ULTRAVIOLET LIGHT AND ITS EFFECT ON GERMINATION, GROWTH, PHYSIOLOGY AND RESPONSES OF COOL SEASON GRASSES.

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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ABSTRACT

The increase of ultraviolet (UV) light levels in the northern hemisphere raises the spectre of possible problems for turfgrass plants due to long term exposure. Cool season turfgrasses which are susceptible to photoinhibition may suffer from a loss of productivity and growth, reducing their ability to sequester carbon. The effect on germination, plant responses in relation to UV absorbing compounds and how turfgrasses were evaluated for loss of quality with regards to linking new technology to visual ratings have had no or limited research.

Enhancing the percentage of Kentucky bluegrass (*Poa pratensis* L.) seed germination and speed could benefit establishment of the grass in a greater range of environmental and geographical conditions. Potential for the use of ultraviolet light to enhance Kentucky bluegrass seed germination exists through exposure to UV light. The effect of ultraviolet light may be lost with seed age. In altering wavelength exposure, there may be an opportunity to enhance the effect.

In measuring turf quality, traditionally human measurement has been the standard method for both color and cover. Color, in particular, is thought to be controlled by pigment changes. In evaluating a total of 51 cultivars of tall fescue (*Schedenorus phoenix* Scop. *Holub*), perennial ryegrass (*Lolium perenne* L.) creeping bentgrass (*Agrostis stolonifera* L.) cv. ‘Penncross’ and ‘L-93’ it was found that nitrogen content is
most crucial in color measurements. Reflective measurements did not correlate with
nitrogen or chlorophyll content. Extract measurements had stronger correlation with
nitrogen content than pigmentation concentration. Current reflective measurement
equipment may not be closely linked to visual rating of turfgrass color possibly due to
variation in leaf surfaces.

There is a difference in response to UV light among grass species. Creeping
bentgrass ‘L-93’ produced increasing anthocyanin in response to UV light. The
characterization of Cyanidin – 3 – O – glucoside was the first reported in the literature in
creeping bentgrass. Carotenoids, zeaxanthin and β-carotene decrease in creeping
bentgrass after exposure to UV-B and turfgrass quality and vegetative production in
bentgrasses decreased to a greater extent than tall fescue or perennial ryegrass. All
grasses have the ability to initially accumulate phenolic compounds and flavonoids in the
tissue most exposed to light, although this still doesn’t mean a prevention in damage to
photosynthetic machinery. Turfgrass recovery and maintenance of optimal photosynthetic
rates will be crucial as breeders try to develop new cultivars that are adapted to higher
levels of UV light.
DEDICATION

To Eddie and Catherine Nangle
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I would like to thank Dr. Dave Gardner for giving me a chance at a PhD, it has been a wonderful life changing experience here and I feel that I have achieved more than I could ever have imagined. The friendship and comments, which were sharp, incredibly witty and sometimes intelligent, were more than I could have asked for. I hope to carry on the good name of the program wherever I may end up in a happy and successful career. Thank you Dr. Metzger, for your invaluable advice and brevity. Dr. Danneberger, your insight and always excellent stories kept me smiling and motivated. Thank you, Dr. Rodriguez-Saona for your patience and willingness to teach and interact with me.

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CHAPTER 1
LITERATURE REVIEW

LIGHT

Plant growth and development depends mainly on energy derived from light (Smith, 1982). The habitat and ecosystem of plants can vary greatly even when there are minor changes in light (Schmitt and Wulff, 1993). There are two properties of light that relate to plant growth and development: light quality and quantity. Light quality can refer to proportions of visible light spread across the 400-700 nm spectra which influences plant growth responses, e.g. changes in the ratio of red light to far red light can influence biomass allocation (Smith, 1994; Maliakal et al., 1999) and light quantity is the actual amount of energy associated with radiation hitting the earth’s surface (Smith, 1994).

There has been an increase in research concerning the effects of ultraviolet light (UV) 100-400 nm on plant growth and development (Caldwell et al., 1989; Mendez et al., 1999; Rowland 2006; Sarkar et al., 2011). This includes UV-C (100-280nm), UV-B (315-280nm) and UV-A (400-315nm). The ozone (O₃) layer in the stratosphere between 15 km and 60 km above the earth filters UV wavelengths to hit the earth’s surface selectively (Rind et al., 1990; Rowland, 2006). Ozone can absorb ultraviolet-B light, while oxygen (O₂) absorbs UV-C light (Caldwell, 1979; Björn et al., 1998). Exposure UV-B is increasing due to the decline of ozone linked to chlorofluorocarbons and the breakage of the O₃ structure by chlorine (Rowland, 2006).
The levels of atmospheric ozone, however, have varied and declined since 1950 with 20% decreases in content in some locations (Björn et al., 1998; Frederick et al., 1989; Kerr and McElroy, 1993; Rowland, 2006). Ozone is also created by UV striking O\textsubscript{2} and splitting it in two followed by the attachment to an unbroken O\textsubscript{2} (Rozema et al., 1997). The incidence of UV radiation varies by season with winter time increases as high 35% per year while there is a 7% increase in summer due to greater ozone loss in colder temperatures catalyzing chlorine depletion of the gas (Kerr and McElroy, 1993; Solomon, 1990). Cloud cover decreases UV radiation by as much as 20% compared to a clear day due mainly to scattering (Lubin and Frederick, 1991).

**ULTRAVIOLET LIGHT**

The discovery of ultraviolet light as a wavelength occurred in 1800 with Ritter (Berg, 2008). The issue of UV radiation hitting the earth’s atmosphere was first noted in 1881 by Hartley when he was able to measure ultraviolet energy hitting the earth’s surface and found it varied depending on altitude (Hartley, 1881). The description of the wavelengths occurred not long after with Hertz who developed a method for measuring microwaves.

The difference in light energy associated with each photon in each UV light portion has led to developments of action spectrums (Caldwell, 1971; Flint and Caldwell, 2003). The energy associated with each portion of the UV range elicits different responses, creating the need for the spectrum. Currently, regions on earth between 40°N and 40°S latitude receive 2-11 kJ m\textsuperscript{-2} d\textsuperscript{-1} of UV radiation and this has the potential to increase due to ozone loss (Taalas et al., 2000; Kakani et al, 2003; Rowland, 2006).
Exposure to UV-B levels along different lines of latitudes vary due to ozone depletion, notably at the poles. Increases in UV levels since 1970 are as great as 130% in the Antarctic in the spring as compared to a 7% increase in northern hemisphere mid-latitudes over the same time period (Madronich et al., 1998). Levels of ozone depleted by 40% in the Antarctic while negligible increases occurred in the mid-latitudes since the 1970s (McKenzie et al., 2003).

Ultraviolet-C and B, impacts DNA through the production of cyclobutane pyrimidine dimers leading to mutations (McLennan, 1987) while UV-B has a greater impact on plant physiology (Stapleton, 1992) and UV-A is considered to have little impact (Flint and Caldwell, 2003). Exposure to UV-B also tends to promote dimerization while UV-A exposure tends to promote repair enzymes such as photolyase which photoreactivate DNA strands by dimer breakage (Strid et al., 1994; Britt, 1996). The increased amount of UV-B is considered most important as it has physiological impact (Caldwell, 1979; Stapleton, 1992). Ultraviolet-B levels are projected to increase by 50-60% from current levels in the northern hemisphere with peak irradiance levels predicted to occur between 2010 and 2019 in the spring time (Taalas et al., 2000). There is variation in the possible increases with seasonal and geographical variations. Alpine regions already have experienced an increase of UV radiation by 1.1% per anum (Blumenthaler and Ambach, 1990) with higher elevations having higher levels of UV-B due to a thinner atmosphere (Caldwell et al., 1980). UV light initially damages the photosynthetic apparatus within the plant. The majority of research indicates that damage
is mainly in photosystem II of the light harvesting complex (Tevini et al., 1988; Bornman, 1989).

The impact of ultraviolet-A light on plants had been noted in various ways including phototrophic responses in the coleoptile tip in wheat (*Triticum* spp.) (Castle, 1935), and control of phytochrome via UV-A light - near blue was proposed by Galston (1949). Pfeiffer (1928) reported that without UV irradiance, plants had longer shoots, thinner leaves and poor root development. Popp and Brown found that there were only negative attributes associated with UV light, (1936). They also disputed the results of Pfeiffer based on lack of replication and poor lighting control. Other studies have demonstrated a decrease in pollen production (Flint and Caldwell, 1984), declination in competitive ability of plants against weeds (Barnes et al., 1990), and reductions in biomass in a range of plants including pines (*Pinaceae* L.), (Tevini et al., 1981; Sullivan and Teramura, 1988). Thinning of the leaf surface and increased levels of flavonoids and phenolics are also found in plants (Tevini et al., 1981; Sarkar et al., 2011). The potential for long term reduction in the ozone layer has driven interest in how long term adaptation of plants will occur in response to increasing UV levels.

There are many causes for the variation of the impact of UV light on plants observed in the literature. Plants with sensitivity to UV-B light produce increased epicuticular wax on the adaxial surface while there is a reduction in leaf thickness in cotton (*Gossypium hirstum* L.). Decreases in mesophyll and palisade tissue also occurs (Kakani et al., 2003). Stomatal densities may also decrease but this is crop dependent and other factors play a role. In saturated CO₂ conditions, treatments of UV-B were more
effective in decreasing stomatal density (Ziska et al., 1992). Altitude changes increase the UV effect on growth habits of plant material. Plant tissue found above 3000 m has increased dark respiration, an earlier effort at reproduction and maintained photosynthetic efficiency compared to plant material from 0 m above sea level when treated with supplemental UV light due to adaptation to the conditions (Ziska et al., 1992). There can be a greater than 40% increase in UV irradiance between the elevations (Caldwell et al., 1980; Sullivan et al., 1992). Variations in levels of ozone with elevation change are considered possible due to regional aerosol conditions solar elevation and also albedo (Pfeifer et al., 2006).

Dilation of thylakoid membranes in \((Pisium sativum\ L.)\) and damage to chloroplast membranes occurred after UV-B irradiation (Brandle et al., 1977). Herbaceous dicots cannot attenuate UV-B in the epidermis with 18-41% of UV-B light reaching the mesophyll layer leaving increased chances for damage to photosynthetic apparatus. Conifer needles, regardless of age, absorb essentially all UV-B light. Grasses and woody dicots are considered intermediate in their ability to protect the mesophyll layer from damage with attenuation in the epidermis ranging from 3-12% (Day et al., 1992). In contrast to the negative effects, tomato ripening can be faster with treatments of UV-B as colored pigments build up in the plant which can absorb the excess energy, (Bacci et al., 1999). Flowering color can be enhanced with UV-B treatments in smoke tree (\(Cotinus coggygria\ ‘Royal purple’\)). The increase in anthocyanin content in the leaf in response to the light treatment resulted in more favorable performance ratings in color (Oren-Shamir and Nissim, 1997).
CAROTENOIDs: LIGHT ABSORBING COMPOUNdS

The potential long term effects are considered to be both anatomical and physiological and plants do possess response mechanisms for dealing with UV-B light. These are mainly antioxidants such as photolyase and super oxide dismutase (Zhang et al., 2005; Sarkar et al., 2011). These systems repair damage caused by excited radicals due to excess energy from UV-B radiation. There are compounds however, that may absorb the light directly and aid the reduction of UV-B light attenuation and energy dissipation in the plant such as flavonoids and carotenoids (Gould, 2004; Rowland, 2006).

Carotenoids are lipid soluble isoprenoid compounds containing C\textsubscript{40} chains (tetraprenoids) which are ubiquitous in nature and have been investigated in relation to human health benefits. Interest exists in looking at the capabilities they can have in plant protection (Bartley and Scolnik, 1995; Müller et al., 2001). Biosynthesis of carotenoids occurs in cellular plastids where they are associated with light harvesting complexes (Kopsell and Kopsell, 2006). The basic C\textsubscript{5} isoprenoid structures are converted to isopentyl diphosphate (IPP) and into dimethylallyl diphosphate (DMAPP). These compounds are substrates for the enzyme geranylgeranyl diphosphate synthase (GGPP) (Cunningham and Gantt, 1998). The enzyme catalyzes GGPP formation which is paired with another GGPP in a head to head condensation reaction creating phytoene (Gross, 1991). The pathway then branches after the conversion of phytoene to lycopene via phytoene desaturase and \( \xi \) carotene desaturase; the following cyclization of the lycopene rings can form carotenoids with two \( \beta \) rings (Fig. 1.1), (zeaxanthin, antheraxanthin,
violaxnathin) or one β ring and one ε ring (lutein, α carotene) on the C40 structures (Fig.
1.1), (Kopsell and Kopsell, 2006).

The reversibility of accumulation of lycopene with increased FR light sources following red light exposure indicates that initial responses in carotenoid synthesis are due in part to phytochrome (Alba et al., 2000). This hypothesis was first thought of early in the 1950’s (Piringer and Heinze, 1954) when accumulations of certain pigments were noted in response to the red light wavelengths. The inability to clarify this result further inhibited work past the 1970’s. The general thought was that this physiological trait occurred which was recently confirmed (Alba et al., 2000).
Figure 1.1: Carotenoid biosynthesis pathway to non-photochemical (NPQ) quenching xanthophyll cycle.
The variations in the carotenoids impact various functions in the plant. Lutein is believed to have a greater impact on light quenching than β carotene (Cuttriss and Pogson, 2004). The xantophyll cycle has received a lot of attention due to its antioxidant capacity and photoprotection element (Bartley and Scolnik, 1995; McElroy et al., 2006). Carotenoids are essential in reducing light photooxidative stress to chlorophyll and the light harvesting complex (Foyer et al., 1994, Moradas-Fereira et al., 1996). The downward energy transfer of singlet oxygen (1O₂) to zeaxanthin reduces the damage it can cause to lipids and cell walls (Owens et al., 1992). In carotenoids with no rings at the end of the structure, such as lycopene, the large amount of double bonds allows for a similar effect (Kopsell and Kopsell, 2006). Light harvesting complexes have the -trans configuration providing efficient singlet energy transfer to chlorophyll molecules. The -cis forms tend to isomerize towards the -trans configuration upon photo excitation. The carotenoids attached to reaction centers are also found to be in –cis conformation and are unaffected by temperature change. This may make them preferred carotenoids for energy dissipation (Lutz et al., 1978; Koyama and Fujii, 1999).

Creeping bentgrass xanthophylls in high light conditions cumulatively increase as a percentage of the total carotenoids in high irradiance but decrease in low irradiance. Neoxanthin and β-carotene decrease in high irradiance but increase in low irradiance (Bell and Danneberger, 1999; McElroy et al., 2006). Testing of creeping bentgrass showed that after up to 24 hours of exposure there was an increase of carotenoids in higher irradiance but a resultant decrease and probable degradation after 168hrs (McElroy et al., 2006). Maximum buildup of lutein, β-carotene and chlorophyll B
occurred at 24 hours in Kale (*Brassica oleracea* L.) but an overall increase in photoperiod also increased total pigment accumulations (Lesfrud et al., 2006). Seeds of certain maize hybrids had lower carotenoid content grown at cooler temperatures (16 °C) (Massacci et al., 1995).

The application of nutrient elements to plants increases carotenoid content. Increasing nitrogen applications to spinach (*Spinacia oleracea* L.) almost doubled the quantity of lutein on a fresh weight basis (Lesfrud et al. 2007). This is a benefit that may already have been realized with current applications of nitrogen but also may allow for improvements in areas which are prone to excess levels of sunlight. The addition of nitrogen to plants increases production of many pigments (Greenwood et al., 1990) until a level of nitrogen toxicity is reached (Carrow et al., 2001). In periods of reduced nitrogen content rice plants may increase their content in an attempt to increase thermal dissipation abilities in responses following decreases in chlorophyll (Huang et al., 2004). The management of turfgrasses on sand based golf greens is dependent on sufficient fertility in a growing medium that is classed as having poor nutrient retention capabilities. Thus poor fertility management may lead to photoinhibition and loss of productivity.

Control or inhibition of carotenoid biosynthesis is a mode of action that has been exploited in the recent development of herbicides. Mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione) has proved useful in controlling common turfgrass weeds (McCurdy et al., 2008; Beam et. al., 2006). Inhibition of certain carotenoids leads to bleaching of the leaf and damage to the photosystems of weeds (McCurdy et al., 2008). The damage to the photosystems then allows for improved turfgrass competition although
there is also potential for damage to turfgrass systems in certain situations (Beam et al., 2006). This form of chemical control is relatively new to the turfgrass industry and suggests the potential for more useful information related to the development and control of secondary metabolites.

There have been efforts to enhance levels of carotenoids in plants. This has been carried out for a variety of reasons including ecological fitness due to reduced photoinhibition and increased human health benefits. Variations occur in tomato in particular providing a wide range of varieties with different carotenoid profiles (Fraser and Bramley, 2004; Ronen et al., 1999; Ronen et al., 2000). Genetic engineering of carotenoids in plants has occurred at an increased rate as our understanding has developed about the pathways. Control is offered with genetic manipulation and allows for a greater understanding and possibility for greater change in compounds (Fraser and Bramley, 2004). Research on the effects of UV-B impact on carotenoid profiles of turfgrass species has not been carried out.

**FLAVONOIDS: UV ABSORBING COMPOUNDS WITH SPECIFIC INTEREST IN ANTHOCYANINS**

Pigments such as flavonoids have specific absorption characteristics in the UV region (Ziska et al., 1992). Flavonoids are compounds defined as having a 15 carbon structure in a form of a C₆ – C₃ –C₆ backbone (Anonymous, 1997). They can be broken down into different pigments which have UV-B absorbing characteristics such as anthocyanins (Gould, 2004). Anthocyanins are a pigment in plants which are associated with various functions. They are derived from shikimic acid via flavonoid formation.
There have been at least 4,000 flavonoids described and many are found in higher plants (Seigler, 1998). Plants use them as a color source for attraction of pollinators which can see in the UV wavelengths and also as a defense mechanism for disease and herbivory (Briscoe and Chittka, 2001; Irani and Grotewold, 2005). They are primarily known for their bright red colors and are used in the food industry as a colorant. In plants, anthocyanin response is related to environmental stresses including drought stress, excessive UV-B conditions, heavy metal toxicity, cold hardiness, insect and fungal attack (Chalker-Scott, 1999; Gould, 2004).

