ABOVE AND BELOW-GROUND MORPHOLOGICAL RESPONSES OF CUCUMBER SEEDLINGS (*Cucumis sativus*) TO ULTRAVIOLET-B RADIATION

THESIS

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ABSTRACT

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The reduction in stratospheric ozone has resulted in an increase in ultraviolet radiation, specifically, UV-B (280-320 nm), incident at the Earth's surface. This increase in UV-B has potential damaging effects on biological organisms. In this study controlled conditions were employed using growth chambers to investigate early morphological responses and timing of these responses on cucumber seedlings (*Cucumis sativus* cv. Burpee Pickler) exposed to UV-B radiation simulating ambient springtime UV-B levels for clear sky conditions in San Marcos, Texas. Specifically, above-ground and below-ground morphology, biomass allocation, and growth responses were investigated in

cucumber seedlings exposed to UV-B radiation. The timing of below-ground responses was then compared to above-ground plant responses. Hypocotyl length ($p < 0.001$) and cotyledon area ($p < 0.001$) were both significantly reduced by UV-B exposure relative to plants that received no UV-B. Primary root length ($p = 0.446$), projected root area ($p = 0.787$), and cotyledon area/weight ratio ($p = 0.367$) were not significantly different between treatments. The projected root area/weight ratio ($p = 0.033$) was significantly greater in plants exposed to UV-B. Thus, the roots of plants exposed to UV-B were apparently thinner or less dense than those of the control (no UV-B) plants. UV-B treated plants had higher early (days 1-2) relative growth rates for root parameters compared to the control plants. However, these early growth rate responses for the UV-B treated plants changed to slower or equal growth rates later (days 4-5) in the experiment. The morphological responses corresponded with a reduction in biomass for all plant parts. However, there was no significant change in the root/shoot ratio between treatments. The below-ground changes in response to UV-B occurred on the third day of UV-B exposure and occurred on the same day as above-ground changes. These findings suggest that root responses to UV-B were not the result of reduction in shoot growth but a consequence of signal transduction between shoots and roots.

INTRODUCTION

The reduction in stratospheric ozone has resulted in an increase in ultraviolet radiation, specifically ultraviolet-B radiation (UV-B; 280-320 nm), incident at the Earth's surface (Frederick et al. 1989, Stolarski et al. 1992, Zavala and Botto 2002). The pronounced reduction in ozone levels at the Southern Hemisphere is mainly due to the release of chlorofluorocarbons (CFCs) and other related compounds into the atmosphere. However, it is unclear what is the cause of the general increase in UV-B elsewhere. The increase in UV-B as opposed to UV-C (250-280 nm) or UV-A (320-400 nm) is a result of the absorption spectrum of stratospheric ozone. Ozone absorbs UV-C so efficiently that even a significant reduction in ozone will not allow the radiation through. Ozone does not absorb UV-A radiation; therefore, it is UV-B radiation levels that change with ozone depletion (Bornman et al. 1997). This increase in UV-B has potentially damaging effects on biological organisms, but effects are highly wavelength dependent. For example, naked DNA shows a peak absorption at 260 nm and absorption decreases sharply with increasing wavelengths (Taylor et al. 1997). Other cellular components, such as membrane lipids and photosystem II (PSII) in plants, show similar spectral sensitivity (Rozema et al. 1997). Exactly how plants receive and interpret light of various spectral qualities remains a question that is still being answered today.

Light is an obvious important external signal for plants. Not only does the light received by a plant "communicate" important information about the plant's surroundings

(e.g., proximity of neighboring plants), but it is also a direct part of the plant's livelihood through photosynthesis (Björn 1994). Therefore, a plant's ability to sense and evaluate light quality and quantity is critical for optimal survival. Plants detect light through cellular components termed photoreceptors (Jenkins et al. 1997). Many different photoreceptors have been identified in plants and are specific for different spectral ranges. The first photoreceptor to be identified was a photoreversible chromoprotein called phytochrome (Briggs and Olney 2001). Phytochrome is a photoreceptor for the red/far red spectrum of light. Regulation of flowering, induction of leaf expansion, and activation of seed germination are all examples of phytochrome-mediated responses in plants. Early belief was that there was a single phytochrome that was responsible for all of these plant responses; however, by 1989 there were five known different phytochromes identified in different plants. The phytochrome photoreceptor system is the most well known of the photoreceptor systems (Briggs and Olney 2001).

The detection of UV-B radiation in plants is not fully understood, nor has there been a photoreceptor identified for UV-B. However, according to Brosche and Strid (2003) in a recent review on UV-B perception in plants, it is likely that there is a UV-B receptor in plants. The best clues about characteristics of a UV-B photoreceptor may be gathered by looking at known UV-A/blue light photoreceptors (Jenkins et al. 1997). Two cryptochromes (cry1 and cry2) and one phototropin are known UV-A/blue light photoreceptors that mediate several different responses in plants (Briggs and Olney 2001). Based on this information it is possible that there are multiple UV-B photoreceptors that are likely responsible for various plant responses. Once UV-B

radiation is perceived, some transduction of the UV-B signal must occur within the plant which then leads to an array of molecular responses by the plant.

One of the best understood and studied areas of molecular responses in plants is in the protection from UV-B. Two main strategies exist in plants for protection from potentially damaging UV-B rays--shielding and repair (Taylor et al. 1997). Shielding involves reflectance of UV radiation through epicuticular waxes and absorbance of radiation by polyphenol compounds, whereas repair mainly refers to repair of damaged DNA before replication occurs. Absorbance of radiation by protective compounds appears to be the primary means of reducing penetration of UV-B radiation within the plant. These UV-absorbing compounds include flavonoids and related phenolics and are found primarily in the upper epidermal layers of leaves where they absorb light between 280 and 380 nm but transmit photosynthetically active radiation (PAR; 400-700 nm). More recently, peroxidases have been noted to aid in protection of PSII from UV-B damage in plants (Jansen et al. 2001). The quantity and location of these compounds appear to differ between different plant groups.

Plants are known to vary in the degree of protection from UV radiation. Of plants sampled from the central Rocky Mountains, herbaceous dicots seemed to be the most ineffective at reducing UV-B penetration into leaves, while conifers were the most effective at eliminating UV-B into needles (Day et al. 1992). Woody dicots and grasses appeared to be intermediate between these two groups in the penetration of UV-B into leaves. These results suggest that differences in UV-B absorbing compounds may be linked to cost-benefit strategies for the different plant groups. For example, conifers keep the same needles for many years and may, therefore, invest more energy and resources

into these protecting compounds, whereas dicots have shorter-lived leaves and may invest less into UV-absorbing compounds (Day et al. 1992). Despite these protection mechanisms, UV-B radiation can still potentially cause cellular damage within plants.

In plants, DNA and components of the photosynthesis system are two cellular structures potentially damaged by exposure to UV-B radiation. DNA that is shielded by cell layers and pigments, as it exists in higher plants, has a peak absorption at 280 nm with minimal DNA damage detected at 405 nm (Quaite et al. 1992). UV-B radiation results in the formation of dimeric and monomeric photoproducts. The most abundant of these photoproducts belong to the dimeric group, specifically, cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts. Other potential damage caused by UV-B radiation includes strand breaks and cross-linking of DNA to proteins. If this damage is not repaired in the DNA molecule, mutations may occur during replication (Strid et al. 1994).

A reduction in photosynthesis rate is a classic plant response resulting from UV-B exposure noted in the literature (Krizek et al. 1998). This reduction could be due to a number of factors, some of which are based on cellular responses. Plants exposed to UV-B have reduced activity and amounts of Rubisco, damage to PSII, decline in activity and amounts of ATP synthase and reduced chlorophyll content (Strid et al. 1994, Baker et al. 1997). Specifically, there is a decrease in the transcription of the Rubisco subunits and the D1 and D2 reaction center proteins in PSII are degraded (Baker et al. 1997, Jansen et al. 2001). However, inhibition in photosynthesis is most prevalent when plants are exposed to high levels of UV-B or unbalanced UV-B relative to other wavelengths, as

often occurs in growth chambers or greenhouses. These cellular responses to UV-B radiation in plants can potentially lead to whole-plant level changes.

UV-B radiation has also been shown to alter plant morphology and growth. Inhibition of stem elongation and reduced leaf area are two properties commonly seen in plants exposed to UV-B radiation (Ballaré et al. 1996). Other negative effects of UV-B on plant growth include reduced biomass, delayed seedling emergence, and premature leaf senescence (Ballaré et al. 1996, Björn et al. 1997). UV-B has also been reported to have positive effects on plants. For example, increased flowering, stimulation of photosynthesis, and increased axillary shoot production are all responses that have been reported after exposure to UV-B radiation (Barnes et al. 1990, Björn et al. 1997). These morphological changes do not always result in significant biomass changes (Searles et al. 1995). Barnes et al. (1990) reported significant leaf blade and internode length reductions in many crop and weed species as a result of UV-B radiation; however, these morphological responses did not result in a corresponding significant total shoot biomass reduction.

