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The Effects of Ultraviolet Radiation on Pigment Production, Growth, and Photochemical Efficiency in *Allium* spp

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To the Graduate Council:

I am submitting herewith a thesis written by Kristin Renee Abney entitled "The Effects of Ultraviolet Radiation on Pigment Production, Growth, and Photochemical Efficiency in *Allium* spp." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Dean A. Kopsell, Major Professor

We have read this thesis and recommend its acceptance:

Carl E. Sams, Svetlana Zivanovic

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis Presented for the

Master of Science Degree at

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Kristin Renee Abney

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Dedication

I would like to dedicate this thesis to my parents, Keith and Lesa Abney, my brothers, Kevin and Kristopher, my grandparents, Bob and Melba Abney, and my aunt, Norma Rogers. I truly appreciate all of your confidence, support and general putting up with me during my education.

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Abstract

In the 1970s, a push for research on the effects of ultraviolet (UV) radiation on food crops began. Since that time, multiple agricultural and horticultural crops have been studied with results showing that the morphological and physical reactions are species dependent. The purpose of these studies to determine how increasing UV radiation affects *Allium fistulosum* L. (scallion onions) and *Allium tuberosum* Rottl. (garlic chives), and how UV radiation affects 16 cultigens of *A. fistulosum*. The effects of UV radiation were determined by shoot height, fresh weight, carotenoid and chlorophyll pigment concentrations, and photochemical efficiency (F_v/F_m). The scallions showed decreases in shoot height and fresh weight in both studies, while the chives showed increases in both shoot height and fresh weight. High performance liquid chromatography showed changes in concentrations of nutritionally important carotenoids like lutein and the xanthophyll carotenoids were noted, while β-carotene concentrations did not change. Changes in chlorophyll *a* and *b* concentrations and ratios were also found. Changes in the xanthophyll cycle were found in the scallion cultigens, indicating irradiation stress. The scallion cultigens were found not to differ much between UV radiation treatments, but there were significant differences among the cultigens. To our knowledge, this is the first study to date that has examined the effects of UV radiation on *Allium* carotenoids.

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Chapter One: Introduction

Ultraviolet Radiation

In 1973, a panel of scientists reported to the Environmental Studies Board that there was a need for research on the effects of ultraviolet (UV) light on food crops (National Academy of Science, 1973). The scientific community was, at the time, worried about how pollution from supersonic transport aircrafts could thin the ozone layer and therefore affect life on earth. However, we now know that the decline in ozone is due to chlorine and bromine containing pollutants entering the atmosphere (Pyle, 1997). Research into the effects of deceased ozone in the atmosphere has continued since the 1970s.

For life to leave the seas and begin on land, primitive plants had to evolve mechanisms to protect themselves from UV radiation. To this day, some algae and even photosynthetic bacteria lack complex flavonoids and instead have other UV-screening compounds (Rozema et al., 2002). These include mycosporine-like amino acids (MAAs) and scytonemins, which filter out UV-C radiation and are only found in cyanobacteria. MAAs and scytonemins can be considered primitive forms of plant protection, since UV-C is unable to penetrate the ozone layer (Rozema et al, 2002). Similar to flavonoids and carotenoids in terrestrial plants, these compounds perform other functions, such as the role of MAAs in reducing freeze damage in alga cells.

Plant Pigments

Carotenoids are secondary plant metabolites which have played significant roles in terrestrial plant evolution (Rozema et al., 2002). These compounds are formed from different plant metabolic pathways. Carotenoids are formed in the isoprenoid pathway by the precursor molecule, melvonic acid (Pallett and Young, 1993). Through multiple isomerizations, the colorless precursor phytoene is produced (Figure C.1; adapted from Kopsell et al., 2009). Phytoene is further metabolized to produce lycopene, β-carotene and eventually the xanthophyll carotenoids. Xanthophyll carotenoids, which include violaxanthin, antheraxanthin, and zeaxanthin, absorb excess light energy and through shifts in concentration, dissipate the energy (Demmig-Adams and Adams, 1993).

Carotenoids protect the light dependent reactions of photosynthesis from excess light energy developed from high-energy UV radiation exposure. Reductions in the ozone layer will allow more penetration of light in the UV-B (280-315 nm) and UV-A (315-400 nm) spectral range. Thus, development of high flavonoid and/or carotenoid concentrations may be an effective plant stress reduction mechanism to eliminate the harmful effects associated with exposure increased UV radiation.

Carotenoid production in plants treated with UV light varies from species to species. Both UV radiation susceptible and non-susceptible *Arabidopsis* plants have shown an increase in carotenoid production under elevated UV radiation. Sorghum (*Sorghum vulgare* Pers.), though, shows no significant changes when exposed to high levels of UV-B light when under treatment for 40 days (Ambashet and Agrawal, 1998). *Rosa hybrida* L. cv. Honesty and *Fuchsia hybrida* cv. Dollarprinzessin showed an

increase in antheraxanthin when exposed to UV-A radiation, but only *Rosa* showed an increase in zeaxanthin (Helsper et al., 2003). Buckwheat (*Fagopyrum tataricum* L. Gaertn.) showed a decrease in carotenoid production between plants grown at under ambient UV-B levels compared with plants grown under increased levels of UV-B radiation (Yao et al., 2006).

Chlorophyll pigment levels also change in response to UV radiation. Both *Rosa hybrida* and *Fuchsia hybrida* showed an increase in chlorophyll *a* and chlorophyll *b* production (Helsper et al., 2003). However, sorghum showed no significant difference in total chlorophyll content between the control and UV treated plants at 20 and 60 days of exposure (Ambasht and Agrawal, 1998). Yao et al. (2006) noted a significant decrease in chlorophyll production for buckwheat (*Fagopyrum tataricum*) as UV-B levels increased from less than ambient levels, ambient levels, and to increased UV-B light.

Physical Effects

Plants exposed to prolonged UV radiation have a range of different physical symptoms. *Arabidopsis* plants exposed to UV light for five days showed a marked difference in dry weight accumulation among susceptible individuals (Rao et al., 1995). Susceptible species of cucumbers (*Cucumis sativus* L.) developed chlorotic lesions after being exposed to UV-A radiation for three days (Adamse and Britz, 1995). Cotton (*Gossypium hirsutum* L.) showed the same response after four to five days of UV-B radiation (Kakani et al., 2003). The chlorotic spots eventually became necrotic. The

leaves exposed to UV-B radiation where also thinner than the leaves grown with no UV-B radiation. Peas (*Pisum sativum* L.), *Commelina communis* L. and rape (*Brassica napus* L.) developed permanent stomatal damage after exposure to UV-B radiation for several weeks (Gonzalez et al., 1996; Nogúes et al., 1999).

Kakani et al. (2003) reported significantly more cuticular wax on the leaves of the cotton plants exposed to UV-B radiation, compared to control plants. However, plants grown at ambient UV light had the highest amount of wax at both squaring and flowering. The wax on these leaves was denser than the wax on the cotton grown at high UV levels. This increase was on the adaxial surface of the leaf, but not on the abaxial side. Gonzalez et al. (1996) showed similar results in pea leaf cuticles. They also found that the UV-B radiation caused a shift in cuticle composition, moving from alcohols to esters and hydrocarbons. There was no significant correlation between amount of UV-B light reflected and the amount of cuticle (Gonzalez et al., 1996).

Saile-Mark and Tevini (1997) also reported a reduction in bush bean (*Phaseolus vulgaris* L.) harvest in the plants that were grown under UV radiation. Kakani et al. (2003) reported smaller flowers on cotton plants exposed to UV light. The cotton plants exposed to high and ambient levels of UV-B light had 33% and 15% less, respectively, anthers compared to the plants not exposed to UV-B light. The plants that did not appear to react could be simply changing biochemical pathways in response to UV radiation (Barnes et al., 1988; Ziska et al., 1992).

All of the cucumbers tested by Adamse and Britz (1995) showed lower leaf dry weights than their control counterparts. Exposure to UV light delayed flowering by one day in bush beans (Saile-Mark and Tevini, 1997). Bush beans also showed a reduction in

height, but the reduction did not correlate with dry weight (Saile-Mark and Tevini, 1997). In comparison to control treatments (no UV radiation), Kakani et al. (2003) reported a stimulation in cotton plant growth under exposure to ambient UV-B radiation levels. However, cotton plants grown under elevated UV radiation were much shorter than both the ambient UV-B light and no UV-B treatment.

Lollo Rosso lettuce 'Revolution' has been shown to be affected by UV radiation as well. Tsormpatsidis et al. (2008) The plants grown under full UV radiation blocking film had an increased above ground dry weight 40% in 2005 versus the plants grown under film that did not block any UV radiation and 122% in 2006. The plants also grown under the UV blocking film also had 28% and 66% more leaves in 2005 and 2006, respectively, than the lettuce grown under the film that blocked no UV radiation. The researchers also found that lettuce grown under standard horticultural film had a dry weight increase over non-UV blocking film of 10% and 34% in 2005 and 2006, respectively. However, there were no significant changes found in the variable and maximum photochemical efficiency of any UV treatment.

Genetic Effects

Changes in secondary nutrient concentrations and physical changes can also be related to genetic factors as well. Genetic variations in carotenoid concentrations have been reported among kale and collard (*Brassica oleracea* L.; Kopsell et al., 2004) cultivars and in phenolic compounds found in different red raspberry cultivars (Anttonen

and Karjalainen, 2005). Onions (*Allium cepa* L.) of different colors can show drastic changes in flavonoid levels. Marotti and Piccaglia (2002) reported that the different cultivars of onions can have significant differences in flavonoid concentrations. According to Price and Rhodes (1997), brown, red and pink onions have over twenty times more quercetin than white onion cultivars.

Blueberries (*Vaccinium* spp) have been found to have differing levels of flavonoids among genotypes. According to Howard et al. (2003), flavonol content in 18 genotypes of southern highbush blueberries (*Vaccinium* spp) and northern highbush blueberries (*Vaccinium corymbosum* L.) were found to range between 1.20 $g \bullet kg^{-1}$ and 0.31 $g \bullet kg^{-1}$ in 2000 and 1.08 $g \bullet kg^{-1}$ and 0.42 $g \bullet kg^{-1}$ in 2001. These same berries were also found to have differing amounts of total phenolics. Howard et al. (2003) found that the genotype A-386's total phenolic content was 2.02 $g \bullet kg^{-1}$ in 2000 while US-407's total phenolic content was 5.86 $g \bullet kg^{-1}$.

Rice (*Oryza sativa* L.) has also been shown to have damaging effects from UV radiation. Caasi-Lit et al. (1997) compared the effects of UV radiation on 16 rice cultivars. The cultivars highly susceptible to UV light showed symptoms of leaf curling and browning while the highly tolerant cultivars showed none of these signs. The susceptible plants also showed damage to cell organelles in the form of ruptured chloroplast envelopes and disrupted granal stacks. It is also interesting to note that the highly tolerant cultivars were shown to have higher levels of phenols than the highly susceptible cultivars.

Alliums

Scallion onions (*Allium fistulosum* L.) and chives (*Allium tuberosum* L.) are two close relatives to the common onion. These crops have been used for centuries by various cultures for flavor attributes and herbal remedies (Craig, 1999). These vegetables and herbal crops are also very well known for high concentrations of secondary plant metabolites, including not only carotenoids and flavonoids, but the sulfur containing compounds like alkyl dimethylthienyl disulfides (Stajner and Varga, 2003; Stajner et al. 2006; Kuo and Ho, 1992).

Metabolites in onion offer health benefits ranging from cancer prevention to improvements in cardiovascular health (Ness and Powles, 1997; Research WCRF/AICR, 1999; Howard and Kritchevsky, 1997). Carotenoids, like lutein and zeaxanthin, help prevent age-related macular eye degeneration (Landrum and Bone, 2001) while other carotenoids, such as lycopene, can help reduce the risk of some cancers (Giovannucci et al., 2002). These compounds work by reacting with reactive oxygen species that attack cellular membranes, proteins, and deoxyribonucleic acid (DNA) (Apel and Hirt, 2004). Scallions can to produce over 1,000 µg lutein + zeaxanthin·100g^{-1} fresh weight and almost 600 µg β-carotene·100g^{-1} fresh weight (USDA, 2007 b). Chives also have high levels of β-carotene, with over 2,500 µg·100g^{-1} fresh weight of leaf tissue, and over 300 µg lutein + zeaxanthin·100g^{-1} fresh weight (USDA, 2007 b).

Onions have also been shown to have high levels of other secondary plant nutrient, flavonoids. Flavonoids can help prevent cardiovascular disease by inhibiting production of low density lipoproteins (LDL) as well as preventing platelet aggregation

and adhesion (Howard and Kritchesky, 1997). Flavonoids have also been shown to work as antioxidants as well (Jordan, 1996). Depending on the cultivar of onion, some can have levels of total flavonoids up to 765.1 $mg \cdot kg^{-1}$) (Marotti and Piccaglia, 2002.) According to Hope et al. (1983), quercetin, an abundant flavonoid in onions, can have antiviral as well as antibacterial properties. Another quality that plants from this genus have is the amount of sulfur-containing compounds. These compounds have been shown to lower the risk of stomach cancer in people who ate onions (Steinmetz and Potter, 1991.)

