agronomy

MDPI

Article

# Pre-Harvest UV-B Radiation and Photosynthetic Photon Flux Density Interactively Affect Plant Photosynthesis, Growth, and Secondary Metabolites Accumulation in Basil (*Ocimum basilicum*) Plants

**Haijie Dou [1], Genhua Niu [2,*] and Mengmeng Gu [3]**

[1] Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843, USA
[2] Department of Horticultural Sciences, Texas A&M AgriLife Research & Extension, The Dallas Center, Texas A&M University, 17360 Coit Road, Dallas, TX 75252, USA
[3] Department of Horticultural Sciences, Texas A&M AgriLife Extension Service, College Station, TX 77843, USA
* Correspondence: gniu@ag.tamu.edu; Tel.: +1-915-859-9111

Received: 19 July 2019; Accepted: 5 August 2019; Published: 7 August 2019


**Abstract:** Phenolic compounds in basil (*Ocimum basilicum*) plants grown under a controlled environment are reduced due to the absence of ultraviolet (UV) radiation and low photosynthetic photon flux density (PPFD). To characterize the optimal UV-B radiation dose and PPFD for enhancing the synthesis of phenolic compounds in basil plants without yield reduction, green and purple basil plants grown at two PPFDs, 160 and 224 μmol·m$^{-2}$·s$^{-1}$, were treated with five UV-B radiation doses including control, 1 h·d$^{-1}$ for 2 days, 2 h·d$^{-1}$ for 2 days, 1 h·d$^{-1}$ for 5 days, and 2 h·d$^{-1}$ for 5 days. Supplemental UV-B radiation suppressed plant growth and resulted in reduced plant yield, while high PPFD increased plant yield. Shoot fresh weight in green and purple basil plants was 12%–51% and 6%–44% lower, respectively, after UV-B treatments compared to control. Concentrations of anthocyanin, phenolics, and flavonoids in green basil leaves increased under all UV-B treatments by 9%–18%, 28%–126%, and 80%–169%, respectively, and the increase was greater under low PPFD compared to high PPFD. In purple basil plants, concentrations of phenolics and flavonoids increased after 2 h·d$^{-1}$ UV-B treatments. Among all treatments, 1 h·d$^{-1}$ for 2 days UV-B radiation under PPFD of 224 μmol·m$^{-2}$·s$^{-1}$ was the optimal condition for green basil production under a controlled environment.

**Keywords:** UVR8; PPFD; dose-dependent; photosynthesis; chlorophyll fluorescence; phenolic compounds

## 1. Introduction

Decreasing arable land, rising urbanization, water scarcity, and climate change exert pressure on agricultural producers [1]. Conventional food production is severely limited by seasonality, unpredictable weather, pests/diseases, and resources such as land and water. Indoor controlled environment agriculture (CEA) systems, which can be built anywhere, have the potential to be a suitable alternative to open field and greenhouse production [2]. However, crops cultivated in indoor CEA systems using artificial lighting are not exposed to ultraviolet radiation. Ultraviolet (UV) radiation is an important environmental signal that initiates plant responses in photosynthetic function, cell division, plant growth, and development [3,4]. In previous studies, UV-B radiation was mainly considered as a stress factor to plants, focusing on the effects of increasing solar UV-B radiation reaching Earth's surface due to stratospheric ozone depletion [5,6]. Recent studies have highlighted

supplemental UV-B radiation as a eustress (i.e., positive stress), and reported that low to moderate UV-B radiation induces a range of favorable processes in plants, such as synthesis of UV-absorbing compounds (anthocyanin, phenolic acids, and flavonoids) and antioxidants (carotenoids, ascorbate, and glucosinolate) [7–9]. These bioactive compounds represent an important source of antioxidant molecules in human diet reducing the risk of cardiovascular diseases, chronic diseases, and specific forms of cancer [10,11].

Manipulation of secondary metabolites in horticultural crops through supplemental UV-B radiation have demonstrated at least two UV-B signaling pathways, which is determined by UV-B radiation dose [11,12]. Under low UV-B radiation dose, the UV-B specific photoreceptor, UV RESISTANCE LOCUS 8 (*UVR8*), initiates an *UVR8*-dependent pathway [13]. Specifically, *UVR8* stimulates gene expression such as CONSTITUTIVELY PHOTOMORPHOGENIC 1 (*COP1*), ENLONGATED HYPOCOTYL 5 (*HY5*), and HY5 HOMOLOG (*HYH*), which play key roles in the synthesis of phenolic compounds, as well as growth retardation such as the inhibition of hypocotyl elongation [14,15]. Under high UV-B radiation dose, UV-B light acts as a damaging agent inducing formation of reactive oxygen species (ROS), causing damage to plant cells, DNA, proteins, and photosynthesis apparatus and, subsequently, negatively affect plant growth and induces synthesis of antioxidants [16,17].

In addition to being dose-dependent, plant responses to supplemental UV radiation also varied among species and cultivars [18]. For example, anthocyanin concentration of red leaf lettuce (*Lactuca sativa*, 'Red Cross') increased by 11% after 12-days UV-A radiation at 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$ prior to harvest (controlled environment, PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) [19]. Synthesis of anthocyanin and other polyphenols in another red leaf lettuce cultivar ('Red Fire', controlled environment, PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) significantly increased after 3-days UV-B radiation at a much lower dose, 1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$ prior to harvest [4]. Furthermore, glucosinolate concentration in 7-day-old broccoli (*Brassica oleracea*) sprouts (controlled environment, PPFD not mentioned) was enhanced by 19% after 1-day UV-B radiation at 7.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 $h \cdot d^{-1}$, compared to 63% enhancement at 10.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 $h \cdot d^{-1}$ [9].

Basil (*Ocimum basilicum*) plants have been considered a source of valuable healthy substances due to their unique flavor and relatively high content of phenolic compounds [20,21]. To improve the yield of high-quality basil, more growers are turning to controlled environment production, which has been proven to be a suitable alternative to open field and greenhouse basil production, due to its high environmental controllability and improved resource utilization efficiency (arable land and clean water) [2,22]. However, crops cultivated in controlled environment systems using artificial lighting are not exposed to UV-B radiation, bearing a direct impact on basil flavor and visual appearance [10]. Meanwhile, considering energy saving, the photosynthetic photon flux density (PPFD) in controlled environment systems is much lower compared to sunlight intensity in open field, resulting in further reduction of secondary plant metabolites [21]. Therefore, there is an increasing interest in the use of supplemental UV-B radiation to enhance the synthesis of health-beneficial phenolic compounds to produce premium quality basil products under controlled environment [3,23,24].

Although some studies investigated the effects of supplemental UV-B radiation on phytochemical accumulation of basil plants, most studies were conducted in the open field or greenhouse using photo-selective film covers, and results varied largely in both biomass production and phenolic contents [25–27]. Meanwhile, most studies only focused on the effects of UV-B radiation on secondary metabolites accumulation, not considering yield reduction caused by UV-B radiation [25,28]. Furthermore, considering the significantly low PPFD used in controlled environment systems, little information is known about the interactive effects between supplemental UV-B radiation and PPFD. Collectively, to identify the optimal combination of UV-B radiation dose and PPFD that enhance concentrations of phenolic compounds without significant yield reduction, further investigation is warranted to characterize the physiological, morphological, and biochemical responses in basil plants to supplemental UV-B radiation and different PPFDs under a controlled environment.

Accordingly, in the present study, we exposed two basil cultivars to five pre-harvest supplemental UV-B radiation doses in order to characterize plant responses to supplemental UV-B radiation under two PPFDs in a controlled environment system. Photosynthetic photon flux density of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for basil plants was selected according to our previous study [21], and a low PPFD of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was selected to test if UV-B radiation can compensate for the reduced accumulation of phenolic compounds in basil plants grown under low PPFD.

## 2. Materials and Methods

### *2.1. Plant Materials and Culture*

Experiments were conducted in a walk-in growth room in Texas AgriLife Research Center at El Paso, TX, USA, from 8 August to 15 September 2017 on green basil 'Improved Genovese Compact' and from 5 September to 19 October 2017 on purple basil 'Red Rubin' (Johnny's Selected Seeds, Winslow, ME, USA), respectively. For both experiments, one basil seed per cell was sown in 72 square cell trays (length 3.86 cm; height 5.72 cm; volume 59 $cm^3$) with Metro-Mix® 360 (peat moss 41%, vermiculite 34%, pine bark 25%, Sun Gro® Horticulture, Bellevue, WA, USA). All trays were put under mist in a greenhouse for germination. Temperature under the mist was maintained at 32.7 °C/22.2 °C day/night. Seedlings were moved out from the mist after the emergence of cotyledons and grown in a greenhouse for two weeks. Temperature and relative humidity in the greenhouse were maintained at 29.1 °C/21.6 °C and 48%/66% day/night, respectively. When one pair of true leaves fully expanded, basil seedlings were transplanted into square pots (length 9.52 cm, height 8.26 cm, and volume 574 $cm^3$) filled with the Metro-Mix® 360, and uniform plants were selected and moved to the walk-in growth room for different treatments.

After transplanting, multi-layer cultivating shelves were used with mechanical mini fans (LS1225A-X, AC Infinity, City of Industry, CA, USA) circulating air to achieve uniform temperatures across treatments. Plant canopy temperature in each treatment was maintained at 23.9 °C/21.2 °C day/night. All plants were manually sub-irrigated with a nutrient solution containing 1.88 $g \cdot L^{-1}$ (277.5 ppm N) 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special, The Scotts Company, Marysville, OH, USA) as needed. The nutrient solution was mixed and stored in a 100-gallon tank with a lid, and the electrical conductivity (EC) and pH were adjusted to 2.0 $dS \cdot m^{-1}$ and 6.0, respectively, using an EC/pH meter (Model B-173, Horiba, Ltd., Japan).

