Technology in Horticulture

ARTICLE

Open Access

https://doi.org/10.48130/TIH-2022-0002

*Technology in Horticulture* **2022**, 2:2

# Daily UV-B treatments and elevated $CO_2$ increases pigment concentrations and net photosynthesis of basil (*Ocimum basilicum* L.)

T. Casey Barickman[1*], Skyler R. Brazel[1], Akanksha Sehgal[2], Charles Hunt Walne[2], and K. Raja Reddy[2]

[1] *Department of Plant and Soil Sciences, Mississippi State University, North Mississippi Research and Extension Center, Verona, MS 38879, USA*
[2] *Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762, USA*
* Corresponding author, E-mail: t.c.barickman@msstate.edu

## Abstract

Anthropogenic emissions have greatly influenced UV-B radiation incidence and $CO_2$ concentration globally. The interactive effects of projected incidence on crops by the end of the century need to be studied to understand the implications. The use of sunlit plant growth chambers in combination with UV-B radiation and $CO_2$ treatments was used to identify the individual and interactive effects on basil 'Genovese' plants. Treatments included 0 and 10 kJ $m^{-2}$ $d^{-1}$ UV-B supplementation at ambient (437 ppm) or elevated (725 ppm) $CO_2$ concentrations. Effects of UV-B by $CO_2$ interactions existed for net photosynthesis, light-adapted maximal quantum efficiency, all plant pigment concentrations, and malondialdehyde. UV-B increased leaf temperature by approximately 1 °C while elevated $CO_2$ concentrations amplified superoxide dismutase and ascorbate peroxidase activity in basil leaves. Despite deleterious impacts on plant health, UV-B radiation is essential for stimulating healthful compounds in basil. Understanding the effects when combined with elevated $CO_2$ is necessary to improve crop production and future research.

**Citation:** Barickman TC, Brazel SR, Sehgal A, Walne CH, Reddy KR. 2022. Daily UV-B treatments and elevated $CO_2$ increases pigment concentrations and net photosynthesis of basil (*Ocimum basilicum* L.). *Technology in Horticulture* 2:2 https://doi.org/10.48130/TIH-2022-0002

## INTRODUCTION

Since the 1950s, there has been a steady increase in ultraviolet radiation to the Earth's surface due to anthropogenic emissions and degradation of the ozone layer[1]. Of the three types of ultraviolet radiation, ultraviolet-B (UV-B) radiation (wavelengths 280–315 nm) is the most harmful type that reaches the Earth's surface, with intensity impacted by altitude, latitude, and time of day[1,2]. In addition, the effects of UV-B light on plant photosynthesis have proven to lower net gains and efficiencies[3]. However, these effects have been variable and highly dependent on the length of time plants are exposed to UV-B[4].

The reduction in photosynthesis is coupled with a decrease in quantum yield efficiency[5] and attributed to the decrease in stomatal conductance[6] and the onset of decreased $CO_2$ assimilation[5,7]. In conjunction with the decline of physiological performance, light-absorbing chlorophylls and protecting carotenoids are reduced in most plants with higher UV-B exposure[6,8]. However, low levels of UV-B radiation can be beneficial to plants. For example, low levels of UV-B can stimulate the production of jasmonate and phenolic compounds offering protection against pathogens and insects[2,9]. Consequently, high levels of UV-B radiation can induce plant stress by increasing the concentrations of reactive oxygen species leading to irreversible cellular and DNA damage[10,11]. Other anthropogenic factors that have led to the increase in ultraviolet radiation have also caused the increase in atmospheric $CO_2$ concentrations[12]. Elevated $CO_2$ concentrations have been shown to increase plant photosynthesis and growth, yet when combined with UV-B radiation, it can reduce and even negates these advantages[13].

Al Jaouni et al.[14] identified that basil grown under elevated $CO_2$ had an increased accumulation of biomass, increased plant photosynthetic rates, and elevated primary and secondary metabolite accumulation. Being a widely used culinary, medicinal, and ornamental plant, basil has exhibited variability in growth and photosynthesis when produced under different light conditions[15]. Although used to protect the crop from light, the use of glazing materials and coverings in the greenhouse production of basil results in reduction in flavor and nutritional properties[16,17]. Basil also responds to environmental stressors like ultraviolet radiation as these stimulate oil gland filling, improving nutritional content[18], increasing regulatory enzymes affecting plant nutrition and growth[17], and increasing phenolic compounds that protect the photosynthesis apparatus[11]. These studies demonstrated that UV-B radiation is essential to maintain the nutritional content of basil, although it may have negative effects on its morphology. Other studies have analyzed variations of UV-B radiation applications on basil[11,19,20]. However, those studies have not investigated continuous UV-B radiation applications when combined with elevated $CO_2$ concentrations and its effects on basil. Thus, the current study's objective is to determine the individual and interactive effects of UV-B radiation and elevated $CO_2$ concentration on basil photosynthetic and biochemical parameters when grown under ambient light.

## MATERIALS AND METHODS

### Plant material and growth conditions

In June–July 2019, an experiment to determine how UV-B and $CO_2$ affect basil biochemical and photosynthesis

parameters was set up in four sunlit controlled environmental chambers, called soil-plant-atmosphere-research (SPAR) units. The SPAR units are located at the Rodney Foil Plant Science research facility of Mississippi State University, Mississippi State, MS, USA. Details of the SPAR unit operations are described in Reddy et al.[21], Zhao et al.[22], and Wijewardana et al.[23]. Average daily solar radiation outside SPAR units ranged from 4.20 to 35.5 MJ $m^{-2}$ $d^{-1}$ with an average value of 25.86 ± 0.92 MJ $m^{-2}$ $d^{-1}$, measured with a pyranometer (Model 4-8; The Eppley Laboratory, Inc., Newport, RI, USA). The Plexiglass chambers on the SPAR units are completely opaque to UV radiation (wavelengths less than 385 nm) and transmit over 95% of incoming PAR (400–700 nm).

