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Individual and Interactive Temporal Implications of UV-B Radiation and Elevated CO_2 on the Morphology of Basil (*Ocimum basilicum* L.)

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Abstract: Temporal and spatial variations in ozone levels and temporal changes in solar radiation greatly influence ultraviolet radiation incidence to crops throughout their growth, yet the interactive effects of CO_2 and UV-B radiation on Basil production under sunlight environmental conditions has not been studied. Basil 'Genovese' plants grown under sunlit plant growth chambers were subjected to a combination of supplemental UV-B (0 and 10 kJ $m^{-2}d^{-1}$) and ambient (420 ppm) and elevated (720 ppm) CO_2 treatments for 38 days after 14 days of germination. UV-B radiation treatments caused a decrease in basil stem branching, fresh mass, and stem dry mass under both CO_2 treatments when harvested after 17 and 38 days of treatment. There was also an increase in basil leaf surface wax under UV-B (10 kJ $m^{-2}d^{-1}$) treatment compared to controls (0 kJ $m^{-2}d^{-1}$). Elevated CO_2 treatments caused a decrease in morphological features, including specific leaf area and fresh mass. Interactive effects between UV-B and CO_2 treatments existed for some morphological features, including plant height, root surface area, and average root diameter. Understanding the impacts that CO_2 and UV-B radiation treatments have on basilcan improve existing varieties for increased tolerance while simultaneously improving yield, plant morphology, and physiology.

Keywords: root surface area; root dry mass; plant height; specific leaf area; epicuticular wax

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1. Introduction

Basil (*Ocimum basilicum* L.) is a widely used herb in gastronomy, ornamental plantings, and medicinal practices, which exhibits variability in growth and photosynthesis when subjected to various lighting conditions [1]. In basil production, glazing materials and coverings used in protected culture production systems are used to protect plants from intense light, but these materials have been shown to reduce the nutrition and flavor of crops [2,3]. An important part of basil production, among other crops, is exposing plants to environmental factors such as ultraviolet radiation due to its stimulation of compounds such as regulatory enzymes that affect plant nutrition and growth [3]. The development of defensive mechanisms to protect against adverse environmental factors, such as ultraviolet radiation, is due to the sessile nature of plants [4]. However, these plant mechanisms are dependent on the exposure intensity and duration of ultraviolet radiation [4].

The concentration of ultraviolet radiation at the Earth's surface has increased steadily since the 1950s, coinciding with the ozone layer's degradation due to anthropogenic emissions [5]. Ultraviolet-A (UV-A), ultraviolet-B (UV-B), and ultraviolet-C (UV-C) are the three components of ultraviolet radiation. Only UV-A and UV-B radiation reach the Earth's

surface, and their individual intensities are influenced by three main factors: altitude, latitude, and time of day [5,6]. Increased intensities of ultraviolet radiation are known to have harmful effects on both human and plant health. The most dangerous radiation that reaches the Earth's surface is UV-B radiation (wavelengths approximately between 280 and 315 nm). UV-B radiation can reduce plant biomass and cause morphological changes in plants, such as reduced seed size, changes in appearance, and siliquae color in canola (*Brassica napus*) [6,7]. Previous research in several species has demonstrated bronzing or browning on the leaf surfaces that subsequently manifests into chlorotic and necrotic tissue [8]. When leaves have sustained exposure to UV-B radiation, they can become cup-shaped and desiccate. In soybean, leaf area and total biomass accumulation were significantly decreased when exposed to 10 kJ m^{-2} d^{-1} [9]. Additionally, Dou et al. [10] demonstrated that basil plants that were treated with increasing exposure to UV-B radiation doses had decreased plant height, width, and leaf area. The damaging effects of UV-B, especially to the leaf tissue, can lower light-dependent plant growth processes by reducing net photosynthesis.