### *2.2. Supplemental Ultraviolet B (UV-B) Radiation and Photosynthetic Photon Flux Density (PPFD) Treatments*

Uniform green and purple basil plants were grown under two PPFDs of 160 and 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod provided by cool white fluorescent lamps (Philips Lighting, Somerset, NJ, USA). Two or five days prior to harvest (basil plant height reaching about 25 cm), UV-B lamps were switched on and basil plants were treated with one of the five UV-B radiation doses including no supplemental UV-B radiation (control), 1 $h \cdot d^{-1}$ for 2 days (1H2D), 2 $h \cdot d^{-1}$ for 2 days (2H2D), 1 $h \cdot d^{-1}$ for 5 days (1H5D), or 2 $h \cdot d^{-1}$ for 5 days (2H5D) with UV-B light intensity at 16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equal to 18.7 $kJ \cdot m^{-2} \cdot h^{-1}$). There were a total of 10 treatments created by the combination of two PPFDs and five UV-B radiation doses, and 12 plants per treatment. Supplemental UV-B radiation treatments were applied from 8:00 in the morning and provided by Philips TL 40W/12 and 20W/12 UV-B broadband lamps (wavelength: 270–400 nm, maximum emission wavelength at 315 nm, Svetila.com d.o.o., Domzale, Slovenia, EU). The cool white fluorescent lamps at PPFD of 160 and 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ radiated low intensity of UV radiation, which was 2.2 and 2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The UV-B light intensity (including UV radiation provided by broadband UV-B lamps and cool white fluorescent lamps) and PPFD in each treatment were measured at 15 cm underneath the lamps at 9 spots using a MU-200 UV radiation meter (Apogee Instruments, Logan, UT, USA) and PS-100 spectroradiometer (Apogee Instruments, Logan, UT, USA), respectively, before placing the plants. To minimize the disproportionate light distribution within each treatment, all plants were systematically rearranged every 3 days.

### *2.3. Measurements*

#### 2.3.1. Growth Parameters

Growth parameters of basil plants such as plant height, width, the number of internodes, leaf area, and yield including shoot fresh weight (FW) and dry weight (DW) were recorded at harvest (on 15 September and 19 October 2017 for green and purple basil plants, respectively). Plant width was calculated as the average of the widest point and its perpendicular width of plant canopy. A leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA) was used to measure the leaf area. Shoot DW was determined after shoot tissues were dried at 80 °C in an oven (Grieve, Round Lake, IL, USA) for 3 days. Specific leaf area (leaf area per unit leaf dry weight) was calculated as an indicator of leaf thickness.

#### 2.3.2. Gas-Exchange Rate, Relative Chlorophyll Concentration, and Chlorophyll Fluorescence

A portable gas exchange analyzer (CIRAS-3, PP Systems International, Amesbury, MA, USA) was used to measure the gas exchange rate, including net photosynthetic rate ($P_n$), transpiration rate (E), and stomatal conductance ($G_s$) of basil leaves at harvest. A PLC3 leaf cuvette with light-emitting diode (LED) light unit (white light, in which the proportions of red, blue, and green light were 38%, 25%, and 37%, respectively) was used. The PPFD, temperature, relative air humidity, and $CO_2$ concentration inside the leaf cuvette were set at 800 μmol·m$^{-2}$·s$^{-1}$, 25 °C, 50%, and 390 μmol·mol$^{-1}$, respectively. The third pair of leaves from the top was used for measuring and measurements were taken until the $P_n$ reached a steady state.

Soil plant analysis development (SPAD) index of basil leaves was recorded on the third pair of leaves from the top at harvest to quantify the relative chlorophyll concentration of basil leaves using a chlorophyll meter SPAD-502 (Konica-Minolta cooperation, Ltd., Osaka, Japan). Three measurements were taken for each leaf and the average was recorded for data analysis.

Chlorophyll fluorescence parameters of basil plants were measured at harvest using a pocket Plant Efficiency Analyzer chlorophyll fluorimeter (PEA, Hansatech Instruments Ltd., Norfolk, UK). The third pair of leaves from the top were dark adapted for at least 30 min prior to the measurement. Minimal fluorescence values ($F_0$) and maximal fluorescence values ($F_m$) in the dark-adapted state were measured, and maximum quantum use efficiency of photosystem II (PSII) in the dark-adapted state was calculated as $F_v/F_m = (F_m - F_0)/F_m$. Performance index (PI ABS, where "ABS" specifies that the reaction centers' density is expressed per absorption), dissipation of energy per cross section ($DI_0$/CS), trapped energy flux per cross section ($TR_0$/CS), and electron transport flux per cross section ($ET_0$/CS) parameters were calculated using the PEA Plus software (V1.10, Hansatech Instruments Ltd., Norfolk, UK).

#### 2.3.3. Secondary Plant Metabolites

Five basil plants were randomly selected for the measurement of concentrations of anthocyanin, phenolics, and flavonoids, and antioxidant capacity of basil leaves at harvest. Fresh basil leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A, Thermo Fisher Scientific, Marietta, OH, USA) at −80 °C until phytochemical evaluation.

Extraction. Approximately 2 g fresh basil leaves were ground in liquid nitrogen and extracted with 15 mL 1% acidified methanol at 4 °C in dark. After overnight extraction, the mixture was centrifuged (Sorvall RC 6 Plus Centrifuge, Thermo Fisher Scientific, Madison, WI, USA) at 13,200 rpm (26,669× *g*) for 15 min, and the supernatant was collected for phytochemical evaluation [29].

Anthocyanin evaluation. Absorbance of the extract was measured at 530 nm using a spectrophotometer (Genesys 10S ultraviolet/Vis, Thermo Fisher Scientific, Madison, WI, USA), and anthocyanin concentration was expressed as mg cyanidin-3-glucoside equivalent per 100 g FW of basil leaves using a molar extinction coefficient of 29,600 [30].

Phenolics evaluation. A modified Folin-Ciocalteu reagent method [29] was used to determine the phenolics concentration of basil leaves: 100 μL extraction sample was added to a mixture of 750 μL

1/10 dilution Folin–Ciocalteau reagent and 150 µL distilled water. After 6 min reaction, 600 µL 7.5% $Na_2CO_3$ was added and the mixture was incubated at 45 °C in a water bath for 10 min before the absorbance was measured at 725 nm using a microplate reader (EL×800, BioTek, Winooski, VT, USA). Results were shown as mg of gallic acid equivalent per g FW of basil leaves.

Flavonoids evaluation. Flavonoid concentration of basil leaves was determined [21] as the following: 20 µL extraction sample was added to a mixture of 85 µL distilled water and 5 µL 5% $NaNO_2$. After 6 min reaction, a 10 µL of 10% $AlCl_3 \cdot 6H_2O$ was added to the mixture. After another 5 min reaction, 35 µL of 1M NaOH and 20 µL distilled water were added to the mixture and the absorbance was measured at 520 nm using the aforementioned microplate reader. Results were shown as mg of (+)-catechin hydrate equivalent per g FW of basil leaves.

Antioxidant capacity evaluation. A 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method [31] was used to determine the antioxidant capacity of basil leaves: 150 µL extracted sample was added to 2.85 mL of $ABTS^+$ solution and incubate at room temperature for 10 min. The absorbance of mixed solution was measured at 734 nm using the aforementioned spectrophotometer. Results were shown as mg of Trolox equivalent antioxidant capacity per 100 g FW of basil leaves.

### *2.4. Statistical Analyses*

Experiments were arranged in a two factors factorial design. Five plants per treatment were randomly selected for measurement. After verifying the significance of the two main factors (UV-B and PPFD) and their interaction (PPFD × UV-B), a one-way analysis of variance among 10 treatments was conducted for green and purple basil plants, respectively, according to Student's *t* method ($p < 0.05$). Some data were pooled from two PPFDs because effect of PPFD was not statistically significant. Pairwise correlations method ($p < 0.05$) was used to test correlations between parameters. All statistical analyses were performed using JMP software (Version 13, SAS Institute Inc., Cary, NC, USA).

## 3. Results

### *3.1. Gas Exchange Rate, Relative Chlorophyll Concentration, and Chlorophyll Fluorescence*

Supplemental UV-B radiation suppressed plant photosynthesis, in which $P_n$, E, and $G_s$ in both basil cultivars were lower compared to plants grown under control, while PPFD showed no effects (Table 1). In green and purple basil leaves, $P_n$, E, and $G_s$ was 68%/70%, 55%/68%, and 65%/76% lower under treatment 2H5D compared to plants grown under control, respectively. Relative chlorophyll concentration of green and purple basil plants was 9%–15% and 6%–8% lower under supplemental UV-B radiation compared to plants grown under control, respectively, while PPFD showed no effect on green basil plants but increased relative chlorophyll concentration in purple basil plants (Figure 1).

Supplemental UV-B radiation inhibited plant chlorophyll fluorescence parameters in green basil plants, including $F_v/F_m$ and PI ABS. However, in purple basil plants, $F_v/F_m$ showed no differences between control and 1H2D treatment, and PI ABS was only lower under the highest UV-B radiation dose, 2H5D treatment (Figure 2A,B). Similarly, $TR_0/CS$ and $ET_0/CS$ in green basil plants were lower after UV-B radiation, while they were not affected by UV-B radiation in purple basil plants (Figure 2D,E). On the contrary, $DI_0/CS$ in purple basil plants was significantly higher under treatments 1H5D and 2H5D, while in green basil plants no treatment effect was observed (Figure 2C). Chlorophyll fluorescence parameters in basil plants were not affected by PPFD.

**Table 1.** Net photosynthetic rate ($P_n$), transpiration rate (E), and stomatal conductance ($G_s$) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under five supplemental UV-B radiation treatments, including no supplemental UV-B radiation (control), 1 h·d$^{-1}$ for 2 days (1H2D), 2 h·d$^{-1}$ for 2 days (2H2D), 1 h·d$^{-1}$ for 5 days (1H5D), and 2 h·d$^{-1}$ for 5 days (2H5D).

| Cultivar | Treatment | $P_n$ (μmol·m$^{-2}$·s$^{-1}$) | | E (mmol·m$^{-2}$·s$^{-1}$) | | $G_s$ (mmol·m$^{-2}$·s$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| Green Basil | Control | 13.2 | a [z] | 2.76 | a | 130 | A |
| | 1H2D | 7.8 | B | 1.74 | bc | 79 | B |
| | 2H2D | 8.5 | B | 1.93 | b | 93 | ab |
| | 1H5D | 7.4 | B | 1.82 | b | 71 | B |
| | 2H5D | 4.2 | C | 1.24 | c | 46 | C |
| Purple Basil | Control | 7.4 | A | 2.73 | A | 131 | A |
| | 1H2D | 4.3 | B | 1.49 | B | 60 | B |
| | 2H2D | 3.1 | C | 1.20 | B | 42 | CD |
| | 1H5D | 3.8 | BC | 1.33 | B | 49 | BC |
| | 2H5D | 2.2 | D | 0.86 | C | 31 | D |

Data were pooled from two photosynthetic photon flux density (PPFD) treatments. [z] Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$).

