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Blue and Red Light Downconversion Film Application Enhances Plant Photosynthetic Performance and Fruit Productivity of *Rubus fruticosus* L. var. Loch Ness

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Abstract: Light downconversion films can modulate incident light wavebands on crops, converting less utilised wavebands in an efficient way. In this experiment, red (conversion of green into red light wavebands), pink (conversion of UV and green into blue and red light but to a smaller degree than red film), and blue (conversion of UV into blue light) light downconversion films were used to cover blackberry plants throughout all phenological stages (from leaf emergence to fruit harvesting). The plants' physiological and biometric performance, and fruit yield and quality were evaluated. Plants under blue and red films showed a higher net photosynthetic rate with +23.1% and +14.9%, respectively, and a higher stomatal conductance with +56.0% and +23.6%, respectively, with respect to controls, maintaining stability across stages, except for a decrease under the red film during fruiting. Both films significantly boosted the fruit yield, with the red film increasing the fruit number (+49.8%) and the blue film enhancing the berry shape (+10.7) and fresh weight (+36.6). Notably, no significant differences in nutraceutical quality, including total flavonoid and anthocyanin content, were observed. These findings suggest that light downconversion films, particularly red and blue films, can effectively enhance the photosynthetic performance and fruit production in blackberry plants without compromising the fruit quality. Future research on this topic should focus on balancing plant growth, fruit productivity, and enhancing fruit nutraceutical properties.

Keywords: light quality; light conversion films; blackberry; photosynthesis; physiological responses; nutraceutical properties

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1. Introduction

Light plays a crucial role in governing plant growth and, consequently, fruit production [1,2]. Thanks to leaf pigments, such as chlorophylls and carotenoids, plants selectively absorb blue-violet light (400–480 nm) and orange-red light (600–700 nm), converting light energy into energy of chemical compounds [3,4]. Clearly, the amount of light is the main environmental factor that regulates plant physiology upon the entire plant life cycle because it is utilised as the energy source for carbon assimilation, but it is also a signal able to activate and/or regulate plant growth and development [5]. Moreover, plant productivity

is affected not only by the intensity (photon flux) and the duration of light (photoperiod), but also by the light quality [6]. For instance, various studies have shown that the enhancement of plant photosynthesis and plant productivity and the increase in leaf pigments and bioactive compounds in fruit is promoted by red and blue light enrichment in outdoor or indoor settings [7,8]. Lauria et al. [8] reported that red light increases the fruit yield, as it can "photomodulate" the strawberry fruit quality and increases the post-harvest tolerance to the fungus *Botrytis cinerea*. In addition, green light influences plant development in the long-term, being able to penetrate deeply in the leaf mesophyll and promote photosynthesis, and is also able to improve the crop yield [9]. Therefore, to increase the plant growth and crop yield, in addition to beneficial nutraceutical compounds in plant-based food, researchers are exploring new effective management strategies to modulate light quality with new light technologies [3,10,11].

Some crops when subjected to artificial light can smoothly fit without compromising the final yield characteristics [11]. However, based on the existing literature, the effect of the light quality on crop production varied in relation to species, but also to variety/cultivar, highlighting contradictory or similar impacts of wavebands [2,12]. For example, for strawberries, red Light Emitting Diode (LED) light improved the net photosynthetic CO_2 assimilation and leaf dry mass in cultivar 'Parous', whereas when blue light was supplemented the number of inflorescences significantly increased in cultivar 'Camarosa' [2]. In the cultivar 'Albion' of the same species the fruit mass increased when the red/blue supplemental lights were utilised [2]. Dale and Blom [12] recorded similar shoot lengths in raspberry cultivars 'Lauren', 'Reveille', and 'Titan' when far-red (FR) light was provided at nighttime.

In addition to LED light employed indoors for plant growth, coloured film applications were designed outdoors to shift sunlight wavebands within the photosynthetically active radiation (PAR) without external energy use [13]. In agriculture, as an alternative to LED lighting, spectral filters [14], light conversion films [15], spectral downconversion films [16], or photoluminescent films [4] are employed in outdoor settings. These technologies alter the sunlight and improve the light quality, thereby influencing plant physiology and the quality of the final product [6,13,17,18]. Light downconversion films are cutting-edge coloured on embedding within the polymer matrix blended with additives, designed to convert ultraviolet (UV) and green light into more photosynthetically efficient wavelengths, such as blue and red light [13,19]. The success of these films hinges on the capability of the light downconversion agent to harness abundant sunlight energy and transmit a well-matched emission wavelength to allow sufficient light to reach the covered plants with less light energy loss [6,20]. In fact, this scientific advancement in a controlled light environment not only accelerates the crop cycle of high-light crops but also demonstrates promise in enhancing the crop yield and overall quality [17,21]. In addition, under light downconversion films the crop yield is improved. For example, the final yield of peppers per unit area increased by 20.34% with a higher fruit weight when grown under a rare earth film with both red and blue light amplification [15]; higher fruit numbers also led to a boosted yield in cucumber by converting part of the green wavelength range to the red wavelength range [22]. Moreover, using the downconversion films employed in this study, a 29.7% yield gain was registered in tomatoes under a red downconversion film [23].

Berries (*Rubus* spp.) are an important source of phytochemicals for human consumption; in particular, phenolics and blackberry plants (*Rubus fruticosus* L.) have long been acknowledged as a nutrient-dense fruit crop [4,24]. The variety Loch Ness is widely cultivated for its high yield and considerable fruit size [25–27].

Given the limited and inconsistent information regarding the impact of light downconversion films on blackberry or, more generally, on crop plants, this study elucidated the effects of three specific types of red, pink and blue films on the physiological characteristics of *R. fruticosus* L. var. Loch Ness blackberry plants. Additionally, this research aimed to assess how these films influence fruit production and quality by analysing the accumulation of polyphenols in the fruit.