There has been recent debate on whether UV-B radiation is a stressor or eustressor, which is highly dependent on intensity and plant response [11]. UV-B radiation at higher levels can act as a stressor by causing increased reactive oxygen species (ROS) concentrations that contribute to irreversible DNA and cellular damage [10,11]. Low UV-B radiation levels can be a eustressor, by causing a cascade of plant responses to adapt to new environments [11]. Despite the deleterious effects UV-B radiation can cause, it can also benefits plant defense against pests and pathogens by increasing jasmonate and phenolic compounds [6,12]. UV-absorbing compounds, such as phenolics, can protect sensitive components from UV-B radiation [13,14]. Studies have also indicated that other environmental factors, such as water and nutrient availability, can also influence how UV-B radiation affects plant growth. For example, interactions between UV-B radiation and environmental stress in field studies can exhibit variability in results due to cross-tolerance stressors [11].

Apart from the increasing ultraviolet radiation levels, other anthropogenic factors change the environment through increasing CO_2 concentrations [15]. The combustion of fossil fuels, industrial processing, and land-use practices are all factors that contribute to increasing atmospheric CO_2 concentrations [16–18]. These processes are expected to cause atmospheric CO_2 levels to reach between 730 and 1020 ppm by 2100 [16]. For most crops, elevated CO_2 concentrations have been shown to increase photosynthesis and plant growth. However, when combined with the detrimental effects UV-B radiation has on plant growth, it can reduce or even negate these advantages [7].

Former studies [18–23] that analyzed the combination of UV-B radiation and elevated CO_2 concentrations have indicated impacts on plant growth and development. In cotton (*Gossypium hirsutum* L.), Zhao et al. [19] observed interactions between UV-B radiation and CO_2 with variability in fruiting, branch number, branch length, and fruit abscission, along with reductions in net photosynthesis. Zhao et al. [20] observed no interactions between UV-B radiation and CO_2 for cotton canopy net photosynthesis and leaf area and detected only individual variability in treatments. Comparable effects with UV-B and elevated CO_2 concentrations were also seen in soybean (*Glycine max* L.). For example, Koti et al. [21] saw reductions in flower length, staminal column length, pollen tube length, pollen production, and pollen germination, whereas Koti et al. [22] observed plant height reductions. Previous research also indicated that basil plants exposed to elevated CO_2 had increased biomass accumulation, improved photosynthesis rates, and increased numerous primary and secondary metabolites [23].

The combination of UV-B and elevated CO_2 has been shown to change the days to veraison and affect photosynthetic parameters in grapes (*Vitis vinifera* cv. Tempranillo) [24], increase leaf wax production in corn (*Zea mays)* [25], and cause widespread effects on various growth and photometric parameters in cowpeas (*Vigna unguiculata* L. Walp.) [26]. Sole applications of UV-B radiation treatments on different sweet potato (*Ipomoea batatas* L. Lam) cultivars displayed various degrees of tolerance, with some having little to no reductions

in yield [27]. However, the combination of UV-B and elevated CO_2 concentrations had non-significant effects on the growth parameters of silver birch (*Betula pendula*) [28] and dark-leaved willow (*Salix myrsinifolia*) [29]. These results indicate widespread variability of response in plants to UV-B radiation between plant species and genotypes.

Previous research on UV-B treatments on basil demonstrated decreased mean leaf area, leaf number, and specific leaf area [3]. In addition, one study demonstrated that basil plants that were grown devoid of UV-B radiation in the greenhouse had decreased leaf-oil glands, which reduced their nutritional contents and healthful benefits [30]. Thus, basil should receive UV-B radiation to maintain nutritional content, despite observed changes in the morphological features. Previous studies with UV-B radiation and basil have used growth chambers with below-ambient supplemental UV-B radiation (same length as photoperiod) [31], short-term UV-B radiation with varying lengths of UV-B exposure [32], and short-term UV-B exposure of above-ambient supplemental UV-B radiation with differing lengths of time [10]. Among others, these studies have not analyzed the effects that a combination of continuous UV-B radiation and elevated CO_2 concentrations would have on basil grown under ambient environmental conditions. The current study's objective was to determine the effects of UV-B radiation and elevated CO_2 concentrations on basil root and shoot morphology parameters when grown under ambient light levels. Thus, UV-B may have detrimental effects on basil morphology and yield while CO_2 will increase basil dry mass (DM).