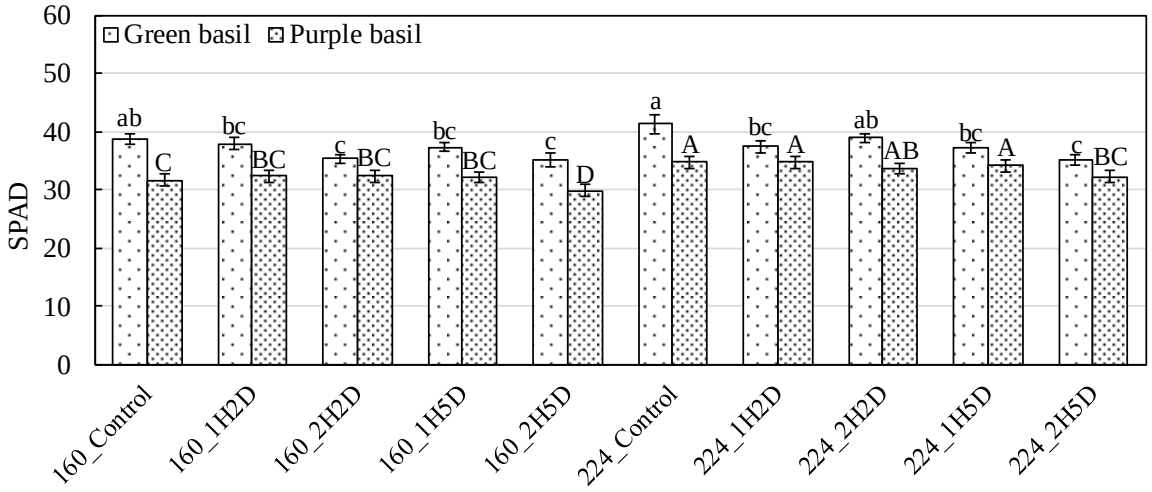


**Figure 1.** Relative chlorophyll concentration (soil plant analysis development (SPAD) index) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants at different treatments. There were 10 treatments created by the combination of two photosynthetic photon flux density (PPFD) of 160 and 224 μmol·m$^{-2}$·s$^{-1}$ and five ultraviolet B (UV-B) radiation treatments, including no supplemental UV-B radiation (control), 1 h·d$^{-1}$ for 2 days (1H2D), 2 h·d$^{-1}$ for 2 days (2H2D), 1 h·d$^{-1}$ for 5 days (1H5D), and 2 h·d$^{-1}$ for 5 days (2H5D). Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$). Bars represent standard errors.

### 3.2. Growth Parameters and Crop Yield

Supplemental UV-B radiation inhibited plant growth in both basil cultivars and performed as lower plant height, width, and leaf area, and the detriment increased with increasing UV-B radiation doses (Table 2). Specifically, under high PPFD (224 μmol·m$^{-2}$·s$^{-1}$), plant height of both basil cultivars was the highest under treatments control and 1H2D, followed by treatments 2H2D and 1H5D, and the lowest under treatment 2H5D. Leaf area of green/purple basil plants was 14%/17%, 28%/30%, 28%/34%, and 44%/44% lower, respectively, under treatments 1H2D, 2H2D, 1H5D, and 2H5D compared to control. Specific leaf area (leaf area per unit leaf dry weight) was calculated and used as an indicator of leaf thickness. In the present study, specific leaf area of both basil cultivars was lower under supplemental UV-B radiation, indicating increased leaf thickness after supplemental UV-B radiation (Table 2). Under

higher UV-B radiation doses such as 1H5D and 2H5D treatments, basil plants also showed leaf bronze, chlorosis, waxy appearance, and premature leaf defoliation (Figure 3).

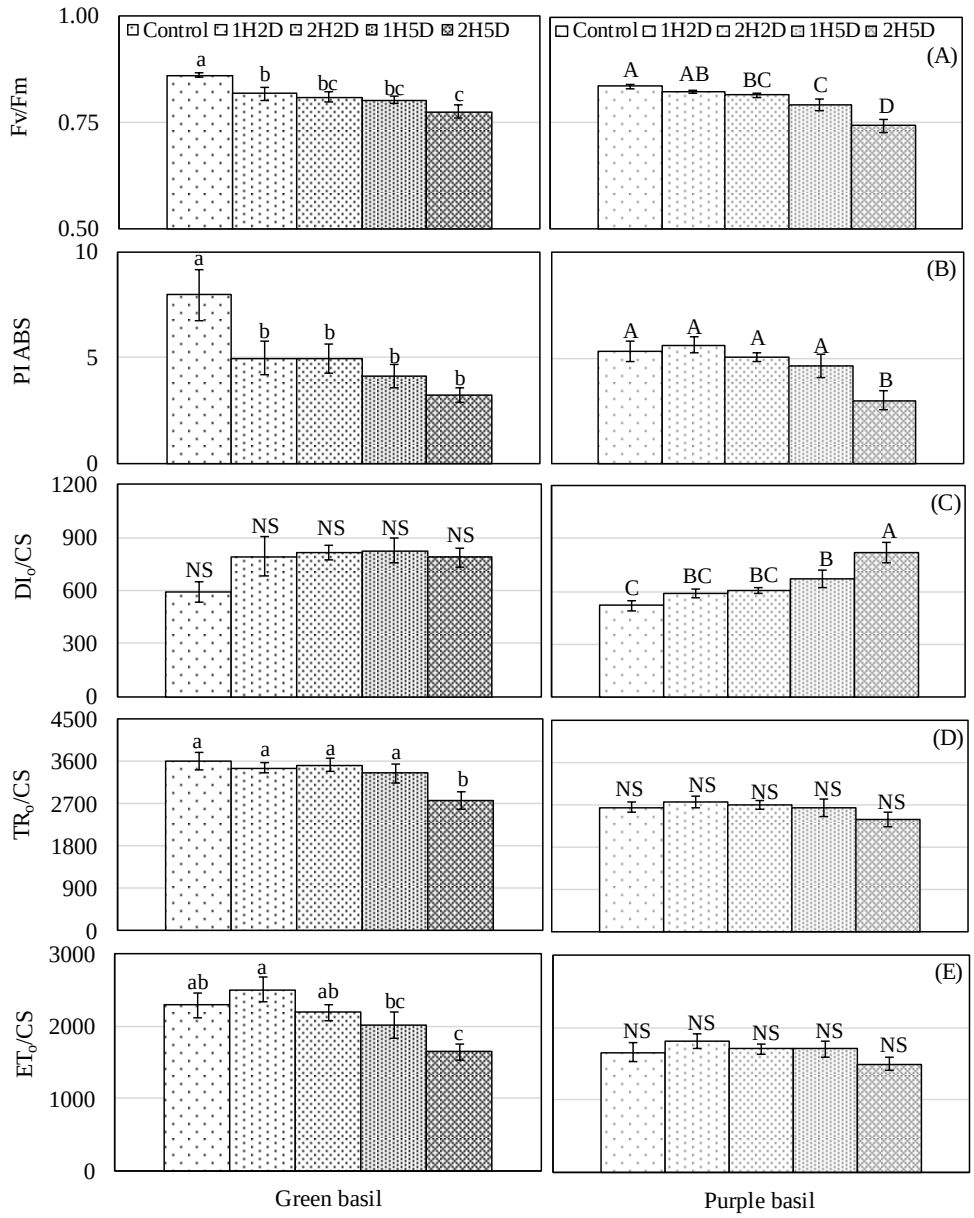


**Figure 2.** Chlorophyll fluorescence parameters, including maximal photochemical efficiency of Photosystem II ($F_v/F_m$) (**A**), performance index (PI ABS, where "ABS" specifies that the reaction centers' density is expressed per absorption) (**B**), dissipation of energy per cross section ($DI_0/CS$) (**C**), trapped energy per cross section ($TR_0/CS$) (**D**), and electron transport flux per cross section ($ET_0/CS$) (**E**) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different supplemental UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D. Data were pooled from two photosynthetic photon flux density (PPFD) treatments. Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$). Bars represent standard errors.

**Table 2.** Plant height, width, leaf area, and specific leaf area of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different treatments. There were 10 treatments created by the combination of two photosynthetic photon flux density (PPFD) levels of 160 and 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and five UV-B irradiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D.

| Cultivar. | Treatment | Height (cm) | | Width (cm) | | Leaf Area ($cm^2$) | | Specific Leaf Area ($cm^2 \cdot g^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| Green Basil | 160_Control | 18.3 | bc [z] | 11.9 | ab | 520 | bc | 531 | a |
| | 160_1H2D | 17.8 | Cd | 12.1 | ab | 453 | cdef | 497 | abc |
| | 160_2H2D | 16.7 | D | 11.6 | bc | 420 | ef | 528 | a |
| | 160_1H5D | 17.2 | Cd | 11.7 | b | 421 | def | 502 | ab |
| | 160_2H5D | 14.4 | E | 10.6 | d | 315 | g | 446 | d |
| | 224_Control | 21.7 | A | 12.1 | ab | 687 | a | 454 | d |
| | 224_1H2D | 21.3 | A | 12.3 | a | 591 | b | 513 | a |
| | 224_2H2D | 19.6 | B | 12.3 | a | 497 | cd | 477 | bcd |
| | 224_1H5D | 19.6 | B | 12.0 | ab | 494 | cde | 466 | cd |
| | 224_2H5D | 16.7 | d | 11.0 | cd | 387 | fg | 450 | d |
| | PPFD | *** | | ** | | *** | | *** | |
| | UV-B | *** | | *** | | *** | | *** | |
| | PPFD × UV-B | NS | | NS | | NS | | ** | |
| Purple Basil | 160_Control | 15.6 | BC | 16.0 | A | 261 | BC | 610 | A |
| | 160_1H2D | 15.4 | C | 15.5 | A | 217 | DE | 553 | BC |
| | 160_2H2D | 15.1 | C | 15.4 | A | 212 | DE | 575 | AB |
| | 160_1H5D | 14.6 | C | 15.2 | A | 221 | D | 545 | BC |
| | 160_2H5D | 12.9 | D | 13.8 | B | 176 | F | 530 | C |
| | 224_Control | 17.7 | A | 16.0 | A | 332 | A | 558 | BC |
| | 224_1H2D | 17.2 | A | 16.1 | A | 274 | B | 542 | BC |
| | 224_2H2D | 16.8 | AB | 15.7 | A | 233 | CD | 531 | CD |
| | 224_1H5D | 15.5 | BC | 16.0 | A | 219 | DE | 534 | C |
| | 224_2H5D | 14.6 | C | 14.0 | B | 187 | EF | 490 | D |
| | PPFD | *** | | NS | | *** | | *** | |
| | UV-B | *** | | *** | | *** | | *** | |
| | PPFD × UV-B | NS | | NS | | * | | NS | |

[z] Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$). Asterisks (*) indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). NS indicates non-significant differences (* $p < 0.05$).