Different species can respond differently to UV-B, which has important implications concerning species-specific sensitivity to UV-B. According to Cybulski III and Peterjohn (1999) two temperate-zone forbs (*Lactuca biennis* and *Oenothera parviflora*) showed opposite trends in biomass production. Under ambient UV-B, *L. biennis* had a 14% reduction in above-ground biomass, while above-ground biomass in *O. parviflora* significantly increased (10.2%). In another study performed on seedlings of three different rangeland weeds (*Cynoglossum officinale*, *Centaurea diffusa*, and *Tragopogon pratensis*) each species seemed to differ in susceptibility to UV-B, with *C.*

officianale being the most susceptible (Furness et al. 1999). Different cultivars within one agricultural species can also respond differently to UV-B. A study performed on eight different bush bean cultivars (four Central and four Southern European) revealed that effects on leaf area and weight parameters differed among the cultivar types. Based on the calculated UV-sensitivity index, this study concluded that the Southern European bush bean cultivars were slightly less UV-sensitive than the Central European cultvivars (Saile-Mark and Tevini 1997).

Probable mechanisms for the morphological responses in plants as a result of UV-B differ depending on the response and for the most part are not well understood. Reduced hypocotyl elongation in sunflower seedlings exposed to UV-B has been attributed to destruction of indole-3-acetic acid (IAA- an auxin) and by formation of growth inhibitors such as 3-methyleneoxindole (3-M) (Ros and Tevini 1995). Also, decreased growth in plants exposed to UV-B has been connected to a possible reduction in photosynthesis rate due to the impact of UV (Krizek et al. 1998). However, Gonzalez et al. (1998) attributed growth inhibition in the pea cultivar Guido to reduction in photosynthetic plant organs (leaves and shoots) resulting in a reduction of overall photosynthesis. In seedlings, a specific UV-B receptor has been implicated in mediating morphological responses (Ballaré et al. 1995a, Shinkle et al. 2004).

One area that has not been studied extensively is the effect of increased UV-B radiation on the root system or below-ground plant growth. There exist two main categories of UV-B root studies: (1) mechanistic studies that expose entire (shoot and root) seedlings, usually etiolated, to UV-B (Mohle and Wellmann 1982, Ballaré et al. 1995a, Ballaré et al. 1995b) and (2) plant morphological studies that predominantly

examine root biomass responses to above-ground plant UV-B exposure. The latter allows for an assessment of biomass allocation in the plant. The research on root biomass and root/shoot ratio reveals inconsistent results. For example, in a field study Zavala and Botto (2002) found that radish tubers increased in biomass when plants were exposed to solar UV-B levels. In contrast, in a field study using supplemental UV-B radiation, Ziska et al. (1993) found a decrease in root biomass and an increase in the root/shoot ratio in cassava plants. Few studies have examined root morphological or structural responses to UV-B. Rhizome growth, root length, and specific root length (an indirect measure of root thickness) are some of the root morphological parameters examined in recent studies (Zaller et al. 2002, Robson et al. 2003). However, as mentioned above, inconsistency in experimental results is a trend seen for other measured plant parameters as well. There are no known studies to date that have examined the timing of these below-ground growth changes in relation to above-ground growth changes.

All of the previously discussed plant cellular and morphological changes in response to UV-B radiation can lead to important ecological changes. Some of the plant community and ecosystem processes that can be affected by increased solar UV-B are decomposition, symbiotic plant-micro-organism relationships, insect herbivory, and competition. Decomposition rates of plant litter exposed to enhanced UV-B may increase due to the photochemical action of UV-B or may decrease due to an increase in lignin content within plant cells combined with a decrease in microbes, bacteria and soil fauna that are involved in breaking down the organic plant litter. A reduction in mycorrhizal infection has also been noted as a potential consequence of plant above-ground exposure to enhanced solar UV-B radiation (Rozema 1999).

Alterations in insect herbivory on plants seems to be based on changes within the plant from UV-B not direct effects of UV-B on the insect. Many studies report a decrease in insect herbivory on the plant, however, a couple of studies have found opposite results with an increase in herbivory (Caldwell et al. 1999).

Competition appears to be the ecosystem process affected most by UV-B induced morphological changes in plants. In a comprehensive study, enhanced UV-B radiation altered the competitive balance between wheat (*Triticum aestivum*) and wild oat (*Avena fatua*) by increasing the competitiveness of wheat. This change in competitive balance was a result of plant morphology changes (leaf insertion heights and leaf blade lengths), which likely altered light acquisition in the canopy (Barnes et al. 1988). Therefore, morphological changes in plant growth as a response to enhanced UV-B radiation can potentially have consequences for community and ecosystem processes.

Changes in below-ground growth in plants exposed to UV-B also have potentially important ecological consequences. One of the main functions of roots is to absorb water and nutrients for the plant to utilize in photosynthesis and other processes. Therefore, changes in root morphology and structure could lead to changes in productivity and eventually in ecological interactions such as competition.

In this study I employed controlled conditions in a growth chamber experiment to investigate early morphological responses and timing of these responses on cucumber (*Cucumis sativus*) seedlings exposed to UV-B radiation simulating springtime ambient levels of UV-B for clear sky conditions in San Marcos, Texas. It is common to use agricultural species in UV radiation studies. Cucumber plants are particularly attractive to use in experiments because they are hearty and grow consistently. Cucumber plants

have been used in previous UV-B studies due to intraspecific differences among the cultivars to UV-B sensitivity and because they exhibit typical responses to UV-B such as, an increase in UV-B absorbing compounds and increases in specific leaf weight (Krizek et al. 1997).

Specifically, I examined the effect of UV-B on shoot and root characteristics of cucumber seedlings to test the hypothesis that UV-B will alter shoot growth and morphology but not root growth or morphology in cucumber seedlings. Similarly, I investigated the effect of UV-B on biomass of cucumber seedlings to test the hypothesis that UV-B will affect leaf/stem biomass partitioning but not root/shoot biomass allocation in cucumber seedlings. In addition, I explored the timing of the shoot and root responses to test the hypothesis that UV-B will have a cumulative effect on these root responses in the cucumber seedlings and will occur after shoot effects.

The time-course comparison of shoot and root responses in the plant can potentially lead to a better understanding of how these below-ground responses are occurring. For example, if the root responses are occurring after the shoot responses then it would be reasonable to conclude that the cause of the root responses would be a reduced carbon supply, potentially from reduced photosynthesis. As previously mentioned, carbon assimilation may be reduced in plants exposed to UV-B because of damage to photosynthetic machinery or because of reduced photosynthetic plant material. In contrast, if the root responses occurred at approximately the same time as the shoot responses it could be deduced that the root responses were a result of a signal transduction from shoot to root. Induction of flavonoid production has been observed in

roots of seedlings exposed to UV-B, therefore, flavonoids could be a possible messenger in signals between the shoot and roots (Mohle and Wellmann 1982).

MATERIALS AND METHODS

Growth Chambers and UV Exposure Protocol

The experiments were performed in two Enconair growth chambers (Model GC-16UV, ECONAIR Ecological Chambers Inc., Pembina, North Dakota) located in the Texas State University – San Marcos Biology Department greenhouse complex. These chambers can be programmed with schedules that allow for control of light and temperature settings. Metal halide (150-Watt) and incandescent bulbs (40-Watt) provided the photosynthetically active radiation (PAR; 400-700 nm) within the chamber. The metal halide and the incandescent bulbs were positioned at the maximum height (1.23 m in chamber one, 1.32 m in chamber two) above the plants during the experiment in order to reduce the amount of heat received by the plants. There was no difference in the amount of photon flux between chambers (Table 1). Ultraviolet radiation was provided by high output fluorescent UV bulbs (1.78 m in length, Model Fs72T12, Ultraviolet Resources International, Cleveland, Ohio) that extended the length of the chamber. These fluorescent UV bulbs were pre-burned for 100 hours to ensure spectral stability. Two UV bulbs were 34.9 cm apart and 50.8 cm above plant level in each chamber. The UV-B treatment effects were achieved by covering the fluorescent UV bulbs with different transparent films. The UV-B treatment consisted of UV bulbs covered in 0.13 mm - thick cellulose acetate (allows UV-B transmittance to 290 nm); while the control (no UV-B) treatment had UV bulbs enclosed in 0.13 mm - thick clear

Table 1. Growth chamber comparison of mean (± S.D.) integrated daily photon flux density (PFD, wavelengths of 400-700 nm) and maximum instantaneous photon flux density values for both experimental trials.

DAILY PFD (mol m^{-2} d^{-1})	**CHAMBER 1**	**CHAMBER 2**
Trial 1	$12.0 \pm 6.7 \times 10^{-2}$	$12.1 \pm 6.6 \times 10^{-2}$
Trial 2	$11.9 \pm 5.2 \times 10^{-2}$	$12.5 \pm 6.3 \times 10^{-2}$
MAXIMUM PFD (µmol m^{-2} s^{-1})		
Trial 1	244.8 ± 2.6	245.9 ± 2.7
Trial 2	242.8 ± 1.6	254.7 ± 1.6

polyester (allows transmittance to 320 nm). Spectral transmittance of the films was verified by measurements using a spectrophotometer (Model DU 640, Beckman Coulter Inc., Gladesville, Australia). The film was replaced on the UV bulbs between the experimental trials due to degradation of the film.