Anthocyanins are naturally found in higher levels in plants such as berries and red fruits (Lohachoompol et. al, 2004). Anthocyanins are found in shoots of plants but in general are predominantly found in the upper epidermis or just beneath it in the mesophyll layer in leaves (Chalker-Scott, 1999). The areas in which they are most often found tend to have greater levels of light exposure (McLure 1975, Chalker-Scott 1999). It has been shown that the synthesis is autonomous to cells (Lancaster et al., 1994). The localization of production would indicate that a specific screen for cell ultra-structure may occur versus whole plant changes.

Anthocyanin synthesis involves approximately seven steps and the pigments are assembled similarly to other flavonoids from two different streams (Bennett and Wallsgrove, 2006). One stream involves the shikimate pathway to produce the amino acid phenylalanine. The other stream produces malonyl-CoA, a C₃ unit from a C₂ unit (acetyl-CoA). These streams meet and are coupled together by the enzyme chalcone synthase (CHS), which forms an intermediate chalcone (Fig. 1.2). The chalcone synthase
is considered to be the first enzyme in all direct flavonoid biosynthesis. The chalcone is subsequently isomerized by the enzyme chalcone isomerase (CHI) to the prototype pigment naringenin (Fig 1.2), (Heller and Forkmann, 1988). Naringenin is oxidized and then further reduced by the enzyme dihydroflavonol 4-reductase (DFR) to the corresponding leucoanthocyanidins. The resulting, unstable anthocyanidins are further coupled to sugar molecules by enzymes like UDP-3-O-glucosyl transferase to yield the final relatively stable anthocyanins (Seigler, 1998). One of the final steps is light controlled and in particular it seems that UV-B light induces increased synthesis (Kubasek et al., 1992).

The initiation of synthesis is thought to be controlled by the light receptors phytochrome or cryptochrome (Mancinelli et al., 1991; Sponga et al 1986). Cryptochrome, a blue light receptor, is inferred to elicit anthocyanin synthesis in tomato seedlings. Blue light applications significantly increased levels of anthocyanins in tomato. The phytochrome response was more significant in cabbage, however, with about 7x efficiency in anthocyanin production with red light (650 – 800nm) applications (Sponga et al., 1986). The initiation response of chalcone synthase to UV-A is mediated by cryptochrome 1 (CRY1) but there is no response to UV-B light via either CRY1 or CRY2 to enhance chalcone synthase, the key flavonoid biosynthesis enzyme (Wade et al., 2001). The interaction between the two light receptors is also possibly important as both phytochrome and cryptochrome converge in a single pool to initiate light responses (Ang et al., 1998). It has been proposed that PHYB, in particular, equilibrates the flux through CRY1 and UV-B signaling pathways (Wade et al., 2001).
Figure 1.2: Anthocyanin biosynthesis pathway.
Plant age affects response to changes in the light and induction of chalcone synthase activity via phytochrome. In 6 days old mustard (*Sinapis hirta* L.) seedlings far red, UV-B and UV-A / blue light induced the CHS transcripts (Kaiser et al., 1995). In older mustard seedlings and mature Arabidopsis leaf tissue, however, there is no phytochrome induction but there was a response due to UV-B and UV-A / blue light treatments (Jackson et al., 1995).

There are 6 main anthocyanins and many others that occur less frequently. The six aglycones (pelargonidin, cyanidin, delphinidin, petunidin, malvidin and peonidin) are widely distributed among angiosperms and gymnosperms (Seigler, 1998). The differences between the various anthocyanins are mainly due to the nature and number of sugars attached to the ‘3 or ‘5 position on the C ring of the structure. The addition of sugars and acids at either of these positions leads to a more stable structure and alters the absorption abilities of the pigments (García-Viguera and Bridle, 1999). Variations can be caused by the nature and number of aliphatic or aromatic acids attached to the sugars (Kong et al., 2003). The variations lead to color changes which can be seen across a whole range of plants. The leaves of red cabbage contain eight acylated cyanidin glucosides while red onion contains four major anthocyanins; cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-malonylglucoside, cyanidin 3-malonyllaminaribioside and four minor ones (Brouillard, 1988; Donner et al., 1997).

There is some argument as to whether anthocyanins are directly related to general stress tolerance or if they are produced as a direct result of the stress under which the plant has been placed. The impact of cold hardiness, changes in light intensity and
wavelengths seems to enhance responses in particular. Photoprotection has been offered as a specific anthocyanin function and hazelnut seeds lacking anthocyanins have died in field conditions versus anthocyanin producing progeny (Mehlenbacher and Thompson, 1991). The anthocyanins also favor absorption of UV-B and green light over blue light with only small quantities of red light being absorbed (McClure, 1975). The absorption of the blue-green light means that there is only red light available to chloroplasts. This is important as it reduces the potential for excessive energy build up (Pietrini and Massacci, 1998). An increased tolerance for photoinhibition in cold acclimated jack pine seed / needles was a result of light attenuation by anthocyanins and increased utilization of light energy (Krol et al., 1995).

Temperature changes elicit many anthocyanin responses. In fall, color changes in leaves lead to increased quantities of carotenoids and anthocyanins giving a range of red, orange and yellow colors. The increase in content has been related to masking of chlorophylls as leaves are senescing thus decreasing potential for photo oxidation damage reducing nutrient retrieval prior to winter-like conditions (Field et. al., 2001). The red leaves are seen when there is de novo synthesis of anthocyanins unlike carotenoids which are revealed with chlorophyll breakdown and there is no increase in levels (Matile et al., 1992). Increased absorption in green yellow light by anthocyanins (Neill and Gould, 1999) may counteract the loss of chlorophyll and attenuate sufficient light to prevent damage as leaves senesce (Field et al., 2001). The reflection of red light may be independent of anthocyanin content and concentration of the pigments is more important than their location (Neill and Gould, 1999).
Temperature also plays a role in the stability of anthocyanins, and they are susceptible to degradation if exposed to prolonged warmer temperatures when used in food colorants (Giusti, personal comm. 2008, Ahmed et al., 2004). In the natural environment the anthocyanins are seen in varying temperatures depending on the plant and the functionality of the anthocyanin. The alterations in temperature are seen as leading to a response also. In cabbage (Brassica oleracea L.) colder temperatures increase light dependent anthocyanin production; however, there is disagreement as to whether the development is a result of temperature effects on the phytochrome system or on chalcone synthase activity related to photoinhibition (Rabino and Mancinelli, 1986).

The pH plays a major role in coloration. Lower pH levels (<2) tend to produce a red color while a pH greater than 4 results in predominantly colorless anthocyanins (Seigler, 1998). The change in plant pH can be caused by a variety of environmental effects including sunlight (Zu-Hua et al., 1990). The pH also affects anthocyanin bases which are stabilized by forming very colorful complexes with metals. The complexes with such minerals as aluminum, iron or magnesium which are extremely susceptible to pH change and have narrow limitations (Seigler, 1998). The extraction and quantification of anthocyanins tends to occur from plant material using a solvent containing a low percentage of hydrochloric or formic acid. This allows samples to be kept at lower pH and reducing the chances of degradation of non-acylated pigments (Kong et al., 2003). Anthocyanins may also have potential to reduce aluminum toxicity. Formation of complexes between anthocyanins and Al³⁺ allows for pigment stabilization and reduces
degradation due to oxygen reactions as well as internal Al\textsuperscript{3+} levels (Moncada et al., 2003).

**PHENOLIC COMPOUNDS**

The class of phenolic compounds is widespread and has been identified in large numbers. Approximately 40\% of the organic carbon is allocated to phenolic compounds in the biosphere (Croteau et al., 2000). Their production is predominantly linked to the shikimate and acetate pathways (Floss, 1997; Hare and Cress, 1997). They are largely derived from phenylalanine and are formed usually in cells close to locations of stress. The enzyme phenylalanine ammonia-lyase (PAL) is used to direct carbons from amino acids into the synthesis of the metabolites. They can be constitutively present (e.g. lignins) or they can be induced (Langebartels et al., 2002). Their production is highly complex and variable as there are so many compounds with multi step reactions required for their synthesis (Mustafa and Verpoorte, 2007). The structures have a minimum of 6 carbons and their structure has an aromatic phenyl ring. There are generally one or more acidic hydroxyl groups attached to the aromatic ring (Croteau et al., 2000).

The action of UV within the photosynthetic apparatus is predominantly involved in the formation of highly excited compounds such as singlet oxygen (Foyer et al., 1994). A majority of phenolic compounds act as scavengers of singlet oxygen and have an ability to donate hydrogen from hydroxyl groups around their aromatic ring to free radicals. This then reduces the impact of oxidation of lipids and other structures within the cell (Foti et al., 1994). This can be used to reduce protein denaturation in the photosynthetic apparatus which undergoes continuous cycles of damage and repair and
can become overwhelmed in high light or enhanced UV-B conditions (Takashi and Murata, 2008). The production of these compounds is stimulated by UV radiation as well as flavonols (Tevini, 1993). The range of compounds means that they can be induced in many situations. High concentrations of phenolic compounds are believed to reduce herbivore attack (Dicke et al., 2003). In Brassicaceae, sinapate esters of phenolics provide protection against UV irradiation due to specificity in UV light wavelength absorption (Sheahan, 1996). Phenolics in epidermal cells have the ability to screen and absorb UV irradiation (Bornman and Teramura, 1993).

Plants do also possess the ability to distribute protective compounds based on where light exposure is highest. In Jersey cudweed (Gnaphalium luteo-album L.), surface concentrations of flavonols increased in response to UV-B radiation (Cuadra et al., 1997). Regular removal of tissue may reduce a plant’s ability to respond to these conditions and enhance the impact of UV-B light stress. Phenolic compounds found in the epidermal layers of leaves are believed to offer screening of elevated light levels to the photosynthetic apparatus (Gould, 2004). The upper part of the plant may alter its pigment composition in response to the light location also (Ju et al., 1999).

MEASUREMENT TECHNIQUES

The ability to separate and identify the compounds has increased as equipment sensitivity and improved structural elucidation developed. This happens via use of spectrophotometry, mass spectrometry, high performance liquid chromatography and nuclear magnetic resonance spectrometry has created better separation techniques which do not damage the compounds during analysis. This has allowed for the creation of a vast
amount of information on these materials and in food sources there is a lot of information known about their structure and molecular weight (Wrolstad and Giusti, 2001). A range of these systems are used to characterize and quantify pigmentation changes. Spectrophotometry is dependent on variations in reflective or transmission properties in compounds based on their reaction to excitation via light energy. The absorption of light leads to a change in energy in bonding electrons (electronic, vibrational, and rotational). The spectra arise when the molecules of interest absorb photons of a specific energy. They remit the energy at a slightly longer wavelength which is then divided out by a monochromator and absorbed by a light sensor. The values are then compared against a set of standards (Hardy, 1938). Spectrophotometers can be used in a pH differential method to quantify specific anthocyanin levels depending on the standards available. There is a very high correlation with the standards (R >0.9), (Lee et al., 2005). Analysis of all pigments in this study could be carried out with spectrophotometry (Wellburn, 1994).

More in-depth analysis can be carried out using high pressure liquid chromatography (HPLC). The process is the separation of chemical substances by partitioning them between two media. The most common form is via a stationary phase and a mobile phase. The first may be a solid or liquid while the second maybe a liquid or gas. The separation of substances depends on a range of situations – compound size, polarity and volatility are some of the common methods. It provides a precise method which can separate compounds which are very similar thus difficult to analyze using other methods. Chromatography itself has many fields and methods such as gas
chromatography, thin layer chromatography, paper chromatography, and gel permeation chromatography (Shackleton, 2010). Specific known wavelengths are again used with a photodiode array along with retention time, order of elution, standards, solvents used in phases and also the extraction methods to characterize and quantify the pigments. Anthocyanins and carotenoids can be separated within the chemical classes using this system and it has become consistent and reliable over time (Hong and Wrolstad, 1990; McCurdy et al., 2008).

Enhancing the separation and developing greater precision requires mass analysis of compounds. This can be carried out using mass spectrometry. It analyzes compounds based on a mass to charge ratio of an analyte. This mass to charge is used in two ways; the charge on the compound and its mass affect its flight through a charged field and depending on this its structure can be determined using time of flight and distance flown.

The compounds are ionized and have a charge added and then analyzed. This occurs simply through a five step process. The loading of samples is carried out usually with an automatic injector or it can be done manually if single samples are being used. The samples are then vaporized undergoing a phase transition from liquid to gas phase. Ionization then occurs via a range of ways – including electrospray and lasers, and this leaves a charged particle which is then accelerated through a larger column which is surrounded by electromagnetic fields. The ions are then deflected by a magnetic field according to their masses. This method has been used successfully to elucidate anthocyanins (McEwen et al., 2005; Pascual-Teresa et al., 2002). These systems allow for further information to be produced on turfgrass changes in response to both biotic and
abiotic stresses. These methods will allow for deeper insight into producing quality turfgrasses with more specific topographical, geological and geographical variances catered for. It will give turfgrass breeders new tools in selecting potential turfgrass cultivars.

The changes that are currently ongoing in the earth’s atmosphere and potential future issues with higher light intensities and alterations in spectral radiation mean that understanding the protective pigment capacities of turfgrass in response to this stress is important. In turfgrass there has been very little work on these pigments. There is a known increase in total anthocyanin concentration in Kentucky bluegrass (*Poa pratensis* L.) in response to UV-B light (Zhang et al., 2005), but there has been no characterization of the pigments. Identification of changes within turfgrass plants based on possible attainable levels of UV-B is important and could aid potential breeding efforts in trying to combat the problem. Their influence on turfgrass color and productivity may not be understood in this situation and this warrants further investigation. The physiological aspect of the plant and the response location is also of importance. It can be expected that tissue receiving more sunlight at the top of the canopy may have a dual purpose. It creates shaded conditions for the understory, reducing potential for light damage while also it could be expected that there is a concentration of protective light pigmentation where the light intensity is greatest. This also may influence carbohydrate allocation and force a change in plant growth habit. A reduction, for instance, in biomass accumulation or tillering would lead to a reduction in recovery capability and thus limiting turfgrass use in certain situations. These questions do pose a learning moment for physiologists and
breeders and the objective of this study is to give insight into what protective pigmentation cool season turfgrasses possess and how plants may or may not use them in relation to a stress response.
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CHAPTER 2

SUBJECTIVE AND OBJECTIVE COLOR RATINGS OF TURFGRASSES AND THEIR LINKS TO PIGMENTATION AND NITROGEN CONCENTRATIONS.

ABSTRACT

Turfgrass color is used to correlate turfgrass responses to management inputs such as fertility treatments and response to environmental stresses. Visual perception of color is considered a crucial measurement of turfgrass quality as it is the quickest method of judging turfgrass performance. The measurement can be subjective; therefore the digital analysis of color has been added as a means to add objectivity to standardize color measurements. The purpose of this study was to test for a correlation between subjective and objective turfgrass color rankings and chlorophyll pigment concentrations in several cool season grasses on an area and volume basis. Fifty one cultivars of six different cool season grasses were used; perennial ryegrass (*Lolium perenne* L.), Kentucky bluegrass (*Poa pratensis* L.), creeping bentgrass (*Agrostis stolonifera* L.), tall fescue (*Schedenorus phoenix* (Scop.) Holub), fine fescues (*Festuca* spp.), and colonial bentgrass (*Agrostis capillaris* L.). Turfgrasses were germinated and maintained in fifteen cm pots for two months. Subjective analysis was then carried out using a visual rating for greenness on a 1-9 scale.
Objective analysis of chlorophyll pigmentation on a mass and area basis was carried out using a handheld colorimeter on the grass surface and a bench top colorimeter on pigment extract. Both systems used the CIELab color measurement system. Tissue nitrogen and chlorophyll content by area and mass were measured. The handheld system measuring reflectance did not correlate to pigmentation concentration ($P=0.05$). Visual rating was closely linked to nitrogen levels and the benchtop system was influenced by pigment concentrations ($P=0.05$). Hand held systems using reflectance type measurements may suffer from excess error due to characteristics such as surface texture and leaf thickness.

INTRODUCTION

Turfgrass color is traditionally ranked on a 1-9 scale with a ranking of one equal to dead brown turf and nine equal to optimal green turf color in the eyes of a rater. However, this is a subjective measurement. In trying to develop a rapid method of measuring turfgrass responses to either stresses or management inputs, researchers have found success with using handheld reflectance systems in field situations to measure plant color. The measurement of plant disease damage and plant nutrient deficiencies have been successfully tested in creeping bentgrass (*Agrostis stolonifera* L.), and corn (*Zea mays* L.), (Nutter et al., 1993; Bausch and Duke, 1996). These systems provided highly correlated data and were shown to have positively correlated (R) values to visual ratings and even tended to be less biased (Nutter et al., 1993). There has been success using systems which are wavelength dependent to indicate biomass status and chlorophyll (680 – 780 nm) content (Filella and Penuelas, 1994). Wavelengths in the red
range 680-780nm are considered the best estimator of leaf area index in (*Capsicum annuum* L.) and (*Phaseolous vulgaris* L.). Water stress can be measured using this technology also, however this was seen only after the stress was well developed (Nutter et al., 1993).

The digital color measurement systems in use have different functions. Digital imaging has been found to be extremely successful ($r^2=0.99$) in correlating Munsell color chips to nitrogen treatments in creeping bentgrass (Karcher and Richardson, 2003). The digital system used is also more precise when compared to traditional methods (Richardson et al., 2001). The digital analysis uses photography and software to develop ranking systems and has the ability to obtain large volumes of data from one picture, and separate out colors with excellent reproducibility. Colorimeters, which depend on a reflective light wavelength and intensity measurement have been used to separate creeping bentgrass cultivars and different nitrogen treatments (Landschoot and Mancino, 2000; Mangiafico and Guillard, 2005). The method used in our trials is offered as an alternative method for turfgrass color analysis which can use either handheld reflective measurements or spectral transmission benchtop measurements and link them to nitrogen measurement in turfgrass.