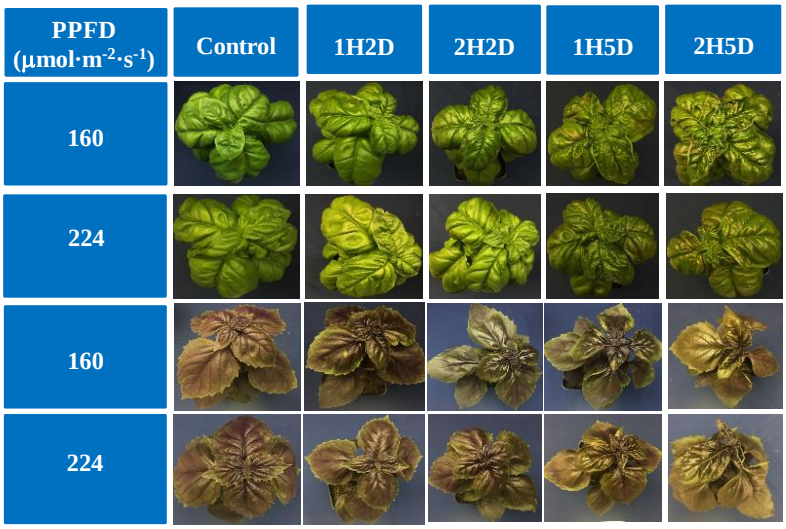


**Figure 3.** Green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different treatments at harvest. There were 10 treatments created by the combination of two photosynthetic photon flux density (PPFD) levels of 160 and 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D.

Shoot FW and DW of green and purple basil plants were generally lower in plants grown under supplemental UV-B treatments, and interactive effects (UV-B × PPFD) were observed on shoot FW ($p$ = 0.01) and shoot DW ($p$ = 0.02) in purple basil plants, while only interactions in shoot DW were observed in green basil plants ($p$ = 0.03). Specifically, under low PPFD (160 μmol·m$^{-2}$·s$^{-1}$), treatment 1H2D showed no effects on shoot FW in green basil plants. So did the 1H2D and 1H5D treatments in purple basil plants, while under high PPFD (224 μmol·m$^{-2}$·s$^{-1}$), shoot FW in both cultivars was lower under UV-B treatments compared to control (Figure 4A,B).

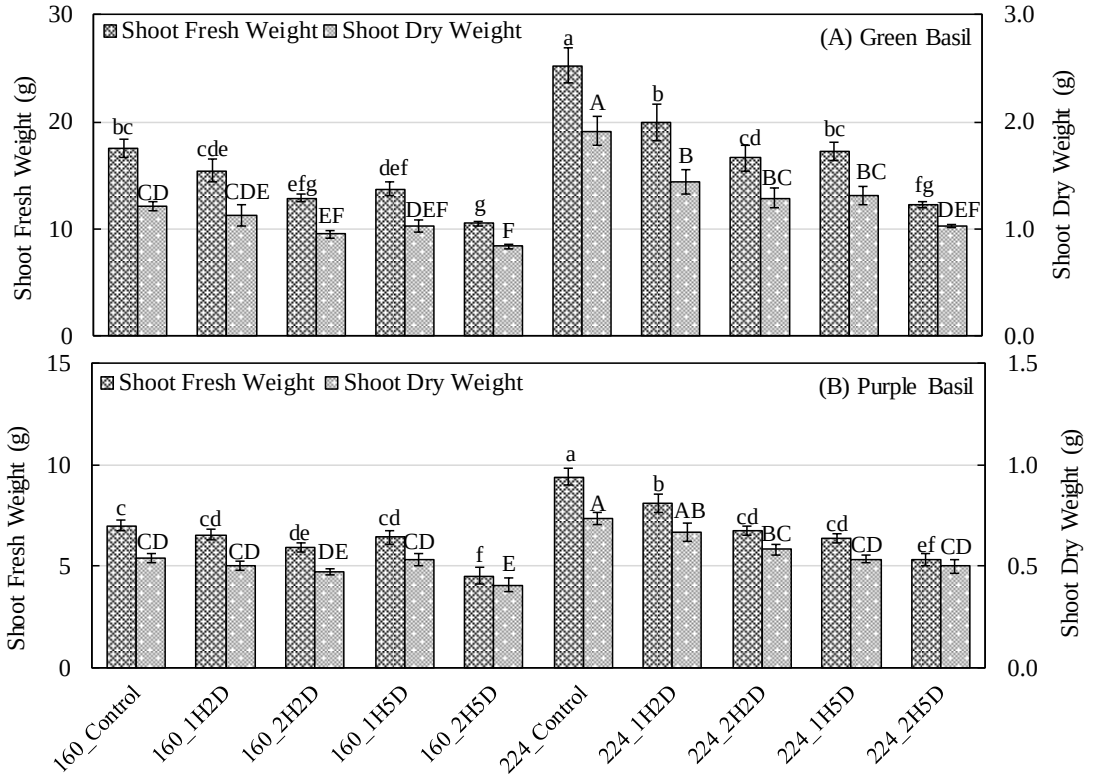


**Figure 4.** Shoot fresh weight and shoot dry weight of green basil 'Improved Genovese Compact' plants (**A**), and purple basil 'Red Rubin' plants (**B**) under different treatments. There were 10 treatments created by the combination of two photosynthetic photon flux density (PPFD) of 160 and 224 μmol·m$^{-2}$·s$^{-1}$ and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D. Means followed by the same lower/upper case letters are not significantly different for green/purple plants, according to Student's $t$ mean comparison ($p < 0.05$). Bars represent standard errors.

Plant height, leaf area, leaf thickness, shoot FW, and shoot DW in both basil cultivars were higher under high PPFD (Table 2, Figure 4A,B). Without supplemental UV-B treatments, plant height, leaf area, leaf thickness, shoot FW, and shoot DW in green/purple basil plants were 16%/12%, 24%/21%, 15%/9%, 44%/34%, and 59%/35% higher under high PPFD (224 μmol·m$^{-2}$·s$^{-1}$) compared to plants grown under low PPFD (160 μmol·m$^{-2}$·s$^{-1}$), respectively.

### 3.3. Secondary Plant Metabolites Accumulation and Antioxidant Capacity

Concentrations of phenolic compounds in green basil plants, including anthocyanin, phenolics, and flavonoids were 9%–23%, 28%–126%, and 80%–169% greater, respectively, after UV-B radiation compared to control (Table 3). Concentrations of anthocyanin and flavonoids in green basil plants were not affected by PPFD, while phenolics concentration was greater under high PPFD (224 μmol·m$^{-2}$·s$^{-1}$). In purple basil plants, only 2 h·d$^{-1}$ UV-B treatments (2H2D and 2H5D) enriched concentrations of phenolics and flavonoids, while UV-B treatments showed no effects on anthocyanin concentration (Table 3). Specifically, under 2H2D and 2H5D treatments, concentrations of phenolics and flavonoids in

purple basil plants were 29%–63% and 37%–79% greater, respectively. Concentrations of anthocyanin and phenolics in purple basil plants were greater under high PPFD (224 μmol·m$^{-2}$·s$^{-1}$), while flavonoid concentration was not affected by PPFD (Table 3).

**Table 3.** Anthocyanin concentration (conc.), phenolics conc., and flavonoids conc. of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different treatments. There were 10 treatments created by the combination of two photosynthetic photon flux density (PPFD) of 160 and 224 μmol·m$^{-2}$·s$^{-1}$ and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D.

| Cultivar | Treatment | Anthocyanin Conc. (mg·100g$^{-1}$ FW) | | Phenolics Conc. (mg·g$^{-1}$ FW) | | Flavonoids Conc. (mg·g$^{-1}$ FW) | |
|---|---|---|---|---|---|---|---|
| Green Basil | 160_Control | 3.19 | d [z] | 1.10 | E | 0.45 | e |
| | 160_1H2D | 3.68 | Abcd | 1.41 | De | 0.92 | cd |
| | 160_2H2D | 3.92 | A | 1.48 | D | 0.81 | d |
| | 160_1H5D | 3.49 | Abcd | 1.68 | Cd | 1.00 | abcd |
| | 160_2H5D | 3.87 | Ab | 2.49 | A | 1.21 | a |
| | 224_Control | 3.29 | Cd | 1.38 | De | 0.54 | e |
| | 224_1H2D | 3.39 | Bcd | 2.06 | B | 0.97 | bcd |
| | 224_2H2D | 3.78 | abc | 1.95 | Bc | 0.99 | abcd |
| | 224_1H5D | 3.35 | bcd | 2.13 | Ab | 1.15 | abc |
| | 224_2H5D | 3.89 | ab | 2.34 | Ab | 1.19 | ab |
| | PPFD | NS | | *** | | NS | |
| | UV-B | ** | | *** | | *** | |
| | PPFD × UV-B | NS | | NS | | NS | |
| Purple Basil | 160_Control | 10.63 | A | 2.06 | CD | 0.94 | CD |
| | 160_1H2D | 11.02 | A | 1.63 | E | 0.82 | D |
| | 160_2H2D | 10.84 | A | 2.66 | B | 1.41 | B |
| | 160_1H5D | 10.74 | A | 2.18 | C | 1.14 | C |
| | 160_2H5D | 10.75 | A | 3.35 | A | 1.68 | A |
| | 224_Control | 10.97 | A | 2.03 | CD | 1.04 | C |
| | 224_1H2D | 11.43 | A | 1.93 | CD | 1.09 | C |
| | 224_2H2D | 10.97 | A | 2.62 | B | 1.49 | B |
| | 224_1H5D | 10.85 | A | 1.85 | DE | 1.03 | C |
| | 224_2H5D | 11.07 | A | 2.85 | B | 1.42 | B |
| | PPFD | * | | * | | NS | |
| | UV-B | NS | | *** | | *** | |
| | PPFD × UV-B | NS | | *** | | ** | |

[z] Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$). Asterisks (*) indicate significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). NS indicates non-significant differences (* $p < 0.05$).