A 3:1 sand/soil (sandy loam; 87% sand, 2% clay, and 11% silt) was used as the soil medium for the polyvinyl-chloride pots (15.2 cm diameter by 30.5 cm height) with 500 g of gravel in the bottom of the pots. Six seeds of basil 'Genovese' (Johnny's Selected Seeds, Winslow, ME, USA) were sown in the pots and then thinned to one plant per pot approximately seven days after emergence. A two-by-two factorial arrangement was used to organize the pots with UV-B and $CO_2$ treatments, with each chamber having three rows of pots and 10 pots per row. Basil plants were randomly assigned to each chamber consisting of 0 kJ $m^{-2}$ $d^{-1}$ and 10 kJ $m^{-2}$ $d^{-1}$ combined with ambient (437 ppm) or elevated (725 ppm) $CO_2$ concentrations. UV-B radiation treatments were initiated 14-days after sowing, or 0 days of treatment (DAT).

Eight fluorescent lamps (UV-313 lamps, Q-Panel Company, Cleveland, OH, USA), with peak wavelength at 313 nm, attached to dimmable 40w ballasts were positioned 0.5 m above the plant canopy, wrapped in calcium diacetate films and changed routinely to filter UV-C radiation, were used to impose UV-B treatments. UV-B treatments occurred from 8:00 to 16:00 daily, with the interception of radiation at the canopy being measured daily with a UVX digital radiometer (UVP Inc., San Gabriel California, CA, USA) and calibrated against an Optronic Laboratories Model 754 spectroradiometer (Optronic Laboratories, Orlando, FL, USA). Non-illuminated bulbs and frames were used in chambers without UV-B radiation treatment.

Pure $CO_2$ from compressed gas cylinders was individually supplied to each chamber through pressure regulators, solenoids, and needle valves with calibrated flow meter[21] and constantly measured by a dedicated infrared gas analyzers (LI-6252, LI-COR Biosciences, Lincoln, NE, USA).

Except for UV-B radiation and $CO_2$, all environmental growth conditions were kept the same throughout the experiment. The daytime temperature of 30 °C was initiated at sunrise, and the night time temperature of 22 °C was initiated 1 h after sunset. Plants were irrigated three times per day (7:00, 12:00, and 17:00) using an automated computer-controlled drip system with full-strength Hoagland's nutrient solution[24] based on treatment-based evapotranspiration values detailed by McKinion & Hodges[25], and modified as described by Reddy et al.[21].

## Gas exchange and chlorophyll fluorescence

Photosynthesis and fluorescence parameters were measured between 10:00 and 12:00 with an LI-6400XT portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) equipped with an integrated fluorescence chamber head (LI-6400-40, LI-COR Biosciences, Lincoln, NE, USA) at 18 DAT. The light intensity (PAR) in the measuring chamber was set to 1,500 µmol $m^{-2}$ $s^{-1,}$ and the relative humidity was set to 50%. The measurement chamber temperature was kept at 30 °C. The $CO_2$ concentration of the leaf chamber was set to the chamber $CO_2$ level, with the flow rate adjusted to 500 mol $s^{-1}$. When the total coefficient of variation (% CV) reached < 0.5%, measurements were recorded. By considering incoming and outgoing flow rates and leaf area, the instrument provides the data for transpiration (E), stomatal conductance ($g_{sw}$), internal $CO_2$ concentration ($C_i$), and electron transport rate (ETR). The internal to external $CO_2$ ratio was calculated by the relationship $C_i/C_a$.

Chlorophyll fluorescence measurements were obtained by providing a saturating flash of light > 8,000 µmol $m^{-2}$ $s^{-1}$ for 0.8 s followed by a dark-flash for 6 s with far-red light pulses to drive photosystem I, draining the electrons of photosystem II. Light-adapted maximal quantum yield of photochemistry ($F_v'/F_m'$) was calculated from chlorophyll fluorescence ($F_m' - F_o'$)/$F_m'$, where $F_o'$ and $F_m'$ are minimal and maximal fluorescence of light saturated leaves. The effective quantum yield of photosystem II photochemistry ($\Phi_{PSII}$) was calculated from chlorophyll fluorescence as ($F_m' - F_s$)/$F_m'$, where $F_s$ is the steady-state fluorescence, and $F_m'$ is the maximal fluorescence, of light saturated leaves. The effective quantum yield of gas exchange measurements ($\Phi_{CO_2}$) was calculated from fluorescence as $(A - A_{dark})/I \cdot \alpha_{leaf}$ where A is assimilation rate, $A_{dark}$ is dark assimilation rate, $I$ is incidence of PAR, and $\alpha_{leaf}$ is leaf absorptance. Photochemical quenching ($q_P$) and non-photochemical quenching ($q_N$) were calculated from chlorophyll fluorescence where ($F_m' - F_s$)($F_m' - F_o'$) and ($F_m - F_m'$)/($F_m - F_o'$), respectively, where $F_m$ is dark-adapted maximal fluorescence.

## Pigment analysis

Plant pigments such as chlorophylls and carotenoids were extracted from freeze-dried tissues according to Kopsell et al. with modifications as described by Brazel et al. using an Agilent 1260 high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA, USA)[26,27].

## Antioxidant and oxidative analysis

### *Malondialdehyde (MDA)*

Lipid peroxidation of membranes was estimated from MDA content, a lipid peroxidation product, using the method described by Heath & Packer[28].

### *Hydrogen peroxide ($H_2O_2$)*

The content of $H_2O_2$ was estimated by the method of Mukherjee & Choudhuri[29].

### *Superoxide dismutase (SOD)*

The activity of SOD was measured following the method of Dhindsa et al.[30].

### *Ascorbic acid (ASC)*

The estimation of ASC was done according to the method of Mukherjee & Choudhuri[29].