A majority of the color-based systems in use are dependent on a reflective measurement of turfgrass leaf surfaces. However, leaf surfaces vary in many ways due to environmental conditions and varietal differences. Tall fescue (*Schedenorus phoenix* (Scop.) Holub.) cultivars have a leaf thickness of between 0.31 and 0.36mm under drought stress and a drought free leaf width of 0.44 cm to 0.52 cm (Fu and Huang, 2004).
Nitrogen content causes variation in tall fescue leaf width from 0.31 cm to 0.75 cm (Rademacher and Nelson, 2001). Colorimeter systems do not have the capability to measure such narrow sizes creating the potential for inaccurate measurements due to different leaf age and different nitrogen concentrations. Upper and lower leaf surfaces also vary in thickness. The upper epidermal surface of Kentucky bluegrass can be 0.58 µm and 1.23 µm on the lower epidermal surface. This thickness variation in the leaf surface plays a role in light absorption, reflection and transmission (Cameron, 1970; Clark and Lister, 1975). Penetration and reflection of light varies by leaf thickness. Treatment by UV-B of alfalfa (*Brassica campestris* L.) resulted in an increase of leaf thickness by 45% - causing an increase in the amount of light back scattered (Bornman and Vogelmann, 1991). Variations across and within turfgrass species leaf thickness could influence reflective type measurements.

Human eyesight uses rods and cones in the retina. The cones are sensitive to short, medium and long wavelengths and work to absorb opposing colors of red, green and blue (Brown and Wald, 1964; Roorda and Williams, 1999). The methods of color measurement by different handheld reflectance systems vary. The systems can use color rankings from the Munsell system (Escadafal et al., 1989), Hunter color systems or CIE Lab scales (Commission Internationale de l'éclairage), (Pattee et al., 1991). The use of the Munsell color system (Munsell, 1915) was designed to rank color by Albert Munsell (Nickerson, 1940). This is considered the initial color system, but it did not transfer consistently into digital reproduction and so more recent systems were developed such as CIE Lab and Hunter scales (Sharma and Trussell, 1997). These two systems attempt to
replicate how the rods and cones work in the retina and are based on a fixed scale so as not to become device dependent and lacking reproducibility (Lemaire et al., 2005).

A handheld colorimeter system uses measurements in different color spaces and $L^*$ is considered a measure of lightness while $a^*$ measures green (-) or red, (+) and $b^*$ is a measure of blue (-) and yellow (+) colors on the wheel. Hue or the color attribute is also measurable in a degree system to fit the color wheel while chromaticity is used to measure the lightness ($100 = \text{white}$) or darkness ($0 = \text{black}$) of the color. This represents a similar system to human eyesight and can be converted into a 3-D image for digital analysis (American Society for Testing and Materials, 1989). The system emits a diffuse uniform light source over a set measuring area that is reflected and collected by an optical fiber cable which enables analysis of color (Minolta, Chroma Meter CR-300 Instruction manual). The light source can be manipulated to equal daylight conditions $D_{65}$ or $C$ which have similar light circumstances but $D_{65}$ is preferred by CIE. There are other illuminant types which can replicate indoor lighting. The handheld systems are fast, simple and field usable (Shimada et al., 2004).

Previous work investigated correlating colorimeter values to creeping bentgrass cultivars using the CIELab system and the Minolta CR-310 handheld colorimeter. The results indicated that the hue angle – the component of the system that describes the true color which is then further influenced by the lightness or chroma of the color – was the most consistent part of the data and correlated favorably with data collected from visual rankings (Landschoot and Mancino, 2000). The data, however, were not linked to any plant characteristic thus offering no ability to replicate the study. CIELab system data can
be transferred to tristimulus based values such as XYZ values and so it offers a possible system that is transferrable between multiple pieces of equipment. This then allows for comparison of the same characteristics across grass types (Reinhard et al., 2001). Trials on creeping bentgrass using the normalized difference vegetation indices (NDVI) system compared to turfgrass pigmentation content indicated an influence on color ratings when nitrogen, magnesium and iron fertilization rates were varied. The variation in the color was only explained 36% of the time by chlorophyll pigmentation and that another factor or combination of factors was responsible for the result (Stiegler et al., 2005).

Extract measurements of chlorophyllous pigments using benchtop colorimeters can also give color measurements based on either transmittance or reflectance dimensions (Ahmed et al., 2000). Transmitted or reflected light beams are collected through a lens which is 8° from perpendicular to the sample surface. The beams are then integrated using a sphere and flash beams. The flash reduces the noise and allows for higher intensity light which normally reduces sensitivity of the pigments due to degradation when exposed to light for an extended period (Joilot and Joilot, 1984). Spectrophotometers have been used to test color differences between grasses extracts (Kavanagh et al., 1985). The transmitted light is then passed through a diffraction grating which separates and measures the wavelengths of light. These systems also possess the ability to measure in L*a*b* color space (Yudin et al., 1987). The use of extracts requires lab time however; this can be a downside to the use of benchtop color measurement due to cost and time while field measurements are easily and quickly carried out.
These systems offer opportunities to standardize color system management and take out subjectivity that can occur between trained and untrained raters of color (Gooding and Gamble, 1990; Landschoot and Mancino, 1997). There, however, needs to be a larger bank of color data compiled with these systems. There are limited data in relation to pigmentation influence on color rating and whether or not surface reflectance measurements or transmission type measurements are more appropriate for color analysis. The objective of this study was to discover how chlorophyll pigments influence color systems analysis and link pigment concentration to a particular trait within the color system to create a baseline of data for breeders and other end users. The study aims to determine what influences human color perception which could be related to stress responses during periods of pigment decline such as exposure to UV-B. It further aims to discover whether there is a correlation between visual rating and color system ratings.

MATERIALS AND METHODS

Plant material

Two separate Turfgrass trials were run from March, 1st 2010 until June, 11th 2010, and June 15th 2010 until September 30th 2010 at The Ohio State University, Howlett Hall greenhouse, 680 Vernon Tharp St., Columbus OH, 43210. Six turfgrass varieties were seeded in a sand based rootzone, of 80/20 volume/volume sand / peat mix. There were 9 cultivars of perennial ryegrass, Kentucky bluegrass, creeping bentgrass, tall fescue, fine fescues, sheeps fescue (*Festuca ovina* L.), creeping red fescue (*Festuca rubra* L.), hard fescue (*Fesutca brevapila* Tracey.). Six cultivars of colonial bentgrass were evaluated.
The grasses were chosen based on their color rankings from national turfgrass evaluation program (NTEP) trial results for 2003 – 2007 for all grasses and based on commercial availability. Cultivars were randomly separated by the rankings of high / intermediate / low (Top 1/3, Middle 1/3 Lowest 1/3) of grasses on the NTEP evaluation results.

Turfgrasses were watered with a mist system initially for 30 seconds every 10 minutes during germination (21 days). Once established, grasses were watered twice daily for 3 minutes. All turfgrass samples were treated with 20-20-20 liquid fertilizer (Scotts Co. Marysville, OH) at a rate of 4.06 kg ha$^{-1}$ on a bi weekly basis. Each grass was then grown out to a height of 10 cm and maintained at this height for two months in a greenhouse with temperatures maintained at ~20° Celsius.

**Data collection**

Turfgrass samples were placed in a completely randomized design in full sun conditions. A total of 44 untrained (not trained in evaluating turfgrass color) and trained (turfgrass researchers with training and experience in ranking turfgrass color) evaluators ranked grasses based on their color. Ranking was set with 1 = brown 6 = satisfactory and 9 = optimal dark green. Turfgrasses were irrigated twice on both days to prevent wilt and plant material was unranked for 1 hour periods post irrigation to prevent any impact of water remaining on leaf surfaces on rankings. On both occasions grasses were in full sun for the total period of time during ranking. A total of forty two surveys were collected between both experiments. Visual ratings by humans were carried out with permission of
institutional review board (IRB project #2010E0291) on June 12th and again on September 22nd 2010.

Turfgrass samples were taken immediately following the visual rating and leaves were collected by area (40 mm$^2$) and on a mass basis (0.05 g) with three subsamples taken from each cultivar and placed in N,N-dimethylformamide (Sigma-Aldrich, St Louis, MO) to extract chlorophyll, (Moran and Porath, 1980). Samples were stored in dark conditions for a period of 48–72 hours after this. Extract was subjected to absorption analysis using a UV-Vis spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD) at wavelengths of 664, 647, 625 and 603 nm. Data were calculated using formulas from Moran, (1982) and chlorophyll totals, chlorophylls A and B were quantified on an area and mass basis. The premise for this was that the hand-held system measures directly on a set area on leaf tissue while benchtop systems require tissue extract for analysis. Correlating color with chlorophyll content based on the area and mass measurements were designed to fit this variation between color measurement systems.

Freshly harvested samples were used for reflectance analysis. Turfgrass leaves were layered four times to create an optically dense stack (Mangiafico and Guillard, 2005), then three light measurements were taken on the top layer (Appendix A), using a Konica Minolta CRM 310 (Konica Minolta Sensing, Ramsey, NJ). The stacking and multiple readings were done to reduce variability due to turfgrass surface irregularity. The layers were then randomly re-ordered and measurements repeated. Samples were re-configured three times for a total of nine color measurements per pot and thirty six
measurements per cultivar. Data were taken for chroma, hue lightness (L*) red/green (±a*) yellow/blue (±b*). Prior to analysis the system was calibrated with a white plate provided by the manufacturer.

Color analysis of extract was carried out using the Hunter Colorquest XE system (Hunter Associates Laboratory Inc., Reston, VA). Turfgrasses were initially tested for spectral transmission properties to assess which volume of turfgrass was suitable to reduce high viscosity or high reflectance levels which could distort data. Turfgrass samples weighing 0.5 g were determined to be most consistent across all grasses used. Turfgrass samples were then placed in 15 mL glass tubes with 10mL of N,N-dimethylformamide. N,N-dimethylformamide was used to extract all color related to chlorophyll pigments due to its high efficiency (Stiegler et al., 2004). Samples were placed in reagent for 48hrs in darkness. Extract was then placed in a 20 mL cuvette with 10 mm path length. Each sample was then tested at illuminant settings of D_65 and transmittance settings with data acquired for chroma, hue lightness (L*) green/red (±a*) yellow/blue (±b*). Prior to analysis, calibration was performed with a white plate provided by manufacturer and dimethylformamide was used as a blank.

Tissue samples from each grass were taken and dried for 48 hrs at 60 °C prior to tissue analysis for percentage nitrogen content at this time (A&L Eastern Laboratories, Richmond, VA).
Data analysis and experimental design

Four replications of each cultivar were used in two repeated trials. The pots were ranked by humans in a completely randomized design in an outdoor setting with full sun on turfgrasses throughout the day. During the second experiment a precipitation event forced the stoppage of ranking, this resumed once water was removed from leaf surface and cloud cover was gone.

Data transformation of a*b* values is required to create the hue value which is in a degree format to fit onto the 3-D color circle associated with the system. The conversion formula is:

\[ \text{degrees (Atan2(b*,a*))} \]

Following this conversion data was tested using robust regression ROBUSTREG analysis for data influence and outliers (Chen, 2002). Normality tests were carried out using the Kolmogorov – Smirnov test while skew and kurtosis were tested also. Data was then analyzed using multivariate principal components analysis with the R program (R Development core team, 2011; Jaisingh et al., 2006) to gain an in initial understanding on variable influence on color. The results of the multivariate analysis were then used to develop regression and correlation data using PROCREG and PROCCORR with SAS (SAS Institute, Cary, NC). Differences between cultivar color, nitrogen content and chlorophyll content were analyzed using PROCGLM. All grasses were analyzed separately and as a completely randomized design.
RESULTS

There was some influence from raw values in the data and outliers were discarded accordingly \((P=0.05)\). Normality tests (Kolmogorov-Smirnov) found the data to be non-normal \((P=0.05)\) and so data was tested for outliers using PROC ROBUSTREG. Data with leverage due to errors in entry or large variability were removed. Kurtosis and skew varied between grasses and also between transmission and reflectance measurements (Table 2.1). Transmission data was distributed normally for fine fescue, colonial bentgrass and Kentucky bluegrass. The data for other three grasses were also normally distributed with tall fescue \((0.04)\) \((P=0.05)\), creeping bentgrass \((0.03)\) and perennial ryegrass \((0.04)\) according to Kolmogorov-Smirnov tests. All grasses, with the exception of fine fescue \((0.03)\) and Kentucky bluegrass \((0.03)\), were found to have normal data for reflectance measurements (Table 2.1). Skew was similar for both transmission and reflectance though no grass had a normal distribution based on kurtosis and skew analysis (Table 2.1).

There was some correlation between hue and chlorophyll B in Kentucky bluegrass \((-0.6)\), colonial bentgrass \((-0.6)\) and creeping bentgrass \((-0.3)\) cultivars for transmission analysis with the bench system (Table 2.2). The negative value obtained correlation is due to the values obtained for the -A* measurements in the green region. Perennial ryegrass, tall fescue and fine fescue had limited to poor correlation with hue, chroma A* and B* values (Table 2.2). The strongest correlation across all pigments and transmission values was -0.6 (Chl B – Hue : Colonial bentgrass / Kentucky bluegrass)
with all grasses except colonial bentgrass having no correlation between a certain pigment and measurement value at least once (Table 2.2).

No, or limited, relationships existed between chlorophyll levels or variations in chlorophyll pigments and visual rating (Table 2.3). Visual rating was closely linked in particular with nitrogen levels in perennial ryegrass (0.7) but there was limited linkage overall, either on an area basis or chlorophyll volume basis between visual rating and chlorophyll content (Table 2.3). Visual analysis and correlation with the reflectance and transmission data was also very limited with data having a correlation of no more than -0.5 which occurred when correlating reflectance characteristics to the visual rating of fine fescue (Table 2.4).

There was a correlation between nitrogen levels and visual rating of green color (Figure 2.1). Lower levels of tissue nitrogen content led to lower rankings within species. Neither pigment concentrations nor nitrogen content influenced CIElab measured reflectance. The transmission analysis was influenced by all of the pigments. Visual rating was independent of transmission and reflectance while N and grass variety had positive correlations with human color rankings (Figure 2.1).

There was a very poor relationship found between reflectance measurements for color and turfgrass pigmentation (Table 2.5). The strongest noted relationship existed between the red/green (A*) component and blue yellow component (B*) of colonial bentgrass with a value of 0.5 and -0.5 for both. Grasses such as tall fescue, perennial ryegrass and fine fescue had an even more limited relationship between pigments and color measurements (Table 2.5). Hue values were not seen to be linked closely to total
chlorophyll in all grasses while reflective analysis did have stronger correlation with Chl b concentrations (Table 2.5).

The a* and b* values were influenced minimally on their own by chlorophyll content in perennial ryegrass, creeping bentgrass and Kentucky bluegrass using both transmission and reflectance methods (Tables 2.2, 2.5). Correlation between turfgrasses fine fescue, tall fescue and colonial bentgrass and chlorophyll was comparatively stronger but the strongest relationship was only found to be 0.5 between chlorophyll B content and A* and B* reflectance measurement (Table 2.5). A correlation of 0.6 and -0.7 was found between nitrogen levels and reflectance measurements for colonial bentgrass and A* and B* (Table 2.6).

The reflectance measurements were linked closest to nitrogen content for A* and B* with the strongest relationships occurring between nitrogen fine fescue, tall fescue and colonial bentgrass (Table 2.6). Nitrogen levels had the strongest relationship in reflectance measured hue for fine fescue, tall fescue and colonial bentgrass (-0.4) and a higher correlation with chroma for the same three grasses (-0.6, -0.5, -0.6). Nitrogen did correlate with colonial bentgrass in transmission measurements for hue (0.8) but transmission analysis in general did not link as closely to nitrogen content in fine fescue or tall fescue. Colonial bentgrass, creeping bentgrass and Kentucky blue grass had stronger links between color characteristics and tissue nitrogen content when using transmission analysis (Table 2.6).

Cultivar differences were noted within grasses. They did not follow visual rating for reflection analysis and there was limited similarity in the rankings from the
transmission measurements (Table 2.4). The visual rating did separate cultivars though not following the same ranking as NTEP evaluations to a large extent. Perennial ryegrass had similar rankings but not values, while tall fescue had limited separation ($P=0.05$) using human data (Table 2.7).

**DISCUSSION AND CONCLUSION**

The correlation between key components of turfgrass color and objective measurements using reflectance and transmission systems in this study was limited. Pigment concentrations in our study had a poor relationship with the handheld measuring system in particular. This is consistent with Stiegler at al., (2005) who stated that pigment concentration could only account for 36% of the variability in turfgrass color and that other factors play a role when using reflective measuring devices. The studies that had success with the reflectance system included variations in nitrogen rates as part of the studies (Landschoot and Mancino, 1997; Mangiafico and Guillard, 2005). The variation in nitrogen rates would presumably enhance differences in turfgrass colors making it more likely that reflection analysis could distinguish differences similar to human eyesight. Forcing of differences was not the aim of this trial so nitrogen rates were not altered.

There was some what more correlation between pigment concentrations in our study and transmission measurements. The use of the extract and use of the spectrophotometer equipment may have been more beneficial for this relationship. In using reflectance systems there also could have been an increase in light scattering from
the leaf surfaces, possibly reducing the precision of the results. The isolation of the pigments through extraction would also have helped the transmission results and offered a clear influence on the results. There may be a greater opportunity to more strongly correlate the reflectance measurements if scattering from the leaf surfaces can be reduced.

Other studies have concentrated on using smooth surface turfgrass leaves such as Kentucky bluegrass and creeping bentgrass. Turfgrass surfaces which are known for varied veination and narrower leaves such as the tall fescue, perennial ryegrass and fine fescues used here offer a problem for attempting to obtain a consistent reflectance type measurement. In tall fescue for instance with a change in ploidy from 4X to 8X, which could have played a role in this study, there was a decrease in vein number on the leaf surface of 14.2 to 10.2 in major veins. Minor veins on average increase from 4.2 to 6.6 with ploidy changes (Byrne et al., 1981). These surface differences and leaf angle play a role in light reflectance properties; abaxial surfaces reflect greater levels of light in grasses than adaxial surfaces. The structure of the epidermal layer (Stiff and Powell, 1974; Thomas and Barber, 1974) which varies in grasses plays a large role in their optical properties (Sheehy, 1975). Glaucous leaves of eucalyptus (*Eucalyptus obliqua* L’Hér.) absorbed only 37% of total radiation while green leaves absorbed 50% of total radiation (Thomas and Barber 1974). These are all possible areas that would affect the use of a handheld system in our study which could reduce its reproducibility.

