotometer (Ultrospec 2100 Pro, GE Healthcare Ltd., Chalfont, Buckinghamshire, UK), the absorbance at 760 nm was assessed and compared with a blank solution lacking fruit extract. The obtained results (n = 4) were reported as mg gallic acid equivalents per g of FW (mg GAE g^{-1} FW).

Total Anthocyanin Content (TAC) Assay

Fruit TAC was evaluated following Giusti and Wrolstad's [33] methodology. A fresh fruit sample (0.1 g) was homogenized using 1% (*v*/*v*) acidified methanolic solution and centrifuged at 10,000× *g* and 4 °C for 15 min to obtain a supernatant to utilize as fruit extract. Then, 100 µL of fruit extract was added to two separate solutions: (i) 900 µL of 0.4 M sodium acetate buffer with a pH of 4.5 and (ii) 900 µL of 0.025 M potassium chloride with a pH of 1. The two combinations were incubated at room temperature for 15 min. The absorbance at 530 and 700 nm was spectrophotometrically measured for both pH homogenates. The calculation for the final absorbance (Af) and for the TAC measurement was performed using the following equations:

$$\text{Af} = (\text{A530} - \text{A700})\text{pH1} - (\text{A530} - \text{A700})\text{pH4}$$

$$\text{TAC} = (\text{Af} \times \text{MW} \times \text{Df})/(\text{E} \times 1 \times \text{Vext} \times \text{Wsample})$$

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

The results (n = 4) were expressed as mg cyanidin 3-glucoside equivalents per g FW (mg cyd-3-glu g^{-1} FW).

Antioxidant Activity

The antioxidant activity of blueberry fruit was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, following Brand-Williams et al.'s [34] methodology and using the same extract utilized for the TPC assay. Briefly, 10 µL of each fruit extract was mixed with 990 µL of 3.12×10^{-5} M DPPH methanolic solution, and 1 mL of DPPH methanolic solution served as the blank solution. At 515 nm, the absorbance was measured spectrophotometrically in comparison to the blank solution. The achieved results (n = 4) were presented as mg of Trolox equivalents (TE) per g FW (mg TE g^{-1} FW).

2.6. Statistical Analysis

Statistical analyses were conducted using GraphPad 8 software (GraphPad, La Jolla, CA, USA). The study involved the evaluation of plant biometric measurements, non-destructive leaf assessments, fruit yield, and quality, as well as the content of bioactive compounds and antioxidant activity. The normality of distribution was verified using the Shapiro–Wilk test with a 95% confidence interval. Percentage data were subjected to arcsine root square transformation.

Then, one-way ANOVA was employed to compare data with the type of film serving as the factor of variation. To assess the effects of film treatment and plant growing time, their interaction on physiological data of gas exchange, and pigment content, a two-way ANOVA was performed. Regarding the fruit yield and quality of fresh blueberries (T0), one-way ANOVA was utilized to examine the impact of LC® films on the fruit parameters. Additionally, for the postharvest experiment, a one-way ANOVA was carried out to evaluate the effects of each film on fruit quality monitoring at T1, T2, and T3. In all ANOVA analyses, to identify differences among means, the least significant difference (LSD) Fisher *post hoc* test was conducted with a confidence level of $p \leq 0.05$.

3. Results

3.1. Plant Biomass and Morphological Measurements

In terms of vegetative growth characteristics, 'Duke' variety blueberry plants grew well under tunnel conditions. However, some distinct differences in growth traits were observed among plants under the three LC® films and the control (Table 2).

The Red film resulted in a higher plant biomass along with significantly higher total leaf weight compared to the control. In addition, plants grown under the Red film showed a significantly lower leaf dry matter than the plants grown under the Cnt film, whereas no differences were found in plants grown under the other two films with respect to the control (Table 2). Specifically, +118.18% of biomass, composed by 0.24 kg plant^{-1} of new shoots and new leaves, was produced during the plant growth under the Red film tunnel in comparison with the control. The number of new shoots was +54% significantly higher in blueberry plants grown under Red film, characterized by (+20%) longer shoots and (+27%) higher shoot diameter compared with the controls. Even the Pink film induced a significant increase in the number of new shoots (+54%) in comparison with the control. As for the plant biomass, mature leaves harvested at the end of the experiment showed higher total leaf weight in plants grown under the Red and Pink films (+120% and +100%, respectively) compared to the control. In addition, the leaves of plants grown under the Red and Blue films exhibited higher leaf area (+47% and +39%, respectively), whereas the Red, Pink, and Blue films induced a higher leaf thickness (+23, +19, and +6%, respectively) in comparison with the control (Table 2).

Table 2. Plant biomass (shoots and leaves), shoot length, diameter, and number per plant, leaf weight, thickness, dry matter, and area of blueberry plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue). Means were subjected to one-way ANOVA, with the type of film as the variability source. Means (±SD) that share the same letter designation are not statistically different at $p \leq 0.05$, as determined by Fisher's least significant difference *post hoc* test.

	Cnt	**Red**	**Pink**	**Blue**
Plant biomass (kg plant^{-1})	0.11 ± 0.03 b	0.24 ± 0.06 a	0.14 ± 0.02 b	0.12 ± <0.01 b
Shoot length (mm)	166.20 ± 4.91 b	199.80 ± 16.82 a	172.00 ± 21.01 b	187.70 ± 7.26 ab
Shoot diameter (mm)	1.60 ± 0.15 b	2.04 ± 0.18 a	1.80 ± 0.18 ab	1.90 ± 0.22 ab
Shoot number (n° plant^{-1})	19.00 ± 4.32 b	29.25 ± 4.92 a	29.25 ± 5.19 a	23.75 ± 6.29 ab
Leaf weight (kg plant^{-1})	0.05 ± 0.01 b	0.11 ± 0.03 a	0.10 ± 0.02 a	0.08 ± 0.01 ab
Leaf thickness (µm)	314.0 ± 14.30 c	379.0 ±18.53 a	367.0 ± 13.37 a	330.0 ± 17.0 b
Leaf dry matter (%)	39.50 ± 3.17 a	35.04 ± 0.81 b	41.62 ± 3.41 a	41.42 ± 2.41 a
Leaf area (cm^2)	70.49 ± 7.58 b	103.60 ± 8.20 a	77.00 ± 6.40 b	98.12 ± 13.81 a

3.2. Gas Exchange and Pigment Measurements

Considering the dependance of plant photosynthetic activity on light spectrum, gas exchange was measured under natural sunlight modulated by the applied LC® covering films under external 400 µmol·mol^{-1} of CO_2 concentration (Figure 2).

The P_n values of blueberry plants grown under Red film doubled from t1 to t2; this strong increase was also observed in plants grown under Pink film (+79.5%), Blue film (+52%), and Cnt film (+68%) (Figure 2a). However, no differences were found at t1 among different treatments, whereas the photosynthetic rate significantly decreased in leaf plants grown under Pink film at stage t2 (−25.9%) in comparison with the Cnt plants.