### *Trehalose*

Trehalose concentration was estimated according to the method of Trevelyan & Harrison[31] and the anthrone method of Brin[32]. The enzymes associated with trehalose metabolism were assayed as per the procedures of Pramanic & Imai[33], with few changes. Trehalose-6-phosphate synthase (TPS) activity was assayed, according to Hottiger et al.[34], which determines the release of UDP from UDP-glucose, involving glucose-6-

phosphate. Trehalose-6-phosphate phosphatase (TPP) activity was assayed according to the method of Klutts et al.[35] by measuring the release of inorganic phosphate from trehalose-6-phosphate. Trehalose activity was determined by activating phosphorylation using cyclic adenosine monophosphate and assayed by measuring the glucose concentration[36].

*Glutathione*

Reduced glutathione (GLTH) was estimated according to the method of Griffith[37]. GLTH content was calculated from a standard graph as described by Griffith[37] and expressed as nmol $g^{-1}$ DM.

### Statistical Analysis

Statistical analysis was performed using SAS (version 9.4; SAS Institute, Cary, NC). PROC GLIMMIXED analysis of variance (ANOVA) followed by mean separation was used to analyze the data. Fixed effects for the experiment consisted of two UV-B light and two $CO_2$ treatments, with replications as random effects. Pooled error terms from the ANOVA table were used as the base for standard errors. UV-B and $CO_2$ treatment differentiation classification was determined by using Duncan's multiple range test ($P \leq 0.05$) based on significant F-values for main effects. Model-based values were used to reflect statistical testing when compared to unequal standard errors from data calculations. Treatment variances were tested to be statistically equal prior to pooling.

## RESULTS

### Gas exchange and chlorophyll fluorescence

The UV-B radiation and elevated $CO_2$ treatments modified plant photosynthesis and fluorescence traits of basil (Tables 1 & 2). The intercellular $CO_2$ concentration for plants grown at elevated $CO_2$ concentrations was more than 40% greater compared to ambient $CO_2$ concentrations. Leaf temperature of plants was influenced by UV-B radiation treatment, and $CO_2$ concentration, where leaf temperatures of plants treated with UV-B were at least 1 °C warmer when compared to non-treated plants. Elevated $CO_2$ treatments also increased leaf temperature slightly. There was a UV-B by $CO_2$ concentration interaction

**Table 1.** Effects of UV-B radiation and $CO_2$ concentration on net photosynthesis ($P_{NET}$), stomatal conductance ($g_{sw}$), internal $CO_2$ ($C_i$), electron transport rate (ETR), transpiration rate (E), leaf temperature ($T_{leaf}$), and intercellular/ambient $CO_2$ ratio ($C_i/C_a$). Measurements were taken on the fourth/fifth fully expanded leaf of plants grown without UV-B radiation (No UV-B) and with UV-B radiation (UV-B) at 437 (ambient [$CO_2$]) and 725 ppm (elevated [$CO_2$]) $CO_2$ concentration between 33 and 35 days of treatment.

| Treatment | $P_{NET}$[1] | $g_{sw}$ | $C_i$ | ETR | E | $T_{leaf}$ | $C_i/C_a$[2] |
|---|---|---|---|---|---|---|---|
| Ambient [$CO_2$] | | | | | | | |
| No UV-B | 24.477$^{b}$ | 0.375$^{a}$ | 295.09$^{b}$ | 187.33$^{a}$ | 6.786$^{a}$ | 30.484$^{c}$ | 0.704$^{a}$ |
| UV-B | 18.949$^{c}$ | 0.315$^{a}$ | 303.50$^{b}$ | 150.69$^{b}$ | 6.504$^{a}$ | 31.761$^{ab}$ | 0.723$^{a}$ |
| Elevated [$CO_2$] | | | | | | | |
| No UV-B | 31.514$^{a}$ | 0.312$^{a}$ | 530.71$^{a}$ | 184.97$^{ab}$ | 6.671$^{a}$ | 31.263$^{bc}$ | 0.737$^{a}$ |
| UV-B | 30.715$^{a}$ | 0.302$^{a}$ | 529.59$^{a}$ | 178.02$^{ab}$ | 6.449$^{a}$ | 32.344$^{a}$ | 0.736$^{a}$ |
| P-Value[3,4] | | | | | | | |
| UV-B | ** | NS | NS | NS | NS | ** | NS |
| $CO_2$ | *** | NS | *** | NS | NS | * | NS |
| UV-B x $CO_2$ | * | NS | NS | NS | NS | NS | NS |

[1] Units: $P_{NET}$ – μmol $CO_2$ $m^{-2}$ $s^{-1}$; gsw – mol $m^{-2}$ $s^{-1}$; Ci – $CO_2$ $\mu mol^{-1}$; ETR – μmol photons $m^{-2}$ $s^{-1}$; E – mmol $H_2O$ $m^{-2}$ $s^{-1}$; $T_{leaf}$ – °C.
[2] The measured intercellular $CO_2$/ambient $CO_2$ of LI-6400XT leaf cuvette.
[3] The standard error of the mean was $P_{NET}$ – 1.318; $g_{sw}$ – 0.302; $C_i$ – 9.601; ETR – 11.247; E – 0.480; $T_{leaf}$ – 0.278; $C_i$/Ca – 0.0206.
[4] NS, *, **, *** indicate non-significant, significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.
Values followed by the same superscript letter are not significantly different within each column.