2. Materials and Methods

2.1. Plant Material and Fruit Yield Measurement

Two-year plants of blackberries (*R. fruticosus* L.) var. Loch Ness were purchased from a local nursery and cultivated at the experimental field of the Department of Agriculture, Food, and Environment, University of Pisa (43°42′16.6″ N, 10°25′36.9″ E). In November 2022, blackberry plants were transplanted in a 12 L pot filled with a substrate mixture of blond and brown peat and perlite (80:20; v:v). The substrate had an apparent dry density of 212 kg m^{-3} and pH 6. Plants were initially fed with a fertiliser Osmocote exact STD (ICL Italy Srl, Milano, Italy) with a dose of 50 g $plant^{-1}$ and regularly irrigated with water. In spring 2023, a total of 24 plants were placed in four different tunnels, each covered with 150 µm thick polyethylene light downconversion films (6 plants per tunnel) provided by Light Cascade® (Clamart, France). Treatments were recorded as Red (conversion of green into red light wavebands), Pink (conversion of UV and green into blue and red light wavebands but to a smaller degree than Red film), and Blue (conversion of UV into blue light wavebands). The control film was a polyethylene film without downconversion technology (Cnt). The films were used to cover open open-ended hoop structure of 6 × 4.15 × 1.9 m. The light spectrum under each light downconversion film (300 to 900 nm) was measured using a spectroradiometer (Ocean HR Series: HR2 Spectrometer, Ocean Optics, Orlando, FL, USA). During the trial period (from March to June 2023), the relative humidity and minimum and maximum temperature data were monitored using a data logger (Tinytag Ultra 2—TGU-4500, Gemini Data Loggers, Chichester, UK) installed under one of the four films (Figure 1). The temperature and relative humidity were not affected by the covering films since the tunnel was open and strictly affected by outside temperature and relative humidity.

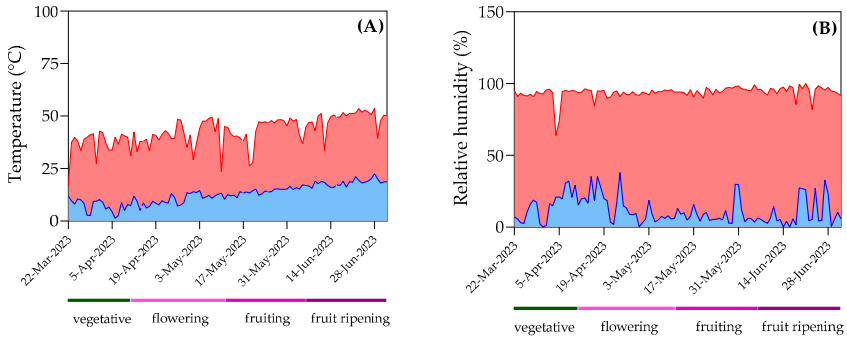

Figure 1. Temperature (**A**) and relative humidity (**B**) values during the trial period under the tunnel structure covered with control film (no light downconversion effect; Cnt), red film (Red), pink film (Pink), and blue film (Blue). Red line represents the maximum temperature/relative humidity whilst blue line represents the minimum temperature/relative humidity.

During the trial period, plant physiological measurements were carried out at three different plant stages (T1, at the end of the vegetative stage; T2, at the end of flowering; and T3, in the middle of fruit ripening). From the start of the fruit ripening stage until the end of plant productivity, blackberry fruits were harvested at the marketable stage, ensuring that fruit from different treatments had reached similar ripening levels (percentage of soluble solid content around 9 and 10 °Brix). All the fruit was counted, weighed, and sized (fruit width in the midpoint and height) to evaluate the overall fruit yield and the fruit number per plant. Moreover, the berry shape index was calculated as the fruit height/fruit width ratio. Then, fruit was differentiated into two homogeneous sub-samples for each treatment. The first sub-sample was used for the organoleptic analyses and the second sub-sample was frozen in liquid nitrogen and stored at −80 °C until biochemical analyses.

2.2. Plant Morphological Parameters

At the fully flowering stage, number of flowers per plant was measured. Then, at the end of the fruit harvest, plant biomass and dry matter (differentiating stem and leaves), stem

length, leaf number, leaf mass area (LMA), leaf thickness, and flower fecundity rate were measured. Stem and leaf dry matter (n = 3) was calculated after drying plant material in an oven (Memmert GmbH Co., KG Universal Oven UN30, Schwabach, Germany) at 105 °C until the constant weight was reached. The total leaf number was counted, and randomly selected leaves were subjected to LMA (n = 3) and leaf thickness (n = 9) measurements. A high-accuracy digital thickness gauge and ImageJ software (version 1.52t, Bethesda, MD, USA) were used to measure the thickness and leaf area. LMA was calculated as the ratio of leaf dry weight to leaf area and expressed as g m^{-2}. Stem length was measured using 5 shoots per plant across 3 plants per treatment (n = 15).

The flower fecundity rate was calculated as follows:

$$\text{Flower fecundity rate (\%)} = (A/B) \times 100$$

where A is the fruit number per plant and B is the flower number per plant.

2.3. Gas Exchange Analysis

At T1, T2, and T3, starting from 11:00 a.m. until 1:00 p.m., fully inflated leaves (n = 7) were randomly selected, and gas exchanges were analysed using an infrared gas analyser LI-6800 system (Li-Cor, Lincoln, NE, USA) at a light intensity of 1400 µmol photons m^{-2} s^{-1}. The CO_2 concentration inside the leaf chamber was set at 400 µmol mol^{-1}, and the flow rate was 500 µmol s^{-1}. This was accomplished by using the CO_2 mixer. The following measurements were made after the system achieved a steady state: net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s). The apparent carboxylation efficiency (P_n/C_i) was calculated by dividing P_n to C_i values.

2.4. Leaf Pigment Content

At T1, T2, and T3, DUALEX® Scientific analyser (Force-A, Orsay, France) was used to analyse the pigment content through non-destructive measurements (n = 30). The measurements were carried out on the adaxial leaf side. The instrument provided values of chlorophyll (Chl) content index, flavonol content (Flav) index, and nitrogen balance index (NBI).

2.5. Fruit Dry Matter, Soluble Solid Content (SSC), and Titratable Acidity (TA)

Fresh fruit was subjected to the dry matter percentage evaluation by calculating the ratio:

$$(\text{Dry weight}/\text{Fresh weight}) \times 100$$

As fresh weight and dry weight stand for the weight value before and after drying fruit material in an oven (Memmert GmbH Co., KG Universal Oven UN30, Schwabach, Germany) at 105 °C until the constant weight was reached. The SSC (n = 9) was determined with a refractometer (ATC, Polsinelli srl, San Giuliano Terme, Italy) and expressed as °Brix. The TA (n = 9) was analysed homogenising 1 g of fresh fruit sample with 30 mL of distilled H_2O, titrated with 0.1 M NaOH, reaching pH 8 through the use of a pH meter (XS Instruments, Modena, Italy). The results were expressed as mg malic acid 100 g^{-1}.