There has been a lot of research in characterizing and quantifying the amounts of secondary plant metabolites in most *Allium* species. However, there has been very little research done to determine the effects of ultraviolet radiation on carotenoids and chlorophyll in *Allium fistulosum* and *Allium tuberosum*. For my project, I will be characterizing and quantifying the concentrations of carotenoids and chlorophylls in scallions and chives as well as differences in appearance, height and weight. I will also be comparing 16 cultigens of scallions for changes in carotenoid and chlorophyll concentrations and for differences under ultraviolet light. I will also be examining the differences in ratios of energy dissipating xanthophyll carotenoids.

Objectives

1) Identify and characterize differences in nutritionally important carotenoids and chlorophylls produced by *Allium fistulosum* and *Allium shenoprasum* under increasing UV radiation.

2) Identify and characterize genetic differences for carotenoid and chlorophyll production among *Allium fistulosum* cultivars in response to increased UV radiation.

3) Examine differences in energy dissipating xanthophyll carotenoid ratios and photosynthesis efficiency under different UV conditions.

4) Examine changes in height and biomass production in *Allium fistulosum* and *Allium shenoprasum.*

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Chapter Two: Identification and quantification of carotenoids and chlorophylls in scallion onions (*Allium fistulosum* L.) and chives (*Allium tuberosum* Rottl.) grown under increasing UV light levels

Abstract

Carotenoids, a class of secondary plant metabolites, not only help maintain mammalian health, but also help prevent damage created by free radicals in both animal and plant species. In this study, two species of *Allium* crops, scallion onions (*Allium fistulosum* L.) and garlic chives (*Allium tuberosum* Rottl.), were grown in controlled environments and were exposed to increasing levels of ultraviolet (UV) radiation. The leaf tissues were analyzed for pigment concentrations by high performance liquid chromatography. Responses to UV radiation for growth parameters of shoot with plant height, fresh weight and photochemical efficiency were also measured. The only carotenoid that differed among UV treatments was lutein in the scallions, which increased in a linear fashion in response to increasing UV radiation. The garlic chives showed linear increases in fresh weight and shoot height as the UV radiation increased, while the scallions demonstrated a quadratic decrease in shoot height and a linear decrease in fresh weight as UV radiation increased. No changes were found in photochemical efficiencies among UV treatments. Linear increases were found in the garlic chives for chlorophylls *a* and *b* and total chlorophylls in response to increasing UV radiation, while the scallions had a quadratic increase in chlorophyll *b* only as UV intensities increased. While the effects of UV radiation have been measured in numerous crop species, there was has been little work to date on *Alliums*.

Introduction

Plant secondary metabolites are compounds that do not directly take part in plant growth and development, but aid in responses to changing environments. Carotenoids, one class of secondary plant metabolites, are receiving attention recently due to reports that they can improve human health (Kopsell and Kopsell, 2006). Carotenoids act as antioxidants, which help quench reactive oxygen species (ROS) created through respiration and prevent damage to cells in the body (Apel and Hirt, 2004). While production of ROS through respiration is common, ultraviolet (UV) radiation can produce ROS as well. Animal species must ingest carotenoids to receive any benefits; however, plants are capable of producing them in response to elevated levels of UV radiation (Jordan, 1996).

Carotenoids have multiple functions in the plant. Some are visible as the yellow-orange colors in flower petals in species like marigolds (*Tagetes erecta* L.); while most are used in protecting the photosynthetic apparatus (Hadden et al., 1999). The xanthophyll carotenoids dissipate excess energy as light harvesting antennae in the photosystem complex (Deming-Adams et al, 1996). According to van Gestel et al. (2005), the light saturation point of *Allium fistulosum* is 1,500 $\mu m \cdot s^{-2} \cdot m^{-2}$. The extra light is captured by xanthophyll carotenoids, and the photons are removed by non-photochemical quenching (Deming-Adams et al., 1996). Under low irradiance, epoxydation occurs, changing zeaxanthin to violaxanthin, via the intermediate antheraxanthin. From here, violaxanthin is converted to neoxanthin, and it can continue

down the pathway to create abscisic acid (Zeevaart and Creelman, 1988). However, under high irradiance, the cycle reverses, and de-epoxydation occurs. It is through these changes that the excess energy is dissipated (Deming-Adams et al., 1996.)

Ultraviolet radiation can affect plant pigment levels. Experiments on sorghum (*Sorghum vulgare* Pers.) have shown an increase in chlorophyll pigments under elevated UV radiation (Ambasht and Agrawal, 1998). Yao et al. (2006) showed increases in not only chlorophylls, but total carotenoids increased in tartary buckwheat (*Fagopyrum tataricum* Gaertn) grown under increased UV radiation. Photochemical efficiency (F_v/F_m) is an indication of photoinhibition and overall plant health and the measure of F_v/F_m can be used to indication radiation stress. One experiment involving Lollo Rosso lettuce (*Lactuca sativa* L. cult. 'Revolution') showed no changes in F_v/F_m in response to UV treatments (Tsormpatsidis et al., 2008).

Multiple studies have shown that while plants can adapt to higher levels of UV radiation, they do so though morphological and physiological changes, in addition to changes in secondary plant metabolites. Kakani et al. (2003a) showed changes in the structure of cotton (*Gossypium hirsutum* L.) plants, like stunted branches and reduced flower area. Tartary buckwheat, another field crop, showed similar changes, like shorter plants and decreased leaf area index (Yao et al., 2006).

While there is a plethora of information about the effects of UV radiation on crops, there have been no studies that look at the effects of UV radiation on carotenoid production in *Allium* crops. This study was designed to measure physiological changes in scallion onions (*Allium fistulosum* L.) and chives (*Allium tuberosum* Rottl.) in response to increased exposure to UV radiation, as well as the effect of increasing UV radiation on

photochemical efficiency. ‘Evergreen Hardy White’ scallion onions and ‘New Belt’ chives will serve as representative *Allium* crop species.

Materials and Methods

Plant Culture

On 2 January 2008, the scallion onions (‘Evergreen Hardy White’) and chives (‘New Belt) were planted for the first part of the experiment. They were planted in 2.5 x 2.5 cm growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and covered with a layer of vermiculite. The scallions were planted 2-3 seeds per cube and the chives were planted 3-4 seeds per cube. The plants were watered twice a day. The seeds were germinated and grown to the second leaf sheath stage in a Model E15 growth chamber (Conviron, Winnipeg, Manitoba) under a 16/8 hour photoperiod at 24°C/20°C day/night, respectively.

At 13 days after planting (DAP), the scallions and chives were thinned to 1 plant per cube. The plants were fertilized with quarter strength Hoagland’s solution (Appendix A) the following day. The scallions and chives received fertilizer solution three times a week until 21 DAP. At 21 DAP, the scallions and chives were fertilized daily with half strength Hoagland’s solution. Each chamber received 350 $\mu m \cdot s^{-2} \cdot m^{-2}$ PAR ± 10%. Measurements were made with a light meter and spectroradiometer (Apogee Nanologger model ANL, Apogee Instruments, Inc., Roseville, Calif.; Spectroradiometer Model SPEC-UV/PAR, Apogee Instruments, Inc., Roseville, Calif.) At 34 DAP, the plants were

transferred to the treatment chambers. Individual chambers represented either 5, 7, 8 or 9 $\mu m \cdot s^{-2} \cdot m^{-2}$ of UV radiation (280-380 nm), supplied by Fluker Farms (Sun-Glow Coil Lantern Fluorescent UVB bulb 15 watt and 20 watt, Fluker Farms, Port Allen, Louis.; Figures C.2-C.5.) Both the scallions and the chives were at the third leaf sheath. The plants were grown in 11-L containers (Rubbermaid, Inc., Wooster, Ohio). Each container was filled with 9 L of half-strength Hoagland's solution. The plants were planted six to a container and put into 2-cm holes spaced at 11 x 9 cm. Water was added daily to bring the solution up to volume and was changed completely every two weeks.

The plants were harvested at 69 DAP. Shoot height and fresh weight measurements were taken at harvest, along with photochemical efficiency (F_v/F_m) measurements. Six plants were harvested from each tub and three measurements were taken from each plant. The F_v/F_m measurements were averaged for the entire tub. The measurements were made at the mid-point of plant height using a modulated fluorometer (OS1-F1 Modulated Fluorometer, Opti Sciences, Hudson, N.H.)The F_v/F_m value is an indication of photoinhibition and overall plant health. The scallion plants were cut to separate the pseudostem and leaf tissue. All plant tissues were then stored in a -80 °C freezer until extraction. All reported measurements are averages per plant.

Carotenoid and Chlorophyll Determination

The tissue pigments were extracted according to Kopsell et al. (2004) and analyzed according to Emenhiser et al (1996). The samples were freeze-dried and ground with a spice grinder (Krups, Millville, NJ). A 0.10g subsample was rehydrated with 0.8mL of ultra pure H_2O. The samples were then incubated at 40ºC for 20 minutes. Then, 0.8mL of ethyl-β-8'-*apo*-carotenotate (Sigma Chemical Co., St. Louis, Mo) was added as

an internal standard to establish extraction efficiency. 2.5 mL of tetrahydrofluran stabilized with 25 mg L^{-1} 2,6-di-*tert*-butyl-4-methoxyphenol (BHT) was added to the sample. Using a Potter-Elvehjem tissue grinding tube (Kontes, Vineland, NJ), the samples was homogenized using ~25 insertions with a pestle attached to a drill press set at 540 rpm. The tubes were immersed in ice to dissipate the heat generated from maceration. The tubes were then centrifuged in a clinical centrifuge for 3 min at 500 g_n. The supernatant was then removed and the pellet was rehydrated with 2 mL tetrahydrofluran. This procedure was repeated until the supernatant extracted was colorless. The combined supernatant was then reduced to 1 mL under a stream of nitrogen gas. The supernatant was then brought up to a final volume of 5 mL with methanol. The samples were then filtered through a 0.2 μm Econofilter PTFE 25/20 polytetrafluoroethylene filter (Agilent Technologies, Wilmington, Del.) using a 5 mL syringe. 2 mL aliquots were put into amber vials and capped prior to high performance liquid chromatography (HPLC) analysis.

Carotenoid and Chlorophyll HPLC Analysis

The samples were run on a 1200 series Agilent HPLC unit with a photodiode array detector (Agilent Technologies, Palo Alto, Calif.) For chromatographic separation, a 250 x 4.6 mm i.d., 5 μm analytical scale polymeric C_{30} reverse phase column (ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, Penn.) was used. The column was equipped with a 10 x 4.0 mm i.d. guard cartridge and holder (ProntoSIL) and was kept at 30ºC using a thermostatted column compartment. All separations were achieved using a mobile phase of 88.99% methanol, 11% methyl-*tert*-butyl ether, and 0.01% triethylamine (v/v). The flow rate was 1.0 mL/min, with a run time of 53 min. There were 2 min of

equilibration prior to the next injection. Eluted compounds from a 10 μm injection loop were detected at 453 nm for carotenoids, the internal standard, and chlorophyll *b* and 652 nm for chlorophyll *a*. The data was collected recorded and integrated using ChemStation Software (Agilent Technologies). Peak assignments for each pigment were performed by comparing retention times and line spectra obtained from the photodiode array detection using external standards. These standards included antheraxanthin, neoxanthin, lutein, violaxanthin and zeaxanthin (Carotenature, Lupsingen, Switzerland), β-carotene, chlorophyll *a* and chlorophyll *b* (Sigma Chemical Co.). The concentrations of the external standards were determined spectrophotometrically using a procedure by Davies and Köst (1988).

Data was analyzed by ANOVA procedure from SAS (Cary, N.C.) Orthogonal polynomials were used to determine changes between UV radiation treatments by partitioning the sums of squares into components that were associated with linear, quadratic and cubic terms (Steel and Torrie, 1980).

Results and Discussion

Physical

The changes in UV light showed that the chives had a significant linear decrease in shoot height ($F = 7.73$; $P = 0.011$) (Table B.1). The scallions showed a significant quadratic increase in height ($F = 5.24$; $P = 0.033$) in response to increasing UV radiation treatments. A decrease in shoot height has been shown in multiple studies as a negative

effect of UV radiation (Ambasht and Agrawal, 1998; Kakani et al., 2003; Yao et al., 2006). However, Al-Oudat et al. (1998) found that broad beans (*Vicia faba*) had an increase in plant height under UV radiation.

The scallions had a significant linear decrease in fresh weight ($F = 24.46$; $P = 0.001$) as UV radiation increased (Table B.2). However, chives had a significant linear increase in fresh weight ($F = 8.51$; $P = 0.019$) under increasing UV radiation. Since the scallions showed decreasing shoot height and fresh weight with increasing UV radiation, the plants were indeed smaller. The chives showed an opposite trend; with increase both shoot height and fresh weight under increasing UV radiation.

There were no changes in F_v/F_m in response to UV treatments in both scallions and chives (Table B.3). Tsormpatsidis et al. (2008) showed no differences in F_v/F_m response to UV radiation treatments of lettuce. In that study, differences were also found between biomass produced in the treatments.

While it may seem contradictory to have two species within the same genus act in opposite ways under UV treatment, it is not uncommon. A study by Yuan et al. (1998) showed decreases in plant height and biomass for spring wheat (*Triticum aestivum* L.) under UV radiation. However, another study looking at durum wheat (*Triticum durum* Desf. var. *Horani)* showed an increase in plant height (Al-Oudat et al., 1998) under increased UV radiation. The biomass of the experimental plants also decreased and increased in these two studies, respectively.

Carotenoids and Chlorophylls

Both leaf and pseudostem tissues in the scallions were analyzed for carotenoid and flavonoid pigments. There were no pigments found in the pseudostem tissue, which has been shown in a previous study by Kopsell et al. (unpublished data).