**Table 2.** Effects of UV-B radiation and $CO_2$ concentration on light-adapted, minimal fluorescence ($F_o'$), light-adapted, maximal fluorescence ($F_m'$), steady-state fluorescence ($F_s$), light-adapted maximal quantum yield of photosystem II photochemistry ($F_v'/F_m'$), effective quantum yield of photosystem II photochemistry ($\Phi_{PSII}$), effective quantum yield of gas exchange measurements ($\Phi_{CO_2}$), photochemical quenching ($q_P$), and non-photochemical quenching ($q_N$). Measurements were taken on the fourth/fifth fully expanded leaf plants grown without UV-B radiation (No UV-B) and with UV-B radiation (UV-B) at 437 (ambient [$CO_2$]) and 725 ppm (elevated [$CO_2$]) $CO_2$ concentration between 33 and 35 days of treatment.

| Treatment | $F_o'$ | $F_m'$ | $F_s$ | $F_v'/F_m'$ | $\Phi_{PSII}$ | $\Phi_{CO_2}$ | $q_P$ | $q_N$ |
|---|---|---|---|---|---|---|---|---|
| Ambient [$CO_2$] | | | | | | | | |
| No UV-B | 448.31$^{a}$ | 840.48$^{ab}$ | 622.16$^{a}$ | 0.466$^{b}$ | 0.261$^{ab}$ | 0.0195$^{b}$ | 0.559$^{ab}$ | 1.875$^{b}$ |
| UV-B | 430.78$^{a}$ | 809.76$^{b}$ | 617.25$^{a}$ | 0.467$^{b}$ | 0.238$^{b}$ | 0.0155$^{c}$ | 0.509$^{b}$ | 1.878$^{b}$ |
| Elevated [$CO_2$] | | | | | | | | |
| No UV-B | 440.77$^{a}$ | 907.91$^{a}$ | 674.12$^{a}$ | 0.513$^{a}$ | 0.259$^{ab}$ | 0.0248$^{a}$ | 0.507$^{b}$ | 2.058$^{a}$ |
| UV-B | 440.13$^{a}$ | 836.64$^{ab}$ | 603.64$^{a}$ | 0.474$^{b}$ | 0.279$^{a}$ | 0.0245$^{a}$ | 0.587$^{a}$ | 1.902$^{b}$ |
| P-Value[1,2] | | | | | | | | |
| UV-B | NS | NS | NS | * | NS | * | NS | * |
| $CO_2$ | NS | NS | NS | ** | NS | *** | NS | ** |
| UV-B x $CO_2$ | NS | NS | NS | * | NS | * | * | * |

[1] The standard error of the mean was $F_o'$ – 9.503; $F_m'$ – 28.161; Fs – 26.365; $F_v'/F_m'$ – 0.00983; $\Phi_{PSII}$ – 0.0111; $\Phi_{CO_2}$ – 0.000976; $q_P$ – 0.0232; $q_N$ – 0.0383.
[2] NS, *, **, *** indicate non-significant, significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.
Values followed by the same superscript letter are not significantly different within each column.

for net photosynthesis ($P_{NET}$), light adapted-maximal quantum yield of photochemistry ($F_v'/F_m'$), effective quantum yield of gas exchange ($\Phi_{CO_2}$), photochemical quenching ($q_P$), and non-photochemical quenching ($q_N$). Net photosynthesis and $\Phi_{CO_2}$ values were reduced by 22.6 and 20.5%, respectively, when treated with UV-B radiation only under ambient $CO_2$ concentrations, but no differences existed under elevated $CO_2$ concentrations. For both $F_v'/F_m'$ and $q_N$, UV-B radiation treatments at ambient $CO_2$ concentrations did not impact values. In contrast, UV-B radiation treatments on plants at elevated $CO_2$ concentrations caused a reduction of 7.6% for both values compared against non-UV-B treated plants. Photochemical quenching ($q_P$) was reduced by UV-B radiation treatment under ambient $CO_2$ concentration and was increased by UV-B radiation treatment under elevated $CO_2$ concentration.

## Pigment concentrations

A UV-B by $CO_2$ interaction existed for all pigment concentrations and ratios evaluated (Table 3). Both zeaxanthin and antheraxanthin exhibited a decrease in concentration when exposed to UV-B radiation only under elevated $CO_2$. The content of neoxanthin and $\beta$-carotene decreased when $CO_2$ was elevated without UV-B treatment, while UV-B treatment caused a dissimilar content increase for both $CO_2$ concentrations. The lutein content decreased when $CO_2$ concentration was elevated under no UV-B treatment, while UV-B treatment increased the content by 15.3% and 63.3% of ambient and elevated $CO_2$ treatments, respectively. The concentrations of violaxanthin, chlorophyll B, chlorophyll A, and total chlorophylls were elevated by exposure to UV-B radiation, with elevated $CO_2$ further increasing pigment concentrations only for UV-B treated plants by 31.4%, 42.2%, 43.0%, and 43.0%, respectively. The total xanthophylls content was elevated for plants treated with UV-B only under ambient $CO_2$ concentration, with no difference under elevated $CO_2$ concentration. The xanthophyll ratio decreased under ambient $CO_2$ when exposed to UV-B treatment, while elevating $CO_2$ further reduced the ratio of UV-B treatment. $CO_2$ did not affect the xanthophyll ratio of plants without UV-B treatment.

## Antioxidant and oxidative concentrations

No differences existed for the hydrogen peroxide, trehalose, or glutathione concentrations of plant samples subjected to $CO_2$ concentrations and UV-B treatment (Table 4). Superoxide dismutase exhibited increased activity when subjected to elevated $CO_2$ regardless of UV-B treatment compared to ambient $CO_2$ levels. Similarly, ascorbate showed a rise in concentration under elevated $CO_2$, but only for plants not subjected to UV-B treatment. For malondialdehyde concentration, a UV-B by $CO_2$ interaction existed where UV-B treatment increased malondialdehyde concentration under ambient $CO_2$, but no difference existed under elevated $CO_2$.

# DISCUSSION

The use of the SPAR units to conduct studies on multiple environmental stresses in a controlled environment is indispensable to expanding our knowledge and research capabilities. Using the SPAR units enables data collection that will help develop crop growth and development models and various physiological responses using field conditions while simultaneously collecting data in a controlled environment. The SPAR units' ability to create an environment of the projected global anthropogenic emission that is estimated to occur in Mississippi by the end of the century with 1,000 ppm $CO_2$ concentration and average UV-B radiation of 10 to 15 kJ $m^{-2}$ $d^{-1}$ allows for a unique insight into current and future environmental conditions[22,38,39].