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The experiment was conducted in four sunlit, controlled environmental chambers known as soil-plant-atmosphere-research (SPAR) units in June–July 2019. The facility is located at the Rodney Foil Plant Science Research Facility of Mississippi State University, Mississippi State, MS, USA. The SPAR chambers can control the air temperature and CO_2 concentrations at determined set points. Each chamber consists of 1.27 cm thick Plexiglas (2.5 m tall by 2.0 m long by 1.5 m wide) that transmits 97% of incoming photosynthetically active radiation (PAR) to pass without spectral variability. During the experiment, the incoming daily solar radiation was measured with a pyranometer (Model 4-8; The Eppley Laboratory Inc., Newport, RI, USA) outside the SPAR units and ranged from 4.2 to 35.5 MJ m^{-2} d^{-1}, with an average value of 25.86 $\pm$ 0.92 MJ m^{-2} d^{-1}. Additional details of the SPAR unit operations and control have been described by Reddy et al. [33] and Wijewardana et al. [34].

Basil 'Genovese' (Johnny's Selected Seeds, Winslow, ME, USA) seeds were sown in polyvinyl-chloride pots (15.2 cm diameter by 30.5 cm height) filled with a soil medium consisting of 3:1 sand/soil classified as a sandy loam (87% sand, 2% clay, and 11% silt) with 500 g of gravel at the bottom of each pot. Multiple seeds were sown in each pot and then thinned to one plant per pot seven days after sowing. Pots were arranged in a randomized complete block design in a two-by-two factorial arrangement with UV-B and CO_2 treatments. Within each of the four SPAR chambers, there were three blocks with ten replications. The environmental growing conditions, except for UV-B and CO_2, remained constant throughout the experiment. The measured average temperature among the units was 26.16 $\pm$ 0.22 °C; the carbon dioxide concentration was 437.5 $\pm$ 1.08 for ambient and 725.52 $\pm$ 7.1 µmol mol^{-1} for elevated treatments, and 9.72 $\pm$ 0.5 kJ m^{-2} d^{-1} for UV-B radiation treatments.

Basil plants were irrigated three times per day through an automated computer-controlled drip system with a full-strength Hoagland's nutrient solution [35]. Irrigation was applied at 07:00, 12:00, and 17:00 h based on evapotranspiration values. Evapotranspiration rates expressed on the ground area (L d^{-1}) throughout the treatment period were measured in each SPAR unit as the cooling coils' rate removed the condensate at 900-s intervals and measured the mass of water [33,36,37].

2.2. UV-B and CO_2 Treatments

UV-B treatments were imposed on the chambers with eight fluorescent lamps (UV-313 lamps, Q-Panel Company, Cleveland, OH, USA), positioned 0.5 m above the plant canopy. The lamps were wrapped in calcium diacetate films to filter UV-C radiation and changed routinely throughout the experiment to account for film degradation. Chambers were kept under ambient light levels, with UV-B treatments occurring from 8:00 to 16:00 h every day. The interception of UV-B radiation at the canopy level was measured daily using a UVX digital radiometer (UVP Inc., San Gabriel, CA, USA). Chambers not receiving UV-B radiation treatments contained non-illuminated bulbs and frames.

The CO_2 concentration [CO_2] of each SPAR unit was measured using infrared gas analyzers (LI-6252, LI-COR Biosciences, Lincoln, NE, USA). Gas samples were drawn from each unit to the field laboratory, where moisture was removed from the sample using refrigerated water (4 °C) and magnesium perchlorate. Individual chamber [CO_2] was maintained by supplying pure CO_2 from compressed gas cylinders through a system of pressure regulators, solenoids, and needle valves with a calibrated flow meter [33].

Plants were randomly assigned to chambers consisting of 0 kJ m^{-2} d^{-1} and 10 kJ m^{-2} d^{-1} combined with ambient (420 ppm) or elevated (720 ppm) CO_2 concentrations. The CO_2 treatments were initiated at sowing. UV-B radiation treatments were initiated at 14 days after sowing when basil plants had two fully expanded leaves. Daytime temperatures were initiated at sunrise and nighttime temperatures one hour after sunset. Throughout the experiment, the temperature was kept at an optimum of 30/22 °C day and night.