For a more accurate assessment of the ability of these handheld systems other pigments may need to be taken into account when working with reflectance
measurements. Flavonoids absorb light up to 430 nm (Saxby, 1964) and thus reach into the visible light range; variations in their concentrations can occur with leaf age and also location in the leaf (Morales et al., 2011). The carotenoids also absorb blue light in the visible spectrum (Zscheile et al., 1942). The pools of carotenoids found in turfgrass including lutein and the xanthophylls can change based on irradiance levels (McElroy et al., 2006). Anthocyanins also absorb light in the 500-530 nm region (Gould, 2004), and may play a role in light wavelengths reflected in that region of PAR. We did not measure these pigments and they may have played a role in creating the variability we found using the handheld system in this study.

Leaf texture may also play a role in the lack of strong data correlation. Grasses which possess leaf hairs and trichomes such as buffalograss (Buchloe dactyloides L.) also offer a problem for this equipment. There may be increased scattering of radiation on turfgrasses thus increasing diffuse reflectance (Gausman and Cardenas, 1969; Chien and Sussex, 1996). Texture influences not only turfgrasses as it has been noted in the food industry that these issues do exist when analyzing color (Giusti and Wrolstad, 2005.)

It could be expected that visual ratings by untrained evaluators in our study would not follow NTEP rankings due to a lack of training and possible differences in light and locations. There can also be variation between trained observers and their color rankings (Landschoot and Mancino, 1997). The value of having trained observers may be lost in this instance with greater argument for use of objective standardized rankings of color. Variability in human eyesight and perspective of color allows for variation in comparison to NTEP trials and also as compared to trained evaluators. Due to location the potential
for visual ratings varying is increased with disparities in light intensity and light angle playing a role in color analysis (Giusti and Wrolstad, 2005). This issue is part of the reason that there is interest in using a standardized system to analyze turfgrass color. The transmission and reflectance methods which offer a standardized color system with CIELab did not follow the visual ratings with NTEP or local visual ratings. This is despite the fact the two systems are designed to replicate human eyesight in standard daylight illumination. This is the first time such a broad scale measurement of turfgrass species and turfgrass cultivars has been tested however, and previous studies did not aim to link results to NTEP trial results.

Visual analysis is linked closest to nitrogen content, which has been noted before. Higher nitrogen rates lead to higher color rankings (Guertal and Evans, 2006; Frank et al., 2004). Transmission and reflection based analysis also linked most strongly with nitrogen although in this research the correlation values of 0.4 are not considered strong. Altering nitrogen rates could lead to a more favorable result (Landschoot and Mancino, 1997). The measurement of nitrogen rather than color may prove to be a better option for future research as a broader database is created for turfgrasses and reflectance-based measurements using the same field equipment or, at minimum, the same scale.
Table 2.1: Normality test results and data processing results for six cool season grasses evaluated for color using transmission and reflection type measurements post ROBUSTREG analysis.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Skew</th>
<th>Kurtosis</th>
<th>Kolmogorov - Smirnov</th>
<th>Skew</th>
<th>Kurtosis</th>
<th>Kolmogorov - Smirnov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine fescue</td>
<td>0.6</td>
<td>2.4</td>
<td>0.05 NS§</td>
<td>0.6</td>
<td>1.5</td>
<td>0.03 *</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>0.7</td>
<td>1.5</td>
<td>0.04 *</td>
<td>0.5</td>
<td>0.6</td>
<td>0.05 NS</td>
</tr>
<tr>
<td>Colonial Bentgrass</td>
<td>0.3</td>
<td>0.8</td>
<td>0.05 NS</td>
<td>0.4</td>
<td>0.5</td>
<td>0.07 NS</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>0.6</td>
<td>0.8</td>
<td>0.03 *</td>
<td>0.6</td>
<td>1.0</td>
<td>0.07 NS</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>0.5</td>
<td>1.4</td>
<td>0.04 *</td>
<td>0.4</td>
<td>0.5</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>0.7</td>
<td>1.3</td>
<td>0.05 NS</td>
<td>0.6</td>
<td>0.7</td>
<td>0.03 *</td>
</tr>
</tbody>
</table>

* Indicates significance in data different from normal at $P=0.05$.
§ NS, Non significant at $P=0.05$.
† Data considered to have skew of 0 are considered normal.
‡ Data considered to have kurtosis of 0 are considered normal.
A measure of color using CIELab system – 3-d color system using lightness / red-green / blue-yellow components.

A measure of intensity of color using CIELab.

The red-green component of the color system.

The blue yellow component of the color system.

Correlation values below 0.7 are considered to have limited relationship between components analyzed.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Chl Total</th>
<th>Chl a</th>
<th>Chl b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hue†</td>
<td>Chroma‡</td>
<td>A§</td>
</tr>
<tr>
<td>Fine fescue</td>
<td>0.1</td>
<td>0.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>0.0</td>
<td>-0.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>Colonial bentgrass</td>
<td>-0.5</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>-0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>0.0</td>
<td>-0.4</td>
<td>-0.2</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>-0.5</td>
<td>-0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chl Total</th>
<th>Chl a</th>
<th>Chl b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hue</td>
<td>Chroma</td>
</tr>
<tr>
<td>Fine fescue</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>0.0</td>
<td>-0.4</td>
</tr>
<tr>
<td>Colonial bentgrass</td>
<td>-0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>-0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>0.0</td>
<td>-0.4</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>-0.4</td>
<td>-0.0</td>
</tr>
</tbody>
</table>

Table 2.2: Pearsons correlation values for chlorophyll pigmentation and transmission characteristics obtained using a Hunter XE colorquest colorimeter on extracts of six cool season turfgrasses.
Correlation values below 0.7 are considered to have limited relationship between components analyzed.

Table 2.3: Pearson's correlation values for chlorophyll pigmentation and tissue nitrogen levels visual rating of six cool season turfgrasses.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Pigment / g(^1)</th>
<th>Pigment / m(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl total</td>
<td>Chl a</td>
</tr>
<tr>
<td>Fine fescue</td>
<td>-0.2(^\dagger)</td>
<td>-0.2</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>-0.1</td>
<td>-0.0</td>
</tr>
<tr>
<td>Colonial bentgrass</td>
<td>-0.2</td>
<td>-0.2</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>-0.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>-0.3</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

\(^\dagger\) Correlation values below 0.7 are considered to have limited relationship between components analyzed.
<table>
<thead>
<tr>
<th>Grass</th>
<th>Transmission</th>
<th>Reflectance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hue†</td>
<td>Chroma‡</td>
</tr>
<tr>
<td>Fine fescue</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Colonial bentgrass</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>0.1</td>
<td>-0.0</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>-0.1</td>
<td>-0.0</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>-0.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

† A measure of color using CIELab system – 3-d color system using lightness / red-green / blue-yellow components.
‡ A measure of intensity of color using CIELab.
§ The red-green component of the color system.
¶ The blue yellow component of the color system.
Correlation values below 0.7 are considered to have limited relationship between components analyzed.

Table 2.4: Pearsons correlation of visual rating to transmission and reflection color characteristics of six cool season turfgrasses.
<table>
<thead>
<tr>
<th>Grass</th>
<th>Chl Total</th>
<th></th>
<th>Chl a</th>
<th></th>
<th>Chl b</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hue†</td>
<td>Chroma‡</td>
<td>A§</td>
<td>B¶</td>
<td>Hue‡</td>
<td>Chroma</td>
</tr>
<tr>
<td>Fine fescue</td>
<td>-0.4</td>
<td>0.2</td>
<td>0.0</td>
<td>-0.4</td>
<td>-0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>-0.1</td>
<td>-0.1</td>
<td>0.2</td>
<td>-0.1</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
<tr>
<td>Colonial bentgrass</td>
<td>-0.2</td>
<td>-0.3</td>
<td>0.4</td>
<td>-0.4</td>
<td>-0.2</td>
<td>-0.3</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>0.0</td>
<td>-0.0</td>
<td>0.0</td>
<td>-0.0</td>
<td>0.0</td>
<td>-0.0</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>0.1</td>
<td>0.1</td>
<td>-0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>0.1</td>
<td>0.0</td>
<td>-0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

† A measure of color using CIELab system – 3-d color system using lightness / red-green / blue-yellow components.
‡ A measure of intensity of color using CIElab.
§ The red-green component of the color system.
¶ The blue yellow component of the color system.
Correlation values below 0.7 are considered to have limited relationship between components analyzed.

Table 2.5: Pearsons correlation values for chlorophyll pigmentation and reflection characteristics obtained using a Konica CR310 handheld colorimeter on surfaces of six cool season turfgrasses.
A measure of color using CIELab system – 3-d color system using lightness / red-green / blue-yellow components.

† A measure of intensity of color using CIELab.
§ The red-green component of the color system.
¶ The blue yellow component of the color system.

Correlation values below 0.7 are considered to have limited relationship between components analyzed.

Table 2.6: Pearsons correlations between reflectance measurements and transmission measurements with tissue nitrogen for 6 cool season turfgrasses.

<table>
<thead>
<tr>
<th>Grass</th>
<th>Reflectance</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hue†</td>
<td>Chroma‡</td>
</tr>
<tr>
<td>Fine fescue</td>
<td>-0.4</td>
<td>-0.6</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>-0.4</td>
<td>-0.5</td>
</tr>
<tr>
<td>Colonial bentgrass</td>
<td>-0.4</td>
<td>-0.6</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>0.1</td>
<td>-0.0</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>-0.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>Cultivar</td>
<td>Colonial bentgrass</td>
<td>Fine fescue</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------</td>
<td>------------</td>
</tr>
<tr>
<td>1</td>
<td>6.4 a†</td>
<td>7.7 ab</td>
</tr>
<tr>
<td>2</td>
<td>5.4 c</td>
<td>6.4 c</td>
</tr>
<tr>
<td>3</td>
<td>6.1 ab</td>
<td>8.0 a</td>
</tr>
<tr>
<td>4</td>
<td>6.4 a</td>
<td>8.0 a</td>
</tr>
<tr>
<td>5</td>
<td>5.2 c</td>
<td>6.1 c</td>
</tr>
<tr>
<td>6</td>
<td>5.6 ab</td>
<td>6.1 c</td>
</tr>
<tr>
<td>7</td>
<td>n/a†</td>
<td>5.5 d</td>
</tr>
<tr>
<td>8</td>
<td>n/a</td>
<td>7.3 b</td>
</tr>
<tr>
<td>9</td>
<td>n/a</td>
<td>7.3 b</td>
</tr>
<tr>
<td>LSD§ (P=0.05)</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

† Data followed by a different letter in each column represent significant differences between cultivars (P=0.05).
‡ Only six cultivars could be found for colonial bentgrass.
§ Means separation carried out using Fishers least protected means (P=0.05).

Table 2.7: Human color rankings for cultivars of six cool grasses. (1= brown / 6 = acceptable / 9 = optimum).
† Directional line indicates influence of component on either transmission or reflection analysis.
PC = principal component.
PC1 = Influence of chlorophyll total, chlorophyll a and b, hue, chroma L* a* b* on transmission color analysis.
PC 2 = Influence of chlorophyll total, chlorophyll a and b, hue, chroma L* a* b* on reflection color analysis.

Figure 2.1: Principal component analysis for turfgrass samples analyzed for color using reflectance and transmission measurements.
REFERENCE


CHAPTER 3

INFLUENCE OF ULTRAVIOLET LIGHT ON GERMINATION RATE AND SPEED OF KENTUCKY BLUEGRASS (Poa pratensis L.)

ABSTRACT

Kentucky bluegrass (Poa pratensis L.) is a commonly used turfgrass in the temperate climates of the United States. The grass is predominantly established from sod, because of its slow time to germinate (21 d). Ultraviolet light has been noted to enhance germination speed of other crops and it may similarly enhance Kentucky Bluegrass germination. Turfgrass seeds from two seed lots were placed in two separate germination chambers and set on an 8hr / day 16hr / night schedule. Supplemental ultraviolet light (11.2 kJ / m² / d⁻¹) was added on a treatment of two 1 hr time periods split over a 3 hour period versus no UV light. The treatments were applied for 21 days. Seed germination was counted at 7, 14 and 21 days. Ultraviolet light increased the germination completeness and speed of germination in the newer but not older seed lot. Seed germination was greater ($P=0.05$) at day 7 and after 21 days there was a greater germination percentage or completeness in ultraviolet light conditions than in control in seed lot one but not lot two. These results suggest that treatment of bluegrass seed with a UV light may enhance germination; however seed age may override the effect.
INTRODUCTION

Turfgrass seed germination is dependent on a number of environmental factors. Manipulating the light environment may be a method to enhance germination speed and completeness. Kentucky bluegrass (Poa pratensis L.) may reach only 60% germination after 28 days and 90% germination after 4 months (Maguire and Steen, 1971). This is a low rate of germination and leads to difficulties in establishment of the turfgrass. Treatments to enhance this have included the use of hydrogen peroxide to increase fresh weight of seed and germination by 20% and 30% respectively (Takabe, 2004). There are limited methods to improve its germination completeness and hence the establishment of Kentucky bluegrass lawns is limited by speed of seed germination. Kentucky bluegrass is considered a favorable turfgrass in the northern United States variety due to its spreading growth habit and desirable color especially when nitrogen rates are increased (Skogley and Ledeboer, 1968).

Methods to enhance germination speed and vigor of grass seedlings include seed placement in favorable moisture conditions prior to seeding. This resulted in a 40hr decrease in germination time of crested wheatgrass (Agropyron cristatum L.) (Keller and Bleak, 1968). The aim of this method is to have the seed active prior to seeding and thus naturally increase the germination rate. Priming seed with initial hydration followed by air drying has proven to increase germination speed and seedling fresh mass (Pill et al., 1997) in Kentucky bluegrass. Osmoconditioning or pre-treating with water, of perennial ryegrass (Lolium perenne L.) seeds increased germination rate and root growth even under suboptimum temperatures (Danneberger et al., 1992). Sonication for 4 minutes
significantly increased germination in orchids (*Calanthe discolor* L.) by 50% (Miyoshi and Mii, 1988). This was due to a perceived requirement for scarification of a thicker seed coat. Different energy treatments using microwaves removes fungal propagules thus reducing the need for fungicide applications and this had no effect on germination rates (Cavalcante and Muchovej, 1993). Gamma radiation of tall fescue (*Schedenorus phoenix* (Scop.) Holub) reduces endophyte levels by ~90% while not affecting germination (Bagegni et al., 1990).

The potential for the use of ultraviolet-B (UV-B) light in promoting germination has received no attention in turfgrass seedling studies. Light is crucial in controlling plant germination in Kentucky bluegrass (Toole and Borthwick, 1971), as well as plant growth and development (Frankhauser and Chory, 1997). Ultraviolet light, (200-400nm) has higher energy compared to photosynthetically active radiation (400-700nm), (PAR). The impact of ultraviolet-C (UV-C) has been found to be inhibitory in sunflower (*Helianthus annuus* L.). This effect, however, can be reversed during dark storage of seeds after the UV-C treatment (Torres et al., 1991). Kale, cabbage, (*Brassica oleracea*), radish (*Raphanus sativus* L.) and agave (*Agave* L) treated with UV-B light germinated more rapidly but subsequent growth was markedly inhibited (Noble, 2002). The number of seeds and dry weight of seeds in *Bromus catharticus* receiving 90% UV-B almost doubled compared to the control (Deckmyn and Impens, 1998). There was, however, not an effect on germination. The objective of this study was to investigate if ultraviolet light could be used as a treatment to speed germination of Kentucky bluegrass and to increase average germination completeness.
MATERIALS AND METHODS

Plant material

Kentucky bluegrass seeds from two different seed lots based on age of the same cultivar ‘Bariris’ were used in this trial (Barenbrug USA, Tangent, OR). Fifty turfgrass seeds were placed in each of 9 petri-dishes (9 cm diam.) for a total 450 seeds per light treatment. Two repeated studies for each lot were carried out in each of two growth chambers with a day / night regime of 8 / 16 hours and temperatures of 25 / 15\degree C. The visible light (400 nm – 700 nm) intensity was measured using cosine corrected light meters (Spectrum Technologies, Plainfield, IL). Ultraviolet light was provided by four Reptisun 10.0 UV overhead fluorescent tubes (Zoo Med Laboratories, San Luis Obispo, CA) in one chamber. The ultraviolet light was measured using a portable IL1350 radiometer with UV-B sensor (International Light, Newburyport, MA). The UV light was then reported as kJ / m\(^{-2}\) / d\(^{-1}\). Each study was conducted for twenty-one days. The lights were switched between chambers for each experiment. The petri dishes were covered in both chambers for 21 hours per day for moisture retention. Seeds were placed on standard blue blotters (Anchor Paper Co., Plymouth, MN), to ensure seed stability and consistent moisture levels. Prior to daily UV treatment initiation, covers were removed to prevent any reflection of UV rays from seed surfaces.

Data collection and analysis

Initial treatment of seed with distilled H\(_2\)O at 5 mL per day occurred and this then decreased to 3 mL per day after 14 d as seed volume and water requirements decreased.
Radicle emergence was used to determine germination and seed count data were collected at 7, 14 and 21 days. Germination rate based on radicle emergence against total seeds used and germination vigor based on seed counts were analyzed. The petri dishes were set up in a randomized complete block design to reduce the impact of lighting variability. Data were analyzed using the general linear models (GLM) procedure of the SAS program (SAS Institute, 2008). The data from both seed lots were pooled from each repetition. Fisher’s protected least significant difference testing (LSD) was used to test for differences in the average germination values and total germination percentages. Data were combined to check for differences between seed lots.