Zeaxanthin was below the detection limits of the HPLC for both plant species. This is a possible consequence to the lower light levels in the controlled environments. No significant differences were found in both the scallions and the chives for antheraxanthin (Table B.4), violaxanthin (Table B.5), neoxanthin (Table B.6) or β-carotene (Table B.7). The chives showed no differences in lutein under increasing UV radiation treatments. One reason that plant pigments did not respond could have been their centers of origin. *A. fistulosum* is believed to be from northern China (Friesen et al., 1999), while *A. tuberosum* is believed to have come from Asia (Brewster, 2008.) This could have an impact, because if plants were originally adapted to areas of higher elevation, then they might not react the same way to UV radiation as a plant native to a lower elevation. The only carotenoid that showed a significant linear increase in response to increasing UV radiation was lutein in the scallion plants ($F = 9.04$; $P = 0.024$; Table B.8).

The chives showed a positive linear increase in chlorophyll *a* ($F = 6.88$; $P = 0.039$) (Table 2.9) and chlorophyll B ($F = 9.09$; $P = 0.024$; Table B.9) in response to increasing UV radiation. While the scallions did not show any changes in chlorophyll *a*, they did show both a linear and a quadratic change for chlorophyll *b*, ($F = 11.24$; $P = 0.003$) and ($F = 7.18$; $P = 0.014$, respectively) in response to increasing UV radiation (Table B.10). While the chives had a positive linear increase in total chlorophylls ($F = 10.04$; $P = 0.0194$) (Table B.11), total chlorophylls in the scallions remained unchanged

among UV radiation treatments. Neither species showed treatment differences in chlorophyll *a* to chlorophyll *b* ratios (Table B.12).

There are several possible reasons why the lutein was the only pigment that showed any change for the scallions, and why the chives showed no treatment changes in concentrations of carotenoids. Lutein is the carotenoid predominantly found in photosystem (PS) II (Demming-Adams et al., 2003.) Several studies have cited this photosystem to be influenced more by UV radiation than PS I (Kakani et al., 2003b.) An increase in lutein concentration in the scallions along with decreased shoot height and biomass could be indicators of higher radiation stress to PS II from the UV treatments imposed in this study. However, we found no indication of changes in lutein production and increased shoot height and biomass in the chives. These results could be inferring that the amount of UV radiation was not enough to stress PS II and it was able to photosynthesize as normal.

Another reason as to why the chives increase in shoot height and biomass could be the angle the leaves on both plants. Visually, the chive leaves seemed to be at a sharper vertical angle than the scallion leaves, especially after the scallions had put out multiple leaves. Research by Day et al. (1992) showed that grasses had much lower depth of UV penetration than did herbaceous dicots, attributing the differences to the leaf angle. While neither plant is a grass or a dicot, this could help explain a lack of response to the UV radiation treatments of the study. An herbaceous dicot is much more likely to have a leaf with horizontal orientation while a blade of grass has an angle that is more perpendicular to the ground, shielding it more from the UV radiation. If the chives were

more shielded from the UV radiation, then they would not need to change the amounts of carotenoids produced and could focus their energy on biomass production.

Other plant antioxidants could be the reason why there was no change in the carotenoids. Another class of plant antioxidants, the flavonoids, has long been regarded as part of the plant's natural defense against UV radiation (Jordan, 1996). These compounds are formed in the epidermis layer of the leaves. If the flavonoids were able to block a significant amount of UV radiation before it reached PS I and PS II, then the plant would not have to change the amount, or flux of its carotenoids.

Another reason could be that there simply was not enough UV radiation to severely affect the carotenoid pathway in the plants. In the spirit of trying to find the best product for the lowest price, we used economical UV bulbs to supplement the UV radiation in this experiment. While we had hoped that the UV light from the bulbs would radiate and bounce of the reflective walls of the chamber, we might not have had as much luck with that as we wished. The light levels used also could have not been different enough to show differences between the light levels and between plant species (Figures C.2-C.5.)

Conclusions

While the majority of carotenoids tested in this experiment did not change between UV light levels or between species, interesting differences were found. The

scallions had decreases in plant biomass and height in response to UV radiation treatments, while the chives demonstrated increases in both areas under the same treatments. The scallions showed a treatment response in shoot tissue lutein concentrations, which the chives did not. While the strength of the lights could be one reason why changes were not seen, the origins of the plants should be considered. Since both of the plants are from elevated areas, they may have a naturally high tolerance to changes in UV radiation and therefore may take higher levels UV radiation to show changes in the plant biochemistry. Further, a more vertical leaf angle orientation in the chives could have limited tissue exposure to the UV treatments.

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Chapter Three: Measurement of genetic variation in pigment composition among different cultigens of *Allium fistulosum* L.

Abstract

Since the discovery that the ozone layer is thinning, there has been a plethora of studies trying to determine the effects of ultraviolet (UV) radiation on food crops and ornamentals. Plants from the *Allium* genus are used world-wide for food and medicinal purposes. In this study, 16 cultigens of scallion onions (*Allium fistulosum* L.) were grown in a greenhouse under and ambient radiation treatments and a UV supplemented treatment to determine impacts of UV radiation on physiological and nutritional components in scallion tissues. The effects of supplemental UV radiation were determined for shoot height, shoot biomass accumulation, photochemical efficiency (F_v/F_m) and the concentrations of shoot tissue carotenoid and chlorophyll pigments. High performance liquid chromatography revealed differences in xanthophyll carotenoids pigments, lutein, and chlorophylls *a* and *b* between radiation treatments and among cultigens. Differences in shoot height and weight were also noted among cultigens and between radiation treatments. Cultigen Pesoenyj responded to supplemental UV radiation with increases in zeaxanthin + antheraxanthin to zeaxanthin + antheraxanthin + violaxanthin, which may indicate a flux in the xanthophyll carotenoids towards de-epoxydation, commonly found under high irradiance stress. Supplemental UV radiation influenced shoot tissue carotenoid concentrations in some, but not all, of the scallion onions. Increases in carotenoid concentrations would be expected to increase crop nutritional values. To our knowledge, this is the first study to demonstrate an influence of UV radiation on shoot tissue carotenoids among scallion cultigens.

Introduction

In the 1970s, scientists discovered that the thinning of the ozone layer was correlated with emissions from man-made chemicals, such as chlorofluorocarbons (CFCs). With the signing and enforcement of the Montreal Protocol, emission of CFCs, which were shown to break down the ozone layer, have been reduced and some believe that the ozone will be able to return to pre-1980s levels by 2050 (Kakani et al., 2003). However, thinning of the ozone layer has resulted in increases in ultraviolet (UV) radiation penetration in the Earth's atmosphere. What remains uncertain is the impact of increased UV radiation on growth and development and nutritional value of cultivated crops (National Academy of Science, 1973). Both UV-A radiation and (380-320 nm) and UV-B radiation (280-320 nm) are able to penetrate the ozone layer. Higher amounts of UV radiation in these ranges may influence the accumulation of plant compounds used to combat light stress. Secondary plant metabolites not only protect plants from excess UV radiation, they can also have the ability to protect humans from UV radiation when translocated to sub-dermal skin tissues (Mares-Perlman et al., 2002).

Fruits and vegetables have varying levels of phytonutrients, in addition to vitamins and minerals. One important class of these phytonutrients is the carotenoids. These compounds help prevent certain types of cancers, and aging eye diseases like macular eye degeneration (Landrum and Bone, 2001). Carotenoids are unsaturated long chain polycarbons that are produced by the plant to help protect the photosynthetic apparatus from high light excitation (Demmig-Adams et al., 1996). *Allium* species

contain carotenoid pigments in leaf tissues (Kopsell et al., unpublished data). *Alliums* also contain different levels of sulfur-containing compounds that also help prevent certain cancers, like stomach cancer (Steinmetz and Potter, 1991.) While all higher plants contain carotenoids, genetic variations for carotenoid accumulations exist both within and among each plant species. Within any given crop species there can be multiple landraces, accessions and cultivars, or collectively, cultigens. These variations are key to advancements in plant development programs for increased nutrition, disease prevention, or other factors. However, different cultigens will react differently under almost any given stress.

Previous studies have demonstrated impacts of UV radiation on plant performance, cellular structures and/or pigment accumulations. Different cultivars of wheat (*Triticum aestivum* L.) have been shown to have varying responses on UV radiation. In a study by Yuan et al. (2000), 20 cultivars of wheat were grown under UV-B radiation stress to determine possible detrimental influences. This study found that most wheat cultivars responded negatively to UV-B radiation; however, several cultivars showed increases in plant height and biomass. Structural changes like ruptured chloroplast envelopes have been noted in UV-sensitive rice cultivars (*Oryza sativa* L.) when grown under UV stress (Caasi-Lit et al., 1997). Increases in UV radiation have resulted in delayed flowering and harvest times among different varieties of bush beans (Saile-Mark and Tevini, 1997). The same study also found that the cultivars had decreases in fruit size and yield when compared to cultivars not grown under UV radiation stress. Tomatoes (*Lycopersicon esculentum* L. cult. 'DRW 5981) grown using UV-B blocking filers showed increases in lycopene and β-carotene, while fruits of the

same variety showed decreases in lycopene, phytoene and phytofluene when grown without the UV-B blocking filters (Giuntini, et al, 2005.) Another cultivar in the same study, HP1, showed more than double the amount of lycopene in tomato fruits when grown under no UV-B radiation. Results from such studies may demonstrate a protective mechanism for some carotenoids against UV radiation.

Allium species can have high levels of nutritionally important secondary plant metabolites, which convey numerous health benefits. For example, bulb onions (*Allium cepa* L.) have been shown to have high levels of flavonols (Price and Rhodes, 1997; Marotti and Piccaglia, 2002). Plants in this genus have been important to multiple cultures of centuries. However, no studies to date have measured the impact of UV radiation on the production of carotenoid compounds in *Alliums*. *Allium fistulosum* is consumed in part for its shoot tissues as well as pseudostem. Carotenoid compounds are present in the shoot tissues of *A. fistulosum*, which conveys nutritional properties when consumed regularly in the diet (Denny and Buttriss, 2007). Therefore, the objectives of this project were to examine both environmental and genetic responses to elevated UV radiation among a large subset of *A. fistulosum* cultigens. Responses were noted for plant height, shoot tissue biomass, F_v/F_m, and concentrations of carotenoids and chlorophyll pigments in the shoot and pseudostem tissue.

Methods and Materials

Plant Culture

On 16 December 2008, 16 *A. fistulosum* accessions were potted in 15 cm pots in a greenhouse in Knoxville, TN (35.96N latitude). The accessions included eight from the USDA-ARS National Plant Germplasm Repository (Geneva, N.Y.) (PI 274254, PI 462345, PI 546343, PI 546228, PI 280562, PI 436539, PI 462357, and G 30393), four cultivars from Seedway, LLC (Hall, N.Y.) (Long White Bunching, Feast, Performer, and Parade) and four cultivars from Johnny's Selected Seeds (Winslow, Maine) (White Spear, Evergreen Hardy White, Deep Purple and Ishikura Improved F1) (Table B.13). The seedlings were watered daily for the duration of the experiment. On 10 January 2009, the seedlings were thinned to two plants per pot and fertilized with Hoagland's solution (Appendix A). Each pot was fertilized once a week for the duration of the experiment with 100 mL of fertilizer solution.

The supplemental UV treatment began on 27 January 2009. The photosynthetically active radiation (PAR) in the greenhouse was 540.5 $\mu m \cdot m^{-2} \cdot s^{-2}$ and the amount of UV was 7.0 $\mu m \cdot m^{-2} \cdot s^{-2}$ (Figures C.6-C.7). Measurements were made with a light meter and spectroradiometer (Apogee Nanologger model ANL, Apogee Instruments, Inc., Roseville, Calf.; Spectroradiometer Model SPEC-UV/PAR, Apogee Instruments, Inc., Roseville, Calf.) . To control pests in the greenhouse, three beneficial insect species were used. *Hypoaspis miles* and *Neoseiulus cucmeris* were used to control

thrips while *Orius insidiosus* was used to help control aphids. These insects were first released on 23 January 2009, and were released every two weeks thereafter.

On 3 March 2009, all of the scallions were harvested. Six plants were harvested from each replication. Fresh weights and plant heights were taken and averaged for each replication. One measure of F_v/F_m was taken from each of the harvest plants at the mid-point of plant height using a modulated fluorometer (OS1-F1 Modulated Fluorometer, Opti Sciences, Hudson, N.H.) The F_v/F_m value is an indication of photoinhibition and overall plant health. All plants were harvested and pseudostem and leaf tissue were separated. The samples were immediately placed in a 20 °C freezer before being moved to a -80 °C freezer.