2.3. Morphophysiological Measurements

Nine plants were harvested at 17 days after treatment (DAT) to obtain phenotypic and growth data while ensuring adequate spacing for all remaining plants (fifteen), which were harvested after 38 DAT. Phenotypic data of basil plants included plant height (HT), node number (NN), branch number (BN), and leaf area (LA), which was measured using a LI-3100 leaf-area meter (LI-COR Biosciences, Lincoln, NE, USA). Plant DM was measured for all plants after oven drying at 75 °C until a constant mass was achieved.

2.4. Root Image Acquisition and Analysis

Roots were cut and separated from the stems and washed thoroughly, avoiding any disturbance to the root system. The longest root length (RL) was determined using a ruler. The cleaned individual root systems were floated in 5 mm of water in a 0.4 by 0.3 m Plexiglas tray. Roots were untangled and separated with a plastic paintbrush to minimize root overlap. The tray was placed on top of a specialized dual-scan optical scanner (Regent Instruments Inc., Québec City, QC, Canada) linked to a computer. Gray-scale root images were acquired by setting the parameters to high accuracy (resolution 800 × 800 dpi). Acquired images were analyzed for the cumulative RL, root surface area, average root diameter, root volume, number of roots, number of roots having laterals, number of tips, number of forks, and crossings using WinRHIZO Pro software (Regent Instruments Inc., Québec City, QC, Canada).

2.5. Specific Leaf Area Estimation

Specific leaf area (SLA) was estimated according to Bannayan et al. [38] to measure the leaf area (cm^2) formed per unit of leaf dry biomass (g). Five (5) leaves randomly selected from the 3rd or 4th leaf from the stem apex from each replication were used, with the total leaf area being measured with the LI-3100 leaf area meter and the leaves dried in a forced-air oven at 75 °C for two days.

2.6. Epicuticular Wax Content Determination

The extraction and quantitative analysis of leaf epicuticular waxes were carried out as per the method of Ebercon et al. [39], with minor modifications as described by Singh et al. [40]. Ten leaf discs constituting an area of 35.36 cm^{-2} from the 3rd or 4th leaf

from the stem apex were cut from three different plants from all the units. Leaf waxes were removed by stirring the leaf disks in 15 mL of chloroform (Sigma–Aldrich, Inc., St. Louis, MO, USA) in a test tube for 20 s. The wax extract was evaporated on a water bath maintained at 80 °C, cooled to room temperature; 5 mL of dichromate reagent was added and further heated on a water bath maintained at 80 °C for 30 min. The reagent was prepared by dissolving 20 g $K_2Cr_2O_7$ in 40 mL of de-ionized water, and the resulting slurry was mixed with 1 L of H_2SO_4 and heated below boiling point until a clear solution was obtained. The samples were removed from the water bath and cooled, and then 12 mL of de-ionized water was added, allowed to stand for 15 min. The color intensity was measured at 590 nm using a Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The wax content was expressed on a leaf area basis (μg cm^{-2}) using a standard curve developed from the same species' wax.

2.7. Statistical Analysis

Statistical analysis was performed using SAS (version 9.4; SAS Institute, Cary, NC, USA) using the PROC GLIMMIXED analysis of variance (ANOVA) followed by mean separation. The experiment's fixed effect consisted of the two UV-B light and two CO_2 treatments, while replications as random effects. Standard errors were based on the pooled error term from the ANOVA table. Duncan's multiple range test ($p \leq 0.05$) was used to differentiate between UV-B and CO_2 treatment classifications when the F-values were significant for main effects. Model-based values were reported rather than the unequal standard error from a data-based calculation because pooled errors reflect the statistical testing conducted. Diagnostic tests were performed to ensure that treatment variances were statistically equal before pooling.

3. Results

In the current study, basil plant height was significantly affected by the interaction of UV-B radiation and CO_2 treatments at 17 DAT. Basil plant height was reduced by 12.6 and 19.1% when basil plants were exposed to 10 kJ m^{-2} d^{-1} UV-B at both [CO_2] treatments, respectively (Table 1). However, there were no differences between the non-UV-B treatments at either CO_2 concentration (Table 1). When basil plants were given time to mature to 38 DAT, the UV-B-treated plants under ambient [CO_2] had the greatest height (Table 2). The elevated UV-B treatment caused reductions in branch number and leaf area regardless of CO_2 treatment (Table 1) at 17 DAT. There were similar trends when the branch number and leaf area were measured at 38 DAT (Table 2). When the number of nodes on the basil plants was measured at 17 and 38 DAT, there were no differences among the treatments.