RESULTS AND DISCUSSION

Treatments of UV totaled 11.2 kJ m\(^{-2}\) d\(^{-1}\) in the UV chamber, and daily PAR was 4.3 mol m\(^{-2}\) d\(^{-1}\). The control chamber had a total PAR of 4.2 mol m\(^{-2}\) d\(^{-1}\) but no detectable level of UV light. There was no supply of UV light in the UV-A region (320 – 400 nm) in either chamber. Due to significant differences (\(P=0.05\)) in overall percentage germination between the two seed lots data were analyzed separately. The newer seed lot which was one year old (Lot 1), had significantly higher (\(P=0.05\)) germination rates compared to the older seed lot (>2 years old) in UV (Table 3.1). There was lower germination rates in control light environment in Lot 1 compared to Lot 2 (Table 3.1). The age of the seed may play a role in the germination percentage change in the UV chamber as has been noted previously where older seeds had reduced germination percentages (Shaidae et al., 1969).
Overall total germination percentage was significantly higher (Figure 3.1) in lot 1 for UV treated seeds compared to control seeds. In tests on lot two UV germination decreased from lot one. The control total germination percentage rates in lot two increased from tests on lot one. This meant there was no overall separation between the germination rates due to the light treatments (Figure 3.2).

Days to germination of lot one was affected by light treatments. Applications of ultraviolet light significantly ($P=0.05$) enhanced seed germination on day 7 compared to the control treatment. The cumulative germination percentage was still higher at day 14 for the UV chamber in lot one but not lot two (Figure 3.1 and 3.2). On day 21 both sets of light treatments were significantly different in lot one. The observation of higher germination on day 7 resulted in significantly faster germination times in the UV treated grass chambers (Figure 3.1). The pattern of greater seed germination ($P=0.05$) on day 7 in the first experiment was also repeated in the second experiment for seed lot one (Figure 1). Seed lot one almost reached germination completeness after 21 days in UV, however data was not collected past this date.

The same results were not observed with the 2nd seed lot. UV treatment did not result in increased germination by day 7 in seed lot 2 (Figure 3.2). The initial difference at day 7 from seed lot one was not noticed in the second seed lot. The second seed lot demonstrated the greatest percentage of germination on day 21 in both experiments and there was no significant difference between light treatments (Figure 3.2). Overall germination decreased in UV conditions in lot 2 compared to lot 1 while there was an increase in germination from lot one to seed lot two in control conditions (Table 3.1).
CONCLUSIONS

The variation between the seed lots means that further work is required in order to conclusively determine if a clear benefit due to UV irradiance exists. The initial results were very promising and agree with data produced from lettuce and wheat seed (Noble, 2002, Wagné, 1966). The amount of published work done with ultraviolet light on turfgrass germination is limited and variability in seed lots due to age and weight has been noted previously regardless of light treatment (Larsen and Andreasen, 2004, Shaidaee et al., 1969). This may be a large part of the reason for the difference in results and a specific cultivar which has more readily available seed lots of similar age would be desirable.

The use of specific wavelengths also requires investigation. The experimental system provided elevated UV-B levels but it also had UV-A wavelengths. This was due to a size restriction with UV-B specific lights. If the study focused solely around the main UV-B wavelengths it may enhance the biologically activity spectrum (Caldwell, 1971). It has been noted in that UV-C (100 nm - 290 nm) does not provide potential for germination enhancement, with inhibition of sunflower germination (*Helianthus annus* L.), (Torres et al., 1991). The wavelengths and energy associated with ultraviolet light may have triggered response from the light receptor phytochrome – A, especially at low fluence rates which irreversibly trigger germination (Shinomura et al., 1996).

Field testing is also a step that has to be considered as well as combing hydration and temperature cycles with the UV irradiation in future studies. It does offer a potential option however to produce enhanced Kentucky bluegrass germination if consistency can
be found with the seed lots. This would allow for greater seasonal use of the grass in home lawn establishment.
<table>
<thead>
<tr>
<th>Lot</th>
<th>Df</th>
<th>Ultraviolet Germination</th>
<th>Control Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td></td>
<td>------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>81.6 a†</td>
<td>60.3 b</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>71.6 b</td>
<td>70.7 a</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>5.8</td>
<td>8.9</td>
<td></td>
</tr>
</tbody>
</table>

† Means followed by different letter were significantly different (P=0.05)

Table 3.1: Germination percentages of Kentucky bluegrass (*Poa pratensis* L.) seed lots treated with PAR (400 – 700 nm) and ultraviolet light (300 – 400 nm).
Figure 3.1: Percentage germination of Kentucky bluegrass (*Poa pratensis* L.) seed treated with photosynthetically active radiation (400 – 700 nm) and ultraviolet light (280 – 480 nm) in newer seed lot one.
Figure 3.2: Percentage germination of Kentucky bluegrass (*Poa pratensis* L.) seed treated with photosynthetically active radiation (400 – 700 nm) and ultraviolet light (280 – 480 nm) in older seed lot two.
REFERENCE


CHAPTER 4

ULTRAVIOLET-B LIGHT INFLUENCE ON COOL SEASON TURFGRASS GROWTH AND MORPHOLOGY

ABSTRACT

Ultraviolet light is a potential issue for plant growth and development due to its effect on photosynthesis and plant productivity. Turfgrass plants may be affected by ultraviolet light causing loss of quality and ability to recover from injury. The objective of this study was to investigate the effects of ultraviolet light on turfgrass productivity and morphology in three cool season grasses. Tall fescue, (Schedonorus phoenix (Scop.) Holub.), creeping bentgrass (Agrostis stolonifera L.), cultivars ‘Penncross’, ‘L-93’ and perennial ryegrass (Lolium perenne L.), were selected due to limited or no information on their growth and development in elevated ultraviolet light conditions at lawn height of 10-12 cm. The impact of ultraviolet light treatment on color, root and shoot biomass and tillering was measured over a four week period of exposure in repeated experiments. Ultraviolet-B light was tested in particular and light levels were measured at 16 kJ m$^{-2}$ d$^{-1}$ biologically effective light in growth chambers programmed for a day/night regime of 14/10 hours. Ultraviolet-B light significantly ($P=0.05$) reduced shoot and root production in all grasses and inhibited tiller production ($P=0.05$). Turfgrasses exposed to this level of ultraviolet-B light at lawn height of cut showed visual damage and significantly lower
color rankings after 2 weeks. The experiment demonstrated that after extended exposure to ultraviolet-B light there is a loss of productivity and color in cool season turfgrasses.

INTRODUCTION

Light plays a crucial role in turfgrass growth and development. It is normally considered most important in the wavelengths of 400-700 nm; photosynthetically active radiation (PAR), (Pons et al., 1993). Light wavelengths can often vary and spectral distribution can fluctuate with environmental conditions (Bell et al., 2000). There is interest in how the degradation of the ozone layer will influence spectral distribution on the earth’s surface (Manney et al., 2011). The decrease in ozone is predicted to lead to a significant increase in exposure to wavelengths in the ultraviolet (UV) range (Kerr and McElroy, 1993). Ultraviolet light is found in the range of 10 – 400 nm and the energy associated with these wavelengths is considered damaging when compared to PAR (Fiscus and Booker, 1995). The UV-B wavelengths which hit the surface of the earth are predicted to increase in springtime radiation by 50-60% from 2010-2020 (Taalas et al., 2000) in the northern hemisphere. The reduction in ozone and a resulting increase in UV irradiance has been linked to chlorofluorocarbons in the atmosphere (Rowland, 1990).

It has been shown that leaf area indices of some Poaceae species can change in response to different levels of biologically effective UV-B irradiance (Caldwell, 1971; Deckmyn and Impens, 1999). Increases in leaf and axillary shoot production in response to UV-B exposure are noted in both monocot crops such as wheat (Triticum aestivum L.), and dicots such as beans (Phaseolus vulgaris L.), (Barnes et al., 1990). Red fescue
(Festuca rubra L.) growth was positively affected by UV-B radiation in all seasons (Deckmyn and Impens, 1999), showing potential for differences in response to exposure. Epidermal thickness alters leaf absorbance properties so much that it is considered an excellent predictor of the effectiveness of a plant’s ability to screen UV-B light (Day, 1993). Root length was inhibited at UV-B irradiances of 2.5 – 5 kJ m$^{-2}$ d$^{-1}$ in Antarctic hairgrass (Deschampsia antartica L.), while there was an increase in tillering but no overall impact in the relative growth rate due to the compensation in tiller production compared to root loss (Rozema et al., 2001). Reductions in stem elongation and leaf expansion in young plants of long spined thorn apple (Datura ferox L.) occur in UV-B light (Ballare et al., 1996). Smaller leaf sizes in white clover (Trifolium repens L.) also have been influential in inferring tolerance to UV-B exposure (Hoffman, 2000). In bean plants, low levels of PAR, combined with UV-B treatments, led to a decrease in leaf area of 47% and a decrease in leaf dry weight of ~25% (Cen and Bornman, 1990). Ultraviolet light exposure is linked to reduced photosynthesis due to direct damage to photosystem II (Brandle et al., 1977), which results in decreased leaf blade and internode lengths and plant productivity.

Varietal differences in rice (Oryza sativa L.) following exposure to UV have been observed. Some cultivars had smaller leaf areas, stunted plant height and a reduction in relative growth rate whereas others showed little response. Plants with rapid growth rates such as squash (Cucurbita pepo L.) tend to be more sensitive to damage while slower growing plants such as peppers (Capsicum frutescens L.) are tolerant of the UV-B irradiance (Smith et al., 2000). These effects were not noted until after 2 weeks of UV-B
treatment (Dai et al., 1992). Perennial ryegrass and orchardgrass (Dactylis glomerata L.) biomass production was reduced in response to comparable spring and summer treatments of elevated UV levels (Deckmyn and Impens, 1999). In poplars (Populus cathayana Rehd.), however, there was no effect on biomass following exposure to different levels of UV-B (Zhang et al., 2011). Variability among plant species in response to UV-B has been noted in review papers and could be related to other issues such as temperature, or concentrations of CO₂ (Krupa and Nickert, 1989). Limited work has been conducted to determine the impact of UV-B on vegetative productivity of turfgrasses that would be used in homelawn situations covering potentially 163,800 km⁻² ± 35,850 km⁻² in the United States (Milesi et al., 2005).

Ultraviolet light has varying effects on quality ratings among the turfgrasses. Chewings fescue (Festuca rubra L. ssp. commutata Gaud.), is considered the most UV-B tolerant turfgrass with Kentucky bluegrass being considered the least tolerant (Zhang and Ervin, 2009). Quality ratings were reduced by as much as 74% for Kentucky bluegrass after 10 days of exposure. There was also cultivar variation in the tolerance of UV-B in this trial. Darker green Kentucky bluegrass cultivars are thought to be also more tolerant of UV-B conditions (Ervin et al., 2004c). In other plants such as apples (Malus domestica) ‘Royal Gala’, increasing the red color post-harvest with the use of UV-B treatments has been seen as a benefit to production (Dong et al., 1995). This response was due to increased production of flavonoid-type pigments. Combined treatments of UV-B and visible light were most effective in enhancing the color in other apple cultivars such
as *Malus domestica* Borkh., cv. ‘Aroma’, (Hagen et al., 2007). The resulting increase in red color on the skin in both cases added value to the apple crop.

Limited research has been conducted to test the effect of UV-B exposure on turfgrasses at higher heights of cut such as home lawns and the estimated area covered by irrigated turfgrass crop covers potentially 163,800 km$^{-2}$ (Milesi et al., 2005). The objective of this study was to test four cool season grasses for their growth rate, tillering and color responses to elevated UV-B conditions predicted to occur in this decade (Taalas et al., 2000). It was hypothesized that thicker-leaved species, such as perennial ryegrass and tall fescue would have greater tolerance to UV-B than thin leaved species like Kentucky bluegrass and creeping bentgrass.

**MATERIALS AND METHODS**

*Sample preparation*

Experiments were initiated in March, 2011 and May, 2011 with no noticeable differences in turfgrasses following establishment in Columbus, Ohio. Turfgrasses were initially seeded into a mason sand rootzone containing >97% sand (Hummel & Co, Trumansburg, NY) as a single seed with structured timing to match germination speeds. The rootzone met United States Golf Association (USGA) classification and had a pH of 7.4 with 2.7% clay silt fraction. Creeping bentgrasses were seeded first into 3.8 cm diameter ‘conetainers’ (Steuwe & Sons, Tangent, OR) and 4 days later perennial ryegrass and tall fescue were seeded. Each conetainer contained a single plant. The plants were placed in a mist house in a ten minute misting cycle of 15 seconds per water application
to maintain moisture. Turfgrasses were grown for 6 weeks and initially treated with Peters 20-20-20 (Scotts-Miracle Gro, Marysville, OH) at a rate of 11.8 kg ha\(^{-1}\) every two weeks. Six weeks after germination all plants were removed and placed in a greenhouse maintained at \(\sim 20^\circ\text{C}\) for four more weeks to allow for maturation. Imidacloprid N-[1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl]nitramide (Bayer Environmental Science, Research Triangle PK, NC) was applied to prevent insect damage. Mefenoxam (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester (Syngenta AG, Greensboro, NC) and thiophanate-methyl (dimethyl 4,4’-o-phenylenebis[3-thioallophanate]) (Cleary Chemicals, Dayton, NJ) were applied for preventative disease control. Water was applied for a 5 minute period daily to prevent wilt, with automated heads using an ESP modular irrigation system (Rain Bird, Azusa, CA). Ten weeks after turfgrass emergence, plants were placed in Conviron E15 growth chambers (Controlled Environment Ltd., Winnipeg, Canada). Plants were left in complete darkness at 20 °C for 24 hours.

Ultraviolet-B treatment was implemented using specific UV-B lamps that have peak light emission at 313 nm, QUV UV-B 313 nm, (Q-Lab Corp., Westlake, OH). The control treatment consisted of only light from fluorescent lamps. The UV-B lamps did not produce any extra visible radiation. Treatment exposure was calculated based on the predicted time of exposure and measured lamp irradiance. Radiation from ultraviolet sources were measured using both UV-B and UV-A sensors attached to an IL1350 photometer (International Light, Newburyport, MA). The radiation is reported in kJ m\(^{-2}\) d\(^{-1}\). Photosynthetic active radiation was measured every 15 minutes for the period of the
study using a cosine corrected light sensor (Spectrum Technologies, Plainfield, IL) and reported as mol m\(^{-2}\) d\(^{-1}\). The chambers were programmed for a day/night length of 14/10 hours. Photosynthetic active radiation (PAR) in the range of 400 – 700 nm was provided in all chambers using 85 watt s\(^{-1}\) high output F12T12-CW.HO fluorescent tubes (General Electric, Fairfield, CT). Water was applied twice daily to prevent wilting. Temperature in each chamber was set to 20°C during light exposure and 17 °C in darkness. Fertility treatments continued for the duration of each study. Turfgrass plants were placed 0.4 m below the lighting in an even spacing.

Data collection and analysis

The experiment was designed as a randomized complete block with weeks being randomized in each block. Under each light regime, there were three blocks with four replications per block of each grass for a total of 12 samples of each grass per measurement. Data were analyzed using the linear model (GLM) method in SAS Institute 9.2 (2008). Means separation was carried out using Fishers protected least significant difference test (\(P=0.05\)) and data were pooled. At the end of each week samples were harvested and separated into roots and shoots, then dried at 70 °C for two days. The shoot: root ratio was then calculated based on dry weight (Gwynn-Jones and Johanson, 1996). Tissue was dried in a VWR 1350F (Univar USA, Redmond, WA) oven in 8.9 x 16.5 cm coin envelopes (Officemax, Naperville, IL). Relative growth rate was calculated based on the formula of Gardner et al., (1985):
RGR = Relative growth weight; lnW2 = natural log weight 2; lnW1 = natural log weight 1; T1 = Time 1; T2 = Time 2.

\[
RGR = \frac{\ln W2 - \ln W1}{T1 - T2}
\]

Tillering was measured by counting daughter plants extending from the crown of each seeded container. Tillers were counted when samples were harvested for each week of treatment at weeks 1, 2, 3 and 4. Tillers were defined as daughter plants arising directly from the crown individual plants. Color rankings were carried out on a 1-9 ranking scale with 1 = dead/brown 9 = dark green 6 = acceptable. The color data were collected prior to trial initiation and weekly during the trial under the same light conditions.

RESULTS

Turfgrasses in the UV-B treated environment received a total of 24 kJ m\(^{-2}\) d\(^{-1}\). Once this was adjusted for UV-B biologically effective radiation the total effective UV-B light seen by turfgrasses (Caldwell, 1971) was decreased to 16 kJ m\(^{-2}\) d\(^{-1}\) (Table 4.1). The daily photosynthetic integral totaled 18.5 mol m\(^{-2}\) d\(^{-1}\) (Table 4.1).

After 3 weeks, growth of all grasses in ultraviolet light was significantly inhibited (\(P=0.05\)) compared to grasses grown in the control light environments. There was, however, a decrease in growth rate in the control chambers after four weeks and so no significant differences were observed. The growth rate in UV conditions, however, had
become negative as turfgrasses began to completely fail (Figure 4.1). There was variability noted within the data, which occurred despite using twelve different samples from each grass at each sampling date.

There was a significant decrease ($P=0.05$) in color quality ratings for all grasses exposed to UV-B (Figure 4.2). There was a 22% decrease in the ‘L-93’ color and a 17% decrease in ‘Penncross’ color. Tall fescue and perennial ryegrass had a loss of 18% and 15% in color quality respectively. After four weeks, both creeping bentgrass cultivars were ranked below acceptable levels and visible damage was clearly seen with browning and yellowing of the leaf surfaces (Figure 4.2), (Appendix B). Dark lesions similar to burn spots were also noted on the UV-treated bentgrass plants in the UV-B treatment. The loss of color was not observed until week two of the experiments and was consistent in both trials (Appendix B). The color differences were consistent after week two until the end of each experiment. There was some reddening of stems and tissue material but this was not consistently observed.