2.6. Fruit Extraction for Flavonoid (TFC) Content and Antioxidant Activity Assays

Frozen fruit samples were subjected to extraction for the determination TFC and the antioxidant activity assays. About 0.1 g of fresh fruit material was combined with 1 mL of methanolic solution 80% (*v*/*v*). The homogenates were subjected to 30 min of sonication at 4 °C. Following this, the homogenates were centrifuged at 4 °C for 10 min at 10,000× *g* using a laboratory centrifuge (MPW 260R, MWP Med. instruments, Warsaw, Poland).

2.7. TFC Analysis

TFC was calculated using the procedure outlined by Silva et al. [27]. In summary, 400 µL of distilled water was added after mixing a volume (100 µL) of the obtained fruit extract with 30 µL of a 5% (*w*/*v*) $NaNO_2$ aqueous solution. Thirty microlitres of 10% (*w*/*v*)

$AlCl_3$ was added after five minutes to initiate the production of the aluminium–flavonoid complex. An amount of NaOH 1 M and 240 µL of distilled water were added to the mixture after 6 min to reach 1 mL of final volume in the cuvette. The blank solution was prepared for each replicate containing all the reagents except for $AlCl_3$ 10% (*w*/*v*) aqueous solution that was replaced with distilled water. The absorbance of samples was registered at 510 nm using a spectrophotometer (Ultrospec 2100 Pro, GE Healthcare Ltd., Chalfont, Buckinghamshire, UK). All the obtained results (n = 15) were explicated as mg rutin equivalents per g fresh weight (mg RE g^{-1} FW).

2.8. Antioxidant Activity and Total Anthocyanin Content (TAC) Assays

The antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [28] and the 2,29-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay [29].

For the DPPH assay, a methanolic solution of 3.12 × 10^{-5} M DPPH was prepared. Then, an amount of each extract was mixed with a specific volume of DPPH solution and the blank was represented by 1 mL of DPPH methanolic solution. The absorbance was spectrophotometrically carried out at 515 nm against blank.

In order to conduct the ABTS test, a 5 mM Na-P buffer solution (pH 7.4) containing 2.5 mM $K_2S_2O_8$ and 7 mM ABTS was made. Subsequently, 950 µL of ABTS solution was combined with 50 µL of fruit extract, and the reaction's kinetics were monitored spectrophotometrically for 90 s at 734 nm. The data obtained (n = 10) from the two experiments measuring antioxidant activity were explicated as mg Trolox equivalents per g FW, or mg TE g^{-1} FW.

For TAC determination, 0.1 g of fresh fruit sample was homogenised with 1% (*v*/*v*) acidified methanolic solution and centrifuged for 15 min at 10,000× *g* and 4 °C. The TAC assay was carried out following a spectrophotometric pH differential method [30]. The acquired data (n = 15) were expressed as mg cyanidin-3-glu g^{-1} FW.

2.9. UHPLC-HR-ESI-Orbitrap/MS Chemical Profile

Approximately 2 g of fruit samples underwent ultrasound-assisted extraction using 6 mL of an 80% (*v*/*v*) aqueous ethanol solution for 15 min at 20 °C to analyse their chemical profile. After centrifugation at 4000× *g* for 5 min, the supernatant from each sample was filtered through Phenex™ Teflon® (PTFE; Phenomenex, Bologna, Italy) filter membranes (0.45 µm pore size, 47 mm diameter). Subsequently, 500 µL of the supernatant was diluted with 500 µL of pure methanol for further analysis. The chemical composition was analysed using ultra-high performance liquid chromatography (UHPLC) equipped with a Vanquish Flex Binary pump, diode array detector (DAD), and a high-resolution mass spectrometer (HR-MS) Q Exactive Plus Orbitrap-based FT-MS with an electrospray ionisation (ESI) source (Thermo Fisher Scientific Inc., Bremen, Germany). The HR-MS spectra were collected in both negative and positive ion modes, covering an *m*/*z* range of 135–2000, with ionisation parameters optimised as previously reported [31]. The data were analysed using Xcalibur 4.1 software.

2.10. Statistical Analysis

The obtained data in terms of plant and leaf morphological parameters, fruit yield and organoleptic or nutraceutical properties, as well as specialised metabolite content, were elaborated and checked for normality of distribution (Shapiro–Wilk test, 95% confidence interval). Then, one-way ANOVA was performed using the film type as the variability factor. The least significant difference (LSD) Fisher post-hoc ($p < 0.05$) test was used to identify significant differences among the effects of the films. The type of film and time were used as variability factors in a two-way ANOVA on physiological data (gas exchange analysis and pigment content). The LSD Fisher post-hoc ($p < 0.05$) test was used to identify significant differences among the treatments. For the statistical analysis, GraphPad software 9 (GraphPad, La Jolla, CA, USA) was used.

3. Results

3.1. Light Downconversion Technology Characteristics

For all three light downconversion films, there was a noticeable absorption of the ultraviolet light range (between 300 and 390 nm; red film: −75.8%; pink film: −75.7%; blue film: −80.8%; Figure 2). When compared to the Cnt film, the blue film dramatically boosted the intensity of the blue light spectrum (in the region of 420–495 nm) by a noteworthy 9% (Figure 2). In contrast, the red film increased the red light spectrum by 7.9% when compared to the Cnt film. This red light spectrum includes wavelengths that range from 600 to 700 nm. It is interesting to note that the pink film outperformed the Cnt film in both the red and blue light spectra, showing gains of 3.9% and 5.6%, respectively.