Pre-experimental measurements were made to determine how long the UV bulbs needed to burn per day and where the plants needed to be placed in the chamber to receive 90-100% UV-B irradiance. For these measurements two UV bulbs covered in acetate, positioned 34.9 cm apart, were set 50.8 cm above the instruments. To determine length of burn time for the UV bulbs, measurements of spectral irradiance were made using a double-monochromator spectroradiometer (Model 752, Optronic Laboratories, Inc., Orlando, Florida) and were compared to measurements calculated from a model (Green 1983). Prior to measurements, the spectroradiometer was calibrated for wavelength accuracy and gain. Wavelength calibrations were performed using mercury vapor lines from a 4-Watt fluorescent lamp (OL 752-150 Dual Calibration and Gain Check Source Module) at 312.9 nm. Gain calibrations were performed over the wavelength interval 280-400 nm using a 200-Watt tungsten-halogen lamp that was traceable to a National Institute of Standards and Technology (NIST) standard. The spectroradiometer was used in both chambers to perform a scan both in the UV spectrum and over the visible spectrum. The raw irradiance measurements from the UV scan performed in chamber 2 were then converted to a biologically effective UV-B radiation ($UV\text{-}B_{BE}$) measurement based on Caldwell's generalized plant action spectrum (Caldwell 1971). The $UV\text{-}B_{BE}$ value was normalized to 300 nm according to this action spectrum. The daily $UV\text{-}B_{BE}$ irradiance value was then calculated for different length burn times of

the UV bulbs. These were compared to the output of the Green (1983) model that was based on ambient springtime UV-B levels for central Texas in the summer, with clear sky conditions. It was determined that the UV bulbs would burn for four hours a day centered around noon, which resulted in a daily UV-B level of 6.2 $kJ \cdot m^{-2} \cdot d^{-1}$ (daily measured instantaneous $UV\text{-}B_{BE} = 428.8\ mW \cdot m^{-2}$).

UV-B measurements were also made using a broadband UV-B sensor (Model SKU 430, Skye Instruments Ltd., Wales, United Kingdom) to determine the placement of the plant racks on the chamber floor. Measurements were taken and the floor was marked evenly spaced across the chamber floor. For each position on the floor the raw voltage readings were then ranked by percentages. The area was then marked on each chamber floor that would receive 90-100% ultraviolet irradiance. During an experiment one plant rack was placed in the marked area on the chamber floor.

During an experiment the schedule of the chamber was programmed to have the metal halide and incandescent bulbs burn for fourteen hours a day. The UV bulbs were set to burn for four hours a day centered around noon. The temperature was set with a daily high of 25°C (when the lights were on) and a daily low of 20°C (when the lights were off). The temperature changes were ramped to increase or decrease by one degree every fifteen minutes. During an experiment, photosynthetically active radiation was measured using quantum sensors (Model LI-190S, LI-COR Inc., Lincoln, Nebraska). UV light was measured using broad-band UV sensors (Model SKU 430, Skye Instruments Ltd., Wales, United Kingdom) connected to the chamber. The broad-band UV measurements were only used to verify that the UV bulbs functioned daily.

Experimental Procedure

The cucumber seed used in the experiments was the Burpee Pickler cultivar obtained from W. Atlee Burpee and Company (Warminster, Pennsylvania). The seeds were soaked in moist paper towels for approximately five hours before planting. This process hastened germination of the seeds once they were planted. Seeds were planted in a 3:1 soil (Baccto potting soil:sand) mixture in super high-density plastic cone-tainers (3.8 cm in diameter, 21.0 cm in depth, 238.8 cm^3, Hummert International, Earth City, Missouri). One seed was planted per cone. The cones were then placed into a rack. Each rack held ninety-eight cone-tainers spaced 4.5 cm apart (Fig. 1). One outer row of cones in each rack served as buffer plants and were not used in measurements. In the first experimental trial each chamber and plant rack was randomly assigned to a treatment type (UV-B exposure or no UV-B exposure, the control). In trial one the UV-B exposure treatment was in chamber one and in trial two UV-B exposure was switched to chamber two. This was done to account for any chamber differences. Once the cones were placed in the chambers the schedule was activated. The soil surface was kept moist by lightly spraying the soil with de-ionized water twice a day. Once plants emerged (cotyledons fully visible) they were watered once a day with 20-40 ml of de-ionized water; the exact amount of water was determined daily as the amount needed to run through. All watering was done after the UV lights were off. Emergence was recorded for all plants, as it was critical to have the plants emerge at approximately the same time. Plants that had not emerged by the harvest date were omitted from the experimental dataset. Harvesting of the plants began after most of the plants had emerged and had received one exposure period (four hours) of UV-B radiation (Fig. 2).

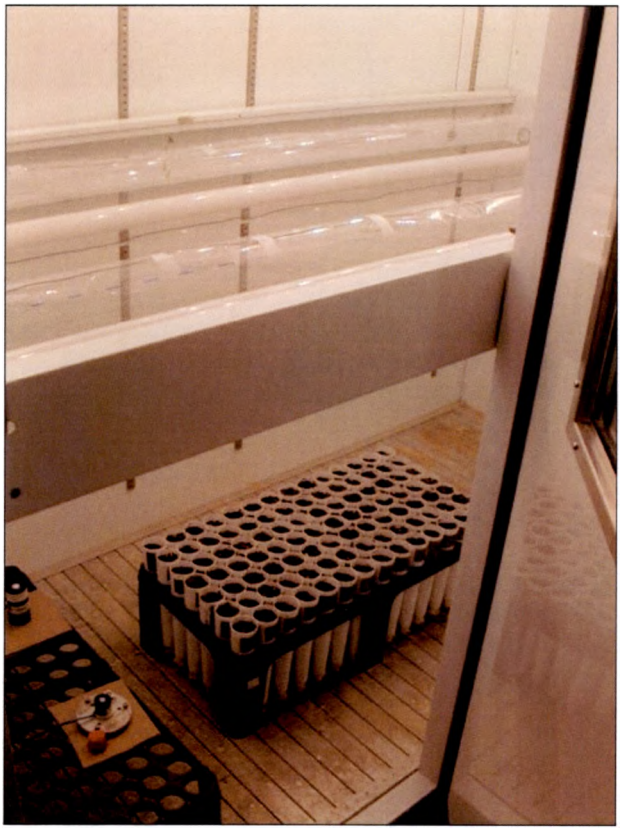

Figure 1. Growth chamber experimental set-up showing pot arrangement, sensor equipment positioning, and UV bulb set-up.

Figure 2. Seedlings in containers just before the first harvest. The seedlings shown are at the same phenological stage of development, with cotyledons fully emerged and expanded.

Twelve plants a day were harvested (n=12) from each treatment for five consecutive days. Plants were harvested two hours after the UV lights went off. When harvested, the entire plant was removed (roots intact) from the cone and dissected into three different parts: intact root system, hypocotyl, and cotyledons (Fig. 3). The roots were washed with water in a sieve with 1 mm openings (Fig. 4). The following measurements were made on each plant at harvest time: primary root length, hypocotyl length, average length and width of cotyledons, and total cotyledon area. Cotyledon area was measured using a leaf area meter (Model 3100, LI-COR Inc., Lincoln, Nebraska). All length measurements were made using digital calipers (Fig. 5). The fresh roots were arranged on clear plastic sheets and then scanned using a flatbed scanner (Fig. 6). The digital images of the roots were saved for later analysis. The root systems, hypocotyl, and cotyledons were dried separately in a drying oven at 80 °C for two days. The plant parts were then weighed for biomass measurements. Cotyledon area/weight ratio, root/shoot ratio, leaf/stem ratio and total plant weight were all calculated from the initial measurements. Scion image analysis software (Beta 4.02 for Windows) was used to obtain a total area value for each root system stored digitally. The area measurement for each root system will be referred to as projected root area. This area measurement is not total surface area because the fresh roots were scanned using a flatbed scanner. Projected root area is used in this study as an estimate of the total size of the root system. Furthermore, a projected root area/weight ratio was then calculated from these data for each root system. Finally, early (1-2 days), late (4-5 days), and total (1-5 days) relative growth rates for all parameters were calculated using calculated means with the following formula:

Figure 3. Removal of the entire intact root system from the container during a harvest.

Figure 4. Manual root washing procedure of an intact root system using gentle water pressure.

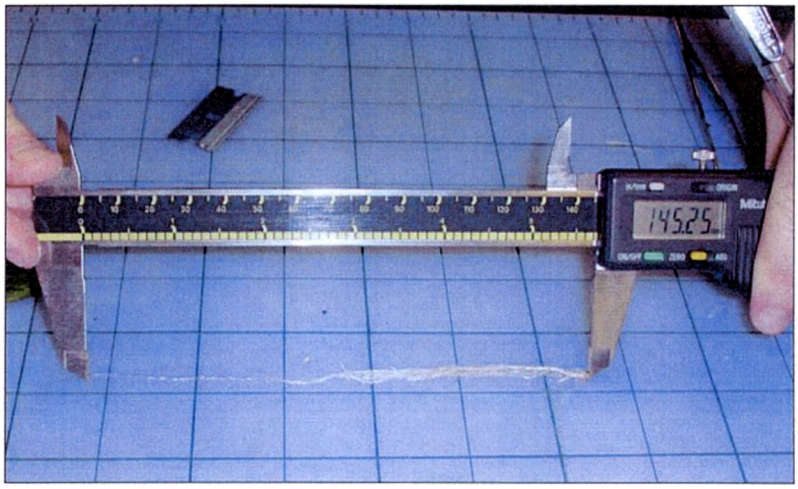

Figure 5. Measurement of the primary root using digital calipers during a harvest.