The total amounts of phytochemicals per plant (i.e., anthocyanin, phenolics, and flavonoids) were calculated by multiplying the phytochemical concentrations by leaf FW per plant (Table 4). Under low PPFD (160 μmol·m$^{-2}$·s$^{-1}$), total amount of anthocyanin in green basil plants was 23% lower under treatment 2H5D compared to control, while total amounts of phenolics and flavonoids were 49%–79%% greater (Table 4). Under high PPFD (224 μmol·m$^{-2}$·s$^{-1}$), total amounts of anthocyanin and phenolics in green basil plants were 15%–39% lower under supplemental UV-B treatments compared to control, while total amount of flavonoids was 43%–44% higher under treatments 1H2D and 1H5D compared to control (Table 4). In purple basil plants, all supplemental UV-B radiation treatments showed negative or no effects on the total amount of phenolic compounds regardless of PPFD (Table 4).

**Table 4.** Total amount of anthocyanin, phenolics, and flavonoids per plant of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different treatments. There were 10 treatments created by the combination of two photosynthetic photon flux density (PPFD) of 160 and 224 μmol·m$^{-2}$·s$^{-1}$ and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D.

| Cultivar. | Treatment | Total Amount of Anthocyanin (mg·plant$^{-1}$) | | Total Amount of Phenolics (mg·plant$^{-1}$) | | Total Amount of Flavonoids (mg·plant$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| Green Basil | 160_Control | 0.47 | cde [z] | 16.0 | d | 6.6 | d |
| | 160_1H2D | 0.47 | Cde | 18.0 | d | 11.8 | b |
| | 160_2H2D | 0.42 | Def | 16.0 | d | 8.8 | cd |
| | 160_1H5D | 0.40 | Ef | 19.2 | cd | 11.4 | bc |
| | 160_2H5D | 0.36 | F | 23.8 | bc | 11.6 | bc |
| | 224_Control | 0.67 | A | 28.4 | ab | 10.8 | bc |
| | 224_1H2D | 0.55 | Bc | 33.2 | a | 15.4 | a |
| | 224_2H2D | 0.59 | Ab | 25.6 | b | 12.8 | ab |
| | 224_1H5D | 0.52 | Bcd | 31.0 | a | 15.6 | a |
| | 224_2H5D | 0.41 | Ef | 24.0 | bc | 12.2 | b |
| Purple Basil | 160_Control | 0.63 | C | 12.0 | BC | 5.6 | DE |
| | 160_1H2D | 0.58 | D | 8.6 | E | 4.2 | F |
| | 160_2H2D | 0.51 | E | 12.6 | BC | 6.0 | CDE |
| | 160_1H5D | 0.57 | D | 11.0 | CD | 5.2 | EF |
| | 160_2H5D | 0.38 | G | 11.4 | BC | 5.6 | DE |
| | 224_Control | 0.83 | A | 15.4 | A | 8.0 | A |
| | 224_1H2D | 0.72 | B | 12.2 | BC | 7.0 | ABC |
| | 224_2H2D | 0.57 | D | 13.0 | B | 7.2 | AB |
| | 224_1H5D | 0.54 | D | 9.4 | DE | 5.4 | DE |
| | 224_2H5D | 0.47 | F | 12.2 | BC | 6.4 | BCD |

[z] Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$).

Antioxidant capacity in basil plants were not affected by PPFDs. Antioxidant capacity in green basil plants was higher under all supplemental UV-B radiation treatments, while it was only higher under 2 h·d$^{-1}$ UV-B treatments (2H2D and 2H5D) in purple basil plants (Figure 5A). Correlation between antioxidant capacity and UV-B radiation doses was analyzed in three terms according to different UV-B radiation patterns, all UV-B treatments (Figure 5A), 1 h·d$^{-1}$ UV-B treatments (1H2D and 1H5D, Figure 5B), and 2 h·d$^{-1}$ UV-B treatments (2H2D and 2H5D, Figure 5C). Antioxidant capacity in green basil plants were all positively related to UV-B radiation doses regardless of radiation patterns, while antioxidant capacity in purple basil plants showed no correlation with 1 h·d$^{-1}$ UV-B radiation treatments (1H2D and 1H5D, $p = 0.1994$).

Correlation between antioxidant capacity with concentrations of phenolic compounds was analyzed in basil plants. In green basil plants, concentrations of anthocyanin, phenolics, and flavonoids were all positively related to antioxidant capacity (Figure 6A). In purple basil plants, concentrations of phenolics and flavonoids were positively related to antioxidant capacity, while anthocyanin concentration showed no relationship ($p = 0.8812$) (Figure 6B).

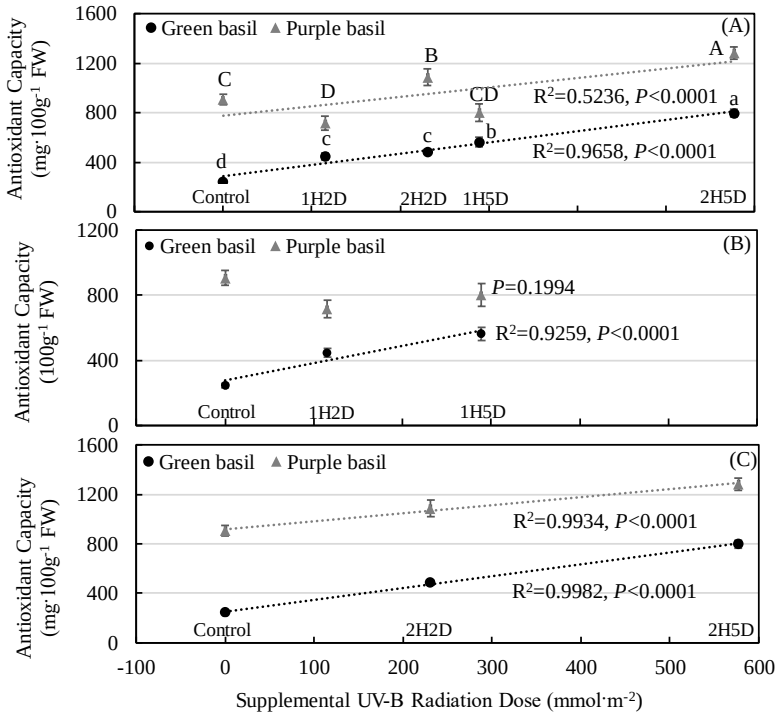


**Figure 5.** Correlation between antioxidant capacity of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants with UV-B radiation doses. Correlation test was conducted in three terms according to different UV-B radiation patterns, five supplemental UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D (**A**), control and 1 $h \cdot d^{-1}$ UV-B radiation treatments (**B**), and control and 2 $h \cdot d^{-1}$ UV-B radiation treatments (**C**). Data were pooled from two photosynthetic photon flux density (PPFD) treatments. Means followed by the same lower/upper case letters are not significantly different for green/purple basil plants, according to Student's *t* mean comparison ($p < 0.05$). Bars represent standard errors. Dashed lines show the regression between antioxidant capacity with supplemental UV-B radiation dose, according to the pairwise correlation method.

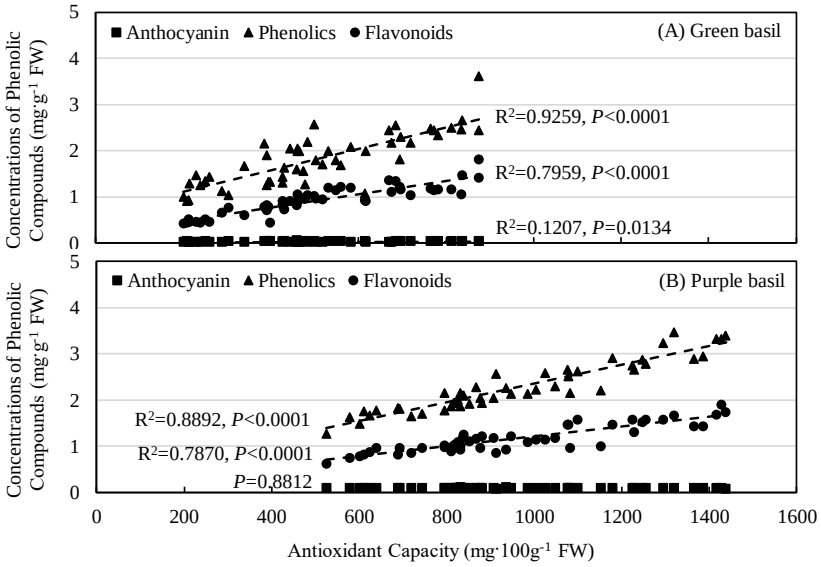


**Figure 6.** Correlation between antioxidant capacity and concentrations of anthocyanin, phenolics, and flavonoids in green basil plants (**A**), and purple basil plants (**B**). Dashed lines show the regression between concentrations of phenolic compounds with antioxidant capacity according to Pairwise Correlation method.

## 4. Discussion

### *4.1. Impacts of UV-B and PPFD on Photosynthesis, Relative Chlorophyll Concentration, and Chlorophyll Fluorescence*

Photosynthesis is one of the most sensitive metabolic processes in plants responding to environmental condition changes, such as supplemental UV-B radiation and PPFD. In the present study, $P_n$ in basil leaves was lower after UV-B radiation, which was mainly caused by the direct damage of PSII components and led to reduced photosynthetic capacity, subsequently decreased $G_s$ [32–34]. Meanwhile, relative chlorophyll content in basil leaves was also lower after UV-B radiation, either through degradation or inhibition of enzymes involved in the chlorophyll biosynthetic pathways [34]. However, compared to depressed photosynthesis and reduced chlorophyll content by supplemental UV-B radiation in our study, a meta-analysis of field studies (more than 450 reports from 62 papers) reported unaffected photosynthesis and chlorophyll content after supplemental UV-B radiation [35]. Differences between our study (controlled environment with artificial lighting) from previous field studies (sunlight) probably resulted from significantly low PPFDs and relatively high UV-B proportion used in our study. Firstly, in controlled environment systems, due to the high cost of powering artificial lighting, lower PPFDs are normally used compared to that of sunlight intensity in an open field. Subsequently, lower PPFDs resulted in depressed photochemical protection system of plants, such as decreased photosynthetic capacity, decreased leaf thickness, and reduced concentrations of UV-absorbing agents [21], which aggravated the negative effects caused by UV-B radiation. Secondly, the damage caused by UV-radiation increases with decreasing UV wavelength, since short UV wavelength has more energy than long UV wavelength [36]. The UV component of sunlight consists of 95% UV-A and 5% UV-B, of which the small portion UV-B radiation shows stronger mutagenic and carcinogenic effects compared to UV-A radiation [36,37]. For example, a less prominent and less long-lasting activation of p53 gene ("guardian of the genome") after UV-A radiation compared to UV-B was observed, suggesting stronger effects of UV-B radiation than UV-A [36]. In the present study, the UV radiation provided by broadband UV-B lamps was mainly UV-B radiation with relatively low UV-A radiation, contributing to aggravated negative effects on plant photosynthesis compared to previous field studies, of which mainly consists of UV-A radiation.