## Gas exchange and chlorophyll fluorescence

For most plants, UV-B radiation can cause a reduction in stomatal conductance that reduces transpiration and photosynthetic processes[40]. Our results indicate that stomatal conductance and transpiration were unaffected by UV-B radiation, which may be due to the phenolic compounds that basil naturally produces in response to UV-B radiation. The phenylpropanoid pathway and associated enzymes are activated when exposed to increased UV-B radiation. This can lead to the production of numerous antioxidants and phenylpropanoids that fill the oil glands and help to protect the plant by absorbing UV-B radiation[18].

**Table 3.** Effects of UV-B radiation and $CO_2$ concentration on carotenoids and chlorophyll concentrations in basil leaves grown in the Soil-Plant-Atmosphere-Research (SPAR) units at Mississippi State University. Leaf samples were taken from basil plants grown without UV-B radiation (No UV-B) and with UV-B radiation (UV-B) at 437 (ambient [$CO_2$]) and 725 ppm (elevated [$CO_2$]) $CO_2$ concentrations between 33 and 35 days of treatment.

| Treatment | Neo[1] | Viol | Anth | Zea | Lut | $\beta$-car | Total Xan[2] | ZA/ZAV[3] | ChlB | ChlA | Total Chl[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ambient [$CO_2$] | | | | | | | | | | | |
| No UV-B | 276.43$^{c}$ | 204.25$^{c}$ | 68.76$^{a}$ | 163.94$^{a}$ | 793.09$^{b}$ | 509.66$^{c}$ | 436.95$^{b}$ | 0.531$^{a}$ | 871.50$^{c}$ | 7005.67$^{c}$ | 7877.17$^{c}$ |
| UV-B | 307.30$^{b}$ | 249.89$^{b}$ | 68.70$^{a}$ | 168.79$^{a}$ | 914.27$^{a}$ | 606.80$^{a}$ | 487.38$^{a}$ | 0.487$^{b}$ | 1010.37$^{b}$ | 9234.86$^{b}$ | 10245$^{b}$ |
| Elevated [$CO_2$] | | | | | | | | | | | |
| No UV-B | 222.36$^{d}$ | 208.03$^{c}$ | 74.30$^{a}$ | 157.75$^{a}$ | 561.09$^{c}$ | 384.95$^{d}$ | 440.08$^{b}$ | 0.526$^{ab}$ | 767.96$^{c}$ | 7132.75$^{c}$ | 7900.70$^{c}$ |
| UV-B | 348.88$^{a}$ | 303.08$^{a}$ | 44.26$^{b}$ | 78.49$^{b}$ | 916.49$^{a}$ | 566.26$^{b}$ | 425.83$^{b}$ | 0.288$^{c}$ | 1329.67$^{a}$ | 12523$^{a}$ | 13853$^{a}$ |
| P-Value[5,6] | | | | | | | | | | | |
| UV-B | *** | *** | ** | *** | *** | *** | NS | *** | *** | *** | *** |
| $CO_2$ | NS | ** | * | *** | *** | *** | * | *** | *** | *** | *** |
| UV-B x $CO_2$ | *** | ** | ** | *** | *** | ** | * | *** | *** | *** | *** |

[1] Neo – Neoxanthin; Viol – Violaxanthin; Anth – Antheraxanthin; Zea – Zeaxanthin; Lut – Lutein; $\beta$-car – Beta carotene; Xan – Xanthophylls; ChlB – Chlorophyll B; ChlA – Chlorophyll A; Chl - Chlorophylls. All values expressed as $\mu g\ g^{-1}$ dry mass, except for ZA/ZAV.
[2] Total Xan – Sum of Viol, Anth, and Zea.
[3] Xanthophyll Cycle Ratio = (Zea and Anth)/(Zea, Anth, and Viol).
[4] Total Chl – Sum of ChlB and ChlA.
[5] The standard error of mean was Neo – 10.99; Vio – 8.83; Anth – 3.77; Zea – 7.90; Lut – 27.19; $\beta$-car – 17.13; Total Xan – 12.72; ZA/ZAV – 0.015; ChlB – 50.99; ChlA – 349.82; Total Chl – 395.77.
[6] NS, *, **, *** indicate non-significant, significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.
Values followed by the same superscript letter are not significantly different within each column.

**Table 4.** Effects of UV-B radiation and $CO_2$ concentration on the concentrations of malondialdehyde, hydrogen peroxide, superoxide dismutase activity, ascorbate acid, trehalose, and glutathione in the concentration of basil leaves. Samples were taken 33 days of treatment from plants grown without UV-B radiation (No UV-B) and with UV-B radiation (UV-B) at 437 (ambient [$CO_2$]) and 725 ppm (elevated [$CO_2$]) $CO_2$ concentration.

| Treatment | Concentration | | | | | |
|---|---|---|---|---|---|---|
| | MDA[1,2] | Perox | SOD | Asc | Tre | Glth |
| Ambient [$CO_2$] | | | | | | |
| No UV-B | 0.008$^b$ | 0.1912$^a$ | 0.0304$^b$ | 0.101$^b$ | 0.089$^b$ | 0.192$^a$ |
| UV-B | 0.053$^a$ | 0.2132$^a$ | 0.0308$^b$ | 0.110$^b$ | 0.110$^{ab}$ | 0.205$^a$ |
| Elevated [$CO_2$] | | | | | | |
| No UV-B | 0.008$^b$ | 0.1832$^a$ | 0.0392$^a$ | 0.191$^a$ | 0.126$^a$ | 0.177$^a$ |
| UV-B | 0.007$^b$ | 0.1914$^a$ | 0.0402$^a$ | 0.119$^b$ | 0.105$^{ab}$ | 0.159$^a$ |
| P-Value[3,4] | | | | | | |
| UV-B | *** | NS | NS | NS | NS | NS |
| $CO_2$ | *** | NS | ** | * | NS | NS |
| UV-B x $CO_2$ | *** | NS | NS | NS | NS | NS |

[1] MDA – Malondialdehyde; Perox – Hydrogen Peroxide; SOD – Superoxide Dismutase; Asc – Ascorbate; Tre – Trehalose; Glth – Glutathione.
[2] Units: MDA – nmol $g^{-1}{\cdot}dw^{-1}$; Perox – µmol $g^{-1}{\cdot}dw^{-1}$; SOD – units $mg^{-1}{\cdot}protein^{-1}$; Asc – nmol $g^{-1}{\cdot}dw^{-1}$; Tre – µmol $g^{-1}{\cdot}dw^{-1}$; Glth – nmol $g^{-1}{\cdot}dw^{-1}$.
[3] The standard error of mean was MDA – 0.00287; Perox – 0.02763; SOD – 0.00212; Asc – 0.02417; Tre – 0.01284; Glth – 0.02096.
[4] NS, *, **, *** indicate non-significant, significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.
Values followed by the same superscript letter are not significantly different within each column.