Carotenoid and Chlorophyll Determination

Tissue pigments were extracted according to Kopsell et al (2004) and analyzed according to Emenhiser et al (1996). The samples were freeze-dried and ground with a mortar and pestle with liquid nitrogen. A 0.10g subsample was rehydrated with 0.8mL of ultra pure H_2O. The samples were then incubated at 40ºC for 20 min. 0.8mL of ethyl-β-8'-*apo*-carotenotate (Sigma Chemical Co., St. Louis, Mo.) was added as an internal standard to establish extraction efficiency. 2.5 mL of tetrahydrofluran stabilized with 25 mg L^{-2} 2, 6-di-*tert*-butyl-4-methoxyphenol (BHT) was added to the sample. Using a Potter-Elvehjem tissue grinding tube (Kontes, Vineland, N.J.), the samples was homogenized using ~25 insertions with a pestle attached to a drill press set at 540 rpm. The tubes were immersed in ice to dissipate the heat generated from maceration. The tubes were then centrifuged in a clinical centrifuge for 3 min at 500 g_n. The supernatant was then removed and the pellet was rehydrated with 2 mL tetrahydrofluran. This

procedure was repeated until the supernatant extracted was colorless. The combined supernatant was then reduced to 1 mL under a stream of nitrogen gas. The supernatant was then brought up to a final volume of 5 mL with methanol. The samples were then filtered through a 0.2 μm Econofilter PTFE 25/20 polytetrafluoroethylene filter (Agilent Technologies, Wilmington, Del.) using a 5 mL syringe. 2 mL aliquots were put into amber vials and capped prior to high performance liquid chromatography (HPLC) analysis.

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chlorophyll *a* and chlorophyll *b* (Sigma Chemical Co.). The concentrations of the external standards were determined spectrophotometrically using a procedure by Davies and Köst (1988).

Statistical analysis was completed using the GLM procedure of SAS (Cary, N.C.) Cultigen means within each treatment were separated by least significant difference (LSD) at $P = 0.05$. Differences between cultigens means between treatments were detected by using t-test ($P = 0.05$) using JMP (SAS, Cary, N.C.)

Results and Discussion

Physical changes under UV radiation

Significant differences were found between the cultigens ($F = 6.67$, $P < 0.0001$; Table B.14), but no differences were found between the UV environments and the interaction between the cultigen and the environments. A *t*-test found a significance change in height of one cultigen, GA-C 76. Long White Bunching had the most plant growth in both the UV supplemented plants and the plants grown without supplemental UV radiation, with 50.38 cm and 49.68 cm respectively. G 30393-06 GI had the shortest final plant height with 35.67 cm of growth. Out of all of the cultigens grown under supplemental UV radiation, Jionji Negi had the least, with 36.20 cm of growth.

There were differences in fresh weight between UV radiation treatments ($F = 238.10$, $P < 0.0001$; Table B.15) and cultigen ($F = 11.09$, $P < 0.0001$), but no difference in treatment and cultigen interaction. T –tests showed cultigens Deep Purple, Feast, GA-

C 76, Ishikura Improved F1, Improved Beltsville Bunching, Jionji, Long White Bunching, Parade, Performer, Pesoenyj, Shounan, White Spear, 274254-05GI and G 30393-06GI all showed decreases in plant fresh weight in response to UV treatments. Cultigens Hardy Evergreen White and Zhang Qui Da Cong did not show any difference between UV treatments. In the plants grown without UV radiation, fresh weights ranged from 76.86 g in Improved Beltsville Bunching to 29.22 g in Pesoenyj. In the plants gown without supplemental UV radiation, the averages ranged from 116.01 g in cultigen Long White Bunching and 62.04 in cultigen Jionji Negi.

Photochemical efficiency showed significant differences between UV treatments ($F = 13.89$, $P = 0.0003$; Table B.16) and cultigen ($F = 2.11$, $P = 0.0152$), but no difference in treatment and cultigen interaction. T-tests showed no difference between cultigens grown under supplemental UV radiation and without UV radiation supplement. All of the plants had F_v/F_m measurements ranging from 0.81 to 0.83.

One previous study by Tsormpatsidis et al. (2008) showed that while Lollo Rosso lettuce (*Lactuca sativa* L.) had decreased vegetative growth when grown under UV light, there was no difference in photochemical efficiency. Another study done with wheat showed that UV radiation decreased photochemical efficiency along with decreases in carotenoid ratios (Lizana et al., 2009). None of the cultigens in this study showed differences in photochemical efficiency, but most of the plants showed differences in plant height and tissue biomass. If so, then that could explain why changes in photochemical efficiency may not have affected plant height.

Carotenoids and Chlorophylls under UV radiation

No carotenoid or chlorophyll pigments were found in the pseudostem of any of the scallion cultigens. This has been shown in a previous study by Kopsell et al. (unpublished data).

Zeaxanthin differed significantly among the scallion cultigens ($F = 4.07$; $P < 0.0001$; Table B.17.) However, there were no significant changes in leaf tissue zeaxanthin in response to the UV treatments or the interaction of the treatments and cultigens. T-tests showed an increase in zeaxanthin in cultigen G 30393-06GI and a decrease in Feast in response to UV treatment. The ranges of zeaxanthin concentrations in the plants grown under supplemental UV light is 0.08 mg•100g fresh weight (FW) in Deep Purple and White Spear to 0.16 mg•100g FW in Improved Beltsville Bunching. Cultigen Pesoenyj had the highest concentration of zeaxanthin among the plants grown without supplemental UV radiation at 0.19 mg•100g FW while Feast and Evergreen Hardy White had the lowest concentration at 0.07 mg•100g FW. Increases in zeaxanthin could be an indication that the plants experienced radiation stress. Plant responses through increased zeaxanthin concentrations would be expected to help dissipate excess energy from the photosystems.

Violaxanthin was shown to respond significantly for both UV radiation treatments ($F = 6.76$; $P = 0.0109$) and cultigen ($F = 4.42$, $P < 0.0001$), but not to changes from the interaction between treatment and cultigen (Table B.18.) *T*-tests showed significant increases in violaxanthin concentrations for cultigen GA-C 76 in response to increased UV radiation. Violaxanthin concentrations under supplemental UV radiation ranged from 2.04 mg•100g FW in GA-C 76 to 0.59 mg•100g FW in Performer. Cultigen Pesoenyj had

the highest concentration of violaxanthin, with 2.35 mg•100g FW in the plants grown without supplemental UV radiation, while G 30393-06 GI had the least with 0.53 mg•100g FW. Increases in violaxanthin in plants grown under UV radiation could suggest that these cultigens may not be as susceptible to UV radiation damage as the other cultigens.

Antheraxanthin, the intermediate compound in xanthophyll cycle, responded significantly to changes in UV radiation treatment ($F = 16.61$; $P < 0.0001$), and by cultigen ($F = 4.68$; $P < 0.0001$; Table B.19.) *T*-test showed no significant changes in antheraxanthin among cultigens grow under UV light and those without supplemental UV radiation. The ranges for antheraxanthin concentrations in plants grown under UV radiation treatment were from1.38 mg•100g FW in Pesoenyj and 0.79 mg•100g FW in 274254-05 GI. In the plants grown without UV radiation, Pesoenyj had the highest antheraxanthin concentration at 1.35 mg•100g FW, while Ishikura Improved F1 had the lowest concentration ay 0.59 mg•100g FW. While changes in this compound cannot be directly tell which way the cycle is fluxing, increases or decreases can help indicate whether epoxydation or de-epoxydation are occurring by if there are increases of zeaxanthin or violaxanthin, respectively.

The ratio of zeaxanthin + antheraxanthin to zeaxanthin + antheraxanthin + violaxanthin (ZA/ZAV) responded significantly to cultigen ($F = 3.01$; $P = 0.0006$; Table B.20), but not to UV radiation treatment or the interaction between treatment and cultigen. *T*-tests showed significant increases in response to supplemental UV light in cultigen Pesoenyj. G 30393-06 GI had the highest ratio of ZA/ZAV of cultigens grown under supplemental UV radiation and Ishikura Improved F1 had the lowest ratio at 0.34.

For the cultigens not grown under UV radiation, Feast had the highest, with a ZA/ZAV ratio at 0.65, and Jionji Negi had the lowest with 0.35.

Changes in the ratio of ZA/ZAV can identify fluxes in the xanthophyll cycle. An increase in this ratio shows a decrease in violaxanthin, which could mean these compounds are undergoing de-epoxydation because of high light energy (Demmig-Adams, 1996.) A study by Niyogi et al. (1998) helped demonstrate the importance of this photoprotection. In this study, mutant *Arabidopsis thaliana* L. were unable to under de-epoxydation and convert violaxanthin to zeaxanthin. This resulted in an increased sensitivity to different light levels. UV radiation has a smaller wavelength and a higher energy than radiation from PAR. Since one role of these compounds is to protect the photosynthetic apparatus, UV radiation stress could cause a flux in the xanthophyll carotenoids as they try to remove and release the excess energy.

Neoxanthin concentration responded significantly to UV radiation treatment ($F = 12.13$; $P = 0.0008$), cultigen ($F = 3.20$; $P = 0.0003$), and to the interaction of UV radiation treatment and cultigen ($F = 2.27$; $P = 0.0092$; Table B.21.) *T*-tests found significant increases in neoxanthin between cultigens Feast, GA-C 76, and G 30393-06 GI when compared to the same cultigens grown without supplemental UV radiation. 'Feast' showed the highest concentrations of neoxanthin under UV radiation treatment at 1.86 mg•100g FW, while it had one of the lower neoxanthin concentrations among other cultigens not grown under UV radiation. Deep Purple had the lowest concentration of neoxanthin at 0.73 mg•100g FW. Pesoenyj showed the highest neoxanthin concentration at 1.96 mg•100g FW compared to the other cultigens not grown under UV light. Hardy

Evergreen White had the lowest of all of the cultigens not grown under supplemental UV radiation at 0.40 mg•100g FW.

The scallions showed significant changes in lutein in response to UV treatment ($F = 17.89$; $P < 0.0001$) and cultigen ($F = 2.34$; $P = 0.0070$; Table B.22.) *T*-tests showed significant increases between two cultigens grown under supplemental UV radiation treatments, Feast and GA-C 76. Pesoenyj had the highest concentrations of lutein, both with and without supplemental UV radiation, at 8.01 and 9.23 mg•100g FW, respectively. Deep Purple had the lowest concentration of lutein among plants that were grown with supplemental UV radiation at 5.04 mg•100g FW, and Feast had the lowest amount of lutein for plants grown without supplemental UV radiation at 4.11 mg•100g FW. Lutein has been shown to be the predominant carotenoid in photosystem (PS) II (Demmig-Adams et al., 1993.) Increases in the lutein concentrations may indicate increased radiation stress of PS II. PS II has been shown in previous studies to be more affected by UV than PS I (Kakani et al., 2003.)

Concentrations of β-carotene showed no changes in response to UV treatment or cultigen (Table B.23.) *T*-tests showed no changes between cultigens as well. Pesoenyj showed the highest amount of β-carotene in plants grown without UV radiation, and 'Ishikura Improved F1' showed the lowest concentration. Ranges for β-carotene for cultigens grown under supplemental UV radiation were between 2.80 mg•100g FW and 0.88 mg•100g FW for Shounan and Evergreen Hardy White, respectively. For the cultigens that were not grown under supplemental UV radiation, the ranges for β-carotene concentration were 3.45 mg•100g FW and 0.64 mg•100g FW. β-carotene has been shown to be the predominant carotenoid in PSI (Demmig-Adams et al., 1996.) With no changes

in β-carotene, it can be reasoned that PS I is not under as much stress from the UV treatment imposed in this study.

Chlorophyll *a* responded significantly to UV radiation treatments (F = 4.35; *P* = 0.0398), but not to cultigen or the interaction between treatment and cultigen (Table B.24.) Feast had the highest concentration of chlorophyll *a* at 59.56 mg•100g FW for cultigens grown under supplemental UV radiation, while Deep Purple had the lowest at 27.75 mg•100g FW. For the cultigens grown without supplemental UV radiation, Pesoenyj had the highest concentration at 63.27 mg•100g FW and Evergreen Hardy White and the lowest at 16.52 mg•100g FW. T-tests found significant increases in tissue chlorophyll *a* in the cultigen Feast when comparing the UV treated plant versus the untreated plant.

The scallions showed significant differences in chlorophyll *b* caused by UV treatment (F = 19.04; *P* < 0.0001) and cultigen (F = 2.08; *P* = 0.0179), but there were no influences from the interaction (Table B.25). *T*-tests showed significant increases in chlorophyll *b* in response to UV radiation for cultigens Feast, GA-C 76 and Shounan. The concentrations of chlorophyll *b* for cultigens grown under supplemental UV radiation ranged from 29.24 mg•100g FW in GA-C 76 to 18.49 mg•100g FW in Improved Beltsville Bunching. For cultigens grown without supplemental UV radiation, the chlorophyll *b* concentrations ranged from 29.74 mg•100g FW for Pesoenyj and 15.78 mg•100g FW in Improved Beltsville Bunching.

Concentrations of total chlorophylls (chlorophyll *a* + chlorophyll *b*) in scallions were found to differ between UV treatment (F = 6.82; *P* = 0.0105), but not among cultigens (Table B.26.) Feast and GA-C 76 were the only scallion cultigens to show

differences between plants grown under the supplemental UV treatment and those not. The ranges of total chlorophyll concentrations ranged from 88.82 mg•100g FW to in Feast and 45.62 mg•100g FW in Zhang Qui Da Cong for plants grown under supplemental UV radiation. For the plants grown without UV treatment, the ranges varied, with 93.01 mg•100g FW in Pesoenyj as the highest and 34.74 mg•100g FW in Evergreen Hardy White as the lowest.

The ratio of chlorophyll *a* to chlorophyll *b* (*a/b*) in the scallions onions showed significant changes by cultigen (F =2.26; $P = 0.0094$), but not for UV radiation treatment (Table B.27.) *T*-tests found that only the cultigen Feast had a higher (*a/b*) ratio under UV radiation. Long White Bunching had the highest (*a/b*) ratio in the plants grown without supplemental UV at 2.25 and GA-C 76 had the lowest at 0.91. But, under UV radiation treatment, Feast has the highest ratio at 2.14, while Improved Beltsville Bunching has the lowest at 1.04.