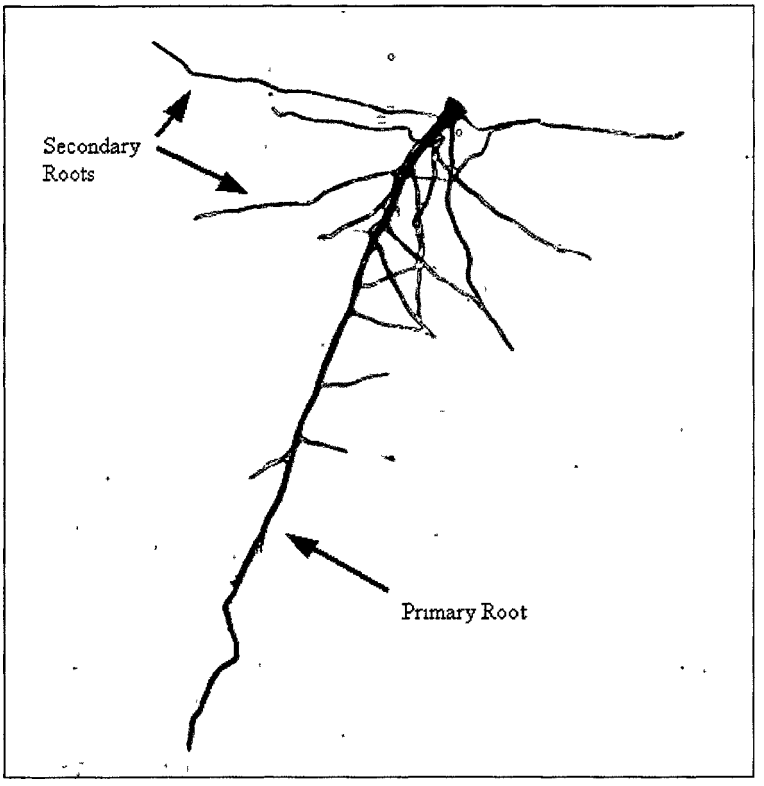

Figure 6. Scanned intact root system from day three of harvest. This image identifies the different developmental root categories—the primary root and secondary roots.

$$RGR = \frac{[(\ln W_2)-(\ln W_1)]}{t_2-t_1}$$

where: W = weight (or other measured parameter)

t = time in days

Statistical Analysis

The data from experimental trials one and two were combined for the UV-B treatment and the control. Therefore, an equal dataset was required in order to remove any bias from either trial. Without manipulation this was not realistic due to the fact that some seeds did not germinate. To acquire an equal dataset, outliers were removed using an unbiased method. The representative parameter to base the elimination from was total plant weight. Standardized values were obtained for each plant weight. Plants were then eliminated if they were greater than two standard deviations away from the mean, regardless of the direction. This allowed for an equal dataset (n=10) for each day, for both experimental trials.

All data were tested for normality and homogeneity of variances. All weight data (hypocotyl, root, cotyledon, shoot, and total plant) and projected root area were log-transformed. The projected root area/weight data were square root-transformed to meet assumptions. Full factorial analysis of variance (ANOVA) tests were run on all variables ($p< 0.05$). For each variable the ANOVA compared treatment effects (UV-B; control, n=100) and time (days 1-5, n=20). Least significant difference comparisons (LSD) were performed on each day for every parameter. LSD comparisons were calculated manually using a pooled error variance from the ANOVA results for the corresponding variable.

All other assumptions testing, transformations, and statistical tests were performed with SPSS statistical software. No statistical analyses were performed on the relative growth rate data since these values were calculated using data means.

RESULTS

Morphological Effects

When averaged over time (five days), total hypocotyl length, cotyledon length, and cotyledon width were significantly reduced by the UV-B treatment relative to the no UV-B controls (Table 2). However, primary root length did not show a significant difference between the UV-B and control treatments. When comparing means between treatments, the greatest percent reduction in length was seen in the hypocotyl of the plant (7.5%). Projected root area was not significantly different between treatments (UV-B, 3.4 cm^2; control, 3.5 cm^2; Table 2). However, the projected root area/weight ratio was significantly greater for UV-B treated plants relative to the controls. Cotyledon area was significantly reduced in plants exposed to UV-B, while the cotyledon area/weight ratio was not significantly different between treatments (Table 2).

Biomass Effects

Root, hypocotyl, cotyledon, shoot, and total plant dry weights were significantly reduced in plants exposed to UV-B (Table 2). When comparing means between treatments, the greatest percent reduction in weight was seen in the root system of the plant (16.0%), while the least percent reduction was in cotyledon weight (11.8%). The root/shoot ratio was not significantly different between the UV-B and control treatments

Table 2. Univariate ANOVA results for total mean (± S.E., n=100, averaged over 5 days) morphological and growth (dry biomass) parameters for each treatment. p = level of significance as determined by ANOVA.

MORPHOLOGICAL PARAMETER	CONTROL	UV-B	p
Primary Root Length (mm)	115.0 ± 4.3	117.8 ± 4.0	0.446
Hypocotyl Length (mm)	28.1 ± 0.5	26.0 ± 0.5	< 0.001
Cotyledon Length (mm)	33.2 ± 1.1	31.7 ± 1.0	0.002
Cotyledon Width (mm)	18.3 ± 0.7	17.1 ± 0.6	< 0.001
Projected Root Area (cm^2)	3.5 ± 0.2	3.4 ± 0.2	0.787
Projected Root Area/Weight (cm^2/g)	729.1 ± 25.3	812.0 ± 30.5	0.033
Cotyledon Area (cm^2)	10.7 ± 0.6	9.3 ± 0.5	< 0.001
Cotyledon Area/Weight (cm^2/g)	292.7 ± 10.6	287.2 ± 9.8	0.367
GROWTH PARAMETER			
Root (mg)	5.1 ± 0.3	4.3 ± 0.3	0.015
Hypocotyl (mg)	4.4 ± 0.1	3.9 ± 0.1	0.001
Cotyledon (mg)	33.8 ± 1.7	21.0 ± 1.3	< 0.001
Shoot (mg)	38.2 ± 1.8	33.6 ± 1.4	< 0.001
Total Plant (mg)	43.3 ± 2.1	38.0 ± 1.6	< 0.001
Root/Shoot	123.0 ± 3.6	120.0 ± 3.4	0.496
Leaf/Stem	7.4 ± 190.0	7.8 ± 220.0	0.080

(Table 2). However, the leaf/stem ratio was marginally significant between treatments indicating an increase in allocation to the leaves in seedlings exposed to UV-B.

Time-Course of Responses

The effect of time, when averaged across the treatments, was significant ($p < 0.001$) for all growth and morphological parameters measured. Length measurements made on the different parts of the seedlings generally showed similar growth trends, with the exception of primary root length (Figs. 7 and 8a). There was no significant difference in primary root length for the treatment by time interaction; therefore, UV-B did not significantly affect primary root length over time. The treatment by time interaction was not significant for hypocotyl length (Fig. 7b). However, days three and five had significantly different treatment means for hypocotyl length. Similarly, the treatment by time interaction was not significant for cotyledon length and width (Fig. 8). Cotyledon length and width showed significant differences between means on days three and five. The treatment by time interaction was significant for cotyledon width (Fig. 8b) indicating that UV-B significantly affected cotyledon width over time. The treatment by time interaction was marginally significant for cotyledon length (Fig. 8a).

Projected root area and the projected root area/weight ratio showed very different temporal responses to the treatments (Fig. 9). Projected root area showed no significant difference between treatment means over the five days, and the treatment by time interaction was not significant (Fig. 9a). Treatment means of projected root area/weight ratio began to diverge after day two, (Fig. 9b) however, only day five had a significant difference between treatment means. Furthermore, the projected root area/weight ratio