For the DM of individual tissues at 17 DAT, the leaf, stem, and root values were reduced under elevated UV-B radiation regardless of CO_2 concentration when compared to non-UV-B-treated plants (Table 1). Additionally, at 17 DAT, elevated CO_2 concentrations caused an increase in DM for only the leaves and stems (Table 1). Interestingly, as the basil plants matured, stem DM was significantly affected by UV-B, root tissue DM was affected by CO_2 concentrations, and leaf DM was not significantly affected by either treatment (Table 3).

Table 1. The height (HT), node number (NN), branch number (BN), leaf area (LA), leaf dry mass (L DM), stem dry mass (ST DM), root dry mass (RT DM), shoot dry mass (SH DM), total dry mass (TTL DM), and root-to-shoot ratio (RS ratio) of basil plants grown without UV-B radiation (No UV-B) and with 10 kJ m^{-2} d^{-1} UV-B radiation (UV-B) at 420 and 720 ppm CO_2 concentration (ambient [CO_2] and elevated [CO_2], respectively) after 17 days of treatment.

Treatment	HT [1]	NN	BN	LA	L DM	ST DM	RT DM	SH DM	TTL DM	RS Ratio [2]
					Ambient [CO_2]					
No UV-B	36.56 a	7.1 a	15.3 a	1223.6 a	4.479 b	2.188 b	0.941 ab	6.667 b	7.608 b	0.140 a
UV-B	29.61 c	6.7 a	11.2 b	960.8 b	3.536 c	1.664 c	0.783 b	5.200 c	5.983 c	0.149 a
					Elevated [CO_2]					
No UV-B	36.61 a	7.0 a	15.3 a	1321.0 a	5.779 a	2.789 a	1.021 a	8.568 a	9.589 a	0.118 b
UV-B	32.00 b	6.9 a	12.3 b	909.3 b	4.757 b	2.368 b	0.868 ab	7.124 b	7.992 b	0.121 b
***p*-Value** [3,4]										
UV-B	***	NS	***	***	**	**	*	**	**	NS
CO_2	*	NS	NS	NS	***	***	NS	***	***	***
UV-B × CO_2	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Plant height units in centimeters; node number and branch number on a per-plant basis; leaf area units in centimeters squared; remaining parameter units are on a gram-per-plant basis, except root shoot ratio. [2] RS ratio—root to shoot ratio (root dry mass in grams/shoot dry mass in grams). [3] The standard error of the mean was HT—0.64; NN—0.16; BN—0.69; LA—65.8; L DM—0.3381; ST DM—0.1379; RT DM—0.074; SH DM—0.456; TTL DM—0.5245; RS Ratio—0.0049. [4] NS, *, **, *** indicate non-significant and significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Values followed by same letter are not significantly different within each column.

Table 2. The height (HT), node number (NN), branch number (BN), leaf area (LA), leaf dry mass (L DM), stem dry mass (ST DM), root dry mass (RT DM), shoot dry mass (SH DM), total dry mass (TTL DM), and root-to-shoot ratio (RS ratio) of basil plants grown without UV-B radiation (No UV-B) and with 10 kJ m^{-2} d^{-1} UV-B radiation (UV-B) at 420 and 720 ppm CO_2 concentration (ambient [CO_2] and elevated [CO_2], respectively) after 38 days of treatment.