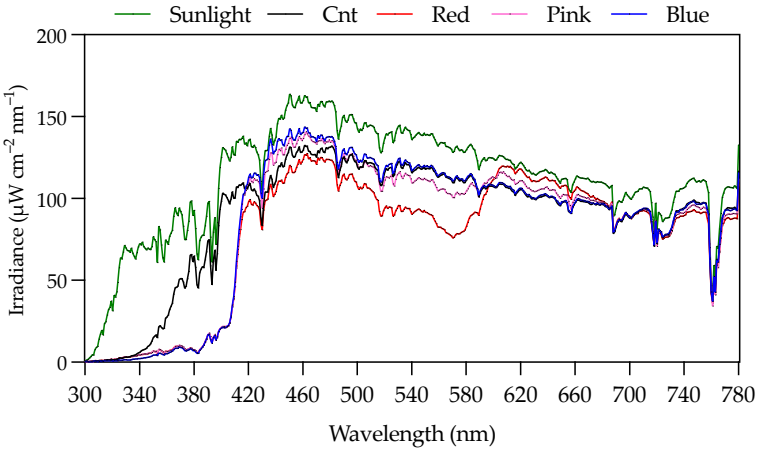

Figure 2. Spectral presentation of the light downconversion red, pink, and blue films in comparison with the control (Cnt; no light downconversion effect) used in the experiment when exposed to sunlight and their effect on the transmitted wavelengths inside the tunnel.

3.2. Results of Plant Morphological Parameters

Table 1 reports the values of the main plant morphological parameters. The plant biomass for plants grown under the three films was not significantly different from the Cnt plants, even though a significant decrease was observed in plant biomass for plants grown under the pink film as compared with the other two films. Similar results were obtained for leaf dry matter that resulted again in lower values for plants grown under the pink film (−10.65%) when compared to Cnt plants. No significant differences were found in terms of stem dry matter (Table 1).

Table 1. Plant biomass, leaf and stem dry matter, leaf mass area (LMA), leaf thickness, stem length, flower number, and flower fecundity rate of blackberry plants grown under control (Cnt), red, pink, and blue films. Means were subjected to one-way ANOVA with type of film as the source of variation. Means flanked by the same letter are not statistically different for $p = 0.05$ after Fisher's least significant difference post-hoc test.

	Cnt	Red	Pink	Blue
Plant biomass (kg plant^{-1})	0.17 ± 0.02 ab	0.20 ± 0.02 a	0.14 ± 0.04 b	0.21 ± 0.02 a
Leaf dry matter (%)	40.0 ± 1.3 a	40.9 ± 1.0 a	35.7 ± 1.4 b	40.5 ± 0.4 a
Stem dry matter (%)	37.8 ± 1.3 a	38.0 ± 5.8 a	34.2 ± 6.0 a	37.6 ±2.1 a
LMA (g m^{-2})	36.3 ± 8.8 ab	30.6 ± 3.1 b	25.7 ± 8.4 b	45.1 ± 4.4 a
Leaf thickness (mm)	0.39 ± 0.01 d	0.51 ± 0.01 a	0.48 ± 0.03 b	0.43 ± 0.01 c
Stem length (m)	1.5 ± 0.1 ab	1.4 ± 0.2 bc	1.5 ± 0.2 a	1.3 ± 0.1 c
Flower number (n° plant^{-1})	289.0 ± 31.8 a	288.2 ± 12.2 a	315.3 ± 13.9 a	206.6 ± 14.0 b
Flower fecundity rate (%)	11.4 ± 2.2 c	28.2 ± 0.7 a	26.6 ± 2.7 a	18.3 ± 3.3 b

The LMA of plants grown under all the light downconversion films were not statistically different when compared to Cnt plants (Table 1). However, plants grown under the blue film exhibited a higher LMA when compared to plants grown under the red and pink films. The leaf thickness was significantly improved in plants under the red film (+31% compared to the Cnt film), followed by those grown under the pink film (+23% compared to Cnt) and then the blue film (+10% compared to Cnt). The stem length was not significantly different in plants under the red and pink film as compared with the control, whereas the blue film induced the shortest stem length (−13.6% when compared to Cnt film).

The flower number was only lower in plants grown under the blue film when compared with Cnt plants and with the other two films. Moreover, in comparison with the control, plants grown under the red and pink films exhibited the highest flower fecundity rate followed by plants grown under the blue film, which also exceeded the control flower fecundity rate.

3.3. Gas Exchange Analysis Results

Figure 3 shows the results of the leaf gas exchange measurements of blackberry plants grown under light downconversion films at T1, T2, and T3. At T1 and T2, plants grown under the blue and red films had higher P_n values than Cnt plants. However, at T3, plants grown under the blue film had higher P_n values (+24.4%) than the Cnt plants, whilst those grown under the red film reported similar P_n values when compared to controls. Plants grown under the pink film exhibited the lowest P_n values at T2 and T3 (Figure 3A). The blue film induced the highest g_s values during all plant stages as compared with other treatments. Plants grown under the red film had similar g_s values to the control plants at T1 and T3, but at T2 they exhibited higher g_s values (+40.82%) than Cnt plants. Plants grown under the pink film had similar g_s values to Cnt plants, except for at T3, where the use of the pink film induced a reduction (−34.11%) in g_s values compared to the Cnt film (Figure 3B).

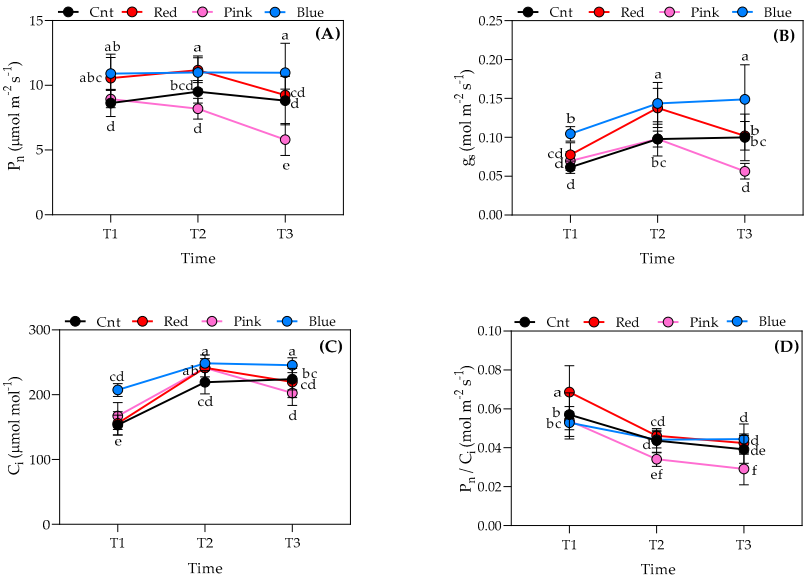

Figure 3. Net photosynthesis (P_n; (**A**)), stomatal conductance (g_s; (**B**)), intercellular CO_2 concentration (Ci; (**C**)), and apparent carboxylation efficiency (P_n/C_i; (**D**)) in control film (no light downconversion effect; Cnt), red film (Red), pink film (Pink), blue film (Blue) of blackberry leaves, measured at vegetative (T1), flowering (T2), and fruiting (T3) stages. Means were subjected to two-way ANOVA with type of film and time as the source of variations. Means flanked by the same letter are not statistically different for $p = 0.05$ after Fisher's least significant difference post-hoc test.