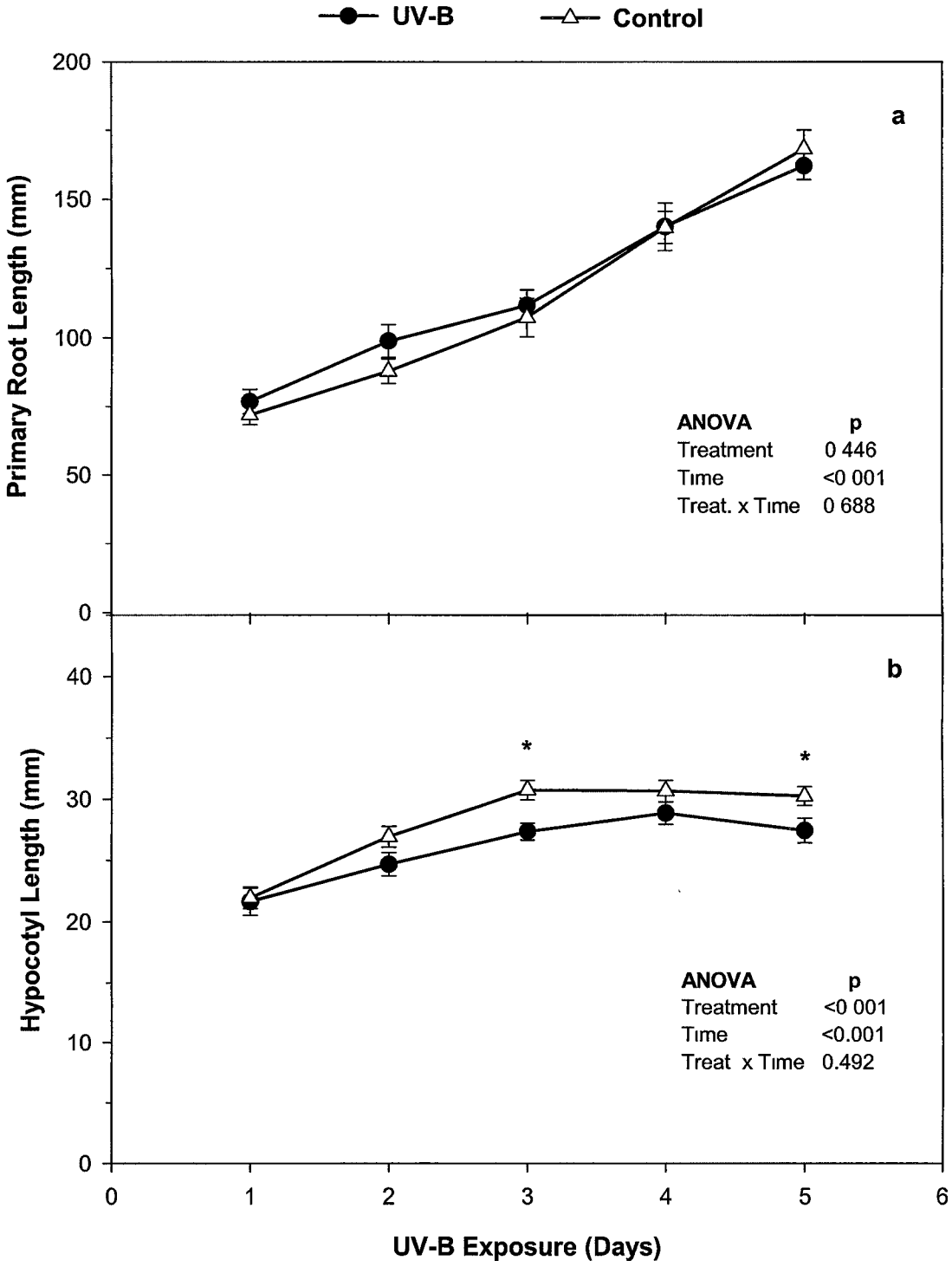

Figure 7. Univariate ANOVA results for daily mean (± S.E., n=20) primary root and hypocotyl length measurements for each treatment. Significantly different means (p= <0.05), as determined by LSD tests, are identified with * above the error bars.

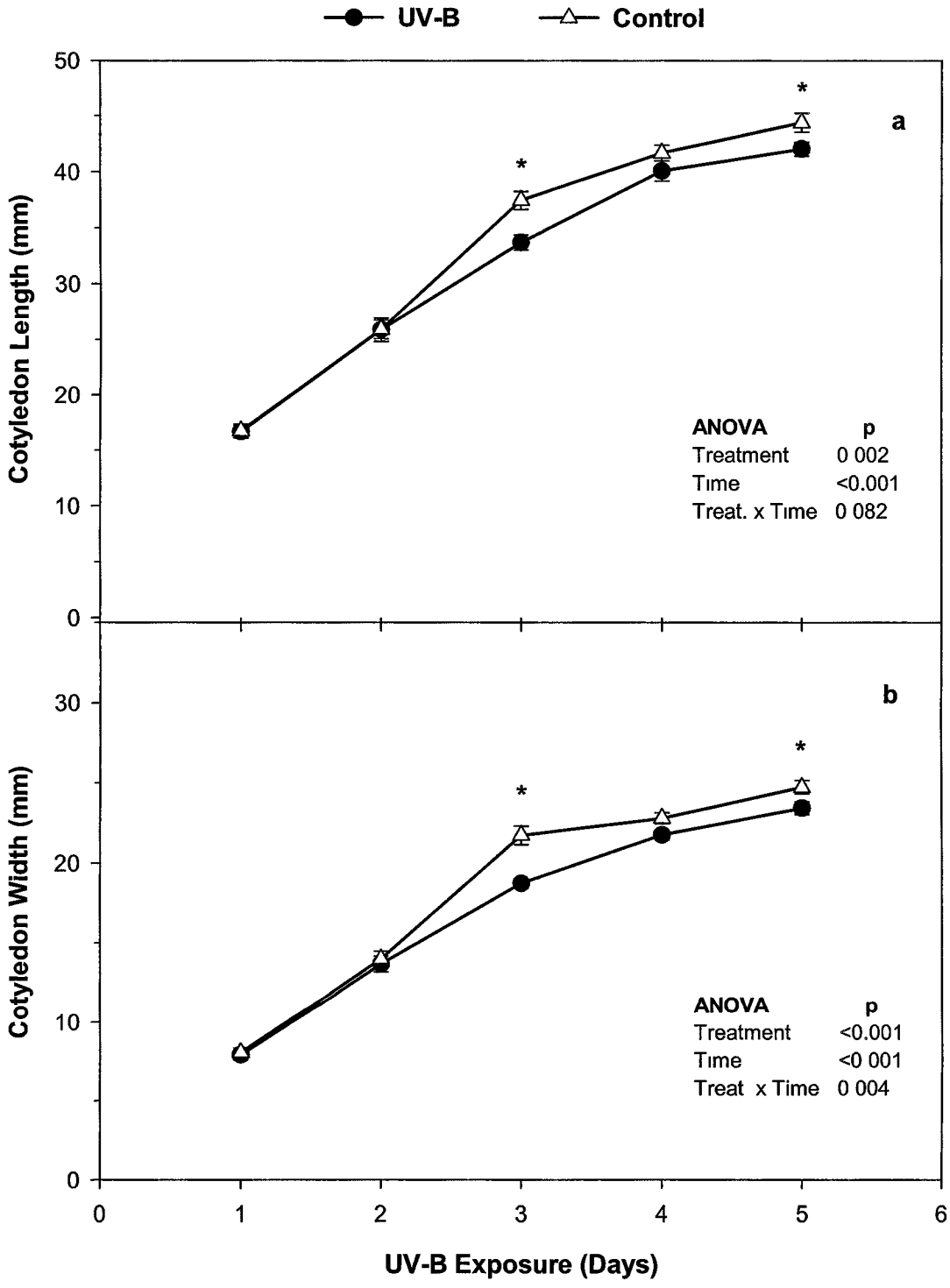

Figure 8. Univariate ANOVA results for daily mean (± S.E., n=20) cotyledon length and width measurements for each treatment. Significantly different means (p= <0.05), as determined by LSD tests, are identified with * above the error bars.