Chlorophyll fluorescence parameters provide precise and objective information with regard to photochemical efficiency and non-photochemical de-excitation involved in the conversion of light energy under different conditions [28,38]. The less reduced Fv/Fm, PI ABS, $TR_0$/CS, and $ET_0$/CS after UV-B radiation in purple basil plants than green basil plants clearly indicate that purple basil plants are more tolerant to UV-B radiation, resulted from its improved capacity to process excess UV-B energy through PSII [39]. Meanwhile, the uninfluenced $DI_0$/CS under UV-B treatments in green basil plants suggests its inability to dissipate absorbed UV-B energy in the form of harmless heat, even under the smallest UV-B radiation dose, 16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 1 $h \cdot d^{-1}$ for 2 days, while purple basil plants coped with excess UV-B energy by increasing heat dissipation. Mosadegh et al. (2018) also reported that the $DI_0$/CS of green basil plants was not affected after 2-weeks UV-B radiation at 68 and 102 $kJ \cdot m^{-2} \cdot d^{-1}$, confirming that green basil plants failed to dissipate UV-B energy as harmless heat [28]. Differences in chlorophyll fluorescence parameters between green and purple basil plants may be due to the relatively higher concentrations of UV-protective antioxidants in purple basil plants such as anthocyanins, phenolics, and flavonoids, which are known to provide plants with strong protection from excess UV-B energy [40].

In our previous study, the gas exchange rate in green basil plants was positively correlated with PPFD [21], while it was not affected in the present study. This may be due to the large variation of $P_n$, E, and $G_s$ caused by UV-B radiation at each PPFD. In green basil plants, $P_n$ ranged from 3.7 to 12.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at low PPFD (160 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and ranged from 4.8 to 13.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at high PPFD (224 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Also, it was observed that the $P_n$ in purple basil plants was much lower compared to the $P_n$ in green basil plants. One hypothesis is that the differences between two cultivars

is due to the lower quantum efficiency of photosynthetically active radiation (PAR) in purple basil plants compared to green basil plants. In purple basil plants, the relatively high concentration of anthocyanins and flavonoids absorbs more PAR light, which decreases the absorption of PAR light by chloroplasts and subsequently decreases the photochemistry energy transferred to reaction centers, resulting in decreased $P_n$ in purple basil plants compared to green basil plants [41].

### *4.2. Impacts of UV-B and PPFD on Growth and Yield*

Plant leaf expansion is invariably inhibited by supplemental UV-B radiation and other leaf morphogenesis changes such as reduced leaf area, increased leaf thickness, and accumulation of leaf surface waxes are also observed across a range of plant species [14,42,43]. Internode length is also a very sensitive growth parameter that responds to UV-B radiation [44]. Kaiserli (2018) reported that most cell-wall elongation genes induced by BRI1-EMS-SUPPRESSOR 1 (BES1) are negatively regulated by UV-B radiation [45]. Meanwhile, the biosynthesis and signaling of plant growth hormone auxin, a key regulator of stem elongation, was also suppressed in arabidopsis (*Arabidopsis thaliana*) and coriander (*Coriandrum sativum*) plants after UV-B radiation, thereby reducing plant stem elongation and promoting a compact phenotype [46]. In the present study, similar results such as reduced leaf area, increased leaf thickness, accumulation of leaf surface waxes, and reduced leaf internode length were observed, which are plant acclimation responses to supplemental UV-B radiation. In addition to protecting plants from receiving excess UV-B energy, these acclimation responses also provide plants with improved tolerance to other adverse environmental conditions, such as heat stress and mechanical handling during postharvest [6,47,48].

Reduced gas exchange rate and leaf expansion, and inhibition of stem elongation of basil plants under supplemental UV-B radiation resulted in a reduction in plant size and yield. The greater yield reduction by the UV-B radiation under high PPFD than low PPFD may be due to its taller plants, which shortened the distance between basil plants and UV-B light tube, resulting in increased UV-B radiation intensity sustained by basil plants, and subsequently severer yield reduction.

### *4.3. Impacts of UV-B and PPFD on Phytochemical Accumulation and Antioxidant Capacity*

Across a range of plant species, phenolic compounds, especially flavonoids, act as efficient UV-screening agents to reduce excess UV light received by photosynthetic tissues to protect plants from possible harm [40,49]. Enhanced accumulation of phenolic compounds by supplemental UV-B radiation has been supported by a large body of experimental evidence [50,51], which was confirmed in this study. Ghasemzadeh et al. (2016) reported that total phenolic and flavonoid content in green basil plants increased by 16% and 85%, respectively, after a 13 $kJ \cdot m^{-2} \cdot h^{-1}$ post-harvest UV-B radiation for 4–10 h, but anthocyanin content was not measured [52]. It was also reported that upon supplemental UV-B radiation, the gene expression of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), two key molecular markers for phenolic compounds biosynthesis increased significantly [46,53]. Noticeably, in the present study, the enhancement of flavonoids and phenolics by UV-B radiation was much greater than anthocyanin. Consistently, antioxidant capacity was significantly correlated with concentrations of phenolics and flavonoids in both basil cultivars, while marginally or not correlated to anthocyanin concentration. This might be due to the higher ROS-scavenging capacity of phenolics and flavonoids than anthocyanins, resulting in more sensitive reactions of phenolics and flavonoids to UV-B radiation [54]. Csepregi et al. (2017) also reported such differential regulation of different phenolic compounds by UV-B radiation, in which quercetins with additional hydroxyl group on ring-B increased up to 10 folds while kaempferol increased 3–4 fold, due to their different ROS-scavenging capacity [55].

Enhancement of phenolic compounds after UV-B radiation was greater in basil plants grown under low PPFD compared to those grown under high PPFD, indicating basil plants are more sensitive to UV-B radiation under low PPFD. In a similar way, Behn et al. (2010) reported that under low PPFD (550 $\mu mol \cdot m^{-2} \cdot s^{-1}$), essential oil quality in peppermint plants was improved in terms of an enhanced

menthone to menthol conversion after UV-B radiation, while not affected by UV-B treatment under high PPFD (1150 μmol·m$^{-2}$·s$^{-1}$) [56]. As mentioned, this may be due to a depressed photochemical and biochemical protection system of plants grown under low PPFD, such as lower leaf thickness and reduced concentrations of UV-absorbing agents [21]. As we hypothesized, concentrations of phenolic compounds in basil plants grown under low PPFD with UV-B radiation was significantly higher compared to those of plants grown under high PPFD without UV-B radiation, suggesting that UV-B radiation could be used as a tool to compensate for reduced accumulation of phenolic compounds in basil plants grown under controlled environment.

Similar to plant responses on chlorophyll fluorescence, different responses in phytochemical accumulation between green and purple basil plants were also observed. Specifically, purple basil plants showed fewer biochemical changes than green basil plants after UV-B radiation, which performed as unaffected anthocyanin concentration and less induction of phenolics and flavonoids. Our hypothesis is that the relatively high concentrations of phenolic compounds in purple basil plants act as potent UV-screening agents as well as free-radical scavengers to protect purple basil plants from excess UV-B light. Under high PPFD without UV-B treatment, concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity in purple basil leaves were 3.33, 1.47, 1.93, 3.72 times those in green basil leaves, respectively. This hypothesis was confirmed by Tattini et al. (2014), in which he reported that purple basil 'Red Rubin' showed lower metabolic cost of photoprotective mechanisms than green basil 'Tigullio' when being moved from 30% to 100% sunlight condition [57].

### *4.4. Impacts of UV-B Radiation Doses and Radiation Patterns on Phytochemical Accumulation and Antioxidant Capacity*

With the radiation doses and different radiation patterns used in the present study, green basil plants were more dose-dependent, while purple basil plants were both dose-dependent and radiation pattern-dependent. Antioxidant capacity in green basil plants was significantly correlated with the UV-B radiation dose for both 1 h·d$^{-1}$ and 2 h·d$^{-1}$ UV-B radiation patterns, while antioxidant capacity in purple basil plants was not affected by 1 h·d$^{-1}$ UV-B radiation treatments. With the similar UV-B radiation dose (1H5D and 2H2D treatments), after 1 h·d$^{-1}$ UV-B radiation treatments, the recovery time until next day treatment (23 h) allowed purple basil plants' signaling and metabolic adaptation to (at least partially) reset to pre-stress level, without increasing phenolic compounds accumulation, while after 2 h·d$^{-1}$ UV-B radiation (recovery time of 22 h until next treatment), purple basil plants failed to recover from UV-B radiation stress and resulted in an overall increase of phenolic compounds to cope with excess UV-B energy. This indicated that radiation patterns play an important role in regulating purple basil responses to UV-B radiation, while radiation dose is the determining factor in regulating green basil biochemical responses. Mosadegh et al. (2018) also reported that with the same UV-B radiation dose of 102 kJ·m$^{-2}$, phenolics concentration of green basil 'Genovese' was the same level regardless of UV-B radiation pattern, continuous 1-d UV-B radiation or discontinuous 6-d UV-B radiation [28]. However, at lower UV-B radiation doses of 8.5, 34, and 68 kJ·m$^{-2}$, when 'Genovese' green basil plants were treated with the same UV-B radiation dose, continuous 1-d UV-B radiation resulted in significant higher phenolics concentration compared to plants treated with discontinuous 6-d UV-B radiation [28]. Thus, plant responses to UV-B radiation in green basil plants may also depend on radiation patterns, which are affected by the total UV-B radiation dose.