For stomatal conductance (g_s), a similar trend to that of P_n was recorded, and the highest values for all treatments were observed at t2 (Figure 2b). At stage t1, no differences in g_s were observed among the treatments and control, while lower values of g_s were recorded in the leaves of plants grown under Red and Pink films at t2 as compared to the Cnt plants (Figure 2b). Overall, Ci did not change in relation to the phenological stage (Figure 2c), but it was evident that the highest value was registered in leaves from plants grown under Pink film at t1 (+24.6%) in comparison with the control.

Leaf chlorophylls were significantly influenced by each light environment beneath the films; significant was also the interaction of the film with the phenological stage of plants (Figure 2d). In fact, at t1, chlorophyll content in leaves of plants grown under LC® films was higher than the values recorded in leaves of controls (Figure 2d). The leaves of plants grown under Red film had the highest chlorophyll content at t1 (+28.09%), whereas no differences were recorded at t2 stage in comparison with the control. Even the leaves of plants grown under Pink film had a higher chlorophyll content than the Cnt at t1 (+12.8%) but significantly lower at t2 (−8.1%). The chlorophyll values in leaves of plants grown under Blue film were significantly higher (+14.6%) than the control at t1, but no differences were found at stage t2 (Figure 2d).

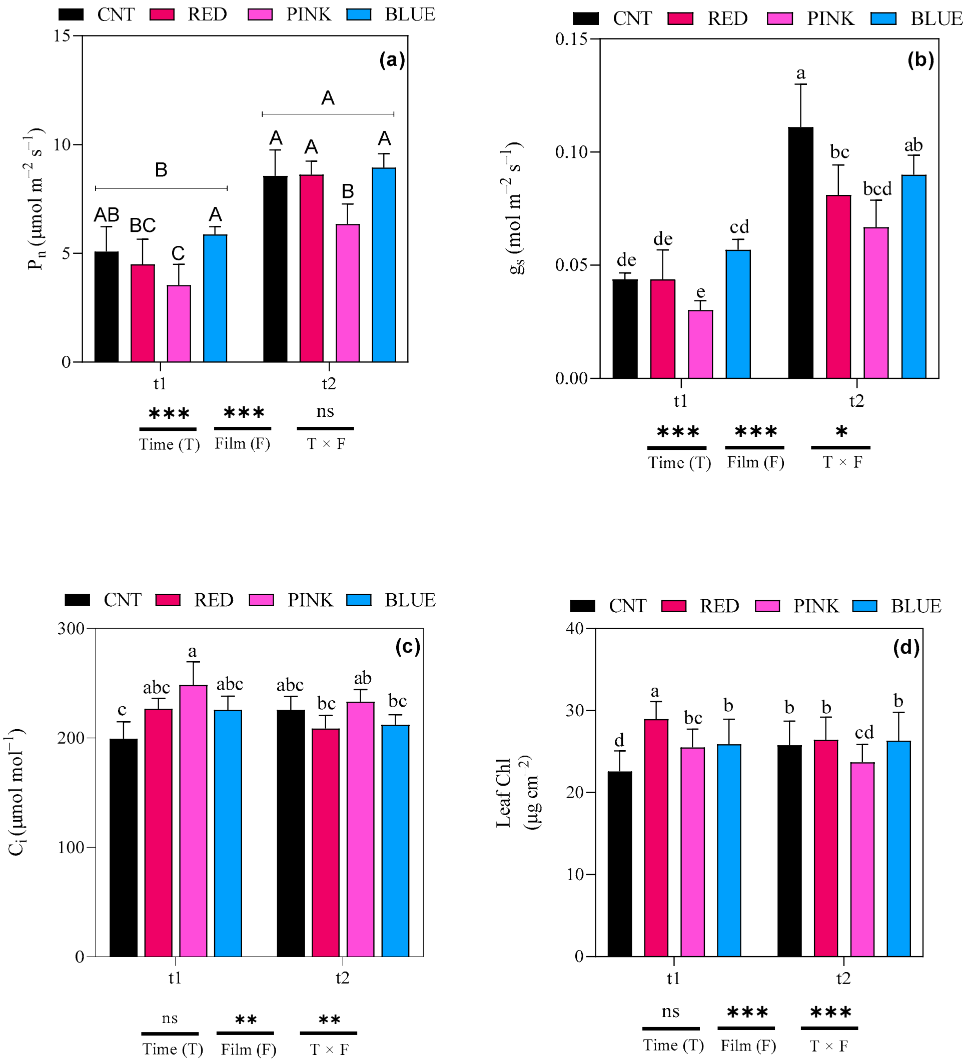

Figure 2. Photosynthetic CO_2 assimilation (P_n; **a**), stomatal conductance (g_s; **b**), intercellular CO_2 concentration (C_i; **c**), and leaf chlorophyll content (Chl; **d**) of blueberry leaves of plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue). Measurements were carried out at 35 days after transplanting (t1) and 55 days after transplanting (t2). Means were subjected to two-way ANOVA with the type of film and time as variability sources and their interaction [ns for no significance, * for $\alpha < 0.05$, ** for $\alpha < 0.01$, and *** for $\alpha < 0.001$]. Means (±SD) that share the same lowercase letter are not statistically different at $p \leq 0.05$, as determined by Fisher's least significant difference *post hoc* test following the significance of the interaction. When the F ratio in the two-way ANOVA interaction was not found to be significant, data were analyzed by one-way ANOVA, using film or time as the variability factor. The presence of different capital letters denotes significant differences for $p < 0.05$ following one-way ANOVA using film or time as a single variability factor.

3.3. *Leaf Thickness Investigation: Anatomical Observations*

Considering the differences among treatments in leaf non-destructive thickness measurements and gas exchange data, anatomical observations on transverse leaf sections were performed to examine the functionality of main four layers, consisting in the upper epidermis, palisade parenchyma, spongy parenchyma, and lower epidermis (Figure 3).

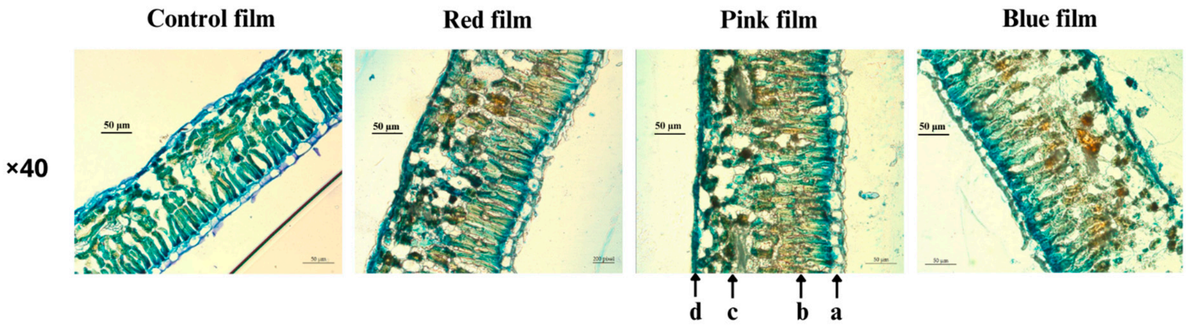

Figure 3. Anatomical observations of leaf transverse sections (40×) of blueberry plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue); a: upper epidermis; b: palisade parenchyma; c: spongy parenchyma; d: lower epidermis.