Another effect of UV-B radiation is the decrease in $P_{NET}$, which was observed only under ambient $CO_2$. The reduced $P_{NET}$ under ambient $CO_2$ may be a result of the impairment of the photosystem II electron transport system, diminished ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and regeneration, the damaging structure of chloroplasts, and the thylakoid membrane[1]. However, no single process, rather a culmination of numerous minor variations under ambient $CO_2$, can be identified as the reason for UV-B reduction based on the non-significant results of $\Phi_{PSII}$, ETR, and chlorophyll fluorescence measurements.

Elevating $CO_2$ resulted in greater $P_{NET}$ regardless of UV-B treatment, increasing internal $CO_2$ ($C_i$) and $\Phi_{CO_2}$ of the leaves compared to ambient $CO_2$. Yet, no difference existed in the $C_i/C_a$ ratio. These results indicate that the amount of $C_i$ for basil plants is directly correlated with ambient (environmental) $CO_2$ within this range. High concentrations of $CO_2$ are stimulatory of higher $P_{NET}$ and suppression of photorespiration due to the increased substrate availability for Rubisco to prefer $CO_2$ over $O_2$[41]. While low $C_i$ concentrations would be inhibitory of Rubisco regeneration, high concentrations can also be inhibitory as Rubisco regeneration rate would become limited by electron transport rate, and further by triose phosphates[41,42]. Although photorespiration is considered an overall energy wasting process, Takahashi & Badger[43] identify that while it limits plant photosynthesis, this limitation may confer protection against damages due to reductions in ETR preventing excessive ROS generation under stressful conditions and dissipating excessive light energy.

While we observed no reductions in ETR rate, we did observe that supplemental $CO_2$ causes an increase in $P_{NET}$, negating some of the harmful effects of UV-B on the photosynthetic apparatus. In contrast, some harmful effects still occur, like those observed with a significant decrease in $F_v'/F_m'$ and $q_N$ of UV-B treated plants at elevated $CO_2$. Although a decline in $\Phi_{PSII}$ would indicate a significant decline in overall photosynthesis, we did observe the decline in $F_v'/F_m'$ and $q_N$ in UV-B and elevated $CO_2$ treatments, which is indicative of some reduction in photosynthetic performance and efficiency. Previous research by Romanatti et al.[44] also demonstrated a sharp decline in dark-adapted quantum efficiency and $q_N$ for eggplants (*Solanum melongena*) when subjected to UV-B radiation.

## Pigment concentrations

Mosadegh et al.[45] found that juvenile basil, when grown at similar UV-B levels (8.5 kJ $m^{-2}$ $d^{-1}$), led to no changes in chlorophyll concentrations but caused decreases in carotenoid concentrations. The reduction in carotenoid concentrations is a typical response in most plants as ultraviolet radiation indirectly affects plant pigments by either inhibiting their synthesis or impacting enzymes in their biosynthetic pathway[40]. However, the results of other researchers are very different from the overall increase among all concentrations of individual pigments that we observed from daily UV-B treatments and may be linked with the age of plants. Previous research by Mosadegh et al.[45] found significant effects of UV-B radiation on plant pigments after only 72 h of exposure, rather than the 28 days of treatment in the current study. Additionally, the long-term exposure of square-wave radiation may have enabled plants to become acclimated, like in the current study. Thus, when plants become acclimated, they may activate protective mechanisms, improving overall plant health and performance, which may enhance plant pigment production.

North et al.[46] identified in *Arabidopsis thaliana* that ABA production directly affects the concentrations of neoxanthin and violaxanthin with little to no influence on other carotenoid pigments. This is due to both neoxanthin and violaxanthin being direct precursors needed for the synthesis of ABA, and both displayed significant increases under UV-B treatment. This increase observed in our study may result from increased demand for abscisic acid (ABA) used to confer UV-B stress protection, which was also observed and concluded as one of the defensive mechanisms against UV-B damage in *Nicotiana attenuate*[47].

## Antioxidant and oxidative concentrations

Malondialdehyde (MDA) is a molecule used to estimate the relative concentration of lipid peroxidation, which is created as a byproduct of reactive oxygen species (ROS) and is a sign of harmful ROS accumulation in plants signifying cellular membrane damage[48,49]. UV-B's effects on MDA accumulation in basil have not been studied, but in cucumber UV-B treatments, increased MDA concentrations as well as marked increases in membrane leakage and reductions in seedling growth significantly reducing crop productivity[49]. Our study only observed an extreme rise in MDA concentration under ambient $CO_2$, UV-B treatment combination, indicating large amounts of lipid peroxidation had occurred and is most likely the result of the UV-B application. However, the lack of elevated MDA under the elevated $CO_2$ and UV-B treatment combination does not necessarily indicate no UV-B damage. Morales & Munné-Bosch[48] identify that MDA accumulation in plants does not always signify stress as it accumulates during periods of plant acclimation to new conditions, and more so is dependent on the length of its accumulation to cause damage in plant membranes.