### *4.5. Implications of Study Findings*

Different plant responses to UV-B radiation are observed in studies conducted in the open field with sunlight than in a controlled environment with artificial lighting, due to different PPFDs and components of UV radiation [13,35,58,59]. The novel finding of the present study is that plants grown under a controlled environment with lower PPFDs are more sensitive to UV-B radiation. Therefore, for future studies under a controlled environment, a lower UV-B radiation dose should be applied to reduce its negative effects on plant photosynthesis, growth, or yield. Furthermore, we see differential

responses in green and purple basil plants to UV-B radiation doses and radiation patterns. Therefore, to better understand plant responses to supplemental UV-B radiation, more plant species/cultivars, lower radiation doses, and different radiation patterns need to be investigated in future studies.

Plant acclimation responses to supplemental UV-B radiation lead to plant cross-protection against other environmental stresses, through photochemical, morphological, and biochemical mechanisms [60]. For example, *UVR8* was recently shown to be involved in regulating thermomorphogenesis, shade-avoidance responses, and plant immunity, underlining the importance of signaling crosstalk among UV-B radiation, hormone, and defense pathways [47,61]. As a result, supplemental UV-B radiation could be used as a tool to improve plant tolerance to other adverse environmental conditions, and interactions between supplemental UV-B radiation and other key environmental factors still need to be studied.

## 5. Conclusions

Results of the present study suggest that a short period of pre-harvest supplemental UV-B radiation could significantly improve phytochemical concentrations in basil plants, and plant responses to UV-B radiation vary among plant cultivars, radiation doses, and radiation patterns. Meanwhile, effects of UV-B radiation on basil plants interacted with PPFDs used in the cultivation system, and high PPFD improved plant tolerance to UV-B radiation. Also, supplemental UV-B radiation could compensate for the reduced accumulation of phenolic compounds in basil plants grown under low PPFD. Therefore, combining plant growth performance, yield, and accumulation of health-promoting phenolic compounds, a pre-harvest UV-B radiation of 1 $h \cdot d^{-1}$ for 2 days under a PPFD of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was recommended for green basil 'Improved Genovese Compact' production under a controlled environment. However, supplemental UV-B radiation doses used in this study decreased the total amount of phenolic compounds in purple basil plants due to yield reduction, and UV-B radiation is not recommended for purple basil 'Red Rubin' production under a controlled environment.

**Author Contributions:** Conceptualization, H.D., G.N. and M.G.; data analysis, H.D.; methodology, H.D., G.N. and M.G.; writing—original draft preparation, H.D.; writing—review and editing, G.N., M.G.

**Funding:** This research was partially funded by the USDA National Institute of Food and Agriculture Hatch project TEX090450, Texas A&M AgriLife Research, and Texas A&M AgriLife Extension Service.

**Acknowledgments:** The authors appreciate the assistance from Youping Sun, Zhanyang Xu, Christina Perez, Triston Hooks, and the student workers at Texas A&M AgriLife Research Center at El Paso, TX.

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

## References

1. Gruda, N.S. Increasing Sustainability of Growing Media Constituents and Stand-Alone Substrates in Soilless Culture Systems. *Agronomy* **2019**, *9*, 298. [CrossRef]
2. Kozai, T.; Niu, G.; Takagaki, M. *Plant Factory—An Indoor Vertical Farming System for Efficient Quality Food Production*, 2nd ed.; Academic Press, Elsevier: New York, NY, USA, 2019; in press.
3. Goto, E.; Hayashi, K.; Furuyama, S.; Hikosaka, S.; Ishigami, Y. Effect of UV Light on Phytochemical Accumulation and Expression of Anthocyanin Biosynthesis Genes in Red Leaf Lettuce. In Proceedings of the VIII International Symposium on Light in Horticulture, East Lansing, MI, USA, 22–26 May 2016; pp. 179–186.
4. Wargent, J.J. UV LEDs in Horticulture: From Biology to Application. In Proceedings of the VIII International Symposium on Light in Horticulture, East Lansing, MI, USA, 22–26 May 2016; pp. 25–32.
5. Caldwell, M.; Flint, S.; Searles, P. Spectral Balance and UV-B Sensitivity of Soybean: A Field Experiment. *Plant. Cell Environ.* **1994**, *17*, 267–276. [CrossRef]
6. Wargent, J.J.; Moore, J.P.; Roland Ennos, A.; Paul, N.D. Ultraviolet Radiation as a Limiting Factor in Leaf Expansion and Development. *Photochem. Photobiol.* **2009**, *85*, 279–286. [CrossRef]

7. Sun, R.; Hikosaka, S.; Goto, E.; Sawada, H.; Saito, T.; Kudo, T.; Ohno, T.; Shibata, T.; Yoshimatsu, K. Effects of UV Irradiation on Plant Growth and Concentrations of Four Medicinal Ingredients in Chinese Licorice (*Glycyrrhiza uralensis*). In Proceedings of the VII International Symposium on Light in Horticultural Systems, Wageningen, The Netherlands, 15–18 October 2012; pp. 643–648.
8. Castagna, A.; Dall'Asta, C.; Chiavaro, E.; Galaverna, G.; Ranieri, A. Effect of Post-harvest UV-B Irradiation on Polyphenol Profile and Antioxidant Activity in Flesh and Peel of Tomato Fruits. *Food Bioprocess. Technol.* **2014**, *7*, 2241–2250. [CrossRef]
9. Moreira-Rodríguez, M.; Nair, V.; Benavides, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. UVA, UVB Light Doses and Harvesting Time Differentially Tailor Glucosinolate and Phenolic Profiles in Broccoli Sprouts. *Molecules* **2017**, *22*, 1065. [CrossRef] [PubMed]
10. Rouphael, Y.; Kyriacou, M.C.; Petropoulos, S.A.; de Pascale, S.; Colla, G. Improving Vegetable Quality in Controlled Environments. *Sci. Hort.* **2018**, *234*, 275–289. [CrossRef]
11. Schreiner, M.; Mewis, I.; Huyskens-Keil, S.; Jansen, M.A.K.; Zrenner, R.; Winkler, J.B.; O'Brien, N.; Krumbein, A. UV-B-induced Secondary Plant Metabolites—Potential Benefits for Plant and Human Health. *Crit. Rev. Plant. Sci.* **2012**, *31*, 229–240. [CrossRef]
12. Dotto, M.; Casati, P. Developmental Reprogramming by UV-B Radiation in Plants. *Plant. Sci.* **2017**, *264*, 96–101. [CrossRef]
13. Henry-Kirk, R.A.; Plunkett, B.; Hall, M.; McGhie, T.; Allan, A.C.; Wargent, J.J.; Espley, R.V. Solar UV Light Regulates Flavonoid Metabolism in Apple (*Malus x domestica*). *Plant. Cell Environ.* **2018**, *41*, 675–688. [CrossRef]
14. Jansen, M.A.; Bornman, J.F. UV-B Radiation: From Generic Stressor to Specific Regulator. *Physiol. Plant.* **2012**, *145*, 501–504. [CrossRef]
15. Höll, J.; Lindner, S.; Walter, H.; Joshi, D.; Poschet, G.; Pfleger, S.; Ziegler, T.; Hell, R.; Bogs, J.; Rausch, T. Impact of Pulsed UV-B Stress Exposure on Plant Performance: How Recovery Periods Stimulate Secondary Metabolism While Reducing Adaptive Growth Attenuation. *Plant. Cell Environ.* **2019**, *42*, 801–814. [CrossRef]
16. Brown, B.A.; Jenkins, G.I. UV-B Signaling Pathways with Different Fluence-rate Response Profiles Are Distinguished in Mature *Arabidopsis* Leaf Tissue by Requirement for UVR8, HY5, and HYH. *Plant. Physiol.* **2008**, *146*, 576–588. [CrossRef]
17. Favory, J.J.; Stec, A.; Gruber, H.; Rizzini, L.; Oravecz, A.; Funk, M.; Albert, A.; Cloix, C.; Jenkins, G.I.; Oakeley, E.J. Interaction of COP1 and UVR8 Regulates UV-B-induced Photomorphogenesis and Stress Acclimation in *Arabidopsis*. *EMBO J.* **2009**, *28*, 591–601. [CrossRef]
18. Alexandru Suchar, V.; Robberecht, R. Integration and Scaling of UV-B Radiation Effects on Plants: The Relative Sensitivity of Growth Forms and Interspecies Interactions. *J. Plant. Ecol.* **2017**, *11*, 656–670. [CrossRef]
19. Li, Q.; Kubota, C. Effects of Supplemental Light Quality on Growth and Phytochemicals of Baby Leaf Lettuce. *Environ. Exper. Bot.* **2009**, *67*, 59–64. [CrossRef]
20. Makri, O.; Kintzios, S. *Ocimum* sp. (Basil): Botany, Cultivation, Pharmaceutical Properties, and Biotechnology. *J. Herbs Spices Med. Plants* **2008**, *13*, 123–150. [CrossRef]
21. Dou, H.; Niu, G.; Gu, M.; Masabni, J.G. Responses of Sweet Basil to Different Daily Light Integrals in Photosynthesis, Morphology, Yield, and Nutritional Quality. *HortScience* **2018**, *53*, 496–503. [CrossRef]
22. Liaros, S.; Botsis, K.; Xydis, G. Technoeconomic Evaluation of Urban Plant Factories: The Case of Basil (*Ocimum basilicum*). *Sci. Total Environ.* **2016**, *554*, 218–227. [CrossRef]
23. Hogewoning, S.; Trouwborst, G.; Meinen, E.; van Ieperen, W. Finding the Optimal Growth-Light Spectrum for Greenhouse Crops. In Proceedings of the the VII International Symposium on Light in Horticultural Systems, Wageningen, The Netherlands, 15–18 October 2012; pp. 357–363.
24. Stutte, G.W. Controlled Environment Production of Medicinal and Aromatic Plants. In *Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization*; Jeliazkov, V.D., Cantrell, C.L., Eds.; ACS Publications: Washington, DC, USA, 2016; pp. 49–63.
25. Johnson, C.B.; Kirby, J.; Naxakis, G.; Pearson, S. Substantial UV-B-mediated Induction of Essential Oils in Sweet Basil (*Ocimum basilicum* L.). *Phytochemistry* **1999**, *51*, 507–510. [CrossRef]
26. Sakalauskaite, J.; Viskelis, P.; Dambrauskiene, E.; Sakalauskiene, S.; Samuoliene, G.; Brazaityte, A.; Duchovskis, P.; Urbonaviciene, D. The Effects of Different UV-B Radiation Intensities on Morphological and Biochemical Characteristics in *Ocimum basilicum* L. *J. Sci. Food Agr.* **2013**, *93*, 1266–1271. [CrossRef]