The sum of all four layers was higher in plants grown under the Pink film in comparison with the control, and no differences were found between the rest of LC® films and the control. Based on the thickness of each component, there were no significant differences in the percentage of upper epidermis and spongy parenchyma in the leaves of plants grown under the three covering films in comparison with the Cnt plants (Table 3).

Table 3. Total thickness and leaf anatomical composition (upper epidermis, palisade parenchyma, spongy parenchyma, and lower epidermis) percentage of leaf sections from blueberry leaves grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue). Means were subjected to one-way ANOVA, with the type of film as the variability source. Means (±SD) that share the same letter designation are not statistically different at $p \leq 0.05$, as determined by Fisher's least significant difference *post hoc* test.

	Cnt	Red	Pink	Blue
Total thickness (µm)	177.8 ± 32.07 b	182.2 ± 81.92 b	276.9 ± 96.83 a	250.3 ± 17.41 ab
Upper epidermis (%)	10.10 ± 1.78 a	10.50 ± 1.10 a	9.52 ± 1.03 a	9.38 ± 1.38 a
Palisade parenchyma (%)	28.38 ± 2.41 b	27.90 ± 2.55 b	36.13 ± 3.48 a	34.45 ± 7.24 a
Spongy parenchyma (%)	50.21 ± 2.91 a	53.80 ± 2.88 a	47.58 ± 3.19 a	49.53 ± 8.11 a
Lower epidermis (%)	8.22 ± 0.68 a	7.19 ± 1.05 ab	6.01 ± 0.85 b	6.31 ± 1.44 b

However, when blueberry plants were grown under Blue and Pink films, a significant increase in palisade parenchyma was recorded when compared to leaves of plants grown under the Cnt film. Conversely, concerning the lower epidermis, both Blue and Pink films induced a significant decrease compared to the control. Finally, plants grown under Red film had a similar ultrastructure to that of Cnt plants.

3.4. Flower Number, Fruit Yield, and Fruit Biometric Measurements

The highest fruit yield that was statistically different from Cnt plants was observed in plants grown under the Blue film (+86.8%) (Table 4). The assessment of the flower number at the full flowering stage showed that plants grown under the Blue film produced a higher and significant number of flowers (+119.3%) than Cnt plants, leading to an +87% increase in the total fruit yield compared to Cnt plants. No significant differences occurred in flower production and the total fruit yield of plants grown under Red and Pink films (Table 4).

The fruit set rate, that indicates that the number of flowers turned into harvested fruit, did not change among the treatments. However, higher fruit numbers were observed in plants grown under the Blue (+84.2%) and Red (+73.2%) films when compared with Cnt plants. Plants grown under Pink film produced a similar amount of fruit per plant than the control did. Finally, the single fruit weight was higher in plants grown under Red film, without any other significant variation in other treatments (Table 4).

Table 4. Flower number, fruit number, fruit set rate, total fruit yield, and single fruit weight of blueberry plants grown under the control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue). Means were subjected to one-way ANOVA, with the type of film as the variability source. Means (±SD) that share the same letter designation are not statistically different at $p \leq 0.05$, as determined by Fisher's least significant difference *post hoc* test.

	Cnt	**Red**	**Pink**	**Blue**
Flower number (n° plant^{-1})	77.12 ± 16.52 bc	103.1± 34.13 b	57.36 ± 20.19 c	169.10 ± 32.29 a
Fruit number (n° plant^{-1})	47.00 ± 6.78 b	81.40 ± 29.42 a	44.40 ± 11.33 b	86.60 ± 18.90 a
Fruit set rate (%)	64.03 ± 6.77 a	63.33 ± 5.98 a	72.98 ± 15.87 a	63.31 ± 6.40 a
Total fruit yield (g plant^{-1})	84.28 ± 19.16 b	98.21 ± 28.31 b	84.15 ± 20.27 b	157.40 ± 8.32 a
Single fruit weight (g plant^{-1})	1.63 ± 0.11 bc	1.80 ± 0.11 a	1.75 ± 0.15 ab	1.57 ± 0.11 c

3.5. Postharvest Experiment

3.5.1. Fresh Blueberry Fruit Characteristics and Quality Traits at Harvest

To assess the visual quality of the harvested blueberry fruit (T0), they were classified into four classes (Supplementary Materials, Figure S4), and the rate of each class per fruit pool was not different among the treatments. A large amount of harvested fruit (43 to 63%) from plants grown under different films was classified into class 3 (fruit diameter between 1.3 and 1.6 cm), with only 4.3% in class 1 (fruit diameter between 2.1 and 2.4 cm) and 5.3% in class 4 (fruit diameter between 1.0 and 1.2 cm).

As class 3 was the most abundant class of harvested blueberries, consequently, 50 fruits from class 3 were allocated in four boxes with a total weight of 90–100 g. At the harvest time, the fruit DM and SSC of fresh blueberries were significantly higher in fruit from plants grown under Blue film when compared with Cnt fruit (+14.7% and +28.5%, respectively; Table 5). In those fruits, the TA was also 2-fold higher in comparison to the Cnt fruit.

Table 5. Fruit dry matter (DM), soluble solids content (SSC), titratable acidity (TA), total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant activity (AA) of fresh blueberry fruit grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue). Means were subjected to one-way ANOVA, with the type of film as the variability source. Means (±SD) that share the same letter designation are not statistically different at $p \leq 0.05$, as determined by Fisher's least significant difference *post hoc* test.

	Cnt	**Red**	**Pink**	**Blue**
DM (%)	10.68 ± 0.20 b	10.74 ± 0.34 b	11.12 ± 0.32 b	12.25 ± 0.74 a
SSC (Brix°)	11.98 ± 1.80 b	11.40 ± 0.85 b	11.95 ± 2.07 b	15.40 ± 1.74 a
TA (mg citric acid g^{-1} FW)	2.86 ± 0.68 b	2.72 ± 0.58 b	3.24 ± 1.05 b	8.87 ± 1.01 a
TPC (mg GAE g^{-1} FW)	3.84 ± 0.89 b	2.25 ± 0.22 c	5.17 ± 0.23 a	4.53 ± 0.34 ab
TAC (mg cyd-3-O glu. eq. g^{-1} FW)	0.91 ± 0.10 a	0.76 ± 0.23 a	0.70 ± 0.17 ab	0.51 ± 0.09 b
AA (mg TE g^{-1} FW)	7.00 ± 1.08 b	7.36 ± 1.42 ab	6.77 ± 0.88 b	8.77 ± 0.71 a

The TPC of fresh blueberries was higher (+34.6%) in the fruit of plants grown under Pink film as compared with the controls but lower (−41.4%) in fruit from plants under Red film (Table 5). The TAC was lower in fruit from plants grown under the Blue film (−34.9%) in comparison with the controls, whereas no statistical differences were found in these compounds between fruit from the controls and the other two films (Table 5). Finally, the antioxidant capacity was higher in fruit from plants grown under Blue film as compared with the controls, but fruit from plants under Red and Pink films were not different from the control (Table 5).