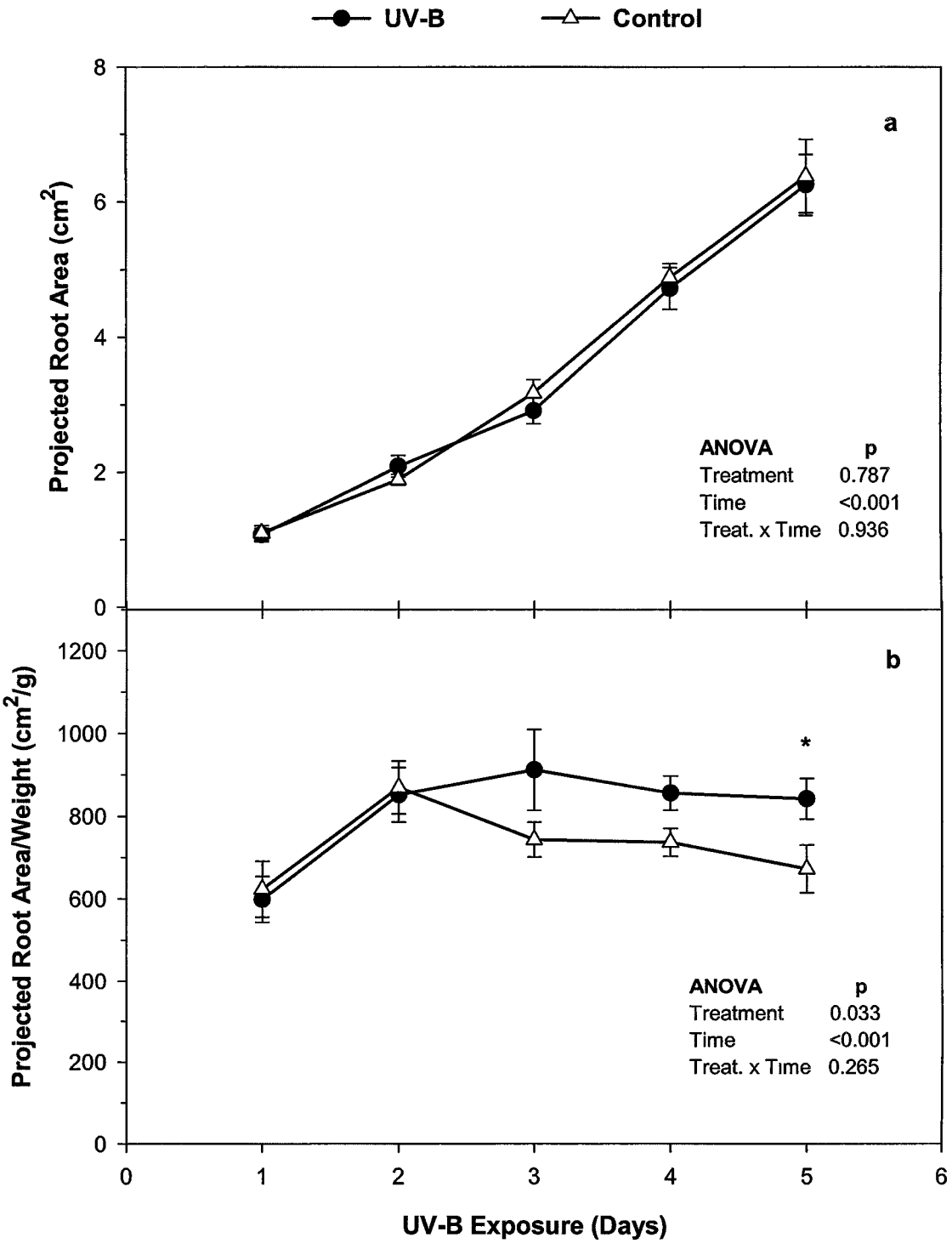

Figure 9. Univariate ANOVA results for daily mean (± S.E., n=20) projected root area and projected root area/weight ratio measurements for each treatment. Significantly different means (p= <0.05), as determined by LSD tests, are identified with * above the error bars.

had no significance for the treatment by time interaction (Fig. 9b). Cotyledon area and cotyledon area/weight ratio also showed different growth trends over time (Fig. 10). The treatment by time interaction was significant for cotyledon area, specifically showing significant differences on days three, four and five (Fig. 10a). The only significant mean treatment difference for the cotyledon area/weight ratio was on day three, however, there was a significant treatment by time interaction. (Fig. 10b).

In general, biomass treatment means were similar at day one and began to diverge after day two (Figs. 11 and 12). Root weight treatment means showed significant differences on days three and five, however, the treatment by time interaction was only marginally significant (Fig. 11a). Days three, four and five indicated significant differences for shoot weight treatment means, and the treatment by time interaction was also significant (Fig. 11b). There was no significance between treatment means for the root/shoot ratio, nor was there a significant treatment by time interaction (Fig. 11c). Hypocotyl weight treatment means differed significantly on days three and five, however, there was no significant treatment by time interaction (Fig. 12a). Cotyledon weight showed significant differences between treatment means on days three, four and five. Also, there was a significant treatment by time interaction (Fig. 12b). Similarly, total plant weight treatment means were significantly different on days three, four, and five. There was also a significant treatment by time interaction (Fig. 12c).

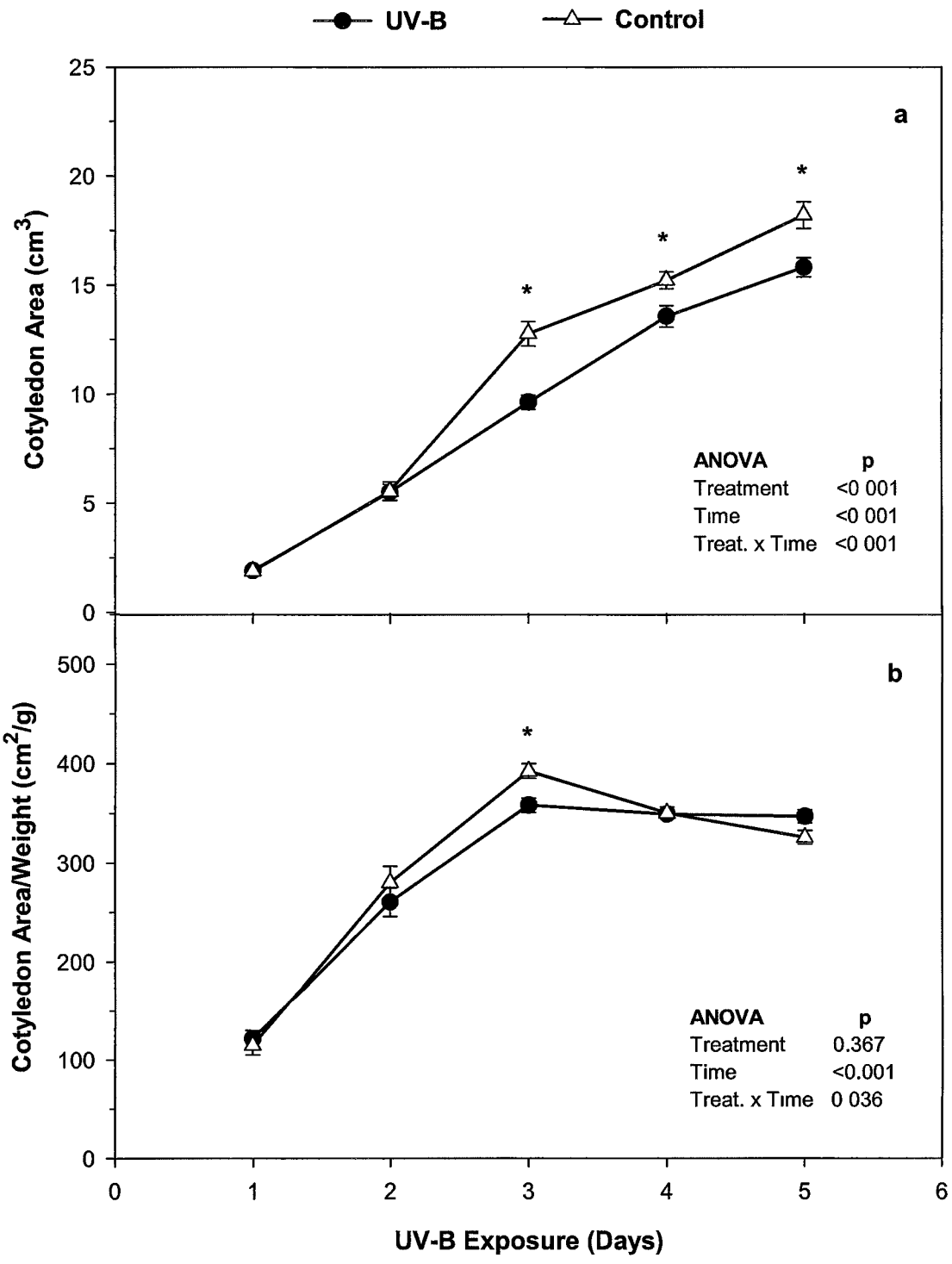

Figure 10. Univariate ANOVA results for daily mean (± S.E., n=20) cotyledon area and area/weight ratio measurements for each treatment. Significantly different means (p= <0.05), as determined by LSD tests, are identified with * above the error bars.