Treatment	HT [1]	NN	BN	LA	L DM	ST DM	RT DM	SH DM	TTL DM	RS Ratio [2]
					Ambient [CO_2]					
No UV-B	61.67 b	10.0 b	29.9 a	6946.3 a	25.032 a	33.049 ab	6.847 b	58.081 ab	64.922 ab	0.1156 b
UV-B	68.97 a	10.0 b	19.7 c	6735.3 a	23.026 a	28.004 b	7.147 ab	51.030 b	57.978 b	0.1376 ab
					Elevated [CO_2]					
No UV-B	60.93 b	10.1 b	29.7 a	8078.9 a	28.393 a	38.733 a	8.511 ab	67.126 a	75.637 a	0.1284 b
UV-B	57.80 b	10.5 a	22.7 b	7369.3 a	25.456 a	30.509 b	9.166 a	55.965 ab	65.131 ab	0.1751 a
***p*-Value** [3,4]										
UV-B	NS	NS	***	NS	NS	*	NS	NS	NS	*
CO_2	***	NS	NS	NS	NS	NS	*	NS	NS	NS
UV-B × CO_2	**	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Plant height units in centimeters; node number and branch number on a per-plant basis; leaf area units in centimeters squared; remaining parameter units are on a gram-per-plant basis, except root shoot ratio. [2] RS ratio—root to shoot ratio (root dry mass in grams/shoot dry mass in grams). [3] The standard error of mean was HT—1.92; NN—0.15; BN—1.03; LA—662.2; L DM—2.362; ST DM—3.171; RT DM—0.957; SH DM—5.482; TTL DM—6.348; RS Ratio—0.0147. [4] NS, *, **, *** indicate non-significant and significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Values followed by same letter are not significantly different within each column.

The root surface area and the average diameter of basil plants were impacted by coupling the UV-B and CO_2 treatments (Table 3). The basil root surface area was reduced by UV-B treatment but recovered when exposed to the elevated CO_2. Moreover, the imposed UV-B treatments decreased the root volume and fork number, regardless of the CO_2 treatment (Table 3). Only the root-to-shoot ratio of the first harvest exhibited reductions when the CO_2 concentration was elevated, with UV-B radiation treatment having no impact (Table 1). The UV-B and CO_2 treatments did not affect the basil RL, total RL, number of root tips, and number of root crossings at 17 DAT. Root morphological measurements were not taken at 38 DAT due to sizing issues with the WinRHIZO equipment.

Table 3. The mean root length (RL), total root length (TRL), surface area (SA), root diameter (RD), root volume (RV), tips (TP), forks (FK), and crossings (CR) of the roots of basil plants grown without UV-B radiation (No UV-B) and with 10 kJ m^{-2} d^{-1} UV-B radiation (UV-B) at 420 and 720 ppm CO_2 concentrations (ambient [CO_2] and elevated [CO_2], respectively) after 17 days of treatment.

Treatment	RL[1]	TRL	SA	RD	RV	TP	FK	CR
				Ambient [CO_2]				
No UV-B	45.11 a	4572.9 a	854.31 a	0.597 ab	14.003 a	10,052 a	38,545 ab	2412.6 b
UV-B	45.44 a	4208.5 ab	600.35 b	0.533 b	9.286 b	10,489 a	30,945 b	2377.4 b
				Elevated [CO_2]				
No UV-B	46.67 a	4159.10 ab	738.59 ab	0.560 ab	15.451 a	12,477 a	46,580 a	3287.8 a
UV-B	42.44 a	3565.30 b	807.88 a	0.618 a	12.722 ab	9726 a	35,863 b	2263.3 b
***p*-Value** [2,3]								
UV-B	NS	NS	NS	NS	**	NS	*	NS
CO_2	NS	NS	NS	NS	NS	NS	NS	NS
UV-B × CO_2	NS	NS	**	*	NS	NS	NS	NS

[1] RL, TRL, and RD on a centimeter-per-plant basis; SA and RV on a cubic centimeter basis; TP, FK, and CR on a number-per-plant basis. [2] The standard error of the mean was RL—2.387; TRL—266.1; SA—54.47; RD—0.027; RV—1.325; TP—1219; FK—3524; CR—262.9. [3] NS, *, ** indicate non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. Values followed by same letter are not significantly different within each column.

In basil plants subjected to 10 kJ m^{-2} d^{-1}, there was increased SLA compared to the non-UV-B treated-plants for both [CO_2] treatments (Table 4). Additionally, an overall reduction in SLA was observed when the CO_2 concentration elevated, regardless of the UV-B treatment (Table 4). Thus, basil plants at ambient CO_2 levels had leaves that were 9.2% larger than UV-B-treated plants. Leaf wax concentrations were significantly affected by the UV-B treatment (Table 4). There was a 14.8% increase in leaf wax concentrations when a 10-kJ $m^{-2}d^{-1}$ UV-B radiation was implemented on the basil plants. Leaf waxes were unaffected by CO_2 concentrations of ambient or elevated treatments.