3.5.2. Postharvest Fruit Storage: Fruit Quality Traits After Storage

Blueberry fruit belonging to class 3 was distributed in packaging boxes and stored in dark conditions at 4 °C and 90–95% relative humidity to mimic fruit conservation after harvesting in agricultural farms or large retailers before the fruit is put up for sale.

As a general trend, upon blueberries' storage, the single fresh fruit weight decreased significantly (Figure 4). Differences were observed in fruit from plants grown under photoluminescent spectrum-converting films, and, in particular, fruit from plants grown under Pink and Blue films registered lower values of fruit weight during storage when compared to the fruit of plants grown under Cnt and Red films.

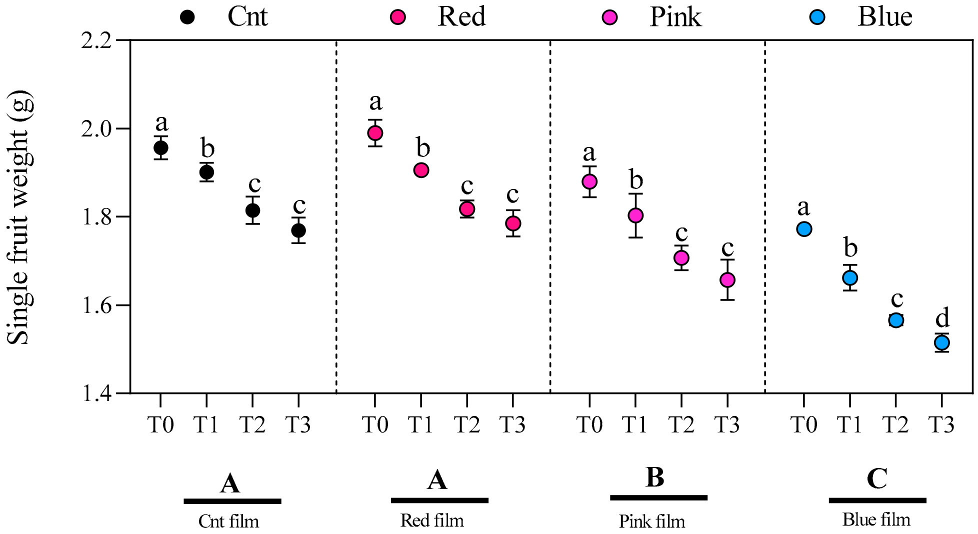

Figure 4. The average weight of blueberry fruit harvested from plants grown under the control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

Concerning the dry matter (Figure 5), fruit from plants under Blue and Pink films had similar contents during the storage times (T0, T1, and T2) with the exception of T3, where the dry matter increased +61.6% in fruit under Blue film and +26.9% in fruit under Pink film in comparison to T0. The fruit of plants grown under Cnt and Red films showed a linear dry matter increase during all storage times except for Cnt fruit at T1 and T2, when they showed similar dry matter contents. However, the highest increase in fruit dry matter was shown in fruit from plants under Blue film and the lowest in fruit from Red and Pink films.

The soluble solids content (Figure 6) in Cnt fruit increased at T2 and T3 when compared to T0, reaching, at the end of storage, a value significantly higher than that recorded at T0 (+37.8%). Similarly, in fruit from plants grown under Red film, an increase was observed at T3, whereas similar values for the SSC were reported in fresh and stored fruit from plants grown under Pink film. Finally, although there was a decrease upon storage in the SSC values of fruit from plants grown under Blue film, similar values were registered between T0 and T3. However, the resulting SSC values were lower in fruit under Red and Pink films during storage when compared to Cnt film (Figure 6).

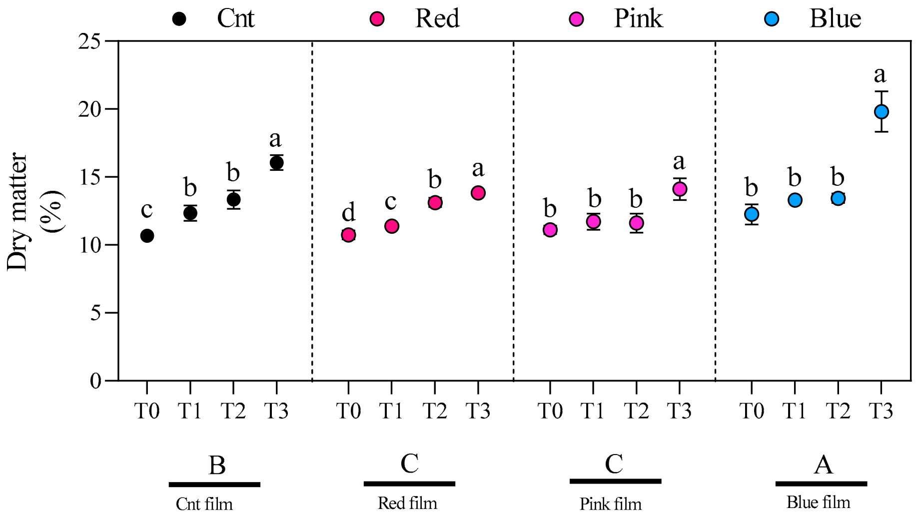

Figure 5. Dry matter percentage of blueberry fruit harvested from plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

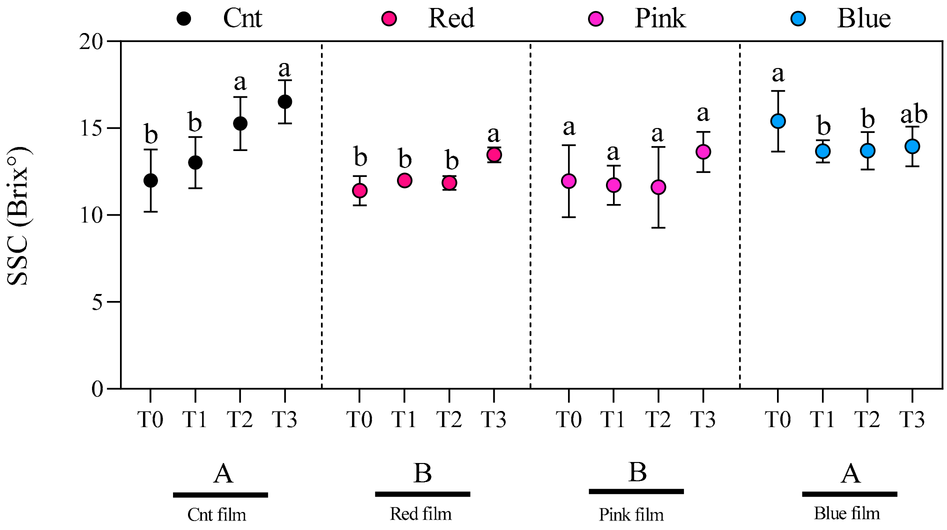

Figure 6. Soluble solids content (SSC) of blueberry fruit harvested from plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