During all plant phenological stages, the blue film led to higher C_i values (+35.22%, +13.35%, and 9.63%, respectively) than the Cnt film (Figure 3C). In T1, plants grown under the red and pink films reported similar C_i values than those grown under the Cnt film, whilst

at T2 the red and pink films induced higher C_i values than the Cnt film (+10.1% and +9.9%, respectively) (Figure 3C). Finally, the red film induced the highest P_n/C_i values at T1, but during the other phenological stages no differences were found when compared to the use of the Cnt film. In all stages, the blue film induced similar P_n/C_i values to the Cnt film, whilst the pink film induced lower P_n/C_i values than the Cnt film only at T2 and T3 (Figure 3D).

3.4. Leaf Pigment Content Results

At T1, the Chl content was lower in plants grown under the red and blue films than in those grown under the Cnt film (Figure 4A). At T2, the pink film induced the highest Chl content, whilst the red and blue films led to a similar Chl content to the Cnt film (Figure 4B). At T3, plants grown under the pink film had the highest Chl values, followed by the red and then the blue films in comparison with those grown under the Cnt film (Figure 4C).

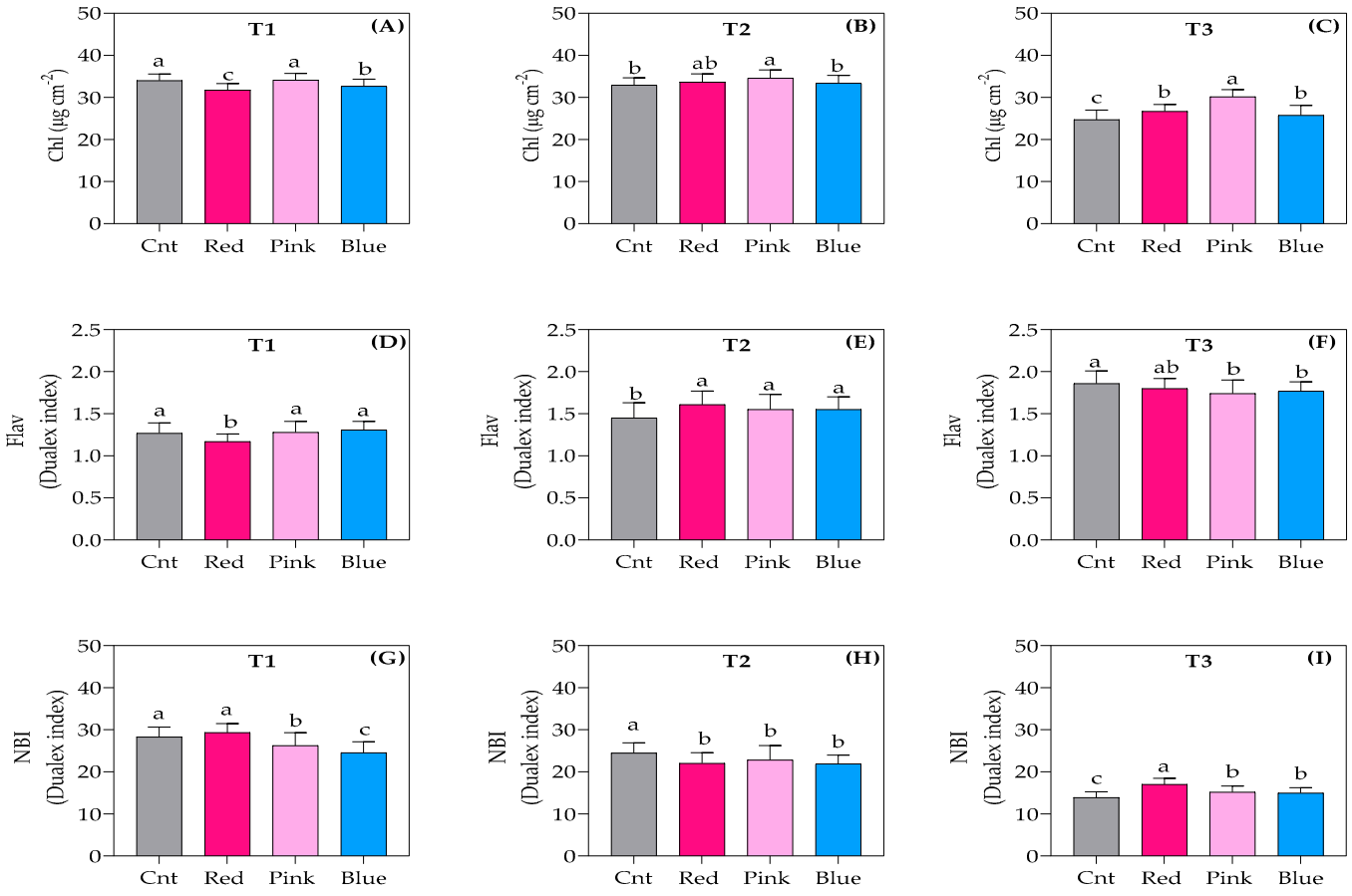

Figure 4. Leaf chlorophyll content index (Chl; (**A**–**C**)), leaf flavonol content index (Flav; (**D**–**F**)) and leaf Nitrogen Balance Index (NBI; (**G**–**I**)) in control film (no light downconversion effect; Cnt), red film (Red), pink film (Pink), and blue film (Blue) of blackberry leaves measured at vegetative (T1), flowering (T2), and fruiting (T3) stages. Means were subjected to one-way ANOVA with type of film as the source of variation. Means flanked by the same letter are not statistically different for $p = 0.05$ after Fisher's least significant difference post-hoc test.