Under UV-B conditions in experiment one there was a clear inhibition ($P=0.05$) of plant tillering in three of the grasses (Figure 4.3). There was a 40% inhibition of tillering in creeping bentgrass ‘L-93’ and 39% decrease in the creeping bentgrass ‘Penncross’ cultivar. Perennial ryegrass was not inhibited significantly in either experiment. In experiment one, tall fescue tillering was significantly inhibited ($P=0.05$) when it was treated with UV-B light. There was a 30% decrease of tall fescue tillering by 30% in experiment one but this did not occur in experiment two. In experiment two, there was an overall decrease in tillering regardless of light environment. The bentgrasses
exposed to UV-B had lower tiller numbers in experiment one but they were not significantly different from the control treatment in experiment two (Figure 4.4). This was possibly due to greater light intensities in the maturation of plants in experiment 2 which occurred two months later in the year. Perennial ryegrass tiller number was not significantly different in response to light regime, between the light treatments. The root : shoot ratio did not change significantly in response to light regime, but the data were highly variable.

DISCUSSION

The decrease in growth rate in the bentgrasses after exposure to UV light has been reported previously (Schmidt and Zhang, 2001). Reducing the efficiency of the photosynthetic machinery due to photolytic damage would effectively reduce plant productivity and explain previous findings. Biological variation and seasonal light changes affect plant maturation and may have affected the turfgrass plants in experiment two. This had an effect on the control plants in experiment two, thus reducing the potential for separation of treatment effects. The potential exists for differences in the leaf surface characteristics of each of the turfgrasses. In particular, differences in leaf thickness are known to alter light absorption and reflection (Cameron, 1970; Clark and Lister, 1975). Exposure to UV-B light can also lead to thickening of the leaf surfaces (Bornman and Vogelmann, 1991), thus affecting plant productivity though not necessarily in a beneficial manner. The difference in productivity between the grasses was also of interest. The two thicker-leafed grasses - tall fescue and perennial ryegrass -
tended to produce greater biomass levels than the thinner-leafed creeping bentgrass. The issue of leaf thickness may be crucial in this situation as a plant tries to maintain normal growth rates in high UV-B conditions.

The inhibition of tillering by UV-B could be considered problematic for turfgrass wear-recovery from daily use as well as regular growth and development. This reduction has been noted before in rice but there were also cultivar differences (Teramura at al., 1991). In that study, 30% of the rice cultivars showed a significant sensitivity to UV-B irradiance. This was also shown in other rice cultivars at ambient levels of UV-B of 8 kJ m\(^{-2}\) d\(^{-1}\), where tillering was reduced occurred in cultivar ‘CL161’ but not in ‘Cocodrie’.

The results were not linked to hormonal or carbohydrate changes in the plants (Mohammed et al., 2007). In Antarctic hairgrass (Deschampsia antarctica), tillering was increased by UV-B; however, the levels of UV-B were not as high as in this study (Rozema et al., 2001). The lack of separation in the root and shoot ratio was somewhat surprising; however, the light levels may have suppressed the growth and allocation of carbohydrate equally. This does not agree with previous results (Gwynn-Jones, 2001), but the light levels were higher in this study and it was conducted over a shorter period of 35 days compared to 45 days.

Light intensity variation plays a role in the overall responses of plants to both UV-B exposure and standard PAR irradiance. Its effect varies regarding the amount of exposure that is received by plants. In lapland reedgrass, (Calamagrostis lapponica) and (Calamagrostis pupurea), levels of UV-B simulating 15% loss of ozone reduced tillering. In light levels simulating 25% ozone depletion, there was an increase in tillering (Gwynn-
Jones and Johanson, 1996). The exposure value equated to 6.5 kJ m\(^{-2}\) d\(^{-1}\), which is lower than turfgrasses were subjected to in this study. Visible light intensities in this study were also lower than normal daily integrals. The effect of UV-B could have been increased due to lower levels of light protecting pigments in the lower visible light conditions (Teramura et al., 1980). In an attempt to gain a consistent turfgrass response, UV-B levels in our study were elevated above normal and this allowed for a clearer insight into the turfgrass response. Another goal of our approach was to subject turfgrasses to equivalents of extended exposures to UV-B irradiation which could simulate a season-long exposure.

The visual evaluation of color and differences between ratings agrees with previous studies (Millington and King, 2010). Turfgrasses may have suffered from probable chlorophyll degradation and this has been connected to a decrease in photochemical efficiency (Ervin et al., 2004c). The loss of color is a principal factor for evaluating turfgrass quality and while the thicker-leaved plants had significant color loss, they still maintained acceptable color levels compared to the two creeping bentgrass cultivars. This measurement can be construed as subjective and future potential use of objective color measurements could offer more accurate and consistent visual representations of the changes. This may be an important factor to consider regarding tolerance of cool season grasses to UV light stress. If the predicted changes in UV light levels occur over time, further research into leaf cuticle thickness and changes in resins and waxes in the cuticular layers will be required. The use of synthetic, reflective type pigments as plant coatings may be an alternative to breeding for increased UV-B tolerance in turfgrasses.
Table 4.1: Lighting conditions in control and ultraviolet treated light environments for turfgrasses grown at The Ohio State University March – April 2011.

<table>
<thead>
<tr>
<th>Chamber</th>
<th>UV-B (280-320nm)</th>
<th>UV-A (320-400nm)</th>
<th>PAR (400-700nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.1</td>
<td>18.6</td>
</tr>
<tr>
<td>UV-Chamber</td>
<td>24.0</td>
<td>0.4</td>
<td>18.4</td>
</tr>
</tbody>
</table>
† Significant difference between grasses grown in ultraviolet and control conditions on this date (\(P=0.05\)).

Figure 4.1: Relative growth rate for four cool season grasses grown in control and enhanced ultraviolet-B light conditions in repeated experiments
† Significant difference between grasses grown in ultraviolet and control conditions on this date ($P=0.05$).

Figure 4.2: Color ratings for four cool season grasses grown in control and enhanced ultraviolet-B light conditions in repeated experiments. (1-9 scale, 1 = brown/dead, 9 = dark green optimal color, 6 = acceptable).
† Bars signify least significant difference between treatments ($P=0.05$).
‡ Histograms with different letters for each grass signify differences at ($P=0.05$).

Figure 4.3: Number of tillers per plant for four cool season grasses grown in control and enhanced ultraviolet-B light conditions in experiment one.
† Bars signify least significant difference between treatments ($P=0.05$).
‡ Histograms with different letters for each grass signify differences at ($P=0.05$).

Figure 4.4: Number of tillers per plant for four cool season grasses grown in control and enhanced ultraviolet-B light conditions in experiment two.
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modeling the biogeochemical cycling of turf grasses in the United States.


CHAPTER 5

PIGMENT CHANGES IN COOL SEASON TURFGRASSES IN RESPONSE TO ULTRAVIOLET-B LIGHT IRRADIANCE

ABSTRACT

Pigments involved in protection of photosynthetic apparatus in high light conditions have potential to aid turfgrasses in periods of enhanced ultraviolet-B (UV-B) radiation. These pigments have light absorbing capacities in the UV-B range (280-320 nm) and also may aid in energy dissipation as an initial protective mechanism from photosystem damage. These pigments and their function in elevated UV-B conditions have received little attention. This study aimed to characterize protective pigment responses to enhanced UV-B conditions in cool-season turfgrass. Three cool season grasses were used - tall fescue (*Schedenorus phoenix* (scop.) *Holub*), perennial ryegrass (*Lolium perenne* L.) and creeping bentgrass (*Agrostis stolonifera* L.) cultivars ‘L93’ and ‘Penncross’. Turfgrasses were treated for pigment responses over a one week period and subjected to 16 kJ m$^{-2}$ d$^{-1}$ of UV-B in growth chambers. The chambers were run on a day / night regime of 14 / 10 hours and plants were subjected to 18.5 mol m$^{-2}$ d$^{-1}$ photosynthetically active radiation (PAR) and 23 / 20 °C. Samples were collected at day
0, 1, 3, 5 and 7, and chlorophyll fluorescence was measured and chlorophyll pigmentation, flavonoids, phenolics, anthocyanins and carotenoids content were determined. Fluorescence measurements and chlorophyll levels decreased significantly ($P=0.05$) in UV-B conditions compared to the control environment. All species possessed significantly ($P=0.05$) higher quantities of total phenolics and flavonoids in the top of the tissue canopy compared to roots or tissue at the soil surface. After day one all of these compounds decreased but there were more significant decreases in UV-B conditions. Anthocyanins were only characterized in creeping bentgrass ‘L93’ with Cyanidin – 3 – O – glucoside making up 60% of the anthocyanins identified. The carotenoids, zeaxanthin and β-carotene declined in total content in UV-B treatments in creeping bentgrass ‘L-93’ and ‘Penncross’ after seven days, but not in perennial ryegrass or tall fescue.

INTRODUCTION

Light drives many photosynthetic responses in plants. High irradiance can lead to photoinhibition and chlorophyll degradation in plants. This may cause a decrease in productivity (Curran et al., 1990; Anderson and Aro, 1994; Gitelson et al., 2006) and leaves plants susceptible to poor recovery from wear and biotic stress. The irradiance that plants receive on a daily basis varies in both quantity and spectral distribution. The light distribution refers to the photosynthetically active radiation (PAR) that occurs in the 400-700 nm wavelengths. It can be measured instantaneously in μmoles m$^{-2}$ s$^{-1}$ (Smith, 1994). The direct effect of wavelength variations differs however from high levels of irradiance. Light low in red light leads to alterations in photoreceptors signaling whole plant and
biochemical changes (Wherley et al., 2005). In Italian ryegrass (*Lolium multiflorum* Lam.), there was reduced tillering while increasing length of leaf blades and reproductive shoots due to increased red light (655 nm – 665 nm) (Casal et al., 1985). On the other end of the spectrum, concentrations of blue light enhanced activity of cryptochrome leading to chalcone synthase induction in *Arabidopsis thaliana*, (Lin et al., 1996). Outside of PAR, other wavelengths such as ultraviolet light (UV) impact plant growth and physiology. Ultraviolet light wavelengths are found between 100 nm and above 400 nm (Rosario et al., 1979).

The effects of these wavelengths vary on plant physiology and growth. Ultraviolet-A increases biomass by 22% in cucumber compared to UV-B wavelengths but standard PAR additionally increased biomass by 14% above UV-A (Krizek et al., 1997). The influence of UV-B is particular is considered to be negative due to development of reactive oxygen species, inhibition of photosynthesis and effects on genetic expression (Mackerness, 1999; Teramura and Sullivan, 1994). Lower regeneration of ribulose bisphosphate, decreases in fluorescence, increased D1 polypeptide turnover within photosystem II have also been linked to UV-B exposure (Greenburg et al., 1989; Strid et al., 1990; Ziska et al., 1993). Pea plants (*Pisium sativum* L.) and cotton (*Gossypium hirsutum* L.) suffer decreases in chlorophyll content when exposed to UV-B (Ziska et al., 1993; Gao et al., 2003). These losses occur within plants particularly from UV-B radiation (Caldwell, 1971). The potential for further damage could increase from 2010-2020. Peak irradiance of UV-B wavelengths are predicted to
occur in this time period prior to degradation of chlorine and bromine containing species (Taalas et al., 2000).

The protective mechanisms that plants possess vary in their action and also in location of response. Plant pigments such as anthocyanins, a class of UV-B absorbing flavonoids are produced in Kentucky bluegrass (Poa pratensis L.), in response to elevated UV-B conditions (Zhang et al., 2005). Anthocyanins are flavonoids which absorb in the UV-B region (Gould, 2004). Polyacylated anthocyanins completely reduced DNA lesions in blue morning glory (Ipomoea tricolor cv. ‘Heavenly Blue’), (Mori et al., 2005). The anthocyanins themselves can vary in their absorption spectrum due to changes in methylation or acylation (Gitz et al., 1998; Do and Cormier, 1991). These changes are affected predominantly by pH alterations within the plant which are light driven (Yin et al., 1990). The actual location of the pigments in the adaxial epidermis of leaf surfaces is also considered crucial for UV protection in coleus (Solenostemon L.) (Burger and Edwards, 1996). There are also other pigments which are used by plants with the aim of reducing light damage to photosynthetic apparatus. The light absorption characteristics are different from anthocyanins but offer light protection.

Carotenoids are another set of compounds which have another role in light protection in high intensity light. A pool of the 40 carbon compounds known as xantophylls alters its composition in creeping bentgrass (Agrostis stolonifera L.) in response to high and low light conditions (Bell and Danneberger, 1999; McElroy et al., 2006). Zeaxanthin is found in larger proportion to violaxanthin in high light conditions but reverts to equal or lower levels in low light conditions. Lutein has also been shown to
be the carotenoid of greatest concentration regardless of light conditions (McElroy et al., 2006). In Kentucky bluegrass treated with UV-B, total carotenoid content decreased over 10 days with recovery occurring after 15 days of treatment completion. There was also a positive correlation (>0.7) between these compounds and turfgrass quality in enhanced UV-B conditions (Zhang et al., 2005). The ability of carotenoids to quench singlet oxygen and other excited free radicals after UV-B treatment (Mathews-Roth, 1996), may be responsible for reduction in damage to LHC in plants and limit the loss of plant quality. The damage to photosynthetic apparatus is used to infer possible reductions in photosynthetic efficiency and this damage has also a positive correlation to total carotenoid content (Zhang et al., 2005).

The repair and defense mechanisms can also include more than pigments. Enzymes which act as catalysts for energy-quenching reactions such as super oxide dismutase build up in response to UV-B exposure in Kentucky bluegrass (Zhang et al., 2005). Glucose-6-phosphate dehydrogenase activity and higher levels of proline occur in creeping bentgrass, Kentucky bluegrass, perennial ryegrass and tall fescue treated with UV-B. A possible repair mechanism allocating resources from glycolysis to the pentose phosphate pathway leads to a buildup in enzymatic activity in turfgrasses exposed to UV-B light (Sarkar et al., 2011). These repair systems stabilize the D1 protein within PSII and offer a method of scavenging excess levels of energy. The systems also help to split up cyclobutane dimers in DNA between two adjacent pyrimidines which are probably formed during UV exposure. Photolyase uses excision repair or recombination repair to complete restoration of DNA damaged from UV-B exposure (Sancar and Sancar, 1988).
The damage to photosynthetic apparatus linked to high energy light within PAR is known as photoinhibition and UV-B light is known to cause similar problems for plants (Friso et al., 1994). The enhanced rate of degradation of both the D1 and D2 proteins inhibits the rate of photosynthesis. The damage to the D2 protein is increased in high UV-B conditions which are not considered normal. In general the D1 protein degrades at a more rapid rate than D2 with the exception where UV-B treatments are imposed in combination with visible light (Jansen et al., 1993). This is due to the UV absorption by the manganese cluster which lies in the oxygen evolving complex. This may act as a component magnifying the protein degradation (Babu et al., 1999; Tyystjärvi, 2008). The extent of the damage may initially be expressed in PSII and then damage will begin to occur in the more robust PSI. The damage in PSII may be construed as an enhanced rate of protein degradation which can be repaired. This damage, however, then leads to damage of PSI which requires complete breakdown and reconstruction and long term damage to PSI (Takahashi and Murata, 2008). The degradation of PSII leads to a buildup of reactive oxygen species and peroxyl radicals with increased lipid peroxidation (Hideg and Vass, 1996; He and Häder, 2002).

In measuring damage to photosynthetic apparatus within plants, damage to photosystem II is most prevalent in relation to UV-B damage (Vass et al., 1996). This damage can be measured indirectly via fluorescence of reaction centers of chlorophyll A (Maxwell and Johnson, 2000). The degradation of the centers leads to a decrease in fluorescence. Chlorophyll content within tissue, which also degrades due to photoinhibition, can also be measured (Strid and Porra, 1992). This degradation in UV-B
conditions is at a higher rate than normal PAR and leads to a decrease in productivity from plants and can occur on a time scale of minutes to hours (Aro et al., 1993). Turfgrass photoinhibition occurs under elevated UV-B light conditions with Kentucky bluegrass suffering significant decreases in the ratio of variable fluorescence to maximal fluorescence (Fv/Fm) (Zhang et al., 2005). This results in a decrease in photosynthetic activity due to increased levels of fluorescence within the photosynthetic apparatus. Under elevated UV-B light conditions it has been shown that photoinhibition also affects the PSII apparatus in tall fescue and perennial ryegrass but not as badly as Kentucky bluegrass (Zhang and Ervin, 2009).

There is a need to elucidate the variations that occur within the plant canopy while also characterizing pigment changes across a range of cool season turfgrasses exposed to UV-B irradiation. The influence of UV-B may create different responses within cool season turfgrasses and how they protect their photosynthetic apparatus in an attempt to retain productivity. The objective of this study was to characterize pigmentation alteration in UV-B conditions and investigate if these changes offer potential for protection from UV-B damage to the photosynthetic apparatus in cool season turfgrasses via energy dissipation and direct absorption of excess light. We hypothesized that turfgrasses would produce higher quantities of protective pigments in response to the UV-B stress. These higher pigment quantities would then allow turfgrasses to retain their photosynthetic capacity during the period of stress. This will aid in reducing summer stress and allow turfgrasses to maintain photosynthetic activity close to normal levels.
MATERIALS AND METHODS

Sample preparation

The studies were conducted in October, 2010 and March, 2011 in Columbus, Ohio. Turfgrasses were initially seeded into a mason sand rootzone >97% sand (Hummel & Co, Trumansburg, NY). The rootzone met USGA requirements and had a pH of 7.4 with a clay silt fraction of less than 5%. The seeding was timed to match germination speeds between the grasses. Creeping bentgrasses were seeded first, and 4 days later perennial ryegrass and tall fescue were seeded into 3.8 cm diameter ‘conetainers’ (Steuwe & Sons, Tangent, OR). Moisture was maintained by placing turfgrasses on a 30 second irrigation cycle every 10 minutes in a mist house. Turfgrasses were allowed to establish for six weeks in the mist house.

Grasses were treated with Peters 20-20-20 (Scotts-Miracle Gro, Marysville, OH) at a rate of 11.8 kg ha-1 every two weeks during this period. After germination, plants were removed and placed in standard greenhouse conditions for a further four weeks to allow for maturation. Turfgrasses were maintained at a height of ~10cm with clippings removed 3 times weekly. Fertility treatments were continued and imidacloprid N-[1-[(6-chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl]nitramide [Bayer Environmental Science, Research Triangle PK, NC] was applied to prevent insect damage. Mefenoxam (R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester [Syngenta AG, Greensboro, NC] and thiophanate-methyl (dimethyl 4,4’-o-phenylenebis[3-thioallophanate]) [Cleary Chemicals, Dayton, NJ] were applied for preventative disease control. Water was applied to prevent wilt, with automated heads
using an ESP modular irrigation system (Rain Bird, Azusa, CA) once daily. Ten weeks after initial turfgrass emergence, plants were placed in Conviron E15 growth chambers (Controlled Environment Ltd., Winnipeg, Canada). To equalize plants they were placed in dark growth chambers at 20 °C. This happened for 24 hours for all plants in both experiments.