Of the remaining antioxidant and oxidative concentrations evaluated, only superoxide dismutase and ascorbate displayed differences among treatments, both of which play a role in removing ROS protecting plants[50]. Yet, the current study's results did not show elevated levels of ROS different between treatments. Elevated levels of these antioxidants would occur when plants are under oxidative stress and confer protection and removal of ROS. Our results show basil plants were only under slight oxidative stress when increasing $CO_2$ concentration, regardless of UV-B treatment; however, like MDA, these concentrations are temporal and change as plants mature and acclimate[51].

## CONCLUSIONS

Although UV-B radiation confers harmful effects on plants, it is essential for crops like basil, which stimulates plant protective mechanisms and pathways to fill oil glands and produce numerous other healthful compounds. Additionally, when UV-B radiation is combined with elevated $CO_2$, at projected levels to be reached by the end of the century due to anthropogenic emissions, it can help to mitigate most of the harmful effects and even improve plant photosynthesis. Thus, when $CO_2$ is combined with environmental stressors like UV-B radiation in our study, it displays the complex nature of plant responses. Furthermore, when combined with information from other sources, the current study's results from analyzing MDA conclude that data from a single point of time is insufficient to determine overall oxidative stress. From these observations, antioxidants and oxidative reactions can change temporally. Therefore, analyzing antioxidants and oxidative reactions will need to be measured multiple times throughout the plant's life cycle to accurately determine its overall oxidative stress response. Thus, while UV-B radiation is essential for basil production but can also harm the plant process, future studies analyzing antioxidant and oxidative concentrations should focus on temporal changes in UV-B radiation rather than single measurements.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Dates

Received 3 March 2022; Accepted 5 May 2022; Published online 14 June 2022

## REFERENCES

1. Prasad PV, Kakani VG, Reddy RK. 2017. Ozone Depletion. In *Encyclopedia of Applied Plant Sciences*, eds. Thomas B, Murray BG, Murphy DJ, Vol. 3. Waltham, MA: Academic Press, Elsevier. pp. 318–26 https://doi.org/10.1016/B978-0-12-394807-6.00015-0
2. Williamson CE, Zepp RG, Lucas RM, Madronich S, Ballare CL, et al. 2014. Solar ultraviolet radiation in a changing climate. *Nature Climate Change* 4:434–41
3. Baroniya SS, Kataria S, Pandey GP, Guruprasad KN. 2014. Growth, photosynthesis and nitrogen metabolism in soybean varieties after exclusion of the UV-B and UV-A/B components of solar radiation. *The Crop Journal* 2:388–97
4. Klem K, Ač A, Holub P, Kováč D, Špunda V, et al. 2012. Interactive effects of PAR and UV radiation on the physiology, morphology and leaf optical properties of two barley varieties. *Environmental and Experimental Botany* 75:52–64
5. Joshi P, Gartia S, Pradhan MK, Panigrahi S, Nayak L, et al. 2013. Acclimation of clusterbean cotyledon to UV-B radiation in the presence of UV-A: partial restoration of photosynthetic energy balance and redox homeostasis. *Acta Physiologiae Plantarum* 35:2323–28
6. Martínez-Lüscher J, Morales F, Delrot S, Sánchez-Díaz M, Gomés E, et al. 2013. Short- and long-term physiological responses of grapevine leaves to UV-B radiation. *Plant Science* 213:114–22
7. Majer P, Hideg É. 2012. Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house. *Plant Physiology and Biochemistry* 50:15–23
8. Singh S, Agrawal SB, Agrawal M. 2015. Responses of pea plants to elevated UV-B radiation at varying nutrient levels: N-metabolism, carbohydrate pool, total phenolics and yield. *Functional Plant Biology* 42:1045–56
9. Ballaré CL, Mazza CA, Austin AT, Pierik R. 2012. Canopy light and plant health. *Plant Physiology* 160:145–55
10. Hideg É, Jansen MAK, Strid Å. 2013. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? *Trends in Plant Sciences* 18:107–15
11. Dou H, Niu G, Gu M. 2019. Pre-harvest UV-B radiation and photosynthetic photon flux density interactively affect plant photosynthesis, growth, and secondary metabolites accumulation in basil (*Ocimum basilicum*) plants. *Agronomy* 9:434
12. Kakani VG, Reddy KR, Zhao D, Sailaja K. 2003. Field crop responses to ultraviolet-B radiation: a review. *Agricultural and Forest Meteorology* 120:191–218
13. Qaderi MM, Reid DM, Yeung EC. 2007. Morphological and physiological responses of canola (*Brassica napus*) siliquas and seeds to UVB and $CO_2$ under controlled environment conditions. *Environmental and Experimental Botany* 60:428–37
14. Al Jaouni S, Saleh AM, Wadaan MAM, Hozzein WN, Selim S, et al. 2018. Elevated $CO_2$ induces a global metabolic change in basil (*Ocimum basilicum* L.) and peppermint (*Mentha piperita* L.) and improves their biological activity. *Journal of Plant Physiology* 224–225:121–31
15. Dou H, Niu G, Gu M, Masabni JG. 2018. Responses of sweet basil to different daily light integrals in photosynthesis, morphology, yield, and nutritional quality. *HortScience* 53:496–503
16. Aphalo PJ, Jansen MAK, McLeod AR, Urban O. 2015. Ultraviolet radiation research: from the field to the laboratory and back. *Plant, Cell & Environment* 38:853–55
17. Johnson CB, Kirby J, Naxakis G, Pearson S. 1999. Substantial UV-B-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). *Phytochemistry* 51:507–10
18. Ioannidis D, Bonner L, Johnson CB. 2002. UV-B is required for normal development of oil glands in *Ocimum basilicum* L. (sweet basil). *Annals of Botany* 90:453–60
19. Sakalauskaitė J, Viškelis P, Duchovskis P, Dambrauskienė E, Sakalauskienė S, et al. 2012. Supplementary UV-B irradiation effects on basil (*Ocimum basilicum* L.) growth and phytochemical properties. *Journal of Food, Agriculture & Environment* 10:342–46
20. Mosadegh H, Trivellini A, Ferrante A, Lucchesini M, Vernieri P, et al. 2018. Applications of UV-B lighting to enhance phenolic accumulation of sweet basil. *Scientia Horticulturae* 229:107–16
21. Reddy KR, Hodges HF, Read JJ, McKinion JM, Baker JT, Tarpley L, Reddy VR. 2001. Soil-Plant-Atmosphere-Research (SPAR) facility: A tool for plant research and modeling. *Biotronics* 30:27–50
22. Zhao D, Reddy KR, Kakani VG, Read JJ, Sullivan JH. 2003. Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environmental conditions. *Plant, Cell & Environment* 26:771–82