Table 4. The leaf dry mass (Leaf DM) and leaf area (LA) used to estimate the specific leaf area (SLA), and the mean epicuticular wax (Wax) of basil plants grown without UV-B radiation (No UV-B) and with 10 kJ m^{-2} d^{-1} UV-B radiation (UV-B) at 420 and 720 ppm CO_2 concentration (ambient [CO_2] and elevated [CO_2], respectively) after 38 days of treatment.

Treatment	Leaf DM [1,2]	LA	SLA	Wax
			Ambient [CO_2]	
No UV-B	0.536 b	0.0123 ab	23.0 bc	20.1 b
UV-B	0.402 c	0.0110 bc	27.4 a	24.2 a
			Elevated [CO_2]	
No UV-B	0.600 a	0.0125 a	20.8 c	18.8 b
UV-B	0.396 c	0.0099 c	25.0 ab	21.5 ab
***p*-Value** [3,4]				
Treatment	***	***	***	*
CO_2	NS	NS	*	NS
UV-B × CO_2	NS	NS	NS	NS

[1] Leaf DM, LA, and SLA are based on 5 plant leaves per replication. [2] Leaf dry mass in grams, leaf area in meters squared, specific leaf area in centimeters squared per gram, epicuticular wax in micrograms per centimeter squared. [3] The standard error of the mean was Leaf DM—0.01997; LA—0.000452; SLA—0.9024; Wax—1.1683. [4] NS, *, *** indicate non-significant and significant at $p \leq 0.05$ and $p \leq 0.001$, respectively. Values followed by same letter are not significantly different within each column.

4. Discussion

Basil plants are exposed to multiple stresses simultaneously during the growing season, which often leads to suboptimal growth and development. To understand the functional relationship of how basil plants' growth, development, and physiological responses interact, controlled environment experiments with multiple stress factors are vital

to advance our knowledge. In addition, the utilization of highly controlled environments, such as the SPAR chambers, allows for the collection of data that are useful for management decisions and the modeling of crop growth in the field environment.

Current projections of environmental CO_2 concentrations are widespread, ranging upwards to nearly 1000 ppm by the end of the century globally [19]. In Mississippi, current ambient UV-B radiation levels occur around 7 kJ m^{-2} d^{-1}, and with the continual atmospheric ozone depletion estimated to reach 30%, would range from 10 kJ m^{-2} d^{-1} to even greater concentrations of around 15 kJ m^{-2} d^{-1} [17,18]. However, it is important to note that UV-B radiation fluctuates throughout the day based on numerous previously mentioned factors. UV-B radiation treatments were applied using square-wave supplementation and were held constant throughout application, which is different than the diurnal nature that plants are exposed to in the environment.

Even though UV-B radiation is a small percentage of the overall solar spectrum, it has been shown to change the morphology of plants. For example, Chang et al. [41] found that exposing basil plants to 3 h per day of UV-B light for two weeks resulted in shorter plants with an increase in dry matter, more axillary branching, and thicker leaves. Conversely, Sakalauskaite et al. [31] found that basil plants exposed to 4 kJ m^{-2} d^{-1} had an increase in plant height compared to plants with no UV-B exposure. In the current study, UV-B reduced the vegetative growth and development in basil except under ambient [CO_2] at the final harvest. However, elevated [CO_2] mitigated most of the adverse effects of UV-B. For example, above-ground morphology, such as height, leaf area, and leaf DM, was mostly affected by UV-B stress, while elevating the [CO_2] increased the indifferences (Tables 1 and 2). The mitigation of both the leaf area and the leaf DM is advantageous as basil leaves are one of the major byproducts, with any decreases impacting yield and prospective profits in the future.

Epicuticular wax was increased under UV-B radiation treatments (Table 4), which was also observed in rapeseed [42] and maize [43]. The observed increase in epicuticular wax is most likely a defensive mechanism used to protect from UV-B radiation damage in basil. Epicuticular wax is found to be elevated in plants exposed to UV-B radiation as it can absorb, reflect, and reduce the radiance received by the plant [5,42] without affecting the PAR reflectance of some species [15]. Epicuticular waxes have not been shown to be negatively associated with basil production, nor has this connection appeared in any publications outside of those associated with this study. It was identified by Ni et al. [42] that thicker wax can lead to greater photosynthetic pigments, which are nutritionally favorable. Overall, epicuticular waxes contain numerous lipophilic substances [42] that may confer healthful benefits, but the wax profile of basil has not been conducted and is not found in literature.