27. Sakalauskaite, J.; Viskelis, P.; Duchovskis, P.; Dambrauskiene, E.; Sakalauskiene, S.; Samuoliene, G.; Brazaityte, A. Supplementary UV-B Irradiation Effects on Basil (*Ocimum basilicum* L.) Growth and Phytochemical Properties. *J. Food Agr. Environ.* **2012**, *10*, 342–346.
28. Mosadegh, H.; Trivellini, A.; Ferrante, A.; Lucchesini, M.; Vernieri, P.; Mensuali, A. Applications of UV-B Lighting to Enhance Phenolic Accumulation of Sweet Basil. *Sci. Hort.* **2018**, *229*, 107–116. [CrossRef]
29. Xu, C.P.; Mou, B.Q. Responses of Spinach to Salinity and Nutrient Deficiency in Growth, Physiology, and Nutritional Value. *J. Amer. Soc. Hort. Sci.* **2016**, *141*, 12–21. [CrossRef]
30. Connor, A.M.; Luby, J.J.; Tong, C.B. Variability in Antioxidant Activity in Blueberry and Correlations Among Different Antioxidant Activity Assays. *J. Am. Soc. Hort. Sci.* **2002**, *127*, 238–244. [CrossRef]
31. Arnao, M.B.; Cano, A.; Acosta, M. The Hydrophilic and Lipophilic Contribution to Total Antioxidant Activity. *Food Chem.* **2001**, *73*, 239–244. [CrossRef]
32. Sullivan, J.H.; Teramura, A.H. Field Study of the Interaction Between Solar Ultraviolet-B Radiation and Drought on Photosynthesis and Growth in Soybean. *Plant. Physiol.* **1990**, *92*, 141–146. [CrossRef]
33. Lidon, F.J.; Reboredo, F.H.; Silva, M.M.A.; Duarte, M.P.; Ramalho, J.C. Impact of UV-B Radiation on Photosynthesis—An Overview. *Emir. J. Food Agr.* **2012**, *24*, 546–556. [CrossRef]
34. Yadav, S.; Shrivastava, A.K.; Agrawal, C.; Sen, S.; Chatterjee, A.; Rai, S.; Rai, R.; Singh, S.; Rai, L. Impact of UV-B Exposure on Phytochrome and Photosynthetic Machinery: From Cyanobacteria to Plants. In *UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth*; Singh, S., Prasad, S.M., Parhar, P., Eds.; John Wiley & Sons, Ltd.: West Sussex, UK, 2017; pp. 259–277.
35. Searles, P.S.; Flint, S.D.; Caldwell, M.M. A Meta-analysis of Plant Field Studies Simulating Stratospheric Ozone Depletion. *Oecologia* **2001**, *127*, 1–10. [CrossRef]
36. Kappes, U.P.; Luo, D.; Potter, M.; Schulmeister, K.; Rünger, T.M. Short- and Long-wave UV light (UVB and UVA) Induce Similar Mutations in Human Skin Cells. *J. Investig. Dermatol.* **2006**, *126*, 667–675. [CrossRef]
37. Ikehata, H.; Ono, T. The Mechanisms of UV Mutagenesis. *J. Radiat. Res.* **2011**, *52*, 115–125. [CrossRef]
38. Strasser, R.J.; Srivastava, A.; Tsimilli-Michael, M. The Fluorescence Transient as a Tool to Characterize and Screen Photosynthetic Samples. In *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*; Yunus, M., Pathre, U., Mohanty, P., Eds.; Taylor and Francis Inc.: New York, NY, USA, 2000; pp. 445–483.
39. Rai, K.; Agrawal, S. Effects of UV-B Radiation on Morphological, Physiological and Biochemical Aspects of Plants: An Overview. *J. Sci. Res.* **2017**, *61*, 87–113.
40. Takahashi, S.; Badger, M.R. Photoprotection in Plants: A New Light on Photosystem II Damage. *Trends Plant. Sci.* **2011**, *16*, 53–60. [CrossRef]
41. Sun, J.; Nishio, J.N.; Vogelmann, T.C. Green Light Drives $CO_2$ Fixation Deep Within Leaves. *Plant. Cell Physiol.* **1998**, *39*, 1020–1026. [CrossRef]
42. Cen, Y.P.; Bornman, J.F. The Effect of Exposure to Enhanced UV-B Radiation on the Penetration of Monochromatic and Polychromatic UV-B Radiation in Leaves of *Brassica napus*. *Physiol. Plant.* **1993**, *87*, 249–255. [CrossRef]
43. Wargent, J.J.; Jordan, B.R. From Ozone Depletion to Agriculture: Understanding the Role of UV Radiation in Sustainable Crop Production. *New Phytol.* **2013**, *197*, 1058–1076. [CrossRef]
44. Zhao, D.; Reddy, K.; Kakani, V.; Read, J.; Sullivan, J. Growth and Physiological Responses of Cotton (*Gossypium hirsutum* L.) to Elevated Carbon Dioxide and Ultraviolet-B Radiation under Controlled Environmental Conditions. *Plant. Cell Environ.* **2003**, *26*, 771–782. [CrossRef]
45. Kaiserli, E. Ultraviolet Rays Light Up Transcriptional Networks Regulating Plant Growth. *Dev. Cell* **2018**, *44*, 409–411. [CrossRef]
46. Fraser, D.P.; Sharma, A.; Fletcher, T.; Budge, S.; Moncrieff, C.; Dodd, A.N.; Franklin, K.A. UV-B Antagonises Shade Avoidance and Increases Levels of the Flavonoid Quercetin in Coriander (*Coriandrum sativum*). *Sci. Rep.* **2017**, *7*, 17758. [CrossRef]
47. Teklemariam, T.; Blake, T.J. Effects of UVB Preconditioning on Heat Tolerance of Cucumber (*Cucumis sativus* L.). *Environ. Exp. Bot.* **2003**, *50*, 169–182. [CrossRef]
48. Kakani, V.; Reddy, K.; Zhao, D.; Mohammed, A. Effects of Ultraviolet-B Radiation on Cotton (*Gossypium hirsutum* L.) Morphology and Anatomy. *Ann. Bot.* **2003**, *91*, 817–826. [CrossRef]
49. Logan, B.A.; Stafstrom, W.C.; Walsh, M.J.; Reblin, J.S.; Gould, K.S. Examining the Photoprotection Hypothesis for Adaxial Foliar Anthocyanin Accumulation by Revisiting Comparisons of Green- and Red-leafed Varieties of Coleus (*Solenostemon scutellarioides*). *Photosyn. Res.* **2015**, *124*, 267–274. [CrossRef]

50. Agati, G.; Tattini, M. Multiple Functional Roles of Flavonoids in Photoprotection. *New Phytol.* **2010**, *186*, 786–793. [CrossRef]
51. Hatier, J.H.B.; Clearwater, M.J.; Gould, K.S. The Functional Significance of Black-pigmented Leaves: Photosynthesis, Photoprotection and Productivity in *Ophiopogon planiscapus* 'Nigrescens'. *PLoS ONE* **2013**, *8*, e67850. [CrossRef]
52. Ghasemzadeh, A.; Ashkani, S.; Baghdadi, A.; Pazoki, A.; Jaafar, H.Z.; Rahmat, A. Improvement in Flavonoids and Phenolic Acids Production and Pharmaceutical Quality of Sweet Basil (*Ocimum basilicum* L.) by Ultraviolet-B Irradiation. *Molecules* **2016**, *21*, 1203. [CrossRef]
53. Rodríguez-Calzada, T.; Qian, M.; Strid, A.; Neugart, S.; Schreiner, M.; Torres-Pacheco, I.; Guevara-González, R.G. Effect of UV-B Radiation on Morphology, Phenolic Compound Production, Gene Expression, and Subsequent Drought Stress Responses in Chili Pepper (*Capsicum annuum* L.). *Plant. Physiol. Biochem.* **2019**, *134*, 94–102. [CrossRef]
54. Hideg, E.; Jansen, M.A.K.; Strid, A. UV-B Exposure, ROS, and Stress: Inseparable Companions or Loosely Linked Associations? *Trends Plant. Sci.* **2013**, *18*, 107–115. [CrossRef]
55. Csepregi, K.; Coffey, A.; Cunningham, N.; Prinsen, E.; Hideg, É.; Jansen, M.A. Developmental Age and UV-B Exposure Co-determine Antioxidant Capacity and Flavonol Accumulation in *Arabidopsis* Leaves. *Environ. Exp. Bot.* **2017**, *140*, 19–25. [CrossRef]
56. Behn, H.; Albert, A.; Marx, F.; Noga, G.; Ulbrich, A. Ultraviolet-B and Photosynthetically Active Radiation Interactively Affect Yield and Pattern of Monoterpenes in Leaves of Peppermint (*Mentha* × *piperita* L.). *J. Agr. Food Chem.* **2010**, *58*, 7361–7367. [CrossRef]
57. Tattini, M.; Landi, M.; Brunetti, C.; Giordano, C.; Remorini, D.; Gould, K.S.; Guidi, L. Epidermal Coumaroyl Anthocyanins Protect Sweet Basil Against Excess Light Stress: Multiple Consequences of Light Attenuation. *Physiol. Plant.* **2014**, *152*, 585–598. [CrossRef]
58. Li, F.R.; Peng, S.L.; Chen, B.M.; Hou, Y.P. A Meta-analysis of the Responses of Woody and Herbaceous Plants to Elevated Ultraviolet-B Radiation. *Acta Oecologica* **2010**, *36*, 1–9. [CrossRef]
59. Wargent, J.J.; Elfadly, E.M.; Moore, J.P.; Paul, N.D. Increased Exposure to UV-B Radiation During Early Development Leads to Enhanced Photoprotection and Improved Long-term Performance in *Lactuca sativa*. *Plant. Cell Environ.* **2011**, *34*, 1401–1413. [CrossRef]
60. Yin, R.; Ulm, R. How Plants Cope with UV-B: From Perception to Response. *Cur. Opin. Plant. Biol.* **2017**, *37*, 42–48. [CrossRef]
61. Schultze, M.; Bilger, W. Acclimation of *Arabidopsis thaliana* to Low Temperature Protects Against Damage of Photosystem II Caused by Exposure to UV-B Radiation at 9 °C. *Plant. Physiol. Biochem.* **2018**, *134*, 73–80. [CrossRef]