At T1, the lowest Flav index was found in plants grown under the red film and no differences were reported for the pink and blue films as compared with the Cnt film (Figure 4D). At T2 (Figure 4E), all light downconversion films induced an increase in the Flav index when compared to the Cnt film, but this behaviour was not detected at T3, when plants under all light downconversion films had a lower Flav index than those under the Cnt film, except for those under the red film, which reported a similar value to control plants (Figure 4F).

At T1, plants under the pink and blue films exhibited lower NBI values than those under the Cnt film (Figure 4G), whilst at T2, all light downconversion films induced lower NBI values than the Cnt film (Figure 4H). At T3, a general decrease in the NBI was observed in plants under all treatments and the red film induced the highest NBI values, followed by the pink and blue films (Figure 4I).

3.5. Fruit Yield and Morphological Parameters

The fruit yield, number, weight, and size showed significant differences among the use of different light downconversion films (Table 2). The fruit yield of plants cultivated under the red and blue films was 1.32- and 1.13-fold higher, respectively, than that of plants grown under the Cnt film, while no differences in terms of fruit yield was observed for plants grown under the pink film. However, a significant and sustained increase in terms of the fruit number was registered exclusively under the red film (+50.2%) as compared with the Cnt film (Table 2).

The blue film led to a significant increase (+26.9%) in fruit weight in comparison with the control, but no differences were observed for the fruit of plants grown under the red and pink films. Moreover, fruit grown under the blue film also showed the highest fruit height and a similar fruit height was recorded in the fruit of plants grown under the red film. With regard to the berry shape index, the blue film induced the highest value as compared with other films that did not differ from each other.

Table 2. Fruit yield, number, weight, width, and height and berry shape of blackberry plants grown under control film (no light downconversion effect; Cnt), red film (Red), pink film (Pink), blue film (Blue). Means were subjected to one-way ANOVA with type of film as the source of variation. Means flanked by the same letter are not statistically different for $p = 0.05$ after Fisher's least significant difference post-hoc test.

	Cnt	**Red**	**Pink**	**Blue**
Fruit yield (g plant^{-1})	80.2 ± 13.4 b	186.0 ± 31.4 a	110.8 ± 33.3 b	171.0 ± 38.7 a
Fruit number (n° plant^{-1})	36.7 ± 4.9 b	73.0 ± 12.2 a	34.7 ± 2.1 b	48.3 ± 5.9 b
Single fruit weight (g)	2.3 ± 0.1 b	2.6 ± 0.4 b	2.4 ± 0.2 b	3.2 ± 0.1 a
Fruit width (mm)	13.8 ± 0.4 a	14.8 ± 1.4 a	13.8 ± 1.0 a	15.6 ± 0.6 a
Fruit height (mm)	15.5 ± 0.7 b	16.7 ± 1.9 ab	15.4 ± 0.5 b	18.1 ± 0.7 a
Berry shape index	1.11 ± 0.01 b	1.14 ± 0.01 b	1.11 ± 0.03 b	1.24 ± 0.07 a

3.6. Fruit Organoleptic Quality

The fruit dry matter, SSC, and TA are reported in Figure 5. Fruit grown under the red film exhibited the highest dry matter, followed by those grown under the pink film, with values higher (+26.8% and +16.7%, respectively) than for the Cnt film. Fruit grown under the blue film had a similar dry matter to the Cnt fruit (Figure 5A). The values of SSC recorded in fruit grown under the red film were significantly higher (+18.7%) than those of fruit grown under the Cnt film, but no differences with the controls were observed for SSC in the fruit of plants grown under the blue and pink films (Figure 5B). All light downconversion films induced a similar TA to the Cnt film.

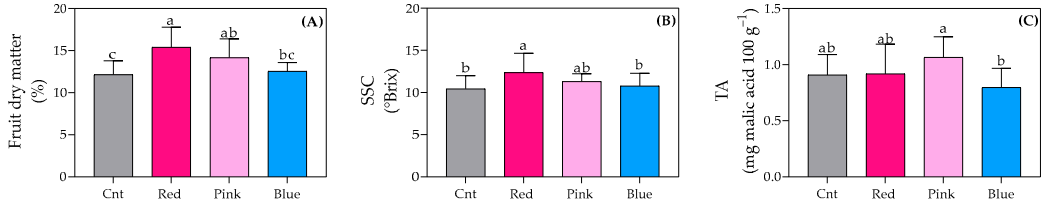

Figure 5. Fruit dry matter (**A**), soluble solid content (SSC; (**B**)), and titratable acidity (TA; (**C**)) of blackberry fruit grown under control film (no light downconversion effect; Cnt), red film (Red), pink film (Pink), and blue film (Blue). Means were subjected to one-way ANOVA with type of film as the source of variations. Means flanked by the same letter are not statistically different for $p = 0.05$ after Fisher's least significant difference post-hoc test.

3.7. Fruit Nutraceutical Quality

Fruit nutraceutical properties of fruit grown under downconversion film are reported in Figure 6. No significant differences were found in terms of TFC, TAC and antioxidant activity analysed using both the two assays DPPH and ABTS among all treatments and the use of Cnt film (Figure 6A–D).