The competition between CO_2 and O_2 for the binding to ribulose-1,5-bisphosphate carboxylase/oxygenase limits the accumulation of non-structural carbohydrates (NSC). However, the elevation of CO_2 induces a growth advantage through increased production of NSC, leading to improved plant growth and production. Previous research has demonstrated that higher NSC availability accounts for higher energy production through an increased respiration rate [44,45].

It was previously demonstrated that basil plants had an increase in biomass [23] when grown at 620 ppm CO_2 compared to plants grown at 360 ppm CO_2. For most of the morphological features in this study, elevating [CO_2] resulted in an increase in values regardless of UV-B radiation treatment, except for the root-to-shoot ratio (RS ratio) (Table 1) and specific leaf area (Table 4). In crops grown for the production of grains or seeds, leaves with large surface areas and low DM are desired to produce NSC used directly for increasing yield. However, in most horticulture crops such as basil, where the consumed materials are leaves, large DM values are desired as this is directly correlated allometrically to fresh mass but not leaf area [46]. Thus, low SLA is desired in basil as the DM in leaves is desired to be maximized over the surface area, as this correlates to greater marketable mass and NSC produced and able to be consumed. Additionally, the partitioning of carbon in

plants is influenced by crop type and environment [47]. Still, its accumulation is preferred in tissues that are to be harvested, marketed, or consumed, such as the leaves in basil, roots of sweet potatoes, or modified stems of onions. The low RS ratio is desired in basil as more carbon and NSC are desired in the shoot tissue than in the root tissue.

Other studies have demonstrated that the interactive effects of elevated CO_2 and UV-B radiation have significant effects on plant morphology in canola [48], corn [25], grapes [24], soybean [22], and tomato [49]. The interactive effects of elevated CO_2 and UV-B radiation for plant height (Table 1), root surface area (Table 3), and root diameter (Table 3) in our study are confirmed by the previous studies' results, except for the plant height at 35 DAT (Table 2). We observed an increase in the basil plant height of UV-B-treated plants under ambient [CO_2] treatment, which is the opposite trend to that observed in the previous research for crops [19,22,23]. Although the plant height for the elevated UV-B treatment group was the lowest at 17 DAT, UV-B radiation application might have been stimulatory only under the ambient [CO_2] treatment. Sakalauskaite et al. [31] also identified this same effect in their study on 'Thai' basil, hypothesizing it as a cultivar response. The 17 and 38 DAT results express that UV-B treatment hindered plant height and later stimulated growth between harvests, but further investigation is necessary to identify this trend and its underlying causes.

5. Conclusions

The combined interaction between elevated [CO_2] and UV-B treatments yielded variability in changes of the shoot and root morphological features of basil and elevated [CO_2]. Different UV-B radiation types negated the effects of each other such as the final plant height. UV-B radiation caused reductions in plant morphological features such as the branch number, stem DM, and root volume while increasing other features, including the wax content, specific leaf area, and root-to-shoot ratio. The latter two were undesirable for basil. Elevating carbon dioxide proved to have little effect on most morphological features except for increasing the final root DM and slightly reducing the specific leaf area, having a more significant impact on juvenile basil plants (17 DAT). The results show that variability exists for the response of basil morphology to UV-B radiation and [CO_2], individually and when combined, with most features being negatively impacted. Thus, in determining the results obtained in the current study, it is evident that climate change events that increase UV-B and CO_2 may have an overall negative effect on basil's growth, development, and morphology. However, increasing CO_2 levels resulted in an increase in basil DM, which ultimately increased its yield mass. Even though CO_2 increased basil's yield mass, other detrimental effects were observed, such as decreased leaf area and increased plant height. Coupling those effects with increasing UV-B only decreases basil's morphological features, rendering it undesirable. These detrimental effects on basil will have a significant impact on basil's economic value.

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