For titratable acidity (Figure 7), an increased peak was observed at T1 in fruit from plants under Cnt, Red, and Blue films, whilst a linear increase was observed in fruit from plants under Pink film, which reported the highest value at T3, with a 2-fold increase between T0 and T3. However, fruit from plants grown under Red film showed another titratable acidity increase at T3, whilst fruit from plants under Cnt and Blue films reported similar or lower titratable acidities between T0 and T3; the highest titratable acidity values were found in fruit from plants under Blue film, followed by those from Red and Pink films, whilst the Cnt film induced the lowest fruit titratable acidity during storage.

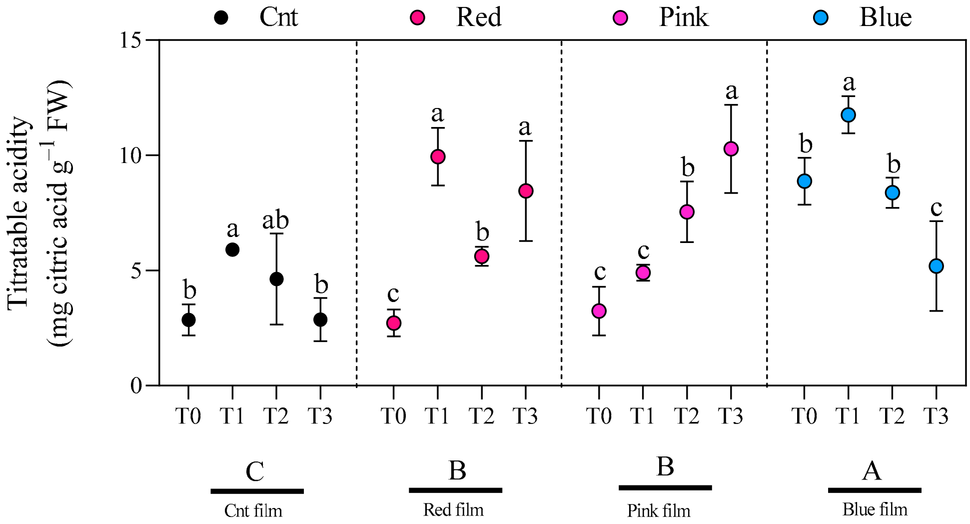

Figure 7. Titratable acidity (TA) of blueberry fruit harvested from plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

3.5.3. Postharvest Fruit Storage: The Nutraceutical Quality

Different trends were found for the TPC in fruit from plants subjected to different coverings and stored for 20 days (Figure 8). Fruit from plants grown under Red film showed an increase in the TPC at T3 in comparison to the value detected at T0 (+42.5, +67.9, and +106.2% at T1, T2, and T3, respectively). Differently, in fruit from plants cultivated under Pink film, an increase in the TPC was detected at T1, followed by a significant decrease at T2 and T3 (−14.3 and −33.0%, respectively, in comparison with T0). Blueberry cultivation under the Blue film did not induce changes upon storage in the TPC of fruit except for a decrease at T2. Similarly, Cnt fruit had similar TPC values at T0 and T1 and increased similar values at T2 and T3. However, fruit from plants under Pink film induced a higher TPC values during storage when compared to those under Red, Blue, and Cnt films, whilst Red film induced the lowest TPC values in stored fruit.

There was a significant effect of each LC® film on the TAC of fresh fruit stored in cold conditions (Figure 9). The Cnt film induced a −52.7% decrease in the TAC at T1 and a −37.9% decrease at T2 when compared with values at T0; however, at the end of the storage, the fruit TAC was similar to the amount recorded in fresh fruit at T0. A TAC decrease was also observed in fruit from plants grown under Red film (−55.9% in T1 and −35.7% in T3 in comparison with T0 values). The most stable was the TAC in fruit from plants under Pink film, with the only exception of a decline (−26.8%) at T2, as compared to T0. The TAC in fresh fruit from plants grown under Blue film did not change upon storage, and only a slight increase was observed at T2. Similar TAC values were observed in fruit from plants under Pink film and Cnt fruit, whereas lower and similar TAC values were observed in fruit from plants under Red and Blue films.

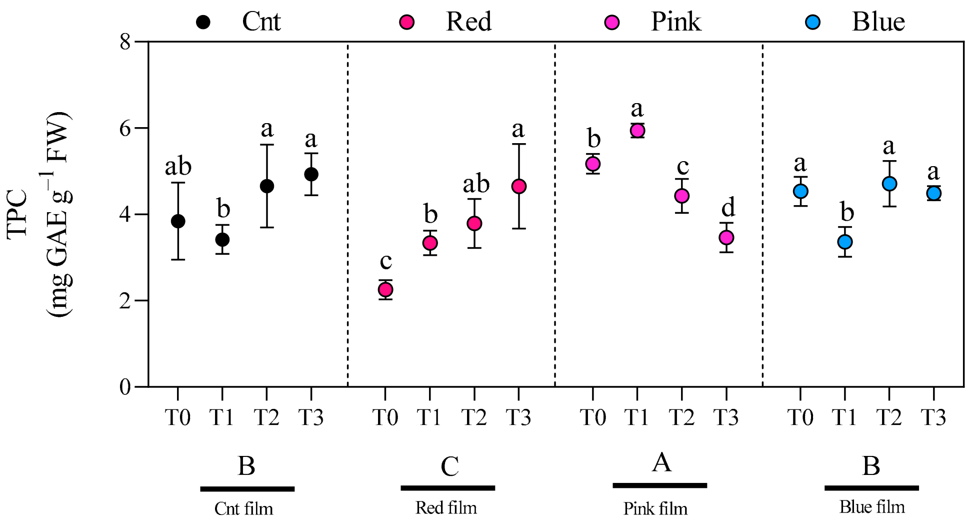

Figure 8. Total phenolic content (TPC) of blueberry fruit harvested from plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