23. Wijewardana C, Hock H, Henry B, Reddy KR. 2015. Screening corn hybrids for cold tolerance using morphological traits for early season seeding. *Crop Science* 55:851–67
24. Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station* 347:2–32
25. McKinion JM, Hodges HF. 1985. Automated system for measurement of evapotranspiration from closed environmental growth chambers. *Transactions of the American Society of Agricultural Engineers* 28:1825–28
26. Brazel SR, Barickman TC, Sams CE. 2021. Short-term waterlogging of kale (*Brassica oleracea* L. var. *acephala*) plants causes a decrease in carotenoids and chlorophylls while increasing nutritionally important glucosinolates. *ISHS Acta Horticulturae* 1329:175–80
27. Kopsell DA, Kopsell DE, Lefsrud MG, Curran-Celentano J, Dukach LE. 2004. Variation in lutein, $\beta$-carotene, and chlorophyll concentrations among *Brassica oleracea* cultigens and seasons. *HortScience* 39:361–64
28. Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125:189–98
29. Mukherjee SP, Choudhuri MA. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. *Physiologia Plantarum* 58:166–70
30. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany* 32:93–101
31. Trevelyan WE, Harrison JS. 1952. Studies on yeast metabolism. 1. Fractionation and microdetermination of cell carbohydrates. *The Biochemical Journal* 50:298–303
32. Brin M. 1966. Transketolase: clinical aspects. In *Methods in Enzymology,* Vol. 9: 377. New York: Academic Press. pp. 506–14 https://doi.org/10.1016/0076-6879(66)09101-8
33. Habibur Rahman Pramanik M, Imai R. 2005. Functional identification of a trehalose 6-phosphate phosphatase gene that is involved in transient induction of trehalose biosynthesis during chilling stress in rice. *Plant Molecular Biology* 58:751–62
34. Hottiger T, Boller T, Wiemken A. 1987. Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in *Saccharomyces cerevisiae* cells subjected to temperature shifts. *FEBS Letters* 220:113–15
35. Klutts S, Pastuszak I, Edavana VK, Thampi P, Pan YT, et al. 2003. Purification, cloning, expression, and properties of mycobacterial trehalose-phosphate phosphatase. *The Journal of Biological Chemistry* 278:2093–100
36. Einig W, Hampp R. 1990. Carbon partitioning in Norway spruce: amounts of fructose 2,6-bisphosphate and of intermediates of starch/sucrose synthesis in relation to needle age and degree of needle loss. *Trees* 4:9–15
37. Griffith OW. 1980. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry* 106:207–12
38. Zhao D, Reddy KR, Kakani VG, Koti S, Gao W. 2005. Physiological causes of cotton fruit abscission under conditions of high temperature and enhanced ultraviolet-B radiation. *Physiologia Plantarum* 124:189–99
39. Kakani VG, Reddy KR, Zhao D, Gao W. 2004. Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. *Physiologia Plantarum* 121:250–57
40. Kataria S, Jajoo A, Guruprasad KN. 2014. Impact of increasing ultraviolet-B (UV-B) radiation on photosynthetic processes. *Journal of Photochemistry and Photobiology B:Biology* 137:55–66
41. Dusenge ME, Duarte AG, Way DA. 2019. Plant carbon metabolism and climate change: elevated $CO_2$ and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist* 221:32–49
42. Sharkey TD, Stitt M, Heineke D, Gerhardt R, Raschke K, et al. 1986. Limitation of photosynthesis by carbon metabolism: II. $O_2$-insenstive $CO_2$ uptake results from limitation of triose phosphate utilization. *Plant Physiology* 81:1123–29
43. Takahashi S, Badger MR. 2011. Photoprotection in plants: a new light on photosystem II damage. *Trends in Plant Science* 16:53–60
44. Romanatti PV, Rocha GA, Veroneze Júnior V, Santos Filho PR, de Souza TC, et al. 2019. Limitation to photosynthesis in leaves of eggplant under UVB according to anatomical changes and alterations on the antioxidant system. *Scientia Horticulturae* 249:449–54
45. Mosadegh H, Trivellini A, Lucchesini M, Ferrante A, Maggini R, et al. 2019. UV-B physiological changes under conditions of distress and eustress in sweet basil. *Plants* 8:396
46. North HM, De Almeida A, Boutin JP, Frey A, To A, et al. 2007. The Arabidopsis ABA-deficient mutant aba4 demonstrates that the major route for stress-induced ABA accumulation is via neoxanthin isomers. *The Plant Journal* 50:810–24
47. Đinh ST, Gális I, Baldwin IT. 2013. UVB radiation and 17-hydroxygeranyllinalool diterpene glycosides provide durable resistance against mirid (*Tupiocoris notatus*) attack in field-grown *Nicotiana attenuata* plants. *Plant, Cell & Environment* 36:590–606
48. Morales M, Munné-Bosch S. 2019. Malondialdehyde: Facts and Artifacts. *Plant Physiology* 180(3):1246–50
49. Teklemariam T, Blake TJ. 2003. Effects of UVB preconditioning on heat tolerance of cucumber (*Cucumis sativus* L.). *Environmental and Experimental Botany* 50:169–82
50. Barickman TC, Adhikari B, Sehgal A, Walne CH, Reddy KR, Gao W. 2021. Drought and elevated $CO_2$ impacts photosynthesis and biochemicals of basil (*Ocimum basilicum* L.). *Stresses* 1:223–37
51. Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohammad Mohsin S, et al. 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. *Antioxidants* 9:681