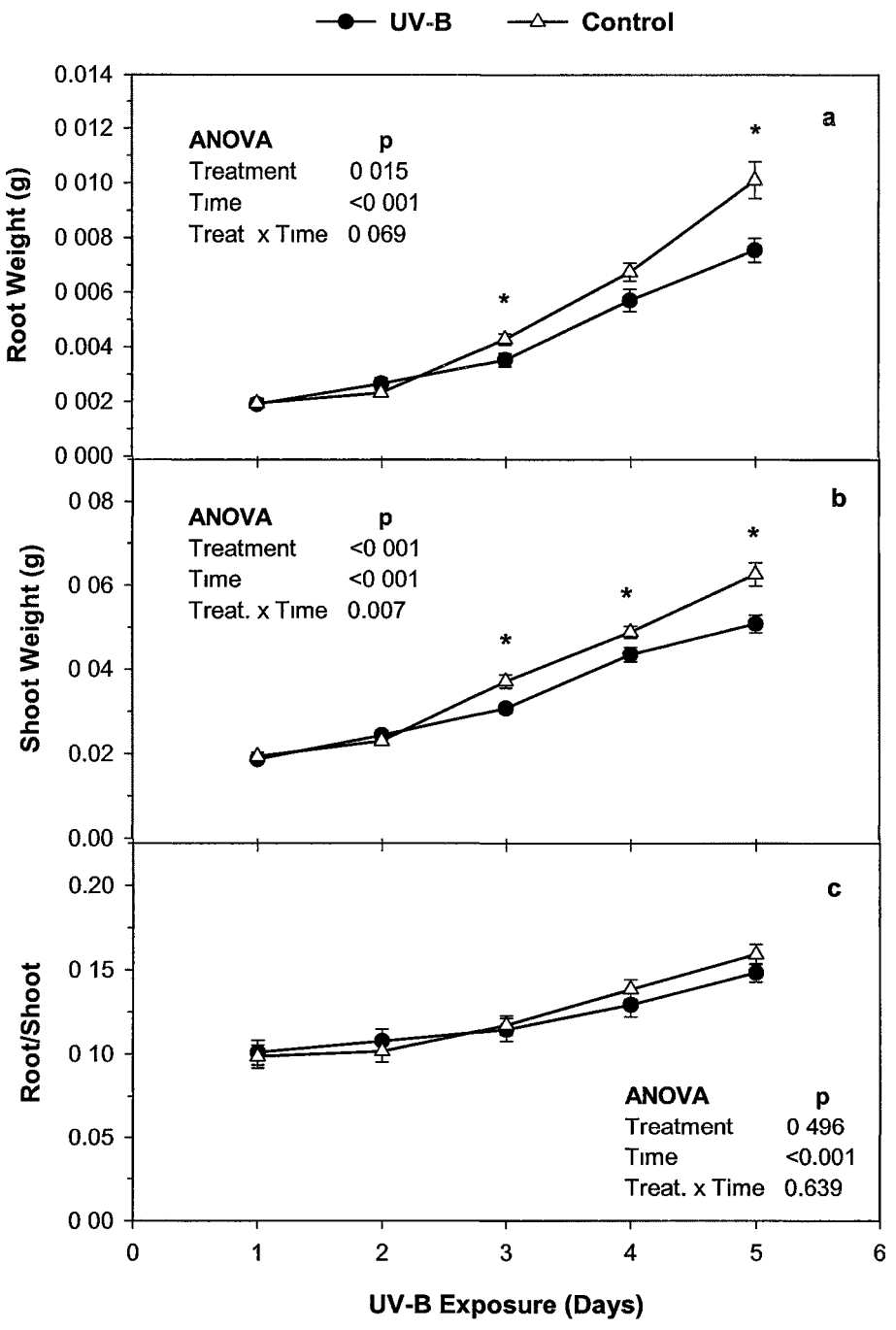

Figure 11. Univariate ANOVA results for daily mean (± S.E., n=20) root and shoot weights and root/shoot ratio measurements for each treatment. Significantly different means (p= <0.05), as determined by LSD tests, are identified with * above the error bars.

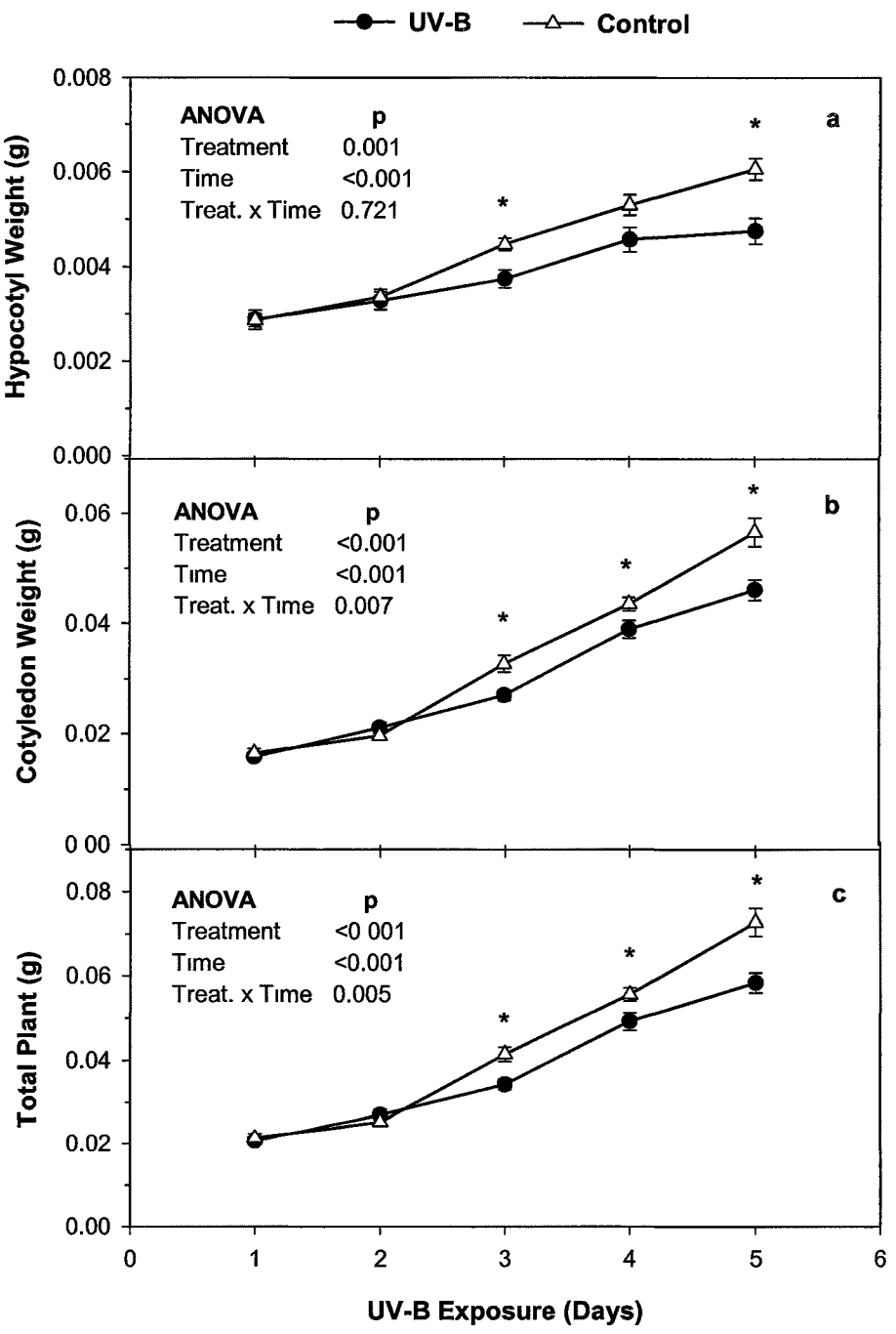

Figure 12. Univariate ANOVA results for daily mean (± S.E., n=20) hypocotyl, cotyledon, and total plant weight measurements for each treatment. Significantly different means (p= <0.05), as determined by LSD tests, are identified with * above the error bars.

Growth Rates

Early growth rates for all root parameters (primary root length, projected root area, and root biomass) were higher for the UV-B treated plants compared to the controls (Table 3). Similarly, the UV-B treated plants had higher early growth rates for all biomass parameters (cotyledon, shoot, and total plant), except for hypocotyl biomass, when compared to the controls. The remaining early growth rates (hypocotyl length, cotyledon length, cotyledon width, and cotyledon area) for the UV-B treated plants were either less than or equal to the controls (Table 3). In contrast, none of the parameters for the UV-B treated plants showed positive or increased late growth rates when compared to the control plants. Between treatments late growth rates were consistently less for the UV-B treatment compared to the control, except for root projected area, cotyledon area, and shoot biomass which were equal between treatments (Table 3). In comparison, there were seven parameters with equal total growth rates between treatments. The exceptions were in hypocotyl length, cotyledon area, root biomass, and hypocotyl biomass which all had lower total growth rates for the UV-B treated plants compared to the controls (Table 3).

Table 3. Early (days 1-2), late (days 4-5), and total (days 1-5) growth rates per day calculated using data means for all parameters measured and for each treatment.

PARAMETER	UV-B TREATMENT			CONTROL		
	Early Rate	Late Rate	Total	Early Rate	Late Rate	Total
Primary Root Length (mm/d)	0.30	0.10	0.20	0.20	0.20	0.20
Hypocotyl Length (mm/d)	0.10	-0.10	0.06	0.20	-0.01	0.10
Cotyledon Length (mm/d)	0.40	0.05	0.20	0.40	0.10	0.20
Cotyledon Width (mm/d)	0.50	0.07	0.30	0.60	0.10	0.30
Projected Root Area (cm^2/d)	0.70	0.30	0.40	0.50	0.30	0.40
Cotyledon Area (cm^2/d)	1.10	0.20	0.50	1.10	0.20	0.60
Root (g/d)	0.30	0.30	0.30	0.20	0.40	0.40
Hypocotyl (g/d)	0.10	0.04	0.10	0.20	0.10	0.20
Cotyledon (g/d)	0.30	0.20	0.30	0.20	0.30	0.30
Shoot (g/d)	0.30	0.20	0.30	0.20	0.20	0.30
Total Plant (g/d)	0.30	0.20	0.30	0.20	0.30	0.30

DISCUSSION

The original hypothesis that UV-B would not affect root growth or morphology was rejected based on results of this study. Cucumber seedlings exposed to UV-B radiation did show significant differences in root growth and morphology compared to the no UV-B control seedlings. However, not all of the root parameters measured were affected by the UV-B treatment. Primary root length and projected root area were not influenced by the UV-B when averaged over time. Seedlings exposed to UV-B radiation had a significantly higher projected root area/weight ratio than the control seedlings. Root biomass, when averaged over time, was significantly reduced in plants exposed to UV-B radiation compared to the control plants. This reduction in root biomass must be explained by some root morphological attribute. For example, the root area or root thickness may be reduced in the plants exposed to UV-B, which would result in root biomass reduction in these plants. The data from this study suggest that the seedlings exposed to UV-B had thinner or less dense roots rather than a reduced size compared to the control seedlings.

Findings from two previous studies that did analyze some aspects of root morphology and production contradict the results from the present study. In a long-term field study (four years) in southern Argentina (Tierra del Fuego), near ambient UV-B reduced root length and increased root thickness in experimental plots of a *Carex* fen ecosystem. However, these changes did not result in a significant change in root biomass

(Zaller et al. 2002). Similarly, in another long-term field experiment located in Tierra del Fuego near ambient UV-B reduced rhizome elongation in *Tetroncium magellanicum* (Robson et al. 2003). The contradictory results between the current study and the two aforementioned studies may be due to the many differences that exist between the studies. Compared to the present study both of these studies were performed on different plant species, over a longer time period, and at different ozone depletion levels. Furthermore, as previously mentioned, throughout the literature results vary depending on species and cultivar types within species for the same parameters.