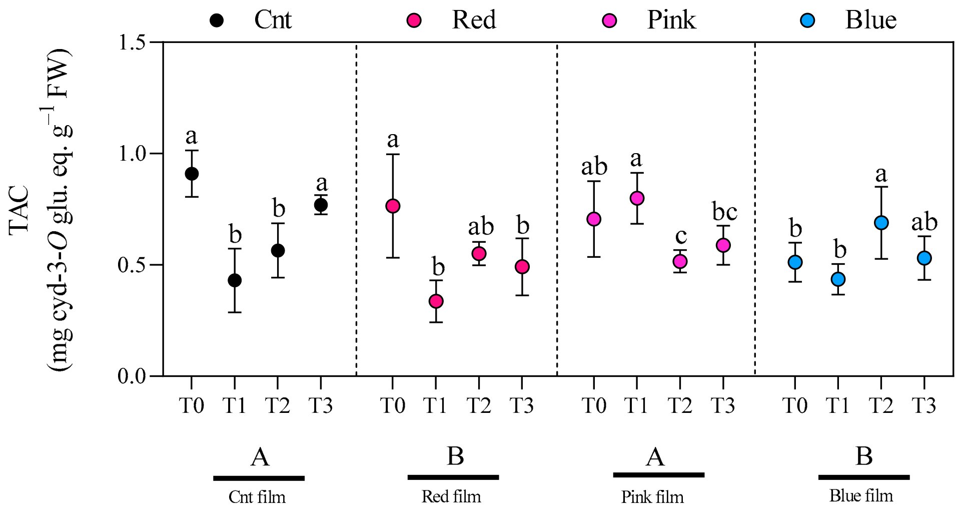

Figure 9. Total anthocyanin content (TAC) of blueberry fruit harvested from plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

Finally, the antioxidant capacity of Cnt fruit gradually increased as compared to T0 (+32.9% at T1, +64.9% at T2, and +38.0% at T3; Figure 10). The antioxidant capacity of fruit from plants under Red film showed a decrease at T2 but with similar values to the other storage times. The antioxidant capacity of fruit from plants grown under the Pink film increased significantly at T1 (+43.7%), but it declined at T3 (−32.4%) as compared to T0. No significant changes in antioxidant capacity were observed in fruit from plants cultivated under Blue films upon storage. The highest antioxidant activity was found in fruit from plants under Blue and Cnt films during storage, whilst the lowest was in fruit from plants under Red and Pink films.

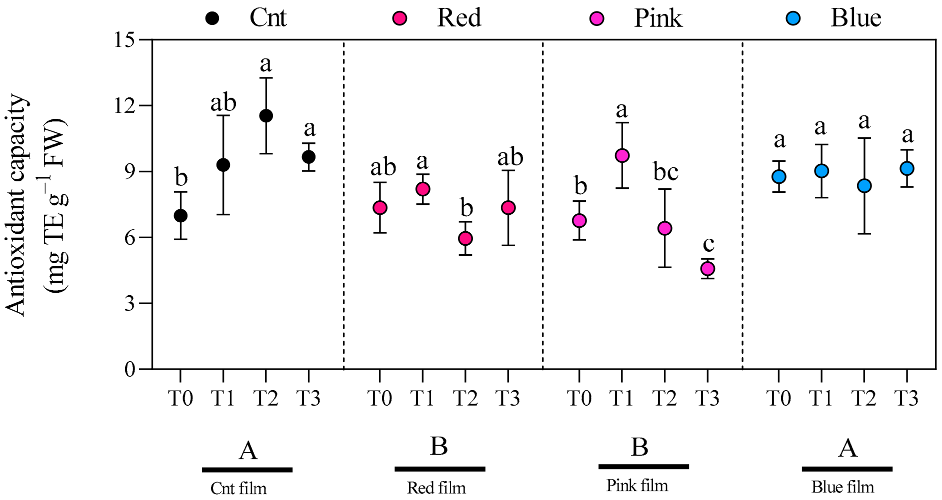

Figure 10. Antioxidant capacity of blueberry fruit harvested from plants grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue) and stored 0 (T0), 6 (T1), 14 (T2), and 20 (T3) days after harvest. Means (±SD) that share different lowercase letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with storage days after harvest as the variability source, as determined by Fisher's least significant difference *post hoc* test. Means (±SD) that share different capital letters are statistically different at $p \leq 0.05$ following one-way ANOVA, with the type of film as the variability source, as determined by the same *post hoc* test.

4. Discussion

Light modulation is important for enhancing plant growth and photosynthesis in controlled environments, which, in turn, ensures high plant and fruit yields, as well as possibly promoting fruit nutritional/nutraceutical quality [35,36]. The modulation of sunlight spectra to stimulate plant responses through light conversion technology represents a promising, yet unexplored tool to achieve these goals [24,25,37,38]. The innovative LC® films represent advanced light-converting technology specifically designed for the agricultural sector, with a promising range of possible applications [26].

The adaptability of LC photoluminescent spectrum-converting films in modifying environmental light conditions has been successfully demonstrated in fruit species such as strawberries and blackberries [24,25]. This study provides new insights into the morpho-anatomical and physio-chemical responses of blueberries to selective light enrichment using three innovative LC films. In addition, the change in nutraceutical value of fruit grown in different light environments was monitored during storage and is presented in the subsequent sections.

4.1. LC® Films Influence Plant Growth Traits Like Shoot Elongation and Leaf Morphology but Have Less Effect Observed on Leaf Gas Exchange

Variations in the ratio of blue and red lights mediate some photomorphogenic characteristics such as shoot elongation and leaf morphology or anatomy (as reviewed by Landi et al. [39]) due to the variations in the activity of photoreceptors such as phytochromes (PHY), able to absorb red and far-red lights, or cryptochromes (CRY) and phototropins (PHO), able to absorb ultraviolet (UV) and blue lights [18,40]. These photoreceptors help plants respond to modulated solar light by photoluminescent films. In fact, the adaptation of blueberry plants under investigation in the present work to the Red film application demonstrated an enhancement in plant biomass that is directly related to the increase in total leaf weight and leaf area. Similarly, Hidaka et al. [40] showed that red photoselective shade film performed better in terms of plant biomass as compared to blue photoselective film in *Vanilla planifolia*. This increase was due to the high red-light portion perceived by plants, even though this kind of film reduced the solar light by 50% [39,40] whilst the photoluminescent spectrum-converting films utilized in the present study did not reduce the incident solar irradiance.