Conclusions

As has been seen in multiple studies, cultigens within a given species can react differently under different stress conditions (Caasi-Lit et al., 1997; Yuan et al., 2000; Giuntini, et al, 2005.) The study showed that UV radiation can affect physiological and morphological traits in scallion onions. Almost every cultigen showed a significant decrease in plant biomass under supplemental UV radiation. On average, all of the cultigens showed decreases in chlorophyll and carotenoid pigments and because of UV

radiation treatment and/or cultigen differences. However, for most of cultigens, the scallions within the same cultigen grown under UV radiation treatment and those grown without it showed no significant changes. Cultigens GA-C 76 and Feast showed the most changes between UV treatments. This is the study that has shown how UV radiation can affect pigment production in *A. fistulosum*.

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Appendix A

Fertilizer Solution:

To make 1 liter of fertilizer, mix 2.5 mL of $Ca(NO_3)_2 \cdot 4H_20$ 1M solution, 2.5 mL of KNO_3 1M solution, 0.5 mL KH_2PO_4, 1 mL $MgSO_4 \cdot 7H_20$, 0.5 mL micronutrient solution and 0.5 mL iron to 800 mL of distilled water. Bring up to 1 L.

To make the micronutrient solution, add 1.43 g of H_3BO_3, 0.90 g of $MnCl_2 \cdot 4H_20$, 0.11 g of $ZnSO_4 \cdot 7H_20$, 0.04 g of $CuSO_4 \cdot 5H_20$, and 0.01 g of $H_2MoO_4 \cdot H_20$ to 800 mL of distilled water. Bring up to 1 L.

To make the iron solution, add 33.3 g of Sprint 138 with 6% iron to 800 mL of distilled water. Bring up to 1 L.

Modified Hoagland's solution, adapted from D.R. Hoagland and D.I. Arnon. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular. 347.

Appendix B

Table B.1. Mean values for shoot tissue height (cm) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Shoot Tissue Height (cm)	
	Scallions	Chives
5	31.06 ± 5.26	6.19 ± 3.02
7	34.80 ± 4.32	6.96 ± 3.75
8	32.71 ± 2.86	8.50 ± 3.10
9	31.56 ± 5.04	7.61 ± 3.97
Contrast		
Linear	NS	P=0.0109*
Quadratic	P=0.0325*	NS
Cubic	NS	NS

NS- not significant; * significant at $P = 0.05$

Table B.2. Mean values for total plant fresh weight (g) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Total Plant Fresh Weight (g)	
	Scallions	Chives
5	37.57 ± 3.00	1.69 ± 0.48
7	39.41 ± 7.32	1.70 ± 0.29
8	31.28 ± 2.62	1.95 ± 0.38
9	28.06 ± 4.30	2.19 ± 0.27
Contrast		
Linear	P=0.0011*	P=0.0194*
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant; *- $P = 0.05$

Table B.3. Mean values for shoot tissue photochemical efficiency (Fv/Fm) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	F_v/F_m	
	Scallions	Chives
5	0.79 ± 0.03	0.81 ± 0.02
7	0.79 ± 0.03	0.79 ± 0.02
8	0.78 ± 0.01	0.79 ± 0.01
9	0.79 ± 0.03	0.79 ± 0.02
Contrast		
Linear	NS	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant; *- $P = 0.05$

Table B.4. Mean values for shoot tissue antheraxanthin (mg/100 g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Antheraxanthin (mg/100g fresh weight)	
	Scallions	Chives
5	1.35 ± 0.45	2.77 ± 1.14
7	1.32 ± 0.57	1.79 ± 0.47
8	1.01 ± 0.29	2.14 ± 0.67
9	1.61 ± 0.33	2.91 ± 0.96
Contrast		
Linear	NS	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant; *- $P = 0.05$

Table B.5. Mean values for shoot tissue violaxanthin (mg/100 g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Violaxanthin (mg/100 g fresh weight)	
	Scallions	Chives
5	1.10 ± 0.45	2.41 ± 0.62
7	1.25 ± 0.49	2.33 ± 0.57
8	0.94 ± 0.40	2.19 ± 0.51
9	1.18 ± 0.68	2.32 ± 0.87
Contrast		
Linear	NS	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant, *- $P = 0.05$

Table B.6. Mean values for shoot tissue neoxanthin (mg/100 g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Neoxanthin (mg/100g fresh weight)	
	Scallions	Chives
5	1.89 ± 0.58	2.93 ± 1.04
7	1.81 ± 0.51	2.92 ± 0.75
8	2.00 ± 0.28	3.21 ± 0.87
9	2.44 ± 0.64	3.52 ± 0.95
Contrast		
Linear	NS	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant, *- $P = 0.05$

Table B.7. Mean values for shoot tissue β-carotene (mg/100g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 μmol·m^{-2}·s^{-2} in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment (μmol·m^{-2}·s^{-2})	β-carotene (mg/100g fresh weight)	
	Scallions	Chives
5	1.80 ± 0.67	2.27 ± 0.63
7	1.53 ± 0.59	1.89 ± 0.60
8	1.75 ± 0.96	2.62 ± 1.17
9	2.09 ± 0.84	2.77 ± 1.79
Contrast		
Linear	NS	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant, *- $P = 0.05$

Table B.8. Mean values for shoot tissue lutein (mg/100g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 μmol·m^{-2}·s^{-2} in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment (μmol·m^{-2}·s^{-2})	Lutein (mg/100g fresh weight)	
	Scallions	Chives
5	6.46 ± 1.41	8.83 ± 1.75
7	6.25 ± 1.43	8.91 ± 1.48
8	6.82 ± 0.87	9.80 ± 2.13
9	8.28 ± 1.34	10.89 ± 2.82
Contrast		
Linear	P=0.0238*	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant; *- P=0.05

Table B.9. Mean values for shoot tissue chlorophyll *a* (mg/100 g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Chlorophyll *a* (mg/100g fresh weight)	
	Scallions	Chives
5	50.76 ± 13.89	81.33 ± 17.76
7	45.30 ± 12.99	75.66 ± 25.47
8	43.82 ± 11.21	85.66 ± 17.55
9	60.44 ± 13.10	97.46 ± 29.04
Contrast		
Linear	NS	P=0.0394*
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant; *- $P = 0.05$

Table B.10. Mean values for shoot tissue chlorophyll *b* (mg/100 g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Chlorophyll *b* (mg/100g fresh weight)	
	Scallions	Chives
5	21.82 ± 4.97	31.65 ± 8.45
7	20.02 ± 4.84	29.02 ± 8.13
8	22.41 ± 3.17	33.13 ± 6.66
9	26.65 ± 4.03	37.31 ± 9.99
Contrast		
Linear	P=0.0029*	P=0.0236*
Quadratic	P=0.0137*	NS
Cubic	NS	NS

NS- not significant; *- $P = 0.05$

Table B.11. Mean values for shoot tissue total chlorophyll (mg/100 g fresh weight) for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Total Chlorophyll (mg/100g fresh weight)	
	Scallions	Chives
5	72.58 ± 18.50	112.98 ± 25.99
7	65.32 ± 17.79	104.68 ± 33.54
8	66.23 ± 11.06	118.79 ± 22.77
9	87.09 ± 14.90	134.76 ± 37.90
Contrast		
Linear	NS	P=0.0428*
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant, *- $P = 0.05$

Table B.12. Mean values for the ratio of shoot tissue chlorophyll *a* to chlorophyll *b* for scallions (*Allium fistulosum* L.) and chives (*A. tuberosum* Rottl.) grown under increasing UV radiation treatments of 5, 7, 8, and 9 $\mu mol \cdot m^{-2} \cdot s^{-2}$ in a controlled environment. Values represent means ± standard deviations of three replications, with 6 plants per replication.

UV radiation treatment ($\mu mol \cdot m^{-2} \cdot s^{-2}$)	Ratio of chlorophyll *a* to *b*	
	Scallions	Chives
5	2.31 ± 0.29	2.60 ± 0.20
7	2.24 ± 0.14	2.57 ± 0.19
8	2.00 ± 0.60	2.62 ± 0.39
9	2.29 ± 0.47	2.63 ± 0.34
Contrast		
Linear	NS	NS
Quadratic	NS	NS
Cubic	NS	NS

NS- not significant

Table B.13. List of *Allium fistulosum* L. cultigens and sources of seeds.

Cultigen	Accession Lot	Seed Source	Source Location
Deep Purple		Johnny's Selected Seed	Winslow, Maine
Evergreen Hardy White		Johnny's Selected Seeds	Winslow, Maine
Feast		Seedway, LLC	Hall, New York
GA-C 76	546343-90U01	USDA-ARS	Geneva, New York
Ishikura Improved F1		Johnny's Selected Seed	Winslow, Maine
Improved Beltsville Bunching	546228-06GI	USDA-ARS	Geneva, New York
Jionji Negi	462345-05GI	USDA-ARS	Geneva, New York
Long White Bunching		Seedway, LLC	Hall, New York
Parade		Seedway, LLC	Hall, New York
Performer		Seedway, LLC	Hall, New York
Pesoenyj	280562-04GI	USDA-ARS	Geneva, New York
Shounan	462357-06GI	USDA-ARS	Geneva, New York
White Spear		Johnny's Selected Seed	Winslow, Maine
Zhang Qui Da Cong	436539-06GI	USDA-ARS	Geneva, New York
274254-05GI	274254-05GI	USDA-ARS	Geneva, New York
G 30393-06GI	G 30393-06GI	USDA-ARS	Geneva, New York

Table B.14. Mean values for shoot tissue height (cm) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	44.71 ± 5.49	45.19 ± 4.52	NS
Evergreen Hardy White	39.11 ± 1.96	41.05 ± 5.38	NS
Feast	38.58 ± 3.24	39.96 ± 2.40	NS
GA-C 76	38.82 ± 1.91	44.13 ± 3.70	$P = 0.0433$*
Ishikura Improved F1	39.63 ± 1.12	41.17 ± 3.13	NS
Improved Beltsville Bunching	44.26 ± 11.69	48.06 ± 2.19	NS
Jionji Negi	36.2 ± 1.82	38.63 ± 1.54	NS
Long White Bunching	49.68 ± 2.09	50.38 ± 4.62	NS
Parade	42.13 ± 2.60	36.03 ± 9.40	NS
Performer	39.37 ± 2.36	39.42 ± 3.10	NS
Pesoenyj	39.44 ± 4.23	44.54 ± 4.03	NS
Shounan	36.72 ± 3.02	37.14 ± 2.54	NS
White Spear	40.06 ± 2.79	42.42 ± 1.72	NS
Zhang Qui Da Cong	36.62 ± 2.88	37.87 ± 3.80	NS
274254-05GI	42.48 ± 1.86	43.79 ± 4.40	NS
G 30393-06GI	36.62 ± 3.40	35.67 ± 1.31	NS
$LSD_{0.05}$	5.77	5.80	

NS- not significant; *- $P = 0.05$

Table B.15. Mean values of shoot tissue fresh weight (g) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	64.54 ± 17.02	97.80 ± 17.44	*P* = 0.0342*
Evergreen Hardy White	38.65 ± 7.80	70.78 ± 8.08	NS
Feast	48.79 ± 15.99	90.46 ± 13.85	*P* = 0.0076*
GA-C 76	41.90 ± 2.66	73.00 ± 15.53	*P* = 0.0076*
Ishikura Improved F1	54.93 ± 9.22	99.80 ± 9.47	*P* = 0.0005*
Improved Beltsville Bunching	76.86 ± 9.82	110.42 ± 19.39	*P* = 0.0214*
Jionji Negi	38.05 ± 4.73	62.04 ± 8.23	*P* = 0.0023*
Long White Bunching	74.07 ± 8.73	116.01 ± 20.24	*P* = 0.0089*
Parade	53.89 ± 10.73	90.81 ± 16.87	*P* = 0.0102*
Performer	54.74 ± 11.08	86.25 ± 16.66	*P* = 0.0198*
Pesoenyj	29.22 ± 9.30	58.76 ± 6.37	*P* = 0.0019*
Shounan	39.17 ± 3.56	63.32 ± 4.02	*P* = 0.0001 *
White Spear	53.16 ± 14.41	87.42 ± 10.58	*P* = 0.0086*
Zhang Qui Da Cong	53.57 ± 19.51	84.72 ± 16.67	NS
274254-05GI	47.63 ± 4.34	84.65 ± 6.98	*P* = 0.0001*
G 30393-06GI	50.96 ± 8.63	81.69 ± 12.01	*P* = 0.0060*
$LSD_{0.05}$	10.72	19.33	