Ultraviolet-B treatment commenced using UV-B lights having peak emission at 313 nm, QUV UV-B 313 nm, (Q-Lab Corp., Westlake, OH). Treatment exposure was calculated using time of exposure multiplied by measured irradiance energy per second. Wavelengths within UV-B and UV-A were measured using an IL1350 photometer (International Light, Newburyport, MA) with specific sensors for each portion of the UV light spectrum. Biologically effective radiation was calculated from spectrum provided by light producers. Ultraviolet radiation is reported in kJ m\(^{-2}\) d\(^{-1}\). Photosynthetically active radiation was instantaneously measured every 15 minutes during the study and reported as mol m\(^{-2}\) d\(^{-1}\) using cosine corrected light sensors (Spectrum Technologies, Plainfield, Il). Light treatments of 14 hours daytime and night time periods of 10 hours were controlled by the chamber. The lamps were pre-lit for 48 hours to prevent any fluctuation in energy output during trials. Photosynthetic active radiation (PAR) which lies in the range of 400 – 700 nm was provided using fluorescent lamps. The same lamps were used in the control and UV chambers, 85 w s\(^{-1}\) high output F12T12-CW.HO fluorescent tubes (General Electric, Fairfield, CT). The turfgrasses were irrigated twice daily to prevent wilt in the chambers. Each chamber was fixed at 20°C during light
periods and 17 °C in darkness. In each chamber, plants were placed 0.4 m below the lighting in an even spacing.

**Chlorophyll content and fluorescence**

The experiment was set up in a randomized complete block design with four blocks and three replications per block for each grass. Analysis was carried out at Day0 (D0), Day1 (D1), Day3 (D3), Day5 (D5) and Day7 (D7) for all data. After each treatment turfgrasses were placed in darkness for one hour. Fluorescence measurements were then taken with 12 total replications for each grass per block to reduce variability. The F_v/F_m values were obtained using a handheld chlorophyll fluorometer OS-30p (Opti-Sciences, Hudson, NH). Leaf tissue from the top 5 cm was used and measured in-vivo with initial 660 nm pulses from an integral probe fluoresced to a photodiode with a 700 – 750 nm bandpass filter. Values were averaged for each block and analyzed separately due to the fact that different turfgrass plants were used for each sample date.

Three turfgrass tissue samples from the top of each grass of 40 mm² were taken and placed into 5 mL of N,N-dimethylformamide (Sigma-Aldrich, St. Louis, MO) for 48 hours in the dark at 4°C. The extract was then placed in 4 mL cuvettes and absorbances were measured at 664, 647, 625, 603 nm. This was carried out using a UV1800 spectrophotometer (Shimadzu Corp., Colombia, MD). Total chlorophyll, chlorophyll A and chlorophyll B were calculated using pre-determined formulae (Moran, 1982). Chlorophyll content was reported in µg m⁻². The rest of the tissue material was separated into the top 5 cm (T5) of tissue, all tissue between the root / shoot interface and the top
cm (T) and roots (R) (Appendix C), and frozen in liquid nitrogen at -210 °C. All samples were then freeze dried using a Virtis ultra 35 LE lyophilizer (The Virtis Co., Gardiner, NY) under a vacuum of 125 mTorr. This ran on an air temperature of -40°C to 10°C over a 20 hour period and maintained at 10°C for a further four hours while tray temperatures were maintained at -20°C. Samples were then stored at -20°C while analysis was completed.

**Total flavonoids**

Soluble flavonoids were measured using the method described by Pourmorad et al., (2006). Samples of 0.035 g were added to 10 mL of methanol and vortexed in 15 mL conical centrifuge tubes and allowed to stand for 10 minutes at room temperature. The samples were centrifuged at 3,400 rpm for 5 minutes. The material stood with an occasional vortex for at least 10 minutes before centrifuging. A standard curve was prepared using quercitin solutions at concentrations of 0, 10, 20, 40 and 50 μg mL in methanol. The extract was then placed into a tube with 1.5 mL CH₃OH, 0.1 mL of 10% AlCl₃, 0.1 mL potassium acetate and 2.8 mL distilled H₂O. The material was vortexed and incubated at room temperature for 30 minutes. The supernatant absorbance was measured at 415 nm with a UV1800U spectrophotometer. Linear regression analysis was used to determine concentrations of flavonoids in each sample. This was carried out for R, T and T5 of all samples to determine concentrations of pigments based on location. This test was performed for each day in both experiments.
Total phenolics

Total phenolics were measured in accordance McDonald et al., (2001). Gallic acid standard curves were made using concentrations of 0, 5, 10, 20, 40 and 80 mg L in MeOH:H₂O, 50:50 v/v. Plant tissue (0.035 g) was placed in 15 mL of methanol and vortexed and let sit for 10 minutes. Tissue sample was then centrifuged for 3 minutes at 3600 rpm. Supernatant (0.5 mL) was collected and placed in a 15 mL tube with 5 mL of 10% folin ciocalteu reagent and 4 mL of 1M Na₂CO₃ and let sit at room temperature for 15 minutes. Total phenol concentration was then determined at absorbance of 765 nm using a UV1800U spectrophotometer. Linear regression analysis was then used to estimate phenol concentrations in each sample on a µg g⁻¹ basis. This was carried out for R, T and T5 of all samples to determine concentrations of pigments based on location. This was carried out for each day in both experiments.

Carotenoid extraction and analysis

Carotenoid extraction was carried out with slight modifications from a method described by Ferruzzi et al., (1998). All experiments were carried out under caution of low light to prevent carotenoid degradation. Tissue sample (0.5 g) was placed in 10 mL of MeOH in a 15 mL centrifuge tube and vortexed for 30 seconds. Samples were then homogenized using a polytron PT10 (Brinkman Institute, Westbury, NY), and then centrifuged for 7 minutes with an Accuspin 3R (Fisher Scientific, Pittsburgh, PA). Supernatant was removed and the pellet was extracted 3 times in 10 mL acetone/hexane 1:1 (v/v). All extracts were pooled in a 50 mL tube, a small volume of NaCl crystals were
added, and the extract was brought up to 50 mL with water and vortexed. The upper hexane layer was brought to 10 mL in a volumetric flask with hexane and transferred to a 25 mL conical flask.

Samples were run through glass wool and Na$_2$SO$_4$ crystals and then saponified to remove the phytol tail from the chlorophyll pigments to allow them to partition into the aqueous phase for removal. Samples (10 mL) were placed in 50 mL conical flasks with 10% KOH (Sigma Aldrich), in a 1:2 v/v with the extract. Flasks were then placed on a benchtop shaker for 2 hours covered in foil. The saponified mixture was then transferred to a 125 mL separatory funnel. The green chlorophyll separated into the lower phase which was removed. The remaining upper phase was washed with 40% Na$_2$SO$_4$ three times. The remaining sample was then dried under nitrogen at less than 2 PSI using a reactive-vap 18780 (Pierce, Rockford, IL). Samples were then resuspended in 2 mL of methyl tert-butyl ether: MeOH 1:1 (v/v) and passed through a 0.22 µm nylon syringe filter (Fisher Scientific, Pittsburgh PA) into 2 mL amber HPLC vials (Waters, Milford, MA).

Sample volumes of 20 µL were injected into an HP1050 series HPLC, with a diode array detector (DAD), were used to quantitate the carotenoids. Samples (20 µL) were injected onto a YMC C30 carotenoid column 4.6 x 250 mm, 3 µm particle size, (Waters, Milford, MA). A gradient system was set up and a tertiary solvent system was used. The gradient was used based on a modified method of Moros et al., (2002), with a flow rate of mL/min. Solvent A consisted of methanol/MTBE/water (81:15:4), solvent B consisted of methanol/MTBE (9:91) and solvent C consisted of MTBE alone. The initial
conditions were 100% A, with a linear increase to 45% A and 55% B over 21 min, changing to 100% C and holding for 2 minutes and returning to 100% A and holding for 5 minutes. Data was collected using HP Chemstation 3D and 453 nm wavelength was used for quantitation of the compounds. External calibration curves of zeaxanthin (CaroteNature, Lupsingen, Switzerland), lutein, β-carotene (Sigma Aldrich), antheraxanthin and violaxanthin (Chromadex, Irving, CA) were used to quantitate the respective carotenoids. Turfgrass samples were used from D0 and D7 of the trials to look at overall changes in concentrations of carotenoids. Samples were taken from the T5 portion of turfgrasses

**Anthocyanins**

Anthocyanins were extracted using a method modified from Giusti et al., (1999). Freeze dried tissue (0.5g) was crushed into a fine powder using a mortar and pestle while suspended in liquid nitrogen. Samples were then placed in 10 mL of 100% acetone in 15 mL tubes, vortexed and left to sit for 5 minutes. Samples were then centrifuged at 3600 rpm for 5 minutes. Supernatant was collected and samples were re-extracted with 0.1% HCL acidified 70:30% acetone:H₂O (V:V). Samples were re-extracted two more times until supernatant was clear. Extract was combined in a 50 mL centrifuge tube and brought to 30 mL. Supernatant and tissue were then combined and passed through a No.1 filter (Whatman, Kent, United Kingdom) to maximize extract collection in a Büchner funnel. This was carried out under vacuum using a Rocker 300 pump (Rocker, Kaohsiung, Taiwan), and samples were collected in a conical flask. To separate water
soluble from lipid soluble compounds, 2:1 volume chloroform was added and samples were sat overnight in dark cool conditions in 125 ml separating funnels. Chloroform and acetone (now mixed together) were removed and the top aqueous portion was collected and retained. To purify the anthocyanins, C-18 cartridges (Biotage, Charlotte, NC) were used. To prepare the cartridges 100% MeOH x2 volumes of cartridge were flushed through under an Alltech 210351 12-port vacuum manifold (Alltech, Deerfield, IL) and washed with 3 volumes of acidified water (0.1% v/v HCL). Samples were then loaded into the cartridge and anthocyanins, sugars and other phenolics were adsorbed onto the C-18 column under vacuum. To remove water soluble compounds (0.01%) acidified water was then passed through the cartridge followed by ethyl acetate to elute non-anthocyanin phenolics. Anthocyanins were finally recovered using v/v 0.01% HCL acidified MeOH in concentrated format. In all stages, the pH of extract was sustained at 2 or lower to retain stability. Samples were collected in 2.5 mL microfuge tubes and spun at 5000 rpm x 5 minutes to remove any debris. Samples were then concentrated using a Büchi R114 rotoevaporator and re suspended in 1 mL distilled deionized water which was acidified with 0.01% HCL.

Samples were passed through a .22 µ filter using 3 mL luer lock syringes and injected into 2 mL amber LCMS vials with 300 µL inserts with springs. Samples were then injected into a LCMS apparatus set up as follows. A CTO-20A (Shimadzu Scientific Inst., Columbia, MD) oven was used to maintain temperatures at 35°C, flow of 0.8 mL min was controlled with LC-20AD – LC pumps, while 50 µL sample injection was managed with a SIL-20AC autosampler. Samples were injected onto a Varian C18-A,
150 x 4.6 mm i.d. column (Varian Inc., Lake Forest CA). Samples were eluted through a SPD-M20A PDA (Shimadzu) with wavelengths 280, 320 and 520 nm simultaneously selected. Spectral analysis of wavelengths between 250 and 700 nm were recorded for all peaks. Solvents for mobile phase were first filtered through .22 µm filters. A binary flow mobile phase consisting of (A) 4.5% formic acid:H₂O v/v and (B) acetonitrile with a flow rate of 0.8 mL min was used. Anthocyanins were separated using a solvent gradient from 5 % B to 15 % in 5 minutes followed by an increase in B to 30 % at 20 minutes. From 20 minutes to 23 minutes solvent B increased to 100 % and was maintained there until 25 minutes. Concentration of B then decreased to 5 % at 28 minutes and sample injection completed after 35 minutes. Samples were then injected into an electron spray mass spectrometer and fragmentation analysis carried out. Due to limited samples only qualitative data was obtained. Known anthocyanin pigments (Chandra et al., 2001) from (Aronia melanocarpa) were injected under the same conditions and used as a reference material (Appendix D). Compound mass, elution order, spectral absorbance and retention time were used to characterize anthocyanins found in turfgrass samples.

Data analysis

Data analysis for chlorophyll, fluorescence, total phenolics and flavonoids was carried out using the general linear model from the SAS program (SAS Inst., 2008). Analysis was carried on block averages for comparison of data from each light environment collected on each specific date. Comparisons between light environments were carried out for each variable on each date because different samples were used for
each date. Carotenoid content for each light environment was calculated as a difference between D0 and D7 to compare overall changes during treatments. The lack of samples due to growth chamber size constrictions for anthocyanin analysis meant that only characterization of these pigments could occur at this time.

RESULTS

Light intensities for the two chambers were very similar within the PAR region of light (400-700 nm). The main difference between chambers was measured in the UV-B region with turfgrasses subjected to 16.9 KJ m\(^{-2}\) d\(^{-1}\) of UV-B\(_{be}\) light in the UV chamber compared to the 0 in the control chamber (Table 5.1).

Fluorescence measurements in all grasses followed the same pattern in both experiments and data was pooled after testing for differences between experiments. In both chambers there was a relative decrease in photosynthetic activity from D0 in all grasses. However, significantly lower fluorescence measurements occurred in chambers that had elevated UV-B conditions (\(P=0.05\)) after D3 and this continued through D7. All grasses suffered greatest decline in F\(_{\text{v}}\) / F\(_{\text{m}}\) up to D5. Perennial ryegrass and “Penncross’ creeping bentgrasses had declines of F\(_{\text{v}}\) / F\(_{\text{m}}\) greater than 10% (Figure 5.1). No grass exhibited complete loss of photosynthetic activity.

Chlorophyll pigmentation exhibited a similar pattern in all grasses in both experiments. Treatment of UV-B resulted in significant declines in total chlorophyll content (\(P=0.05\)). The decreases were noted after D1 in both perennial ryegrass and tall fescue. This separation between control and UV treated grasses did not occur until D3 in
the creeping bentgrass cultivars. There were also declines in the control chamber in all grasses; however, the decline in chlorophyll content in the control chambers was faster in the bentgrass cultivars (Figure 5.2). Overall, chlorophyll levels were higher on an area basis in perennial ryegrass and tall fescue compared to the creeping bentgrass cultivars. The change in chlorophyll A and B also followed the same pattern. Significant declines ($P=0.05$) occurred in both of these chlorophyll pigments in UV-B conditions (Appendix E).

The distribution of both phenolics and flavonoids were similar across all grasses regardless of light environment. This occurred in both experiments. Turfgrasses exhibited the ability to accumulate significantly higher ($P=0.05$) quantities of both phenolics and flavonoids in the T5 portion of the tissue sample. There was almost a 50% difference in total phenolics between the T5 and T in creeping bentgrass ‘L-93’ in control conditions at the end of the trial. On that same date there was an 85% difference between T5 and R tissues in ‘L-93’ (Figure 5.3). There was an initial increase in phenolic content in both portions of tissue on D1 and this was then followed by a decline. Flavonoids also exhibited similar significant separation with T5 tissue containing significantly greater concentrations. In perennial ryegrass, concentrations of total flavonoids varied between T5 and T tissue was as much as 50% after D7 (Figure 5.4). There was for the most part a spike in response after day 1 but this was followed by a decline in concentration in both parts of the plant tissue.

The overall concentration of flavonoids increased on D1 in both light environments. This response was then followed by a decline in all grasses until D5 when
quantities then leveled off. There was significant decreases in all grasses in UV-B irradiated conditions at D7. There was on average a 27.5% decrease in all grasses at D7 (Figure 5.5). A similar decrease was observed in both light environments in total phenolic concentrations (Data not shown). The difference at D7 between control and UV-B chambers was only significantly different in the two creeping bentgrass cultivars (Data not shown).

Anthocyanin responses to UV-B exposure were very limited. Extracts of tissue from D1, D3 and D5 yielded no data when passed through HPLC conditions at 520 – 530 nm. This occurred in all grasses for both sets of light environments and in both experiments. In D7 tissue from creeping bentgrass ‘L-93’, HPLC analysis indicated that the presence of anthocyanins sufficiently strong for detection at 520 nm (Figure 5.6) and 320 nm. Electrospray ionization of compounds resulted in masses for four main pigments in the ‘L-93’ tissue. The elution order and approximate retention time was similar to *Aronia* samples which were also injected and checked in previous literature (Chandra et al., 2001). This information was then used to characterizing the anthocyanins found in creeping bentgrass ‘L-93’ (Table 5.2). First eluted compound at 8.4 minutes was cyanidin 3 – O – glucoside which was followed by peonidin 3 – O – galactoside at 9.95 minutes. At 11.05 minutes cyanidin 3 – O – malonyl glycoside was detected and this was followed by cyanidin 3 – O – succinyl glucoside at 13.25 minutes (Figure 5.6). No other grasses displayed detectable anthocyanin increases at D7 in either light environment.

Using internal spikes to quantify potential pigment degradation resulted in noted loss of antheraxanthin, lutein and violaxanthin due to saponification. Zeaxanthin and β-
carotene were identified based on absorbance, peak shape, elution order and retention time linked to standards (Figure 5.7). Zeaxanthin eluted at 11.1 minutes while β-carotene eluted at 20.1 minutes. Peaks that did not fit any of standards available eluted at 16, 19 and 24 minutes (Figure 5.7). Degradation of β-carotene was ~10% through saponification and zeaxanthin had ~15% degradation. Saponification was required to separate chlorophyll pigments from carotenoids prior to HPLC analysis. There was no difference in carotenoid content between light environments at D0 or D7. There was however greater degradation in the UV environment from D0 to D7 with significant decreases in β-carotene content in both of the bentgrasses (Appendix F) in the UV chamber. Perennial ryegrass and tall fescue (Appendix G) did not have such a significant loss of β-carotene in UV conditions (Figure 5.8a). Creeping bentgrass ‘Penncross’ also had significant β-carotene loss between samples from D0 to D7 in control light conditions (Data not shown). Creeping bentgrass ‘L-93’ had significant losses ($P=0.05$) of zeaxanthin in both UV and control chambers. No other grass exhibited this loss of zeaxanthin and this did not happen in the control chamber (Figure 5.8b). There was no difference in zeaxanthin content between UV and control treated turfgrasses at D7.