UV-B radiation did cause a significant difference in shoot growth and morphology in plants compared to the control treatment plants. Based on these results the hypothesis that predicted UV-B will alter shoot growth and morphology in cucumber seedlings was not rejected. Hypocotyl length was significantly reduced in seedlings exposed to UV-B compared to the control seedlings. Cotyledon area was also significantly reduced in UV-B exposed seedlings, but there was no significant difference in the cotyledon area/weight ratio between treatments. These above-ground morphological responses were associated with a significant reduction in shoot biomass between treatments. Similar shoot responses have been observed in both greenhouse and field studies. In a greenhouse study, Teramura and Sullivan (1987) found reduced plant height and leaf area in soybean plants. Similarly, inhibited stem elongation and reduced leaf expansion were reported in a field study on *Datura ferox* in Buenos Aires Argentina (Ballaré et al. 1996). Furthermore, both of these studies found reduced total plant biomass in plants exposed to UV-B.

The cause of the reduced above-ground growth observed in this study is not fully understood. As previously mentioned, there are two possible mechanisms for growth reduction: (1) damage to the photosynthetic machinery of the plant and (2) reduced leaf area which results in a reduction in carbon gain. Photosynthetic rates were not measured in the present study and thus it is not possible to determine whether UV-B-induced reductions in biomass were the result of the partial inhibition in photosynthesis. The fact that biomass reductions occurred with morphological changes, however, suggests that these two responses may be linked.

UV-B radiation did not cause a difference in biomass allocation patterns in plants compared to the control treatment plants; therefore, the hypothesis that stated that UV-B would not effect root/shoot biomass allocation in cucumber seedlings was not rejected. The root/shoot ratio, when averaged over time, was not significantly different between treatments. However, individually the root and shoot weights when averaged over time were significantly reduced in plants exposed to UV-B compared to the control plants. These results indicate that the biomass reduction in plants exposed to UV-B was proportionate between the roots and the shoots. Similar results were reported for four different cultivars of cucumber exposed to ambient solar UV-B (Krizek et al. 1997). Similarly, in a greenhouse study on sixteen different rice cultivars, Teramura et al. (1991) found that UV-B did not have a significant effect on root/shoot ratio. According to the results of the present study, these plants are not shifting more carbon to the root system to further growth in the "non-exposed" portion of the plant. These results possibly signify that the UV-B effects are integrated over the entire plant and that there are not available nutrients or carbon to shift belowground.

The original hypothesis that UV-B would affect leaf/stem biomass allocation in cucumber seedlings was supported by this study. A marginal significant increase was observed in the leaf/stem ratio in seedlings exposed to UV-B compared to the control seedlings. These results indicate an increase in biomass allocation to the leaves. In contrast, in a greenhouse study using various rangeland weeds, seedlings of *Cynoglossum officinale* exposed to UV-B exhibited a reduced leaf/stem ratio. However, other species in the same study showed no significant change in leaf/stem ratio (Furness et al. 1999). Again, this contradiction suggests that this response may be species-specific.

The time-course data on the growth of the plants only support the first part of the original hypothesis that UV-B would have a cumulative effect on the below-ground growth responses in cucumber seedlings and that these responses would occur after shoot effects. The results from the growth data reveal that most of the changes in the above-ground plant growth began occurring on day three. Similarly, changes in root biomass began on day three as well. There was no significant difference between treatments for the root area/weight ratio until day five. However, the trend was apparent on day three but was not significant due to high variance. These results show that the changes in above-ground and below-ground growth did mostly occur after two days (apparent on day three) of UV-B exposure and were nearly simultaneous. To date, no UV studies have compared the timing of early growth responses between above and below-ground growth. A few studies have examined early hypocotyl growth responses in seedlings exposed to UV-B. Inhibited hypocotyl length has been shown in de-etiolated tomato, sunflower, and cucumber seedlings only after a few hours of UV-B exposure (Ballaré et al. 1995b, Ros and Tevini 1995). Perhaps the reason why effects were not seen in the plants until the

third day in the present study was because the experimental design of this study allowed for the plants to germinate, emerge, and grow under a balanced spectrum. This would potentially allow for the emerging seedlings to produce UV-B protective compounds. These circumstances contrast the aforementioned studies that used dark or dim light grown seedlings then exposed the etiolated seedlings to the UV radiation conditions. These experimental design differences could account for the delayed seedling response observed in the present study.

The somewhat simultaneous changes in above and below-ground growth could mean that there is some signal that is transmitted from the exposed above-ground plant portion to the below-ground portion of the plant which does not receive UV-B. If these below-ground growth changes were primarily a direct result of reduced photosynthesis it would seem that the changes would be delayed when compared to the above-ground growth. Support for flavonoids acting as a chemical signal between plant shoot and root material comes from studies where seedling roots produce such chemicals. Dill seedling roots have been found to produce flavonoids in response to UV-B exposure (Mohle and Wellmann 1982). Therefore, flavonoids could be one possible chemical signal responsible for communication between above-ground and below-ground plant growth. Another line of evidence points toward light quality as a potential signal within the plant. Sun et al. (2003) found that vascular tissue in woody plants can conduct light. Their study indicated that light is conducted from the stem towards the roots and probably contributes to photomorphogenic responses within them.

Data from the present study also indicate early effects on the relative growth rate for plants exposed to UV-B. Unfortunately, no statistical analysis could be performed on

the growth rate data and thus it is not possible to draw robust conclusions based on these results. However, these data do show some interesting trends. The root parameters and most of the biomass parameters (cotyledon, shoot, and total plant) showed increased early growth rates for the UV-B treated plants when compared to the no-UV-B control plants. However, these positive growth rate responses disappeared in the late growth rates. Specifically, UV-B treated plants either exhibited slower or equal late growth rates when compared to the control plants. These results suggest that there may be some interesting growth rate responses occurring early on in plants exposed to UV-B. Furthermore, these data support further exploration of this topic.

Plant responses to UV-B can be categorized into two main groups based on the cause of the response. Responses can be grouped as photomorphogenic or non-photomorphogenic. Photomorphogenic responses have been characterized as non-damaging morphological changes in response to light quality. Non-photomporphogenic responses have been described as resulting from UV-B induced cellular damage (Shinkle et al. 2004). The present study did not examine any mechanistic parameters so no real conclusions can be drawn about the nature of the responses observed; however, some comments can be made based on the trends observed in the results.

In the present study the morphological responses observed did occur together with changes in biomass or growth between treatments. These results provide indirect evidence towards non-photomorphogenic morphological responses. However, this statement is made with caution due to the complexities involved with the mechanisms behind common morphological responses. Hypocotyl inhibition is one common morphological response that has been considered a photomorphogenic response. This is

based on the reduced growth resulting from alterations in growth regulators or from a UV-B receptor (Shinkle et al. 2004). In contrast, reduced morphological responses, such as leaf area, may be non-photomorphogenic in nature and result from cellular damage. These UV-B responses are influenced by many factors, including the spectral balance, in particular the ratio between UV-B and visible light, used in the design of the study.

The responses observed in the present study were from seedlings grown in growth chambers using a relatively low daily UV-B dose. Growth chamber experiments have been criticized throughout the literature for having low levels of PAR compared to UV-B. These conditions tend to accentuate UV-B responses in plants (Tevini and Teramura 1989). However, in the present study relatively low daily UV-B doses were used and no visible damage was observed in the seedlings (i.e., chlorosis or burn lesions). Furthermore, in a field exclusion study using cucumber similar results were found for plant fraction biomass, stem height, and leaf area (Krizek et al. 1997). Nonetheless, it is unknown whether similar responses to those observed in the present study would be found under field conditions.

This study offers many possibilities for future experiments. A similar study could be performed in the field using ambient UV-B and over a longer time period. Other root morphological and structural attributes, including root branching, density and surface area, could be studied in the plants. The extent of root branching would provide interesting insight into nutrient acquisition. This could provide more detailed and potentially more meaningful results. Finally, a similar study could be performed using other species of plants and even a comparison of different cultivar types.

In conclusion, the results of this study indicate that seedling roots were affected by above-ground exposure of UV-B. The fact that these below-ground changes occurred after only two treatments of UV-B and were nearly simultaneous with above-ground changes are very interesting results. This could indicate that an internal signal may be moving within the plant in response to little UV-B exposure. This study should be a precursor for future studies that could examine the physiology or mechanisms behind this connection between above and below-ground growth in plants exposed to UV-B.

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VITA

Kristy Diane Barker Scott impatiently arrived, for which her mother was grateful, early in the morning on February 20, 1978, in Houston, Texas. She moved to her childhood home, Buda, Texas, when she was three years old. She lived in her beloved home in Leisurewoods and attended school in the Hays School District until she graduated high school in 1996. She then moved on to college at Texas State University-San Marcos, where she met her love in summer Botany class. Here she also earned a Bachelor of Science in Biology with a minor in Chemistry. She took a short breather to do some substitute teaching and catch up on vital television shows before entering graduate school. Kristy was married to her love, Brian Scott, during her graduate career and lived happily in New Braunfels, Texas, right down the road from historic Gruene, with her husband and wild feline, Ripley.

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