NS- not significant; *- *P* = 0.05

Table B.16. Mean values of the efficiency of photosynthesis (Fv/Fm) in shoot tissue for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	0.81 ± 0.01	0.82 ± 0.00	NS
Evergreen Hardy White	0.82 ± 0.01	0.82 ± 0.00	NS
Feast	0.82 ± 0.00	0.83 ± 0.01	NS
GA-C 76	0.82 ± 0.01	0.82 ± 0.00	NS
Ishikura Improved F1	0.82 ± 0.01	0.82 ± 0.01	NS
Improved Beltsville Bunching	0.82 ± 0.00	0.82 ± 0.01	NS
Jionji Negi	0.82 ± 0.00	0.82 ± 0.01	NS
Long White Bunching	0.82 ± 0.01	0.82 ± 0.00	NS
Parade	0.82 ± 0.01	0.83 ± 0.01	NS
Performer	0.82 ± 0.01	0.82 ± 0.01	NS
Pesoenyj	0.81 ± 0.00	0.82 ± 0.01	NS
Shounan	0.82 ± 0.01	0.82 ± 0.00	NS
White Spear	0.82 ± 0.00	0.82 ± 0.00	NS
Zhang Qui Da Cong	0.83 ± 0.00	0.83 ± 0.01	NS
274254-05GI	0.81 ± 0.01	0.81 ± 0.01	NS
G 30393-06GI	0.81 ± 0.01	0.82 ± 0.01	NS
$LSD_{0.05}$	0.01	NS	

NS- not significant; *- $P = 0.05$

Table B.17. Mean values for shoot tissue zeaxanthin (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 µmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	0.08 ± 0.02	0.10 ± 0.03	NS
Evergreen Hardy White	0.10 ± 0.03	0.07 ± 0.02	NS
Feast	0.11 ± 0,01	0.07 ± 0.01	P = 0.0096*
GA-C 76	0.12 ± 0.02	0.08 ± 0.02	NS
Ishikura Improved F1	0.10 ± 0.05	0.09 ± 0.02	NS
Improved Beltsville Bunching	0.16 ± 0.02	0.15 ± 0.04	NS
Jionji Negi	0.12 ± 0.06	0.13 ± 0.03	NS
Long White Bunching	0.12 ± 0.03	0.11 ± 0.03	NS
Parade	0.10 ± 0.02	0.11 ± 0.02	NS
Performer	0.09 ± 0.02	0.10 ± 0.02	NS
Pesoenyj	0.12 ± 0.03	0.19 ± 0.06	NS
Shounan	0.13 ± 0.05	0.09 ± 0.01	NS
White Spear	0.08 ± 0.02	0.08 ± 0.03	NS
Zhang Qui Da Cong	0.11 ± 0.04	0.12 ± 0.02	NS
274254-05GI	0.13 ±0.04	0.15 ± 0.03	NS
G 30393-06GI	0.13 ± 0.03	0.08 ± 0.02	P = 0.0162*
$LSD_{0.05}$	NS	0.04	

NS- not significant; *- P = 0.05

Table B.18. Mean values for shoot tissue violaxanthin (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 µmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	1.25 ± 0.36	0.88 ± 0.42	NS
Evergreen Hardy White	1.73 ± 0.61	1.20 ± 0.25	NS
Feast	1.35 ± 0.82	0.66 ± 0.58	NS
GA-C 76	2.04 ± 0.19	1.34 ± 0.20	$P = 0.0022$*
Ishikura Improved F1	1.75 ± 0.94	1.10 ± 0.38	NS
Improved Beltsville Bunching	1.62 ± 0.54	1.25 ± 0.14	NS
Jionji Negi	1.54 ± 0.25	1.51 ± 0.35	NS
Long White Bunching	0.89 ± 0.18	0.81 ± 0.42	NS
Parade	1.23 ± 0.50	1.02 ± 0.61	NS
Performer	0.59 ± 0.52	1.45 ± 0.60	NS
Pesoenyj	1.93 ± 0.33	2.35 ± 0.82	NS
Shounan	1.87 ± 0.81	1.04 ± 0.36	NS
White Spear	1.00 ± 0.60	0.77 ± 0.47	NS
Zhang Qui Da Cong	1.29 ± 0.48	1.30 ± 0.41	NS
274254-05GI	1.64 ± 0.42	1.27 ± 0.52	NS
G 30393-06GI	0.60 ± 0.51	0.50 ± 0.43	NS
$LSD_{0.05}$	0.74	0.70	

NS- not significant; *- $P = 0.05$

Table B.19. Mean values for shoot tissue antheraxanthin (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	1.07 ± 0.31	0.78 ± 0.23	NS
Evergreen Hardy White	1.03 ± 0.18	0.78 ± 0.14	NS
Feast	1.15 ± 0.27	0.92 ± 0.19	NS
GA-C 76	1.92 ± 0.65	1.27 ± 0.47	NS
Ishikura Improved F1	0.81 ± 0.50	0.59 ± 0.13	NS
Improved Beltsville Bunching	0.99 ± 0.38	0.72 ± 0.21	NS
Jionji Negi	1.20 ± 0.25	0.78 ± 0.50	NS
Long White Bunching	0.82 ± 0.13	0.89 ± 0.14	NS
Parade	0.86 ± 0.16	0.79 ± 0.24	NS
Performer	0.87 ± 0.06	0.85 ± 0.32	NS
Pesoenyj	1.38 ± 0.30	1.35 ± 0.52	NS
Shounan	1.18 ± 0.40	0.63 ± 0.30	NS
White Spear	0.74 ± 0.13	0.60 ± 0.09	NS
Zhang Qui Da Cong	0.81 ± 0.30	0.74 ± 0.06	NS
274254-05GI	0.79 ± 0.14	0.71 ± 0.33	NS
G 30393-06GI	1.07 ± 0.35	0.67 ± 0.14	NS
$LSD_{0.05}$	0.44	0.44	

NS- not significant; *- $P = 0.05$

Table B.20. Mean values for shoot tissue ratios of zeaxanthin + antheraxanthin to zeaxanthin + antheraxanthin + violaxanthin for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	0.48 ± 0.01	0.51 ± 0.19	NS
Evergreen Hardy White	0.41 ± 0.06	0.41 ± 0.01	NS
Feast	0.51 ± 0.16	0.65 ± 0.21	NS
GA-C 76	0.49 ± 0.07	0.49 ± 0.10	NS
Ishikura Improved F1	0.34 ± 0.03	0.40 ± 0.08	NS
Improved Beltsville Bunching	0.41 ± 0.03	0.41 ± 0.07	NS
Jionji Negi	0.46 ± 0.07	0.35 ± 0.15	NS
Long White Bunching	0.52 ± 0.04	0.58 ± 0.17	NS
Parade	0.45 ± 0.10	0.50 ± 0.24	NS
Performer	0.66 ± 0.17	0.40 ± 0.02	NS
Pesoenyj	0.44 ± 0.03	0.39 ± 0.01	P = 0.0217*
Shounan	0.42 ± 0.03	0.40 ± 0.03	NS
White Spear	0.50 ± 0.21	0.51 ± 0.22	NS
Zhang Qui Da Cong	0.42 ± 0.05	0.41 ± 0.08	NS
274254-05GI	0.36 ± 0.06	0.40 ± 0.00	NS
G 30393-06GI	0.69 ± 0.20	0.55 ± 0.12	NS
$LSD_{0.05}$	0.15	0.20	

NS- not significant; *- P = 0.05

Table B.21. Mean values for shoot tissue neoxanthin (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 µmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	0.73 ± 0.51	1.04 ± 0.59	NS
Evergreen Hardy White	0.74 ± 0.47	0.40 ± 0.18	NS
Feast	2.09 ± 0.48	0.79 ± 0.57	P = 0.0130*
GA-C 76	1.53 ± 0.32	0.66 ± 0.18	P = 0.0031*
Ishikura Improved F1	0.63 ± 0.43	0.63 ± 0.30	NS
Improved Beltsville Bunching	0.82 ± 0.89	0.60 ± 0.16	NS
Jionji Negi	0.92 ± 0.25	0.91 ± 0.36	NS
Long White Bunching	1.76 ± 0.26	1.47 ± 0.35	NS
Parade	1.46 ± 0.81	0.87 ± 0.36	NS
Performer	1.14 ± 0.90	0.75 ± 0.47	NS
Pesoenyj	1.03 ± 0.19	1.96 ± 0.78	NS
Shounan	1.32 ± 0.71	0.54 ± 0.32	NS
White Spear	1.21 ± 0.62	0.93 ± 0.63	NS
Zhang Qui Da Cong	0.75 ± 0.42	0.65 ± 0.29	NS
274254-05GI	0.84 ± 0.37	0.72 ± 0.45	NS
G 30393-06GI	1.86 ± 0.44	0.85 ± 0.62	P = 0.0383*
$LSD_{0.05}$	0.78	0.67	

NS- not significant; *- P = 0.05

Table B.22. Mean values for shoot tissue lutein (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 µmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	5.04 ± 1.48	5.38 ± 0.73	NS
Evergreen Hardy White	7.10 ± 2.86	5.10 ± 1.49	NS
Feast	7.66 ± 0.90	4.11 ± 0.54	P = 0.0005*
GA-C 76	7.66 ± 0.38	5.57 ± 0.67	P = 0.0017*
Ishikura Improved F1	6.35 ± 3.18	4.80 ± 1.20	NS
Improved Beltsville Bunching	6.95 ± 1.34	5.62 ± 0.65	NS
Jionji Negi	7.35 ± 1.65	6.21 ± 1.49	NS
Long White Bunching	6.00 ± 0.58	5.05 ± 1.17	NS
Parade	6.36 ± 1.17	6.04 ± 1.47	NS
Performer	6.33 ± 0.96	5.60 ± 2.36	NS
Pesoenyj	8.01 ± 1.21	9.23 ± 2.59	NS
Shounan	7.66 ± 2.83	5.03 ± 1.26	NS
White Spear	6.08 ± 0.94	4.70 ± 1.37	NS
Zhang Qui Da Cong	5.65 ± 1.44	5.31 ± 1.47	NS
274254-05GI	6.18 ± 0.79	5.33 ± 1.82	NS
G 30393-06GI	6.02 ± 1.18	4.42 ± 0.69	NS
$LSD_{0.05}$	NS	2.14	

NS- not significant; *- P = 0.05

Table B.23. Mean values for shoot tissue β-carotene (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 µmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	1.07 ± 0.81	1.09 ± 0.30	NS
Evergreen Hardy White	0.88 ± 0.71	0.64 ± 0.23	NS
Feast	1.85 ± 0.85	1.04 ± 0.74	NS
GA-C 76	1.48 ± 0.27	1.17 ± 0.09	NS
Ishikura Improved F1	2.20 ± 2.59	0.78 ± 0.41	NS
Improved Beltsville Bunching	1.64 ± 0.95	1.39 ± 0.30	NS
Jionji Negi	1.74 ± 1.20	2.29 ± 1.32	NS
Long White Bunching	1.69 ± 0.29	1.94 ± 0.56	NS
Parade	1.26 ± 0.14	2.54 ± 1.23	NS
Performer	1.28 ± 0.48	2.38 ± 1.65	NS
Pesoenyj	1.87 ± 0.20	3.45 ± 2.39	NS
Shounan	2.80 ± 1.46	1.10 ± 0.54	NS
White Spear	1.49 ± 0.51	1.08 ± 0.60	NS
Zhang Qui Da Cong	1.05 ± 0.24	1.20 ± 0.57	NS
274254-05GI	1.81 ±0.86	1.86 ± 1.57	NS
G 30393-06GI	1.86 ± 0.77	1.28 ± 0.67	NS
$LSD_{0.05}$	NS	1.54	

NS- not significant; *- $P = 0.05$

Table B.24. Mean values for shoot tissue chlorophyll *a* (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	27.75 ± 22.11	25.26 ± 5.58	NS
Evergreen Hardy White	31.38 ± 21.14	16.52 ± 3.18	NS
Feast	59.56 ± 20.34	19.00 ± 10.54	$P = 0.0122$*
GA-C 76	46.98 ± 16.36	17.22 ± 10.56	NS
Ishikura Improved F1	49.86 ± 53.63	23.08 ± 5.94	NS
Improved Beltsville Bunching	23.90 ± 28.40	20.63 ± 8.85	NS
Jionji Negi	48.31 ± 23.35	47.18 ± 18.86	NS
Long White Bunching	36.85 ± 6.638	37.18 ± 7.41	NS
Parade	38.57 ± 13.16	44.40 ± 18.75	NS
Performer	36.80 ± 3.06	49.58 ± 31.87	NS
Pesoenyj	36.78 ± 10.15	63.27 ± 36.88	NS
Shounan	44.31 ± 24.94	20.59 ± 19.13	NS
White Spear	30.41 ± 9.85	18.21 ± 9.61	NS
Zhang Qui Da Cong	25.25 ± 18.28	27.28 ± 13.48	NS
274254-05GI	36.63 ± 23.05	39.95 ± 18.14	NS
G 30393-06GI	39.76 ± 11.31	22.58 ± 13.83	NS
$LSD_{0.05}$	NS	25.99	

NS- not significant; *- $P = 0.05$

Table B.25. Mean values for shoot tissue chlorophyll *b* (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	20.59 ± 4.43	16.86 ± 2.36	NS
Evergreen Hardy White	25.50 ± 5.05	18.22 ± 0.70	NS
Feast	27.27 ± 4.04	17.72 ± 1.86	$P = 0.0051$*
GA-C 76	29.24 ± 7.07	18.13 ± 1.29	$P = 0.0213$*
Ishikura Improved F1	25.59 ± 11.62	17.60 ± 4.67	NS
Improved Beltsville Bunching	18.49 ± 7.60	15.78 ± 1.02	NS
Jionji Negi	25.45 ± 3.12	20.85 ± 4.77	NS
Long White Bunching	19.70 ± 2.81	17.07 ± 2.65	NS
Parade	21.22 ± 4.05	19.92 ± 4.35	NS
Performer	23.02 ± 2.13	20.94 ± 7.09	NS
Pesoenyj	26.31 ± 2.37	29.74 ± 8.74	NS
Shounan	24.26 ± 8.29	16.63 ± 7.61	NS
White Spear	20.76 ± 1.94	17.75 ± 0.95	$P = 0.0330$*
Zhang Qui Da Cong	20.37 ± 7.41	19.83 ± 4.18	NS
274254-05GI	18.87 ± 5.31	18.65 ± 4.85	NS
G 30393-06GI	21.34 ± 4.09	16.98 ± 3.70	NS
$LSD_{0.05}$	NS	6.75	