DISCUSSION

The decrease in photosynthetic activity in our study occurred regardless of grass and agreed with previous data in cool season turfgrass (Zhang et al., 2005; Sarkar et al., 2011). This loss of activity in our study could be attributed to the decrease in phenolics or also loss of enzymes used for repair of the photosynthetic apparatus. The decrease in
carotenoid pigments may also have played a role in the decrease in photosynthetic activity in this study. The loss after D1 of UV the absorbing compounds would indicate that the photosynthetic apparatus would have been suffering damage at the same time. This was born out in the results show using the fluorecence measurements.

The loss of chlorophyll pigmentation in this study was despite taller turfgrasses having a dense canopy. The loss of chlorophyll coincided with the decrease in fluorescence also and this would then lead to a loss of biomass productivity as has been noted in wheat (*Triticum aestivum* L.) which had a decline of 18% (Correia et al., 1999) in response to elevated UV-B levels. Degradation of chlorophyll is a common response to UV-B radiation in plants. Kentucky bluegrass has exhibited this response before as have sunflower cotyledons and other plants (Zhang et al., 2005; Costa et al., 2002; Tevini et al., 1981). The energy associated with the photons in the UV-B wavelengths (3.94 – 4.43 electronvolts) is considered strong enough to destroy many chemical bonds and it may have contributed directly to the chlorophyll degredation (Kovács and Keresztes, 2002).

The variation in distribution of phenolics and flavonoids in our study could be attributed to two causes. Turfgrass plants in such a dense canopy may have led to self-shading thus the elevated levels in the upper canopy of these UV absorbing compounds is a response to the light conditions in the grasses tested. This argument is further strengthened by the lack of response and much lower levels of each set of compounds in the root tissue (Buer and Muday, 2004). Similar distributions of flavonols have been noted (Cortell and Kennedy, 2006) and also in phenolic compounds in roots and leaf tissue of cow lily (*Nuphar advena*) and large leaf pondweed (*Potamogeton amplifolius*).
L.) (Cronin and Lodge, 2003). The second cause could relate to age of the leaf tissue which can be more susceptible to damage from UV-B exposure when it is younger (Lois, 1994). We feel this distribution of compounds in our study is due to a light response as material was clipped 3 days before trial initiation to reduce the influence of leaf age.

The actual quantities of phenolics and flavonoids had an initial increase from D0 to D1 in both light environments in our study. This type of response is considered a plant protective response in an attempt by the plant to reduce the impact of the UV-B wavelengths on the photosynthetic apparatus. Phenolics have antioxidant capacity and offer a method to reduce oxidized radicals and reduce damage to the photosynthetic apparatus (Duval et al., 1999). Mutant Arabidopsis plants unable to accumulate flavonoids are highly susceptible to UV damage (Landry et al., 1995). There was then a decline in flavonoid and phenolic levels in all grasses, with greater declines for both in UV-B. This loss may be related to an inability of turfgrass plants to allocate enough energy to retain initial response levels due to lower levels of visible light in the chambers (Teramura et al., 1980). The UV-B exposure may have overwhelmed the plants capability to repair itself and recover from free radical damage in this study.

The potential benefit of anthocyanins was only seen after seven days of exposure only in the ‘L-93’ cultivar of the creeping bentgrass in our study. This delay in production was after a decline in photosynthetic activity and so the benefit was both delayed and limited. Increased anthocyanins have been found in Kentucky bluegrass in response to UV-B exposure (Zhang et al., 2005). The UV-B absorption capabilities of anthocyanins are argued as a benefit and that plants possessing this response are
acclimated to UV-B stress (Gould, 2004). There is some argument to the photoprotective capabilities of these compounds as only marginally higher photosynthetic efficiency was found in red Kermes oak (*Quercus coccifera* L.) leaves compared to greener ones (Karageorgou and Manetas, 2006). Also limited or no photoprotective value was found in myrtle plants with anthocyanins in the epidermis (Woodall and Stewart, 1998).

In our study the creeping bentgrass cultivar ‘L-93’ only exhibited such a response while ‘Penncross’ did not. The variety differences in anthocyanin response noted in our study have also been noted before though not in turfgrass. In maize (*Zea mays* L.), of seven varieties tested for UV-B response, only two varieties responded with anthocyanin production while one other showed a strong anthocyanin development response to far red light (Beggs and Wellmann, 1985). The other reason that both the perennial ryegrass and tall fescue may have had limited response in this study was that epidermal thickness plays a large role in determining the transmission of light into the epidermis. Tall fescue has large variations in its thickness and it can significantly alter its leaf thickness from 0.31 cm to 0.36 cm in response to drought (Fu and Huang, 2004). This may require much larger consideration for UV-B screening effectiveness (Day, 1993) combined with pigment concentrations.

The concentration of β-carotene decreased in both light environments in our study but the decrease was greatest in UV conditions and also only in the bentgrass species. The loss in β-carotene content has also been linked to a loss of photosynthetic activity in *Dunlaliella* cells exposed to UV-B (White and Jahnke, 2002). This loss of an important pigment may relate to the loss of quality and also the greater loss of photosynthetic
activity in the UV chamber-treated bentgrasses. Zeaxanthin decreases have occurred over extended periods of UV-B exposure in maize (Carletti et al., 2003). There have been initial increases in content also but possible changes in energy allocation may reduce their levels after prolonged exposure. This degradation of both carotenoids reduces the turfgrass capabilities to moderate damage from reactive oxygen species. The impact of carotenoids may be crucial in high UV conditions due to their capabilities of energy dissipation and is particularly important for the thinner leaf bentgrasses. Further work completing the xantophyll cycle response and also quantifying and characterizing these compounds is required to gain a fuller understanding of their value.

The loss of xantophylls due to saponification has been observed in prior studies (Kimura et al., 1990). It is the first time that the problem has been reported in turfgrass and may reduce the capability to cleanly separate carotenoid and chlorophyllous pigments when using HPLC analysis. The alkalinity of the methanolic KOH leads to hydrolysis of the compounds; however, it was thought that the lower percentage of KOH would reduce the problem (de Sá and Rodriguez-Amaya, 2003).

CONCLUSION

The loss of photosynthetic activity in turfgrasses observed in this experiment due to exposure to UV-B was expected. It was interesting to note a decrease in quantity of UV-B absorbing pigments over extended exposure. However, the combination of high UV and low visible light levels may have played a role in this. The loss of carotenoids in the creeping bentgrasses is of interest as is the production of anthocyanins in ‘L-93’
creeping bentgrass due to their UV absorbing abilities. The reduction in carotenoids would lead to an increase in highly energized products thus requiring some form of antioxidant response and also a compound that absorbs the excess light. Immediate breeding responses for a crop that covering potentially 163,800 km$^{-2}$ (Milesi et al., 2005), of cultivated land are not possible. In an attempt to address the problem rapidly, the use of synthetic coatings may offer a practical response and tool for turfgrass managers. This first characterization and extraction of turfgrass anthocyanins also offers some potential for further research in turfgrass stress physiology. Further work on carotenoid characterization and chromatogram publication is also required due to their importance in light stress and new herbicide research.
Table 5.1: Light intensity and light distribution for Conviron E15 growth chambers illuminated using high output F12T12-CW.HO fluorescent tubes and QUV UV-B 313 nm UV-B lights.

<table>
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<tr>
<th>Light</th>
<th>Control</th>
<th>Ultraviolet-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR (400-700 nm)</td>
<td>26.2 mol m^{-2} d^{-1}</td>
<td>25.9 mol m^{-2} d^{-1}</td>
</tr>
<tr>
<td>UV-A (400 – 320 nm)</td>
<td>1.0 KJ m^{-2} d^{-1}</td>
<td>0.8 KJ m^{-2} d^{-1}</td>
</tr>
<tr>
<td>UV-B_{\text{be}}} (320 – 280 nm)</td>
<td>0.0 KJ m^{-2} d^{-1}</td>
<td>16.9 KJ m^{-2} d^{-1}</td>
</tr>
</tbody>
</table>

*Biologically effective radiation within UV-B wavelengths based on Caldwell, (1971).
<table>
<thead>
<tr>
<th>Compound</th>
<th>$T_r$ (min)$^\dagger$</th>
<th>[M] $m / z^{‡}$</th>
<th>Fragments $m / z^{§}$</th>
<th>UV – vis nm$^{¶}$</th>
<th>Area % (520 nm)$^{#}$</th>
<th>Identity</th>
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<tbody>
<tr>
<td>1</td>
<td>8.42</td>
<td>449</td>
<td>287</td>
<td>280, 516</td>
<td>60.5</td>
<td>Cyanidin 3 – O – glucoside</td>
</tr>
<tr>
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<td>9.95</td>
<td>463</td>
<td>301</td>
<td>324, 516</td>
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<td>Peonidin 3 – O – galactoside</td>
</tr>
<tr>
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<td>11.05</td>
<td>535</td>
<td>287</td>
<td>281, 519</td>
<td>27.5</td>
<td>Cyanidin 3 – O – malonyl glycoside</td>
</tr>
<tr>
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<td>13.25</td>
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<td>287</td>
<td>329, 516</td>
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<td>Cyanidin 3 – O – succinyl glycoside</td>
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</table>

$^\dagger$ Retention time.
$^‡$ Mass of whole compound.
$^§$ Breakdown of compound sizes after passage through mass spectrometer.
$^{¶}$ Peak absorption of compounds in PAR and UV regions.
$^{#}$ Percentage of total compounds identified.

Table 5.2: Anthocyanin pigmentation detected in creeping bentgrass ‘L-93’ after 7 days of enhanced UV-B treatments.
Figure 5.1: $F_v / F_m$ values for Perennial Ryegrass and Creeping Bentgrass ‘Penncross’ treated with UV-B light (280 – 315 nm) and PAR light (400 – 700 nm) conditions over seven days.

*† Significantly different at ($P=0.05$)
*† Significantly different at \((P=0.05)\)

Figure 5.2: Total chlorophyll content for Tall Fescue and Creeping Bentgrass ‘L-93’ treated with UV-B light (280 – 315 nm) and PAR light (400 – 700 nm) conditions over seven days.
*† Significantly different at ($P=0.05$) between Roots (r), Top (T) and Top 5 cm (T5) tissue.

Figure 5.3: Distribution of phenolics in creeping bentgrass ‘L-93’ after treatment with both UV-B light (280 – 315 nm) and PAR light (400 – 700 nm). Data was pooled as all grasses had the same response.
*† Significantly different at ($P=0.05$) between Top (T) and Top 5 cm (T5) tissue

Figure 5.4: Distribution of total flavonoids in perennial ryegrass tissue after treatment with both UV-B light (280 – 315 nm) and PAR light (400 – 700 nm). Data was pooled as all grasses had the same response.
† Significant difference occurred in flavonoid content for each grass on each day where letters are different. Significance at ($P=0.05$).

Figure 5.5: Turfgrass flavonoid content in Creeping bentgrass, Tall Fescue and Perennial Ryegrass grown in UV-B light (280 – 315 nm) and PAR light (400 – 700 nm) D0 to D7.
† Retention times, mass, spectral information and order of elution provided in Table 5.2.

Figure 5.6: Anthocyanin profile of creeping bentgrass ‘L-93’ exposed for 7 days to UV-B light. Produced from HPLC analysis using C$_{18}$ column and light absorbance measured at 520 nm using a PDA.
† 1 – Zeaxanthin; 2 – β - carotene.

Figure 5.7: Chromatogram of Perennial ryegrass carotenoids with absorbance at 453 nm. Data produce using C30 column and PDA connected to Chemstation software.
† Means followed by different letters significantly different ($P=0.0$).

Figure 5.8. β-carotene (top) and zeaxanthin (bottom) content in creeping bentgrass, tall fescue and perennial ryegrass exposed to UV-B light D0 – D7.
REFERENCE


144
Karageorgou, P. and Y. Manetas. 2006. The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol. 26:613-621.


CHAPTER 6

OVERALL CONCLUSIONS ON UV-B INFLUENCE ON TURFGRASS PIGMENTS AND GROWTH

ABSTRACT

The studies conducted for this project have yielded a range of insights as to the effects of UV-B on different cool season turfgrasses. In looking at growth and germination there is an applied element to the turfgrass knowledge which adds to the expanding base of information in place. Investigating the changes in pigmentation and developing new information and areas of research has created potential for new turfgrass cultivar breeding and also offers insight into physiological changes that previously were undocumented in turfgrass. Overall conclusions and ideas for future work are offered.
DISCUSSION

The influence of UV-B light from these studies on turfgrass is varied. From the initial growth and germination stage to maturity and canopy effects, there were some benefits and some negative impacts. The use of UV to enhance Kentucky bluegrass seed germination offers the potential to increase chances of successful establishment in a broader range of environmental conditions. Further studies, including field research, should be conducted in this area as this was the first trial using UV light and Kentucky bluegrass seed. There were also some issues with both the chambers and the lighting. Seed age seemed to reduce the effectiveness of UV light in seed germination overriding any benefit. The exposure of Kentucky bluegrass seedlings to UV did not seem to damage seed coats prior to germination and further work on long term exposure of Kentucky bluegrass seedlings to UV is warranted. Enzymatic responses and breakdown of starch reserves undoubtedly will paint a clearer picture for the method’s potential use.

Investigating what affects turfgrass color raised some interesting questions. Previous research suggests that adding nitrogen darkens turfgrass. The pigment correlation or lack of it in cultivars which were not forced by different rates of nitrogen applications was somewhat surprising as plants were not chlorotic and seemed dark green in the trial. Nitrogen was supplied at a single rate and thus this would explain the green color on its own. The data analysis indicated that the hand-held system which depended on light reflection failed to be influenced by pigment content and did not correlate to human visual quality rankings. This is somewhat of a concern as handheld systems are becoming prevalent and may mean that a whole new ranking database will need to be
built up if they are going to become standard. There was a relatively strong relationship found between both systems and leaf tissue nitrogen content, thus handheld systems measuring nitrogen might be more appropriate. The extract analysis undoubtedly offered more information and links to pigment changes; however its cost and time delay is problematic. Other pigments could also play a role in color which we did not account for of course. Pigments such as carotenoids absorb light and may completely reduce reflection of light in the blue region. This would account for the fact that the systems could not separate out green colors. Trained evaluators, using the long standard NTEP +9 scale, may still be the most consistent and precise method for color rankings.

Increasing UV-B light in this situation on mature stands of cool season turfgrasses produced some interesting and exciting responses. Characterizing anthocyanins for the first time in turfgrass is a mile stone and offers a whole new area of research in turfgrass. Potential lies for pigments in a range of areas and if turfgrasses can be controlled in a way that naturally enhances pigment production, then a potential for improved stress tolerance exists. The result here was not overly consistent; however, by varying light intensity and possibly temperature then opportunity exists for quantification and greater characterization of the pigments. The perennial habit of turf also offers a possibility to be harvested with the aim of using the pigments possibly as natural food colorants or light absorbing dyes. The characterization of the impact of UV-B on specific carotenoid content is also a first in turfgrass management and shows how important these pigments are in grass. Reductions in carotenoid content and the publication of chromatograms are a first within the turfgrass research area on carotenoids. As herbicide modes of action
continue to diversify into bleaching and photoinhibition due to damage to photosynthetic apparatus, knowledge of these pigments is required within grasses to offer potential for selective control.

The turfgrass decline in both the UV-B and control chambers is of interest. In summer periods PAR light intensity is far higher than was found in these studies and so turfgrass undergoes a daily stress of photo-inhibition. This low light however is a problem which occurs when using growth chambers, depending on the grass, turfgrasses may still be susceptible to photoinhibition in chambers. The photoinhibition can vary between rapidly repaired dynamic photoinhibition to chronic photoinhibition leaving turfgrass with a reduced capacity for optimal productivity. This is particularly true for C3 turfgrasses, and during the period of the year when opportunities for carbon sequestration are greatest due to higher light levels. This undoubtedly plays into a loss of quality during stressful periods of the year. It also may reduce turfgrass plant’s competitive ability with weedy grasses and further work is currently ongoing looking into this acclimation during seasonal changes in light.

The difference between the quality of grasses that have a shiny leaf surface and thicker leaves compared to the thin-leaved bentgrasses is also of interest. Perennial ryegrass and tall fescue which are considered to have shiny leaf surfaces were not as affected as the bentgrasses and so reflective characteristics of the leaf surface may be important. Limited attention has been paid to turfgrass leaf epidermal content changes in high light conditions. This may become more of an interest going forward as new herbicides aim for selectivity with greater potential for damage to target species due to an
ability to penetrate leaf surfaces. It is also of concern for herbicide use in summer periods where potential damage to non-target species can occur due to changes in epidermal surfaces. Reducing the impact of higher UV-B levels becomes more important from a plant health standpoint with the likely increase in levels penetrating the ozone in the next ten years (2010-2020).
APPENDIX A: HANDHELD REFLECTANCE MEASUREMENTS OF TURFGRASS TISSUE
APPENDIX B: COLOR OF LEAVES OF CREEPING BENTGRASS (Agrostis stolonifera L.) TISUE AFTER EXPOSURE TO UV-B LIGHT
APPENDIX C: CHROMATOGRAM CAROTENOIDS STANDARDS

158
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<th>#</th>
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APPENDIX D: CHROMATOGRAM CHOKEBERRY *ARONIA*
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APPENDIX E: CHLOROPHYLL A CONTENT AFTER D7 UV TREATMENT
CREEPING BENTGRASS ‘L-93’
Chlorophyll A Creeping bentgrass 'Penncross'

Chlorophyll A content (µg m⁻²) vs Day

- UV
- CTRL
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APPENDIX G: TALL FESCUE CAROTENOID CHROMATOGRAM
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