Light penetration within leaves is significantly influenced by both red and blue light environments, affecting leaf anatomy [41]. While only a few studies have investigated the use of photoluminescent spectrum-converting films, here, our results are discussed in the context of the existing literature on the use of colored LED lighting while acknowledging the distinct optical properties of colored films compared to LED lamps. Zhang et al. [36] reported that LED light with a high red/blue ratio boosts leaf total thickness and, particularly, the spongy parenchyma of cotton seedlings. In the present study, leaf thickness was measured at the field as macro-morphological data followed by a second measurement using the total thickness of leaf anatomical components. The latter measurement was performed for leaf composition functional assessment in relation to gas exchange and leaf pigment content data. Plants grown under Red and Pink films showed higher leaf thickness than the other treatments of control and Blue film-treated plants, attributing this finding to an equivalent increase in all measured anatomical components (upper and lower epidermises, palisade, and spongy parenchyma). However, the spongy parenchyma did not show significant differences compared to the other treatments. This increase in leaf thickness was not coupled with a dry mass accumulation, while in tomato plants grown in greenhouse conditions, supplemented red light positively affected leaf dry mass accumulation [42]. This discrepancy between species may be related to different allocation strategies adopted to manage perceived light quality. The structure of leaves is vital for influencing how carbon dioxide (CO_2) moves and how light is distributed within them, which, in turn, affects the efficiency of photosynthesis. The palisade tissue located near the surface of the leaf allows light to penetrate more deeply, while the spongy mesophyll scatters light to promote even absorption among chloroplasts and lessen variations in light exposure [36]. Leaves grown under Blue film showed greater thickness of the palisade parenchyma proportion, which may contribute to an increase in chloroplast density [43]. This occurrence could contribute to explaining the increment of chlorophyll levels observed in plants grown under Blue film, as this leaf tissue has been characterized by a higher density of chloroplasts [43]. Despite being less densely packed with chloroplasts compared to palisade parenchyma, the spaces in spongy parenchyma allow for efficient diffusion of gases such as CO_2 and O_2 within the leaf [44]. In our study, no significant differences in the spongy parenchyma were detected among treatments. Thereafter, these results were aligned with the unchanged intercellular leaf CO_2 concentration performance of plants grown under all LC films during the green fruit stage (t2). Additionally, our results showed that leaf photosynthetic capacity from the flowering and shoot growth stage (t1) to the green fruit stage (t2) was unaffected by the Red or Blue films. Also, although light variation occurred with the three LC films, the stomatal conductance decreased during t2 stage in plants grown under LC films compared to the Cnt plants. These gas exchange results were not consistent with previous research, where both Red and Blue films enhanced the photosynthetic capacity in strawberry and blackberry plants, with the Blue film showing higher g_s values in both crops compared to controls, suggesting that the LC films have species-specific effects among the tested berry species [24,25]. In fact, other studies on light conversion films have shown a positive effect on photosynthetic responses among an array of species [40,45]. Within the context of in vitro culture, Ge et al. [46] reported notable upregulation of genes related to capturing and transferring light energy in photosynthesis of *A. thaliana* grown under light conversion film characterized by dual-emission properties of both red and blue lights (similar conversion effect to the Pink film in our study), in comparison to plantlets subjected to Cnt film. Conversely, another study [35] has indicated that radishes exhibited increased photosynthetic rates when grown under Red and Blue films when measured during the early morning hours. Moreover, Pink film induced a reduction in the photosynthetic performances (P_n and g_s) combined with the maintenance of chlorophyll levels. This indicates that the photosynthetic machinery was intact and that low photosynthesis is linked to impaired biochemical processes, as already observed in *Rubus fruticosus* L. var. Loch Ness in a previous work by the same authors [25]. Indeed, leaf nitrogen was efficiently distributed, which maintained leaf chlorophyll content, so no impaired light capture or photoprotection are expected in plants grown under Pink film [41]. Instead, El Horri et al. [25] attributed this plant behavior to both stomatal and biochemical limitations, which can lead to the accumulation of CO_2 within the leaf intercellular airspaces, even under conditions of low g_s values. Biochemical constraints may involve limitations in the Calvin–Benson cycle as well as other CO_2-assimilating pathways. These include mechanisms such as the co-regulation of g_s and P_n and the modulatory roles of CO_2, abscisic acid, and hydrogen peroxide [25].

4.2. Red Films Promoted Fruit Yield and New Shoot Numbers

The biology of berry species such as blueberries, classified as perennial crops, highlights the importance of shoot elongation and expansion in supporting flowering for the subsequent [14]. Our results indicate that the application of Red and Pink films before bud burst significantly stimulates the vegetative buds' sprout and growth, as demonstrated by an increase in the number, elongation, and diameter of shoots compared to the Cnt film. These findings agree with those of Cho et al. [47], who showed that red light supports the vegetative phase of blueberry plants by promoting the differentiation of axillary buds into vegetative buds through the activation of phytochromes. Similarly, increasing the proportion of red light facilitated growth of the stem length and thickness of blueberry tissue-cultured seedlings, while blue-light supplementation encouraged the formation of more leaves, as reported by Zhang et al. [43].

In some studies, light wavelengths such as red, blue, and UV-B have been shown to stimulate the carbohydrate partitioning between fruit and vegetative growth [12,13,30]. Specific light exposure can influence physiological processes that alter carbohydrate allocation to the developing fruit, such as far-red light that directs carbohydrates to stems while reducing allocation to leaves and roots [30]. This has been observed in blueberry plants grown under Red film, where the red-light portion has been enhanced. We noticed that although the highest biomass produced was recorded in plants grown under the Red film, the fruit yield remained statistically comparable to that of Cnt plants. Consequently, our findings suggest that Red film application allowed the plants to allocate carbohydrates toward fruit and vegetative growth similarly, thereby maintaining fruit production and, at the same time, improving plant carryover capacity to produce fruiting shoots for the next year. This is in accordance with another study that reported that red artificial light encouraged vegetative growth of blueberry plants, while blue light encouraged flower production for year-round production [47]. In fact, although plants were transplanted under LC® films after flower buds' differentiation and a similar fruit set rate between treatments, our plants grown under the Blue film produced significantly higher fruit yields, closely associated with high flower production and an elevated fruit number. In contrast, the same plants exhibited reduced plant biomass with maintained shoot growth. The modulated solar light produced by the Blue film provided optimal lighting conditions for our experiment with *V. corymbosum*. This aspect clearly enhanced light-use efficiency, as evidenced by the relationship between accumulated total dry weights and intercepted light intensity, as it was similar between treatments and the Cnt. Consistent with the findings of Yang et al. [48], selecting and adjusting the optimal lighting conditions for improved leaf area exposed to light are keys to improved plant performance achieved through increased photosynthesis and higher light-use efficiency, whereas in another experiment, red photo-selective shading nets resulted in a delayed harvest time without negatively impacting the return bloom, yield, or fruit quality in *Vaccinium corymbosum* cv. Elliott [49].