NS- not significant; *- $P = 0.05$

Table B.26. Mean values for shoot tissue total chlorophyll (chlorophyll *a* + chlorophyll b) (mg/100 g fresh weight) for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 µmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	48.34 ± 25.58	42.13 ± 7.45	NS
Evergreen Hardy White	56.88 ± 26.06	34.74 ± 3.53	NS
Feast	86.82 ± 24.31	36.72 ± 10.99	*P* = 0.0094*
GA-C 76	76.22 ± 23.32	35.35 ± 11.52	*P* = 0.0200*
Ishikura Improved F1	74.45 ± 65.13	40.68 ± 2.55	NS
Improved Beltsville Bunching	42.38 ± 35.98	36.41 ± 9.75	NS
Jionji Negi	73.77 ± 25.98	68.03 ± 23.35	NS
Long White Bunching	56.54 ± 9.18	54.25 ± 6.25	NS
Parade	59.79 ± 17.18	64.32 ± 23.00	NS
Performer	59.82 ± 4.75	70.51 ± 38.92	NS
Pesoenyj	26.31 ± 2.37	93.01 ± 45.61	NS
Shounan	68.57 ± 32.56	37.22 ± 26.68	NS
White Spear	51.17 ± 10.72	35.36 ± 8.91	NS
Zhang Qui Da Cong	45.62 ± 25.61	47.12 ± 17.60	NS
274254-05GI	55.50 ± 28.33	58.60 ± 21.46	NS
G 30393-06GI	61.10 ± 15.12	39.56 ± 15.96	NS
$LSD_{0.05}$	NS	31.60	

NS- not significant; *- *P* = 0.05

Table B.27. Mean values of the ratio of chlorophyll *a* to chlorophyll *b* in shoot tissues for *Allium fistulosum* L. cultigens grown under supplemental UV light (7.0 μmol·m^{-2}·s^{-2}; UV) or ambient (control) light in a greenhouse in Knoxville, TN (35.96N latitude). Values represent means ± standard deviations of four replications.

	UV	Control	Significance
Deep Purple	1.25 ± 0.90	1.50 ± 0.23	NS
Evergreen Hardy White	1.15 ± 0.56	0.91 ± 0.16	NS
Feast	2.14 ± 0.43	1.07 ± 0.63	$P = 0.0311$*
GA-C 76	1.58 ± 0.18	0.93 ± 0.51	NS
Ishikura Improved F1	1.69 ± 1.06	1.45 ± 0.70	NS
Improved Beltsville Bunching	1.04 ± 0.92	1.29 ± 0.50	NS
Jionji Negi	1.85 ± 0.70	2.21 ± 0.50	NS
Long White Bunching	1.86 ± 0.06	2.25 ± 0.68	NS
Parade	1.77 ± 0.33	2.16 ± 0.48	NS
Performer	1.60 ± 0.11	2.07 ± 1.19	NS
Pesoenyj	1.40 ± 0.42	1.95 ± 0.80	NS
Shounan	1.78 ± 0.52	1.08 ± 0.53	NS
White Spear	1.46 ± 0.42	1.08 ± 0.62	NS
Zhang Qui Da Cong	1.11 ± 0.50	1.32 ± 0.46	NS
274254-05GI	1.80 ± 0.71	2.14 ± 0.81	NS
G 30393-06GI	1.84 ± 0.31	1.32 ± 0.64	NS
$LSD_{0.05}$	NS	0.96	

NS- not significant; *- $P = 0.05$

Appendix C

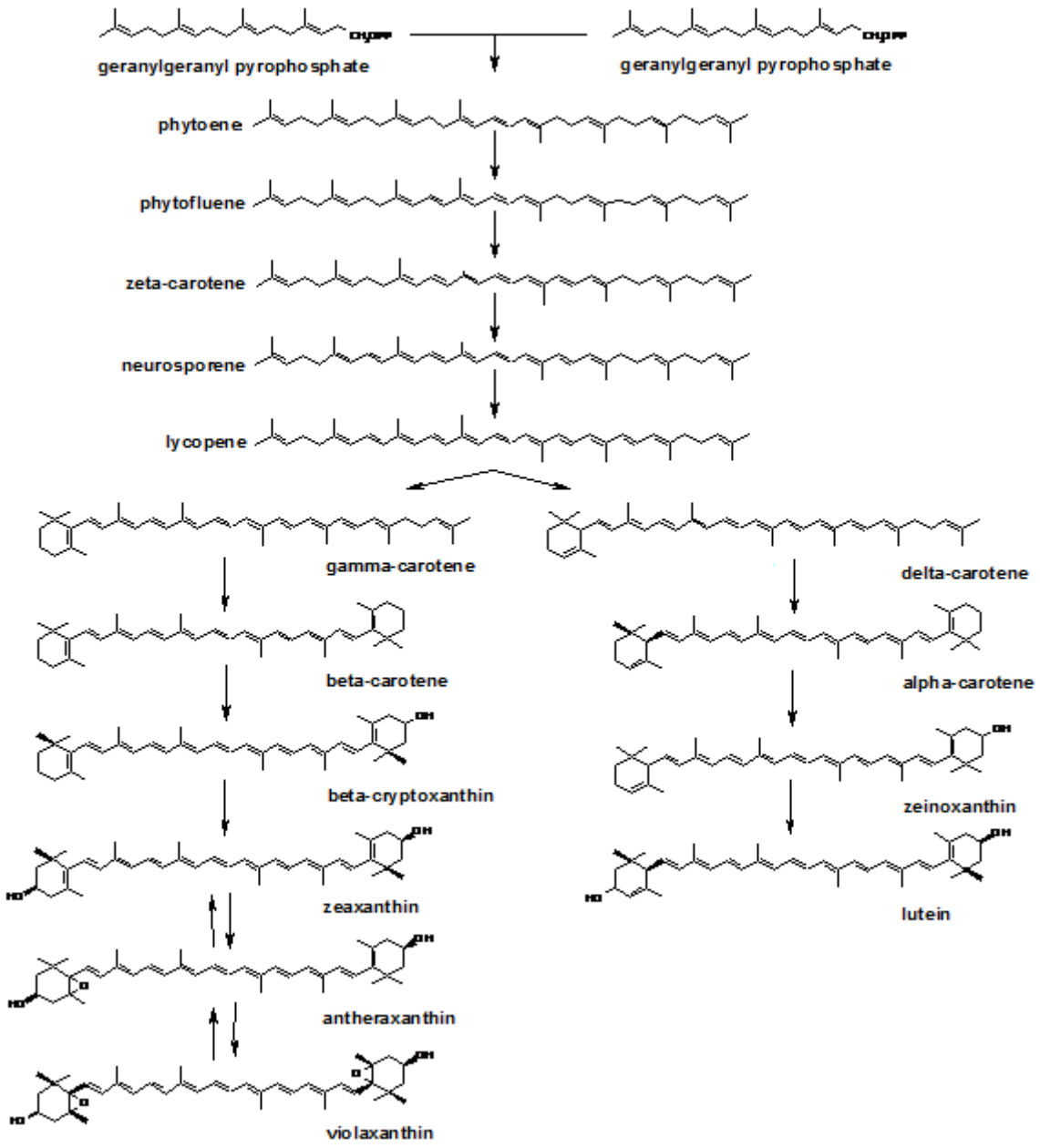

Figure C.1. A simplified carotenoid biosynthetic pathway in plants. The C_{20} geranylgeranyl pyrophosphate (GGPP) is the immediate precursor for carotenoid biosynthesis formed from three molecules.

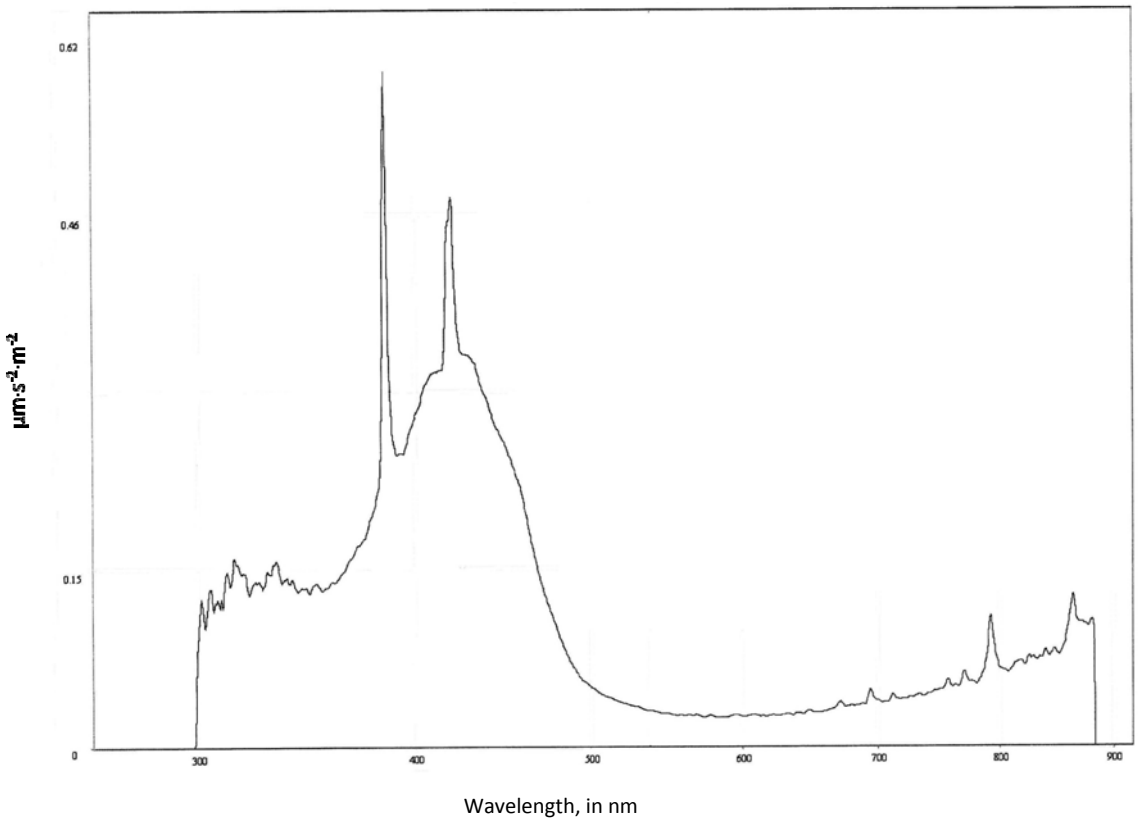

Figure C.2. Spectroradiometer reading of chamber with 350 µm·s^{-2}·m^{-2} PAR with no supplemental UV radiation.

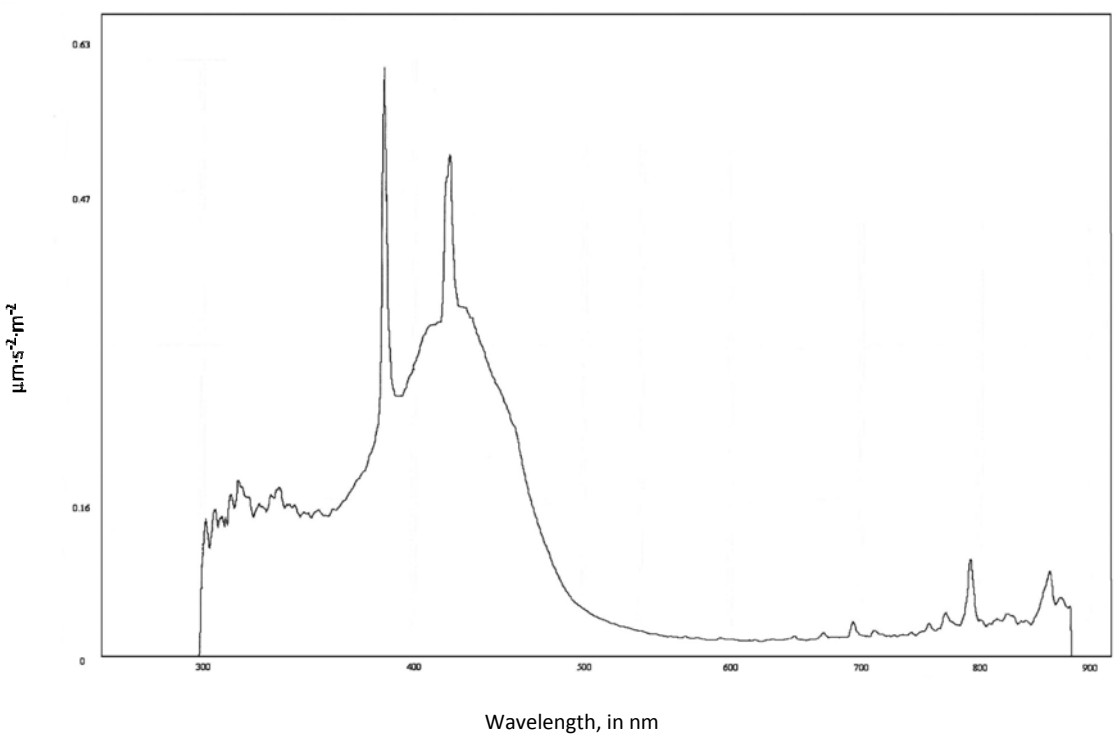

Figure C.3. Spectroradiometer reading of chamber with 350 µm·s^{-2}·m^{-2} PAR + 5 µm·s^{-2}·m^{-2} supplemental UV radiation.