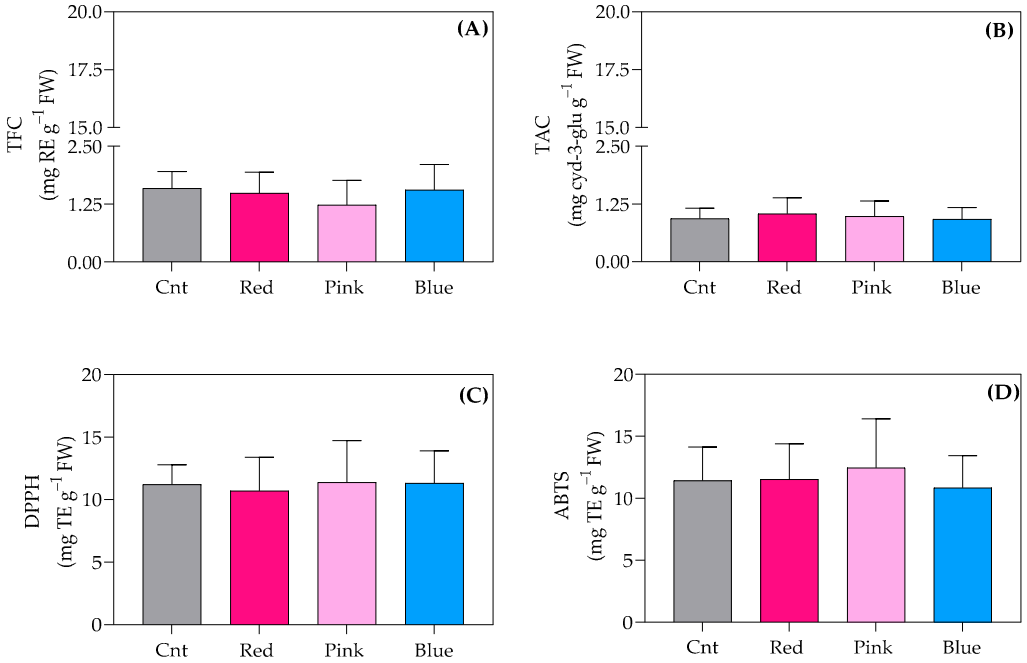

Figure 6. Total flavonoid content (TFC; (**A**)), total anthocyanin content (TAC; (**B**)), and antioxidant activity (DPPH (**C**) and ABTS (**D**)) of blackberry fruit grown under control film (no light downconversion effect; Cnt), red film (Red), pink film (Pink), and blue film (Blue). Means were subjected to one-way ANOVA with type of film as the source of variation. The absence of letters on the bars means the absence of significant differences among films for $p = 0.05$ after Fisher's least significant difference post-hoc test.

3.8. Chemical Fingerprint of Fruit Using UHPLC-HR-ESI-Orbitrap/MS Analysis

The obtained LC-MS chromatograms exhibited similar qualitative profiles among all analysed blackberry samples (Supplementary Material—Figures S1 and S2). Comparing retention times, molecular formula, measured accurate mass, HR full mass spectra, and fragmentation patterns with data reported in the literature [25,32–35] and considering a mass error < 5 ppm on the molecular formula, a total of 43 compounds were tentatively identified as specialised metabolites of blackberry fruit. Tables S1 and S2 report the identified molecules that exhibited significant differences among treatments and belong to different chemical classes: catechins and proanthocyanidins (in the form of dimers and

trimers), ellagic acid conjugates, ellagitannins (especially sanguiin isomers), flavonoids (quercetin derivatives), hydroxycinnamic acids (*p*-coumaric and chlorogenic acids), and anthocyanins (cyanidin, pelargonidin, and delphinidin glycosides). The use of films did not yield many benefits compared to the Cnt film in the production of other metabolites. Indeed, fruit grown under the pink and red films showed a significant decrease in the content of anthocyanins, especially cyanidin derivatives (cyanidin 3-*O*-glucoside, cyanidin xyloside, cyanidin malonylhexoside), compared with Cnt fruit. Moreover, once plants were subjected to blue, red or pink films, a significant decrease in the content of ellagic acid derivatives, ellagitannins, and flavonoids was observed compared to the Cnt film. Nonetheless, the use of blue and pink films promoted the biosynthesis and accumulation of chlorogenic acid isomers and epicatechin, respectively, compared to the Cnt film.

4. Discussion

4.1. Red and Blue Films Preserved Plant Morphology, Boosting Photosynthesis and Flower Fertility

The light spectrum variations in terms of quantity can notably affect plant growth in different species (as reviewed by Landi et al. [7]). Indeed, thanks to the alteration of sunlight amount perceived by crops, plant developmental and growth pathways are activated to mediate root growth, flowering time, or shade avoidance responses mainly through phytochromes (red light receptors) and to modulate phototropism, chloroplast movement, and stomatal opening mainly through cryptochromes (blue light receptors) [36,37]. Despite these well-known aspects, in the present experiment, few significant differences were observed in blackberry plants in terms of plant growth parameters among the red or blue light downconversion films compared to the Cnt one, but the light downconversion developed by the films utilised in the present work demonstrated major effects on flowering and fruit productivity. Plants grown under the red and pink film produced a similar number of flowers per plant but had a higher flower fecundity percentage compared to those grown under the Cnt film. In contrast, plants grown under the blue film had fewer flowers than the Cnt group but exhibited higher fertility, resulting in a similar number of fruits per plant as the Cnt plants. The registered fecundity pattern might be due to a greater attraction of pollinating insects to the colour of the film similar to that of flowers. The visual effect of flowers displayed in colour is a pivotal signal in plant–pollinator interactions and the colour of the film can be attractive to pollinators [38].

The photosynthetic performance of blackberry plants is conducive to plant growth and physiologic performance during three phenological phases of blackberry growth, flowering, and fruiting [19]. In the present study, during all three phenological stages, the blackberry plants grown under a blue film outperformed the Cnt plants in terms of CO_2 assimilation, which was also justified by higher and more stable g_s values (Figure 3). These results were confirmed by our previous study on strawberry plants [17]. The results obtained in both studies indicate that plants grown under a blue film improved the CO_2 assimilation rate due to the increase in stomatal conductance. The comparison between the effect of a supplemental or a monochromatic light environment and the use of light downconversion films of the present experiment results was forced by the lack of literature regarding the effect of these films on crop physiology and productivity. For example, Lauria et al. [39] observed higher g_s and C_i in green and leafed basil plants grown under supplemental blue light in a greenhouse when compared to control plants, demonstrating an increased stomatal number and, consequently, a higher stomatal density that induced higher g_s values. Moreover, Dewir et al. [36] observed a higher stomata aperture in blackberry micro-shoots grown in vitro in a fully LED light environment composed of 2:1 blue/red ratio. Both these experiments attributed the obtained results to the role of blue photoreceptors such as cryptochromes [37,40]. For red film, the higher P_n values observed at the vegetative stage might be due to the increased efficiency in the photo-assimilation of CO_2 pathways, as suggested by the increase in the apparent carboxylation efficiency (P_n/C_i). In contrast, during the flowering stage, the high P_n values observed in plants under the red film can be traced back to the stomatal opening. Moreover, a noticeable