4.3. Red Film Application Maintained Fruit Quality Traits and Nutraceutical Postharvest Fruit Quality

The existing research predominantly focuses on postharvest management strategies such as on the application of artificial light prior to or during storage [10,30]. In addition, preharvest treatment applications are proven to significantly influence fruit quality at harvest and during postharvest storage. They are mostly applied on fruit to delay softening and increase phenolic content [29,30]. However, there is limited information available on the application of modulated light at the field level. Therefore, we chose to harvest blueberry fruit grown under LC film based on the visual commercial ripening index of fully colored fruit, which was utilized for storage experiments. Most of the fruits, with no differences among the treatments, were classified as class 3, indicating that LC films did not have a significant impact on their size. Within fruit group, blueberries are not classified as typical climacteric fruit, as they lack the characteristic ethylene surge observed in other fruits [16,20]. Instead, their respiration rate rises in response to storage conditions that stimulate ethylene production, resulting in softening and decay, which reduces their firmness and nutritional value [16,20]. Effective storage of blueberries is also influenced by the conditions under which they were grown, as these factors play a significant role in maintaining their visual appeal and overall quality [1,3].

Across all treatments, blueberry fruit underwent a typical loss in weight during storage combined with an increase in dry matter. The increase in dry matter is likely attributed to the conversion and degradation of carbohydrates into sugars and other soluble compounds during the primary metabolic processes of blueberries associated with the reduction of moisture content due to fruit respiration processes [50]. Additionally, metabolic changes during postharvest storage in ripe fruit are linked to a decline in overall quality, but the responsible biochemical pathways are still not fully understood. One of the main parameters linked to organoleptic characteristics, such as TA, decreased during storage of fruit from plants grown under Blue film. Probably, its decline combined with maintained SSC during storage is linked to the utilization of fruit organic acids in respiratory processes, as supposed by Cai et al. [51].

The nutraceutical fruit quality during storage can also be a light-dependent process [52]. Based on our findings, although low TPC was found in blueberry fruit grown under Red film, the values of these compounds increased during cold storage. This increase is likely attributed to the interconversion of organic acids and carbohydrates into phenolic compounds [7]. Considering the linear increase in fruit dry matter from T0 to T3 in fruit grown under the Red film, the moisture loss can also lead to an increase in metabolic concentration. The management of light quality and intensity is an abiotic factor that can enhance the expression of genes related to phenylpropanoid biosynthesis, leading to the increased production of phenolic compounds [3,10]. Blue light generally promotes the accumulation of a variety of phenolic compounds and activates the corresponding biosynthetic genes. Meanwhile, red and white lights can specifically increase the levels of certain phenylpropanoids, including ferulic acid, kaempferol, and rosmarinic acid, depending on the type of plant and its stage of development [53].

Overall, regardless of the storage time, the fruit grown under the Pink film contained a higher TAC content than the control. Differently, the Red film induced a decrease in anthocyanins in fresh fruit upon storage. However, following the storage experiment, most treated fruit maintained its TAC until T3. This preservation of anthocyanins may be linked to the acidity levels present in fresh blueberries, which creates suitable conditions for anthocyanin stability during storage [5]. During the ripening process of similar blueberry crops (*V. myrtillus* L.), the presence of anthocyanins was mainly due to a significant increase in the accumulation of delphinidin glycosides [15]. Other results have been reported by Zoratti et al. [22] in the blueberries cv. 'Brigitta' ripened under blue (blue/red ratio of 0.75) and red (blue/red ratio between 0.49 and 0.52) photoselective nets. The content of TAC was like a control net, and the lowest values were registered in fruit grown under a black net [22]. Furthermore, kiwifruit that received 23% shading from red nets showed a decline in polyphenol concentration and antioxidant activity at the time of harvest. This decrease is likely linked to a reduction in the UV component present in both total and scattered light beneath the nets.

According to Zifkin et al. [54], anthocyanins play a significant role in the antioxidant activity of blueberry fruit cv. 'Rubel'. Similarly, Mesa et al. [55] reported that postharvest application of blue LED light improved the antioxidant capacity of 'Valencia' oranges. A similar effect was observed in the present study, where blueberry fruit cv. 'Northland' from plants grown under the Blue film exhibited a high antioxidant capacity at the start of storage, which remained stable throughout all the periods. Also, this TPC and TAC stability of fruit grown under Blue film during postharvest is linked to the role of these molecules in UV-to-blue conversion, which may upregulate antioxidant pathways [29]. However, fruit from plants grown under the Pink film showed antioxidant capacity values fluctuation, especially during the first days of storage. This pattern is difficult to explain, even though it is probable that the ripening stage of some fruit was sometimes different.

5. Conclusions

In conclusion, light down-conversion technology is an effective method to modify solar light in covered facilities. However, due to the unique properties of each LC®, precise knowledge of diverse crop responses is required for a profitable application. In this respect, as complementary research to the previous applications of LC® on strawberries [19] and blackberries [20], our results suggest that the use of LC films may be additionally useful to improve crop growth and to boost fruit yield of blueberry var. Duke. Under controlled tunnel conditions with single-season evaluation, Red spectrum-conversion films showed increased plant biomass, although this requires validation through multi-season studies with proper experimental replication before commercial recommendation. The new developed shoots are potentially enhancing carryover capabilities for the following growing season. Additionally, the Blue film can increase berry production and enhance the nutritional value of the fruit. Both Blue and Red films also effectively address postharvest limitations, maintaining the fruit nutraceutical profile. The findings of this study contribute valuable insights into the response of blueberry plants to red- and blue-light supplementation via light down-conversion technology. Several limitations constrain the applicability of these findings: (1) Single-season data cannot predict consistent performance across varying environmental conditions. (2) Energy balance analysis is needed to determine net photosynthetic benefit. While the initial results suggest potential benefits of spectrum-conversion technology in berry production, commercial implementation requires (1) multi-year validation studies across diverse growing conditions and (2) comprehensive economic analysis comparing costs with LED alternatives. Future research priorities include (1) multi-cultivar and multi-season validation studies; (2) molecular analysis of photoreceptor pathway activation; (3) economic modeling for commercial feasibility; (4) life-cycle assessment of film technology vs. LED alternatives; and (5) consumer preference evaluation for spectrum-modified fruit products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15071708/s1, Figure S1: Spectral presentation of the light conversion films (Red, Pink, and Blue) in comparison with the control polyethylene film (Cnt) employed in the experiment when exposed to sunlight and their effects on the transmitted wavelengths inside the tunnel; Figure S2: Spectral presentation of the difference rates of light transmittance under light conversion films (Red, Pink, and Blue) relative to the control polyethylene film (Cnt) employed in the experiment when exposed to sunlight; Figure S3: Timeline of blueberry plant development under light conversion films, in total 108 days after transplantation (DAT), and during the fruit postharvest experiment, in total 20 days after harvest (DAH); Figure S4: Blueberry fruits grown under control (Cnt), Red film (Red), Pink film (Pink), and Blue film (Blue). (a) Fresh fruits classified into class 1 (fruit diameter between 2.1 and 2.4 cm), class 2 (fruit diameter between 1.7 and 2.0 cm), class 3 (fruit diameter between 1.3 and 1.6 cm), and class 4 (fruit diameter between 1.0 and 1.2 cm). (b) Fruit state after 26 days of harvest and stored in dark conditions under 4 °C and 90–95% relative humidity.

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