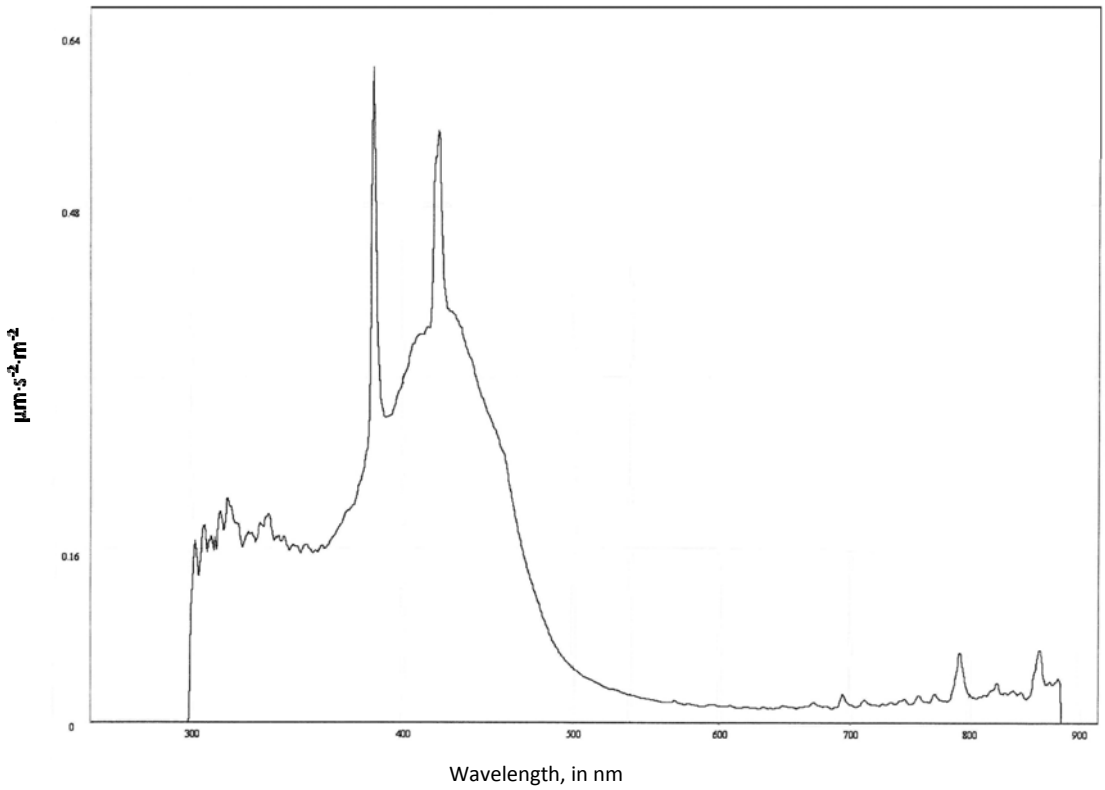

Figure C.4. Spectroradiometer reading of chamber with 350 µm·s^{-2}·m^{-2} PAR + 7 µm·s^{-2}·m^{-2} supplemental UV radiation.

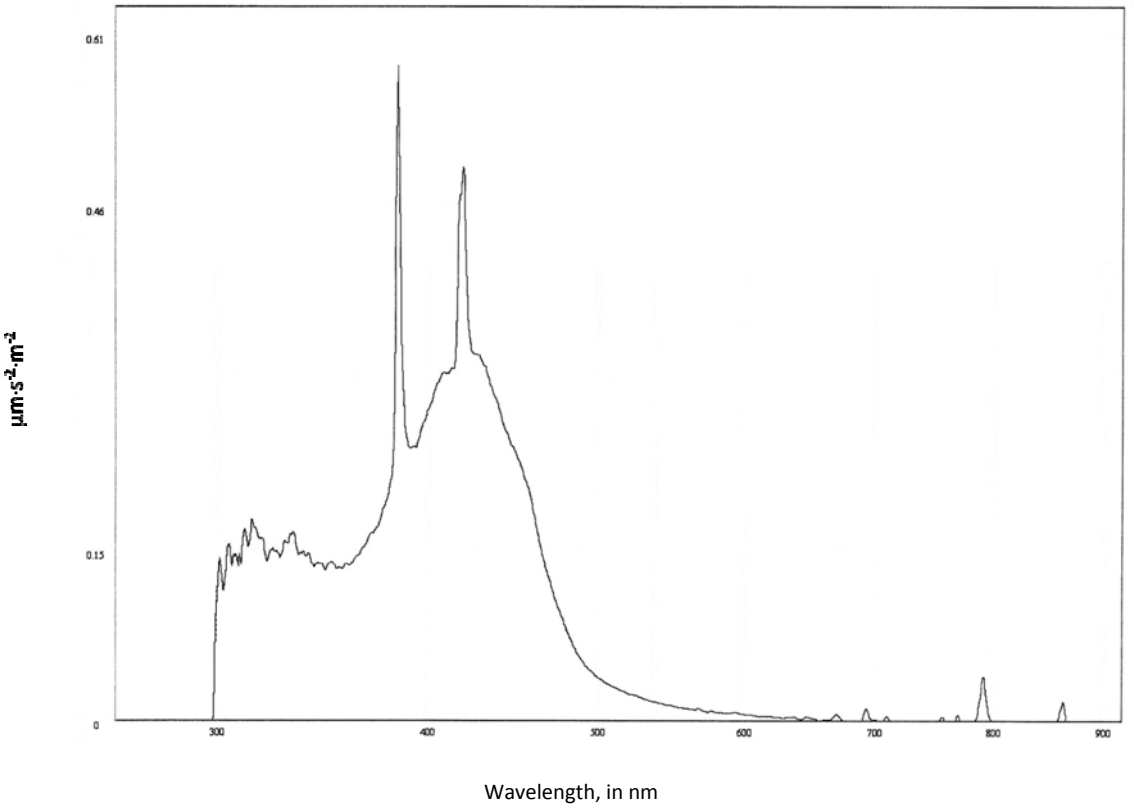

Figure C.5. Spectroradiometer reading of chamber with 350 µm·s^{-2}·m^{-2} PAR + 9 µm·s^{-2}·m^{-2} supplemental UV radiation.

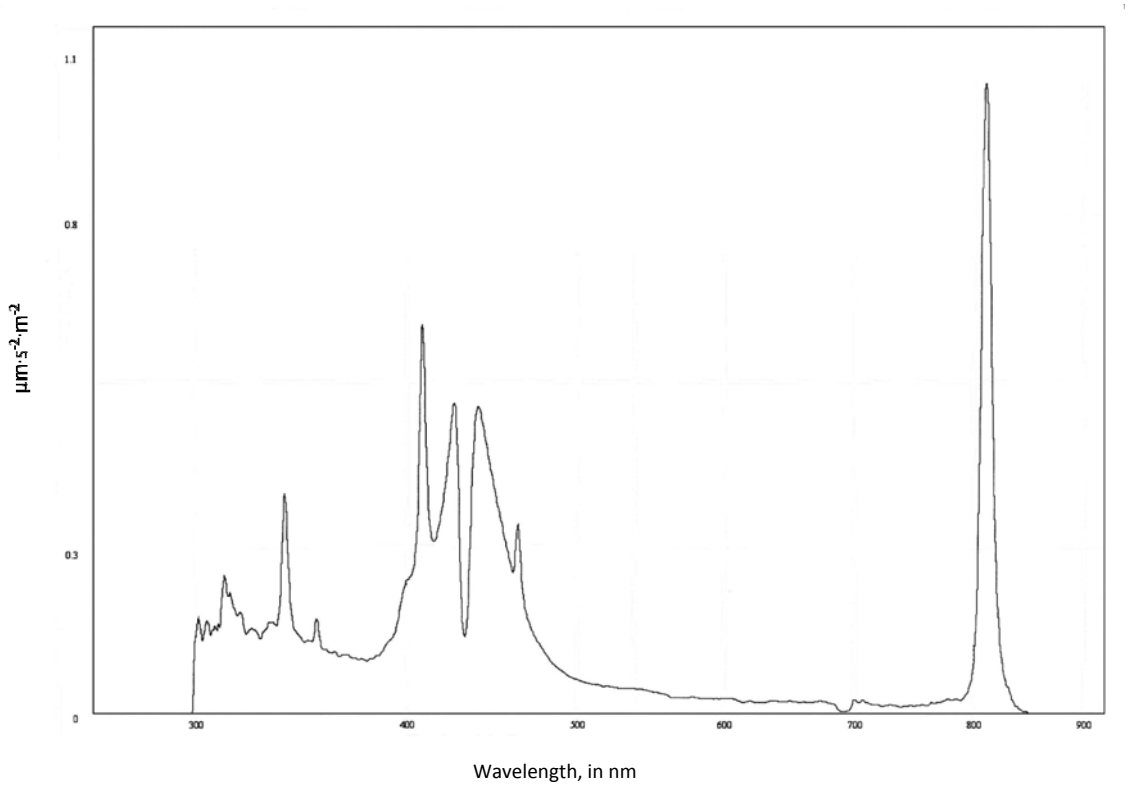

Figure C.6. Spectroradiometer reading of greenhouse with 540.5 µm·s^{-2}·m^{-2} PAR with no supplemental UV radiation.

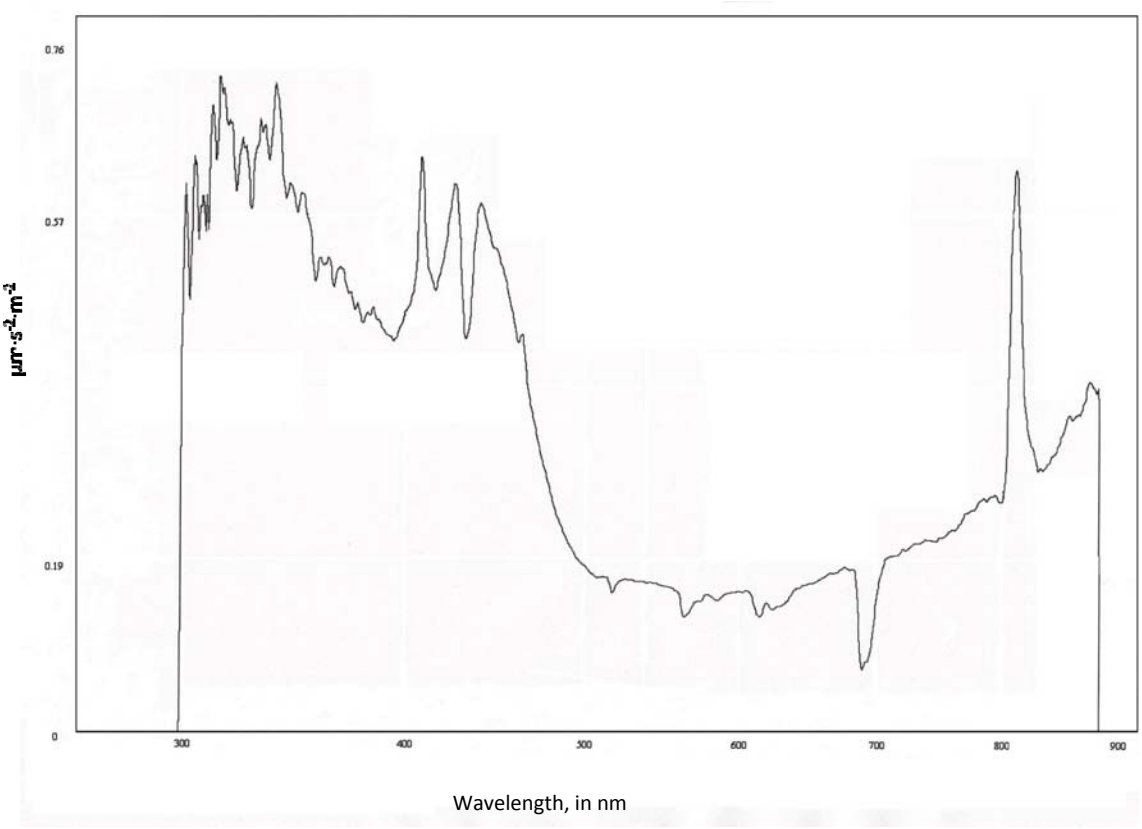

Figure C.7. Spectroradiometer reading of greenhouse with 540.5 $\mu m \cdot s^{-2} \cdot m^{-2}$ PAR + 7 $\mu m \cdot s^{-2} \cdot m^{-2}$ supplemental UV radiation.

Vita

Kristin Renee Abney was born on January 6, 1985 in Tulsa, Oklahoma. She attended The University of Georgia and completed her Bachelor's of Science in Agriculture in 2007. She began her Master's of Science in horticulture that same year under the direction of Dean Kopsell. Upon completion, she hopes to attend The University of Georgia for her PhD in horticulture with a focus on postharvest physiology.