photosynthesis rate was also a driver for biomass accumulation in cabbage and lettuce seedlings thanks to the increase in the fraction of orange-red sunlight of the red film [40]. In our case, the reduction in terms of UV light thanks to the selective ability of the red and blue films might have positively influenced the plant photosynthetic performance. In fact, blackberry plants were not exposed to UV radiation known to inhibit the level of growth stimulators, consequently reducing plant growth [41]. Other authors found similar results in plants grown in a supplemental red light environment [42]. These authors found an increase in photosynthetic performance in mini-cucumber seedlings grown in a greenhouse environment enriched with a 4:1 red/blue light ratio (220 μmol m^{-2} s^{-1}) and a photoperiod of 12 h. Some authors, by using photoselective films and analysing basic growth parameters in different plant species, evidenced an increase in the chlorophyll content in leaves of *Eustoma grandiflorum* and *Thymus vulgaris*, partially attributing these results to the role of phytochromes [43,44]. These findings confirmed our results in terms of chlorophyll content under the red film (at T2 and T3). The pink film induced similar P_n and g_s values to the Cnt film at T1 and T2, whilst at T3 blackberry plants under the pink film showed lower P_n and g_s values than the Cnt film combined with similar C_i values. This behaviour could be explained by the presence of stomatal and biochemical limitations which can induce an accumulation of CO_2 in the leaf intercellular airspace, despite low g_s values. The biochemical limitations could involve the Calvin–Benson cycle, but also other CO_2 biochemical mechanisms engaging available intercellular CO_2 such as the co-regulation of g_s and P_n, the roles of CO_2, abscisic acid, and H_2O_2 (as reviewed by Damour et al. [45]).

4.2. Red and Blue Films Did Not Alter Blackberry Fruit Organoleptic and Nutraceutical Quality, Enhancing Fruit Productivity

The fruit productivity findings evidenced that blackberry plants grown under both blue and red films registered an almost 2-fold higher fruit yield than controls thanks to an enhanced berry shape index for the blue film and an increased fruit number for the red film. This result is remarkable in terms of the economic sustainability of the use of downconversion films. More specifically, fruit grown under the blue film had a greater berry shape index and a higher fruit weight compared to fruit grown under the Cnt film, combined with a lower dry matter content. This behaviour could be a result of the low density of flowers per plant allowed by larger internodes that gave fruit more space to develop in height, consequently obtaining an improved berry shape compared to Cnt fruit. Similar results were found by Yoshida et al. [46]. Indeed, these authors noted an advanced flowering of strawberry plants under monochromatic blue light together with a significant increase in the fruit yield [46]. Contrastingly, the berry shape index of fruit grown under the red film was not different from that of Cnt fruit, but the fruit number doubled and the fruit dry matter increased. These findings were confirmed by Thwe et al. [47], who observed that tomato fruit grown under a red film was significantly larger, with a 13% increase in the fruit yield.

Phytochemical studies of *R. fruticosus* fruit reported a highly complex and varying pool of bioactive compounds [25,33]. For example, an analysis of methanolic extracts of blackberry fruit showed several nutraceutical compounds belonging to the main classes of phenols, and chlorophyll breakdown products [33]. In this study, both the TFC and the TAC of fruit grown under all downconversion films did not exhibit any significant differences in comparison with the Cnt blackberry fruit. These results were also confirmed by the metabolomic profile, which reported no significant differences for 14 identified metabolites and a significant decrease in flavonoid and anthocyanin identified compounds, especially in fruit from plants grown under the pink film (Tables S1 and S2). Therefore, we can also affirm that the light downconversion films did not enable blackberries to have antioxidant activity variation in contrast with the control. These results could be linked to the blackberry variety Loch Ness, as supported by the findings of Huang et al. [48], who analysed different blackberry varieties and blackberry–raspberry hybrids, and showed

a specific correlation between the antioxidant activity and the rest of the fruit secondary metabolites contents. For example, the total antioxidant capacity of Boysen was positively correlated with the flavonoid and glutathione contents, whereas the total antioxidant capacity of Hull was positively correlated with the phenolic compounds [48]. Despite the non-significant differences in terms of flavonoids, anthocyanins, and antioxidant activity, and the decrease in the accumulation of specific secondary metabolites in fruit under the pink film, a positive result in terms of antioxidant compound accumulation is the increase in the epicatechin content. Indeed, this compound is considered as the most frequent flavonol in blackberry fruit along with catechin and their polymerised forms [49]. However, the homogeneity of results in terms of the accumulation of secondary metabolites in fruit under all films and the increase in the fruit yield under the red and blue films is an interesting and promising result in the adoption of these coloured films.

5. Conclusions

The present experiment was an opportunity to evaluate sunlight alteration conditions using light downconversion films in which blackberry plants were grown. The response of *R. fruticosus* var. Loch Ness in terms of photosynthetic activity and the subsequent effect on fruit production and nutritional composition properties in blackberries when grown under light downconversion films was detected. In terms of overall responses of *R. fruticosus* var. Loch Ness to the applied light downconversion films, the red and blue films succeeded in improving the leaf photosynthesis and overall fruit biomass. In this regard, the fruit production boost was manifested in terms of fruit number under the red film, whereas the blue film improved the fruit berry shape without manifesting any direct alteration on the nutraceutical fruit quality. These results validate the promising efficiency of the employment of blue and red films in berry fruit growing settings to enhance the fruit production and photosynthetic performance of blackberry plants. Further research could be useful to find a compromise between the plant grown and fruit productivity and the enhancement of nutraceutical properties of the fruit, and, in this direction, the use of coverings at different plant stages, avoiding the use of light downconversion films during all plant stages, can be an opportunity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10101046/s1, Figure S1: LC-MS chromatograms; Figure S2: LC-MS profiles; Table S1: Compounds tentatively identified through UHPLC-HR-ESI-Orbitrap/MS analysis in *Rubus fruticosus* L. fruit of plants grown under control film (no light downconversion effect; Cnt), Red film (Red), Pink film (Pink), Blue film (Blue); Table S2: Anthocyanins tentatively identified using UHPLC-HR-ESI-Orbitrap/MS analyses in *Rubus fruticosus* L. fruit of plants grown under control film (no light downconversion effect; Cnt), Red film (Red), Pink film (Pink), Blue film (Blue).

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